Plenary Session 1

Traumatic brain injury / spinal cord injury – achievements and failures

1. Spinal cord injury – achievements and failures (Invited lecture)

C.H. Tator (Toronto, CDN)

Patients with spinal cord injury (SCI) now receive dramatically improved diagnostic tests and treatment, and most attain a near normal life span. Achievements include better first aid, organized units for acute care and rehabilitation, better understanding of the underlying pathophysiology, including the preliminary and secondary injury, precise imaging of the spinal cord and column with MRI and CT, more effective pharmacotherapy and surgical techniques for decompression and fusion, and increased attention to prevention of complications. Unfortunately, our failures remain so great that most patients with major SCI recover very little neurological function. There are still major deficiencies in our knowledge of the pathophysiology of SCI, and disappointing results with current pharmacotherapy. Many promising treatments in laboratory animals fail to achieve similar results in humans, and there have been insufficient randomized control trials. Although safe and effective surgical treatments are available, none has been proven to enhance neurological recovery partly due to the lack of well designed clinical trials. Finally, regeneration of lost neurons or axons in human SCI remains an unrealized goal. However, there is good reason to believe that these deficiencies can be overcome.

2. Traumatic brain injury – achievements and failures (Invited lecture)

G.M. Teasdale (Glasgow, UK)

The last decades of the 20th Century saw unprecedented advances in knowledge of head injuries and their impact on the brain. The achievements include recognition of the immense scale of the problem in the acute stage and the nature of the long-term sequelae, the clarification of different mechanisms and patterns of injury through interaction between biomechanical and structural investigations, widespread acceptance of simple standard methods for assessment of severity and outcome, the identification of potentially avoidable mortality and morbidity, secondary insults and how targeting of treatment against these, leads to improved outcome. The failures to some extent, are the converse of the achievements. Prevention of injury is possible but efforts are patchy, inadequate and incomplete. The substantial understanding gained from experimental work of the events at the molecular and cellular level has yet to be translated into undoubted clinical benefit. Severe head injuries are managed vigorously, intensively, with increasing sophisticated approaches yet rigorous objective data to establish an evidence based approach is in short supply. Perhaps the main achievement, and the key weapon in attacking failures, is the increasing collaboration translating into interdisciplinary and multidisciplinary research efforts, so clearly expressed in International Neurotrauma Symposia.

Regular Session 1

Cell and gene therapy, stem cell biology

3. Neurotrophic factors for motoneurons (Invited lecture)

M. Sendtner, M. Digby, U. Schweizer, J. Gunnersen, G. Pei, B. Holtmann, S. Wiese (Wuerzburg, D)

In higher vertebrates, motoneurons are generated in excess during embryonic development, and a significant proportion of the newly generated cells that have made functional contact with their target field are eliminated. During development and postnatal life, motoneurons are maintained by neurotrophic factors which are produced by skeletal muscle and glial cells. A variety of such factors which are members of various gene families have been identified. Gene inactivation by homologous recombination has shown that these factors play together in supporting survival of these cells, both during embryonic and postnatal development. Mice in which factors of the CNTF/LIF/CT-1 gene family or corresponding receptor components are eliminated have shown that these factors are important for developmental survival of significant proportions of these cells. Surprisingly, cell type-specific elimination of STAT-3, a major signal transducer of CNTF/LIF/CT-1 action in neurons, does not enhance developmental motoneuron degeneration. This suggests that alternative signaling pathways are important for survival of embryonic motoneurons. Investigations with isolated motoneurons, sensory and sympathetic neurons have shown that exposure of these isolated neurons to neurotrophic factors (neurotrophins or CNTF) leads to a more than 25fold upregulation of members of the IAP/ITA family. The avian ITA is homologous to the baculoviral and mammalian inhibitor of apoptosis (IAP proteins), which can prevent apoptosis by inhibition of specific caspases. Overexpression of ITA in primary neurons supports survival of these cells in the absence of neurotrophic factors, and ita antisense constructs inhibited NGF-mediated survival. These data indicate that the upregulation of members of the IAP/ITA family is an essential signaling event for survival of primary neurons in response to neurotrophic factors.

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4. Building brains – from stem cells to synapses (Invited lecture)
R. McKay (Bethesda, USA)

The identification of stem cells in the fetal and adult mammalian brain has many scientific and clinical consequences. The evidence for a common stem cell generating the central and peripheral nervous system (CNS+PNS) will be presented. As stem cells can be obtained in large numbers they provide ideal systems to analyze the pathways that control fate choice. Available data suggest that both contact dependent and soluble signals control the differentiation of stem cells. The similarity between these mechanisms in fetal and adult stem cells will be discussed. Data will be presented showing that embryonic stem cells can be manipulated to generate CNS stem cells and the terminal neuronal and glial fates of the CNS. It is important to determine if stem cells give rise to functional neurons. Neuronal differentiation is a complex process that requires interactions between different cells over several days. The events controlling the different steps in neuronal differentiation have been defined in tissue culture. We have defined the effects on neurons of identified signals released by glial cells. When neurons interact with glial cells neurotrophins activate glutamatergic and GABA-ergic synapses. Neurotrophins have the same effects on excitatory and inhibitory synapse activation on neurons derived from hippocampal stem cells and on cultured hippocampal neurons that differentiate directly without stem cell expansion. This result shows that stem cells can generate synaptically active neurons and suggests that hippocampal stem cells generate the appropriate neuron types. These results show that the events controlling the birth and death of neurons are increasingly understood. The clinical potential for this technology is increasingly recognized. In our group we have focussed on clinical models of neurodegenerative diseases. Experiments in tissue culture and in animal models will be used to illustrate how control of the origin of neuronal and glial cells will give new insight into Parkinson's disease, Alzheimer's disease and demyelinating disease. Our knowledge of CNS stem cells and later steps in neuronal differentiation may be used to develop cell therapies but will also have an important impact on new pharmacological treatments for brain disease.

5. Preferential regeneration of axons of large retinal ganglion cells into peripheral nerve grafts in adult mice, and enhancement of regrowth by ciliary neurotrophic factor
Q. Cui, A.R. Harvey (Perth, AUS)

We describe an adult mouse model of CNS regeneration in which autologous peripheral nerve (PN) is transplanted onto transected optic nerve. We examined 1) whether intravitreal injection of ciliary neurotrophic factor (CNTF) increased the number of regrowing retinal ganglion cells (RGCs) and 2) what types of RGCs regenerated axons into grafts. All surgery was carried out under halothane anesthesia. The survival time was 3 weeks. Regenerating RGCs were retrogradely labeled with fluorogold (FG) which was applied at the distal end of the PN grafts 2 days before sacrifice. In mice with temporal CNTF eye injections there was a 2.5-fold increase in the number of fluorogold (FG)-labeled regenerating RGCs (mean=342; n=6) compared with sham-injected mice (mean=133; n=8). This increase in RGC number was mostly restricted to temporal retina, suggesting that CNTF had localized growth-promoting effects after intravitreal injections. Consistent with this, greater numbers of regenerating RGCs (mean=1198; n=8) were seen across the entire retina in mice that received both nasal and temporal CNTF injections. The majority of cells that regrew axons into PN grafts, especially in the middle and peripheral parts of the retina, had large somata and dendritic fields, probably corresponding to type-I RGCs. Most of the smaller regenerating RGCs (presumably type II and III) were located more centrally, in the vicinity of the optic disc. Compared with the smaller RGCs, we estimated that type-I cells were at least 10-15 times more likely to regrow an axon into a PN graft, indicating a heterogeneous response to axotomy in the adult mouse visual system. These data thus provide a baseline for further studies, utilizing genetically modified mouse strains, aimed at discovering factors/genes that are important in axon regeneration in the adult mammalian CNS.

6. Intrathecal administration of epidermal growth factor and fibroblast growth factor 2 promotes ependymal proliferation and functional recovery after spinal cord injury in adult rats
A. Kojima, C.H. Tator (Toronto, CDN)

To investigate the effect of epidermal growth factor (EGF) and fibroblast growth factor 2 (FGF2) on spinal cord injury (SCI), we administered EGF, FGF2, EGF plus FGF2, or artificial cerebrospinal fluid (aCSF) intrathecally at a flow rate of 0.5 ul/hr (15 ng/hr of EGF or FGF2) after mild (2.4 g) or moderate (20 g) clip compression injury at T1 in adult rats for 3 or 14 days (n=3-7/group). Bromodeoxyuridine (BrdU) was injected intraperitoneally daily, and the proliferative activity in the ependyma at T1 was evaluated by the BrdU labeling index (LI). Functional recovery was assessed weekly by the Basso, Beattie, and Bresnahan (BBB) rating scale. Histological assessment after moderate injury was based on the percent cavitation at the epicenter. At 3 days after

mild or moderate injury, there were no significant differences in LI among groups. At 14 days after mild injury, the LI in the ependymal cells in the EGF plus FGF2 group (46.1 ± 9.1 %) was significantly higher than in the aCSF (16.6 ± 4.8 %), EGF (19.4 ± 2.8 %), or FGF2 (19.6 ± 4.8 %) groups ($p<0.05$). At 14 days after moderate injury, the LI in the ependymal cells in the EGF plus FGF2 group (42.6 ± 6.5 %) was significantly higher than in the aCSF group (20.5 ± 5.6 %) ($p<0.05$). As well, the percent cavitation in the EGF plus FGF2 group (18.2 ± 3.8 %) was lower than in the aCSF group (35.0 ± 10.4 %), although the difference was not significant ($p>0.05$). In the moderately injured groups at 3 weeks, the BBB score in the EGF plus FGF2 group was higher than in the aCSF group ($p<0.05$). Thus, the intrathecal administration of EGF plus FGF2 for 14 days promoted the expansion of ependymal cells and functional recovery after SCI in adult rats.

8. Spinal cord progenitor cells – regulation by growth factors and injury
P.J. Horner, L.L. Horky, B.R. Benish, F.H. Gage (La Jolla, USA)

We have identified a highly proliferative and abundant progenitor population in the intact spinal cord. These cells express NG2 and represent 40-50% of all BrdU incorporating cells in the adult intact spinal cord. In the present experiments we show that this population responds to epidermal and fibroblast growth factor (EGF and FGF) differently. Adult Fisher rats were given 12 single daily injections of BrdU (50mg/Kg) and then sacrificed 1 day or 4 weeks later. At the beginning of the experiment, animals were fitted with mini-osmotic pumps and catheters into the lateral ventricle. These two week pumps contained either EGF, FGF, or artificial CSF. At 1 day, FGF increased proliferation of ependymal cells and also the survival of APC expressing oligodendrocytes in the central region of the spinal cord. EGF increased proliferation of ependymal cells, NG2 expressing progenitors and APC expressing glia throughout the spinal cord. By 4 weeks, significant loss of APC cells occurred in the EGF but not FGF treated rats. In a group of animals, a dorsal hemisection was performed at T8 at day 1. Rapidly proliferating cells were lost at 1 day post-injury and with only limited regrowth at 4 weeks. These data indicate that progenitor cells in situ can be manipulated with exogenous growth factors, but that these cells may be exquisitely sensitive to injury. (Supported by Christopher Reeve Paralysis Foundation, The Lookout Fund, and NO1-NS-6-2348.)

9. Spontaneous corticospinal axonal plasticity and functional recovery after adult CNS lesions
N. Weidner, A. Ner, N. Salimi, M.H. Tuszynski (Regensburg, D; San Diego, USA)

It is commonly believed that little spontaneous structural and functional plasticity occurs after injury to the adult mammalian central nervous system. Nonetheless, various degrees of spontaneous functional recovery are not uncommon after acute human brain or spinal cord injury. To investigate potential naturally-occurring compensatory mechanisms for CNS injury, lesions of defined components of the corticospinal motor pathway were made in adult rats in the rostral cervical spinal cord or caudal medulla. Following lesions of more than 95% of the corticospinal motor pathway, functional recovery occurred that was paralleled by a spontaneous and significant increase in the number of corticospinal axon terminals on medial motoneuron pools in the cervical spinal cord. Motor performance highly correlated with the formation of new connections. These findings demonstrate the presence of significant structural and functional plasticity after injury to the adult central nervous system. Spontaneous plasticity mechanisms may be useful targets for enhancing recovery after adult CNS injury. (Supported by Canadian Spinal Research Organization, Swiss Institute International de Recherche en Paraplegie, and the NIH.)

Regular Session 2
Neurotrauma models

10. Significance of transgenic biology models in neurotrauma research (Invited lecture)
P.H. Chan (Palo Alto, USA)

A rapid increase in the need to explore the molecular basis of cellular function, injury and repair in the brain has led neuroscientists to employ transgenic and knockout mutant animal technology. Over the past two decades, studies involving transgenic and knockout mutant mice have led to insights into the regulation of gene expression, the process of tumorigenesis and sexual alteration, the functioning of the immune system, and the molecular basis of cystic fibrosis, aging and Alzheimer's disease. With the development and availability of these animals, mechanisms of signaling pathways, neuronal injury, apoptosis and infarction have been elucidated in cerebral ischemia and trauma in the past decade. The majority of the studies involved transgenic and knockout mice of CuZnSOD, mitochondrial manganese SOD (SOD2), nitric oxide synthases, and genes involving receptor function and inflammation. We have demonstrated

that transgenic mice and rats that overexpress SOD1 are resistant to neuronal injury after transient focal and global cerebral ischemia, whereas Sod1 and Sod2 knockout mice are highly susceptible to ischemic infarction and neuronal apoptosis. In trauma studies, we have demonstrated that overexpression of human CuZnSOD (SOD1) reduced edema and infarction, improved motor functions and alterations of c-fos and Hsp70 mRNA expression in mice after contusion injury. Reduction in brain edema, blood-brain barrier (BBB), extravasation of Evans blue, lesion volume and neurological deficits, as well as reduction in the activation of metalloproteinases, occurred in SOD1 transgenic mice compared with wild-type littermates. These data have led us to suggest that transgenic animals are extremely useful tools for elucidating cellular and molecular mechanisms underlying signaling pathways, neuronal death, BBB alterations and neurological deficits in brain trauma.

11. Spinal cord injury induced in rat by local injection of colchicine

P. Zanoli, M. Baraldi (Modena, I)

The intraspinal cord (i.s.c.) injection of colchicine (2-5 ug/rat) at the lumbar level induces urine retention and bladder hypertrophy together with hindlimb paralysis. The onset of these phenomena occurs 24-48 h after the injection. A slow spontaneous recovery leads to normal bladder contraction within 4 weeks; also a functional improvement from sensorimotor deficit occurs in the same time. Hence this experimental model was used as a tool to study the neurochemical mechanisms of the impairment in the micturition reflex and in the sensorimotor function of hindlimb, as well as to test new pharmacological treatments. Several neuropeptides have been shown to modulate the micturition reflex in the spinal cord : we described a dramatic reduction in the level of Substance P in the lumbar tract of spinal cord of colchicine treated rats in comparison with controls. In parallel we found a reduced presence of the NMDA-related receptors labeled by 3H-TCP and of sigma receptors labelled by 3H-SKF 10047 associated to an increased presence of calcium binding sites, labelled by 3H-PN200-110, suggesting hence an alteration in the glutamatergic system in this model. From the pharmacological point of view, we tested the ability of different drugs to improve the recovery of bladder activity and of sensorimotor function, by means of cystometrographic analysis and of a sensorimotor test battery, carried out on different days after colchicine injection. In rats i.s.c. treated with 2 ug of colchicine, the administration of Nerve Growth Factor and monosialoganglioside GM1 normalizes urine output within 3 days and improves recovery of bladder contraction. In rats i.s.c. treated with 5 ug of colchicine, we demonstrated that, while NMDA competitive antagonist MK 801 failed to exert a positive effect, calcium channel antagonist (PY 108-068) strongly facilatate the sensorimotor recovery of colchicine treated rats.

12. Morphology of traumatically induced axonal injury in a biological model

D. Spagnoli, G. Tomei, L. Bello, N. Grimoldi (Milan, I)

Diffuse axonal injury is a well-recognized feature of traumatic brain injury. To provide more insights on the pathogenesis of early response of the axon to the traumatic insults we investigated the electron microscopic finding in a biological model (optic nerve of the guinea pig)of focal axonal injury. Specimens of the optic nerves were taken from animals killed after 6,12,24 hours and one week from injury. The temporal axonal modifications observed in our model were as follows: 1) early ultrastructural modifications (6th hr) at the level of cytoskeleton elements: the microtubules and neurofilaments were partly fragmented and bent on their-self with loss of their normal orientation. Later on (12th, 24th hr), areas of large accumulation of organelles were accompanied by a complete disintegration of microtubules and neurofilaments at the level of the enlargement documented along the long axis of the axon. At the level of axonal swelling, a detachment and a rupture of the axolemma from the myelin sheath was noticed along a large accumulation (24th hr) of mitochondria, vesicular structures and multivesicular bodies. 2) One week after trauma, along with the presence of axoplasm free myelin cylinders and normal axons, uniform enlargements of axons were noticed. No organelle accumulation were evident and the axon showed the presence of an accumulation of microtubules and neurofilaments that were regularly arranged and longitudinally oriented. The hypothesis that “traumatically induced axonal damage is triggered first by focal intra-axonal change involving the neurofilament subunits” can be confirmed in our biological model of focal axonal injury. The early effect of direct mechanical traumatic strain of the optic nerve axons is a derangement of cytoskeleton elements without impairment of the myelin sheath. The following modification of the axoplasmic transport and organelle accumulation is a direct consequence of this modification.

13. Functional studies of an in vitro trauma injury model – role of gap junctional communication

M.V. Frantseva, Y. Adamchik, D. McFabe, C.C.G. Naus, J.L. Perez Velazquez (Toronto, CDN)

A model of mechanical trauma injury was used to assess the role of gap junctional communication in spreading cell death after the impact damage. Impact

injury was achieved by dropping a weight of 0.137g from a height of 4 mm onto hippocampal organotypic cultured slices. The initial localised impact damage, assessed by propidium iodide fluorescence, spread 24, 48 and 72 hours after the injury and reached 11% cell death at 24 hours and 35% at 72. The gap junctional blockers carbenoxolone (150 μM) and octanol (50 μM) significantly reduced the spread of the cell death in our trauma model. Higher concentrations of octanol (200 μM) were found to be toxic. To determine whether specific connexins (the proteins that form gap junctions) are involved in spreading the damage, we used organotypic brain slices prepared from mice with a mutation for connexin43 (a connexin present mostly in astrocytes). Cell death spread to a similar extent in slices from wild type, heterozygote (that express less than half of connexin43 expressed by wild-type) and knockout mice. Functional alterations in synaptic transmission in the CA1-CA3 pyramidal layers of the hippocampal slice after the impact injury was assessed by electrophysiological field recordings. Synaptic transmission was abolished immediately after the trauma injury in 12 of 14 slices tested, recovered to some extent in 30% of the slices after 24 hours, and was completely lost in all slices at 48 hours after the impact. Even though carbenoxolone did not alter the immediate effects of the injury on synaptic transmission in the slices (n=10), neurotransmission recovered by 24 hours in 80% of the slices and was still preserved in 40% of the slices by 48 hours. These results indicate that gap junctional communication plays a critical role in the spread of trauma-induced injury in brain tissue and that these models of moderate mechanical trauma could be potentially exploited to investigate neuroprotective strategies.

14. Characterization of the neuronal somatic response to axotomy

R.H. Singleton, J.P. Zhu, J.R. Stone, J.T. Povlishock (Richmond, USA)

Traumatic axonal injury (TAI) is a consistent feature of traumatic brain injury (TBI) that contributes to both the morbidity and mortality associated with closed head injury. Although significant data regarding the pathologic progression of traumatically injured axons exist, little is known about the response of the neuronal somata whose axons have been damaged. To address this issue, twenty Sprague-Dawley rats were injured using the well-characterized fluid percussion model (FPI) of TBI. At varying time points postinjury (30m-24h), animals were perfused and their brains sectioned for light and electron microscopic immunohistochemistry. Antibodies to the C-terminus of APP, a well-recognized marker of TAI that has been shown to be more specific and produce less background staining than other antibodies targeting the N-terminus (Stone, et al., (2000). Brain Research. In Press.), were utilized to permit identification of disconnected axons in continuity with their sustaining somata. Upon LM analysis, numerous axotomized neurons were visualized in the lateral thalamic nuclei at all time points, while in the dorsolateral neocortex they were observed as early as 2 hours postinjury. Additionally, in the hilus of the dentate gyrus axotomized neurons were evident as early as 4 hours postinjury. Contrary to expectations, EM analysis of axotomized somata revealed no overt cytoskeletal or nuclear change shortly after injury. Accordingly, double-labeling strategies targeting specific cascades linked to neuronal damage and death were employed to further characterize the somatic reaction to injury. The results of these immunohistochemical studies as well as their implications for the investigation of TBI will be discussed. (Supported by NIH Grants NS 20193 and T32 NS7288.)

15. Dynamic neuronal and glial reactions in the brain to a single exposure to a very short-lasting, intense impulse noise

A. Säljö, K.G. Haglid, A. Hamberger, H.-A. Hansson (Göteborg, S)

A model system was developed for the assessment of effects of a singel exposure to intense impulse noise for as short time as 2 milliseconds. The model system simulate a closed head brain injury but is not associated with any gross injury, nor any skull or neck rotation. Anaestethized rats were affixed to a shelf in a tube, and exposed to intense impulse noise at 198 dB or 202 dB [corresponding to pressure waves with a positive peak 154–240 kPa, duration 2.0–2.5 msec]. No gross damage, nor any bleedings could be seen, neither any light microscopically recognizable brain changes after routine staining from 2 h to 3 weeks. Immunohistochemical examination revealed distinct changes in e.g. the cytoskeleton of nerve cells throughout the brain as well as impaired axonal transport. There was an abnormally increased eexpression of immediate eraly gene products, e.g. c-jun, deposition of amyloid precursor proteins and nerve cell apoptosis. Microglial cells appeared to an increased extent, evident after 18 h and most prominent after 2 days. Hypertrophic astrocytes were seen from 2 days after the exposure, e.g. superficially in the brain cortex, in the hippocampus and in the brain stem. The astrogliosis was extensive after 7 days and remained evident for at least 3 weeks. The blood brain barrier was intact to macromolecules such as Evans blue labelled albumin. Vascular endothelial cells showed altered patterns of junctional proteins and other markers. We conclude that the exposure of rodents to short-lasting impulse noise (pressure

waves) in the absence of skull and neck rotation may be associated with distinct brain cell reactions even in the absence of signs of gross injury or bleedings.

16. Correlation of lesion volume and brain swelling from a focal brain trauma

J. Eriskat, M. Fürst, M. Stoffel, A. Baethmann (Munich, Bonn, D)

Introduction: Brain edema and secondary growth of a traumatic brain tissue necrosis are important manifestations of secondary brain damage, and of prognostic significance in severe head injury. Aim of the current study was to analyze the interdependency of the resulting brain swelling from the size of the focal traumatic lesion.
Materials and methods: Male Sprague-Dawley rats (300±30g) were intubated and mechanically ventilated with halothane/nitrous oxide/oxygen. Following fixation of the skull in a stereotactic frame a trephination was made over the left parietal cortex for induction of a highly standardized cold lesion. Different injury severities were achieved by variing the contact time of the cooled copper-cylinder (Ø 5 mm, -68°C) and the exposed cortex (i.e. 5s, 10s, 15s, 20s, 25s, 30s). Animals were randomized into 12 experimental groups. Hemispheric swelling was measured by gravimetry in groups A1-A6 (n=4-8) 24 hrs after lesion (5s to 30s). In animals of groups B1-B6 (n=5-7) the volume of necrosis was planimetrically assessed in histological serial sections obtained 24 hrs after trauma (5s to 30s). Hemispheric swelling in the latter experiments was calculated as the difference of the histomorphometrically measured volume of the ipsi- and contralateral hemisphere.
Results: In groups A1-A6 hemispheric swelling was increasing with prolonged contact times from 7.7±0.4% (5s) to a maximum of 9.9±0.5% (25s). Longer contact times (30s) were not more effective to increase hemispheric swelling. The contact times and extent of swelling were linearly correlated between 5s and 25s (r^2=0.96, $p<0.01$). The volume of necrosis in groups B1-B6 increased from 35.7±3.7mm³ (5s) to 106.3±10.3mm³ (30s). There was again a linear correlation between contact time (i.e. injury severity) and volume of necrosis (r^2=0.98, $p<0.01$). The histomorphometrically assessed hemispheric swelling in groups B1-B6 ranged from 8.6±1.0% to 9.1±0.7%, yet without a statistical relationship to the contact time (r^2=0.03).
Conclusion: As seen, the lesion volume could be increased in a reproducible manner from 35.7 up to 106.3mm³ by extending the contact times of the cooling device and cerebral cortex. Hemispheric swelling predominantly due to vasogenic brain edema was expanding in relationship with the volume of necrosis. Hemispheric swelling assessed by histomorphometry, however, did not correlate with the trauma severity or hemispheric swelling studied by gravimetry. This indicates a superior sensitivity of the gravimetric vs. planimetric determination of brain swelling. (Supported by BMBF-Verbund Neurotrauma München, FKZ 9030911.)

Regular Session 3
Progressive degeneration, subacute and chronic injury

17. Prion neurotoxicity and neuroinvasion (Invited lecture)

A. Aguzzi, S. Brandner, I. Hegyi, C. Röckl, M.B. Fischer, A. Behrens, M. Glatzel, P. Parizek, M.A. Klein (Zurich, CH)

A wealth of evidence points to the identity of PrPSc with the prion, the transmissi-ble agent causing spongiform encephalopathies (TSEs). To address the question of CNS pathogenesis, we grafted neuroectoderm from mice which overexpress PrPC into the brain of scrapie-resistant PrP-deficient mice, and inoculated it with scrapie prions. Infected grafts developed scrapie and contained high amounts of PrPSc and infectivity, while neighbouring cells remained unaffected. The host life span was not reduced. Therefore, availability of endogenous PrPC to the infectious agent, rather than deposition of PrPSc, correlates with scrapie neurotoxicity in vivo. We then addressed the spread of prions from peripheral sites to the CNS, by trans-planting neuroectoderm from overexpressing PrP to the brain of Prnpo/o recipients. Scrapie was not detected in grafts after intraocular (i.o.), intraperitoneal (i.p.), or subcutaneous (s.c.) inoculation. Immunity to PrP developed in several animals soon after grafting, but anti-PrP titers did not influence the course of the disease after i.c. inoculation, and no transport of i.o. infectivity was detected in animals tolerant to PrP. Adoptive transfer of PrP-expressing bone marrow cells restored prion replication in the spleen, but did not reconstitute neuroinvasion via i.p. route. These results indicate that PrPC supports infectious spread from the periph-ery to the CNS, and imply that neuroinvasion depends on the neuroimmune inter-face. B-lymphocytes are crucial for neuroinvasion, independently of whether they express PrPC or not: B-cell deficient mice resist i.p. inoculation of prions, and in-fectibility is restored upon transfer of PrP-positive or negative B-cells. B-cells sup-port maintenance of follicular dendritic cells by presenting lymphotoxin-a/b trimers to them: interference with this pathway by administration of soluble lym-photoxin-b receptors appears to impair lymphoid replication of

prions. This may indicate a target for post-exposure prophylaxis.

18. Ventricular shunting in an animal model of fetal-onset hydrocephalus – hypothalamic alterations and functional outcome

J.P. McAllister, R.M. Abdolvahabi, J.A. Mitchell, D.M. Lawson, F.G. Diaz, A.I. Canady (Detroit, USA)

Children treated for hydrocephalus exhibit precocious puberty and amenorrhea, but the pathogenic mechanism for these deficits is unknown. Therefore, we studied the effects of progressive hydrocephalus and its treatment on the hypothalamic gonadotropin releasing hormone (GnRH) system and reproductive function. We treated 183 H-Tx rats (established model of perinatal onset hydrocephalus) with ventricular shunts at 6 (early shunts) and 12 (late shunts) days of age. Successfully shunted animals (18 early shunts and 18 late shunts) were sacrificed at 21 or 50 days of age, and compared to age-matched control (n=52) and untreated hydrocephalic (n=14) littermates. In untreated animals, hypothalamic GnRH protein levels were significantly ($p<0.05$) increased at 21 days (9.17 vs 0.97 pg/ng total protein). In addition, serum LH levels in the untreated animals increased 173% at 21 days. However, GnRH immunoreactive fibers were unaffected until 50 days of age. Early and late shunting normalized hypothalamic GnRH and serum LH protein levels by day 50 but not by day 21. Functional studies revealed normal vaginal opening and estrus cycles for animals shunted successfully at day 6 (early shunts). On the other hand, the late shunted group and shunt failures exhibited delayed vaginal opening and estrus cycle irregularities. Therefore, in this model hydrocephalus affects the function of the hypothalamic GnRH system early on without compelling morphological changes. Moreover, early shunting is more effective in restoring reproductive function in the hydrocephalic animals. Damage to the hypothalamic GnRH system may be the common denominator for hydrocephalus associated reproductive abnormalities.

19. Activation of cyclo-oxygenase-2 contributes to cognitive and behavioral dysfunction following diffuse traumatic injury in rats

I. Cernak, C. O'Connor, G.P. Hamlin, R. Vink (Townsville, AUS)

It is now known that much of the brain damage produced by head impact is not the result of the initial trauma, but rather develops over a period of hours to days after the primary event. One of the processes that may play a significant role in the development of delayed brain injury is posttraumatic inflammation. During posttraumatic inflammation, metabolic products of arachidonic acid known as prostanoids are released and exacerbate the injury process. Prostanoid synthesis is regulated by the enzyme cyclo-oxygenase (COX) that is present in at least two isoforms: COX-1, the constitutive form, and COX-2, the inducible form. Expression of COX-2 has recently been shown to be an important determinant of the cytotoxicity connected with inflammation. In this study, we examine the temporal and spatial profiles of COX-2 expression and its importance in motor, cognitive and behavioural dysfunction following diffuse axonal injury (DAI) in rats. Furthemore, the effects of nimesulide, a COX-2 inhibitor, were examined. Adult, male Sprague-Dawley rats were injured using the 2-metre impact acceleration model of DAI. One group of animals received nimesulide (6 mg/kg i.p/.) starting 30 minutes after injury, later administered every day once. Over the 10 days posttraumatic period, motor outcome was assessed by the rotarod test, cognitive deficit was estimated using Barnes circular maze, while behavioural characteristics were observed by open field test. At preselected time points after injury, animals were killed and the expression of COX-2 was measured in parietal cortex, amygdala and hippocampus by Western blotting techniques. After 24 h of DAI, the significant cognitive and motor deficit was attenuated in nimesulide-treated rats, which was comparable with the alterations in COX-2 expression in brain structures. These results indicate the involvement of COX-2 in DAI-induced cognitive and motor dysfunction.

20. Exacerabation of brain injury in the mice lacking the glutamate transporter GLT-1

T. Suzuki, K. Shima, H. Nawashiro, K. Tanaka (Tokorozawa, Tokyo, J)

Our previous studies have demonstrated that neuronal damage after traumatic brain injury (TBI) is caused mainly by massive glutamate release that activates NMDA. In the present study, we used the mutant mice deficient in GLT-1(-/-), an astrocytic glutamate transporter. GLT-1(-/-) mice and controls (GLT-1(+/+)) were subjected to lateral fluid-percussion(FP) brain injury (0.6 atm) or sham injury and sacrificed at 48 hours post-injury. And extracellur glutamate was monitored in real time using an enzyme electrode biosensor following FP brain injury in the mice. Specific hippocampal regions examined were CA1.CA3, the hilus and dentate granular cell layer. Lateral FP injury resulted in the formation of a contusion in the injury cortex. The lesion of cortical injury in mutant mice was a significantly greater than that of control mice. Brain injured mutant mice revealed markedly increased cell loss in be involved in the processes of glutamate-mediated neurotoxicity after TBI.

21. AIF-1 expression defines a proliferating and motile microglia/macrophage subpopulation following spinal cord injury in rats

J.M. Schwab, E. Frei, I. Klusman, L. Schnell, M.E. Schwab, H.J. Schluesener (Tuebingen, D; Zurich, CH)

Microglial cells are of monocytic origin and are among the first and dominant cell type to respond to several types of CNS injury. Following calcium influx, microglial activation leads to a variety of cellular responses, such as proliferation, increased or de-novo expression of immune related molecules and release of cytotoxic and neurotrophic mediators. Allograft inflammatory factor-1 (AIF-1) is a highly conserved, calcium binding peptide, associated with microglia activation processes in the brain. Here we have analyzed the expression of AIF-1 following spinal cord injury within the lesion site and in remote brain regions. Following spinal cord injury, AIF-1+ microglia/macrophages accumulated in pan-necrotic areas confined to the lesion core already at day 1 ($p<0.0001$) post injury, culminated at day 3 and decreased until day 7, a situation characterized by infiltrating blood borne macrophages and lymphocytes. In remote areas of Wallerian degeneration and delayed neuronal death a more discrete and delayed activation pattern of AIF-1+ microglia/macrophages was observed peaking at day 14 ($p<0.0001$). Frequently, AIF-1+ cells co-expressed the proliferating cell nuclear antigen, PCNA, and moved towards a peri-neuronal position. AIF-1 expression preceded phagocytic activity indicated by ED1 expression. Thus it appears, that AIF-1+ microglia/macrophages are the among the earliest cells to respond to spinal cord injury. Our results suggest a role of AIF-1 in the Ca2+ dependent initiation of the early microglial response leading to activation, motility and proliferation constituting an essential part of the acute CNS response to injury. AIF-1 may be essential to modulate microgliosis and therefore be associated with velocity of tissue debris removal, myelin degradation, recruitment of oligodendrocytes and re-organisation of CNS architecture.

22. Depression of early glucose hypermetabolism precedes cortical neuronal death after lateral fluid percussion injury in rats

T. Kuroiwa, X. Jiang, L. Qian, B. Tominaga, T. Nariai, K. Hirakawa, K. Ohno (Bunkyo-ku, Tokyo, J)

We investigated changes in cerebral blood flow and energy metabolism in rats developing cortical neuronal death following traumatic brain injury (TBI). The animals were subjected to lateral fluid percussion injury, local cerebral blood flow (lCBF), glucose utilization (lCGU), and activity of succinic dehydrogenase (SDH) – a mitochondrial enzyme of tricarboxylic acid cycle – were measured over 2 weeks of post-trauma and compared to the histological changes. Post-traumatic brain evolved a transient and diffuse increase in lCGU at 1 h post-trauma (178±15 in the contralateral parietal cortex, % of control), which was followed by a significant decrease at 6 h (78±2) and thereafter. The transient increase in lCGU was depressed in the ipsilateral parietal cortex (65±19), which was followed by a further decrease at 6 h (33±5) and thereafter.. Neuronal death was evident in the ipsilateral parietal cortex at 2 w post-trauma but not in the other cortical areas. lCBF decreased at 1 h, reached the lowest levels in most of the cortical areas at 6 h and remained low over 2 w post-trauma. SDH activity significantly decreased at 1 h post-trauma in most of the cortical areas. SDH activities were the lowest at 6 h and 24 h post-trauma, and gradually returned to the baseline levels. These results indicated that simultaneous development of decreased rCBF, mitochondrial dysfunction via the TCA cycle and depressed glucose hypermetabolism under hyperglycolytic conditions in the early period after injury may produce unfavorable effects on the post-traumatic brain tissue. A therapeutic window for preventing the slowly progressing neuronal death may exist within 6 h after TBI.

22A. Proteasome inhibition – a novel target for anti-stroke therapy

P. Elliott, I. Shah, K. Lees, C. Pien, F. Tortella, J. Adams (Cambridge, USA; Glasgow, UK; Silver Spring, USA)

The inflammatory component of ischemia-induced cellular injury is substantial and well documented. Many of the pro-inflammatory mediators such as cytokines and cell adhesion molecules that are responsible for the development of the cerebral infarct are known to be under the control of NF-kB. The activity of NF-kB is itself tightly regulated through the multi-catalytic enzyme known as the proteasome. PS-519 is a highly selective and novel small molecule that inhibits the proteasome. Preclinical evaluation has shown that PS-519 to be active in multiple animal models of cerebral and cardiac ischemia over a range of doses. Importantly, anti-ischemic activity was seen when the drug was given as an intravenous bolus at the time of reperfusion and even after reperfusion has been established in these models. The toxicity profile of PS-519 following repeat dosing has been completed in rats and dogs and the side effect profile determined to be acceptable for the start of Phase I clinical trials. Furthermore, during the evaluation of PS-519 an ex vivo assay was also developed to allow monitoring of drug activity in both animals and human

volunteers. This assay is being used to guide dose escalation. To date, PS-519 has been given to over 30 normal male volunteers as a single intravenous bolus. No drug-related adverse events have been reported and as such PS-519 appears to be well tolerated. Using the ex vivo assay, doses of PS-519 eliciting significant dose-related inhibition (>50%) of the proteasome have been given without incident. Critically, this level of inhibition is in excess of levels seen in preclinical models in which PS-519 was shown to be active. The further clinical evaluation of PS-519 is planned.

Special Luncheon Seminar 1

Novel developments in safety engineering for car design (sponsored by BMW Group, Dept. Safety Engineering)

LS23. Accident injury prevention – cooperation of medicine and engineering

O. Pieske, G. Lob, W. Lange, G. Messner (Munich, D)

Road traffic accidents are the most common source of severe injuries. Effective injury prevention is only possible if the injury pathomechanism is clarified. Therefore we established an interdisciplinary study involving trauma surgeons (Department of Traumatology, Ludwig-Maximilians-University) and engineers (BMW Department of Accident Analysis) to perform in-depth analyses of severe real world accidents. The results are continuously integrated in the design of future vehicles and safety features. The first part of this presentation demonstrates the technical methods of accident reconstruction and vehicle deformation analysis, and includes the medical evaluation of occupant injuries and kinematics. Methods are explained using the example of a severe side collision resulting from a driver running a red light. The second part of the presentation demonstrates the development of passive safety features in frontal collisions, which is the predominant collision type. From the start, safety belt usage has led to a tremendous reduction in severe injuries. Further intelligent belt modification (e.g., geometry, pre-tensioning and force-limitation supported this positive result. The combination of belt and front airbag has led to the well-accepted standard of accident injury prevention especially with regard to the head and cervical spine, as well as to the chest and abdomen. The third part of this presentation presents occupant kinematics in side collisions, which are known to cause the most severe accident-related injuries to the head/cervical spine and chest. A unique side airbag system, consisting of thorax and head protection system (HPS), will be presented. Standardized side crash tests have demonstrated the beneficial effect of the thorax bag, with a Thorax Trauma Index (TTI) reduction of 30%. To evaluate the effectiveness of HPS, worst case side-impact tests with dummy occupants were carried out both with and without HPS. Test vehicles were accelerated into a rigid pole at a speed of approximately 20 MPH in accordance with a draft test procedure developed by ISO (ISO/CC22/SC10/WG3/N121). In these tests, the HPS reduced the "Head Injury Criterion" (HIC) from 4700 to 600, significantly far below the critical HIC of 1000. In conclusion, the interdisciplinary cooperation of trauma surgeons with the automobile industry can result in effective injury prevention in order to fulfill our basic rule: an ounce of prevention is worth a pound of cure.

LS24. Driver assistance systems

D. Frank (Munich, D)

As a result of improved vehicle safety, the number of deaths in traffic accidents has been substantially reduced in the past two decades. Secondary Safety, which refers to systems intended to reduce accident injuries, has achieved a high level of effectiveness. Additionally, systems for accident avoidance (Primary Safety) have been increasingly installed in vehicles. In this area, however, there is still a great potential for further development. This presentation discusses existing as well as possible future Driver Assistance Systems which should contribute substantially to increased Primary Safety.

LS25. Legal requirements – head impact tests in vehicles

A. Buchauer (Munich, D)

US head impact protection regulations, the most extensive from the legal standpoint, require that during head impact inside the vehicle interior above the door line, a prescribed head acceleration value must not be exceeded. This is specified by the HIC (Head Impact Criterion). The dimensionless HIC value is an index based on the integral of the resultant head acceleration over time and represents a criterion for evaluating the risk of head injury. Using test equipment known as the MGA device, occupant protection testing of vehicle interiors can be performed to ensure compliance with the requirements of Federal Motor Vehicle Safety Standard (FMVSS) Nr. 201. The standard specifies that the test device must protect a free motion headform (Hybrid III dummy head) onto parts of the vehicle interior above the door line at specified angles with a velocity of approximately 24 km/h / 15 mph. The law requires only the testing of certain prescribed surface-related points within the entire upper roof area. However, the

internal BMW goal is compliance with these legal requirements at every conceivable point in this area.

Plenary Session 2
Cell injury and death

26. Mechanisms of tissue injury from acute ischemic insults (Invited lecture)
K.A. Hossmann (Cologne, D)

Acute injury damages the brain in two only partly related ways: directly by the injurious impact, and indirectly by secondary events occurring during the recovery from this injury. In ischemia, the immediate injury is a function of the threshold relationship between blood flow and metabolic disturbances, with energy metabolism ceasing at flow rates below 15% of control. With ongoing ischemia, the threshold of energy metabolism gradually increases until – within 6-12 hours – it merges with the much higher threshold of protein synthesis suppression, indicating expansion of the infarct core into the metabolic penumbra. If the brain is resupplied with blood after ischemia, some of the injury is reversed, depending on the severity and duration of the preceding ischemia, on one hand, and on the quality of reperfusion, on the other. In the absence of no-reflow and/or postischemic hypoperfusion, energy metabolism is restored after normothermic circulatory arrest of at least one hour but delayed post-ischemic injury may evolve in the vulnerable and – with increasing duration of ischemia – also in the resistant parts of the brain. The latency of this injury increases with decreasing ischemia time and, after 30 minute transient MCA occlusion, may be as long as 3 weeks. Delayed neuronal injury during or after ischemia has been attributed, among others, to calcium, zinc or glutamate toxicity, acidosis, free radical-mediated injury, dysfunction of endoplasmic reticulum or mitochondria, programmed cell death or the PARP-mediated injury cascade. However, any of these mediators of delayed death seems to prevail in some but not in other experimental models of brain ischemia. To substantiate this notion, the evolution of ischemic brain injury is presented in four models of focal brain ischemia: permanent and transient MCA occlusion induced by intraluminal suture insertion, and permanent and transient MCA occlusion following clot embolism with or without rt-PA induced thrombolysis. The evolution of injury is evaluated by MRI and multiparametric imaging of blood flow, pH, energy metabolism, protein synthesis, single and double strand DNA fragmentations, edema formation and multiple genomic expression patterns. The data indicate that each of these models presents a different topical pathophysiology which cannot be readily associated with a unified hypothesis of ischemic cell death. These observations explain the poor responsiveness of clinical stroke to unifactorial pharmacological interventions, and stress the need for a multifaceted therapy of ischemic injury.

27. Subcellular and cellular injury vs. tissue damage from acute cerebral insults (Invited lecture)
B.K. Siesjö (Honolulu, USA)

Tissue damage after ischemia or trauma is triggered by events elicited during the acute insult. These usually involve a reduction of the phosphorylation potential (ATP · ADP-1 · Pi-1), release of excitatory amino acids, and loss of ion homeostasis. Key events are activation of surface receptors and influx of calcium into cells. As a result of this, proteins and lipids are degraded, protein phosphorylation is altered, and new genes are expressed. Some of the changes thus elicited, e.g. the induction of enzymes such as iNOS and COX-2, can give rise to delayed changes of an inflammatory and immunological nature. Calcium influx and/or oxidative stress may also alter the membrane properties of mitochondria, making them prone to dysfunction, and to release of mediators of cell death such as cytochrome c or apoptosis-inducing factor (AIF). The important pathogenetic role played by delayed mitochondrial dysfunction is underscored by the effects of drugs or genes that serve to stabilize mitochondrial membranes.

Regular Session 4
Cytokines, role of inflammatory response, immunology

28. Inflammation cells and mediators in central nervous system injury – the Janus face of inflammation in injury and repair (Invited lecture)
G.Z. Feuerstein (Wilmington, USA)

At the onset of the 21st century, stroke is the third leading cause of death in most developed countries and the primary cardiovascular cause of death in Japan and China. The health burden of the disease is staggering as loss of productive life inflicts heavy toll on patients, their families and the society at large. Yet, this disease has no effective therapeutic beyond a limited treatment (<5% of patients) with thrombolytics that carry significant adverse effect such as intracerebral hemorrhages. This situation is prominent in spite of intense research efforts and numerous clinical trials attempting to develop drugs that reduce morbidity and mortality from stroke. So far, the efforts of drug development for stroke targeted modulators of ion channels (Ca+2, Na+), scavengers of oxygen radicals and antagonists of excitotoxic neurotrans-

mitters (primarily the various glutamate and glycine receptors). However, all clinical trials that tested the efficacy of such agents failed so far due to lack of efficacy, adverse effects or other development difficulties. Debates on the reasons for this grim reality have been a focus of intense debates in numerous meetings where the possible opinions for failed trials cited: 1. wrong animal models; 2. wrong mechanism of action; 3. poor clinical design; 4. inadequate 'therapeutic window' in preclinical studies and 5. Pharmacokinetics issues. While this debate is ongoing, the stroke research community seems to have been disenchanted from the 'classical' targets for drug development (vide supra) as evidenced by the emerging hope that other avenues such as 'reconstruction', 'apoptosis' and 'inflammation' are better pastures from which new and promising molecular targets will yield the expected therapeutic success. In this brief report, the role of inflammation in ischemic brain injury will be reviewed with special attention to both detrimental and salutary role the inflammatory reaction may carry.

29. Inflammatory response to traumatic brain injury – beyond the darkside (Invited lecture)

P.M. Kochanek, R.S.B. Clark, C.E. Dixon, T.M. Carlos, L.W. Jenkins, S.T. DeKosky, S.H. Graham, P.D. Adelson, D.W. Marion (Pittsburgh, USA)

The perspective of clinicians and scientists on the role of the inflammation in mediating secondary damage after traumatic brain injury (TBI) has changed greatly over the past 20 years. The traditional view of the brain as an immunologically privileged site, even after injury, has evolved through several phases. Evidence will be presented showing that a number of laboratories, including our own, have shown a broad array of robust inflammatory reactions to TBI-particularly near the injury site. Subsequently, as reflected by reports of beneficial effects of inhibiting selected aspects of the inflammatory cascade, a number of laboratories have shown that inflammation mediates secondary damage. However, recent studies by our group and others suggest that the role of the inflammatory response to TBI is broad in scope and involves a complex interplay of deleterious and beneficial effects-including key links with regeneration/plasticity. Recent studies also indicate that for specific inflammatory mediators, issues related to timing, dose and location critically influence whether detrimental or beneficial effects are produced. Putative clinical strategies for optimal manipulation of the inflammatory response will be discussed. Support: NS 30318 from NINDS, the University of Pittsburgh CIRCL/CDC, and the Laerdal Foundation.

30. Toxic effects of tumor necrosis factor alpha are enhanced by reactive oxygen species

V. Trembovler, S. Abu-Raya, P. Lazarovici, E. Shohami (Jerusalem, IL)

Tumor Necrosis Factor (TNFa) plays a dual role in the pathophysiology of brain injury. Whereas long-term post traumatic damage in TNFa receptor (-/-) mice is greater than in the corresponding wild type, early post-injury pharmacological inhibition of TNF was shown to be beneficial. We have proposed that within the early post-injury phase, the excess of TNFa is deleterious since it activates toxic signals in concert with other mediators such as reactive oxygen species (ROS) which accumulate within minutes after injury. In this study we compared the toxicity of oxygen glucose deprivation (OGD) to that of TNFa and ROS, acting alone or in combination, in a model system of PC12 cells. The protective effects of the multi-mechanistic dexanabinol, (HU-211) acting as NMDA antagonist, TNFa inhibitor and antioxidant was examined. PC12 cells were exposed for 4h to glucose oxygen deprivation followed by 18h of reoxygenation (OGD), to TNFa (1-100 ng/ml), to H2O2 (1-300 mM) or to both mediators, each at sub-toxic concentrations. The three insults (OGD, TNFa, H2O2) in a dose-dependent manner induced release of LDH, indicating cell death, and accumulation of PGE2, suggesting activation of COX2, an NF-kB-regulated enzyme. A linear correlation (R=0.836) was found between the levels of LDH and PGE2 in 16 different combinations within the concentration range of 0-25ng/ml and 0-300 mM for TNF and H2O2 respectively. TNFa exerts toxicity above 50 ng/ml, and H2O2 above 150 mM, however, when together, much lower levels (10-20 ng/ml and 30 mM, respectively) were shown to induce toxicity, similar to that produced by OGD. HU-211 effectively protected the cells, with significant inhibition of LDH leakage accompanied by reduction in PGE2 release. These findings corroborate our previous report that ROS inhibit TNFa-mediated NF-kB activation and support the hypothesis on the cooperative toxic effect of TNFa and ROS, which are both elevated within minutes to hours after brain injury.

31. Upregulation of IL-8 / MIP-2 and ICAM-1 after severe traumatic brain injury occurs by distinct inducers and with different kinetics

V.I. Otto, P.F. Stahel, M. Rancan, O. Trentz, T. Kossmann, M.C. Morganti-Kossmann (Zurich, CH)

Blood-brain-barrier (BBB) disruption after traumatic brain injury (TBI) is mediated by neutrophil accumulation, release of chemokines (e.g. IL-8) and expression of adhesion molecules (e.g. ICAM-1). In patients with severe TBI, we have previously shown

that elevated cerebrospinal fluid (CSF) IL-8 and soluble (s)ICAM-1 are associated with BBB dysfunction. In this study, we analyzed the upregulation of IL-8 / its murine analogue MIP-2 and of ICAM-1 with regard to the inducers and kinetics in three systems, (1) patients with severe TBI, (2) mice lacking the genes for tumor necrosis factor (TNF) / lymphotoxin-alpha subjected to experimental closed head injury, and (3) cultured murine microvascular endothelial cells (MVEC) and astrocytes, both cell types being part of the BBB. In CSF of 7 patients, IL-8 and sICAM-1 were elevated over 19 days after severe TBI, while their common inducer TNF exceeded normal values on 9 of 19 days. In brain homogenates from TNF/LT-alpha -/- and wild type (wt) mice, MIP-2 levels were increased 4h after TBI returning to base line after 7 days while ICAM-1 expression was only elevated at day 7. The ICAM-1 levels in wt exceeded those in TNF/LT-alpha -/- mice. In vitro stimulation of astrocytes and MVEC with TNF simultaneously induced the release of MIP-2 reaching saturation by 4-8h and of sICAM-1 increasing continuously from 2-4h to 12h. Augmented sICAM-1 production correlated with enhanced membrane-bound (m)ICAM-1 expression. In addition, sICAM-1 induced MIP-2 secretion by astrocytes and MVEC rising steadily from 2h to 12h. When compared to induction by TNF, MIP-2 secretion triggered by sICAM-1 was slower, but more abundant. In conclusion, TNF seems to be an important inducer of the late upregulation of ICAM-1 after TBI, concomitantly affecting its membrane-bound and soluble form. For MIP-2 upregulation after TBI, TNF seems to play a minor role. Our in vitro data suggest that instead, sICAM-1 may be a major inducer of IL-8 / MIP-2.

32. The effect of additional brain injury on the inflammatory mediator response after trauma
T. Hensler, B. Bouillon, D. Rixen, M. Raum, E. Neugebauer (Cologne, D)

Different mediators of inflammation are systemically detectable after trauma and may help to assess the patients immunological situation for further therapeutic treatment. A prospective clinical study was undertaken to assess the influence of additional brain injury on systemic interleukin (IL)-6, IL-10, soluble tumor necrosis factor receptor (sTNFR) and procalcitonin (PCT) concentrations. 36 patients with an isolated severe head trauma (SHT), 49 patients with SHT and polytrauma and 52 patients with polytrauma without SHT were included in our study. Blood samples were taken on day 1 to 10 (additional samples every 6 hours during the first 3 days), and on days 14, 21, and 28. As for control the blood samples from 34 healthy blood donors were analysed. ELISA-technique was used to detect the plasmatic IL-6, IL-10 and sTNFR-levels and an immunolu-minometric assay was used for PCT-detection. IL-6, IL-10 and the sTNFRp55/p75 ratio were significantly elevated in all 3 injury groups within 3 hours of trauma. Lowest initial levels were detected in patients with an isolated SHT (Injury severity score; ISS: 18.1±5.6). No relevant difference could be found for polytrauma patients with (ISS: 35.3±9.6) or without additional SHT (ISS: 25.5±11.7), though relevant differences in the ISS. Maximum PCT-values from 12-54 hours after trauma were found in patients with multiple injuries without SHT (106 ng/ml), with additional SHT (21 ng/ml) and to a minor degree in patients with an isolated SHT (10 ng/ml). PCT-levels were highest in patients with an ISS greater than 40 points. We found a high correlation of PCT to sTNFRp75 (r=0.54), to sTNFR p55 (r=0.52) and to the development of MOF (r=0.33). Our data clearly demonstrate that IL-6, IL-10 as well as the sTNFRs are directly elevated in trauma patients with no effect of additional SHT. PCT is a good predictor for posttraumatic MOF and correlated best with high sTNFR-levels (Supported by the German Ministry of Education and Research (FKZ 01 KO 9517).)

Regular Session 5
Metabolism, vascular mechanisms

33. Function and derangements of cerebral energy metabolism in brain injury (Invited lecture)
D.A. Hovda (Los Angeles, USA)

Following traumatic brain injury, changes in cerebral metabolism were thought to be relevant primarily in terms of reflecting the decreases in neurological functions. Consequently, the rationale for monitoring cerebral metabolism after injury was to notify physicians of potential secondary insults associated with cerebral ischemia and/or hypoxia. However, recent studies suggest that the metabolic demands of the brain following injury reflects an ongoing injury process as well as provides important insight into the degree and extent of cellular vulnerability. There is now considerable evidence indicating that the ionic fluxes induced by trauma to the brain contribute to t dynamic changes in local cerebral metabolic rates for glucose as well as reducing the ability of cells to respirate oxygen. Taken together with the uncoupling of cerebral blood flow to metabolism, these events place cells which have survived the initial insult into a state of energy crisis making them vulnerable to secondary insults. A brief review of this concept of trauma-induced energy crisis will be presented with specific emphasis on regional measurements in both animals and patients utilizing positron emission tomography following traumatic brain injury.

(Supported by NS30308, NS27544 and the Lind Lawrence Foundation.)

34. Brain damage in neurotrauma – role of perfusion and metabolism (Invited lecture)

M. Ginsberg, L. Belayev, W. Zhao, Y. Liu, O. Alonso, R. Busto (Miami, USA)

We have applied advanced 3D autoradiographic image-processing methods to study derangements of local cerebral blood flow (LCBF) and glucose utilization (LCMRglu) in a rat model of moderate parasagittal fluid-percussion traumatic brain injury (TBI). One hour post-trauma, ipsilateral LCBF is moderately depressed while LCMRglu is elevated by ~1.5-fold in many cortical and subcortical sites. This results in striking elevations of the metabolism/flow ratio ("uncoupling").(1) Sites of uncoupling correspond to regions of histological vulnerability (e.g., hippocampus, cortex). Our recent studies have shown that moderate hypothermia (30°C x 3h) (2) and high-dose human albumin therapy (2.5 g/kg) are both neuroprotective in this model. However, their effects on glucose metabolism and blood flow differ. Hypothermia post-TBI generally fails to affect LCMRglu but significantly reduces LCBF (relative to the 37°C condition), thereby accentuating uncoupling. (3) By contrast, albumin therapy significantly reduces (by ~½) the metabolism > flow uncoupling otherwise expected at 60 min post-TBI. On the basis of this pathophysiological distinction, we suspect that albumin therapy may prove to be more efficacious than hypothermia in the therapy of TBI. Finally, we have shown that, 2 months after TBI, ipsilateral forebrain LCMRglu is still suppressed by ~40-45%, and whisker stimulation fails to induce the expected metabolic activation of cortex or thalamus ipsilateral to prior trauma.(4). (Supported by NS 05820 and NS30291.)

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35. Superoxide generation links NOC/oFQ release to impaired NMDA cerebrovasodilation after newborn pig brain injury

W.M. Armstead (Philadelphia, USA)

Although N-methyl-D-aspartate (NMDA) receptor activation contributes to altered cerebrovascular regulation following brain injury, the effects of such injury on the vascular response to NMDA itself is uncertain. The opioid nociceptin/orphanin FQ (NOC/oFQ) elicits pial artery dilation in a prostaglandin dependent manner and is released into CSF following fluid percussion brain injury (FPI). Generation of superoxide anion (O-2) occurs after FPI and a byproduct of cyclooxygenase metabolism is the generation of O-2. This study was designed to determine whether NOC/oFQ generates O-2, which, in turn, could link NOC/oFQ release to impaired NMDA induced dilation after FPI in piglets. Superoxide dismutase (SOD) -inhibitable nitroblue tetrazolium (NBT) reduction was determined as an index of O-2 generation. Under non brain injury conditions, topical NOC/oFQ (10-10M, CSF concentration after FPI) increased SOD-inhibitable NBT reduction from 1±1 to 20±3 pmol/mm². Indomethacin (5 mg/kg iv) blunted such NBT reduction (1±1 to 6±2 pmol/mm²) while the NOC/oFQ receptor antagonist, [F/G] NOC/oFQ (1-13) NH2 (10-6M) blocked NBT reduction. [F/G] NOC/oFQ (1-13) NH2 and indomethacin also blunted the NBT reduction observed after FPI (1±1 to 15±1 vs 1±1 to 4±1 pmol/mm² for sham and NOC/oFQ antagonist treated animals). NMDA (10-8, 10-6M) induced pial artery dilation was reversed to vasoconstriction following FPI and [F/G] NOC/oFQ (1-13) NH2 attenuated such vasoconstriction (sham, 9±1 and 16±1 vs FPI, -7±1 and -12±1 vs FPI – [F/G] NOC/oFQ (1-13) NH2 pretreated, -2±1 and -3±1%). Indomethacin, polyethylene glycol SOD and catalase also partially restored NMDA dilation. These data show that NOC/oFQ, in concentrations present in CSF following FPI, increased O-2 production in a cyclooxygenase dependent manner, contributes to such production after FPI, and contributes to impaired NMDA dilation after FPI. These data suggest, therefore, that cyclooxygenase dependent O-2 generation links NOC/oFQ release to impaired NMDA induced cerebrovasodilation after brain injury.

36. Hyperglycolysis following human traumatic brain injury as demonstrated by a modified Kety-Schmidt Method

T.C. Glenn, D.F. Kelly, P.M. Vespa, M. Oertel, D.A. Hovda, S. Matharu, M. Etchepare, N.A. Martin (Los Angeles, USA)

Diminished cerebral oxidative metabolism is common in patients after severe TBI. In order to maintain energy production it is postulated that cells will increase anaerobic glycolysis resulting in periods of hyperglycolysis. To test this hypothesis, 22 TBI patients enrolled from August 1988 through April 2000 (median initial GCS 7, M:F 17:5, mean age 36±14) were serially studied (mean 5±3 days) with daily arterial and jugular venous samples and 133Xenon CBF studies to determine cerebral metabolic rates (CMR) of oxygen and glucose. Hyperglycolysis was

defined in absolute and relative terms: CMRglucose >7.72 mg/100g/min (historical mean + 2 SD), or a decreased metabolic ratio (MR) CMRO2/CMRglucose <0.58 (historical mean – 2 SD), respectively. CMRO2 was below normal (<3.0 ml/100g/min, historical mean – 2 SD) in all patients (mean =1.25±0.4, n=93 samples). Conversely, 6 patients (27.3%) exhibited at least one episode of absolute hyperglycolysis (mean 10.7±2.8 mg/100ml/min). In 5 of these patients this occurred within 65 hours after injury. CMRglucose was negatively correlated with time after injury r= -0.23, p=0.03, Pearson correlation. All patients except one had a minimum of a single episode of relative hyperglycolysis; 65.9% of all calculated MRs were less than 0.58 (n=90 calculations). Neither MR nor CMRO2 correlated with post-injury time. However, CMRO2 did correlate with GCS at the time of study and 6 month Glasgow Outcome Score (GOS, n=17 patients), R=0.48, p=0.001, and R=0.40, p=0.001, respectively, Spearman correlation. Yet, 4 of the 6 patients with absolute hyperglycolysis had a poor outcome and the one patient without either absolute or relative hyperglycolysis had a 6 month GOS of 5 (good recovery). In conclusion, global hyperglycolysis is a frequent finding following TBI. This confirms our previous P.E.T. findings in TBI patients that showed acute periods of hyperglycolysis (Bergsneider, 1997). It remains to be determined if the acute state of glucose utilization is a determinant of outcome. (Supported by NS30308.)

37. Microcirculatory changes in focal cerebral ischemia and reperfusion

J. Beck, J. Eckhoff, A. Baethmann, E. Uhl (Munich, D)

Introduction: A compromised perfusion and inflammation contribute to secondary brain damage from ischemia or trauma. Objective of the current study was to assess the microcirculatory changes in the early reperfusion period after focal cerebral ischemia (FCI) including leukocyte-endothelium interactions (LEI), functional capillary density (FCD), and patency of microvessels.
Methods: For assessment of the microcirculation a transdural window was implanted over the parietal cortex of Mongolian gerbils. FCI was induced by transient (2h) ligation of the left common carotid artery. Gerbils with a decrease in regional cerebral blood flow (rCBF) as measured by laser-Doppler-fluxmetry to less than 10% of baseline (n=8) were compared with sham operated animals (n=6). The brain surface was observed before, during and after ischemia up to 3 h of reperfusion by intravital microscopy, utilizing fluorescence labeled dextran for contrast enhancement of blood vessels and Rhodamine 6G for labeling of leukocytes.
Results: FCI led to a significant increase of the number of rolling leukocytes [n/100 microm*min] at 5 min (7.7 +/- 4.2) and 30 min (6.4 +/- 2.4) of reperfusion as compared to control animals, however, declining thereafter. The number of leukocytes (stickers) attached to the endothelial surface was also increased shortly after ischemia, albeit without reaching statistical significance. There was a significant decrease in FCD from 168 +/- 10 /cm before ischemia to 7 +/- 5 /cm after vessel occlusion. FCD did not recover during the whole reperfusion period (180 min), remaining at 15 +/- 11 /cm. The number of perfused postcapillary venules (8-20 µm in diameter) decreased to 44% +/- 18% until the end of the ischemic period. Recovery of the venular perfusion was delayed until 60 min of reperfusion.
Conclusion: In FCI activation of leukocytes is limited to the immediate reperfusion period from 5-30 min after ischemia. FCD was significantly reduced and did not recover during the whole observation period, while reperfusion of small venules was delayed for 1h. The currently observed disturbances of the postischemic cerebral microcirculation are most likely involved in the maturation of ischemic brain damage and may, thus, be considered as a therapeutic target.

Regular Session 6
Experimental therapy developments

38. Laboratory therapy developments for neurotrauma (Invited lecture)

W.D. Dietrich (Florida, USA)

The pathophysiology of traumatic brain and spinal cord injury (SCI) is complex, and several therapeutic strategies have been reported that provide various degrees of protection in models of injury. Pharmacological treatments including steroids, growth factors, receptor blockers, radical scavengers and inhibitors of lipid peroxidation, anti-inflammatory cytokines, and agents that target ionic perturbations are actively being investigated. Hypothermia, alone or in combination with pharmacotherapy, remains a leading candidate for the treatment of neurotrauma. Mechanistic studies have demonstrated that hypothermia attenuates many injury processes and prevents episodes of post-traumatic hyperthermia. Following SCI, moderate hypothermia improves locomotor function and reduces overall contusion volume. Likewise, following traumatic brain injury (TBI), hypothermia improves sensorimotor and cognitive function while reducing contusion volume, selected neuronal necrosis, vascular permeability and axonal damage. Recently, the importance of clinically relevant secondary injury mechanisms, including hypoxia and hypotension, with regard to traumatic

outcome have been emphasized. Whether established therapeutic strategies are neuroprotective in models complicated by secondary injury mechanisms is an important question. In this regard, recent data indicate that post-traumatic hypothermia leads to significant improvement in histopathological outcome in a TBI model complicated by secondary hypoxia. Several factors, including the duration and degree of hypothermia and rewarming conditions following therapeutic hypothermia appear to be important in this outcome.

39. Concurrent calpain and caspase-3 activation following oxygen-glucose deprivation (Invited lecture)

R.L. Hayes, J.K. Newcomb, B.R. Pike, X. Zhao, K.K.W. Wang, D.K. Anderson (Gainesville, Houston, Ann Arbor, USA)

Experiments employed oxygen-glucose deprivation (OGD), an in vitro model of ischemia, to determine the relative contributions of caspase-3 and calpain to apoptotic and necrotic cell death following OGD in primary septo-hippocampal co-cultures. Data collected from cells subjected to OGD were supplemented by observations from pharmacological insults producing exclusively necrotic (maitotoxin) or apoptotic (staurosporine) cell death phenotypes in this culture system. Cell viability and cell death phenotypes following OGD were characterized. Cell markers (NeuN and MAP2 or GFAP) assessed the effects of OGD on neuronal and astroglial viability, respectively. Calpain or caspase-3 mediated proteolysis of a-spectrin was examined using Western blot techniques. Activation of these proteases in individual cells phenotypically characterized as apoptotic and necrotic was also evaluated by using antibodies specific for calpain or caspase-3 mediated breakdown products to a-spectrin. Administration of appropriate caspase-3 and calpain inhibitors examined the effects of protease inhibition on cell death. OGD produced expression of apoptotic cell death phenotypes primarily in neurons, with relatively little damage to astroglia. Although Western blot data suggested greater proteolysis of a-spectrin by calpain than caspase-3, co-activation of both proteases was usually detected in cells exhibiting apoptotic or necrotic cell death phenotypes. Inhibition of calpain and caspase-3 activity decreased LDH release following OGD. These data demonstrate that both calpain and caspase-3 contribute to the expression of apoptotic cell death phenotypes following OGD, and that calpain could potentially have a larger role in the expression of apoptotic cell death than previously thought. Future studies must examine whether calpain and caspase-3 contribute to cell death via independent or interactive mechanisms. (Supported by NIH R01 NS21458; F32 NS10584; and Navy Research Grant N0014-97-1-1064.)

40. Melatonin augments endogenous brain reducing capacity and protects mice after closed head injury

S.M. Beni, R. Kohen, R.J. Reiter, E. Shohami (Jerusalem, IL; San Antonio, USA)

Melatonin, the main pineal hormone, acts as free radical scavenger and an antioxidant in humans and in experimental models. Closed head injury (CHI) is associated with increased production of reactive oxygen species (ROS), which mediate secondary tissue damage. The brain combats oxidative stress by defense mechanisms such as enzymes and low molecular weight antioxidants (LMWA), and its ability to elevate LMWA in response to CHI correlates well with functional outcome. This study was designed to evaluate the effect of melatonin on clinical outcome and brain reducing capacity after experimental CHI. Mice were subjected to CHI or sham surgery as previously described and Neurological Severity Score (NSS) was evaluated 1h later. Mice were treated with either vehicle (5% ethanolic saline, V group) or melatonin (1, 5 or 10 mg/kg ip; M group). NSS was evaluated again at 24h and 4d. Brain LMWA (types and levels) were analyzed by cyclic voltammetry: oxidation potentials and anodic currents were assessed. NSS1h was similar in both V and M groups (7.06±1.62 and 6.73±1.39 respectively). A significant decrease in NSS, indicating faster recovery, appeared in the M (5mg/kg) treated group as compared to the V group at 24h ($p<0.02$) and 4d ($p=0.05$) after CHI. At 24h LMWA levels were higher in the M, as compared to V group. At 4d post CHI, overall reducing capacity of brain tissue decreased as compared to pre-CHI levels and to those at 24 h after CHI. However, M group still maintained 10-fold higher levels of LMWA compared to V group. No difference in LMWA was found after treatment with 1mg/kg M while in the 10mg/kg group LMWA was lower than in V group ($p<0.05$). The neuroprotective effect of melatonin was maximal at 5mg/kg. This effect was associated with elevation in brain LMWA levels. Considering its short half-life and long-lasting effect, we suggest that melatonin augments brain total reducing capacity. (Supported by the David R. Bloom Center for Pharmacy, the Hebrew University School of Pharmacy, Jerusalem/Israel.)

41. Cyclosporin A improves brain tissue oxygen consumption and learning/memory performance after lateral fluid percussion injury in rats

B. Alessandri, J. Zhu, M. DeFord, J. Levasseur, D. Chao, R. Bullock (Mainz, D; Richmond, USA)

Introduction: Traumatic brain injury (TBI) triggers a complex pathophysiological cascade, leading to cell death. A major factor in the pathogenesis of TBI is the overload of cells with calcium, causing the opening of mitochondrial permeability transition pores (MPTP), which consequently inhibits normal mitochondrial function. The immunosuppressant Cyclosporin A (CsA) has been shown to block MPTPs,.and to be neuroprotective in ischemia and TBI. However, the translation of these effects on mitochondrial function, and into behavioral endpoints has not been investigated thoroughly.
Methods: We tested the effect of a clinically relevant CsA dose of 0.375 mg/kg (= 3 mg/kg/24hrs; infused for 3 hours) and a higher "known" neuroprotective dose of 18.75 mg/kg on brain tissue O2 consumption, on motor and cognitive performance, and on neuronal cell death in the hippocampal CA3 region following lateral fluid percussion injury (FPI) in rats.
Results: CsA at both concentrations abolished the 25% decrease in O2 consumption, seen in saline-treated animals at 5 hours post-FPI. Furthermore, the lower dose of CsA ameliorated acute motor deficits (days 1-5 post-FPI) and learning and memory impairments in a 'Morris water maze' on days 11-15 post-FPI. This low dose also reduced neuronal cell loss in CA3. The higher dose of CsA improved cognitive performance and reduced neuronal cell loss only slightly, when compared to the saline-treated FPI group.
Conclusion: These results indicate, that CsA at low concentrations improved motor and cognitive behavior probably through a direct effect on mitochondrial function and neuronal cell death.

42. Inhibition of neurogenic inflammation improves motor and cognitive outcome following diffuse traumatic brain injury

R. Vink, X. Hu, C. Bennett, I. Cernak, A.J. Nimmo (Townsville, AUS)

It has been recognised that the neurogenic component of inflammation plays a significant role in the formation of oedema in peripheral tissues. This neurogenic component involves the local release of neuropeptides from the peripheral terminals of primary afferent neurones (C-fibres). These neuropeptides have significant vascular effects causing vasodilation and increased vascular permeability. The aim of this study was to examine whether the neuropeptides released from C-fibres might play a role in the formation of cerebral oedema and the development of motor and cognitive deficits following diffuse traumatic brain injury. Adult male Sprague-Dawley rats were pre-treated with capsaicin (125 mg/kg s.c.), which causes a depletion of neurotransmitter from C-fibres lasting approximately 28 days, or equal volume saline. At 14 days after capsaicin pre-treatment, pentobarbital anaesthetised animals were injured using the 2 metre impact-acceleration model of diffuse brain trauma. Subgroups of animals were assessed for brain oedema (wet weight/dry weight), BBB permeability (Evans Blue), cognitive outcome (Barnes maze) and motor outcome (Rotarod) for up to 2 weeks posttrauma. In vehicle treated animals, brain injury resulted in significant impairments of motor and cognitive outcome that was associated with increased BBB permeability and oedema development. In contrast, capsaicin pre-treated animals exhibited little motor and cognitive impairment after trauma, and no significant BBB permeability or oedema development. Postinjury treatment with an NK1 selective substance P antagonist (N-acetyl-L-tryptophan; 246 mg/kg i.v.) at 30 minutes after trauma also significantly improved all postinjury parameters to a degree that was not significantly different from capsaicin pre-treated animals. We conclude that neurogenic inflammation significantly contributes to the development of oedema and posttraumatic deficits after diffuse brain trauma and that substance P antagonists may represent a novel therapeutic approach to traumatic brain injury.

Regular Session 7
Apoptosis, necrosis

43. Temporal profile and cell subtype distribution of activated caspase-3 following experimental TBI

R. Beer, G. Franz, R. Hayes, B. Pike, A. Srinivasan, A. Kampfl (Innsbruck, A; Gainesville, La Jolla, USA)

We investigated the temporal expression and cell subtype distribution of activated caspase-3 following cortical impact-induced TBI in rats. The animals were killed and examined for protein expression of the p18 subunit of caspase-3 at intervals from 6 hours to 14 days after injury. In addition, we also investigated the effect of caspase-3 activation on proteolysis of the cytoskeletal protein alpha-spectrin. Increased protein levels of p18 and the caspase-3 specific 120 kD breakdown product to alpha-spectrin were seen in the cortex ipsilateral to the injury site from 6 hours to 72 hours after the trauma. Immunohistological examinations revealed increased expression of p18 in neurons, astrocytes and oligodendrocytes from 6 hours to 72 hours following impact injury. Quantitative analysis of caspase-3 positive cells revealed that the number of caspase-3 positive neurons exceeded the number of caspase-3 positive glia cells from 6 hours to 72 hours after injury. Moreover, concurrent assessment of nuclear histopathology using hematoxylin identified p18 immunopositive cells exhibiting apoptotic-like morphological profiles in the

cortex ipsilateral to the injury site. In contrast, no evidence of increased p18 expression or alpha-spectrin proteolysis was seen in the ipsilateral hippocampus, contralateral cortex and hippocampus up to 14 days after the impact. Our results are the first to demonstrate the concurrent expression of activated caspase-3 in different CNS cells after traumatic brain injury in the rat. Our findings also suggest a contributory role of activated caspase-3 in neuronal and glial apoptotic degeneration after experimental TBI in vivo. (Supported by grants from the Austrian Science Fund (FWF; P12287-MED), the National Institutes of Health (NIH R01 NS40182), the US Army (DAMD17-9-1-9565), and University of Pittsburgh Navy Project (N00014-99-1-0765).)

44. Regulation of apoptotic cell death in traumatic spinal cord injury

J.E. Springer, R.D. Azbill, S.A. Nottingham, S.E. Kennedy, P.E. Knapp (Lexington, USA)

Traumatic injury to the spinal cord initiates a host of pathophysiological events that are secondary to the initial insult. Soon after injury, the damaged spinal cord is exposed to numerous secondary insults leading to neuronal dysfunction and death mediated, in part, by glutamate-related excitotoxicity and oxidative stress. Glutamate-mediated excitotoxicity and oxidative stress are commonly associated with necrotic cell death in the CNS. However, recent studies suggest that disruption of mitochondrial function by glutamate and oxidative stress events leads to apoptotic cell death. We recently found that spinal cord injury results in rapid activation of the caspase-3 apoptotic cascade in neurons within the injury site and oligodendroglia distant to the injury site. We now report that activation of caspase-3 in neurons is initiated by the release of excitotoxic levels of glutamate soon after the injury. In addition, the activation of caspase-3 is dependent, in part, on activation of the Ca2+-sensitive phosphatase, calcineurin, which dephosphorylates BAD, a pro-apoptotic member of the bcl-2 gene family. Once dephosphorylated, BAD redistributes to the outer mitochondrial membrane where it binds to and inhibits the actions of Bcl-xL, an anti-apoptotic protein that regulates cytochrome c release, the first step in activation of the caspase-3 apoptotic cascade. These findings implicate glutamate-mediated calcineurin activation and BAD dephosphorylation as potential therapeutic targets for inhibiting caspase-3 activation and neuronal apoptosis in traumatic spinal cord injury. (Supported by PHS grants NS30248, NS40015, and the Kentucky Spinal Cord and Head Injury Research Trust.)

45. Post-traumatic apoptosis in the spinal cord – role of the FAS and P75 death receptor pathways

S. Casha, W.R. Yu, M.G. Fehlings (Toronto, CDN)

Apoptosis or programmed cell death has been implicated in the pathophysiology of neurotrauma. In the present study, we examined the cellular distribution of apoptosis and the role of the FAS and p75 death receptor signaling pathways in this process in a rat clip compression model of spinal cord injury at C7-T1. Extensive apoptotic cell death was observed using DNA agarose electrophoresis, electron microscopy and TUNEL. The cells undergoing apoptosis were principally oligodendrocytes (63% of TUNEL nuclei by double labeling with CNPase). Temporally, the number of apoptotic cells rose gradually to day 7 and decreased at day 14 post-injury. Due to the susceptibility of oligodendrocytes to apoptosis after spinal cord injury we examined the role of the FAS and P75 death receptor pathways. By western blotting we found both FAS and P75 deceased initially following injury, and then gradually rose until day 7. At day 14, FAS levels decreased, while P75 remained elevated. By double labeling immunohistochemistry we observed both FAS and P75 on oligodendrocytes and microglia after injury, as well as on TUNEL positive cells. We examined caspase 8 and caspase 3 cleavage/activation on western blots. Caspase 8 was activated by day 1 post-injury and peaked at day 7 similar to FAS expression and the temporal profile of apoptotic cells. Caspase 3 was largely activated by day 1 post-injury and remained so until day 7, at day 14, activation was decreased. FLIP-L, an endogenous inhibitor of caspase 8, on western blotting was seen to decrease at times of maximum apoptosis. This study provides evidence for the involvement of FAS, P75 and the caspases they activate in glial apoptosis after spinal cord injury. (Funded by an Ontario Premier's Research Excellence Award and the Cervical Spine Research Society.)

46. Cold lesion-induced neurotrauma in rats – evidence against apoptotic cell death involved in tissue damage

M. Barth, U. Hübner, P. Schmiedek, Y. Oulmi (Mannheim, D)

Apoptotic cell death often detected by terminal transferase mediated d-UTP nick end labeling (TUNEL) has been implicated in the pathogenesis of neurotrauma, both experimentally and clinically. In the present study the TUNEL technique was used to characterize time course, distribution, and ultrastructural morphology of cell damage in the cold lesion model of neurotrauma. In Sprague-Dawley rats a right-sided craniotomy was performed and a lesion

induced by applying a cold probe (diameter, 5 mm; temperature: -68°C; duration, 10 sec) to the exposed dura as described previously [1]. Rats were sacrificed by in situ perfusion 1-72 h after lesion. In coronal sections TUNEL signal was visualized with diaminobenzidine for light microscopy or immunogold for electron microscopy. Quantification of the TUNEL signal was performed in serial equidistant sections encompassing the whole lesion area using a computer controlled quantification unit. The ultrastructure of TUNEL-stained cells was studied in ultrathin sections (approx. 50 nm) by transmission electron microscopy. Occurrence of TUNEL-positive cells started as early as 1 h after lesion with the maximum number reached at 3 h. All cells were located within the lesion area. Thereafter, the number decreased gradually reaching approximately 10% of maximum number at 36 hours after lesion. At that time point lesion volume had reached it maximal extension. Ultrastructurally, TUNEL positive cells displayed signs of necrotic cell death such as rupture of the nuclear envelope, chromatin protrusion and disintegration of cell organels. The present results suggest necrosis as the main avenue of cell death after cold lesion to the exposed dura. Thus, DNA fragmentation appears to be a general indicator of cell death rather than a specific marker for apoptosis in this model of neurotrauma.

References: [1] Schilling L, Wahl M (1994) Effects of antihistaminics on experimental brain edema. Acta Neurochir. Suppl. 60: 79-82.

47. Prolonged calpain activation in regions of tissue atrophy after traumatic brain injury

B.R. Pike, E. Johnson, J. Flint, C. Glenn, R.L. Hayes (Gainesville, USA)

Our laboratory previously reported that the cysteine protease calpain is rapidly activated after traumatic brain injury (TBI) and remains active for up to 7 days in hippocampus and up to 14 days in thalamus (Pike et al., NeuroReport (1998):9:2437-2442). The purpose of this investigation was to examine calpain activation in brain regions up to one-month post-TBI, and to correlate calpain activation patterns with neuronal cell loss and tissue atrophy by H&E staining.

Methods: Homogenized cortical, hippocampal, thalamic, and striatal tissue samples from sham-injured and TBI (1.6 mm or 2.0 mm lateral cortical impact) animals were separated by gel electrophoresis. Immunoblots were probed with an antibody that specifically recognizes the calpain and caspase-3 generated fragments to the cytoskeletal protein alpha-spectrin (280 kDa). Animals at identical time-points were stained for H&E to examine overt levels of cell loss.

Results: Calpain-specific a-spectrin fragmentation (145 kDa) was detected at the earliest time-point assayed (3h) in all brain regions except striatum after 1.6 or 2.0 mm TBI. The 145 kDa fragment was no longer detectable in cortex and hippocampus by 14 days post-injury. Interestingly, the 145 kDa fragment was still detectable in thalamus after 1.6 mm, but not 2.0 mm injury at 28 days post-injury. The pattern of calpain activation closely paralleled the pattern of neuronal atrophy in these regions as assayed by H&E staining. Increases in the caspase-3 specific fragment to alpha-spectrin (120 kDa) was not detected at any post-injury time point.

Discussion: Calpain is activated for much longer than has been previously reported after TBI and the thalamus appears susceptible to sustained neuronal degeneration after TBI. Thus, calpain inhibition may be an appropriate therapeutic strategy for both acute and chronic post-injury intervention. However, examination of calpain's putative role in cellular repair after TBI is also warranted. (Supported by NIH R01-NS 21458, NIH F32-NS 10584, Navy Research Grant N0014-97-1-1064, and The University of Florida Brain and Spinal Cord Rehabilitation Trust Fund.)

48. Neuroprotection profile of the novel proteasome inhibitor PS-519 in an experimental rat model of focal stroke and reperfusion injury

F. Tortella, J. Adams, P. Elliott (Silver Spring, Cambridge, USA)

Comprehensive testing of the proteasome inhibitor PS-519 has been conducted in a rat model of transient focal cerebral ischemia where rats are subjected to 2 hr of localized ischemia using the filament method of middle cerebral artery occlusion (MCAo) followed by either 22 or 70 hr of reperfusion. Infarct volumes, neurological testing, cortical EEG function, and leukocyte infiltration are assessed. PS-519 was initially given at 2 hr post-MCAo as a single i.v. bolus. In later studies the therapeutic window was estimated by delaying treatment for 4 or 6 hr. In normal or MCAo rats PS-519 has no significant effect on physiological parameters, including temperature, measured out to 6 hr. Dose-response analysis of infarct volumes at 24 hr have shown that PS-519 neuroprotection approaches 60% and is accompanied by a decrease in infiltrating neutrophils and clinical evaluations demonstrating significant improvements in neurological function and EEG activity. In the 72 hr recovery model, infarction was reduced 40% and significant improvements in clinical outcome were again measured. Consistent with the PS-519 neuroprotection, considerable reductions in both neutrophil and macrophage infiltration have been measured at 72 hr. Delaying PS-519 treatment 4 hr (but not 6 hr) also results in significant

neuroprotection. Our studies have established that postinjury therapy with PS-519 mitigates infarction and improves neurological recovery in brain injured rats, an effect due in part to a reduction in the leukocyte inflammatory response. Critically, similar results have been replicated in multiple independent models of ischemia.

Regular Session 8

Gene expression in pathobiology

49. Role of gene expression in the pathobiology of acute brain insults (Invited lecture)

T. Wieloch, D. Chin, G. Gido, K.C. McFarland, T. Melcher (Lund, S)

Following brain injury, brain cells may react by activating protective andregenerative processes leading to survival and repair of the cells and functional recovery. If the insult is severe, brain cells may still survive, but recovery of function may be poor, and eventually lead to delay cell death. In cells where no repair occurs or repair is unsuccessful, a persistent change in cell signaling is observed, leading to among others aberrant activation of gene programs. If these genes are translated into proteins, an imbalance in a multitude of cellular functions can be envisaged, eventually leading to cell death. Studies of gene expression have so far been be devoted to a few genes or classes of gene. We have analyzed changes in gene expression of the rat brain following global and focal cerebral ischemia, at various time points following reperfusion, by combining de novo differential cloning with cDNA microarray techniques. Over 2,000 genes, many without recognized function were identified as induced at different time points. Principal component nalysis of microarray data was used to derive both spatial and temporal gene expression patterns in various ischemia models. The results demonstrate complex patterns of gene activation in the brain following injury, including genes involved in synaptic remodeling, cell repair and cell death.

50. Influence of genetic make-up on outcome from head injury (Invited lecture)

D.I. Graham, J.A.R. Nicoll (Glasgow, UK)

There is a significant association between a history of head injury with loss of consciousness and the risk of subsequent neurodegenrative changes, e.g. deposition of Ab-PP plaques, in 30% of cases. A possible mechanism to explain the link might be the polymorphism of the apolipoprotein E (APOE) gene. Support for the hypothesis has now been provided by survival studies that have shown patients with apoE e4 to have a worse outcome after traumatic brain injury. A small study (n=16) of patients with prolonged post-traumatic coma revealed a higher frequency of apoE e4 among patients who did not recover consciousness than those who did, and a prospectively recruited series of patients admitted to a Neurosurgical Unit (n=93) found 57% of patients with apoE e4 had an unfavourable outcome 6 months after injury. Of potential relevance to longer term outcome is the role of APoE in the delivery of lipids to neurons required for neurite outgrowth and synaptogenesis, clearance of degeneration products, microglial activation, and maintenance of the cholinergic system.

51. Differential gene expression in the central and peripheral nervous system of the rat after axotomy

K. Pech, S. Breuer, A.B. Schmitt, A. Buss, F.W. Schwaiger, G.W. Kreutzberg, J. Noth (Aachen, Martinsried, D)

Spinal cord injury leads to complex cellular and molecular changes contributing to the failure of any functionally significant regeneration of severed central axons. In contrast, injury of the peripheral nervous system leads to functional reinnervation. To identify genes which might influence the fate of injured neurons, we have investigated axotomy-induced gene expression in neurons of the red nucleus and Clarke´s nucleus after spinal cord hemisection and in the facial nucleus after transection of the facial nerve. Seven days after injury, RNA was purified from ipsilateral and contralateral tissues. By reverse transcription, cDNAs were generated and analyzed by DD-PCR. Differentially expressed cDNA bands were isolated, cloned and sequenced. At present about 120 clones have been analyzed: 100 without any significant homology with known sequences and 20 fragments coding for known proteins involved in signal transduction, fatty acid synthesis or energy metabolism. To confirm the differential expression of these gene fragments, riboprobes were generated for in situ hybridization. This technique demonstrates the anatomical and cellular distribution and may reveal a functional role of the gene fragments. Interesting gene candidates will be selected for further studies, including the identification of their full sequence. In conclusion, the comparison of gene expression between regenerating and non-regenerating systems allows the identification of genes which are of potential significance for neuronal and glial plasticity after injury. (Supported by grants from the DFG (Na 289/2-3 and Schm 1304/3-1.)

52. Effects of methylprednisolone on gene expression in acute spinal cord injury
R.P. Hart, Y. Ji, J. Liu, W. Huang, W. Young (Piscataway, Newark, USA)

Acute contusion injury of spinal cord results in a complex program of gene regulation including inflammation, stress response, wound repair and regrowth. We have used gene arrays to determine patterns of gene responses associated with injury and the effect of the standard methylprednisolone (MP) treatment on acute injury. Rats were contused using the MASCIS impactor, then injected with 30 mg/kg MP or saline. Two hours following injury, segments of spinal cord were removed from the site of injury and used to prepare RNA. Probe cDNA was hybridized in triplicate with Clontech Atlas 1.2 macroarrays containing 1,176 known gene probes. Results indicate that only 20 genes are significantly regulated by injury over control ($p<0.05$ by Student's t test), and that 38 genes are significantly different when comparing saline-infused injured to MP-treated injured rats. Clustering analysis indicates that MP increases relative expression of several metabolic functions, and decreases several signaling genes, including NPY and TRH. To identify all genes that are regulated following acute spinal cord injury, we have used subtractive hybridization. cDNA was prepared from rats injured for 0-24 hrs and subtracted using a PCR-based subtraction protocol. Regulation was confirmed using virtual Northern blots. Few genes appear to be increased after acute injury, but a large group was found to be diminished. Identification of gene expression patterns following spinal cord injury provides new insight into injury responses and aid in identifying new therapies. (Supported by the Christopher Reeve Paralysis Foundation.)

53. Effects of ApoE genotype on outcome of head injury – relationship to age
G.M. Teasdale, G. Murray, J. Nicoll, H. Fiddes, C. McLaughlin, C. Dobson, S. Swiatek, E. Stewart (Glasgow, Edinburgh, UK)

Objectives: To test the concept (1) that genetic factors influence outcome from head injury.
Design: Prospective identification of patients with a head injury admitted to a Neurosurgical Unit, collection of demographic and clinical data Apolipoprotein E (ApoE) genotyping by polymerate chain reaction PCR and follow-up at 6 months.
Subjects: Nine hundred and eighty four subjects, age range 0-93 years (mean 37 years), 33% with ApoE e4 allele.
Outcome measures: Glasgow Outcome Scale assigned by structured interview (2).
Results: Although patients with an ApoE e4 gene had slightly less serious injuries, they had a poorer outcome (favourable outcome 64% vs 67%). Taking severity into account, the relationship was significant (odds ratio 1.60: p=.005). The effects was strongly related to age (p=0.007). Thus, possession of ApoE e4 allele reduced the prospect of a favourable outcome to a relatively greater extent in children (from 94% to 83%) than in adults (from 60% to 58%), but the effect was significant in both age groups.
Conclusion: An association between ApoE genotype and outcome is confirmed; the greater influence in younger patients suggests the effect is expressed through the processes of repair and recovery.
References: (1) Teasdale et al 1997. Lancet 350; pp 1069. (2) Wilson et al 1998. J of Neurotrauma 15; pp 573.

Regular Session 9
Membrane biology and pathology

54. Molecular mechanisms of myelination as studied in knock-out mouse mutants (Invited lecture)
M. Schachner (Hamburg, D)

Analysis of mouse mutants deficient in the genes for the major peripheral myelin protein P0 (1) and the minor myelin-associated glycoprotein MAG of the central and peripheral nervous system (2), has yielded important insights into disease processes in the human. Analysis of the mouse mutant has shown that the consequences of ablation of one gene can lead to abnormal regulation in the expression of other myelin components. In the P0 knock-out mutant myelination is defective, thus attributing to P0 an essential role in the formation of myelin. Mutations in the P0 gene in the human lead to the mild and severe forms of Charcot-Marie-Tooth disease type 1 b. The functional consequences of P0 mutations may be indirect, since the abnormally high expression of NCAM, MAG, PLP, tenascin-C and the p75 NGF receptor in the mouse mutant could be the cause rather than the consequence of one or more aspects of the myelin abnormality. On the other hand, it is conceivable that the ability of some Schwann cells to engage in a limited extent of myelin formation may rather be due to the abnormal expression of, for instance, NCAM and MAG in the mutant. Loss of distal axons and sensory Merkel cells and degeneration of skeletal muscle in P0 knock-out mice indicate that denervation, particularly of hindlimbs takes place in P0 knock-out mice (3). Immune deficiency in heterozygous P0 knock-out animals that normally show a late onset of myefin degeneration leads to improved myelin maintenance(4). – Analysis of double

knock-out mutants are helpful in assessing the contribution of individual molecules to the abnormal phenotype. Mutant animals deficient in both P0 and myelin basic protein show that the two molecules are involved in formation of the major dense line of myelin. – In contrast to the P0 knock-out mutant, the MAG knock-out mutant shows comparatively normal myelin formation, with degeneration of myelin in the peripheral nervous system after three months of age and a late onset of myelination with subtle morphological abnormalities of myelin in the central nervous system. Degeneration of myelin at later ages shows that MAG is involved in the maintenance of myelin. NCAM is a candidate for functional compensation of MAG, since it is overexpressed at sites that contain MAG in wild type mice. Double knock-out mutants for MAG and NCAM show accelerated degeneration of myelin. – The combined observations from these exemplary mutations suggest that multiple molecular mechanisms may be operative during formation and maintenance of myelin and that partial compensation may work in some instances, but not in others.

References: (1) Giese et al. (1992) Cell 71, 565-576. (2) Montag et al. (1994) Neuron 13, 229-246. (3) Frei et al. (1999) J Neurosci 19, 6058-6067. (4) Schmid et al. (2000) J Neurosci 20, 729-735.

55. Derangements of membrane biology in cerebral contusion – a clinical perspective (Invited lecture)

Y. Katayama, T. Kawamata (Tokyo, J)

Some patients with cerebral contusions exhibit a non-hemorrhagic mass effect within the period of initial 24 to 48 hours post-trauma. Such a mass effect is sometimes so severe that surgical excision of contused brain tissue is the only therapy which can avoid death. The precise mechanism of the non-hemorrhagic mass effect is not yet clearly determined. Cerebral contusion is composed of two components that are distinguishable histopathologically: an area of contusion necrosis proper and a peri-contusion area. Cellular responses in these two areas are entirely different. All cellular elements in the contusion necrosis proper uniformly undergo shrinkage, disintegration and homogenization. The maximum extent of this area becomes evident at approximately 6 hours post-trauma. In contrast, the peri-contusion area exhibits microthrombosis as well as numerous swollen cells. This area contributes secondary growth of contusion necrosis during the period between 12 and 24 hours post-trauma. We discuss several lines of clinical and experimental evidence which indicate that (1) the area of contusion necrosis proper comprises a major component of the severe cerebral contusion observed clinically, and (2) the contusion necrosis proper leads to a large amount of water accumulation through the synergistic effects of high osmotic potential of the necrotic brain tissue and cellular swelling of the peri-contusion area. We suggest that the osmotic potential of the necrotic brain tissue represents an unique mechanism underlying the mass effect of severe cerebral contusions. The most effective therapy for early massive edema caused by cerebral contusion may be surgical excision of the area of contusion necrosis proper.

56. The effect of group II and III metabotropic glutamate receptors on neuronal injury in a rodent model of traumatic brain injury

M. Zwienenberg, Q.Z. Gong, R.F. Berman, J.P. Muizelaar, B.G. Lyeth (Sacramento, USA)

Objectives: The role of metabotropic glutamate receptor (mGluR) activation after traumatic brain injury (TBI) is not well known.In vitro studies suggest that Group II and III mGluR may mediate neuroprotection, and these receptors may thus be a potential target in the development of therapeutic strategies. To further elucidate the role of group II and III mGluR, we examined the effects of two selective agonists on neuronal degeneration after in vivo TBI.

Methods: 54 male Spraque-Dawley rats were subjected to lateral fluid percussion brain injury followed by injection of DCGIV (2-(2',3') dicarboxycyclopropyl-glycine: group II) or PPG ((R,S) -4- phosphonophenylglycine: group III) in the CA 2-3 area of the hippocampus. The following dosing regimens were used in each study. DCGIV: vehicle (n=7), 20 fmol (n=8), 100 fmol (n=6), and 500 fmol(n=6). PPG: vehicle (n=7), 8nmol (n=5), 40 nmol (n=7), and 200 nmol (n=7). All animals were sacrificed 24 hours after TBI. Four 50 mm brain sections were obtained from each animal and stained with the fluorochrome Fluoro-Jade. The number of Fluoro-Jade positive, degenerating neurons in the CA 2-3 area of the hippocampus of each brain section was counted.

Results: The highest dose of DCGIV significantly reduced the number of Fluoro-Jade positive neurons ($p<0.001$). Lower doses of DCGIV were associated with a decreased but not statistically significant number of Fluoro-Jade positive neurons. In contrast, no difference in the number of Fluoro-Jade positive neurons was found between vehicle and the drug PPG.

Conclusion: Modulation of group II metabotropic glutamate receptors with a selective agonist protects neurons against in vivo TBI. These receptors may thus be a promising target for future neuroprotective drugs. The role of group III receptors is uncertain and further studies are needed to evaluate their potential as targets for therapeutic agents in TBI.

57. In vitro binding of [11C]flumazenil after parasagittal fluid percussion brain injury in rats.
S. Sihver, N. Marklund, B. Långström, L. Hillered, Y. Watanabe, M. Bergström (Uppsala, S; Osaka, J)

Background: [11C]Flumazenil binds to the central benzodiazepine receptor (cBZR)-gamma-aminobutyric acid (GABA) A receptor complex. GABA, a major inhibitory neurotransmitter, is important in reducing glutamate-induced neurotoxicity in traumatic brain injury (TBI) [1]. The [11C]flumazenil binding is reduced early after focal ischemia and reflects irreversible neuronal damage in experimental ischemia and in acute stroke patients [2]. PET with [11C]flumazenil can identify the extent of neuronal damage in vegetative state and predict the possibility of recovery of consciousness and function [3]. In the present study the dissociation constant (Kd) and maximum number of cBZR (Bmax) of [11C]flumazenil binding to cBZR were studied after parasagittal fluid percussion (FP) brain injury in rats.
Methods: Male SD rats (400 g) were subjected to FP injury [4] causing cortical and diffuse subcortical loss of neurons by 12 hrs from impact [5]. The animals were decapitated 2 or 12 hrs (n=3) after FP injury. After decapitation, quantitative frozen section autoradiography [6] was performed by incubating brain sections at various concentrations of [11C]flumazenil. Images were created and analysed using storage phosphor imaging plates, and Phosphor Imager with ImageQuant software.
Results: At 12 hrs a significant ($p<0.05$) 32% and 24% decrease appeared in cBZR number both in trauma site cortex and in underlying hippocampus, respectively.
Conclusion: The decrease in cBZR after TBI might reflect subsequent neuronal loss, and be involved in the development of cellular dysfunction eg via diminished inhibition of TBI induced glutamate-mediated excitotoxicity.
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58. Decreased N-Methyl D-Aspartate Receptor (NMDAR) activity after developmental fluid percussion injury (FPI) demonstrated by changes in subunit composition
C.C. Giza, S.M. Lee, T.J. Kremen, D.A. Hovda (Los Angeles, USA)

NMDARs play an important role in neuroplasticity and their activity correlates with subunit composition. A decrease in the ratio of NR2A:NR2B subunits is associated with enhanced glutamate-induced ionic conductance and improved cognition. Conversely, NMDAR dysfunction early in life can lead to a reduction in potential neuroplasticity. We have recently shown that mild-moderate lateral FPI sustained during development attentuates neuroplasticity induced by rearing in an enriched environment. We hypothesized that developmental FPI decreases NMDAR function by altering subunit composition. Postnatal day 19 rats were divided into sham (n=18) and FPI (n=20) groups. On post-FPI days 1, 4, 7 and 14 left and right parietal cortex homogenates were prepared. For each time point, sham (n=5, except day 14 n=3) and FPI (n=5) samples (10mcg protein/lane) were analyzed with 3 Western blots, using antibodies to NMDAR subunits NR1, NR2A and NR2B. When comparing the average signal of FPI animals to that of age-matched shams, no significant differences were detected at post-FPI days 1, 7 and 14. On post-FPI day 4, however, the parietal cortex ipsilateral to FPI exhibited a significant elevation of NR2A (255+/-37%, $p=0.01$) with no change in NR1 or NR2B, a relative increase of 2.5:1 in the NR2A:NR2B ratio. At the same time point, in contralateral parietal cortex, all 3 subunits were increased (NR1 296+/-53%, NR2A 173+/-27%, NR2B 171+/-28%) compared to shams, with no change in the relative NR2A:NR2B ratio. The increase in NR2A:NR2B ratio within the injured cortex leads to a decrease in NMDAR function and may explain the loss of plasticity seen in injured pups. (Supported by NS30308, NS37365, NS27544 and the Lind Lawrence Foundation.)

Special Seminar 2

Role of neurochemical markers in the management of head injury (sponsored by Byk-Sangtec, Dietzenbach, D)

LS59. Protein S-100B and neuron specific enolase (NSE) – physiological and analytical aspects
R.A. Sherwood (London, UK)

The S100 family of proteins are primarily acidic calcium binding proteins. There are two members of this group of proteins that have attracted interest in the medical community – S100A1 and S100B. These are composed of combinations of two subunits a and b, containing 91-93 aminoacids and sharing 58% sequence homology. S100A1 comprises two a subunits and S100B two b subunits. Initially it was believed that the S100 proteins were only located in the brain, however, it is now known that they have a wide tissue distribution. S100A1 is found in myocardial cells and various glands, including the

adrenal gland, the pancreas, salivary and sweat glands. It is also found in skeletal muscle predominantly associated with slow-twitch muscle fibres. In humans the highest concentrations of S100B are found in the brain, primarily in glial cells, being 30-100 fold higher than in other tissues. S100B is also found in melanocytes and has been shown to be of value as a tumour marker for malignant melanoma. An elevated serum concentration of S100B in a patient with melanoma following removal of the primary lesion almost invariably indicates metastatic disease and the concentration varies with tumour burden. S100B appears to be renally cleared from the circulation and may be falsely elevated in patients with severe renal dysfunction. Neurone specific enolase (NSE) is a 78 kD glycolytic enzyme which originates predominantly from the cytoplasm of neurones and neuroendocrine cells. NSE is released from the brain following trauma, strokes and in various neurological disorders. Increased serum NSE concentrations can also be found in patients with small-cell lung cancer, neuroblastoma and other tumours derived from neuroendocrine cells. A disadvantage of NSE is the higher concentration in red blood cells compared to serum which means that haemolysis in a blood sample results in a falsely elevated NSE concentration. Methods for measurement of NSE in body fluids have been in existence for many years. Most methods for NSE are now automated non-isotopic (e.g. enzyme or chemilumiscent labels) immunoassays. Early methods for protein S100B were based on particle counting immunoassays and were relatively insensitive with most normal subjects having undetectable S100B concentrations in serum. Subsequently a radioimmunoassay was introduced with substantially greater sensitivity and this has now been largely superceded by an automated chemiluminescent assay. This has permitted S100B to be measured in normal subjects and a greater understanding of the biological variation of the protein is being achieved.

LS60. Release patterns of S-100B and NSE after traumatic brain injury (TBI) and their relation to intracranial pathology as demonstrated in CT imaging

M. Herrmann, M.L. Fork, C. Grubich, N. Curio, S. Jost, H. Synowitz (Dietzenbach, D)

Objectives: Meanwhile, a huge number of studies give evidence that release patterns of biochemical markers of brain damage are associated with clinical outcome after TBI. Here, we present data that additionally show a significant association between intracranial pathology as demonstrated in cranial computed tomography (CCT) and posttraumatic serum concentration of protein S-100B and NSE. Furthermore, we demonstrate that early S-100B values have a high prognostic value with respect to the short- and long-term neurobehavioral correlates of TBI.
Materials and methods: From a consecutive series of 167 patients admitted to the Dept. of Neurosurgery NSE and S-100B concentrations were analysed in serial venous blood samples taken one to three days after TBI. Standardized neurological examination and plani- and volumetric evaluation of computerized tomography scans were performed in all patients. In a subgroup of patients, we additionally conducted detailed neuropsychological assessment in the postacute stage and 6 months after TBI.
Results: Both, patients with and without visible intracranial pathology in CT scans presented highly increased concentrations of NSE and S-100B after TBI and a significant decrease in the follow-up blood samples. Release patterns of S-100B and NSE differed in patients with primary cortical contusions, diffuse axonal injury (DAI), and signs of increased intracraniall pressure (ICP). Patients with short- and long-term neuropsychological disorders presented significantly higher NSE and S-100B serum concentrations and a significantly longer release of both markers Patients with DAI tended to present more persisting and more severe neuropsychological deficits at the follow-up examination.
Conclusion: Our data show that the early release patterns of NSE and S-100B may mirror different pathophysiological consequences of traumatic brain injury. Taken into account that particularly DAI is underestimated in the evaluation of CCT images the present data indicate that the analysis of release patterns of biochemical markers of brain damage might help to identify subgroups of patients highly at risk for persisting neurobehavioral disorders

LS61. Clinical evaluation of serum S-100B protein as a routine laboratory test for brain cell damage in neurocritical care patients

A. Raabe, O. Kopetsch, J. Lang, V. Seifert (Frankfurt am Main, D)

Objectives: There is growing interest in a biochemical marker to monitor primary and secondary brain cell damage after head injury, subarachnoid haemorrhage and stroke. In our study, we have investigated the use of serum S-100B protein, which is regarded as one of the most promising biochemical markers of glial cell damage. The objective of our study was to test whether serum S-100B is useful as a routine biochemical monitoring of secondary brain insults in neurocritical care patients.
Methods: One-hundred neurocritical care patients with different intracranial diseases were included in our study. Serum S-100B protein was measured daily

using a immunoluminometric assay (LIAISONâ, Byk-Sangtec Diagnostica, Dietzenbach, Germany). Sensitivity, specificity, positive and negative predictive value of S-100B increases for the occurrence of neurological complications and outcome were calculated.
Results: 68 patients (68 %) showed primarily increased values due to their neurological disease before or after surgery. In 18 patients, a secondary increase in S-100B of more than 0.5 µg/l was observed. All patients with secondary increases in S-100B showed neurological deterioration, which was in 12 patients due to rebleeding, contusion enlargement or ischaemia. In 4 patients with neurological deterioration, the cause for secondary increase remained unknown. There was no severe neurological complication without a concomitant increase in S-100B.
Conclusion: Secondary increased values highly correlated with both outcome and neurological complications such as brain ischaemia, infarction, postoperative haemorrhage, progressive brain oedema or contusion. Serum S-100B protein may have diagnostic potential in neurointensive care patients, especially in patients after head injury and subarachnoid haemorrhage.

LS62. The clinical value of protein S-100 measurements after minor head injury
T. Ingebrigtsen (Tromsø, N)

The severity of traumatic brain injury after minor head injury (MHI) is difficult to assess, because specific measures of the presence and severity of such injuries have been unavailable. S-100ß is a calcium-binding protein synthesized in astroglial cells of the central nervous system. It is normally present in serum in very low concentrations. We studied MHI patients with Glasgow Coma Scale scores of 13 to 15. Serum levels of S-100ß were analyzed with an immunoradiometric assay kit (detection limit, 0.2 µg/L). A pilot study showed detectable serum levels in 20%. Patients with increased S-100ß levels in serum reported an increased frequency of post-concussion symptoms and showed impaired neuropsychological performance. In a detailed study of 50 MHI patients with normal computed tomography (CT) scans of the brain, serum levels of S-100ß was measured at admittance and hourly thereafter until 12 hours after injury. Magnetic resonance imaging (MRI) was performed within 48 hours, and neuropsychological follow-up was conducted at 3 months postinjury. Fourteen patients (28%) had detectable serum levels. The proportion of patients with detectable serum levels were significantly higher when MRI revealed a brain contusion. In patients with detectable serum levels, we observed impaired neuropsychological functioning on measures of attention, memory and information processing speed. In a further study 182 patients admitted to three different Scandinavian neurotrauma centers, increased serum level of S-100 protein was seen in 69 (38%). CT scan demonstrated intracranial pathology in 10 (5%). The proportion of patients with detectable serum level was significantly higher among those with intracranial pathology (90%) compared to those without (35%). The negative predictive value of an undetectable S-100 level was 0.99. We observed an increased frequency of post-concussion symptoms among patients with detectable serum levels. In conclusion, determination of S-100ß protein in serum provides a valid measure of the presence and severity of traumatic brain injury. After MHI, detectable serum levels are related to a brain contusion revealed by MRI or CT, increased frequency of post-concussion symptoms andn impaired neuropsychological functioning.

LS63. Assessment of S-100B in head trauma patients – comparison between results obtained from LIA-mat® and LIAISON®
P. Biberthaler, T. Mussack, S. Eich, C. Gippner-Steppert, W. Mutschler, M. Jochum (Munich, D)

Background: Measurement of specific glial protein S-100B in patients after minor head trauma (MHT) has been suggested as a potential tool to identify high-risk patients. These investigations were performed using the LIA-mat® immunoluminometric assay (Byk-Sangtec-Diagnostica). However, the results obtained with this test have no real bedside therapeutic consequences due to the long sample (serum) collection time and duration of the assay procedure (ca. 180 min). Recently, the fully automated LIAISON®-system has been introduced as a rapid device (<60 min) for S-100B measurement. Hence, the aim of this study was to compare data obtained from LIA-mat® with those from LIAISON® in head trauma patients using citrate plasma instead of serum to further shorten the data collection time.
Methods: In our prospective study 44 patients after minor head trauma (MHT) were enclosed presenting a GCS of 13-15 and at least one of the following symptoms: amnesia, loss of consciousness, nausea, vomitus and severe headache. In addition, 7 patients suffering from severe head trauma (SHT) were enrolled as positive controls. Blood samples were drawn on admission. Serum and citrate plasma concentrations of S-100B were measured using the manual LIAmat® and fully automated LIAISON® test (Byk-Sangtec-Diagnostica). Due to the lower detection limit of 0.1ng/ml with the LIA-mat® method only MHT samples above that concentration were considered for calculations. For comparison of both methods linear

regression, correlation coefficient and Spearman´s rank order correlation were calculated.
Results: Out of the 44 MHT patient samples, 25 were above 0.1ng/ml in the serum group and 28 in the citrate plasma group whereas all values from SHT were above the detection level. In the MHT group, the mean S-100B concentration assessed with the LIA-mat® amounted to 0.7±0.2ng/ml both in serum and plasma, whereas using the LIAISON®-test the mean concentration was 0.4±0.1ng/ml in serum and 0.5±0.1ng/ml in plasma. In the SHT group, the LIA-mat® method quantified 7.7±4ng/ml in serum and 8.6±4ng/ml in plasma. Applying the LIAISON®-test mean S-100B serum concentration was 4.7±3ng/ml and mean plasma concentration 5.6±3ng/ml. Comparing both methods the regression equations for the MHT patients were y=0.48x+0.08 in serum and y=0.78x±0 in citrate samples and for the SHT patients y=0.63x-0.13 in serum and y=0.66x-0.06 in citrate samples, respectively. The r^2-values ranged between 0.93 and 0.99 and Spearman´s coefficient was always below 0.001. All data sets passed normality tests and constant variances tests.
Conclusion: Our study clearly demonstrates, that S-100B values obtained using both methods are highly significantly correlated, although the LIAISON® method measures lower absolute levels than the manual LIA-mat® procedure. Moreover, use of citrate plasma leads to higher values, shortens data collection time and provides even superior correlation parameters of both methods compared to serum. Yet, further data have to show the reliability of the LIAISON® method using plasma instead of serum as a bed-side tool to identify high-risk head trauma patients.

Plenary Session 3

BMBF-Research Consortium „Neuro-traumatology and Neuropsychological Rehabilitation“ (sponsored by the German Federal Ministry of Education and Research)

64. A system analysis of the pre- and early hospital care in severe head injury in Bavaria

A. Baethmann, A. Wirth, D. Chapuis, A. Schlesinger-Raab, and Study Group (Munich, D)

A system analysis of the pre- and early clinical management of patients with severe head injury was carried out in Southern Bavaria (catchment area: 5.6 mio) on a population based level. 1448 cases suspected with severe head injury were initially enrolled, severe head injury was confirmed in 528 of the hospitalized cases. 289 patients were dying prior to hospital admission on the scene, 217 of those were autopsied confirming TBI in no less than 214. Of this subpopulation, head injury was causal for a fatal outcome in ca. 80 %. Falls preferentially affecting patients above 45 years were the most frequent cause of head injury, followed by car- and bicycle accidents. TBI was isolated in 2/3 of the population while combined with multiple trauma in 1/3. The patient management beginning at the scene was documented upon alarm of the dispatch center until conclusion of the acute clinical phase, including assessment of prognosis-relevant intervals, as e.g. time until intubation, or until establishment of the CT-diagnosis, among others. The preclinical rescue was concluded within 49 min in 75 % of cases, admission to the hospital within 74 min. Acute pre- and early clinical procedures were terminated after 3 hrs 20 min in 75 %, the time point of admission to the ICU or operation theatre. Mortality of the hospitalized cases was 45.6 % with a mean survival of 19 days. When considering the prehospital outcome in addition, mortality was almost 60%. Risk factors predicting a poor prognosis identified by logistic regression ($p<0.05$) were: advanced age – followed by abnormal pupillary response – lesion severity in CT-scan including brain stem injury, brain swelling, subdural hematoma – arterial hypotension (systolic < 80 mmHg) and a low GCS at the scene, while additional polytrauma was not affecting the prognosis. The present data albeit demonstrating a still unacceptably poor outcome from severe head injury, indicate a high pre- and early clinical management efficiency. It is suggested that outcome is preferentially influenced by the primary irreversible brain lesion from trauma as compared to the secondary complications. (Supported by bmb+f FKZ 01 K0 9704 9030 914.)

65. The neural basis of paradoxical movements

T. Schenk, B. Baur, N. Mai, K. Bötzel (Munich, D)

We recently examined a patient with post-traumatic ataxia who was unable to reach for a stationary object but was perfectly able to reach for rapidly moving objects. This phenomenon, called paradoxical movement, is also observed in patients with Parkinson's disease (PD). We studied the effect of stimulating the basal ganglia in six patients with Parkinson's disease in order to understand the neural basis of paradoxical movements. The patients had neurostimulators implanted bilaterally in the globus pallidum internum. The patients and six healthy age-matched subjects (controls) were instructed to reach and grasp for a target object that was either stationary or moving at a certain speed (0.5 and 1.0 m/s). Two motor-driven linear axes were used to produce target motion within a plane. A 3-D movement registration system (Fa. Zebris) was used to record the hand and

arm movements. PD patients were examined with the neurostimulator switched off or on. While reaching speed for the stationary object in the stimulator off-condition was clearly reduced in PD patients compared with that of healthy subjects, reaching speed of the PD patients increased with speed of the target objects and almost normalized for rapidly moving objects (0.7-1.0 m/s). These results confirm that moving target objects can also be used to evoke paradoxical movements in PD patients. Reaching was clearly faster for the stationary object when the neurostimulator was switched on. However, neurostimulation had no effect when the target object was moving. Thus conditions that evoke paradoxical movements are not affected by the modulation of neural activity in the basal ganglia. This finding suggests that paradoxical movements involve a second cortico-spinal motor loop that bypasses the basal ganglia.

66. The incidence of severe brain trauma in Cologne – results of an epidemiological study

B. Bouillon, M. Raum, B. Buchheister, R. Lefering, H. Fach, D. Liebler, E. Clemens, E. Neugebauer (Cologne, D)

Epidemiological data on the incidence, the prehospital and hospital care and the outcome of traumatic brain injury in Germany are scarce. It is therefore difficult to estimate the importance of this injury with respect to magnitude as well as effectiveness and efficiency of therapeutic concepts. We therefore planned a study that was supposed to provide population based epidemiological data on severe brain trauma from the site of the accident until discharge from hospital. All 90.000 prehospital emergencies that were cared for by emergency physicians in Cologne (EMS) from 1990 until 1996 were reviewed for identification of severe brain trauma. Their clinical course was documented using standard charts and patients were included if they had their accident within the city of Cologne and fullfilled the final inclusion criteria of GCS£8 or AISHead³3. 518 eligible patients were identified of whom 453 had complete datasets (follow-up 87%). Univariate statistical analysis was performed for all relevant variables. The main study endpoints were incidence and outcome of severe brain trauma. The annual incidence of severe brain trauma was 7.4/ 100.000. The average age was 38 years and 73% of the patients were male. 55% of injuries were motor vehicle related. 44% of the studypopulation suffered from multiple injuries. 91% of injured were reached by EMS within 10 minutes. Two third of patients had cardiorespiratory dysfunction and 45% pupil abnormalities on site. 97% had iv lines started in the field and 71% were intubated prehospitally. The average time from accident to hospital admission was 60 minutes. 43% had skull fractures, 44% cerebral edema and 67% intracranial bleeding. 70% had non-operative treatment. The average time on ventilator was 6.7 days and average length of stay was 9.6 days on ICU and 18.5 days in hospital. The overall mortality rate was 50%, 58% of deaths occurring within the prehospital setting. Mortality rates increased with ISS³24 and age³60 years. Hospital mortality increased from 29% to 69% if prehospital hypotension was present. The incidence of severe brain trauma in Cologne in this study was significantly lower than expected from the literature. Most severe brain trauma patients are treated non-operatively. The overall mortality was high, especially in the prehospital setting. Secondary injury due to prehospital hypotension seems to be high. (Supported by the Federal Ministry of Education and Research, Joint Project "Neurotrauma – NRW", FKZ: 01 KO 9808.)

67. Investigations of glial and neuronal reactions in the human spinal cord after lesions of descending tracts

A.B. Schmitt, A. Buss, S. Breuer, S. Brook, G.W. Kreutzberg, J. Noth (Aachen, D)

Little is known about the cellular responses in human spinal cord after lesions of descending tracts. Microglial and neuronal reactions were investigated on post mortem human spinal cord tissue of 20 patients who died after traumatic spinal cord injury or brain infraction with a range of survival times. Immunohistochemistry and PCR were applied to study lesion-induced responses of microglia/macrophages. Caudal to the spinal cord injury or brain infarction, a strong increase in the number of activated microglial cells was observed within the denervated intermediate gray matter and ventral horn of patients who died shortly after the insult (4-14 days). After longer survival times (5 weeks to 4 months), MHC class II-immunoreactivity (MHC II-IR) was reduced in the grey matter but very abundant in the white matter, co-localizing with the macrophage marker CD68 within the corticospinal tract. No T- or B-cell invasion or involvement of co-stimulatory B7 molecules could be observed. Our results suggest that the up-regulation of MHC II on microglia (which lack the expression of B7 molecules) may be responsible for the prevention of a T-cell response, thus protecting the spinal cord from secondary tissue damage. The subsequent expression of CD68 by microglia most likely reflects the switch in a macrophagic role, phagocytosing pre-synaptic terminals in target regions of descending fibre tracts at early time points and removing degenerating axons and myelin within descending tracts at later survival times, during Wallerian degeneration. Since C-Jun and GAP-43 seem to play a key role in the process of regeneration, in situ

hybridization studies were performed on patients who died after spinal cord trauma. These results demonstrate that axotomized Clarke`s nucleus neurons up-regulate GAP-43 and C-Jun mRNA after short survival times. This confirms our experimental data possibly reflecting a regenerative attempt which fails over time.

68. Differential patterns of microglial cell activation in human traumatic brain injury and ischemia

R. Meyermann, H.J. Schluessener, R. Beschorner (Tuebingen, D)

Microglial cells are sensitive sensors to a variety of stressors to the brain. Activation of microglia is considered a crucial factor in development and recovery from brain lesions. In contrast to animal models, only few markers are available to perform comparative analysis of microglial cell and macrophage activation antigens in tissue sections. In this study, a library of 47 human brains from patients with traumatic brain injury and ischemia and 20 control normal brains were analysed. Microglial cell/brain macrophage activation was assessed by various antibodies, notably against the Ca2+-binding members of the S-100 supergene family MRP8 and MRP14, and against allograft inflammatory factor-1 (AIF-1). Expression of this putatively Ca2+-binding peptide is upregulated in microglial cells during experimental inflammation and degenerative diseases of the rodent CNS (6). We observed that AIF-1 was significantly upregulated in ischemia, but not in TBI (1,3). This is in contrast to MRP8, but not MRP14, that is selectively upregulated in the early phase of ischemia (until day 3) and during the delayed phase of TBI (from day 3 and later) (2-5). As a practical consequence expression of MRP8 by brain macrophages/microglial cells can be used in forensic medicine to distinguish between traumatic and ischemic brain injury.

69. Functional restitution of traumatic brain injuries in children

M. Illert, J. Kuhtz-Buschbeck, B. Leplow, M. Lehnung, M. Gölge, H. Stolze, and Study Group (Kiel, D)

In children the situation for restitution of motor and cognitive functions after TBI differs from that in adults for various reasons: – TBI affects functions already developed and influences their further development; – the brain of children may be particular susceptible for brain injuries at certain phases during the development; – it is often assumed that the not fully matured brain may have a better potential for restitution than that of adults. Before this background the re-search project has concentrated on four major topics: (1) Development of motor functions and their restitution after TBI (prehension movements; locomotion on a treadmill and in space); (2) development of spatial orientation and memory and their restitution after TBI; (3) effects of severity and age at injury on cognitive development, neuropsychological functions and psycho-social adjustment; (4) target reaching and object manipulation after lesions of defined de-scending motor tracts: experimental studies in cats. The presentation will focus on the investigated sensorimotor and cognitive functions and intro-duce the tests developed and adapted to study the respective modalities. Results on the devel-opment of these function obtained in more than 140 children in the age range between 13 months and 12 years will be presented. These data can serve as standards at various age levels. Investigations in children after TBI show specific revovery curves for the cognitive and motor functions displaying different recovery speeds. The data demonstrate that the developed tests are highly sensitive to monitor restitution of brain functions. (Funded in the Research Program "Gesundheit 2000" by the German BMBF, FKZ 01KO9512.)

70. Traumatic injury to the developing brain: neuropathological and molecular characterization

C. Ikonomidou, P. Bittigau (Berlin, D)

71. Compensatory swelling of retinal ganglion cells predicts recovery of vision after optic nerve crush

B.A. Sabel, V. Rousseau (Magdeburg, D)

Diffuse axonal injury after neurotrauma can be simulated in the adult rat by partial optic nerve crush (ONC). Immediately after such injury rats have severe visual deficits, but within 2-3 weeks they can recover some of their lost visual functions, as long as a minimum number of cells survive the injury. To evaluate the role of the surviving cells in recovery of vision, we have now applied in vivo confocal neuroimaging microscopy (ICON) to correlate morphological alterations of retinal ganglion cells (RGCs) repeatedly in vivo with behavioral performance over time. After rats had learned to perform a visual contrast discrimination task, their RGCs were labeled retrogradely with fluorescent beads. Animals then received either no ONC, a complete axotomy or bilateral mild, moderate or severe ONC. ICON was then applied every five days for 40 days while vision was assessed in parallel with an automated contrast discrimination task on the four remaining days. When ONC was severe, most of the RGCs died after having undergone a fast and massive soma swelling which was accompanied by no

recovery of vision. In contrast, after a mild or moderate crush, about 30% of the RGCs survived, half of which showed a slow and moderate, compensatory cell swelling which is distinctly different from cell death associated swelling. The correlation analysis between cell size changes and behavioral performance revealed the following: The relative number of surviving cells which do not swell correlates with behavioral performance a few days after the injury, but not at post-operative days 23 and 38. At these later time points, but not early on, the relative number of "compensatory neurons" and the extent of their diameter increase correlates very highly (r= 0.96), in time course and extent, with subsequent recovery of contrast discrimination performance. Thus, recovery of vision can be predicted with high accuracy with this simple, morphological criterion. In summary, "compensatory neurons" showing moderate soma swelling contribute in a prominent way to recovery of vision, providing an important structural substrate for neuronal tissue repair. Key words: vision, plasticity, retinal ganglion cells, recovery, ICON-microscopy, contrast discrimination. (Supported by grants from the Deutsche Forschungsgemeinschaft (DFG), by the State of Sachsen-Anhalt and by the Ministry of Education and Research (BMBF, Neurotraumaverbund Magdeburg/Berlin, TP A2).)

72. Optimization of sacral anterior root stimulation (SARS) by the application of multichannel-generated, quasitrapezoidal pulses in an anodal block technique in a canine model

C. Seif, P.-M. Braun, A. Sotelino, J. Weiss, K.-P. Juenemann (Mannheim, D)

Introduction and objectives: After spinal cord injury with loss of bladder function, reservoir function and voiding control can be restored by sacral anterior root stimulation (SARS) with deafferentation. Previous studies with modified, size-adapted Finetech Brindley electrodes revealed very good results in selective sphincter blockade and simultaneous bladder stimulation using modified quasitrapezoidal (QT) pulses in an anodal block technique. The aim of this study was to apply four QT-pulses, determined in previous trials, by means of a multichannel bladder stimulator.
Materials and methods: In acute animal trials, lumbal laminectomy (L4-L7) and sacral deafferentation (S1-S4) were performed in 6 male anaesthetized foxhounds. The sacral anterior root S2 was placed into a modified tripolar Brindley electrode. Two mono- and two biphasic quasitrapezoidal (QT) pulses were applied in uni- and bilateral trials. Sphincter pressure was urodynamically monitored. Current parameters that demonstrated the best sphincter pressure reduction in unilateral application were utilized for multichannel stimulation. Bilateral measurements were analyzed, evaluated and compared with the multichannel sphincter pressure results. A two-channel current source (Fraunhofer Institute, IMBT) with two direct arbitrary programmable signal channels initiated stimulation. Each anterior root was stimulated separately.
Results: A selective urethral sphincter blockade was achieved in all QT series. In bilateral stimulation trials the average sphincter pressure could be reduced to 8.05 % of its maximum value. In multichanal stimulation sphincter pressure reduction to 4.78 % was seen. For maximal sphincter blockade the average current applied (1.1 to 1.2 mA) was the same with multichannel stimulation and with bilateral stimulation.
Conclusion: It is possible to achieve selective urethral sphincter relaxation with the application of quasitrapezoidal pulses in an anodal block stimulation technique. The application of individually specified current parameters to each sacral nerves enables better sphincter blockade than bilateral stimulation using the same parameters for each side with the application of the same amount of current. Multichannel stimulation is essential in the development of an neurostimulator that will induce coordinated and synergic bladder contractions. In the near future it will be possible to adjust the signal and current in this stimulator for simultaneous parameter application in accordance with bladder filling.

73. The possible role of nitric oxide for the development of urinary bladder hyperreflexia and central pain in rats with chronic spinal cord injury

P. Trudrung, P. Callsen-Cencic, U. Wirth, S. Mense (Heidelberg, D)

Hyperreflexia of the lower urinary tract (LUT) and chronic pain close to the level of the lesion after spinal cord injury (SCI) have been hypothesised to develop following a reduction in the activity of inhibitory mechanisms in the spinal cord. Previous work from our laboratory has shown that the local release of nitric oxide (NO) exerts a tonic inhibitory influence on neurones processing nociceptive information in the spinal cord dorsal horn (DH). The aim of the present study was to analyse if there are alterations in the number of NO-synthesising neurones in lumbosacral segments related to the innervation of the LUT and in the vicinity of a complete chronic SCI which, if the is a reduction in number, might contribute to hyperreflexia in the LUT and to the generation of pain close to the level of the lesion. Female Sprague-Dawley rats (250-300g) were deeply anaesthesised and – under aseptic conditions – either completely spinalised at the level T9-T10 or sham-operated (laminectomy only). Postoperatively, animals were closely monitored for

any signs of distress, their urinary bladders were evacuated manually twice a day until automatic micturition occured, they received antibiotics and analgetics. After six weeks survival time, serial sections of the lumbosacral spinal cord, sections of the thoracic spinal cord rostral and caudal to the lesion site, and sections from corresponding segments of sham-operated controls were analysed for the presence of NO-synthesising neurones. Cell numbers were counted and statistically evaluated. In lumbosacral spinal cord segments related to the innervation of the LUT the number of NO-synthesising neurones was significantly decreased in comparison to sham-operated controls. The number of NO-synthesising neurones was also reduced on both sides of the lesion, the reduction being statistically more significant rostral to the lesion site. The decrease in number of NO-synthesising neurones in lumbosacral segments of the spinal cord involved in the innervation of the LUT might contribute to the increased activity in LUT reflex pathways which is almost regularly expressed after SCI. In a similar way, the reduction of NO-synthesising neurones in the vicinity, especially rostral to a chronic SCI, might contribute to a considerable decrease in the tonic inhibition of neurones processing nociceptive information in the DH and, therefore, to an increase in the perception of pain in segments close to the chronic SCI. The involvement of the neurotransmitter / modulator NO could explain why customary methods of treatment are rather insufficient in patients with urinary hyperreflexia and pain following spinal cord lesions. An increase in spinal NO production could contribute a new method of treatment of these disorders. (Supported by the BMBF, grant 01KO9504.)

Special Luncheon Seminar 3

Inhibition of the bradykinin B2-receptor in acute brain insults (sponsored by Laboratoires Fournier, Daix, F)

LS74. LF 16-0687 Ms – a novel non-peptide bradykinin B2 receptor antagonist

D. Pruneau, J.L. Paquet, J.M. Luccarini, E. Defrêne, C. Fouchet, R.M. Franck, B. Loillier, C. Robert, B. Cremers, M. Vernier, P. Dodey (Daix, F)

Bradykinin (BK) and kallidin are endogenous 9- and 10-aminoacid peptides produced from the kallikrein-mediated cleavage of high and low molecular weight kininogens, respectively. Although kinins are maintained at low levels by enzymatic degradation, they may be locally increased following traumatic brain injury (TBI), then contributing to the development of brain edema and secondary neurological lesions. This occurs through the activation of B2 receptors coupled to the release of secondary mediators including nitric oxide and PGE2. In the present study, we compared the pharmacological profile of the non-peptide B2 receptor antagonist, LF 16-0687 Ms and of CP-0127 (Bradycor), a peptide B2 receptor antagonist. The binding affinity (Ki) of LF 16-0687 Ms and Bradycor for the cloned human stably expressed in HEK 293 cells was 0.67 nM and 313 nM, respectively. In addition, the functional potency (pA2) of LF 16-0687 Ms and Bradycor evaluated against BK-induced contractions of isolated human umbilical vein was 9.1 and 5.7 respectively and mouse bladder was 8.4 and 7.7, respectively. Although species-difference in affinity and potency was noted for both compounds, it appears that Bradycor is more than 1000 fold less potent than LF 16-0687 Ms as an antagonist of the human B2 receptor.

Moreover, we found that BK (100 nM) produced a calcium signal in primary cultures of embryonic rat cortical neurons which was inhibited by LF 16-0687 Ms with an IC50 of 3 nM. Since a positive trend was noted with Bradycor in two clinical trials conducted in severe head trauma patients, we believe that testing of a non-peptide B2 receptor antagonist which is more potent is justified. In this respect, LF 16-0687 Ms has been evaluated in various rodent models of traumatic brain injury and showed promising results.

LS75. Neuroprotective effect of LF 16-0687 Ms, a bradykinin B2 receptor antagonist, on a model of traumatic brain injury in mice

C. Verrecchia, C. Mésenge, M. Plotkine (Paris, F)

Pathological conditions such as brain trauma or ischemia are associated to cerebral edema. Bradykinin is one of the major mediators of brain edema. In the present study, we investigated the effect of LF 16-0687 Ms, a new nonpeptide B2 receptor antagonist (1), on neurological deficit and cerebral edema in a mouse model of closed-head injury (2). Four hours after traumatic brain injury (TBI), the neurological deficit was evaluated by a grip test and edema formation was measured by the brain water content. In addition, the therapeutic window of LF 16-0687 Ms on the neurological consequences of TBI was determined. Control mice had a grip score of 28.2 ± 0.8 s. TBI led to a significant reduction of the grip score (8.0 ± 1.5 s, $P<0.001$) and to a significant edema formation (80.10 ± 0.77 % versus 79.29 ± 0.37 % in uninjured mice, $P<0.001$). When LF 16-0687 Ms was given s.c., 30 min after TBI, at 0.3, 1, 3 and 10 mg/kg, the grip score was significantly enhanced (respectively, 14.3 ± 2.6 s, $P< 0.05$; 20.9 ± 2.1 s, $P<0.001$; 20.5 ± 2.2 s, $P<0.001$ and 14.3 ± 2.4 s, $P<0.05$). The neuroprotective effect observed at 1 and

3 mg/kg was associated with a reduction of brain edema (respectively 79.70 ± 0.16 %, P<0.05 and 79.57± 0.09 %, P<0.01). At 30 mg/kg, LF 16-0687 Ms had no effect on the grip score. The effect elicited by LF 16-0687 Ms (3 mg/kg) on post-traumatic neurological deficit was still present when treatment was delayed 1 or 2 h after injury. The present data show that LF 16-0687 Ms improves the neurological outcome after TBI, suggesting that B2 receptor activation contributes to the post-traumatic neurological deficit. The neuroprotection is present when treatment is delayed until 2 hours. However, this neuroprotective activity is not always associated to an antiedema effect. Although the mechanism of neuroprotection is not completely understood, these data suggest that B2 receptor antagonism could be a new therapeutic target for the treatment of acute TBI.
References: (1) Pruneau et al. (1999) Immunopharmacology 43: 187-194. (2) Mésenge et al. (1996) J. Neurotrauma 13: 209-214.

LS76. Effect of a novel non-peptide bradykinin B2 receptor antagonist (LF 16-0687 Ms) on vasogenic brain edema in the rat

N. Plesnila, J. Schulz, J. Eriskat, M. Stoffel, A. Baethmann (Charlestown, USA; Munich, D)

Bradykinin is an important factor in the formation of vasogenic brain edema by increasing vascular permeability through the activation of endothelial B2 receptors. The present study examined the effect of a novel non-peptide B2 receptor antagonist, LF 16-0687 Ms, on post-traumatic vasogenic brain edema in rats. Male Sprague-Dawley rats (250-300 g b.w) were anesthetized with halothane (0.8%). After exposure of the left parietal cortex, a cryogenic lesion was induced by a copper cylinder (Æ 5 mm, -68°C, 15 s). The animals (n=10/group) were randomly assigned to i.v. treatment with 10 µg/kg/min, 100 µg/kg/min LF 16-0687 Ms or its vehicle (0.9% NaCl). The infusion started 10 min before trauma and lasted for 24 h. LF 16-0687 Ms 10 µg/kg/min or its vehicle (n=10/group) was also given starting 30 or 60 min after injury. At 24 h, rats were sacrificed, the brain was removed and the hemispheres separated. Determination of water content of each hemisphere was performed by the wet-dry method and a swelling index was calculated. In the vehicle-treated group the traumatized hemisphere had a water content of 81.5 ± 0.3% (± SD) vs 80.0 ± 0.3% for the contralateral hemisphere (p<0.001). The traumatized hemisphere swelled by 9.3 ± 1.1%. After 100 µg/kg/min LF 16-0687 Ms, the water content was reduced in both hemispheres (traumatized: 80.8 ± 0.8%; contralateral: 79.6 ± 0.6%). The hemispheric swelling was 7.4 ± 1.3% (n.s.). LF 16-0687 Ms at 10 µg/kg/min reduced the water content in the traumatized hemisphere (81.3 ± 0.6%) and decreased brain swelling to 6.4 ± 1.3% (p<0.05). However, when treatment with LF 16-0687 Ms at 10 µg/kg/min was started 30 or 60 min after trauma no reduction of brain water content or hemispheric swelling could be observed. These results show, that administration of LF 16-0687 Ms at a low dose decreased vasogenic brain edema from a cortical lesion when applied prior to but not after injury. These findings support an important role of B2 receptors in the development of vasogenic brain edema.

LS77. The selective bradykinin B2 receptor antagonist LF 16-0687 Ms significantly reduces traumatic brain edema in rats

J.F. Stover, N.K. Dohse, A.W. Unterberg (Berlin, D)

Activation of bradykinin B2 receptors contributes to vasogenic brain edema formation. Therefore, blocking of B2 receptors, primarily located on endothelial cells, should decrease edema formation. Anti-edematous effect of the novel nonpeptide B2 receptor antagonist LF 16-0687 Ms was investigated in 40 brain-injured rats. Following controlled cortical impact injury, rats received a single subcutaneous dose of no (NaCl), low (3 mg/kg body weight) or high dose (30 mg/kg) of LF 16-0687 Ms at 5 min after trauma. After 24 hours, brain swelling and mediators of edema formation (CSF taurine: volume regulation and hypoxanthine: ATP-degradation) were determined. Both the low and high dosages of LF 16-0687 Ms significantly reduced brain swelling by 25 and 27% respectively (p<0.03). Mediators of edema formation, i.e. CSF taurine and hypoxanthine levels were significantly decreased following single administration of LF 16-0687 Ms (p<0.005). Under the present study design, single administration of LF 16-0687 Ms successfully reduced post-traumatic brain swelling and decreased taurine and hypoxanthine levels. Following traumatic brain injury, selective inhibition of B2 receptors with LF 16-0687 Ms is a promising anti-edematous target.

LS78. A clinical trial of a bradykinin antagonist (Bradycor™ (Deltibant CP1027)) in severe traumatic brain injury

A. Marmarou, J. Nichols, J. Burgess, D. Newell, J. Troha, D. Burnham, L. Pitts (Richmond, USA)

The neuroprotective effects of bradykinin antagonists have been well demonstrated in the laboratory setting. In this report, we document the experience in the clinical setting where a prospective, randomized, double blind trial of a bradykinin antagonist CP-0127 (Bradycor) was conducted in North America in severely head injured patients. (n=139). The primary objective was to assess the efficacy of a continuous infusion of Bradycor in preventing ICP (intra-cranial pressure) elevation. Other efficacy measures included

the effect of Bradycor on the therapy required to treat ICP, mortality and functional outcome. A secondary objective was to assess the safety of Bradycor in patients with severe brain injury. Patients were followed for the first 14 days of hospitalization with long term outcome assessed at 3 and 6 months after injury. The drug was well tolerated in this patient population and no adverse events were attributable to the compound. Although positive trends were seen for ICP and therapy intensity level in the Bradycor group, these differences analyzed on a daily basis were significant only for the more severely injured patients on days 4 and 5. Patients treated with Bradycor showed a 10.3% improvement in outcome at 3 months and a 12% improvement in outcome at 6 months. (p=0.26). The positive trends seen in ICP, neuropsychological tests and Glasgow outcome score provide supportive evidence of a neuroprotective role for bradkinin antagonists in the head injured population.

LS79. LF 16-0687 Ms, a non-peptide B2 receptor antagonist, reduces cerebral edema and improves neurological outcome after closed head trauma in rats

Y. Shapira, I. Asa, A.A. Artru, Y. Ivashkova, S. Shikanov, D. Pruneau (Beer-Sheva, IL)

Bradykinin is a potent dilator of the brain vasculature which also opens the blood brain barrier. It activates the arachidonic acid cascade, induces the synthesis and release of free radicals in the vascular wall and stimulates glial cells to release glutamate and aspartate. It was shown that bradykinin suppression by kallikrein inhibition decreased cerebral edema and specific inhibition of the kinin-kallikrein system resulted in an attenuation of vasogenic brain edema in a rabbit model of cortical cold lesion. In the present study, we evaluated the effect of LF 16-0687 Ms, a novel nonpeptide bradykinin B2 receptor antagonist, on closed head trauma (CHT) in rats. Rats were anesthetized with halothane and were randomly assigned to treatment subcutaneously (SC) by LF 16-0687 Ms or its vehicle (NaCl 0.9 %) either with (Gr 3-7) or without (Gr 1-2) CHT. LF 16-0687 Ms or its vehicle (Gr 1, 3) was injected at 1 mg/kg (Gr 4) and 3 mg/kg (Gr 6) 1 h after CHT and at 1 mg/kg x 2 (Gr 5) and 3 mg/kg x 2 (Gr 7), 1 and 8 h after CHT. Neurological severity score (NSS) was evaluated at 1 h, and 24 h following CHT. Twenty four hours following CHT, brains were removed and water content of the hemispheres was determined. LF 16-0687Ms at 1 mg/kg x 2 (Gr 5), and 3 mg/kg (Gr 6) reduced significantly edema of the injured hemisphere by 70%. There was no significant change of blood pressure. There was a significant improvement in neurological outcome in all treated groups except for Gr 4 (1 mg/kg). These results showed that blockade of bradykinin B2 receptor represents an effective approach to reduce cerebral edema and to improve neurological outcome following traumatic brain injury without affecting blood pressure. Further studies are needed to assess the role of bradykinin in the development of vasogenic and cellular edema.

Plenary Session 4

Regeneration, reorganization and repair, neurotrophins

80. Inflammation in the injured spinal cord (Invited lecture)

L. Schnell, D.C. Anthony, V.H. Perry, M.E. Schwab (Zurich, CH; Southampton, UK)

Traumatic lesions in the CNS are accompanied by a complex cascade of reactions, leading not only to the immediate loss of parenchymal cells, but also to the development of areas of secondary damage and to the initiation of an acute inflammatory response. Comparative studies in brain and spinal cord have shown that a stronger inflammatory reaction is evoked in the cord, reflected by leukocyte numbers, astrocyte activation and the extent of the BBB breakdown. There was no evidence that differences in the expression of endothelial cell adhesion molecules could account for these compartment-specific differences. Experiments with minimally-invasive injections of the pro-inflammatory cytokines IL-1 and TNF made clear that the stronger inflammatory reaction evoked in the spinal cord was not simply related to the mechanical disruption of the more prominent vasculature found in this CNS compartment. To understand to what extent inflammatory cells promote or limit secondary injury, we manipulated leukocyte infiltration by either whole body irradiation or selective depletion of leukocyte populations using specific antibodies. While the use of radiation achieved a reduction in the extent of the BBB breakdown, the secondary cell death area remained unchanged. Surprisingly, the use of leukocyte-specific depletion not only failed to reduce the area of plasma extravasation, but also led to an increase in the cell death area. These results cast doubt on the previously assumed importance of neutrophils and monocytes in post-traumatic secondary tissue loss, and emphasize the fact that our understanding of the beneficial and/or detrimental influence of inflammatory processes after CNS lesion is still far from being clear.

81. Actual development of regeneration and repair in spinal cord injury (Invited lecture)
A. Privat (Montpellier, F)

Current strategies in traumatic spinal cord injury involve the well known triad: neuroprotection-regeneration-substitutive therapy. Regeneration of injured central neurons is now considered as possible, due to progress in the analysis and overcome of inhibitory factors. If inhibition of the regenerative capacity of axons in the white matter appears to be due to specific oligodendrocyte proteins (NOGO, MAG) it appeared that the main obstacle to long distance regeneration and reconstruction of circuits resides in the astrocytic scar. Efforts have been directed at circumventing it by the use of transplants of permissive cells (Schwann cells, olfactory bulb ensheathing cells, tanycytes) but also by the analysis of the role of cell coat and extracellular matrix molecules of the scar, thanks to transgenic mouse models. Finally, substitutive therapy could be based on the best use of sub-lesional spinal centers responsible of specific behaviour – such as the central pattern generator for locomotion – as target for cell (embryonic, stem cells, engineered cells) therapy and for gene therapy. (Supported by Inserm, IRME, and Verticale.)

Regular Session 10
Neuroprotection, hypothermia

82. Current state of clinical neuroprotection in severe head injury (Invited lecture)
G.L. Clifton (Houston, USA)

Background: Hypothermia treatment for brain injury produced consistently positive results in laboratory studies and in small clinical studies, but a definitive test of efficacy was needed. The National Acute Brain Injury Study: Hypothermia, a randomized prospective, multi-center trial of surface-induced hypothermia versus normothermia was performed.
Methods: Hypothermia (33ºC) was initiated in patients age 16-65 in coma from brain injury (Glasgow Coma Score 3-8) within six hours of injury and maintained for 48 hours. Management was consistent with the Guidelines for the Management of Severe Head Injury. Glasgow Outcome Scale was measured at six months.
Results: Final enrollment was 392 patients. Patients treated with hypothermia had a higher percentage of hospital days with complications, fewer instances of elevated intracranial pressure and no improvement in outcome. Twenty-eight percent of patients were hypothermic on admission (<35°C). For all admission temperatures, patients treated with hypothermia age >45 years (n=52) had a 20 percent increase in poor outcomes, associated with increased medical complications (P=0.04). In 81 patients age <45 years admitted with temperature <35ºC, maintenance of hypothermia was associated with a 24 percent improvement in poor outcomes (p=0.02). Patients who were normothermic on admission had no treatment effect.
Conclusion: In patients with severe head injury and age >45 years, hypothermia is associated with worse outcome regardless of admission temperature, but in patients with hypothermia on admission and age <45 years, maintenance of hypothermia is associated with improved outcome. Patients normothermic on admission were likely treated with hypothermia too late.

82A. CBF and metabolism in acute phase of severe head injury – a comment on hypothermic therapy
U. Ito, H. Tomita, O. Tone, M. Hara (Tokyo, J)

Introduction: Hypothermic patients in the acute phase of head injury have generally been considered to have a poor outcome. Recently, in some institutions, artificial hypothermia has been applied to patients during the acute phase of head injury. Using the Kety-Schmidt method, we have measured cerebral blood flow and metabolism (CMRO2) of patients for 8 days after severe head injury, and compared the results with the outcome of each patient.
Patients and methods: We have measured CBF and CMRO2 during the first week after head injury in 59 patients whose Glasgow Coma Scale (GCS) was less than 9 on admission. Outcome was assessed by the Glasgow Outcome Scale (GOS) 6 months after injury. We divided the patients into two groups on the basis of the GOS: those with good recovery and moderate disability were categorized as Fair Outcome (FO), and those with severe disability, or who became vegetative or died, as Poor Outcome (PO). CBF and CMRO2 were measured by the nitrous oxide (N2O) inhalation method, analyzing blood N2O and O2 content. CBF values were adjusted to those when PaCO2 was 34 mmHg. Relative brain ischemia was defined as CMRO2 more than, and CBF less than the mean ± SD of the control group (n=10). Each value given is the average ± SD.
Results: FO group: In 33 patients, the GCS was 6.4 ± 1.4, CBF was 51.9 ± 10.5 ~ 64.5 ± 30.3 (ml/100g/min), and CMRO2 was 3.01 ± 1.18 ~ 4.19 ± 2.29 (ml/100g/min) during the first week. PO group: In 26 patients, the GCS was 5.4 ± 1.7, CBF was 31.8 ± 8.1 ~ 50.0 ± 10.0, and CMRO2 was 1.97 ± 1.07 ~ 2.93 ± 1.71. The scatter graph of CBF against CMRO2 of all patients showed a well coupled relationship between these parameters. CBF and CMRO2 of all patients in

the PO group were lower than the average values of the control group. Relative ischemia was found in the brain of one patient in the FO group at one day after head injury, and in one patient in the PO group at 6 days after head injury.
Conclusion: All patients in the PO group had a lower CBF and metabolism during the first week after severe head injury. Only two patients suffered from relative brain ischemia. Hypothermia, which causes a further reduction of CBF and metabolism, seems to be an unfavorable factor during the initial acute phase of severe head injury.

83. Hemodynamics during moderate therapeutic hypothermia after traumatic brain injury
J.L. Nates, E.R. Miller, G.L. Clifton (Houston, USA)

Despite increased use of moderate hypothermia (33oC), hemodynamic aspects of this therapy are still unclear. Most studies have focused on neurologic effects of hypothermia, while hemodynamics have been overlooked. The National Acute Brain Injury Study: Hypothermia (NABIS:H) was a randomized multi-center study with 392 patients in two groups, standard management at normothermia (N) or standard management at hypothermia (H) for 48 hours. Patients age 16-65 were enrolled with a Glasgow Coma Score < 8. Abbreviated Injury Scores (AIS>4) were used to exclude patients with multiple trauma. There was no difference in Glasgow Outcome Score at six months. A subgroup of 40 patients from one center in NABIS:H was reviewed (H 19, N 21). Age, sex, diagnosis, injury severity score (ISS), heart rate (HR), mean arterial pressure (MAP), cardiac output (CO), systemic vascular resistance (SVR), pulmonary artery wedge pressure (PAWP), and fluid balance (FB) were examined. Data were analyzed using the unpaired t-test, with $p<0.05$ as significant. In this subgroup, the groups were well matched for age (H 27.3+12, N 30.4+12; NS), admission GCS (H 5.5, N 5.3; NS), and ISS (H 27.8+9, N 30.8+7; NS), although more males were in the hypothermia group (H 84%, N 57%). Hypothermia patients showed decreasing trends in MAP ($p<0.007$) and SVR ($p<0.03$) and increasing trends in HR ($p<0.0000$) and CO ($p<0.001$). Fluid balance on Day 2 was significantly greater in the hypothermia group. The only significant difference seen in the normothermia group was an increasing trend in MAP ($p<0.002$). We found major cardiovascular changes on Days 2 and 4 in hypothermia patients. From the hypothermic period (Days 1 and 2) through rewarming (Day 3) and post-rewarming (Day 4), hypothermia patients showed increases in HR and CO and decreases in MAP and SVR. These findings have implications for fluid and vasopressor therapy in conjunction with therapeutic hypothermia.

84. Critical physiologic variables over the first 96 hours of moderate hypothermia for severe traumatic brain injury
E.R. Miller, J.L. Nates, G.L. Clifton (Houston, USA)

Introduction: Intracranial pressure (ICP), mean arterial pressure (MAP), and cerebral perfusion pressure (CPP) have significant effects on outcome from severe TBI. Moderate hypothermia (33o C) is a promising addition for treatment of severe TBI as a neuroprotective agent and for its effect on ICP.
Materials and methods: The National Acute Brain Injury Study: Hypothermia (NABIS:H) was a randomized multi-center study with 392 patients in two groups, standard management at normothermia (N) or standard management at hypothermia (H) for 48 hours. Patients age 16-65 were enrolled with a Glasgow Coma Score < 8. Abbreviated Injury Scores (AIS>4) were used to exclude patients with multiple trauma. There was no difference in Glasgow Outcome Score at six months. Age, GCS, injury severity score (ISS), ICP, Therapy Intensity Level (TIL), MAP, and CPP were examined. Data were analyzed using the unpaired t-test, with $p<0.05$ as significant.
Results: The groups were well matched for age (H 31.7+12, N 32.3+13; NS), admission GCS (H 5.5, N 5.6; NS), and Injury Severity Score (H 28.3+9, N 27.8+8; NS). There was no difference in mean ICP for any day; however, mean MAP and CPP differed on Days 1, 3, and 4. There were also significant differences in the percentage of patients with ICP>25, ICP>30, CPP<50, and MAP<70.
Conclusion: Hypothermia significantly reduced occurrence of ICP>25 (Days 1 and 2) and ICP>30 (all days). Induction of hypothermia (Day 1) was associated with a higher MAP and fewer patients with MAP<70 or CPP<50. During rewarming (Day 3) and post-rewarming (Day 4), hypothermic patients were more likely to have MAP<70 and CPP<50 and required more therapy for ICP (TIL). This occurred despite aggressive volume and vasopressor therapy.

85. Combination drug therapy and hypothermia after focal cerebral ischemia in the rat – evidence for efficacy in a post-treatment regimen
S. Zausinger, K. Schöller, A. Baethmann, R. Schmid-Elsaesser (Munich, D)

Purpose: We have recently demonstrated that pretreatment with magnesium (calcium- and glutamate-antagonist) and tirilazad (antioxidant) in combination with intraischemic mild hypothermia offers superior neuroprotective efficacy in a rat model of focal reversible cerebral ischemia. In the present study we investigated the therapeutic efficacy of this pathophysiologically orientated treatment strategy with

a posttreatment regimen to define its role for stroke patients.
Materials and methods: 28 Sprague-Dawley rats were subjected to 90 min of MCA occlusion by an intraluminal filament. Animals were randomly assigned to four groups (n=7 each): (I) normothermic vehicle-treated controls; (II) MgCl (2x1mM/kg) and tirilazad (2x3mg/kg) + hypothermia (33°C) beginning at 0 hours; (III) 1 hour; or (IV) 3 hours after induction of ischemia. Drugs were given in one hour intervals and hypothermia was maintained for 2 hours. Neurological deficits and body weight were assessed daily. Infarct size was planimetrically determined on postoperative day 7.
Results: Combination therapy with MgCl and tirilazad + mild hypothermia significantly reduced infarct volume compared to normothermic controls by -72%, -57%, and -65% if applied at 0 hours, 1 hour, and 3 hours after induction of ischemia, respectively. All treatment groups showed significantly less neurological deficits and a trend towards better weight gain.
Conclusion: Postreatment with this pathophysiologically orientated combination therapy using clinically licensed drugs such as magnesium and tirilazad + mild hypothermia is highly efficacious with a therapeutic window of at least 3 hours. Further experiments with larger groups and longer postischemic intervals are carried out at the moment.

86. Blockade of N-type voltage-sensitive calcium channels is neuroprotective in a rat LFP model of traumatic brain injury
R.F. Berman, L.L. Lee, J.P. Muizelaar, B.G. Lyeth (Davis, USA)

Calcium is an important intracellular regulator of many critical processes required for normal cell function. Under normal physiological conditions the resting free intracellular calcium concentration is maintained at very low levels (e.g., ~100 nM) relative to extracellular calcium (e.g., 1.2 mM). Traumatic brain injury (TBI) can disrupt calcium homeostasis, leading to an excessive amount of calcium entering the cell. This abnormal accumulation of intracellular calcium is thought to contribute to several biochemical mechanisms of secondary neuronal injury, including free radical production, activation of proteolytic enzymes (e.g., calpain) and induction of apoptotic pathways. The activation of voltage sensitive calcium channels (VSCC's) by TBI is thought to provide an important route of entry for calcium into damaged neurons and glia. Therefore, drugs that block specific calcium channels have been hypothesized to be neuroprotective. In the present study, the potential neuroprotective effects of a specific N-type voltage gated calcium channel blocker, SNX-185, was examined. SNX-185 is the synthetic form of omega-conotoxin TVIA isolated from the fish eating marine snail Conus tulipa. Traumatic brain injury was induced in anesthetized rats using the lateral fluid percussion (LFP) (2.15 ± 0.05 atm) injury model. Immediately after injury 50, 100 or 200 picomoles of SNX-185, or ACSF-vehicle was injected into the CA3 subregion of the hippocampus (bregma -3.0mm, 3.0mm lateral to midline and 3.1mm ventral). Drug or vehicle was delivered in a volume of 20 microliters over a 1 hr interval via a 25 ga injection needle attached to a programable microsyringe. At 24 hr after TBI, the animals were euthanized and perfused transcardially with 4% paraformaldehyde. The degree of degeneration was visualized in brain sections using Fluoro-Jade staining, a histofluorescent marker for degenerating neurons. Cells were counted from CA2/3 region of the hippocampus (4 sections, between 2.3-3.8) by two "blinded" individuals. Compared to controls, rats treated with both 100 and 200 picomoles of SNX-185 showed a significant decrease in neuronal degeneration. No significant neuroprotection was observed in rats treated with 50 picomoles of SNX-185. Neurobehavioral protection was also observed in separate groups of animals following SNX-185 treatment for motor and cognitive performance. Our data indicate that blockade of N-type voltage sensitive calcium channels can be neuroprotective, and that compounds such as SNX-185 may have important therapeutic potential. (Supported by NIH NS 39090.)

87. Polymorphonuclear leukocyte accumulation in cerebral contusion – effects of vinblastin and anti-P-selectin antibody on contusion-induced edema formation
N. Aoyama, T. Mori, T. Kawamata, Y. Katayama (Tokyo, J)

Introduction: Previous studies have demonstrated that polymorphonuclear leukocytes (PMNs) accumulate in the injured brain, and this may cause secondary tissue damage in cerebral ischemia or trauma. In the present study, to determine whether or not PMN accumulation is associated with contusion edema, we evaluated the PMN accumulation and edema formation in a rat model of cerebral contusion, and tested the effects of vinblastin which induces leukocytopenia, and monoclonal P-selectin antibody which blocks endothelial leukocyte adhesion.
Materials and methods: Cortical contusion was induced in male Wistar rats using a controlled cortical impact device. The animals were divided into three groups: 1) a non-treated control group (saline, 1.5 ml/kg iv), 2) a leukocytopenia group (vinblastin, 0.7 mg/kg iv), and 3) an anti-P-selectin group (anti-P-selectin monoclonal antibody, 1.5 mg/kg iv). Edema

formation was evaluated in the central and peripheral areas of contusion by the specific gravity method at 1, 8, and 24 hours after injury. Brain coronal sections were sliced and stained with hematoxylin-eosin. PMN accumulation was estimated by counting the total PMNs in the peripheral area of contusion in five high-power fields (x 400) under a microscope.
Results: The non-treated control group revealed progressive PMN accumulation and edema formation. The edema formation was significantly attenuated in both the leukocytopenia and anti-P-selectin groups ($p<0.05$). The PMN accumulation was significantly reduced in the leukocytopenia group (31± 3/five fields; $p<0.05$), and in the anti-P-selectin group (30± 4/five fields; $p<0.05$), as compared to the non-treated control group (41± 6/five fields).
Conclusion: The present findings suggest that PMN accumulation and adhesion are involved in the development of edema formation in cerebral contusion. Attenuation of PMN adhesion may help to secondary cell damage following traumatic brain injury.

Regular Session 11
Pediatric head injury

88. Pediatric head injury – differences from and similarities with that in adults (Invited lecture)
H.E. James (San Diego, USA)

Pediatric head injury has unique issues in patient diagnosis, management and outcome, that differ from that of adult head injury. One of the most unique componenets of pediatric head injury that separates it from the adult head injury is that of non-accidental trauma. In non-accidental trauma, repeated secondary insults may well affect diagnosis, management and outcome. Age related aspects will determine a greater or lesser degree of cranial cervical junction injuries (disproportionate cranial size to trunk in infancy and early childhood), brain maturation, and apoptosis and neuronal recovery may be of significant importance in recovery and outcome. Other factors are underlying congenital anomalies, physiological factors such as cerebrovascular reactivity and blood flow. The support systems needed for the management of head injury in infants children will vary significantly from that of the adults in neuro imaging, specialized pediatric critical care needs, nursing needs, and acute rehabilitation. Neurological recovery in non-accidental trauma is uniformly poor when compared to accidental trauma. This may be due to the secondary insults, additional hypoxia and ischemia that may occur from repeated trauma and/or asphyxation. Pediatric head injury has its own specific needs in reference to rehabilitation, community re-entry programs, and educational needs for school re-entry.

89. Outcome of closed head injury in children – a comparison between children and adults
T. Tokutomi, T. Kuramoto, Y. Takeuchi, T. Yuge, M. Shigemori (Kurume, J)

Outcome after severe head injury has been shown in some studies to be better in children than in adults. To examine the factors that affect the favourable outcome in children, the severity and the type of intracranial lesion of head-injured children was compared with adults in a consecutive series of 815 closed head injury patients (543 of GCSÅÖ8; 82 children and 461 adults). WISC or WAIS-R test was administered to the patietns who made a GR or MD by GOS. The outcome of severe head-injured children was better than that of adults, however, the outcome were similar between children and adults in the GCS matched subgroups. Patietns with diffuse injury or epidural hematoma had a significantly better outcome than patients with subdural hematoma or intracerebral hematoma. The rate of patients with a GCS<5 was significantly low in children and the incidence of subdural hematoma or intracerebral hematoma was significantly high in adults. These factors associated with a better outcome in children. IQ scores were higher in children than adults at a few months after injury, but became similar at 1 year. We conclude that the reason for favourable outcome in children is that fatal injury or hematoma are less in children. Recovery of cognitive function in children is not so well.

90. Trauma Infant Neurological Score (TINS) is superior to children's coma scores in evaluating infants younger than 2 years after head injury
L. Beni-Adani, D. Asher, S. Constantini, S. Beni (Tel-Aviv, Jerusalem, IL)

Objectives: To develop a new Trauma-Infant-Neurological-Score (TINS) that would serve as a prognostic tool as well as an optimal guideline for performing CT in head-injured infants younger than 2y old.
Methods: TINS scores from 1(best) to 10 points (worst). It abandons the problematic parameters (mostly of the verbal part) of Children's Coma Scales, and includes the following: Trauma mechanism; Intubation (the infant being intubated or not); Neurological exam: pupils, motor-deficit and alertness (in a simple normal-abnormal grading); and presence of Subgaleal-hematoma. TINS has been applied on 312 infants, in parallel with CCS, using CT scanning as the gold-standard for detecting intra-cranial

abnormalities, and correlating both scores with outcome.
Results: mean age was12m (+7m), 181male (58%) and 147 female infants, mean CCS 13+3, and TINS 3+2. Outcome was good in 92.3%, mild deficit in 1.7%, severe deficit in 2% and mortality of 4%. Infants with TINS=1 had all good outcome, and none needed operation. High score of TINS correlated with higher incidence of pathological CT and worse outcome ($p<0.005$). In the minor-moderate head-injury group TINS correlated better than CCS with presence of surgical pathologies and outcome.. In infants with CCS 14-15 it was more sensitive in detecting intra-cranial pathologies on CT.
Discussion: Infants are a high risk group in head trauma, mainly due to lack of proper methods of neurological evaluation. None of the previously proposed Children's GCS was accurate in predicting outcome or in introducing optimal guideline for CT in head-injured infants. TINS was found superior to CCS probably because it uses objective parameters, some of which are not included in previously used scores.
Conclusion: TINS should be the new standardized method in evaluating neurological severity in infants after head trauma. In the previously healthy infant, TINS of 2 and above should be the guideline for performing a CT scan.

91. Volume of focal brain lesions and hippocampal formation in relation to memory function after closed head injury in children

G. Di Stefano, J. Bachevalier, H.S. Levin, J.X. Song, R.S. Scheibel, J.M. Fletcher (Bern, CH; Houston, USA)

Objectives: (1)A study of verbal learning and memory in children who had sustained a closed head injury (CHI) at least three months earlier. (2)To relate memory function to focal brain lesion and hippocampal formation volumes using morphometric analysis of magnetic resonance imaging (MRI).
Methods: A group of 245 children who had been hospitalized for CHI graded by the Glasgow Coma Scale (GCS), including 161 severe and 84 mild CHI patients completed the California Verbal Learning Test (CVLT) and underwent MRI which was analyzed for focal brain lesion volume independently of memory test data. MRI with 1.5 mm coronal slices obtained in subsets of 25 severe and 25 mild CHI patients were analyzed for hippocampal formation volume (HV). Interoperator reliability in morphometry was satisfactory.
Results: CHI severity and age at study significantly affected memory performance. Regression analysis showed that bifrontal, left frontal, and right frontal lesion volumes incremented prediction of various learning and memory indices after entering the GCS score and age into the model. Extrafrontal lesion volume did not contribute to predicting memory performance.
Conclusion: Prefrontal lesions contribute to residual impairment of learning and memory after severe CHI in children. Although effects of CHI on HV might be difficult to demonstrate in nonfatal pediatric CHI, further investigation using functional brain imaging could potentially demonstrate hippocampal dysfunction.

92. Does maturation matter? Developmental models of traumatic brain injury

A.-C. Duhaime, S.S. Margulies, S.R. Durham, R. Raghupathi (Philadelphia, USA)

Objectives: To learn whether the immature gyrencephalic brain responds differently to traumatic brain injury compared to adults, we have developed two different piglet models to study the response of the brain at different stages of development, including models of focal contusional injury and angular deceleration. Understanding developmental differences is of importance because treatments designed and tested in adults might have different efficacy or might even be counterproductive at different stages of maturation.
Methods: Piglets at 5 days (infant), 1 month ("toddler"), and 4 months (adolescent) have been studied. The contusion model was precisely scaled for the changes in mass and dimensions of the brain with growth in order to deliver comparable injury inputs at each age. In this model, strain distribution was held constant among ages by varying both diameter and depth of indentation to rapidly displace approximately 1% of total brain volume. This technique produced displacement of comparable structures including grey and white matter, and produced a histologically visible frontal lesion with no associated clinical deficits. Acute physiologic variables including cerebral blood flow (CBF) as well as lesion size at 7 days post-injury were measured. In the second model, angular deceleration non-impact injuries with 110 degrees of axial angulation at peak angular velocities of 230-280 rad/sec were applied to each age, with histology analyzed at 6 hours post-injury.
Results: Using the scaled focal contusion model, a striking age-dependent difference in lesion size was obtained, with the youngest subjects having the smallest lesions (0.8, 8, and 21% of hemisphere area injured, respectively). While most physiologic variables remained stable, CBF was lowest at baseline in the youngest animals, and rose after injury, while the older groups declined slightly. In the inertial model, younger animals demonstrated less widespread axonal injury compared to older subjects.

Conclusion: Because of its developmental and morphologic similarity to human infants, the piglet brain offers insight into the pathophysiology of brain injury during immaturity. These studies demonstrate that the immature brain, rather than being "fragile", is remarkably resistant to mechanical injury. Ongoing studies using MRI and immunohistochemical techniques are helping to delineate whether these age-dependent differences represent improved neuronal survivability or better repair or regeneration processes in the young.

93. Traumatic brain injury affects reorganization of the dentate gyrus following deafferentation in the developing brain

M.L. Prins, J.T. Povlishock, L.L. Phillips (Richmond, USA)

The current study was designed to address the long-term effects of traumatic brain injury (TBI) on plasticity and reorganization in the developing brain. While established models of TBI generate insults with both neuroexcitation and deafferentation, the effects are too diffuse to examine post-injury plasticity. Instead a unique combined injury model (Phillips et al., 1994) was employed to examine the effects of TBI on the synaptic reorganization of the dentate gyrus following entorhinal cortical lesion in the developing rat. Postnatal day 28 (P28) rats were given either sham, central fluid percussion (FP), unilateral entorhinal cortical (UEC) lesion, or FP+UEC injury. Cognitive performance was assessed in the Morris water maze (MWM) between 11-15 days post-injury and the brains were processed for synaptophysin (SYN) immunohistochemistry to assess reorganization within the dentate gyrus. The MWM results reveal that FP or UEC lesions delivered independently do not produce significant latency deficits in P28 rats. However, when these injuries are combined they produce significant deficits in the MWM. SYN labeling shows adult trilaminar pattern in sham and FP animals and UEC lesions resulted in increased SYN in the outer molecular layer (OML) of the dentate gyrus. By contrast, the animals subjected to the combined insult revealed no SYN staining in this OML region. These results suggest that TBI does affect the reorganization pattern within the dentate gyrus of juvenile subjects and that this may have an adverse effect on the subsequent behavioral outcome in the developing brain. (Supported by NSO7288-13.)

94. Reactive and regenerative cellular changes following in vivo and in vitro experimental axotomy of mature cortical neurons

J. Vickers, P. Adlard, B. Coleman, T. Dickson, I. Jacobs, C. King (Hobart, AUS)

We examined the reactive and regenerative cellular changes following axotomy in in vivo and in vitro experimental models. For the in vivo model, a 25 gauge blunt needle was inserted into the cortex of anaesthetised adult rats. After 1 day post-injury (PI), reactive changes in axons included bulb- and ring-like accumulations of neurofilament (NF) immunolabelling surrounding the needle tract. Ultrastructurally, these corresponded to dilated and collapsed axons containing whorls of NFs, abnormal mitochondria and other organelles, but few microtubules. From 3-7 days PI, sprouting axons, strongly immunoreactive for NFs, were localised to the tract margins and amongst the microglia that filled the space between the tract edges. Sprouting axons were ultrastructurally characterised by a high density of NFs with a bulbous ending lacking these filaments. Co-injection of Taxol, a microtubule-stabilising drug, significantly reduced the density of reactive changes at 1 day PI. The in vitro model involved the culturing of cortical neurons in Neurobasal medium for 3 weeks followed by the transection of axonal bundles. At 1 day PI, NF-labelled bulb and ring-like structures were present within transected axonal bundles, which were replaced by sprouting axons immunolabelled for NFs and GAP-43 at 2-3 days PI. Video microscopy confirmed that the sprouting processes emerged from the transected axon. However, the leading edge of the sprouting axon contained GAP-43 immunolabelling whereas NFs were localised behind the growth tip. These studies show that axotomized mature cortical neurons are capable of a substantial sprouting response, and that alterations in cytoskeletal proteins have a particular role in the reactive and regenerative morphological changes. NFs may provide a stabilising or propulsive force for the sprouting axon.

Regular Session 12

Neuromonitoring, functional imaging

95. Advanced neuromonitoring including systemic physiology for the clinical management of severe head injury (Invited lecture)

C. Robertson (Houston, USA)

Technology has rapidly advanced our ability to monitor the brain in critically ill trauma patients. It is now possible to monitor tissue concentrations of pO2, pCO2, pH as well as energy metabolites such as glucose and lactate, and neurotransmitters such as glutamate. It is possible to monitor cerebral blood flow, both globally and regionally. Electrical activity, both spontaneous and evoked, can be monitored. Intracranial compliance can be monitored. In addition computer technology has rapidly increased the ability to trend both the actual measured data, and to

calculate sophisticated prediction models based on the measured data. Clearly these new technologies have added to our understanding of the physiology of human traumatic brain injury. It is less clear which of these monitors will leave the category of research monitoring and find real usefulness in the clinical management of patients. One reality that these newer monitoring devices have defined is that trauma induces a heterogeneous condition in the brain, and that therapies directed at a single injury mechanism may not have an overall benefit. Another reality is that the injury processes associated with brain trauma evolve over time, and that therapy directed at an individual injury mechanism may be very appropriate on day 1, but have an adverse effect on day 3 or 4 postinjury. Because of these findings, the trend in neuro critical care is to attempt to individualize treatment for each patient. At the present time, a reasonable approach is to monitor patients for systemic parameters that may cause secondary ischemia in the brain, such as blood pressure, central venous or pulmonary artery pressure, pulse oximetry, end-tidal CO2, and to monitor intracranial pressure and possibly global cerebral oxygenation. If regional hypoperfusion is present or suspected, a local pO2 probe placed in the area of hypoperfusion, may provide additional information.

96. Presence and future of neuromonitoring in severe brain injury (Invited lecture)

A. Unterberg (Berlin, D)

Monitoring of severely head injured patients aims at detecting and/or preventing secondary insults to the brain, at individually guiding therapy and at prognosticating final neurological outcome. During the last decades, basic clinical and general intensive care monitoring has been supplemented by monitoring of intracranial pressure (ICP), cerebral perfusion pressure (CPP), of electrical activity (EEG) and pathways (evoked potentials), cerebral oxygenation and blood flow as well as energy metabolism. At present, monitoring and guided therapy of increased ICP and decreased CPP are the only widely accepted, but not generally performed modalities. Even in countries with highly advanced health care systems, ICP and CPP monitoring are installed in only ca. 70% of severely head injured patients. Nevertheless, they are regarded mainstays. Whereas EEG monitoring did not prove useful, evoked potentials may be helpful in prognostication. Monitoring of cerebral oxygenation may be helpful to target therapy, especially hyperventilation, although it still has to be proven to favourably affect outcome. In the near future, probes will be available that monitor numerous parameters online, like ICP, tissue PO2, PCO2, PH and local blood flow. Already today, it is possible to combine such probes with monitoring of local metabolism by microdialysis. Coming goals will be to clarify which parameters of multimodality monitoring are truely necessary and/or helpful to manage severely head injured patients and where to monitor these, in normal, vital or in perifocal, damaged tissue.

97. Functional imaging in brain trauma (Invited lecture)

A. Marmarou, P. Fatouros, S. Signoretti, A. Beaumont (Richmond, USA)

The development of more sophisticated magnetic resonance techniques for imaging of brain have enabled researchers to explore the cellular and neurochemical changes that take place following severe brain injury non-invasively. In-vivo diffusion weighted imaging (DWI) is a relatively new MR technique which by employing strong magnetic field gradients is sensitive to the random molecular translation of protons and is an ideal method for investigating the self-diffusion of water. Using this method, it is possible to label the water molecules by manipulating the magnetization of the hydrogen nuclei without interfering with the diffusion process. This is important as clinical studies have shown that water accumulation and not vascular engorgement is responsible for traumatic brain swelling and subsequent rise in ICP. Calculation of the apparent diffusion coefficient (ADC) allows the differentiation of the type of edema Moreover, the application of magnetic resonance spectroscopy allows detection of the proton spectra and a measure of N-Acetyl-Aspartate, which is synthesized by the mitochondria, found exclusively in neurons, and is released with brain injury. Results show that ADC is reduced by 50 % in regions of brain swelling signifying a predominant cellular edema. This is associated with a concommitant reduction in NAA. We conclude that mitochondrial dysfunction may play a significant role in the subsequent development of brain swelling.

98. Monitoring of serum S-100B protein in severe head injury

A. Raabe, V. Seifert (Frankfurt am Main, D)

Objectives: Despite the recent progress in monitoring secondary brain insults after head injury, it is still difficult to objectify ongoing secondary brain damage occurring at a cellular level. We have investigated serum protein S-100B as a biochemical marker of cellular damage.
Methods: 100 patients with severe head injury (GCS score <9) were included in this prospective study. Venous blood samples were obtained as soon as possible after admission and every 24 hours for a maximum of 10 consecutive days. Peak levels and

time course of serum levels of S-100B protein were compared to outcome after 6 month, clinical variables and initial computed tomography findings.
Results: Patients who died had significantly higher serum S-100B values compared to those who survived (median 2.6 µg/l versus 0.6 µg/l, p<0.0001, Mann-Whitney U test). There was a strong association between S-100B and CT findings. Using logistic regression analysis in a model with age, GCS, ICP and CT findings S-100B was an independent predictor of outcome. Patients with poor outcome had higher initial values compared to patients with good outcome. Moreover, the average time for returning to baseline levels increased with decreasing neurological outcome.
Conclusion: S-100B might be a promising serum marker for assessing the time course of secondary damage after severe head injury. S-100 might serve as a surrogate outcome parameter for testing neuro-protective measures. (Supported by BMBF project Neurotrauma/Neuropsychology Magdeburg/Berlin.)

99. Relationship between neurochemical parameters and outcome in severe head-injured patients

B. Alessandri, T. Clausen, A. Khaldi, H.F. Young, R. Bullock (Mainz, D; Richmond, USA)

Introduction: Traumatic brain injury (TBI) causes disturbeances in energy metabolism, due to glutamate release, ionic shifts, and reduced CBF. Glucose (Gluc) and oxygen (O2) are the main substrates for the brain to produce energy in form of ATP, that is needed to restore ionic homeostasis and glutamate re-uptake. Thus, low extracellular Gluc and tissue ptiO2 maybe strongly correlated with outcome after TBI. Therefore wie investigated the relationship of different Gluc and O2 levels on 3-month outcome and neurochemical parameters.
Methods: Patients (n=34) with severe contusion injury were monitored using micordialysis and neurotrend for glucose, lactate, glutamate and ptiO2, and separated into a good (GOS 1-2; n=18) and poor outcome group (GOS 4-5; n=16). Low Gluc and O2 were defined as <50µM, and <20mmHg, respectively. Data were analyzed as mean (±sem) of the entire monitoring period or as mean of values at defined conditions which were labelled as ´Normal´ (Gluc>50µM and ptiO2>20mmHg), hyypoxic´ (>50µM, <20mmHg), ´Ischemic´ (<50µM, <20mmHg) and ´Hypermetabolic´ (>50µM, >20mmHg).
Results: Poor outcome patients had lower mean values of glucose (351±30 vs. 565±29), lactate (654±66 vs 756±41), and higher values of glutamate (17.8±5.2 vs 5.3±0.7) when compared with good outcome patients. Under ´Normal´conditions in the time-course of monitoring, only glucose was significantly lower in the poor outcome group (626±45 vs 846±31). ´Hypoxia´ caused a massive increase of glutamate to 58±19.4µM in the poor outcome group (Good: 13.5±4µM) and higher lactate (760±65 vs 721±89). If ´Ischemia´ occurred, glutamate and lactate increased to 96±29µM and 248±101µM in the poor outcome group, whereas lactate decrease to 78±39µM in the good outcome group. ´Hypermetabolism´ reduced lactate in both groups massively to 127±66 (poor) and 257±50µM (good).
Conclusion: These data indicate that the combination of glucose, glutamate, lactate and oxygen provides reliable information about biochemical processes in the brain and the outcome after severe head injury.

100. Extracellular cortical histidine in head-injured patients measured using microdialysis correlates with outcome

J. Goodman, S. Gopinath, A. Valadka, C. Robertson (Houston, USA)

Objectives: Excitatory amino acids have been studied extensively as contributors to damage in head injury. Measuring 26 amino acids in microdialysis samples from head injured patients, we found that the only other amino acid that differed between survivors and non-survivors was histidine.
Methods: 86 head-injured patients with Glasgow Coma Scores less than 8 were studied using intracortical microdialysis. Microdialysis probes were placed under direct visualization into grossly uninjured cerebral cortex and sterile physiologic saline was pumped through the probe at 2 microliter/minute. Amino acid analysis was performed by HPLC on samples obtained from 30 minute epochs.
Results: 1905 microdialysate samples (average 22/patient) were analyzed. None of the 26 amino acids analyzed showed a relationship to nonsurvival other than glutamate, aspartate and histidine. Mean histidine was 5.7 microM (3.3-9.7 microM, interquartile) in survivors and 9.2 microM (5.8-43.9 microM) in non-survivors (p=0.01).
Conclusion: Elevated extracellular cerebral cortical histidine is associated with mortality in head injured patients. Histidine is the precursor of the neurotransmitter/neuromodulator histamine which is involved in a wide range of autonomic, vegetative, and arousal functions. In addition, histidine itself is an endogenous anti-oxidant with significant neuroprotective capability. Elevation of histidine in nonsurvivors may reflect a failure of central histamine synthesis and failed anti-oxidant defenses. Study of histidine metabolism in head injury is appropriate as numerous histamine system pharmaceuticals are already available. (Supported by NIH NINDS PO1 NS27616. Human Subject Institutional Review Board approval applied to all aspects of this project.)

101. Monitoring by intracerebral microdialysis in patients with SAH

F. Staub, R. Graf, M. Köchling, N. Klug, W.-D. Heiss (Cologne, D)

Objectives: On the search for putative indicators of primary and secondary brain damage multiple metabolites were measured in the dialysate of patients with subarachnoid hemorrhage (SAH) in order to elucidate their significance for the outcome as well as their temporal profile of liberation.
Methods: Microdialysis-probes were placed into a frontal lobe of 10 patients with aneurysmal SAH for 4.6±0.5 days along with a ventriculostomy catheter for CSF drainage. Amino acids, metabolites of glycolysis, purines, catecholamines and nitric oxide (NO) oxidation biproducts were determined by HPLC. Metabolite levels in the dialysate were compared with patients' outcome according to the Glasgow outcome scale (GOS) three month after ictus.
Results: When average peak concentrations in the dialysate of patients with a favourable- (GOS IV-V) and unfavourable outcome (GOS I-III) were compared, significantly higher levels of excitatory amino acids (EAA), taurine, lactate and nitrite but not of purines and catecholamines were found in patients with a poor course ($p<0.05$). The significantly increased levels of amino acids in patients with a poor outcome followed a bi-phasic course with maximum concentrations on the first and second or the seventh day after the insult, respectively ($p<0.01$).
Conclusion: The data confirm the usefulness of EAA and lactate as major parameters for neurochemical monitoring in patients threatened by acute cerebral disorders, however substances such as taurine or nitrite might be also predictive. Release of these substances in the brain might be particularly relevant for the development of secondary brain damage after SAH. (Supported by the Köln Fortune Programm, University of Cologne (37/98).)

Special Luncheon Seminar 4
Large simple trials in head injury – the CRASH trial (sponsored by Pharmacia & Upjohn)

LS102. Corticosteroids in head injury

It's time for a large simple randomised trial
The global epidemic of head injuries is only just beginning. Currently over a million people die each year from brain injuries, and a similar number are disabled, often with profound effects on the quality of their lives. 1. Road crashes account for most of the injuries, and car use is rapidly increasing in many countries. By 2020 road crashes will, it is estimated, have moved from ninth to third in the world ranking of disease burden as measured in disability adjusted life years, and second in developing countries. 2. Identifying effective treatments for head injury is thus of global health importance. Corticosteroids have been used to treat severe head injury for over 30 years, though recently their value has been questioned because of the failure to demonstrate effectiveness in randomised trials. 3. Nevertheless, corticosteroids continue to be used widely, albeit inconsistently. Two 1996 British surveys, one of nursing staff in 39 neurosurgical intensive care units and one of medical directors in 44 neurosurgical intensive care units, found that corticosteroids were used to treat head injury in 49% and 14% of units respectively. 4,5. If a treatment as simple and widely practicable as corticosteroids produced just a moderate benefit this would be worth while. If, for example, they reduced the absolute risk of death by 2% (say from 15% to 13% dead) and reduced the risk of permanent disability by a similar amount then treatment of 500 000 patients would avoid 10 000 deaths and prevent 10 000 permanent disabilities. Such a benefit would be impossible to show reliably without evidence from large randomised trials. If 10 000 patients were randomly allocated to receive a corticosteroid infusion and 10 000 a placebo infusion then a 2% absolute reduction in the risk of death or disability should be detectable and a 3% reduction would certainly be detectable. By contrast, a trial of only 2000 patients would probably miss such differences. Reliable refutation of benefit is of equal importance, as it would protect patients currently treated with corticosteroids from any adverse effects.
So far all the randomised trials of corticosteroids in head injury have been too small to demonstrate or refute the possibility of moderate but clinically important benefits or harm from corticosteroids: the largest included only a few hundred patients, and even in aggregate they have included only about 2000. 6. As a result, the use of corticosteroids in head injury has waxed and waned over time, with extensive variations in practice. Evidence of benefit from corticosteroids in acute spinal cord injury has renewed interest in their role in brain injury. The second US national acute spinal cord injury study (NASCIS 2) compared 24 hours of corticosteroid (methylprednisolone) with placebo in 333 patients with acute spinal cord injury. 7. At six months patients who had received corticosteroids within eight hours of injury had greater improvement in motor function and in sensation to pinprick and touch. Similar results were reported in a Japanese trial of 151 patients who received the same regimen. 7. More recent trials of methylprednisolone in acute spinal cord injury have indicated slightly more neurological recovery with 48 than with 24 hours of treatment. 7. On the basis of

these results high dose methylprednisolone is now widely used in acute spinal cord injury. The dose of corticosteroid used in these randomised trials was based on results from animal studies showing that methylprednisolone can reduce post-traumatic neuronal degeneration after spinal cord injury, with 30 mg/kg body weight being required for maximal effect. High dose methylprednisolone has also been shown to reduce post-traumatic neuronal degeneration and improve outcome in animals with head injury: 30 mg/kg methylprednisolone enhanced recovery in mice that were subjected to moderately severe brain injury when given five minutes after injury. 8. To date only two small randomised trials of high dose (30 mg/kg) methylprednisolone have been performed in head injury. 9,10. In both there was a non-significant reduction in the risk of death in the methylprednisolone treated group, but because the trials were small the effectiveness of high dose methylprednisolone in head injury remains uncertain (pooled risk difference 3% lower mortality, 95% confidence interval 14% lower to 9% higher). Results from animal studies also suggest that early administration of corticosteroid is important for maximal effect. Because axonal disruption after acute trauma of the central nervous system does not occur for several hours, there may be an early phase when neurological deficit is reversible. 11. Timing of corticosteroid administration has also been shown to be important in acute spinal cord injury. 7. The administration of corticosteroids in many of the existing trials in head injury, however, may have been outside this window of opportunity. The CRASH trial (corticosteroid randomisation after significant head injury) is a large simple randomised placebo controlled trial of the effect of early administration of a 48 hour infusion of corticosteroid (methylprednisolone) on the risk of death and disability after head injury (www.crash.ucl.ac.uk). The results of the trial will inform clinical decision making in an area of increasing public health importance. Reliable demonstration of a benefit from corticosteroids has the potential to avoid thousands of deaths and disabilities. Similarly, the reliable refutation of any benefit would protect thousands of patients from possible side effects. However, the trial requires many thousands of patients with acute head injury to be randomised. This will be possible only if hundreds of doctors and nurses collaborate in the participating emergency departments. Management of the increasing global burden of head injury must be addressed in a similar way to that adopted so successfully in ischaemic heart disease. Prevention and the understanding of basic pathophysiology must be complemented by well conducted large simple trials.

On behalf of the CRASH trial management group: CRASH Trial Coordinating Centre, Department of Epidemiology and Public Health, Institute of Child Health, London WC1N 1EH (CRASH@ich.ucl.ac.uk)

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Plenary Session 5
Spinal cord injury research

103. Regeneration, reorganization and repair in spinal cord injury (Invited lecture)

M.E. Schwab (Zurich, CH)

Newborn spinal cord and brain have an enormous capacity to reorganize anatomically and compensate for functional deficits in response to lesions. The decline of these abilities to the low adult level parallels CNS maturation and in particular myelin formation during the postnatal period. We have shown that CNS myelin contains potent neurite growth inhibitory

molecules, among which Nogo-A, a membrane protein and antigen of mAB IN-1. Applying the inhibitor neutralizing AB IN-1 to adult spinal cord lesioned rats induces sprouting and long distance regeneration of transected corticospinal axons. In addition, cortical-bulbar connections and rubrospinal fibers sprouts and reorganize. Behaviorally, a high degree of functional recovery can be observed in lesioned mAB IN-1 treated rats (locomotion, grid walk, grasping of food pellets). Electrophysiology in animals with bilateral transections of the corticospinal tracts shows a "take over" of the targets of the corticospinal tract by sprouting rubrospinal fibers and the establishment of a fast cortico-rubro-motoneuron connection. These results show an unexpectedly high level of plasticity and regeneration capacity in the adult mammalian CNS under specific conditions, i.e. after neutralization of the neurite growth inhibitor Nogo-A.

104. Spinal cord injury similarities with and differences from traumatic brain injury (Invited lecture)

M.G. Fehlings (Toronto, CDN)

The pathophysiology of neurotrauma, including traumatic brain and spinal cord injury (TBI and SCI) involves an initial or "primary" mechanical insult followed by a complex series of molecular and cellular events termed the "secondary injury" which include ischemia, rises in intracellular Na+ and Ca++, glutamate toxicity; free-radical mediated cell damage and apoptosis (1-4). Secondary axonal degeneration after neurotrauma has been linked to intracellular entry of cations including Na+ and Ca++ (Fig 1), reverse operation of the Na+-Ca++ exchanger, activation of calpain which degrades neurofilament proteins and free radical mediated peroxidative injury. There is increasing evidence that neurons and glia may also undergo apoptosis, or programmed cell death, after TBI and SCI. Stimuli for apoptosis after neurotrauma may include TNF or FAS receptor stimulation, heat shock proteins, reactive oxygen species and glutamate-mediated excitoxicity. Although, TBI and SCI share many similar features in pathophysiology, the relative importance of the secondary injury mechanisms differ in these two forms of neurotrauma. In particular, TBI involves a very diverse spectrum of injuries, with varying degrees of ischemia, glutamate toxicity and axonal degeneration which are difficult to model. SCI predominantly involves axonal disconnection with additional loss of local neuronal circuits. Chronic maladaptive neuronal and glial changes occurs with all forms of neurotrauma, although the clinical importance in SCI appears greater due to the development of neuropathic pain and autonomic dysfunction. Ultimately, combination therapy directed at several mechanisms of secondary injury will be required to achieve improved clinical outcomes for both SCI and TBI. Regenerative approaches targeted at restoring neuronal circuits and axonal tracts will need to designed in a manner specific to the underlying lesion.

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105. Advances in the surgical management of spinal cord injury (Invited lecture)

Y.S. Kim, Y.E. Cho, D.K. Chin, B.H. Jin (Seoul, KOR)

Objectives: The cervical spine is the most mobile segment of the vertebral column and there may be many kind of cervical spinal injury. Unfortunately severe neurological deficit is the most common presentation and the prognosis is poor. Many forms of treatment for the cervical spinal injury have been reported, however, the outcome is not still satisfactory. We reviewed the type of injuries, treatment methods and outcomes to determine the best treatment option.
Methods: Total 658 operations on spinal cord injury were performed in our Spine Center during last 13 years. Among them, cervical injuries were 558 cases (85%), thoracic injuries were 54 cases (8%), and lumbarsacral injuries were 46 cases (7%). In the cervical cord injury, 125(22.5%) were high cervical injuries and odontoid process fracture (36.5%) and C2 pedicle fracture (19.1%) were common type. The rest of them were subaxial cervical cord injuries (433: 77.6%), in which compression fracture and fracture & dislocation were common type.
Results: In high cervical injuries, neurological deficits were more frequently observed in C1/2 dislocation compared to other type of injury. Forty-five (36%) cases were treated surgically and surgical methods were occipito-cervical fusion (7cases), transoral fusion (1case), odontoid screw fixation (3cases), and C1/2 posterior fusion (34cases). In subaxial lesions, surgical treatment was performed in 238(55%) cases and anterior interbody fusion was performed in 133(47%) cases.
Conclusion: The cervical spinal cord injuries are very critical situation, and the management of them is also important. With the advances in spinal instrumentation, there have been significant changes in the concept of spinal surgery. Therefore, it is a

pertinent matter to review the recent advances in the surgical management of spinal injury.

Regular Session 13

Novel molecular mechanisms of axonal injury, current and future treatment possibilities in SCI

106. Guidelines of operative vs. non-operative treatment in spinal cord injury (Invited lecture)

A. Holtz (Uppsala, S)

Spinal cord injury following trauma to the spine results in human suffering for each individual, independent of the severity of the neurological deficit. The management of the acute spinal cord injured patients has in most countries changed from a purely conservative treatment advocated 20-30 years ago to a more active policy of today with early surgery and neurointensive care. Some of the the-rapeutic improvements comes from animal experiments and is based on the theory of secondary injury mechanisms. Such secondary injury is the result of a cascade of biochemical and pathophysiological events set in motion by the trauma and may cause the neurological deterioration seen in some patients in the period immediately following injury. The non-surgical intensive care guidelines in the treatment of the "software", i.e. spinal cord, are well established. These well accepted guidelines includes a se-cured adequate respiration, establishment of an adequate circulation and maintained perfusion to the spinal cord as well as pharmacological treatments. However, the indications and timing of surgery following spinal cord injury are still controversial, except in patients with ongoing neurological deterioration and a simultaneous compression of the spinal canal by hematoma, disc material or bone fragments. Early spinal surgery is performed for various reasons, (i) to prevent or alleviate secondary clinical complications, (ii) to decompress the spinal cord and restore the stability of the spine in order to facilitate rehabilitation and (iii) to improve the neurological restitution. Of course, the most importent issue is the role of acute decompressive surgery in improving neurological outcome. Several animal experimental studies have shown beneficial effects on the neurological outcome following decompression. However, no prospective controlled study have been performed with the same time limits as presented by the NASCICS studies. We therefore have to rely on retrospective studies comparing the result of surgical and non-surgical treatment on e.g. secondary complications, neurological outcome and length of hospital stay. A survey of experimental and clinical studies affecting the outcome of various clinical outcome measures will be presented. In addition, practical guidelines for the overall treatment will be discussed with special reference to surgical intervention.

107. Novel molecular mechanisms of axonal injury (Invited lecture)

J.T. Povlishock (Richmond, USA)

While most understand that traumatically induced axonal change (TAI) involves delayed disconnection of the injured axon, most do not appreciate that the initiating pathogenesis of this delayed axotomy varies. In this presentation, I provide evidence from our laboratories focusing on brain injured rodents, pigs and humans to demonstrate differing intra axonal initiating pathologies which are the result of differences in fiber size and type as well as the degree of injury. In some large caliber axons mechanoporation of the axolemma will be described in concert with the activation of a cysteine protease cascade. This cysteine protease cascade will be shown to participate in progressive intra axonal cytoskeletal degradation leading to disconnection without overt swelling due to a conversion of anterograde to retrograde axoplasmic transport. The implications of this non-swelling for the potential underestimation of the number of damaged axons in animals and man will be discussed. Evidence for primary cytoskeletal change leading to axonal swelling without overt axolemmal perturbation will also be presented together with the consideration of nodal change in small caliber axons that potentially involves both primary cytoskeletal and axolemmal alteration. Therapies targeting specific features of TAI will also be addressed. The benefits of early posttraumatic hypothermia will be presented together with evidence that gradual rewarming is further protective whereas rapid rewarming exacerbates axonal injury. Lastly, the therapeutic benefits of two members of the immunophilin family, FK506 and cyclosporin A, will be evaluated in terms of their abilities to blunt specific features of the above described TAI. It will be shown that the actions of these drugs in inhibiting the opening of the mitochondrial permeability transition pore as well as inhibiting calcineurin provide axonal protection via the attenuation of the cysteine protease cascade and cytoskeletal stabilization.

108. Intraspinal transplantation – update of ongoing clinical study (Invited lecture)

E.D. Wirth, P.J. Reier, R.G. Fessler, D.K. Anderson (Gainesville, USA)

In an initial attempt to extend a plethora of intraspinal transplan-tation studies in animals to humans, we are transplanting human embryonic spinal cord (ESC) tissue into patients with progressive post-traumatic

syringomyelia (PTS). Studies in rats and cats have demonstrated that solid and suspension grafts of embryonic tissue from several areas of the CNS survive intraspinal grafting, mature, and integrates with the host's spinal cord. The primary goal of our clinical pilot study is to investigate the feasibility and safety of human ESC allografts and to assess if these allografts will survive, grow, and serve as a plug to fill the spinal defects thereby preventing further expansion of the syrinx. To date, 6 males and one female, 45-66 years of age, who sustained a SCI at thoracic levels between 12-31 years ago and who were diagnosed with PTS 2-17 years after their original injury, have received solid and/or suspension transplants of 7-8 human ESC that were 6-9 week gestational age following drainage of the syringes (1-30 cm in length) and detethering of intradural adhesions. All patients were immunosuppressed with cyclosporine for 6 months. The patients are evaluated twice pre-operatively and at 8 intervals post-operative for 2 years using MRI and neurophysiological tests in addition to measures of disability, pain, and neurological impairment. To date, there have been no complications related to the transplant procedure, MR images show evidence of solid tissue in 6 of 7 graft sites, and sensory and motor scores have remained stable in 6 patients whereas 1 patient has demonstrated substantial improvement in locomotor function. While it is still too early to draw firm conclusions, these data suggest that allografts of ESC tissue can be safely introduced into post-traumatic syringes and that partial obliteration of these cysts can be achieved for at least 22 months.

109. C-jun expression in axotomized Clarke's nucleus and red nucleus neurons
K. Rache, S. Breuer, K. Pech, A. Buss, J. Noth, A.B. Schmitt (Aachen, D)

Interruption of mature axons activates a cascade of events in neuronal cell bodies which leads to various outcomes from functional regeneration in the PNS to the failure of any significant regeneration in the CNS. One factor which seems to play an important role in the molecular programs after axotomy is the transcription factor c-jun. This molecule is able to bind to a specific DNA-sequence (AP1-binding site) of neuronal genes such as GAP-43 and may regulate their expression. It is known, that axotomized rubrospinal neurons induce GAP-43 and c-jun after high cervical lesions, but not after low thoracic lesions. To test the functional relevance of this c-jun up-regulation, we investigated the expression of its activated, phosphorylated form, phospho-c-jun. Phosphorylation of c-jun was first seen 2 days after high cervical lesions in a subpopulation of rubral neurons and lasted for at least 8 weeks. No phosphorylation was detected in these neurons after low thoracic lesions. Recently, we demonstrated that axotomized Clarke's nucleus neurons express c-jun and phospho-c-jun after a low thoracic hemisection. Interestingly, these neurons also express c-jun and phospho-c-jun after high cervical lesions. This up-regulation starts approximately 7 days after injury and can still be detected at 21 days. These results indicate that Clarke's nucleus neurons do not obey the "near-far-principle" in response to axotomy such as rubral neurons. One possible explanation might be that Clarke's nucleus neurons undergo degeneration whereas rubral neurons undergo atrophy after injury. (Supported by grants from the DFG (Na 289/2-3 and Schm 1304/3-1).)

110. Tissue plasminogen activator increases neuronal damage after spinal cord injury
Y. Abe, H. Nakamura, O. Yoshino, T. Kimura (Toyama, J)

Plasmin is converted from its zymogen plasminogen by tissue plasminogen activator (tPA) and degrades many components of the extracellular matrix. tPA-deficient mice has been demonstrated to be resistant to excitotoxin-induced neuronal degeneration in the brain. Because excitotoxin-induced neuronal damage also relates to spinal cord injury (SCI), tPA was thought to increase neurodegeneration after SCI. To investigate the effects of tPA on neuronal damage after SCI, we examined the differences in behavioral outcome, histological damage, and apoptotic cells between wild-type and tPA-deficient mice using newly developed spinal cord impactor. Locomotor recovery was greater in tPA-deficient mice than in wild-type mice throughout the experimental period. From myelin-stained sections, both white and gray matter were maintained in tPA-deficient mice better than in wild-type mice. From TUNEL-stained sections, the number of apoptotic cells, 24 hours after SCI, was larger in wild-type mice than in tPA-deficient mice. These results suggested that tPA increased neuronal damage via apoptosis and the inhibition of tPA/plasmin proteolitic cascade will be effective in neuroprotection after SCI.

Regular Session 14
Cell injury

111. Use of complementary in vitro and in vivo models to study mechanisms of injury induced cell death (Invited lecture)
A.I. Faden (Washington, USA)

It is increasingly clear that both necrosis and apoptosis contribute to posttraumatic neurological

dysfunction. Although clinically relevant animal models have been used to examine basic mechanisms involved in trauma induced cell death, complementary in vitro models offer substantial advantages for elucidating specific signal transduction pathways involved. We have developed several types of mechanical injury models using both rat and mouse cortical neuronal or neuronal/glial cultures. One of the most useful has been an electromagnetically controlled punch device that produces 28 parallel cuts in individual wells of 96-well microplates. This system is highly reproducable and has been very well characterized with regard to secondary neuronal injury. It has been used to study glutamate receptor mediated mechanisms of cell death, including both ionotropic and metabotropic receptors, as well as signal transduction mechanisms involved for groups I, II and III mGluR modulation of traumatic cell death. In such studies the pharmacological profiles have been remarkably similar to those found with fluid percussion induced trauma in rats. A variation on this model includes mild metabolic compromise using low doses of 3-nitropropionic acid; this results in both NMDA receptor mediated necrosis and caspase-3 mediated apoptosis in the same cultures. The latter model has been used to demonstrate the additive neuroprotective effects of combination treatment with the NMDA antagonist MK-801 and the caspase inhibitors BAF and z-DEVD-fmk. More recently, we have begun to use these models to study cultures from selected knock-outs for genes involved in apoptosis. Because these in vitro models use 96-well plates, they readily permit extensive dose-response studies for multiple structurally different drugs that may modulate a specific receptor or pathway, something not feasible with in vivo models.

112. Trophic factors and CNS transplantation in experimental traumatic brain injury (Invited lecture)

T. McIntosh (Philadelphia, USA)

The mechanisms underlying secondary or delayed cell death following traumatic brain injury are poorly understood. Recent evidence from experimental models suggests that widespread neuronal loss is progressive and continues in selectively vulnerable brain regions for months to years after the initial insult. The mechanisms underlying delayed cell death are believed to result, in part, from the release or activation of endogenous "autodestructive" pathways induced by the traumatic injury. The development of sophisticated neurochemical, histopathological and molecular techniques to study animal models of TBI have enabled researchers to begin to explore the cellular and genomic pathways that mediate cell damage and death. This new knowledge has stimulated the development of novel therapeutic agents designed to modify gene expression, synthesis, release, receptor or functional activity of these pathological factors with subsequent attenuation of cellular damage and improvement in behavioral function. This talk represents a compendium of recent studies suggesting that modification of post-traumatic neurochemical and cellular events with targeted pharmacotherapy can promote functional recovery following traumatic injury to the central nervous system.

113. Assessment of white matter injury following middle cerebral artery occlusion (MCAO) in the rat

E.A. Irving, S.J. Hadingham, A.A. Parsons (Harlow, UK; Philadelphia, USA)

Recently it has been shown that in addition to neurones, axons and their associated oligodendrocytes exhibit structural damage rapidly in response to an ischaemic challenge. The aim of this study was to further investigate white matter damage from 6h to 2 wk. following MCAO in the rat. MCAO was induced in rats using the intraluminal filament technique. Sham operated animals underwent surgery but not occlusion. Six, 24h or 2 wk. following the onset of ischaemia, brains were processed for histological staining, Tau 1, and APP immunohistochemistry (n=4/group). Six and 24h following MCAO, the density of Tau 1-positive oligodendrocytes was increased in the ipsilateral external capsule, corpus callosum and caudate nucleus, compared to normal tissue. This increased number of Tau1-positive oligodendrocytes was accompanied by APP accumulation in axons. In contrast, 2 wk following MCAO, axonal Tau 1 and APP staining was decreased in the white matter bundles of the caudate nucleus and in an area of the external capsule encompassed by the lesion. Luxol fast blue staining confirmed axonal/myelin loss within these areas. Adjacent to this area, APP accumulation was apparent, however significant numbers of Tau 1 positive oligodendrocytes were not detected. Interestingly, up to 2 weeks following MCAO, both increased Tau1-positive oligodendrocytes and APP accumulation were detected in regions such as the internal capsule and throughout various thalamic nuclei distant from the lesion core. Increased Tau 1 in oligodendrocytes and accumulation of APP in axons may reflect tissue "at risk" following MCAO and provide a marker of secondary ischaemic damage. However, loss of Tau 1 and APP immunostaining in the axonal compartment may provide sensitive markers of white matter infarction.

114. Quantitative analysis of axonal cytoskeletal responses after nondisruptive axonal injury followed by short term hypothermia and rewarming

W.L. William (Glasgow, UK)

Recent evidence has suggested that posttraumatic hypothermia is neuroprotective in traumatic brain injury (TBI).But the optimal timescale for posttraumatic hypothermia and rewarming has not been determined. We have previously demonstrated that cooling for 4 hours after injury in a model of traumatic axonal injury (TAI) results in amelioration of pathology in injured axons (J. Neurotrauma 16, 1225). We decided to monitor the effects of short term cooling followed by rewarming upon the development of axonal pathology after nondisruptive axonal injury. The right optic nerves of adult guinea pigs were exposed to controlled, transient tensile strain. Animals were either maintained normothermic for 4 hrs, had hypothermia induced for 4 hrs or were cooled for 2 hrs and then rewarmed for 2 hrs before being killed. All material was processed for quantitative electron microscopy of changes in the organisation of the axonal cytoskeleton. The right optic nerve of adult guinea pigs were exposed to controlled, transient tensile strain. Animals were either maintained normothermic for 4 hrs, had hypothermia induced for 4 hrs or were cooled for 2 hrs and then rewarmed for 2 hrs before being killed. All material was processed for quantitative electron microscopy of changes in the organisation of the axonal cytoskeleton. There was not compaction of neurofilaments following short term hypothermia and rewarming. But there was loss of axonal microtubules from the internode ($p<0.05$). With posttraumatic hypothermia there was neither loss of microtubules or compaction of neurofilaments. Thus short term hypothermia is neuroprotective for neurofilament responses after TBI but not for microtubular responses. We provide the first evidence for a differential amelioration of posttraumatic responses between different components of the axonal cytoskeleton. We suggest that the development of posttraumatic therapy after TAI may therefore be more complex than first appreciated.

115. The lipid peroxidation by-product 4-hydroxynonenal is involved in white matter damage following brain injury

E. McCracken, V. Valeriani, T. Jover, K. Horsburgh, J. McCulloch, D. Dewar (Glasgow, UK)

To investigate if the lipid peroxidation by-product 4-hydroxynonenal (4-HNE) is involved in white matter damage following brain injury a number of methods were employed. Firstly, we investigated if 4-HNE was present in the rat brain following permanent cerebral focal ischaemia by means of immunohistochemistry using a polyclonal antibody raised against 4-HNE Michael adducts. Secondly, fresh brain tissue dissected from the corpus callosum of a group of head-injured patients and controls was analysed using a lipid peroxidation assay to determine if there were alterations in the levels of lipid peroxidation by-products. Thirdly, exogenous 4-HNE was stereotactically injected into the subcortical white matter of male adult rats and tissue sections analysed by immunohistochemistry using amyloid precursor protein (APP) to determine the presence of axonal damage. Finally, to determine if 4-HNE was toxic to oligodendrocytes, 1, 10 or 50mM 4-HNE was added to cultured cells. 4-HNE immunoreactivity was increased in neuronal perikarya and within axons at the boundary of the ischaemic lesion in rats. Levels of 4-HNE and malondaldehyde were significantly increased in the head-injured group compared to the control group. Intracerebral injection of 4-HNE produced a lesion in the subcortical white matter and overlying grey matter compared to the vehicle treated group. APP immunostaining demonstrated disruption of axons along the corpus callosum and into the contralateral hemisphere. Following a 4hr incubation period 4-HNE resulted in a concentration-dependent increase oligodendrocyte death in culture. The data indicate that exogenous 4-HNE is toxic to both axons and oligodendrocytes, that endogenous 4-HNE is present following experimental cerebral ischaemia in rats and that lipid peroxidation by-products are elevated in human white matter following head injury.

Regular Session 15
SCI/TBI – Prognostication, epidemiology, databases

116. Moderate head injury (MHI) – a review on definition, databases, outcome and prognostic factors (Invited lecture)

R. Braakman, A.I.R. Maas, Ch. Hukkelhoven (Berkel-Enschot, NL)

Since the introduction by Rimel in 1982 of the term MHI based on a GCS sum score of 9-12, the definition has been amended in various directions. Many prefer e.g. to include GCS 13, which has a similar incidence of lesions on CT and at surgery. These changes have confounded the comparison between studies on MHI. At present the classification based on the GCS is not practical because in many countries HI patients are already put on the ventilator before they have been thoroughly classified. The amount of literature on MHI is small in comparison with that on severe and mild HI. The mortality rate in MHI is a useful indicator of the efficacy of local HI management. In most surviving

patients cognitive impairment often persists for years after injury. A recent prospective Dutch study confirms previous studies that the outcome of surviving patients with MHI is more determined by the duration of PTA and by early behavioural disturbances, in combination with early CT findings which disclose focal lesions, than by the GCS on admission. MHI patients with impaired cognitive outcome have significantly more frontotemporal abnormalities with imaging techniques, and early behavioural problems such as agitation and restlesssness. The inclusion of patients with MHI in randomized trials on neuroprotective drugs in order to speed up recruitment, is futile unless the dichotomous categorisation of outcome based on the GOS scale has been replaced by other statistical, preferably ordinal,scales involving the results of neuropsychological tests concerning memory, attention and speed of information processing after 6 or 12 months. Psychosocial outcome often does not stabilize within 3 to 5 years.

117. Traumatic brain injury (TBI)- Israeli National Trauma Registry

E. Peles, V. Barell, V. Boiko, G. Kaplan (Tel-Hashomer, IL)

TBI has been established as a category in reporting systems. Uniform data systems case definition has been suggested for hospital discharge surveillance systems based on ICD-9-CM diagnostic codes. Included specific mention of intracranial injury including contusion, laceration, hemorrhage, fractures and concussion. Inspection of data from the Israeli Trauma Registry suggested that 2 diagnostic groups of different severity and outcome unjustifiably combined. Aim: To evaluate the validity of sub-dividing TBI into 2 discrete groups, using the presence of specific mention of intracranial injury and/or loss of consciousness for more than one hour as the definition of definite TBI. Possible TBI includes skull fractures with no mention of intracranial injury and/or concussion with no loss of consciousness.
Methods: The study population includes all traumatic injuries admitted to hospital, dying in the ER or transferred to other hospitals and recorded in the 1998 Trauma Registry in all 6 level I trauma centers in Israel and two level II centers.
Results: Of the 16574 casualties recorded in 1998, 4971 (30%) had diagnoses of TBI. Thirty-one percent were definite TBI and 69% possible. Thirty percent of the definite group had Glasgow Coma Scale of 8 or less compared to 2% in the possible group. The same pattern was demonstrated by all commonly used severity scores; for example: 26% of definite TBI cases had a Revised Trauma Score of 10 or less but only 3% of those with possible TBI. Although those classified definite TBI were only 10% of all casualties recorded in the Registry, they were responsible for 56% of the inpatient deaths and 48% of all ICU days. 29% of those with definite TBI underwent head surgery as compared to 6% of those with possible TBI.
Conclusion: Significant differences in severity, hospital resource use and immediate outcome between the groups support the validity of sub-dividing the TBI classification into definite and possible categories.

118. The end of severe head injury in car crashes – how soon?

T.A. Gennarelli, F. Pintar, N. Yoganandan (Milwaukee, USA)

The United States National Automotive Sampling System (NASS) collects a statistical survey of detailed crash and injury data and normalizes these data to the national population. The NASS databases from 1991 through 1998 were analyzed for drivers and front passengers of cars involved in frontal crashes regarding the presence and severity of head injury as measured by the Abbreviated Injury Scale (AIS). Of the 7.2 million frontal occupants, the incidence of head injury was 2.6% (189,240) ranging from 1.6-18% as crash speed increased. However when fully restrained by seat belts and airbags, only 1% of 975,000 incurred head injuries. More importantly, there were only 0.14% serious head injuries (AIS >3) whereas unrestrained occupants were injured 6-7 times more frequently (7% 1.1M) and more severely (0.76% AIS>3). In this eight year period, there were no AIS=5 head injuries and only 34 AIS=4 head injuries (AIS4 and 5 roughly correspond to GCS<9)that occurred to fully restrained occupants when the crash speed was <35mph. This suggests that current airbag-seatbelt restraints are highly effective in preventing and mitigating severe head injury in frontal crashes. This may partly explain the apparant decrease in vehicular head injuries seen in the USA. If new or improved restraint systems are developed for frontal and other crash types, prevention of almost all severe head injuries may become a reality, especially when restraint use becomes universal.

119. Traumatic brain injuries in two regions of Estonia (preliminary data)

N. Popova, I. Drikkit, T. Asser (Tartu, EE)

Objectives: To identify all traumatic brain injury cases that occurred during 1999 among the residents of the University town of Tartu situated in the agricultural area and the industrial town of Kohtla-Järve. To compare the differences of brain injury epidemiology between these two socially and economically different regions.

Materials and methods: Our study included all physician-diagnosed brain injury cases treated as outpatients or hospitalised; also cases with autopsy evidence of brain injury were included. The cases were identified on the basis of medical documentation. Glasgow Coma Scale score on admission was used to classify the severity of the brain injury.
Results: The incidence of brain injuries in Kohtla-Järve was more than four times higher than in Tartu. The male-female ratio was 2.8:1 for Tartu and 1.8:1 for Kohtla-Järve. The mean age was 37.4 and 34.3 years accordingly. The causes of the injuries in Tartu were falls in 37.5%, transport related accidents in 39.9% and assaults in 19.3% of cases, in Kohtla-Järve these causes comprised accordingly 36.1%, 13.0% and 39.8%. Alcohol intoxication was diagnosed in 31.8% and 25.7% of victims. The majority of brain injuries were mild providing 71.6% of cases in Tartu and 88.2% in Kohtla-Järve. Patients were delivered by ambulance in 66.4% of cases in Tartu and in 29% of cases in Kohtla-Järve; 29.2% and 65.1% sought for medical care directly at the emergency departments or family doctor offices; 59.5% and 36.4% of patients were hospitalised. Neurosurgical interventions were performed in 20.1% and 6.1% cases. A fracture of cranial bones was presented in 23.9% and 8.5% of cases. Case fatality rate was 14.4% and 5.7% cases accordingly.
Conclusion: Our preliminary results show that brain injuries occur mainly in males. The main age at the moment of injury is higher than in other studies. There is a difference between incidence, distribution of cases and severity of injuries in the two regions.

120. Progressive hemorrhagic injury after TBI – predictors and consequences

M. Oertel, D. Kelly, D. McArthur, J. Lee, T. Glenn, N. Martin (Los Angeles, USA)

Progressive post-traumatic brain hemorrhage seen on serial CT scans is often noted but its risk factors and consequences are unclear. In total 134 patients (mean age 34.4+/-14.6 yrs, median GCS 8 (range 3-15), M:F=108:26) had 2 CT scans within 14 hours of injury and had an ICP monitor. The mean time from injury to first CT was 2.0+/-1.7 hrs, and from the first to second CT was 6.7+/-3.7 hrs. The diagnosis of progressive hemorrhagic injury (PHI) was determined by comparing the first and second CTs and categorized as epidural (EDH), subdural (SDH), intracerebral hematoma/contusion (ICH), or subarachnoid (SAH). Over the first 10 days post-injury, ICP was recorded hourly and treated if >20 mmHg. The daily mean ICP and total hours of ICP>20 mmHg were calculated. Logistic regression analyses and t-tests were performed. PHI was found in 42.5% of patients and in 49% of patients scanned within 2 hours of injury. Of the 57 patients with PHI, 87.7% had their first CT within 2 hours of injury and only one had PHI documented if the first CT was performed over 6 hours post-injury. The progressive lesions were ICH/contusions, SAH, EDH and SDH in 77%, 25%, 14% and 11% of instances, respectively. Logistic regression analyses identified the time from injury to first CT as a predictor of PHI, whereas, coagulation parameters (PT, PTT, platelets), ethanol level, blood pressure and body temperature prior to second CT were not predictive. Patients with PHI had a higher mean ICP (15+/-7 vs. 12+/-6 mm Hg; $p<0.01$) and longer periods of ICP>20 mmHg (49+/-62 hours vs. 24+/-53 hours; $p<0.05$) during the first 10 days post injury. This analysis indicates that PHI occurs in almost half of patients who have a CT within 2 hours of injury, occurs most frequently in cerebral contusions and is associated with subsequent ICP elevations. Time from injury to first CT appears to be a key determinant of PHI.

121. Do CT scan abnormalities predict ICP and CPP course? – Pilot data from the European Brain Trauma Foundation database

E. Bagiella, C. Hesdorffer, R. Zimmermann, W. Hauser, J. Maeda, J. Ghajar, R. Hartl (New York, USA)

Little is known about the relationship between initial CT scan findings and ICP and CPP course over the first 10 days following traumatic brain injury (TBI). We used pilot data from the Brain Trauma Foundation TBI Study that was initiated in 1997 in 5 European countries to examine these relationships. At each hospital a Head CT scans done within 12 h of injury were scanned, digitized, sent to BTF via internet, and centrally read by 3 neuroradiologists: findings were categorized according to Marshall et al. Hourly ICP and CPP were recorded over 10 days. Repeated measures ANOVA was used to analyze data, treating the patient as a random effect. A total of 174 patients were studied. 113 (64.9%) admission CT scans were available. Among patients with admission CT scans, ICP was monitored in 48 (42.5%) of patients. 55 (50.0%) demonstrated diffuse injury types I-IV, 43 (39.1%) had evacuated mass lesions, and 12 (10.9%) showed non-evacuated mass lesions. 58 (51.3%) had a lesion volume <25 ml and 55 (48.7%) had a lesion volume >25 ml. Hourly ICP was higher and CPP lower among those with small compared to large lesions during day 1 to day 7 after which the trend reversed. Over 10 days, hourly ICP was significantly related to CSF drainage, volume, hour and day; hourly CPP was significantly related to mannitol, volume, hour and day. This BTF-TBI study introduces a new Internet-based database. We show a relationship between CT lesion size and course of ICP and CPP. These data

stress the importance of ICP monitoring in TBI patients.

Regular Session 16
Management of TBI/SCI, guidelines

122. Management of TBI – surveillance systems, databases, epidemiology (Invited lecture)
L.F. Marshall (San Diego, USA)

The modern era of head injury research began with the first detailed epidemiologic study comparing outcome of brain injury in three countries by Jennett and Braakmann. This study which was the first to take significant care to match patients, served as a model for many many later studies. The development of computerized data collection technologies as epitomized by the Traumatic Coma Databank has led to a new standard for clinical investigation in patients suffering from brain injury. From such investigations have come detailed descriptions of outcome in patient populations with an increasing emphasis on matching patients on a variety of input measures as well as on outcome. These include the Glasgow Coma Scale, the status of the pupils, the pattern of brain damage as evidenced on CT and more recently on MRI, and a more detailed look at the overall true outcome of patients in terms of better measures of their cognitive and behavioral impairments. Pharmacologic trials of new agents to treat head injury have led to further modifications of such databases in an attempt to provide answers to the prime question as to whether or not a drug in fact is efficacious in brain injury, but as a secondary product of such investigations, is a literal treasure of new information has come forth as well. In addition, collection of large epidiemiotogic databases from specific regions such as the UK, Munich, and San Diego have provided useful information as to the characteristics unique to each region, and the influence of a variety of factors including pre-hospital systems, physician availability, etc. that have been useful in attempting to design ideal systems for patient care. The availability of large databases has allowed us to recognize the importance of varying patterns of subarachnoid hemorrhage and the role of intracranial hypertension versus cerebral perfusion pressure in the management of patients with severe brain injudes, The importance of sound epidemiologic investigations and the use of composite databases will be illustrated and discussed.

123. Management guidelines – their practical utilization (Invited lecture)
A. Marmarou, R. Narayan, American Brain Injury Consortium (Richmond, USA)

In July of 1995, the AANS in collaboration with the Brain Injury Foundation released a set of guidelines for management of head injury. By March of 1996, the guidelines were distributed to members of the AANS and by November of the same year, they were published in the Journal of Neurotrauma. It was also in March of 1996 that the American Brain Injury Consortium (ABIC) initiated clinical trials in traumatic brain injury with several pharmaceutical companies. The ABIC developed procedures for reviewing the management of each patient to insure protocol compliance. This also gave the ABIC the opportunity to review patient management and determine the degree to which management was in compliance with AANS guidelines. A total of 326 head injured patients of Glasgow Coma Score (GCS) ranging from GCS 3 to 8 treated by 84 level I trauma centers were entered into this study. During the entire study period 190 of the 326 patients reviewed (58.6 %) were classified as in compliance with AANS head injury guidelines. The greater number of deviations were attributed to the use of sustained aggressive hyperventilation and management of low cerebral perfusion pressure. This study supports the notion that publication of the guidelines unto itself does not insure that the information is filtering to the neurosurgical residents, intensivists, anesthesiologists and trauma surgeons who are intimately involved in the care of the head injured patient.

124. Do US trauma centers comply with guidelines for the management of severe TBI?
C. Hesdorffer, J. Ghajar, L. Iacona (New York, USA)

Background: Evidence-based guidelines for the management of severe traumatic brain injury (TBI) were published in 1995 yet information regarding their implementation is limited. We surveyed U.S. trauma centers in 1999-2000 to evaluate guideline implementation.
Methods: Head nurses at all designated US trauma centers caring for adults with TBI in their ICU were surveyed. Hospitals transferring patients from their emergency department were excluded. We queried: number of TBI admissions; compliance with guidelines for intracranial pressure (ICP) monitoring, ICP treatment, ICP technology, hyperventilation, mannitol, and glucocorticoids; and degree of compliance.
Results: Of 924 centers identified, 828 participated (90%), and 429 hospitals with ICUs caring for TBI patients were surveyed; 38% level I, 54.6% level 2, and 7.4% level 3. Compliance differed significantly by trauma center level for ICP monitoring indications, mannitol use, and glucocorticoids (level 1 centers complied best). Compliance did not differ for ICP treatment threshold, ICP monitoring technology, and hyperventilation (60% compliance for the latter two

guidelines). Full compliance was observed in 25.8% of level 1 centers, 9.8% of level 2 centers, and 6.3% of level 3 centers ($p<0.0001$). In our previous survey in 1991 of 219 centers from 45 states, we found that 39.7% of centers complied with ICP monitoring indications; the current survey shows 50.8%.
Conclusion: Although evidence-based guidelines have existed since 1995, our survey suggests that huge gaps exist between the guidelines and their implementation. Focus must turn to active education of US trauma centers.

125. Management strategies for focal traumatic brain contusions

P.K. Narotam, N.N. Nathoo, P.V Govender, J.R. Van Dellen (Winnipeg, CDN; Durban, ZA; Preston, UK)

Background: A management dilemma has always existed in the management of traumatic brain contusions which comprise a significant percentage of patients who arrive at the hospital awake and even in a relatively stable neurological condition but subsequently die, frequently from a dynamic process of uncontrolled expansion of the contusion leading to raised intracranial pressure and herniation. Consensus is seldom obtained amongst a group of neurosurgeons as to the correct interventions timing or technique. The clinical course of these patients is often unpredictable and the cause of the high mortality (30-40%). In a 15 year study involving >1600 patients with focal traumatic brain contusions, we report on the efficacy of various treatment modalities i.e. clinical observation; intracranial pressure monitoring (ICPM) with medical therapy (mannitol &/ hyperventilation) with or without secondary surgery; and primary surgery.
Methods: In a retrospective analyses, undertaken from 1983 to 1987, the effect of contusion management on patient outcome (mortality) was determined. From this experience, a contusion management protocol was devised (1987) and implemented from 1988 onwards. The effectiveness of the "protocol" was validated prospectively by examining its impact on contusion mortality.
Results: Pre-protocol: Between 1983-5, 243 patients were evaluated. At that time, no clear management guidelines were used. A contusion management mortality was 19.3% was found. Over the next 2 years (1985-7) ICPMs were placed to identify the "at risk" patients. 30% of the 127 patients developed RICP. A 50% reduction in mortality to 9.4% occurred. Protocol Era: From the above experience, several clinical and CT scan criteria as indication for immediate surgery, ICPM or clinical observation were established and implemented prospectively over a 10 year period (1988-97) in 1237 patients. With the use of the contusion management protocol, the acute contusion management mortality reduced even further to 2.67%! Protocol violations (50%) or deaths prior to surgery (25%) accounted for most of the mortality.
Conclusion: The implementation of a management protocol using well defined clinical and CT criteria have significantly reduced the mortality associated with the management of focal contusions and have removed the unpredictability in treating these treacherous lesions.

126. The open lung approach in patients with brain injury

L. Schürer, S. Wolf, H.A. Trost, Ch.B. Lumenta (Munich, D)

Objectives: Acute Lung injury and ARDS are common comorbities after traumatic brain injury, intracranial hemorrhage and SAH. Recent studies on ARDS in a mixed population addressed the beneficial effect of low tidal volume ventilation to prevent ventilator induced lung injury. One concept going even further is the so called Open Lung Approach, which consists of opening atelectatic alveoli with recruiting maneuvers followed by ventilation on low tidal volumes combined with an elevated PEEP level to prevent recurring lung tissue collapse. However, in most neurosurgical departments high PEEP levels are refused due to the potential risk of intracranial deterioration.
Methods: We present the clinical course of twelve patients with known intracranial pathology, either from traumatic brain injury, SAH or intracranial bleeding and concomitant ARDS or acute lung injury which was treated according to the Open Lung concept.
Results: The mean oxygenation index (paO_2/FiO_2) increased from 135±85 to 322±62 measured 24 hours after the first recruitment maneuver ($p<0.001$). Mean PEEP level used after the first recruiting maneuver was 15.0±3.1 mmHg. Although $paCO_2$ levels increased moderately (36.4±5.1 mmHg vs. 40.4±4.4 mmHg, $p<0.001$), comparison of mean ICP values as well as peak ICP values 24 hours before and after the first recruitment revealed no significant change (14.8±8.1 mmHg vs. 13.7±6.4 mmHg mean ICP, n.s., resp. 17.7±10.9 mmHg vs. 17.4±7.2 mmHg peak ICP, n.s.). No patient had to be withdrawn from Open Lung ventilation due to an uncontrolled ICP rise.
Conclusion: With careful neuromonitoring, there is no reason to withhold Open Lung ventilation in neurosurgical patients. In our feasibility analysis, this approach was as safe and beneficial as in other ARDS patients. The potential risk of intracranial deterioration due to critical ICP values seems far lower than the achievable benefit and decrease of mortality due to improved oxygenation.

Regular Session 17
SCI/TBI rehabilitation

127. Advances in the understanding of mechanisms underlying neuropsychological disturbances from acute brain insults (Invited lecture)

B. Preilowski (Tübingen, D)

Our knowledge about the neuropsychological sequelae of acute brain damage differs from that which we have gained about the consequences of vascular accidents and surgical interventions. The reasons are important variations in the clinical populations, such as with regard to age and sex distributions and – most importantly – to the great variability of the kinds of trauma and the resulting complexity of primary, secondary and tertiary brain pathological processes as well as the types of accompanying neuronal damage. Also, from the dominant clinical pictures of acute brain injury, such as for example short- and long-term post-comatose deficits, the types of questions posed to the neuropsychologist often differ from those presenting themselves in the context of other brain pathology. Thus, instead of emphasizing mechanisms of circumscribed sensory-motor and cognitive functions in explaining the performance of patients, the concern is more directed towards the role of underlying basic functions and states such as activation, attention, motivation and emotion. Furthermore, acute brain injuries are often accompanied by non-medical, e.g. legal issues. This is the case especially with so-called mild head trauma without clear-cut neurological signs of brain damage. Here the possibility of "non-organic" causes, of aggravation or simulation has to be considered as well as possible physiological mechanisms of more subtle kind, for instance, trauma related changes in transmitter balance, or indirect influences, such as post-traumatic stress influences and the effects of peripherally caused pain on brain functions. Finally, this review will touch upon the problem of long-term consequences of early traumatic brain injury and changes in the appreciation of early and life-long brain plasticity.

128. Mechanisms of neuropsychological rehabilitation in acute cerebral insults (Invited lecture)

E. Pöppel (Munich, D)

With respect to rehabilitation or restitution of function several potential mechanisms have to be distinguished. At present it is unclear which mechanisms are dominant in the rehabilitation process. Improvement of function can be due to a redundancy of functional representation based on divergence of axonal projections within a circumscribed region. Alternatively, multiple represenations (as in the two hemispheres) might be of importance. A third morphological basis might be provided by axonal sprouting. Traditionally, removing diaschisis has been discussed, i.e. a loss of funtion has been considered as an imbalance in an excitatory/inhibitory network. What might be the compensatory local mechanisms for restitution of function? Post-synaptic sensitivity can be improved, pre-synaptic activity is enhanced, trophic factors play a role, new synaptic contacts might be formed or silent synapses might be switched on again. Thus, there are various morphological, systemic and local factors that are involved in the restitution of function.

129. Rehabilitation in spinal cord injury – neuroprostheses, weight supported locomotion (Invited lecture)

V. Dietz, M. Wirz, G. Colombo (Zurich, CH)

Following central motor lesions two forms of reorganisation can be observed which lead to improved mobility: 1. The development of spastic muscle tone, and 2. the activation of spinal locomotor centres induced by specific treadmill training. Tension development is different from normal during spastic gait and appears to be independent of exaggerated monosynaptic stretch reflexes. Exaggerated stretch reflexes are associated with an absence or reduction of functionally essential polysynaptic reflexes. When supraspinal control of spinal reflexes is impaired, the inhibition of monosynaptic reflexes is missing in addition to a reduced facilitation of polysynaptic reflexes. Therefore, overall leg muscle activity becomes reduced and less well modulated in patients with spasticity. Electrophysiological and histological studies have shown that a transformation of motor units takes place following central motor lesions with the consequence that regulation of muscle tone is achieved at a lower level of neuronal organization which in turn enables the patient to walk. Based on observations of the locomotor capacity of the spinal cat, recent studies have indicated that spinal locomotor centres can be activated and trained in patients with complete or incomplete paraplegia when the body is partially unloaded. However, the level of electromyographic activity in the gastrocnemius (the main antigravity muscle during gait) is considerably lower in the patients compared to healthy subjects. During the course of a daily locomotor training program, the amplitude of gastrocnemius electromyographic activity increases significantly during the stance phase, while inappropriate tibialis anterior activation decreases. Patients with incomplete paraplegia benefit from such training programms such that their walking ability on a

stationary surface improves. This article reviews the pathophysiology and functional significance of spastic muscle tone and the effects of treadmill training on the locomotor patterns underlying new attempts to improve the mobility of patients with paraplegia.

130. Neuroorthoptic treatment in brain-damaged patients with disturbed convergent fusion

G. Kerkhoff, G. Eberle-Strauss, E. Haaf, N. Rettinger (Munich, D)

Some 30% of head trauma victims or patients with cerebro-vascular lesions show severe and persistent disorders of convergent fusion which prevent binocular vision, impair reading and PC-work and lead to eye-strain and headache. To investigate whether these patients can benefit from a repetitive visual training of convergent fusion using binocular devices, we studied 25 patients with acquired brain damage and severely impaired convergent fusion. Training consisted of 12 (mean, range: 8-16) treatment sessions (à 50 min) over a period of 6 weeks. Before treatment onset a 3-4-week baseline period without fusional treatment, but with cognitive and physical rehabilitation training, was implemented to separate treatment-induced effects from spontaneous recovery or unspecific effects arising from other treatments. The mean follow-up period after cessation of treatment was 3 months. The main outcome measures were: convergent fusional range (in cm/m), stereo acuity (in arc seconds), subjective reading duration (in min), visual acuity of both eyes for the near and far viewing distance, as well as accommodation. None of the 25 patients showed a significant improvement in the fusional range during the no-treatment baseline period, while 18 of 25 patients showed a significant improvement during treatment in convergent fusion, stereoacuity, reading duration, as well as a significant reduction in subjective symptoms (eye-strain, headaches). All improvements remained stable at follow-up. In contrast, accommodation and visual acuity did not improve, which excludes unspecific effects. We conclude that systematic, fusional training can significantly improve convergent fusion in the majority of patients with brain lesions.

131. Long term follow-up of patients following traumatic brain injury with proton magnetic resonance spectroscopy

M.R. Garnett, A.M. Blamire, R.G. Corkill, B. Rajagopalan, T.A.D. Cadoux-Hudson, P. Styles (Oxford, UK)

Neurological recovery following traumatic brain injury (TBI) is protracted with early conventional imaging correlating weakly with clinical outcome. To investigate the processes that take place during this period 27 patients (mean age 37 years) have been studied with an initial mean delay from TBI of 11 days (a), and then followed up at 6.2 months (b) and 24.5 months (c). At each time point conventional MRI (T1 and T2 weighted imaging) and proton magnetic resonance spectroscopy (MRS; single voxel technique, echo time 30 ms, repetition time 3000 ms) were performed. The voxel was localised to frontal lobe white matter that appeared normal on conventional MRI. Spectra were analysed for relative amounts of N-Acetylaspartate (NAA), creatine (Cr) and choline (Cho) compounds. Results were compared to 20 control subjects (mean age 37 years). Clinical outcome was assessed using the Glasgow outcome score and the disability rating scale. At each time point the NAA/Cr was significantly ($p<0.05$) reduced (a 1.31 (0.21) (mean (sd)), b 1.23 (0.25), c 1.32 (0.15), controls 1.44 (0.12)) and the Cho/Cr significantly increased (a 0.84 (0.20), b 0.83 (0.23), c 0.77 (0.12), controls 0.66 (0.07)) compared to controls. The NAA/Cr decreased significantly between 11 days and 6.2 months with some evidence of recovery at 24.5 months. The NAA/Cr assessed at 11 days significantly correlated with the clinical outcome of the patients at 6.2.months and 24.5 months. The reduction in NAA/Cr would be in keeping with significant injury to the normal appearing frontal white matter tracts in these patients with evidence for ongoing injury between 11 days and 6.2 months. This could be explained by diffuse axonal injury at the time of injury together with subsequent Wallerian degeneration. The increase in Cho/Cr would be consistent with altered membrane metabolism, possibly allowing for cellular repair. Alternatively the increase in Cho/Cr could reflect an altered cellular population secondary to neuronal fallout and a reactive astrocytosis.

Plenary Session 6
Mild and moderate brain injury

132. Cognition and memory deficits from mild and moderate traumatic brain injury (Invited lecture)

H. Levin, S. McCauley, J. Song, C. Boake, C. Contant (Houston, USA)

Cognition and memory were studied 3 months after mild (n=33) to moderate (n=4) traumatic brain injury (TBI) in 37 adults (mean age 35.3 years;mean education = 10.8 years) and compared to 39 adults who had general traumatic injury (GT) without TBI (mean age 37.2 years; mean education=11.1 years). Males comprised 70% of the TBI and 67% of the GT groups. Patients who met eligibility criteria were recruited from consecutive admissions to emergency

department. No significant differences were found in memory (Selective Reminding and Rey Complex Figure),processing speed (Paced Auditory Serial Addition and Symbol Digit Modalities), or problem solving (Wisconsin Card Sorting). Findings with a comparison group of extracranially injured patients confirm previous controlled studies showing recovery of cognition and memory to a normal level by 3 months after mild TBI. (Supported by a grant from Centers for Disease Control.)

133. Brain injuries in sports (Invited lecture)
T.A. Gennarelli (Milwaukee, USA)

Brain injuries occurring during a scholastic, amateur or professional sporting event have been known for ages, but there is growing appreciation for the impairments that they produce. Although catastrophic injuries leading to death or severe disability occur, they are relatively unusual. These occur principally in speed related sports such as motor racing, horse riding and alpine skiing, but also in boxing where acute subdural hematoma is the most common fatal injury. Much more frequently are concussive events. These occur in virtually every type of sporting event. Of greatest recent concern are multiple concussions occurring in the same player. These repetitive brain injuries are providing evidence for cumulative effects after injury since many players do not return to normality after incurring several concussions. A test-retest paradigm using a short battery of tests has been used in professional American football and ice hockey players. This and other systems to determine when a player should return to play will be discussed, but to date, none is based on a firm scientific foundation. Sports injuries remain a fruitful area to conduct future clinical research.

Regular Session 18

Clinical trials (failure or poor design), outcome measures

134. Clinical trial design in severe head injury – current problems, future strategies (Invited lecture)
A. Maas (Rotterdam, NL)

Over the past decade many neuroprotective agents have been developed with the hope of being able to improve outcome in patients with acute cerebral disorders, such as stroke, head injury and subarachnoid hemorrhage. Unfortunately, in the field of head injury none of the phase III trials performed have convincingly demonstrated efficacy in the overall population. A common misconception is that consequently these agents are ineffective. Such has however not been proven and some trials show evidence of efficacy in subgroups of the population studied. The negative results, as reported in the overall population may well, at least in part, be caused by problems in clinical trial design and analysis. Problems identified include heterogeneity of the population, use of the dichotomized GOS and difficulties of showing a possible treatment effect in relation to a strong prognostic effect. The effect of heterogeneity is less as trials include more patients, but large mega-trials are impractical in head injury. Imbalances between placebo and treated groups, due to heterogeneity, may be resolved by pre-randomization stratification for multiple variables, or by post hoc adjustment on analysis, pre-specified in the protocol. Primary endpoint in most clinical trials conducted has been the GOS dichotomized into unfavorable versus favorable. The hypothesis in trials was to demonstrate an absolute improvement in favorable outcome by 10% in the treated population. First, such an expectation may be considered over-optimistic, and secondly, we have demonstrated that a substantial and clinically relevant change in outcome distribution may not be reflected in a significant way in the dichotomized GOS. A particular point of concern is the difficulty in showing a treatment effect in relation to a strong prognostic effect. Head injury trials include patients who will do poorly "whatever you attempt" as well as those who "will do well no matter what you do". The currently used enrollment and exclusion criteria are effective in reducing the number of patients with a poorer prognosis, but conversely the chance of favorable outcome or survival is greatly increased. The risk estimate, obtained from prognostic modeling studies should therefore be taken into account in the design and analysis of trials. In seeking for solutions the crux of the question is whether the risk profile should be used to target analysis to patients with an intermediate risk profile, excluding patients with a high prognostic effect, or whether the primary outcome measure for efficacy should be differentiated according to the risk profile. It may be concluded that a major change in our approach to clinical trial design and analysis is required in order to minimize the risk of discarding potentially beneficial treatments, due to insufficiencies of methodology employed. It is our duty and task as investigators to convince the regulatory authorities of the need for these changes, supporting our recommendations on basis of hard evidence.

135. NIH initiative on clinical drug trial problems in traumatic brain injury (Invited lecture)
M. Walker (Bethesda, USA)

136. Prerequisites for outcome measures in clinical trials
S.C. Choi (Richmond, USA)

The prerequisites for the primary outcome measures in clinical trials of severe neurotrauma are discussed. The prerequisites include the following: the measure must be ordinal; the potential treatment effect should be easy to interpret; misclassification (i.e., incorrect classification) rate of outcome categories must be low; and measure must be completely determined in all patients. These prerequisites are discussed using the outcome measures including the Glasgow Outcome Scale (GOS), Disability Rating Scale (DRS), Functional Independence Measure (FIM) and various neuropsychological measures. In general, composite outcome measures such as DRS and FIM are not ordinal, and it is difficult to interpret the treatment effect. A measure that satisfies all the prerequisites is the GOS. A new study on the effect of misclassfications is also presented. The power decreases with increasing misclassification rates. One implication of this finding is illustrated using the results from a recently conducted hypothermia trial. Finally, it is suggested that some anomalous results of compostie measures are likely due to a combination of unordinality and misclassification rates

137. Protec – a dose-effect study of gacyclidine in patients with severe traumatic brain injury
M. Tadie, P. D'Arbigny, B. Vigue, J.M. Mathe, G. Loubert, M. Hurth, P. Carli, J. Delcour (Paris, Nantes, Garches, Amiens, F)

Gacyclidine is a non competitive NMDA antagonist, in clinical development. 48 patients presenting with confirmed severe traumatic brain injury (TBI) with a Glasgow Coma Scale (GCS) from 4 to 8 inclusive were enrolled within 2 hours after the trauma in a multicenter French clinical study. Patients were assigned to placebo or one of the 3 doses of gacyclidine: 0.005 mg/kg, 0.01 mg/kg, 0.02 mg/kg injected IV and repeated once after 4 hours. The primary endpoint was the Glasgow outcome scale (GOS) at 3 months and one year. The GOS was dichotomized into favorable and unfavorable outcome. Taking into account the a priori defined outcome prognosis factors: lesion severity on the CT-scan, occurrence of low systolic blood pressure on day of injury, GCS prior to enrollment, time interval accident-treatment first administration, a dose-effect is observed. If the limited number of patients does not allow to reach the significance threshold, the magnitude of the difference between the placebo group (20% of favorable outcome) and the group treated with the best dose 0.02 mg/kg (41 % of favorable outcome) leads to double the rate of favorable outcome. With the regimen of administration tested gacyclidine was well tolerated. Gacyclidine appears to be a promising treatment for patients presenting with TBI, to be confirmed in phase III studies.

138. Can potential neuro-protective drugs be administered within an appropriate therapeutic time window? Early experience from the international randomized controlled trial of corticosteroids in head injury – the CRASH trial
J. Wasserberg (Birmingham, UK)

Introduction: Early administration of neuroprotective agents is important for maximal effect in CNS trauma, as demonstrated in the North American spinal cord injury (NASCIS) trials. This study reports the feasibility of entering head injured patients into a randomised trial within three hours of injury.
Design: A randomised controlled trial of a 48 hour infusion of methylprednisolone or placebo in head injured adults. Patients are randomised, in the emergency room or neurosurgical unit using telephone randomisation.
Subjects: Head injured adults with a Glasgow coma score of 14 or less in whom the doctor is "substantially uncertain" whether corticosteroids are indicated.
Outcome measures: Time from injury to randomisation based on data from the randomisation service.
Results: Between April 1999 and April 2000 a total of 420 patients had been randomised in seven countries. Thirty nine percent (164/420) of patients were randomised within one hour of injury, 66% of patients (277/420) were randomised within three hours of injury. The remaining 143 patients were randomised between 3 and 8 hours. Time to randomisation was not affected by the severity of head injury.
Conclusion: Corticosteroid was given within three hours of injury in most eligible patients. If the trial shows benefit from corticosteroids then administration within a three hour time window would be acheivable.

139. Predictors of early assessment of outcome after severe head injury and multiple trauma with head injury
U. Lehmann, E. Rickels, M. Lorenz, S. Zech, M. Winny, U. Molitoris, H. Pape (Hannover, D)

Backgrounds: Primary brain damage is caused by accident and mechanism like hemorrhage and hypoxia or metabolic changes caused by specific injury pattern may secondarily increase the extent of brain damage.
Methods: Prospective study from 3/95 to 7/97, n=83 patients with inclusion criteria of AIS head ³ 3 points, patients were separated into a group with isolated

head injury (HI) and a group with head injury and an additional injury in another region with an AIS value ³ 3 points (MT). Outcome results were assesssed by the Glasgow Outcome Scale (GOS) 12 months after injury and classified into GOS 4 and 5 for good (G2=HI; G4=MT) and for bad (G1=HI; G3=MT) outcome. The parameters of initial GCS, pupillary response, systolic blood pressure (RRsys), oxygene saturation (O-Sat) at the scene of accident, ISS, hemoglobin, Quick's test, partial thromboplastin time (PTT), standard bicarbonate (HCO3) and temperature after arrival at the hospital and amount of blood transfusion including the first phase in the OR. Comparison between groups was tested by ANOVA, the predictive value was compared by sensitivity, specificity and Odd ratio.
Results: 57% (G2) of the patients with isolated head injury (HI) had a good, 43% (G1) a bad outcome and 41% (G4) of patients with head injury and multiple trauma (MT) a good, 59% (G3) a bad outcome. Initially assessed GCS was 5.2±2.1 (G1), 10.3±4.0 (G2), 6.0±3.8 (G3) and 8.4±4.0 (G4); p<0.01. Injury severity score (ISS) was 37±23 (G1), 22±7 (G2), 41±11 (G3) and 32±9 (G4); p<0.01. RRsys measured at the scene of accident was 117±39 (G1), 123±33 (G2), 99±38 (G3) and 106±27 (G4); p<0.05. O-Sat didn't show significant differences between patient groups; 91±11 (G2), 93±11 (G4) vs. 93±7 (G1), 97±3 (G3); p< 0.55. Due to hemorrhage hemoglobin (g/dl) was lowered in G3 (8.8±2.4) in comparison to G1 (11.1±2.4), G2 (12.3±2.2) and G4 (10.4±1.9) and the amount of blood transfusions was significantly higher (G1: 0.2±0.1; G2: 0.6±0.5; G3: 2.4±2.1; G4: 1.5±1.4; p<0.01). PTT time was significantly impaired in patients with bad outcome (G1: 62±53; G2: 35±6; G3: 96±70; G4: 38±8; p<0,01). Core temperature was significantly decreased in G3 (33.5±2.0) in comparison to G1 (35.8±1.9), G2 (35.8±1.2) and G4 (36.1±1.2). HCO3 was significantly lowered in patients with bad outcome (G1: 19.6±4.7 vs. G2: 22.8±3.0 and G3: 18.3±4.7 vs. G4: 22.2±1.6). Highest percentage of correct prediction was achieved by pupillary response with SE=(67%; HI vs. 92% MT), SP=(82%; HI vs. 85% MT) and OR=(9.0; HI vs. 60.5 MT). The best predictor after arrival at the emergency room was PTT with SE=(100%; HI vs. 83%; MT), SP=(72%; HI vs. 82%; MT) and OR=(15.4; HI vs. 22.5; MT).
Conclusion: The hemorrhagic component was the decisive factor for posttraumatic changes and reveal itself in clinical practice by quantity of blood transfusion. Suitable single predictors to estimate outcome were the pupillary resonse and the PTT.

140. Reliability of postal questionnaires for the Glasgow Outcome Scale
J.T.L. Wilson, P. Edwards, G.M. Teasdale (Stirling, London, Glasgow, UK)

Large scale clinical trials in head injury require an assessment of outcome which is easy to administer and yet reliable. We developed a structured interview for the Glasgow Outcome Scale (1) and showed that this improves the reliability of the GOS. However, an interview even by telephone may be impractical in large scale studies of outcome. The present study investigated whether postal questionnaires could provide a satisfactory assessment of outcome. We designed two questionnaires: (a) a simple one page questionnaire giving a rating on the 5-point GOS; (b) a more complex three page questionnaire for the 8-point Extended GOS. The questionnaires could be completed by either the patient or a carer. We studied the main properties of the questionnaires in two ways: each postal questionnaire was compared to a structured interview by telephone using the 8-point GOS, and each was administered twice to determine test-retest reliability. The studies were carried out in separate groups of head injured patients consisting of 32 to 38 cases. Weighted kappa values for the comparison with the telephone interview were .72 for the 5-point questionnaire, and .91 for the 8-point questionnaire. Weighted kappa values for the test-retest comparison were .92 for the 5-point scale, and .94 for the 8-point scale. The weighted kappa values indicate good to excellent agreement for all comparisons. There was no evidence that the simpler questionnaire had superior properties. We conclude that outcome after head may be reliably assessed using postal questionnaires.
References: (1) Wilson JTL, Pettigrew LEL, Teasdale GM. Structured interviews for the Glasgow Outcome Scale and Extended Glasgow Outcome Scale. J Neurotrauma 1998;15:573-585.

141. The GM1 ganglioside acute spinal cord injury (SCI) study I – baseline observations and their influence on outcomes
F.H. Geisler, F.C. Dorsay, G. Grieco, S. Piva, D. Poonian, R. Fiorentini (Chicago, Barrett Park, New York, Rockville, Washington, USA; Abano Terme, I)

Objectives: To assess the influence of baseline observations on motor and sensory outcomes and fraction of marked recovery.
Background: GM1, a cell membrane component abundant in the CNS, has shown acute neuroprotective and long-term regenerative effects in experimental ischemia and injury.
Design and methods: This was a double-blind parallel study of placebo vs "low-dose" GM1 vs "high-dose" GM1, stratified by injury level (cervical vs thoracic), age (= vs > 29 years old) and Baseline severity in 28 North American centers followed for one year. The NASCIS II MPSS dosing regimen was started =8 hours post-injury, followed by study medication

starting =72 hours post-injury. The "low-dose" regimen was 300mg iv, then 56 daily iv/im doses of 100mg; the "high-dose" regimen was twice as much. Marked Recovery was defined as a=2-grade improvement in the modified Benzel scale post-Baseline vs the Baseline ASIA Impairment Scale (AIS). Motor scores sensory scores and bowel and bladder functions were also assessed.
Results: Of 797 patients enrolled, 760 were analyzable. Of these 63.4%, 17.2%, and 19.3% were in severity groups A, B, and C+D, respectively. Overall, 77.4%, 39.7%, and 15.6% of patients in Baseline severity strata A, B, and C+D respectively did not improve = 1 Benzel/AIS grade at Week 26. Overall 10.6%, 41.2%, and 77.6% of patients in Baseline severity strata A, B, and C+D respectively showed Marked Recovery at Week 26. The distributions of motor score changes and sensory score changes were highly non-normal.
Conclusion: Functional outcome as assessed by change in Benzel/AIS grade, motor score changes, and sensory score changes, was highly dependent on Baseline severity. Severity group A patients, nearly 2/3 of the study population, demonstrated a "floor" effect with little chance of recovery of function. Severity group C+D patients demonstrated a "ceiling" effect with a high probability of substantial recovery.

Regular Session 19
Nerve grafting and bridging

142. Strategies to improve outcome after spinal cord injury (Invited lecture)
M.B. Bunge (Miami, USA)

Improving outcome after SCI will require strategies to (1) save as much tissue as possible immediately after injury and to (2) promote axonal regeneration accross the injury and (3) beyond to approriate targets. New experiments have addressed all these goals, using both complete transection and weight-drop lesion models in adult Fischer rat thoracic spinal cord. For (1), a combination of methylprednisolone and IL-10 reduces cavity volume, protecting gray but not white matter after contusion injury. The combination is more effective than the compounds tested singly. For (2), also after contusion, transplanting Schwann cells (SCs) and olfactory ensheathing glia into the cavity at 1 wk reduces tissue loss and promotes sparing/regeneration of propriospinal and brainstem axons and decreases CST dieback. For (3), adenoviral vector-mediated delivery of BDNF and NT-3 7 mm distal to a SC graft in a complete transection gap leads to improved axon extension caudal to the graft. Over a 12 wk period, the BBB scores revealed that hindlimb function in the neurotrophin group is significantly improved compared with the LacZ group (7.6 vs. 5.3). These strategies could be combined to test whether return of function is further improved. (Supported by The Christopher Reeve Paralysis Foundation; NIH 09923; NWO-GMW Pioneer Grant to J. Verhaagen; and the Miami Project.)

143. Role of PTEN in traumatic and ischemic neural injury
Q. Wan, C. Hu, M.V. Frantseva, R. Nashmi, P.L. Carlen, M.G. Fehlings (Toronto, CDN)

PTEN, a novel tumor-suppressor and phosphatase, has been implicated in the regulation of cell growth, cell cycle progression, cell survival, extracellular matrix interactions, and cell migration. Strong evidence demonstrates that high levels of PTEN expression result in apoptotic cell death and that inactivation or deficiency of PTEN activity increases cell survival and proliferation. However, little is known about the function of PTEN in the central nervous system (CNS). We have recently obtained evidence that the PTEN protein is mainly expressed in neurons in rat brain and spinal cord and that the expression of PTEN is markedly decreased after traumatic rat spinal cord injury and hypoxic/aglycemic hippocampus insult. Interestingly, we found that the reduction of PTEN was accompanied with an increased phosphorylation level of the serine/threonine kinase Akt, a crucial anti-apoptotic and cell survival-promoting factor. Subsequently, using a PTEN-deficient cell line transfected with PTEN cDNA, we confirmed that down-regulation of PTEN contributed to the increased phosphorylation levels of Akt as compared to non-transfected PTEN-deficient cells after traumatic injury. Together, our studies provide evidence indicating that the down-regulation of PTEN may play an important role in enhancing cell survival, suggesting that alterations in PTEN signaling may represent a novel therapeutic target to enhance neuronal survival after nervous system injury.

144. Neuron-glia Cell Adhesion Molecule (NgCAM) enhances functional recovery after spinal cord contusion injury
W. Huang, J. Haspel, M. Grumet, W. Young (Piscataway, USA)

The aim of this study was to determine whether NgCAM (the chicken homologue of L1) could improve functional recovery after a spinal cord contusion injury. We made soluble recombinant NgCAM-Fc by fusing the extracellular portion of chicken NgCAM to human immunoglobulin Fc (Haspel et al, J. Neurobiol, 42:287-302, 2000), and applied the NgCAM-Fc (250 µg/ml) intrathecally to rat spinal cords. The spinal cords were injured with a 25.0 mm injury using the

NYU impactor. After 3-month survival, neuroanatomical tracing and immunohistological methods were used to examine the growth of axons. The results suggest that intrathecal administration of NgCAM-Fc (n=12) significantly (repeated measure ANOVA, $p<0.05$) improved functional recovery. NgCAM treated rats showed frequent to consistent plantar stepping (BBB, 9.88±0.4) while saline treated rats (n=12) only showed sweeping movements or body weight support in stance only (BBB, 8.42±0.2). NgCAM-Fc administration led an increases in the extent of axonal growth within and beyond the injury site. Unexpectedly, NgCAM-Fc treatment induced higher autophagia incidence than the control group, suggesting that NgCAM-Fc treated rats may develop an early sensory recovery or pain. These results indicate that exogenously applied NgCAM-Fc may enhance locomotor recovery and axonal growth in spinal cord contusion injury.

145. Plasticity of cortical connectivity after frontal traumatic brain injury

P. Stude, M. Ahrenkiel, D. Osenberg, W. Ischebeck, D. Stolke, M. Keidel (Essen, Hattingen, D)

In the present study we tested the following hypothesis: A MRI-defined brain lesion after traumatic frontal brain injury leads to disturbances of the neuronal network and it is compensated by changes in the functional strength of coupling between neuronal populations of different brain areas and by a reorganisation of the temporal pattern of cortical interregional coupling. Thus, the aim of the study was to demonstrate an altered spatio-temporal pattern of neuronal coupling between the contralateral ´active´ motorcortex and all other areas in relation to a simple voluntary movement (self paced, brisk abductions of the right index finger). In 22 patients (42 ± 14,8 yrs., 24 ± 16,6 months after traumatic brain injury) and in 20 normal subjects (27,2 ± 5,7 yrs.) the electroencephalogram was recorded from 30 electrodes according to the 10-20 system. In relation to movement onset absolute and relative changes in coherence, as a measure of the functional coupling strength, were analysed. Recordings of identical channel-pairs under a controlled resting condition served as reference. The significance of the difference between both groups was tested by a multiple t-Test. Analysing the cohort of healthy subjects, we observed movement related increases of coherence in the alpha-, beta1- and beta2-band between the ´active´ motor cortex and ipsilateral frontal regions followed by coupling of both homologous motor cortices. Patients with traumatic frontal brain lesions (in the chronic state) showed a hemispherical lateralisation of the activity preponderance of the contralateral motor cortex with a decrease of the interhemispherical coupling between both motor cortices and with a functional extension of the contralateral motor cortex by strengthening the neuronal coupling of the ´active´ motor cortex with the premotor-frontal area. Since different brain areas were coupled in an distinct temporal pattern to preparate, execute and evaluate movements, the results support the idea that neuronal populations form a specific movement-related neuronal network implicating connectivity encoding of voluntary movements. We conclude that a traumatic frontal brain lesion is functionally reorganized by a plasticity of cortical connectivity by an increase or decrease of coupling-strength between different neuronal populations. (Supported by German Ministry of Education and Research, BMBF# 01K09807/2.)

146. Bioartificial nerve grafting – histological findings on implantation of embryonic spinal nervous tissue and a polyimide sieve probe on degenerating and regenerating rat sciatic nerves

P.M. Klinge, K. Wewetzer, U. Meyer, S. Groos, M. Samii, T. Brinker (Hannover, St. Ingbert, D)

To evaluate a concept of bioartificial grafting for improved functional restoration of degenerating peripheral nerves that enables electrical stimulation of grafted embryonic spinal neurons via a flexible polyimide multi-channel sieve electrode. A suspension of at least 20-30µl embryonic neurons (E14) were grafted onto the degenerating sciatic nerve stump of 10 adult rats. The neurons were implanted within a containment (autologous vein graft) that was sutured onto the nerve. With Nissl- and neurofilament-immunostaining the vitality of the graft was investigated after 4 and 10 weeks. In further 10 rats, a flexible polyimide sieve probe (sieve area with 2 mm diameter, about 670 via holes of 40µm diameter) was a-traumatically sutured onto the regenerating proximal stump of a transected sciatic nerve. Long-term bio-stability and axonal sprouting was investigated by assessment of neuroma formation with H&E-staining and neurofilament-immunostaining after 1 and 9 months. Within the vein-containment the implanted embryonic neurons survived to a considerable degree, however, immunoreactivity to neurofilaments was steadily increasing over a period of 10 weeks demonstrating the vitality of the nerve graft by neurite outgrowth into the transected degenerating nerve. Neurofilament staining of the nerve-polyimide area showed regular neurite sprouting through the entire sieve-interface which dramatic increase after 9 months. During the observation time, however, no signs of its degradation were observed. Both, the histological findings on the viability of the embryonic spinal nerve graft and the in-vivo behavior of the polyimide sieve probe establish the concept of a biohybrid graft for improved functional restoration of

degenerating peripheral nerves and their target muscles.

147. A flexible polyimide-sieve-electrode for interfacing severed peripheral nerves – immunohistological characterization of axonal sprouting and reactive tissue changes after long term implantation to the transected rat sciatic nerve

P.M. Klinge, K. Wewetzer, K. Haastert, M. Samii, T. Brinker (Hannover, D)

The aim of the present investigation was to provide a detailed histological analysis of axonal sprouting and biocompatibility after implantation of a novel flexible sieve electrode suitable for electrical contacting and stimulation of regenerating peripheral nerves. In 10 adult rats, a flexible polyimide sieve probe (sieve area:2 mm diameter; about 657 via holes of 40µm diameter) which can be manufactured with integrated multi-channel electrodes was a-traumatically sutured onto the proximal stump of the transected sciatic nerve. Long-term bio-stability and axonal sprouting was investigated after 1,6 and 11 months implantation using monoclonal antibodies against neurofilaments (68,160 and 200kD), cell type-specific antibodies against Schwann cells (27C7,PO,10B3) and macrophages (ED2, CD11b). Massive neurite growth through the sieve electrode occurred. The number of penetrating axonal processes steadily increased to about 19000 neurites eleven months after implantation. Parallel to this increase was the expression of myelin markers like P0, whereas non-myelin-forming Schwann-Cells were found to stagnate indicating an intact neuron-Schwann cell interaction. Since lesion related antigens, like the tenascin-C-like antigen 10B3 and hematogenous macrophages were transiently up-regulated and returned to basal levels after 11 months, growths pattern simulating physiological nerve development and regeneration were found at the sieve interface. At no time signs of degradation were observed. The introduced flexible polyimide implant is permissive for substantial neurite growth and its “capacity” is sufficient for electrical contacting peripheral nerves. Since it furthermore showed excellent long-term stability, the used electrode is a promising new tool for interfacing peripheral nerves concerning future prosthetic approaches.

148. Role of macrophages and serum complement in experimental immunological demyelination of the CNS

J.K. Dayer, J.A. Bourque, J.D. Steeves (Vancouver, CDN)

Complement mediated, antibody directed, immunological demyelination of the CNS has been shown to be an effective method of promoting axonal regrowth in the CNS. This is due to myelin removal, surrounding the CNS injury site, thereby overcoming myelin-associated inhibitors of axonal growth. Previous data indicates that myelin removal requires the presence of both antibody and complement, and is macrophage mediated. We now present data that further explores the role of complement in initiating a macrophage response, and the significance of macrophages in clearing myelin. Depletion of Factor B (alternative pathway) does not prevent demyelination, indicating a limited role of this pathway in immunological demyelination. Macrophages are observed, with typical phagocytotic morphology, suggesting that absence of Factor B does not inhibit phagocytotic activity. On removal of C3 protein (common to both classical and alternative pathways) or C4 protein (classical pathway) normal myelin was observed. The removal of C3 from serum complement results in failure of macrophage activation, and subsequent preservation of myelin. This is due to the lack of production of C3 derivatives, which activate macrophages, suggesting that the classical pathway has a fundamental role in demyelination. The role of macrophages, and their interaction with the classical pathway, was further examined by systemic monocyte depletion. Using liposomes containing dichloromethylene diphosphate to deplete blood monocytes, we inhibited monocyte entry to the CNS. We observed (1) absence of Ox42+ macrophages, (2) preservation of myelin, (3) decreased GFAP+-IR at the infusion site and (4) reduction in the size of damage at the infusion site. This suggests that macrophages are the primary mediators of myelin phagocytosis in the immunologically myelin-suppressed cord, through their interaction with opsonized tissue. Also, macrophages may play a role in the progression of astrogliosis and subsequent injury cavitation.

Regular Session 20

Cerebral blood flow

149. Cerebral microcirculation and blood flow in severe head injury (Invited lecture)

P. Muizelaar (Sacramento, USA)

150. Effects of mannitol administration on cerebral metabolism and oxygenation in severely head-injured patients

O.W. Sakowitz, A.S. Sarrafzadeh, J.F. Stover, W.R. Lanksch, A.W. Unterberg (Berlin, D)

To investigate whether mannitol treatment of intracranial hypertension in severe head injury (SHI) improves cerebral metabolism and oxygenation. The ability of osmotic agents to lower elevated intracranial pressure (ICP) has been extensively documented. However, blood-brain barrier (BBB) disruption may theoretically interfere with beneficial effects of i.v.-application. Multiparametric cerebral monitoring (MCM), consisting of intraparenchymal ICP measurement, tissue oximetry (ptiO2) and microdialysis (MD) in the less injured hemisphere, was initiated in 6 male SHI patients (age: 24-63 yrs, range; GCS <9). A total of 14 mannitol boli (20%, 0.5g/kg, 20 min infusion time) were administered to treat elevated ICP (>20 mmHg). MCM time courses following mannitol infusion were digitally recorded for 120 min. Microdialysates were assayed immediately for extracellular concentrations of glucose, lactate, pyruvate and glutamate. Elevated ICP was lowered successfully in all cases. Treatment effect was maximal 40 min after start of infusion (26+/-6 mmHg to 18+/-3 mmHg, $p<0.05$) and lasted up to 100 min. At the same time cerebral oxygenation remained unaffected (21+/-8 mmHg to 23+/-9 mmHg, n.s.). Microdialysate concentrations of all analytes rose unspecifically by 10-40% from baseline with maximum concentrations reached 40-60 min after start of infusion. Mannitol is an effective treatment option to lower increased ICP. At an ICP of 20-30 mmHg it does not affect cerebral oxygenation. The unspecific increases of extracellular fluid metabolites may be explained by the concentrating effect of cerebral water reduction.

151. Cortical hypoperfusion precedes hyperperfusion following controlled cortical impact injury in rats

U. Thomale, K. Schaser, A. Unterberg, J. Stover (Berlin, D)

Impaired cerebral perfusion contributes to tissue damage following traumatic brain injury. In this longitudinal study persistence of reduced cortical perfusion employing laser Doppler flowmetry and intravital microscopy using orthogonal polarization spectral imaging (CytoscanTM) were investigated in six rats following controlled cortical impact injury (CCII). Before, 30 min, 4, 24, and 48 hours after CCII, perfusion in pericontusional and non-traumatized cortex was determined moving a laser Doppler probe in 50 x 0.2 mm steps over the traumatized hemisphere. Diameter and flow velocity in arterioles and venules were assessed by intravital microscopy. Differences of results (mean ± SEM) are significant at $p< 0.05$. Cortical perfusion was significantly diminished by 33% at 4 hours followed by a significant increase by 43 and 107% at 24 and 48 hours after CCII. Intravital microscopy revealed corresponding changes: 30 minutes and 4 hours after CCII, vessel diameter and flow velocity of arterioles was significantly reduced by 30 and 17%, respectively. In venules flow velocity was significantly decreased by 51% with unchanged vessel diameter. By 24 and 48 hours, vessel diameter was significantly increased in arterioles (+ 53%) and venules (+ 71%), coinciding with significantly increased flow velocities. In venules, development of microthrombosis leading to complete stasis in the early phase was followed by spontaneous reperfusion at 48 hours. Cortical hypoperfusion found within the early phase following CCII is related to vasospasm and microthrombosis and seems reversible as it precedes a long lasting phase of hyperperfusion. Changes in tissue mediators (acidosis, NO, endothelin) could account for these findings.

152. The role of nitric oxide in cerebral reactivity to CO2 after severe head injury

Y. Furuya, R. Hlatky, A.B. Valadka, J.C. Goodman, R.G. Grossman, C.S. Robertson (Houston, USA)

Objectives: Nitric oxide (NO) has been observed to mediate the vasodilatation that occurs with hypercapnia in experimental models. To test the hypothesis that NO plays a role in cerebral vasomotor reactivity after severe head injury, the relationship between NO metabolites (nitrate and nitrite) level measured in microdialysates and CO2 reactivity of middle cerebral artery measured by transcranial Doppler methods was examined.
Methods: Twenty four severely head injury patients (Glasgow motor score < 6) who had a microdialysis probe (CMA 70, CMA/Microdialysis AB) in place during days 1-5 after injury were enrolled in this study. Microdialysis samples were collected every 30 minutes and the NO metabolites were measured in each sample using NO chemiluminescence following vanadium stripping. In twenty patients of 24, CO2 reactivity was tested every 12 hours. Other parameters such as intracranial pressure (ICP), SjO2, cerebral perfusion pressure (CPP) and end tidal CO2 (EtCO2) were collected every an hour.
Results: Subjects could be divided a high and a low NO group by their average concentration of NO metabolites in the dialysates samples. The high NO group had continuously high concentration of NO metabolites from day 1 (mean 61 +/- 7 micromol/L) and the low NO group had low concentration of NO metabolites on day 1 (mean 19 +/- 10 micromol/L). The high NO group had significantly a higher CO2 reactivity at day 1, and tended to have a higher AVDO2, lower ICP, higher CPP and lower flow velocity of MCA than the low NO group with time

course (group effect $p<0.05$). There was no significant difference in $EtCO_2$ or SjO_2 between the two groups. *Conclusion:* These studies are consistent with experiment data which suggest that impaired CO_2 reactivity after TBI may be due to low concentrations of NO, either because of rapid inactivation or because of reduced production of NO.

153. Patterns of SPECT blood flow deficits and cognitive performance after head injury

J.T.L. Wilson, E.A. Stamatakis, D.J. Wyper, G.M. Teasdale (Stirling, Glasgow, UK)

The purpose of the study was to identify patterns of SPECT blood flow deficits early and late after head injury, and investigate whether blood flow abnormalities were related to cognitive test performance. SPECT and MRI scans of 62 patients were obtained during the acute stage and at follow-up 5 to 10 months post-injury. Patients were aged 18 to 60 at the time of injury, and had no previous history of head injury, intracranial operation, psychiatric illness treated by hospitalisation, treatment for alcoholism or drug abuse, epilepsy, or mental handicap. SPECT imaging of was performed using a maximum dose of 500MBq 99Tcm-HMPAO (Ceretec), injected intravenously with the patients supine on the scanning couch under resting conditions. A group of 32 SPECT scans from non-head injured patients was used for comparison with the head injured group. A battery of neuropsychological tests was completed by head injured patients on the same occasion as follow-up SPECT imaging. Statistical Parametric Mapping (SPM) (1) was used to identify SPECT lesions and to relate blood flow deficits to test performance. All SPECT scans were spatially normalized to Talairach space using a 12-point linear affine transformation. They were smoothed with an isotropic 12mm FWHM Gaussian filter and were normalised with proportional scaling to account for blood flow and tracer uptake variations in individuals. Lesion identification was achieved by testing against the 32 controls in SPM with a replication of conditionsexperiment. The results were obtained at $p<0.01$. MRI images were analysed in a ROI fashion using software provided by the manufacturer of the scanner. In order to assess the relationship of neuropsychological scores to blood flow, a covariates only type analysis was used in SPM with the score of the individual being the covariate of interest and age the confounding covariate. SPECT detected more extensive abnormality than MRI in both acute and follow- up imaging. SPM allowed patterns of blood flow deficits to be detected in individual cases, and visualised overall distributions of deficits. The findings indicate that SPM can be used to relate blood flow variation in localised regions to cognitive test performance after head injury.

154. Loss of vasoreactivity following traumatic brain injury is attenuated by L-type calcium channel blocker

T. Maeda, D.A. Hovda, S.M. Lee (Los Angeles, USA)

Abnormal cerebrovascular responses to traumatic brain injury (TBI) may play an important role in the development of secondary cell damages and may influence neurological outcome. Recent studies have demonstrated that regional cerebral blood flow (rCBF) and vasoreactivity markedly decrease following both experimental and clinical TBI. However, the mechanisms responsible for these post-injury events are not yet fully understood. In the present study, the effect of verapamil, an L-type calcium channel blocker, on post-traumatic rCBF changes was evaluated following fluid percussion injury (FPI). Anesthetized Sprague-Dawley rats (250-300 g, n =15) were given a left parietal FPI of moderate severity (2.2-2.4 atm). Immediately following FPI, all animals received slow i.v. infusions of verapamil (200 µg/kg/min) and norepinephrine to maintain mean arterial blood pressure. In other FPI animals (n=15), SI cortices were stimulated bilaterally (300 µA, 100 ms duration at 1 Hz) to induce cortical spreading depression using chronically-implanted tungsten electrodes during CBF measurements. Following FPI, basal CBF was reduced throughout the ipsilateral cortex (mean ± SEM, 72.5 ± 4.7%) within 1 hr. Verapamil increased basal rCBF in FPI cases by 119.6 ± 7.6% compared to no treatment. In electrically stimulated cases, verapamil administration resulted in 466.1 ± 11.5% increase in rCBF when compared to non-treated animals, indicating that metabolic autoregulation was intact. These results indicate that vasoconstriction and loss of vasoreactivity may be responsible for the majority of post-traumatic hemodynamic depression. We conclude that the restoration of vasoreactivity following TBI may be as simple as returning cerebral vessels to their normal caliber. (Supported by NINDS grants NS 37363, NS 30308 and NS27544.)

155. Temporal profile of uncoupled neuronal activity and cortical blood flow after controlled cortical impact injury in rats

J. Stover, O. Sakowitz, G. Flügge, A. Unterberg (Berlin, Göttingen, D)

Following traumatic brain injury increased neuronal activity together with decreased cerebral perfusion contribute to tissue damage. Changes in cortical perfusion, neuronal activity, NMDA receptor binding, CSF glutamate, and brain edema were investigated after controlled cortical impact injury (CCII) in 20 rats. Cortical perfusion and neuronal activity were determined before, 5-30 minutes, 4, and 24 hours

after CCII by laser Doppler flowmetry and quantitative EEG. Glutamate receptor binding [(in vitro receptor autoradiography (125I-MK-801)], CSF glutamate and brain swelling were measured at 4 and 24 hours. Differences of results (mean ± SEM) are rated significant at $p < 0.05$. Within 30 minutes after trauma, cortical perfusion and neuronal activity were reduced by 20% and 54% compared to pre-trauma levels. At 4 hours, cortical perfusion was significantly decreased to 50% while neuronal activity was significantly increased by 60%. At 24 hours, cortical perfusion had reached pre-trauma levels again while neuronal activity remained significantly elevated by 40%. Specific glutamate receptor binding was decreased within the contusion but remained unchanged in the pericontusional cortex. Pathologically increased CSF glutamate levels reflected sustained neuronal activity, reaching from 16 ± 3 at 4 hours to 33 ± 4 microM at 24 hours. Brain swelling significantly increased over time (4 h: 3.4 ± 0.9; 24 h: 7.2 ± 2.2%). During the acute phase following CCII, neuronal activity is uncoupled from cortical blood flow. Simultaneous occurrence of reduced cortical perfusion and sustained neuronal activity with increased glutamate release and regionally unchanged glutamate receptor binding seem to contribute to evolving tissue damage.

Special Luncheon Seminar 5

Nimodipine in head injury: results of the HIT IV study (sponsored by Bayer AG)

LS155A. Nimodipine in head injury: results of the HIT IV study

Head injury remains a major cause of morbidity and mortality. There are few interventions that have been shown to substantially alter the prognosis. Subarachnoid haemorhage (SAH) on computerised tomography (CT) scan after head injury confers a disadvantage and is an important and independent factor in predicting outcome. Previous clinical trials using nimodipine in head injured patients have had mixed results. A European head injury trial (HIT II) showed a trend towards a favourable outcome in patients who had tSAH on CT scan. This notwithstanding, a subsequent prospective trial in German centres enrolling 123 patients with tSAH demonstrated on initial CT scan was carried out (HIT III). Outcome was assessed by means of the Glasgow Outcome Score (GOS) at 6 months. The patients treated with nimodipine had a significantly less unfavourable outcome at 6 months than the placebo-treated patients when assessed on an intention to treat basis. However, there was concern that the number of patients was relatively small, and this prompted a further study with a larger number of patients. The HIT IV study was constructed on a similar basis to the HIT II and HIT III studies. It was a randomised, double blind, placebo-controlled, multinational, multicentre study designed to prove the efficacy and safety of nimodipine in tSAH associated with mild to moderate head injury. The symposium will present the rationale and design of the study, as well as the detailed demographic, efficacy and safety data.

Regular Session 21

Autonomic dysfunction and peripheral nerve injury

156. Spinal cord injury induces plasticity in somatic and autonomic reflex circuits (Invited lecture)

J.C. Bresnahan, G.M. Holmes, M.S. Beattie (Columbus, USA)

The loss of descending axons after spinal cord injury (SCI) provides the possibility of reorganization in segmental circuitry caudal to the injury. Axonal and synaptic sprouting has long been thought to play a role in changes in spinal reflexes after injury and may promote recovery and/or produce dysfunctional outcomes such as spasticity, dysreflexia and chronic pain. Changes in synaptic inputs to both parasympathetic and sympathetic circuits will be described and correlated with alterations in eliminative and sexual function, and cardiovascular (autonomic dysreflexia) and respiratory function. Alterations in the functions of these circuits after complete spinal cord transection will be described and compared to recovery after spinal cord contusion injuries, and to recovery of locomotor function. (Supported by NIH grant NS-31193.)

157. Managements of severe plexus lesions – experimental and clinical approaches (Invited lecture)

T. Carlstedt (Stanmore, UK)

One of the most devastating nerve injuries is a lesion of the brachial or lumbosacral plexus. Restoration of limb function and control of pain after such injuries are formidable tasks. In the severe plexus lesion,one or several spinal nerve roots have ruptured or been avulsed from the spinal cord. This is a spinal cord injury and therefore considered not amenable to treatment. A long series of animal experiments has,however demonstrated an unexpected capacity for survival and regeneration of motoneurons within the spinal cord after re-implantation of the avulsed roots.In primates a substanial recovery of function could be seen due to implantation of avulsed ventral roots.After an immediate root implantation the

population of motoneurons was rescued from cell death.Different functional types of neurons had been attracted to regrow axons to the implanted root as judged by their position in the ventral horn.Deficient target specificity was correlated to co-contractions or synkinesis.Eventually a functional dexterity was regained. In the first ten human cases of intraspinal brachial plexus repair regeneration of motoneurons from the spinal cord to denervated muscles could be demonstrated in most patients. Useful function with muscle power that could overcome gravity was noted in three of the ten cases.Magnetic brain stimulation showed a normal amplitude and latency from motor cortex to reinnervated muscle.A certain degree of synkinesis compromised function initially. Eventually co-contractions were reduced, probably due to plasticity within the central nervous system.Pain was appreciated to be alleviated as motor function was restored. In these successful cases there was also,surprisingly, some return of sensory function.Sensory stimulation(thermal,mechanical) within avulsed dermatomes was perceived abnormally and/or experienced at remote sites.There was some return of joint position sense.The mechanism of the sensory recovery is uncertain. A short time lag between injury and surgery was recognised as most important for a successful outcome in the treatment of this spinal cord injury.

158. Administration of neurotrophic factors for protection of motoneurons after nerve root avulsion

E. Lang, N. Plesnila, A. Baethmann, G. Hofmann, M. Sendtner (Murnau, Munich, Wuerzburg, D; Boston, USA)

Introduction: Until now a satisfying conservative or operative therapy is not available for traumatic lesions of the spinal cord with paraplegia and nerve root avulsion. By administration of neurotrophic factors and replantation of nerve roots into the spinal cord therapeutic progress could be reached. This point is the objectice of the current investigations.
Methods: Ventral nerve root avulsion was induced in the spinal cord C7- segment in rabbits. The animals were subjected to perfusion fixation after a survival period of one and three weeks. The spinal cord segments (from C6 to C8 were embedded in paraffin. Serial sections were stained by NISSL. The number of viable alpha-motoneurons was determined and compared with that of the opposite healthy side.
Results: More than 80% of motoneurons were lost in segment C7, while 55% in segments C6-C8 after nerve root avulsion between one and three weeks after trauma. Local application of CNTF (ciliary neurotrophic factor) or BDNF (brain derived neurotrophic factor) with fibrin glue at the lesion side afforded an attenuation of the loss of motoneurons to 17.1% (CNTF) or 25.3% (BDNF) after one week survival and nearly the same after three weeks survival.
Conclusion: Loss of 55% of motoneurons in segment C6-C8 after root avulsion without further therapy takes place within the first week after trauma. Local administration of neurotrophic factors may enhance survival of affected motoneurons after nerve root avulsion significantly. Further improvements might be expected from nerve root replantation into the spinal cord.

159. Changes in N-methyl-D-aspartate receptor (NMDAR) subunit composition after Fluid Percussion (FP) injury appear to prepare the hippocampus for neuroplasticity in adult rats

C.L. Osteen, C.C. Giza, D.A. Hovda (Los Angeles, USA)

The NMDAR is known to be involved in the injury-induced ionic flux and secondary cell death following traumatic brain injury (TBI). To determine if the NMDAR is altered in the adult rat brain following TBI, Western blotting was performed using antibodies to NR1, NR2A, and NR2B subunits. Rats were subjected to a sham (n=3) or mild-moderate lateral FP (n=3) injury under isoflurane anesthesia and 4-5 days after injury homogenates of cortex (CX) and hippocampus (HIP) were prepared. Samples (10 mcg protein/lane) were processed by double-labeled Western blots (NR1/NR2A and NR1/NR2B) and volumetric analysis of bands was conducted for the CX and HIP ipsilateral (L) and contralateral (R) to the site of fluid pulse administration. Paired comparisons of sham and FP rats were performed. The NR1 subunit is an estimate of the total number of functional NMDARs. In the injured group, NR1 was decreased in 3/3 comparisons in the L CX (-37.59 +/- 25.15%) and R CX (-30.99 +/- 26.62%), while findings in the L and R HIP were not significant. After normalizing by NR1 signal, the NR2A:NR2B ratio was calculated. This ratio directly reflects the sensitivity and conductance of the NMDA receptor, with a smaller ratio being correlated with enhanced plasticity. While no difference existed between sham and injured rats in the L and R CX, the NR2A:NR2B ratio was decreased in injured rats in 3/3 comparisons in the L HIP (-26.74 +/- 1.58%) and R HIP (-35.94 +/- 20.37%). This data suggests that the NMDAR exhibits structural alterations 4-5 days after TBI. The NR1 subunit seems to be downregulated in the CX, decreasing excitotoxic potential, while the subunit composition of the NMDAR in the HIP seems to shift to favor post-injury plasticity. (Supported by NS30308 & NS27544.)

Regular Session 22

Pathophysiology of TBI/SCI

160. Experimental application of A1- agonists in spinal cord ischemia

G.Z. Sufianova, L.A. Usov, A.A. Suphyanov, S.S. Golubev, L. Raevskaya, A.G. Schapkin, O.N. Smekalina (Irkutsk, RUS)

Acute vascular pathology of spinal cord is an actual problem of modern medicine. Purpose of this work was study protective action of selective agonists of A1-adenosine receptors – cyclopentyladenosine (CPA) and cyclogecsyladenosine (CGA). The work is carried out on 33 rats, weighing 100-160 g.. Transitory ischemia of lumbar division of spinal cord was created by the complete abdominal aorta and its branches occlusion. For this group of rats (N =10) through both femoral arteries under surface anaesthesia introduced occluders before level of diaphragm (on depth 6-7 refer to). Through left common carotid artery on direction to occluders introduced polymeric substance, capable be fixed on the occluders. Occluders were extracted after 45 minutes. During the whole period ischemia each 5 minutes the estimation deep, tactile, temperature and pain sensitivity on 6 ball scales, offered by us. On time of steady degree of reduction of sensitivity was conducted the estimation of neurological deteriorations. Through 48-72 hours the spinal cord was extracted and fixed in 96 % alcohol. Were studied the transverse cuts of spinal cord at a level of lumbar bulge, stained by hematoxylin-eosine. To the second group rats (N=13) CPA were introduced intracerebroventriculary in the dose 25 mkg/kg 60 minutes before introducing polymeric substance (occurrence of spinal cord ischemia). To the third group rats (N=10) CGA were introduced intracerebroventriculary in the dose 25 mkg/kg 60 minutes before occurrence of spinal cord ischemia. Data were analysed using at Student's test. Macroscopic and microscopic investigation revealed ischemic change of all organs of abdominal cavity, retroperitoneal space and lumbar area at modelling ischemia. At rats was noted paraplegia, anaesthesia and atrophia of lumbar area and limbs muscles. Histological examination revealed remarkably constant pattern of ischemic spinal cord damage, characterised by severe ischemic changes in most neurones. In the first group of rats, time of appearance of reduction of reaction for deep, tactile, temperature and pain sensitivity accordingly are 18.5, 15 and 17.5 minutes. Time of appearance of reduction of sensitivity for groups of animals with ischemia on a background of CPA action accordingly are 30.8, 25.4 and 29.2 minutes. In the third group rats, times of appearance of reduction of sensitivity accordingly are 22.0, 19.5 and 23.0 minutes. The differences with control animals group (the first group of rats) were statistically reliable ($P<0.01$). Histopathological changing of the neurones and degree of ischemia herewith there more denominated. The results of work allow to make a conclusion about protective action of À1-agonists at spinal cord ischemia. It will by perspective using À1-agonists in clinical practice for treatment vascular myelopathy, complicated injure of spinal cord, during neurosurgical spinal cord operations, vascular operations at aorta and aortagraphy.

161. Survival analysis of time to first jugular venous oxygen desaturation

C.F. Contant, C.S. Robertson (Houston, USA)

The results of a clinical trial to compare two treatment protocols for the maintenance of cerebral blood flow were reported recently(Robertson and others 1999). In this paper we demonstrated a significant difference between the rate of occurrence of one or more jugular venous oxygen (SjvO2) desaturations in two treated groups of patients. In the CBF-targeted protocol, cerebral perfusion pressure was kept > 70 mm Hg, and pCO2 was kept approximately 35 mm Hg. In the ICP-targeted protocol, cerebral perfusion pressure was kept > 50 mm Hg, and hyperventilation to a pCO2 of 25-30 mm Hg was used to treat intracranial hypertension. There were significantly fewer events in the CBF-targeted group. A logistic regression model indicated several variables beside the treatment protocol were significantly related to the occurrence of an event. The analyses presented here focused on comparison of the time-to-first desaturation between the two treatment groups. Kaplan-Meier, log-rank and Cox Proportional Hazard models were used to examine the data. One hundred and eighty-nine patients from the original trial were included in the analysis. Of the 100 CBF-targeted patients, 26 experienced one or more events, while 42 of the 89 patients in the ICP protocol experienced events. By 24 hours after admission to the ICU, the Kaplan-Meier estimate of survival was 0.84 in the CBF group and 0.66 in the ICP patients. The Kaplan-Meier mean survival estimates for the CBF group was 443 hours, while it was only 210 for the ICP group. The log-rank comparison of the two survival curves was significant at $p<0.0034$. A Cox Proportional Hazards model was then fit by adding a set of covariates which had individually demonstrated a significant difference between the two treatment groups in the original paper (Table 5 of (Robertson and others 1999)). Where values recorded at 24 hours were used in the original analyses, the value recorded at admission to the emergency center was used in the current analysis. Additionally, the analysis was stratified by time-block, which represented different groups of

residents working in the intensive care unit. Unlike the earlier analyses, none of the covariates was significantly related to the time to first event. When all the covariates were entered into the model, the treatment effect was significant at $p<0.005$. After removing the covariates in a stepwise fashion, the treatment effect was still significant at $p<0.005$. No significant deviation from the proportional hazards assumption was seen ($p=0.55$). These results indicate that the covariates which influence whether a patient will ever experience a desaturation are not important in determining the timing of the event. Robertson CS, Valadka AB, Hannay HJ, Contant CF, Gopinath SP, Cormio M, Uzura M, Grossman RG. 1999. Prevention of secondary ischemic insults after severe head injury. Crit Care Med 27(10):2086-95.

162. Combination drug therapy and mild hypothermia in a rat model of permanent focal cerebral ischemia

K. Schöller, S. Zausinger, A. Baethmann, R. Schmid-Elsaesser (Munich, D)

Purpose: We have recently demonstrated the superior neuroprotective efficacy of combination therapy with magnesium (calcium- and glutamate-antagonist), tirilazad (antioxidant) and mild hypothermia in a rat model of focal cerebral ischemia and subsequent reperfusion. In the present study we investigated this pathophysiologically orientated treatment strategy under conditions of permanent focal cerebral ischemia.
Methods: 29 Sprague-Dawley rats (250-300g) were intubated and subjected to permanent MCA occlusion by an intraluminal filament. Animals were randomly assigned to one of two groups: (1) vehicle (n=12); or (2) MgCl (2x1mM/kg) and tirilazad (2x3mg/kg) + hypothermia (33°C) (n=17). Drugs were given intravenously 30 min before and one hour after induction of ischemia. Hypothermia was maintained for 2 hours after induction of ischemia. Local cerebral blood flow was measured for 3 hours by continuous laser Doppler flowmetry. Neurological deficits and body weight were assessed daily. On postoperative day 7, brains were perfusion-fixed, HE-stained, and infarct size was planimetrically determined.
Results: High mortality rates within the first 3 days were observed in both vehicle- (41%) and combination-treated groups (47%). Treated animals showed a better neurological recovery on days 6 and 7 ($p<0.05$) and showed a trend towards better postoperative weight gain compared with vehicle-treated controls. There was no significant difference in infarct size between groups.
Conclusion: Combination therapy with MgCl, tirilazad and mild hypothermia seems to exert its neuroprotective properties primarily under conditions of limited ischemia, possibly by ameliorating reperfusion injury. The high mortality rates within the first days suggest that extensive brain swelling occurs after permanent focal cerebral ischemia in this model, which cannot be prevented by this therapeutical approach.

163. The influence of APOE genotype on intracranial contusion size after head injury

L.T. Dunn, I. Liaquat, A.R. Nicoll, G.M. Teasdale (Glasgow, UK)

Background and objectives: Possession of one or more APOE-epsilon 4 alleles has been shown to be associated with worse outcome 6 months after head injury (1). This study assessed whether poseesion of an APOE-epsilon 4 allele influences the size of post-traumatic intracranial contusions.
Subjects: 97 patients admitted to a neurosurgical centre between September 1995 and July 1999 with contusions evident on initial CT scan performed within 72 hours of injury were identified. 28 patients were excluded because an accurate time of injury was not known.
Methods: A computerised volumetric assessment of high and low-density components of the contusions was performed blind to genotype. APOE genotypes were determined by polymerase chain reaction (PCR) amplification and restriction enzyme digestion. Outcome was assessed at 6 months using the Glasgow Outcome Scale.
Results: 17 patients had one or more epsilon 4 alleles- a similar frequency to that found in the non-injured general population. Patients with epsilon 4 alleles had larger high density contusion volumes than those without (median 22.9 cc vs 7.35 cc; Mann Whitney U test $p<0.05$). The epsilon 4 allele patients also had larger low density and total contusion volumes (low density- 30.6 cc vs. 15.05 cc; total 55.3 cc vs. 24.4cc) although these differences just failed to reach significance. The epsilon 4 allele patients had worse outcome at 6 months (Mann Whitney U test $p<0.01$). The age, time from injury to CT scan and severity of injury assessed by admission Glasgow Coma Score was similar in the two groups.
Conclusion: Possession of an APOE epsilon 4 allele is associated with larger intracranial contusions after blunt head injury. This may be one of the factors contributing to poorer outcome after injury in this group.
References: 1. Teasdale et al. (1997) Lancet 350; 1069-1071.

164. Oligodendroglia and oligodendrocyte precursor cells upregulate Kv 1.4 K+ channel gene expression after chronic spinal cord injury

L. Edwards, O.T. Jones, P.H. Backx, C.A. Ackerly, L.E. Becker, M.G. Fehlings (Toronto, CDN)

K+ channel expression is associated with cell proliferation in many types of glial cells in culture. Little is known about the molecular identity of K+ channels in spinal cord glia in vivo and how these cells react to injury. In this study we examined the changes in expression and cellular localization of the K+ channel protein Kv 1.4 with spinal cord injury (SCI). Injury was performed on adult Wistar rats by compressing the spinal cord at T7-T8 with a modified aneurysm clip (closing force of 23g) for 1 minute. Western blotting was used to quantify Kv 1.4 protein expression 1 and 6 weeks after SCI. Localization of Kv 1.4 protein was investigated by immunohistochemistry. Immunoelectron microscopy was used to quantify single cell Kv 1.4 protein expression and in situ hybridization was performed to quantify Kv1.4 mRNA expression after SCI. Western blotting revealed that Kv 1.4 protein as significantly increased in the chronically injured spinal cord compared to controls and 1 week post injury. Kv 1.4 is localized to CNPase positive oligodendrocytes and GFAP positive astrocytes in normal and injured spinal cord white matter. In the gray matter, Kv 1.4 is localized to the peri-nuclear region of neuronal somata. Immunoelectron microscopy showed Kv 1.4 protein levels in oligodendrocytes was increased 6-fold and in situ hybridization showed that Kv 1.4 mRNA was increased by 2-4 fold after SCI. In support of previous findings in culture we found Kv 1.4 was localized to oligodendrocyte precursor cells labeled with NG2 chondroitin sulfate proteoglycan. Furthermore, the number of Kv 1.4 expressing glial cells was significantly increased after SCI. These results suggest that with SCI, there is an upregulation of Kv 1.4 gene and protein expression, which is associated with OPC proliferation. Funding provided by MRC (LE &MGF) and an Ontario Ministry of Health Career Scientist Award (MGF).

165. Resolution of vasogenic brain edema at 48 h after intracerebral hemorrhage

J. Lehmberg, S. Fonk, M. Fürst, A. Baethmann, H.J. Reulen, E. Uhl (Munich, D)

Introduction: Vasogenic edema fluid spreading from acute intracerebral hemorrhage is drained into the subarachnoid space (SAS) and ventricular system (VS). The edema fluid accumulates than to a higher extent in the SAS as compared to VS. In order to study the edema clearance at a later time point chronic experiments were conducted from 48 until 56 h after intracerebral hemorrhage.
Methods: Intracerebral hematoma was induced by injection of 0.5ml (10 ml/h) of autologous blood into the left frontal lobe of anesthetized, spontaneously breathing rabbits (n=10). Animals were allowed to wake up thereafter, but were reanaesthetized and mechanically ventilated 48 h later. Fluorescein (MW 376) was administered continuously i.v. (20mmol/l, 1 ml/kg/h), Texas Red-Albumin (MW 66.000) as bolus (0.18 pmol). A closed cranial window was implanted above the left parietal cortex for superfusion of the brain with artificial cerebrospinal fluid to study resolution of the fluorescence labels via SAS. Clearance into the VS was examined by ventriculo-cisternal perfusion. Effluates were collected every 30 min for 8 hrs following injection of edema markers, their concentrations were measured by spectrophotometry.
Results: Fluorescein appeared simultaneously at 90 min in SAS and VS after onset of i.v. infusion, while clearance of Texas-Red-albumin was not observed. At termination of the experiment (8hrs), fluorescein (mean +/- SD) accumulated at an amount of 1.16 +/- 0.68 pmol in the SAS, while at 2.49 +/- 1.77 pmol in the VS.
Conclusion: The data demonstrate that vasogenic edema spreading from chronic intracerebral hematoma is also drained into the SAS and VS. At 48 to 56 h, in contrast to 0 to 8 h, edema clearance into the VS seems to be more pronounced than into the SAS. As in the acute experiments, the blood-brain barrier was found to be open only for the low molecular weight marker. (Supported by BMBF-Verbund "Neurotrauma" Munich, FKZ: 01K09704.)

Regular Session 23
Special Young Investigator Session

166. Lens-derived crystallins of the beta- and gamma- super family promote axonal regeneration within the adult optic nerve

D. Fischer, S. Thanos (Münster, D)

Traumatically induced injuries to the mature CNS lead to impairments that remain incurable because central nerve cells are unable to regenerate their axons and therefore die. We hypothesise that crystallins, which are phylogenetically conserved lens proteins having specific functions during cellular processes of stress management, are potential candidates for nerve repair. The present study examined whether lens-derived crystallins exert growth-promoting activity in the injured retinal ganglion cell axons of adult rats. Crystallins of the water-soluble fraction of the beta-/gamma-super family were purified by using gel

filtration chromatography. Both fractions were tested for their ability to promote neuritogenesis in three experimental paradigms. In vitro, in the organotypic retinal cultures, in the grafting paradigm wherein the cut optic nerve was replaced with a sciatic nerve segment, and in vivo on suturing the optic nerve back after it had been cut. Both beta- and gamma-crystallins were able to dramatically enhance the number of regenerating axons, and to increase the velocity of growth cone movement by a factor 2,3. In the second paradigm of replacing the optic nerve in vivo, significantly more ganglion cells regenerated their axons within a peripheral nerve graft when purified beta- and gamma-crystallins were injected intravitreally. In the third paradigm of regeneration in situ, axons of ganglion cells, that had been cut, grew within the distal optic nerve stump over distances of more than 4.5 mm within 2 weeks and reached the optic chiasm after 4 weeks. The results show that the lens crystallins constitute a novel class of potent neuritogenic factors with potential therapeutic relevance in human neurodegenerative diseases. Second, they are the first externally applicable small proteins, which allow cut axons to override the inhibitory environment of the CNS and grow within the white matter over several millimetres.

167. Spatio-temporal induction of in situ gelatinase activity and matrix metalloproteinases in response to spinal cord injury: A potential target for neuroprotection and axonal regrowth

Y. Duchossoy, S. Arnaud, S. Feldblum (Paris, F)

Mediators of extracellular matrix proteins degradation, the matrix metalloproteinases (MMPs), involved in inflammation as well as in facilitation of process outgrowth by oligodendrocytes are interesting targets for neuroprotection and axonal regrowth. Recent data reported their activation after seizures, cerebral ischemia and spinal cord injury (SCI). The present study was designed to correlate at cellular level the gelatinase activity, by in situ zymography, with the immunodetection of MMP-2 (gelatinase A) and MMP-9 (gelatinase B), in a rat spinal cord closed contusion model, sharing homologies with human SCI. To establish the profile of gelatinase activation, animals were sacrificed, early after injury (1h, 4h, 1d, 2d) and at later time (7d, 15d, 30d and 60d). A sensitive method of in situ zymography was developped to localize the gelatinolytic activity in 16 mm cryostat sections. The spinal sections were incubated with fluorescein-quenched DQ gelatine (50 mg/ml) for 12h. The gelatin digestion yielded cleaved fluorescent peptides enabling the detection of gelatinolytic activity at cellular level. In parallel the spatio-temporal expression of MMP-2 and MMP-9 was assessed. A strong gelatinase activity was detected at the lesion site in infiltrated cells, vascular structures, neurons and in tissue undergoing necrosis. This activity occurs within the first week post-injury and remained visible up to one month. A significant reduction of gelatinase activity in presence of MMPs inhibitors, along with a strong immunodetection of MMP-2 and MMP-9 at the site of injury suggested their contribution to the gelatinase activity. MMP-9 detected specifically in lesioned spinal cord was visible as early as 4h and up to 1 month post SCI in neurons and glial cells (oligodendrocytes and astrocytes). The present data suggest the involvement of MMPs after SCI in the clearance of debris, as well as in the structural reorganisation of the injured spinal cord. (Supported by NEUROLAB.)

168. Fas/Fas ligand-mediated apoptosis affects the neurologic insult after acute experimental spinal cord contusion in mice

O. Yoshino, H. Matsuno, H. Nakamura, Y. Abe, T. Kimura (Toyama, J)

Apoptosis is a form of programmed cell death seen in a variety of developmental and disease states. Recently, the presence of apoptosis in spinal cord injury following a contusion has been reported in rats and monkeys. Fas, which is a well-known apoptosis-associated cell surface molecule, is detected in the central nervous system. The main objective of this study is to determine whether Fas-Fas ligand interactions participate in apoptosis after spinal cord injury. We examined the differences in functional deficits and histopathological changes, including terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick end labeling (TUNEL) and immunohistochemistry for Fas and Fas ligand identification, between lpr mice which had a mutation in the Fas gene and wild-type mice after spinal cord contusion using a newly developed spinal cord impactor. Locomotor recovery was similar in two groups until 2 weeks after contusion, and was greater in lpr mice than in wild type 3 weeks later after contusion. The number of TUNEL-positive cells was larger in wild type than in lpr mice. Immunohistochemical study showed Fas-positive cells and Fas ligand-positive cells in spinal cord after contusion. The results of our experiments showed that Fas-Fas ligand interactions participated in apoptosis after spinal cord injury.

169. Intrathecal elevation of interleukin-18 following traumatic brain injury – a clinical and experimental study

P.F. Stahel, D. Perez, V.I. Otto, O. Trentz, M.C. Morganti-Kossmann, T. Kossmann (Zurich, CH)

Interleukin (IL)-18 is a novel cytokine with potent pro-inflammatory properties, such as induction of Th1-cytokine production and regulation of cell-mediated immune responses. IL-18 is functionally closely related to IL-12, a cytokine which we have previously shown to be elevated in the cerebrospinal fluid (CSF) of patients with severe head trauma (Stahel et al., Neurosci. Lett. 1998, 249:123-6). In order to further evaluate the pro-inflammatory mediators contributing to the intracranial inflammation following traumatic brain injury (TBI), we determined the concentrations of IL-18 by ELISA in ventricular CSF and serum of TBI patients (n=10) and in brain homogenates from mice subjected to experimental closed head injury (n=24). IL-18 levels were significantly elevated in the CSF compared to matched serum samples in 80% of the patients for up to 14 days after trauma ($P<0.05$). In some patients, the daily IL-18 CSF concentrations exceeded the corresponding serum levels by more than 15-fold, with peak values of 391 pg/ml. In brain-injured mice of the C57BL/6 strain, elevated IL-18 concentrations were detected in brain homogenates at t=4h (4.21 ± 0.43 ng/mg protein; mean ± SD), 24h (3.73 ± 1.57 ng/mg protein), and 7 days after trauma (5.29 ± 1.05 ng/mg protein), compared to sham-operated animals (2.98 ± 0.62 ng/mg protein). Interestingly, preliminary data demonstrate a significant attenuation of intracerebral IL-18 levels (0.39 ± 0.25 ng/mg protein) within 24h after intrathecal injection of 200 ng murine recombinant TNF. Altogether, these data demonstrate for the first time elevated IL-18 levels in the intrathecal compartment after severe TBI, both in human and experimental studies. Furthermore, TNF seems to represent an important suppressor of intracerebral IL-18, acting by mechanisms which have not been elucidated yet.

170. Evidence of cerebral ischemia following traumatic brain injury

J.P. Coles, T.D. Fryer, E.J. Williams, D.A. Parry, J.C. Matthews, T. Donovan, D. Day, F. Aigbrhio, J.C. Clark, J.D. Pickard, D.K. Menon (Cambridge, UK)

We have previously used positron emission tomography (PET) to show that cerebral blood flow (CBF) reductions following head injury (GCS<12) represent ischaemia, rather than coupled hypoperfusion due to hypometabolism (1). This abstract compares our results to arteriojugular blood monitoring techniques. Eleven patients were studied within 24 hours of severe (n=6) and moderate (n=5) closed head injury, with imaging of CBF and oxygen extraction fraction (OEF). Voxels on calculated brain images were binned with respect to their OEF. One subject was excluded from analysis due to the presence of a possible arteriovenous fistula. Ischaemic brain volume (IBV; calculated as the sum of voxels in bins with OEF>75%) was compared with global measures of cerebral oxygenation, including jugular bulb oximetry (SjvO2), arteriojugular oxygen content differences (AJDO2), arteriojugular oxygen saturation differences (AJDSO2 : cerebral extraction of oxygen), lactate oxygen index (LOI) and the modified LOI. Compared to control data there was an increase in OEF, with an IBV of 8±7% (mean ± SD). However, there was marked heterogeneity in the volume of ischaemic brain (range 1-20%). While one subject with an IBV of 20% displayed an SjO2 of 49% and an AJDSO2 of 51%, in general, SjO2, AJDO2, AJDSO2, LOI and mLOI did not reach ischaemic thresholds (mean values 62+8%, 5+1 ml/dl, 36±9%, 0.03±0.03 and 0.03±0.02 respectively). SjO2 and AJDSO2 values correlated with IBV ($r2=0.644$, $p<0.0001$ and $r2=0.772$, $p=0.0001$) Early cerebral ischaemia is a variable but significant problem following both moderate and severe closed head injury. Commonly used thresholds for global ischaemia in head injury may miss regional ischaemia.

References: 1. Coles JP et al., J Neurosurg Anesth 2000 (In Press).

171. Secondary growth of a traumatic brain lesion in mice

R. Sircar, J. Eriskat, H. Gumprecht, A. Baethmann (Munich, D)

Introduction: As previously observed, a focal brain tissue necrosis induced by trauma is expanding within 24 h up to 300% in rabbits while to 150% in rats. In order to study underlying mechanisms in mice with selective deletions of specific genes (knock- out), the model of a highly standardized focal brain lesion was adapted for mice for analysis of the secondary growth of necrosis.

Materials and methods: Three groups of male C57/BL6 mice (25-30g) were subjected to halothane/ N2O anesthesia. To ensure stable systemic conditions, one group (n=8) remained in anesthesia for 3h with monitoring of the arterial blood pressure and blood gases via a catheter implanted in the carotid artery. Animals of the other two groups (n=8) were subjected to trephination of the skull without damaging the underlying dura. A highly standardized focal lesion of the right parietal cortex was induced by cold injury. One group of animals (baseline) was sacrificed 10 min after trauma, the other animals were allowed to wake up and were reanesthetized 24h later. For histology animals were transcardially perfused with 4% paraformaldehyde and the brain was removed. The NISSL-stained serial sections were digitized, and the area of necrosis was planimetrically measured for calculation of the necrosis volume. Data are given as mean ± SEM.

Results: Animals of the long term anesthesia group had a MAP of 80± 10 mmHg throughout; the arterial blood gases were also in a physiological range. At 10 min after trauma, animals had a volume of necrosis of 1.30± 0.12 mm3,the maximal lesion area was 1.47± 0.13 mm2. At 24h, the volume of necrosis had expanded to 2.01± 0.23 mm3, the maximal lesion area to 1.95± 0.20 mm2, respectively ($p < 0.05$).
Conclusion: The current lesion model adapted to mouse brain allows a highly reproducible induction of a traumatic necrosis under maintenance of physiological systemic conditions. As seen, the volume of necrosis was expanding by more than 50% within 24 hours. The reproducibility of the model makes possible to explore mechanisms of secondary growth of the brain lesion with consideration of underlying moleculargenetic mechanisms. (Supported by BMBF- Verbund Neurotrauma, München, FKZ 9030911.)

Plenary Session 7
Novel therapeutic strategies

172. Novel therapeutical strategies in traumatic brain injury (Invited lecture)
R. Bullock (Richmond, USA)

173. Novel therapeutic strategies in spinal cord injury (Invited lecture)
W. Young (Piscataway, USA)

The decade of the 1990's overturned several well-established dogmas in spinal cord injury. Long considered to be irreversible by clinicians and scientists, spinal cord injury was viewed with great pessimism at the beginning of the decade. During the decade, five classes of therapies were shown to improve neurological and functional recovery in spinal cord injury:
1. Neuroprotective therapies. In 1990, the National Acute Spinal Cord Injury Study (NASCIS) reported that high-dose methylprednisolone improved neurologic recovery when given within 8 hours after injury. The first neuroprotective therapy shown to be effective in humans, the discovery established the concept of progressive secondary tissue damage and the need for find better neuroprotective therapies for central nervous system injuries.
2. Reparative therapies. Several recent studies, however, suggest that the inflammatory response in the injured spinal cord may serve reparative functions. For example, activated macrophages and lymphocytes may improve recovery when transplanted into the spinal cord. Recent studies indicate beneficial effects of inoculating animals to stimulate antibody production before spinal cord injury.
3. Restorative therapies. Many therapies improve function of surviving axons at the injury site. These include 4-aminopyridine which improve conduction and synaptic efficacy of emyelinated axons. Several cell transplants have been shown to remyelinate spinal cord axons, including Schwann cells, oligodendroglial precursor cells (O2A), and neural stem cells.
4. Regeneration therapies. Several laboratory studies showed that treatments can induce regeneration in the spinal cord. These include the neurite growth inhibitor Nogo antibody (IN-1), peripheral nerve bridges, olfactory ensheathing glia (OEG), application of neurotrophins (NGF, BDNF, and NT-3) directly or through genetically modified cells, and even therapeutic inoculation of animals to stimulate endogenous antibodies that promote remyelination (M1) and regeneration.
5. Rehabilitation therapies. Recent studies suggest that neurological deficits in spinal cord injury may result from "learned non-use" and that intensive "forced-use" rehabilitation paradigms can reverse such deficits. Weight-supported ambulation training, functional electrical stimulation, biofeedback training, and exercise therapies can produce surprising functional recovery in people who have been paralyzed for many years.
The challenge that faces us in the coming decade is optimization and combination of these therapies for clinical trial. The number and variety of therapeutic approaches will require innovative therapy validation approaches. We have powerful new tools to diagnose, study, and manipulate the response of the spinal cord to injury and for recovery of specific function. These include high-resolution imaging of the spinal cord and gene chips to do large-scale surveys of gene expression associated with the injury response, repair, remyelination, pain, spasticity, and regeneration. We can modify cellular response to injury with ex vivo and in vivo gene therapy to stimulate expression of cytokines, cellular adhesion and guidance molecules. We can use therapeutic vaccines to stimulate desired humoral and cellular immune responses to injury. Even neuronal replacement therapy may be possible through both human or animal stem cell transplants. It is thus a brave new world.

Poster Session 1

Cell injury

P174. Local cyanide perfusion decreases cerebral oxygen consumption and increases brain tissue oxygen tension in a feline model

T. Clausen, J. E. Levasseur, A. Zauner, A. C. Rice, R. Bullock (Richmond, USA)

Recent studies indicate that mitochondrial failure may play an important role in the pathogenesis of brain damage following severe traumatic brain injury (TBI). However it is not clear yet how mitochondrial dysfunction affects cerebral oxygen metabolism. In order to evaluate the impact of "isolated" mitochondrial failure on local cerebral oxygen tension and oxygen consumption we blocked oxidative mitochondrial metabolism using local perfusion with cyanide solution via a microdialysis probe in different concentrations in seven cats (10 – 100 mmol/l). Local brain tissue oxygen tension (ptiO2) was monitored with a Neurotrend sensor system. In addition, brain tissue samples were taken at defined time points and cerebral tissue oxygen consumption was measured in a "Cartesian diver" microrespirometric system. Brain tissue oxygen tension increased significantly from 31 ±9 mmHg (baseline) to 84 ±30 mmHg after 60 min cyanide perfusion ($p<0.05$). Cerebral tissue oxygen consumption decreased significantly from 14.45 ±3.91 ml/h/mg to 12.11 ±2.67 ml/h/mg for the same period ($p<0.05$), and remained low after discontinuation of cyanide perfusion: 10.83 ±1.74 ml/h/mg at 40 minutes after stopping the cyanide perfusion. These results show that mitochondrial failure decreases cerebral oxygen consumption and that brain tissue oxygen tension concomitantly increases during normal oxygen delivery. The study furthermore showed the ability of the Neurotrend system to detect changes in ptiO2, even restricted to small tissue volumes, consistently. More specific studies of mitochondrial function after TBI may provide more insight into the mechanisms that ultimately lead to secondary injury after human TBI.

P175. Early astrocyte loss precedes neuronal degeneration after traumatic brain injury

B. Lyeth, X. Zhao, T. Hallam, P. Muizelaar, R. Berman (Davis, USA)

Neuronal-glial interactions contribute to normal brain function and provide important maintenance of the brain's extracellular environment. Damage to glial cells following traumatic brain injury (TBI) could therefore be an important contributing factor to neuronal injury. We examined the early fate of astrocytes and neurons after TBI in rats. Sixteen rats were euthanized at 1, 2, 4, or 8 hrs after moderate, 2.2 atmosphere lateral fluid percussion TBI. Following transcardial perfusion with 4% paraformaldehyde, brains were sectioned (50 microns) and ipsilateral and contralateral hippocampal and parietal cortex were examined in coronal sections from -2.3 to -4.2 mm relative to bregma. Adjacent sections were processed with markers for either astrocytes or neuronal degeneration. Astrocytes were visualized using glial fibrillary acidic protein (GFAP) immunohistochemistry; neuronal degeneration was visualized using Fluoro-Jade (FJ) histofluorescence. At 1 hr, there was a moderate loss of GFAP immunoreactivity in ipsilateral hippocampal CA3 and parietal cortex with some loss of astrocyte structural integrity of remaining cells. The number of healthy astrocytes decreased progressively over time with extensive astrocyte loss at 8 hrs after TBI. At 1 hr, lightly stained FJ-positive neurons were scattered in the ipsilateral CA3 and parietal cortex. The intensity and number of FJ-positive neurons progressively increased over time with moderate numbers of degenerating neurons evident in the ipsilateral hippocampal CA3 and parietal cortex at 8 hrs after TBI. We conclude that astrocyte loss precedes early neuronal degeneration inthe hippocampus and cortex after TBI. The data suggest that loss of supporting glial cell may contribute to subsequent neuronal degeneration. (Supported by NIH 29995.)

P176. Slowly progressing and widespread mitochondrial dysfunction after lateral fluid percussion injury in rats

T. Kuroiwa, X. Jiang, L. Duan, M. Aoyagi, K. Hirakawa, K. Ohno (Bunkyo-ku, Tokyo, J)

We investigated distribution and time course of mitochondrial dysfunction after traumatic brain injury (TBI). Lateral fluid percussion injury with low, moderate or high pressure was induced in rats, time course and regional change in the activity of succinic dehydrogenase (SDH) – a mitochondrial enzyme of tricarboxylic acid cycle, was examined and compared to the histological outcome. SDH activity (% of control) decreased slowly over 72 h of post-trauma to 51±9 and 78±18 in the ipsilateral parietal and occipital cortex, 82±6 and 79±8 in the ipsilateral hippocampal CA1 and CA2-3 sectors, respectively. The area of reduced SDH activity gradually extended to the whole ipsilateral cortex and hippocampus. The speed of reduction paralleled to the severity of impact. Neuronal death was evident in the ipsilateral parietal cortex and hippocampal CA2-3 sector at 3 d and 2 w post-trauma, respectively. Present study revealed slowly progressing and widespread mitochondrial dysfunction after TBI. The speed of dysfunction was dependent on the severity of impact. Neuronal death occurred in the regions evolving severe mitochondrial

dysfunction. The observed slowly progressing mitocondrial dysfunction is probably an important process leading to post-traumatic neuronal death.

P177. White matter damage following systemic injection of the mitochondrial inhibitor 3-nitropropionic acid

E. McCracken, D. Dewar, J. Hunter (Glasgow, UK)

To determine whether oxidative stress is a major pathogenic mediator involved in axonal cytoskeletal breakdown following brain injury we systemically injected the mitochondrial inhibitor 3-nitropropionic acid (3-NPA), a known generator of free radicals, into male adult rats. Animals received an intraperitoneal injection of 10, 15, 20 or 30mg/kg 3-NPA or vehicle and were transcardially perfused with 4% paraformaldehyde 24 hours later. Tissue sections were stained for histology and immunostained for axonally transport proteins, amyloid precursor protein (APP) and SNAP-25. Adjacent sections were also stained with an antibody raised against manganese-superoxide dismutase (Mn-SOD) to determine the presence of oxidative stress in this model. Accumulation of either APP or SNAP-25 was detected after 3-NPA injection within the myelinated fibre tracts of the striatum. Semi-quantitative analysis of stained sections using a scoring method demonstrated that in the vehicle treated group there was no immunoreactivity present in axons. There was a concentration dependent increase in the amount and distribution of axonal damage reflected by APP and SNAP-25 accumulation in the 3-NPA treated animals. Axonal pathology was anatomically coincident with the neuronal lesion. All animals in the study had a basal level of Mn-SOD in the external capsule and striatum, however a concentration dependent increase in Mn-SOD immunoreactivity was evident within the striatum of 3-NPA treated rats. The data suggests that the oxidative stress cascade may play a pivotal role in the breakdown of the axonal cytoskeleton following brain injury.

P178. Cellular response in hydrocephalus resembles brain damage after neurotrauma

P.M. Klinge, A. Mühlendyck, W. Luedemann, S. Groos, M. Samii, T. Brinker (Hannover, D)

To study regional and temporal profile of glial and neuronal reaction to induction of kaolin hydrocephalus in adult rats. 20 (5 controls) adult rats were immunohistologically (kryosections) investigated 2, 4, 6 and 8 weeks after injection of 0.01 ml kaolin into the cisterna magna and kryosections were performed. Enzymatic changes were analysed by glutamatergic transmission activity (GLDH) in astrocytes and immunohistochemistry included nitrous oxide synthetic activity (nNOS) in neurones, glial fibrillary acidic protein (GFAP), 68 kd neurofilament protein (NF68), synaptophysin (SYN 38) and bFGF. Staining results were quantified by computed imaging analysis. In the acute phase, after 2 weeks global increases in astrocytic GLDH-activity were observed with highest increases in the CA2 section (30.7±7.4 vs. 15.3±4.2 density; $p<0.01$) parallel to an increase of nNos-activity in cortical and hippocampal pyramidal neurones (CA1 and CA3). In CA1, staining of neurofilament 86 (4.5±0.2 vs. 6.5±0.13%; $p<0.01$) and synaptophysin (0.03±0.01 vs. 0.1±0.05%; $p<0.05$) was decreased. In the chronic phase sustained structural changes occurred: GFAP immunoreactivity steadily decreased in overall hippocampal (4.3±1.0 vs. 5.9±1.5%; $p<0.05$) and cortical astrocytes (0.15±0.03 vs. 0.5±0.1%; $p<0.01$). NF68 immunoreactivity in cortical pyramidal neurones increased (7.5±0.9% vs. 6.1±1.1%; $p<0.01$) indicating neurofilament degradation paralleled by compensatory increases of synaptophysin in CA3/CA4 (0.35±0.01% vs. 0.12±0.02%; $p<0.05$). The onset of bFGF-activity in cortical neurones indicated severe neuronal injury. The observed temporal and regional profile in the hydrocephalic condition with initial global toxic cellular reactions and chronic neuronal damage that was most pronounced in selective vulnerable areas resemble mechanisms of secondary brain damage as found in traumatic brain injury.

P179. Cyclosporin A prevents N-acetylaspartate reduction and improves energetic metabolism following diffuse experimental traumatic brain injury

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Introduction: N-acetylaspartate (NAA), a well-established biochemical marker of neuronal damage, is synthesized by the mitochondria and its reduction, following traumatic brain injury (TBI), has been related to cellular energetic impairment, secondary to mitochondrial dysfunction. The objective of this study was to demonstrate that following diffuse TBI, CsA prevents early neuronal biochemical damage and improves mitochondrial energetic metabolism.
Methods: Sprague-Dawley rats.were randomly divided into the following experimental groups: sham (n=8), injured vehicle-treated (n=6) and injured drug-treated (0.15 cc, intrathecal administration of 25mg/ml CsA, 30 minutes post-injury; n=6). Quantitative HPLC analysis of whole brain samples was studied 6 hours after Impact Acceleration injury for levels of NAA and ATP. Results Following trauma, in vehicle-treated group, NAA was reduced by 32% compared to sham ($p< 0.0001$); concurrently ATP showed a 44%

reduction (p< 0.0001). In the CsA-treated group was significantly elevated compared to trauma controls. (p <0.0001). ATP levels were also significantly elevated, showing a 30% increase with respect to the trauma vehicle-treated group (p<0.005).
Conclusion: Early post-injury, intrathecal administration of Cyclosporin A is an effective therapy for blunting post-traumatic NAA reduction and, most importantly, promoted significant improvement of energetic metabolism, preserving ATP. These findings, coupled with published effects of CsA (1,2) contribute to the notion that CsA achieves neuroprotection via the preservation of mitochondrial integrity.
References: 1. Sullivan PG, Thompson MB and Scheff SW. Cyclosporin A attenuates acute mitochondrial dysfunction following traumatic brain injury. Exp Neurol: 160, 226-34, 1999. 2. Okonkwo DO, Povlishock JT. An intrathecal bolus of cyclosporin A before injury preserves mitochondrial integrity and attenuates axonal disruption in traumatic brain injury. J Cereb Blood Flow Metab: 19, 443-51, 1999.

P180. Regional changes of glutamate metabolism as monitored by glutamate dehydrogenase activity following focal brain injury

M.U. Schuhmann, M. Scardelly, W. Lüdemann, P. Klinge, S. Thomas, M. Samii, T. Brinker (Hannover, D)

We investigated the regional differences and the time course of glutamate metabolism by means of enzyme histochemistry following controlled cortical impact injury (CCII) in rats. 66 Sprague Dawley rats (250-350g) were investigated at 10min, 1h, 4h, 24h, and 1 week after injury. CCII (impactor Æ5mm, velocity 4m/s, 2.5mm depth) was applied to 40 rats, 26 underwent Sham operation. Glutamate dehydrogenase (Gldh) was stained in frozen sections cutting through the centre of contusion. Areas investigated bilaterally were hippocampal CA1 & CA2/3 sections, cortex adjacent to contusion (Cort), internal capsule (IC) and thalamus (Thal). Gldh activity was quantified by determining percentage of stained area within field of view. Differences were defined if p>0.05 in nonparametric test. In Sham we saw a sign. bilateral mean activity increase of 280% with peak at 1h and return to 10min levels within 1 week in CA1, CA2/3 and Cort. Following CCII changes in CA1 & CA2/3 were similar ipsi- and contra-laterally to lesion. A constant activity increase with peaks of 265-430% at 24h was seen. Differences to Sham existed at 4h, 24h & 1w. Activity increased in cortex ipsi- by 824% and contra-lat. by 590% with peak at 24h returning at 1w. Differences to Sham existed at 4h & 24h ipsi- and 24h contralat. In Thal and IC no changes over time & vs. Sham were seen. The glutamate generating enzyme Gldh is predominantly localised in astrocytes, which supply neurons with glutamate. After CCII a significant, bilaterally similar, activity increase of Gldh is seen in the hippocampus. In cortex the increase is sign. more prominent on the injured side. The time course of enzyme activity is fundamentally different to that of extracellular glutamate concentrations as determined by microdialysis or total glutamate determined by MR spectroscopy. It parallels however the time course of oedema formation and cerebral swelling.

P181. Quantitative non-invasive metabolic monitoring using proton magnetic resonance spectroscopy after focal brain injury

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We quantified & monitored brain metabolites over 4 weeks following controlled cortical impact injury (CCII) using volume-selective proton magnetic resonance spectroscopy (1H-MRS). 20 Sprague Dawley rats were investigated at 1h, 4h, 24h, 1 week & 4 weeks after injury. CCII (4m/s, 2.5mm depth) was applied to 14 rats, 6 underwent Sham operation. Using a 4.7 T Bruker Biospec coronal T2-weighted RARE_8 images were acquired. A 3mm3 voxel for 1H-MRS (PRESS) was placed below the contusion and analogously on the contralateral side, covering hippocampus/basal ganglia. 122metabolite spectra were analysed for Inositol (Ino), Cholin (Cho), Creatine + Phosphocreatine (Cr), Glutamate/Glutamine (Glx), N-Acetylaspartat (NAA), and Lactate (Lac). Peaks were quantified according to Provencher's method. p>0.05 in nonparametric test defined differences. Ipsilateral to injury Cho, Ino and Lac peaked with a max. increase (vs. 1h) of 40%, 15% and 137% respectively at 1 week. Changes were significant vs. Sham and the contralateral side. For Glx an 20% increase was detected at 1h vs. the contralateral side, however not vs. Sham. NAA was depressed by max 23% at 24h, returned to normal at 1w and was decreased by 16% at 4w. Changes were significant vs. Sham and contralateral side. Lac was only detected on the injured side. In conclusion 1H-MRS quantified changes of energy, membrane and transmitter metabolism in the area below brain contusion. Except for Glx these changes were long-lasting and had not resolved at 4w. The persisting lactate increase seemed unrelated to hypoxia and might indicate long-lasting derangement of energy metabolism. NAA showed an early and late depression. Further study is necessary to elucidate the exact role of metabolites like Ino, Cho and NAA.

P182. Effect of hypoxia on traumatic brain injury in the rat

Y. Matsushita (Tokorozawa, J)

Our previous work showed that hippocampal CA3 damage following traumatic brain injury (TBI) with hypoxia in the rat. The present study was designed to determine the effects of posttraumatic hypoxia on extracellular glutamate (Glu) and histopathological consequences. Sprague-Dawley rats were subjected to moderate lateral fluid-percussion brain injury. The animals were divided into four groups: TBI alone, TBI followed by hypoxia, hypoxia alone, and sham. Extracellular Glu was monitored in real-time using an enzyme electrode biosensor. Seventy-two hours after the insult, coronal brain sections were used for TTC staining. Immunostaining for caspase-3 and TUNEL was also done at 1, 6, 24 and 72 hrs post-insult. When hypoxia was combined with TBI, prolonged efflux of Glu and significantly larger contusion volume were observed. Immunohistochemical analysis demonstrated an increased number of both the caspase-3- and TUNEL-positive cells at 72 hours post-insult. These results suggest that TBI with hypoxia induced the prolonged efflux of Glu, which resulted in more cortical damage due to necrosis and apoptosis.

P183. Influence of oxidative stress on glial cell volume control in vitro

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Introduction: Oxidative stress is a significant factor of cell death from traumatic or ischemic brain injury. Besides, induction of cell swelling with breakdown of membrane permeability might be considered in addition. Objective of the present experiments was to assess the cell volume response of suspended glial cells during exposure to reactive oxygen species.
Materials and methods: C6 glioma cells were harvested from culture and suspended in a temperature-, pH- and pO2- controlled incubation chamber. The cell volume was measured by flow cytometry. After a control period, oxidative stress was induced by addition of H_2O_2 at different concentrations (0.1, 1.0, 5.0 mM). The cell volume was measured before and during 120 min of H_2O_2 exposure.
Results: Under control conditions and upon administration of 0.1 mM H_2O_2, cell volume remained unchanged, while it was decreasing to 94.8 ± 1.3 % of control ($p<0.05$) following exposure to 1.0 mM H_2O_2. Subsequently, however, the cell volume was recovering to the normal level and expanding thereafter. Initial shrinking of cells was also observed upon administration of 5.0 mM H_2O_2 which, however, was followed by a more pronounced cell swelling response, finally reaching 105.1 ± 1.7 % baseline ($p<0.05$).
Discussion: The present findings provide novel information at the quantitative level of how oxidative stress (exposure to H_2O_2) affects maintenance of the volume of in vitro suspended glial cells. Unexpectedly, cell volume was initially shrinking upon administration of H_2O_2, which was followed by a moderate swelling response, notwithstanding that rather high dose levels were studied. The findings may, thus, question the role of ROS in the formation of cytotoxic cell swelling. It can not be excluded, however, that ROS in combination with other cytotoxic agents, e.g. glutamate or arachidonic acid is involved in cytotoxic brain edema. (Supported by faculty program ´Förderung von Forschung und Lehre` of the University of Munich.)

Poster Session 2

Neurotrauma models 1

P185. Impaired motor learning and diffuse axonal damage in motor and visual systems of the rat following traumatic brain injury

Y. Ding, B. Yao, Q. Lai, J.P. McAllister, F.G. Diaz (Detroit, USA)

Cognitive-motor functioning or motor skill learning is impaired in humans following traumatic brain injury. An understanding of the mechanisms involved in disorders of motor skill learning is essential for any effective rehabilitation. The specific goals of this study were to examine motor learning disorders, and their relationship to pathological changes in adult rats with mild to moderate closed head injury induced by impact acceleration (weight drop). Motor learning deficits were determined by comparing the ability to complete a series of complex motor learning tasks (parallel bar traversing, foot placing, ladder climbing, and rope climbing) with simple motor activity (determined by runway traversing and beam balance). The extent of neuronal damage was determined using silver impregnation (FD NeuroSilverTM Kit I). At all post-injury time points (days 1 to 14), statistically significant deficits were observed in all motor learning tasks. Performance improved with time, but never reached control levels. In contrast, no deficits were found in simple motor activity. Histologically, axonal degeneration was widely distributed in several brain areas that relate to motor learning, including the white matter of sensorimotor cortex, corpus callosum, striatum, thalamus and cerebellum. Additionally, severely damaged axons were observed in the primary visual pathway, including the optic chiasm, optic tract, lateral geniculate nuclei, and superior

colliculus. This study has suggested that the impaired motor learning could be caused by mild to moderate brain injury in the experimental animal model; and this behavorial deficit could be attributed to a diffuse axonal injury distributed both in the motor and the visual systems.

P186. Cerebral metabolism, acid-base homeostasis, and oxygenation after traumatic brain injury and secondary hypoxic injury in a feline model

A. Zauner, T. Clausen, A. C. Rice, J. E. Levasseur, H. F. Young, R. Bullock (Richmond, USA)

In several neuroscience centers clinical microdialysis and continuous brain tissue oxygen, carbon dioxide and pH monitoring are performed. The interpretation of the data thus obtained is difficult, since the relationship between primary and secondary insults and the severity of injury and the effects on metabolites and oxygen tension are not known. The aim of this study was to simultaneously evaluate early changes of cerebral metabolism, acid-base homeostasis and oxygenation and their relationship after traumatic brain injury (TBI) and secondary injury in a larger, gyrencephalic brain. A fluid percussion injury (FPI; mean pressure: 2.5 atm) was performed in 9 cats. Cerebral extracellular fluid (ECF) concentration of glucose, lactate, pyruvate, glutamate and glycerol, as well as tissue pH, oxygen tension (ptiO2) and carbon dioxide tension (ptiCO2) were measured simultaneously using microdialysis and the Neurotrend system. To test the effect of secondary hypoxic injury on these parameters, the cats were then ventilated with 10% oxygen for one hour, starting two hours after the initial injury. One hour after FPI, ptiO2 had decreased from baseline (33 ± 4 mmHg) to 5 ± 3 mmHg, ptiCO2 had increased from 55 ± 2 mmHg (baseline) to 80 ± 8 mmHg post-TBI, and cerebral pH had fallen from 7.10 ± 0.06 (baseline) to 6.85 ± 0.10 after TBI (all, $p<0.05$). During hypoxia, ptiO2 and pH decreased further, to 0 mmHg and 6.56 ± 0.14 after 40 min of hypoxia, respectively ($p<0.05$), while ptiCO2 remained high at 83 ± 18 mmHg. ECF lactate significantly increased from baseline (513 ± 69 mM) to 804 ± 72 mM, 30 min after FPI ($p<0.05$). Secondary hypoxic injury caused a further large increase in cerebral lactate to 3219 ± 650 mM after 40 min of hypoxia ($p<0.05$). ECF glycerol increased as well, from 35 ± 9 mM to 53 ± 7 mM at 30 min after FPI ($p<0.05$), and further rose to 167 ± 104 mM after 40 min of hypoxia ($p<0.05$). However, hypoxia did not cause a decrease in ECF pyruvate concentrations. In this study, cerebral metabolism, oxygen supply, and acid base balance were severely compromised, ultra-early after TBI, and they declined further if hypoxia was present. The complexity of the pathophysiological changes and their interactions after human TBI suggests that in most patients early monitoring reveals the metabolic effects of prior TBI, exacerbated by hypoxic events.

P187. Transient cortical decrease of glutamate uptake protein GLT-1 in iron induced epilepsy

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Posttraumatic late epilepsy (PTE) develops in approximately 5 % of patients with traumatic brain injury (TBI). Several risk-factors have been identified for the development of PTE, but the mechanisms are to a large extent unknown. Seizures are associated with elevated extracellular levels of glutamate in patients as well as in animal models, presumably due to increased synaptic release. Increased extracellular glutamate levels are also observed in the brain following TBI but the cause of this is less clear. The extracellular homeostasis of glutamate is normally maintained by efficient uptake of glutamate into astrocytes through specific transport proteins. A disturbed glutamate uptake could contribute to the high levels of glutamate after TBI and during seizures. In this pilot-study we used quantitative immuno-blotting techniques to measure the cortical levels of astrocytic glutamate uptake proteins, GLT-1 and GLAST, in a post-traumatic epilepsy model induced by ferrous chloride injection in the cortex of rats. The levels of GLT-1 were significantly lower in epileptic rats than in controls, day 1 and 5 after induction, but not at 3 months. GFAP levels increased with time in the epileptic model, indicating reactive gliosis. The levels of GLAST and the neurone specific protein beta-tubulin III remained unchanged compared to controls. EEG at 3 months showed epileptiform activity in the animals that received ferrous chloride, thus validating the model. The transient decrease of GLT-1 could play a role in the epileptogenesis in the studied model and the increasing gliosis might be another factor in the development of chronic epilepsy.

P188. Recovery experiments of IL- 1 beta and IL- 6 by means of an in vitro microdialysis-perfusion system

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Introduction: Cells involved in the inflammatory responses are regulated by the actions of cytokines. Interleukin- 1 beta (IL- 1 beta) and IL- 6 are thought to play a pivotal role in regulating inflammatory responses in traumatic brain injury (TBI). Recently, intracerebral microdialysis has become available to monitor physiological and pathophysiological changes in chemical processes occurring in (human) brain.

Until now, no published data are available in detecting cytokines by means of microdialysis. Initially, we performed recovery experiments of cytokines in vitro to ascertain extraction efficiency at different flow rates and to avoid pitfalls in executing in vivo experiments in the future. The objective of our study was to investigate the membrane-specific features in measuring cytokines in an in vitro microdialysis-perfusion system.
Methods: Recovery experiments for cytokines across a polyether sulfon microdialysis probe were performed in a medium consisting of artificial cerebrospinal fluid and IL- 1 beta and IL- 6 in concentrations of 10 ng/ml and 50 ng/ml respectively. Two different flow rates of 0.5 microl/min and 2.0 microl/min were used. Dialysate samples of 100 microliter were collected in 150 microliter High Performance ELISA (HPE) –buffer solution, and stored until assay at –20 C. Possible wash out effects were measured after transfering the microdialysis probe to a sterile medium. Dialysates were assayed for cytokine content using enzyme-linked immunosorbent-assay (ELISA). In a subsequent experiment, bovine serum albumine (BSA) was added as well to the medium as to the perfusion fluid in an attempt to reduce possible sticking of cytokines to the membrane. All samples were collected in duplo using two separated circuits running simultaneously.
Results: A flow rate of 2.0 microl/min resulted in a mean recovery rate of 0.78% (range: 0.55%–0.97%). Decreased flow rate of 0.5 microl/min resulted in an increased mean recovery rate of 6.05% (range: 5.97%–6.13%). Cytokines sticking at the membrane leads to a statistically significant decrease in the apparent extraction efficiency of both IL- 1 beta and IL- 6. Addition of BSA to the medium and to the perfusion fluid resulted in a 3 to 14 fold increase in the extraction efficiency probably by preventing non-specific binding of cytokines to the membrane or tubing. In all wash out samples, concentrations of IL-1 beta and IL- 6 were under the ELISA detection limit.
Conclusion: Microdialysis by using a polyether sulfon membrane allows recovery of cytokines under in vitro conditions. Extraction efficiency is dependent on flow rate. Addition of a carrier protein to the microdialysis fluid markedly enhances the apparent extraction efficiency of cytokines and thereby facilitates the detection of cytokines by microdialysis. In our opinion microdialysis may prove valueable in monitoring local cytokine production in TBI.

P189. Heterogeneous responses of endothelium and foot processes of astrocytes in blood-brain barrier damaged from cortical contusion in rats
W. Poon, H. Ng, Y. Chan (Hong Kong, PRC)

To assess the time course and extent of responses of endothelia and foot process of astrocytes, main components of BBB to severe TBI, a modified impact-acceleration model was used for producing both focal cortical contusion and widespread DAI simultaneously. A total 32 rats were randomly divided into seven groups: sham, 1hour, 4hours, 1day, 3days, 6days and 11days post-injury. Immunohistochemistry was performed in adjacent serial paraffin sections of injured brain, using following three antibodies: 1) anti-endogenous IgG, detecting BBB disruption, 2) anti-Endothelial Barrier Antigen (EBA), marker of integrity of endothelia 3) anti-Aquaporin 4 (AQP4), marker for normal function of foot process of astrocytes. The area of negative immunostaining was quantified by image analysis (CAS 200). BBB damages detected by IgG immunostaining were mostly concentrated in cortical contusions, and in small extent of the marginal regions of ipsilateral hippocampus and brain stem. Within the contusion, loss of AQP4 expression was prominent at 4 hours (5/6), peaked at 1day(5/6) and re-expression started from 3days(2/5) and afterwards; meanwhile, loss of EBA began at 1 hour, peaked at 4hours, re-expression started from 1day (4/6) and later. Paired comparison of adjacent serial sections from the same contusion demonstrated that heterogeneous responses also occurred in contusions with larger AQP4(-) area (>0.5 mm2). The AQP4(-) area (1.68±1.21 mm2) was significantly larger than EBA(-) area (0.47±0.31 mm2); (mean±SD, P=0.015, paired t test) in the same contusion. However, there is no significant difference of immunostaining pattern in other diffusely injured regions between the AQP4 and EBA. We conclude that heterogeneous response can be evoked in contusion under the same mechanical injury loading. The recovery of disturbed endothelial cell is faster than that of dysfunction of foot process in contusion. The foot process of astrocyte is more vulnerable to mechanical injury. We did not demonstrate any significant BBB dysfunction in regions with diffuse axonal injury.

P190. A 4-axes model of the stress response
A. Kunz (Boston, USA)

This paper proposes an expanded 4-axes model of the physio-logical stress axis to help identify cross-cultural potential risk factors for traumatic brain injury (TBI), and its sequella, and to augment the relevant evolving international public health surveillance in the past decade. The 4-axes stress response model includes the interrelated fast-acting hypothalamic-pituitary-adrenal (HPA) axis, the intermediately fast-acting pineal-opioid (PO) axis, the slow-acting glucose-gluta- mine-GABA (GGG) axis, and the hippocampal-cortical (HC) learning/memory axis. An example of the significance of the interrelated- ness in

this expanded 4-axes complexity is the high density of corti- sol receptors in the hippocampus, and the co-localization of gluta- mate and opioid peptides in the hippocampus. Also, plasma glucose levels have now been identified as an early marker to predict survival after TBI, since the brain tissue is continually permissive to glucose. Two relevant studies (published abstracts) by Stickgold, et al, address the cognitive impairments seen even in mild TBI: damage to the HC (hippocampal-prefrontal cortex) memory axis. In the first study, tetris game experts, tetris game novices, and tetris trained amnesiacs scored similarly in recall learning when awakened from slow wave sleep. Since the amnesiac group had no recent (hippo- campus) recall, the results established a dual system (hippocampal and neocortical) for memory and learning, whose path, from hippo- campus to prefrontal cortex, is unidirectional. In the second study, it was found that a minimum of 6 hours sleep, preferably 8, was need- ed for memory consolidation, and that an "all-nighter" severely blighted memory consolidation even post 3rd day. An indirect value of the expanded 4-axes stress response model is that it is easier to identify multiple points at which risk factors for TBI act, for example, alcohol intoxication, whose correlation with TBI has been confirmed, p=.002. Alcohol disregulates the 4-axes stress response at two points: by potentiating the glutamate receptor and by inhibiting the GABA receptor. As a leading cause of disability and death, TBI is a towering, un- resolved, worldwide public health concern. In an expanded stress response model, ongoing surveillance for the underdiagnosed TBI, for improvement of the consequences of TBI, and for understanding risks for prevention can augment our evolving effective efforts.

P191. Dynamic neuronal and glial cell reactions in the brain after closed head injury by exposure to rotational forces and to pressure waves

A. Hamberger, Y.-L. Huang, M. Runnerstam, F. Bao, K.G. Haglid, H.-A. Hansson (Göteborg, S)

Human victims exposed to closed head injuries due to rotational acceleration (e.g. strong forces to the body and face, traffic accidents) or due to blasts (e.g. explosions) may suffer from not only acute, usually transient symptoms but may as well lately develop chronic, complex neuropsychiatric disorders. Traditional explanations of post-traumatic stress disorder have revolved around a physiological theory. An alternative explanation could be that a major cause to the disorder is structural damage to the central nervous system. The aim of the present study was to elucidate whether a single exposure of a body to rotational acceleration could cause brain damage. Anaesthetized rabbits were exposed to rotational acceleration of the head (peak 212 krad/s2, 0.95 ms pulse). The effects of the deceleration was eliminated. A pneumatic system was used to impact a titanium cap, cemented to the skull. The rabbits appeared unaffected with respect to general behavior, posture, movement and food consumption. There was no gross injury such as fractures or lacerations. Subarachnoidal hemorrhages were recognized in all animals on the basal brain, from the optic nerve to the cervical spinal cord. There was a transient activation of immediate early gene products. Astrogliosis and neurofilament abnormalities were revealed during the first week, the latter indicating disturbed axonal transport, and the development of diffuse axonal injuriy (DAI). Neuronal apoptosis was evident. The concentration of extracellular glutamate increased. We conclude that the exposure of a body to rotational acceleration is associated with distinct acute cell reactions in the brain, in areas important for the memory and learning, and may as well affect and impair the brain function in the long run for victims, tentatively resulting in e.g. post-traumatic stress disorder symptoms.

P192. Role of extracellular potassium on rapid and widespread microglial activation following traumatic brain injury – a study using rat brain slice injury model

M. Fukushima, Y. Katayama, M. Koshinaga (Tokyo, J)

We have previously demonstrated that rapid and widespread microglial activation observed following traumatic brain injury (TBI) can not be attributed exclusively to the infiltration of blood-borne monocytes or molecules by using the brain slice paradigm which excludes the various constituents of circulating blood. The present study was undertaken to elucidate the trigger of such microglial activation, the effects of excitatory amino acids (EAAs) antagonists and potassium channel blockers on microglial activation were examined. Controlled cortical impact injury was applied to adult rat brain slices and the complement receptor (CR3) expression and morphological transformation were evaluated by OX42 immunohistochemistry. At 10 min following injury, activated microglia with intense CR3 expression appeared throughout the hemisphere exclusively on the injury side. This rapid and widespread microglial activation was effectively blocked when either kynurenic acid (the broad-spectrum EAA antagonist) or Cs+(an blocker of inwardly potassium channels) was applied to the incubation medium prior to the injury induction. In contrast, AP5 (a NMDA receptor antagonist), DNQX (an AMPA-KA receptor antagonist), TTX (a voltage-gated sodium channel blocker) or 4-aminopyridine (an blocker of outwardly potassium channels) respectively did not show similar effects on microglial activation.These findings indicate

that the increase in extracellular pottasium concomitant with traumatic depolarization may be the earliest stimulant which trigger the microglial activation following TBI.

P193. Evaluation of the intracranial reserve exhaustion by application of physiological loadings in an experimental model of intracranial hematoma

J. Andrychowski, Z. Czernicki, J. Bogucki, M. Glowacki (Warsaw, PL)

Objectives: Previous methods to evaluate intracranial reserve reserve exhaustion(IRE) either utilize imaging modalities or are associated with application of invasive techniques. Instead of of external infusion physiological loadings (changes in body position, Valsalva test, apnea) are applied.
Methods: As a model of intracranial haematoma silicone application into white matter of the cats brain hemispher was used. Animals were divided into 4 groups based on the rate of silicone infusion and volume of the applied silicone.Twenty one experiments were performed including 5 in the control group.During applicaton of physiological loadings values of ICP,BVF-TCD,ABP,VBP and pCO2 were monitored.Pulse related pressure changes and high frequency centroid were also analyzed.
*Results:*In groups with the highest level of IRE considerable decrease in blood flow velocity evaluated by TCD was observed during application of physiological loadings.
Conclusion: Physiological loadings could be used to evaluate the level of IRE in clinical practice. Under physiological loadings ICP changes correlated with BFV changes are of important diagnostic value in the evaluation of the IRE level.

P194. Axonal damage in a lamb model of pediatric traumatic head injury after different types of head impact

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The aim of this study was to compare the distribution and severity of axonal injury (AI) (1) in the immature lamb brain following nonpenetrating impacts to the temporal, frontal and occipital regions of the unconstrained head, (2) in lamb and adult sheep brains by temporal nonpenetrating impacts, and (3) after penetrating and nonpenetrating head impacts in lambs. Studies were conducted on anaesthetised, ventilated and physiologically monitored, 4-5-week-old lambs and 2-year-old ewes and heads were impacted with penetrating and nonpenetrating humane stunners (captive bolt pistols). Two hours post-impact, brains were perfusion-fixed with 4% paraformaldehyde and, following neuropathological assessment according to a standard protocol, whole coronal sections of the cerebral hemispheres, brainstem and cerebellum were stained for amyloid precursor protein (APP), a sensitive marker of AI. Microscopic evaluation of AI was then performed using a quantitative grid system. The results showed that AI in all lamb brains was widely distributed and (1) was most severe after frontal impacts, followed by occipital and then temporal impacts in decreasing order of magnitude, (2) the pattern of injury was similar to that in the adult sheep brain following temporal impacts, and (3) of comparable severity after penetrating and nonpenetrating head impacts.

Poster Session 3
Regeneration

P195. Rubrospinal neurons have undergone massive atrophy, but not death, and can be stimulated by BDNF to regenerate one year after cervical axotomy in the adult rat

C. Messerer, N.R. Kobayashi, J. Liu, G.W. Hiebert, W. Tetzlaff (Vancouver, CDN)

Adult rat rubrospinal neurons have been reported to undergo massive atrophy after axotomy in the cervical spinal cord and between 40-50% are reported dead by 3-4 months. Unfortunately, due to the massive atrophy of the rubrospinal neurons it is very difficult to discriminate a shrunken neuron from a glial cell in cresyl violet stained sections. We have therefore used the neuron specific antibody, NeuN, and counted rubrospinal neurons one year after axotomy in the cervical spinal cord using the disector method. This counting method requires a series of sections and a cell scores if it no longer appears in the adjacent section. We established that counting every 4th section (i.e. 3-4; 7-8; 11-12; 15-16; 19-20; 23-24) yielded cell counts ranging plus/minus 20% around the "true" value (=every section counted) and a mean error of less than 5%. We found that the neuronal antigen NeuN was still detectable in highly atrophic rubrospinal neurons as late as one year after spinal cord injury. Many NeuN stained cell profiles were not detectable in cresyl violet staining. Using the disector method we found comparable numbers of NeuN positive rubrospinal neurons on the axotomized and non-axotomized side of the red nucleus. Infusion of BDNF for 7 days at the end of the 10 months period (almost completely) reversed the atrophy of the chronically axotomized RSN. Again the number of NeuN stained neurons on the axotomized and BDNF treated side was not reduced. Treatment with BDNF also stimulated the expression of GAP-43 and Ta-1 in the chronically injured RSN. Moreover, we report that

the chronically injured neurons by application of BDNF to their cell bodies can be stimulated to regenerate into peripheral nerve transplants grafted one year after cervical injury. (Supported by PVA, MRC of Canada and Rick Hansen Neurotrauma Initiative.)

P196. BDNF applied to the motor cortex promotes growth of intact corticospinal fibers into the cervical spinal cord denervated by unilateral pyramidotomy

G.W. Hiebert, L.T. McPhail, W. Tetzlaff (Vancouver, CDN)

Previous experiments in our lab have shown that administration of BDNF to the motor cortex facilitates sprouting of corticospinal neurons in the region immediately rostral to a thoracic spinal cord injury. In the present experiment we investigate whether application of BDNF to intact corticospinal neurons results in axonal growth into a denervated region of the cervical spinal cord. A unilateral pyramidotomy was performed to remove half of the corticospinal input to the spinal cord. At the same time as the lesion, a cannula was implanted into the intact motor cortex (contralateral to the lesion). The cannula infused BDNF (1.0 µg/µl, 0.5 µl/hr) into the parenchyma for 14 days. The tracer Micro-ruby was injected into 6 sites of the intact motor cortex to label the corticospinal axons. The tissue was assessed 28 days after injury. Fluorescent images of the labeled corticospinal fibers at the cervical level (C6) were captured with a digital camera. Using Adobe Photoshop the labeled fibers within the grey matter contralateral to the normal innervation (i.e. within the denervated region) were manually traced and their cumulative length expressed as a total number of pixels. Initial results indicate that animals treated with BDNF into the cortex had 2.5 times as many pixels as compared to saline treated control animals. These data show that treatment of the cell body can stimulate intact neurons, which project below the level of a neural injury, to grow into denervated areas of the cord. Whether or not this growth facilitates behavioral recovery (or compensation) is currently under investigation. (Supported by the MRC of Canada, SCRF (PVA) and the Rick Hansen Institute.)

P197. Structural reorganization of the retinal ganglion cells and the superior colliculus following optic nerve crush in adult rats

T. Rohatgi, M. Arndt, J. Hanke, U. Lendeckel, S. Ansorge, B.A. Sabel (Magdeburg, D)

It is known that 3 weeks after optic nerve crush (ONC) in adult rats recovery of discrimination of visual stimuli occur. Visual recovery after diffuse injury of the CNS in adult mammalians should be accomplished with certain amount of repair in the axons of the corresponding neuronal cells. To further investigate this point, quantitative RT-PCR was used to detect changes in the gene expression of different cytoskeletal elements of the retinal ganglion cells (RGCs). Therefore, we examined 12 weeks old hooded rats 2 days, 4 days, and 8 days after ONC. Beside this, the superior colliculus was examined at the same time course. Despite the structural damage of the optic nerve due to crush, the RGCs and the corresponding superior colliculus seem to exhibit a high degree of repair mechanisms at the cellular level indicated that changes in the cellular expression of the cytoskeletal elements correlates with axonal repair after ONC. (Supported by the BMBF Neuroverbund-projekt Magdeburg B4/B5.)

P198. Internal axon repair after optic nerve crush in adult rats

J. Hanke, U. Schröder, B.A. Sabel (Magdeburg, D)

It is known that after a diffuse axonal injury of the optic nerve in adult rats about 15% of the retinal ganglion cells survive. After initial blindness the adult animals recover discrimination of visual stimuli in a behavioral test after 3 weeks. The anterograde transport of Mini Ruby intraocularly applied recovers 3 weeks after lesion distal to the place of crush, while Taxol inhibits this transport. At the same time point the heavy neurofilament is completely restored in the axons of the surviving retinal ganglion cells. The filaments appear wavelike and/or squeezed after the optic nerve crush up to day 8 but first signs of the heavy neurofilament restoration in the fibers of the optic nerve are seen at day 12 after the lesion of the optic nerve. After injection of horseradishperoxidase into the superior colliculus of the rat after optic nerve crush, we can detect recovered retrograde transport within 3 weeks. These results give strong evidence for an internal axonrepair after diffuse axonal injury. In the future it will be of great importance to know how we can manipulate the surviving RGCs to get a faster functional recovery of the injured axons. (Supported by the BMBF Neurotraumaverbund Magdeburg/Berlin A2.)

P199. Normal day light-cycle improves the recovery of retinal ganglion cells (RGCs) after optical nerve crush (ONC)

H. Tietgens, K. Gans, J. Hanke, B. Sabel (Magdeburg, D)

In-Vivo Confocal Neuroimaging (ICON) has been technically improved by modification of some elements of Confocal Laser Scan Microscope (CLSM). Investigations with ICON have shown that 3 types of RGCs can be detected after ONC: (1) RGCs

without change in fluorescence intensity (FI) and cell diameter; (2) RGCs with moderate change in FI and cell diameter and (3) dying cells with a massive increase in FI and cell diameter. For determination of the influence of light support on the survival of RGCs after ONC we examined animals in 12/12 h light/dark cycle (n=5) and in 1/23 h light/dark cycle (n=5). After 1 week adaptation at the rhythm of life the RGCs are marked retrogradely with the calcium marker Oregon Green BAPTA injected into the corresponding superior colliculus. RGCs were visualized for 11 weeks after ONC with the ICON method. We detected that a lack of light stimulation of the retina in animals living in the dark led to a clearly reduced number of active RGCs. The time course of active RGCs was similar in both animal groups, but in the group of animals held in the dark-cycle the number of active RGCs decreased for approximately 30-40%. Changing of the life-rhythm from 1/23 h to 12/12 h light/dark cycle 7 weeks after ONC showed no increase in the number of active RGCs. Future investigations will show if an active light stimulation of the RGCs will contribute to restitution after ONC. (Supported by the Deutsche Forschungsgemeinshaft.)

P200. Reorganization of retrograde axonal transport after optic nerve crush of adult rats visualized by ICON technique

V. Nahmmacher, J. Hanke, B.A. Sabel (Magdeburg, D)

We investigated the recovery of a retrogradely transported fluorescence marker in rats after optic nerve crush (ONC). Therefore, we held adult hooded rats in two groups: the first group lived in a 12h/12h day/night cycle while the other were held in a 1h/23h cycle, water and food ad libitum. The corresponding retinal ganglion cells (RGC) of all rats were retrogradely labelled by injection in the right superior colliculus 5 days before and the left superior colliculus 10 days after optic nerve crush (ONC). The ONC was made with an forceps distance of 0.2 mm (mild crush). The retinas of the rats were observed by In-Vivo Confocal Neuroimaging (ICON) technique (Sabel et al. Nature Medicine 3:244 1997) which made it possible to investigate the same retina of one animal for several times. Images were taken every 5 days for 10 weeks except injection-days, while the rats were anaesthetised with Rompun, Ketanest and Vetranquil.The images taken at various ICON sessions for one retina were compared with each other for detection of alterations in the appearance of the RGCs. The RGCs in the left eyes of the animals which were stained before ONC survived to about 15% after injury. In the right eyes where the injection of the fluorescence marker was made after ONC we detected the first stained RGCs 20 days after injury. This indicated that the retrograde axonal transport after ONC is interrupted and delayed until day 20 after ONC where it takes function again. (Supported by the BMBF Neurotraumaverbund Magdeburg/Berlin A2.)

P201. Primate olfactory ensheathing glia – anatomy, harvest methods, culture, and characterization

J.D. Guest, A. Marcillo (Miami, USA)

Olfactory ensheathing glia (OEG) are candidate cells to support CNS regeneration. Looking toward an autologous OEG transplantation in a primate model, we compared the in vivo and in vitro characteristics of cells from the olfactory bulb (OB) and nasal mucosa of Macaca fasicularis using immunostaining and electron microscopy. OB were obtained via a medial orbital craniotomy; olfactory mucosa was from nasal turbinates of euthanized primates. Dissociated OB and mucosal cells were expanded on collagen in serum-containing media with heregulin and forskolin. OEG were selectively concentrated using anti-primate p75 antibody panning, and assessed by EM and with anti-GFAP, p75 and S-100.

Results: In vivo, OEG somas were concentrated superficial to glomeruli in the anterior and ventral OB, with thin OEG processes surrounding multiple tightly packed axons in the nerve fiber layer. Some OEG contained loosely bundled intermediate filaments both in vitro and in vivo. OEG processes devoid of extracellular matrix were closely associated with astrocytes and myelin-forming oligodendrocytes. Primate OEG have distinctive nuclei and cigar-shaped mitochondria. After plating on collagen, p75 selected significantly expanded in number, resulting in a yield of more than 2.5 x 106 cells/ bulb after three passages (n=10). Panned cells were 85 +/- 5% OEG (S100+ and p75+), with approximately 40% of p75+ cells clearly GFAP+. Cells remained viable in culture up to 10 wk, then began to detach. Abundant cellular inclusions, apparently lysosomes, indicated that culture conditions were not optimal. p75+ cells from the primate olfactory mucosa had features identical to OB-OEG, but the cellular yield per gram of tissue was lower.

Conclusion: Primate OB-OEG survive and proliferate in tissue culture. Similar cells can be derived from the olfactory mucosa, which is clinically more accessible. We are currently assessing the ability of these cells to support regeneration of injured spinal axons. (Supported by the Miami Project and the Roy Travis Foundation.)

Poster Session 4

Intensive care and neuromonitoring 1

P202. Cerebral perfusion pressure and cerebral oxygenation in severely head injured patients

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To assess the effects of standard treatment of intracranial hypertension in patients with severe head injury (SHI) 58 severely head injured patients (GCS £ 8) were observed. Arterial blood pressure, intracranial pressure (ICP), cerebral perfusion pressure (CPP), regional cerebral oxygenation (rSO2) were studied during 24 hours after trauma. The cerebral oxygenation was determined with noninvasive technique (near infrared transcranial spectroscopy). Various treatment interventions: elevation of CPP with dopamine (n=37), mannitol infusion (n=21) and controlled hyperventilation (n=27) were performed. All patients were divided into 2 groups according to Glasgow outcome scale (GOS). In patients with GOS 1-2 the decrease of CPP from 53.9±5.4 to 45.6±8.7 mm Hg owing to rising ICP took place. The applying of dopamine, mannitol and hyperventilation in this group did not provide, nevertheless, the adequate CPP. The parameters of a cerebral oxygenation reflecting cerebral blood flow also reduced from 43.2±4.6 to 37.4±6.1%. Patients of second group (GOS 4-5) indicated intact cerebral autoregulation in all cases. Elevation of CPP from 54.2±3.7 to 65.6±4.1 mm Hg with dopamine in this group significantly improved rSO2 from 57.9±6.1 to 68.3±6.7%. Mannitol normalised ICP and CPP but in some patients did not affect rSO2. Hyperventilation (PCO2 – 21±3 mm Hg) normalised ICP and CPP, but significantly reduced rSO2 (to 41.4±5.3%).
Conclusion: 1). In patients with intact cerebral autoregulation (GOS 4-5) the applying of dopamine provides adequate CPP and cerebral oxygenation despite of rising ICP. 2). In patients with damage cerebral autoregulation (GOS 1-2) dopamine, mannitol and induced arterial hypocapnia are inefficient. 3). All interventions for CPP elevation should be conducted under the cerebral oxygenation monitoring.

P203. Gradual progression of intracranial hypertension in traumatic coma

St.M. Iencean (Iasi, RO)

This study is based on 330 patients with traumatic coma for at least 24 hours. It must be differentiated the cases with hematoma (epidural,subdural,cerebral hematoma) and cerebral laceration with hematoma, the cases withlimited traumatic lesions and cerebral swelling and the cases with diffuse cerebral traumatic edema. The mechanisms of intracranial hypertension (ICH) in all these three conditions are differently but the sequence of progression of ICH is self-same, although it can evolve with distinct rapidity. Initially the increase in intracranial pressure (ICP) appears as an allarm signal,then the elevated ICP is accompanied by symptoms as ICH syndrome and later on the symptomatology in ICH aggravates and it can exceed the symptomatology of the initial traumatic lesion. This third stage of ICH is the acute form of ICH (decompensated) when the ICH appears as an acute disease with individual evolution. This three-phase evolution characterizes ICH like a dynamic system because its progression is defined by determined mechanisms but unstable as the evolutionary complexity is individually. The cerebral CT or MRI and the diagnosis of increased ICP as early as possible are the means for a good treatment.

P204. Alteration in autonomic nervous function from life to death with severe head injury – evaluation with heart rate variability

C.-F. Su, T.B.J. Kuo, H.-I. Chen (Hualien, PRC)

To investigate the effect of head injury on sympathetic and parasympathetic activity in different levels of brain damage, we used power spectrum analysis of heart rate variability (PSA of HRV) to evaluate the autonomic balance. Twenty-three women and 67 men with head injury were studied. We grouped the patients to 5 levels to differentiate the effect of different brain damage according to brain stem reflex, pupil dilatation, and Glasgow coma scales (GCS). Precordial lead II EKG was recorded for 5 min when the patient was admitted and repeated at the first 3 days. These data were transferred to personal computer in which the frequency domain analysis of short-term, stationary RR intervals could be analyzed. They were subsequently divided into very low frequency power (VLF, 0.003 to 0.04 Hz), low-frequency power (LF, 0.04 to 0.15 Hz) and high-frequency power (HF, 0.15 to 0.40 Hz). In addition, the ratio of LF to HF (LF/HF), LF power in normalized units (LF%) and HF power in normalized units (HF%) were counted. Group I (GCS, 15) patients showed normal HF, LF, HF% and LF%. Group II (GCS, 9-14) patients, HF power of HRV was diminished but the LF and LF% increased when compared with the group I patients. In group III (GCS, 3-8, no dilatation of pupils) and group IV (unilateral or bilateral dilatation of pupils without brain death), reduction of HF or HF% and marked increase of the LF component and the LF% were evident. In group V (brain death) all levels of the frequency domain power were markedly diminished or completely disappeared. In head injury patients with preserved brain stem function, the increased

intracranial pressure and contusion of brain may activate the sympathetic drive and tends to suppress the parasympathetic function. However, either LF or HF components were totally diminished when the brain death happened.

P205. Relationship between the somatosensory evoked potential and cerebral oxidative metabolism following severe head injury

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Introduction: The utility of the somatosensory evoked potential (SEP) is recognized in the assessment of a severe head injury. However, it is not clear whether deteriorations in the SEP are due to the evolution of primary axonal injuries or secondary insults (such as ischaemia or intracranial hypertension) or both. We have investigated the relationship between the amplitude of the SEP and the vascular/metabolic state of the injured brain between day one and eleven, post injury.
Methods: Fourteen patients with acute head injuries were studied at least once by positron emission tomography (PET) using triple-15O and 18F-fluorodeoxyglucose protocols to obtain values of regional cerebral blood flow (rCBF), cerebral oxygen metabolism (rCMRO2), oxygen extraction fraction (rOEF) and glucose metabolism. During PET studies SEP responses to increments in stimulation intensity were obtained bilaterally. PET images were transformed into standard stereotactic space and entered into an anatomical region of interest analysis. The median amplitude of the SEP primary cortical response and mean values of rCBF, rCMRO2 and rOEF for the left and right parietal regions were entered into a regression analysis.
Results: rCMRO2 ($p<0.001$) and rOEF (range 22-62%, $p<0.05$) were found to be positively associated with SEP amplitude across the group. Analysis of rOEF and rCBF failed to identify any parietal regions with substantial energy depletion (mean rOEF 37.4% S.D. 9.9%). No relation was found between SEP amplitude and rCBF or intracranial pressure. Analysis detected a region of parietal hyperglycolysis in one patient in whom no effect on SEP amplitude was observed.
Conclusion: We found SEP amplitude to be significantly associated with the residual oxidative metabolism of the injured brain. A relationship with the occurrence of secondary insults was not found.

P206. Persistently poor continuous quantitative EEG percentage alpha variability can determine outcome early after traumatic brain injury

P. Vespa, N. Martin, V. Nenov, M. Nuwer, T. Glenn, M. Bergsneider, D. Kelly, D. Becker, D. Hovda (Los Angeles, USA)

Introduction: Prognosis after traumatic brain injury is difficult but continuous electroencephalographic monitoring (cEEG) may assist in early prognosis.
Methods: Prospectively 89 traumatic brain injured patients (GCS 3-13) underwent cEEG. A 10 electrode, 14 channel bipolar and referential montage was used with automated quantitative EEG trending of percent alpha (PA) activity and percent alpha variability (PAV). The timing and extent of the worst PAV were compared to discharge Glasgow outcome score.
Results: There were 65 males and 24 females with a median GCS 6. The mean duration of cEEG was 7.5 ± 4.2 days. There was poor correlation between admission GCS and GOS ($r=0.20$). In patients with poor Glasgow outcome score (GOS) 1-2, the PAV remained poor (0.13 ± 0.99). At a mean of 2.6 days ± 2.9 after injury, the worst score of PAV was able to discriminate between those patients with a poor discharge GOS (1-2) [PAV 0.075 ± 0.045] and those with a good GOS (4-5) [PAV 0.12 ± 0.068] ($p<0.01$). The worst PAV score of “Poor” (PA<.11) was 97% sensitive in determining poor outcome (GOS 1-2) and the final PAV score of “Good” (>.18) was 81 % specific for a good outcome (GOS 4-5). The positive predictive value (PPV) of 0.85 whereas the GCS was had a PPV 0.65. PAV correlated with the initial CT scan lesion burden and was sensitive in indicating development of new CT lesions ($p<0.05$) with subcortical lesions influencing PAV independently.
Conclusion: cEEG monitoring can provide early prognosis (within 3 days of injury)in traumatic brain injury patients and predict good outcome. PAV remained poor in patients who died, whereas PAV improved in the ICU in those patients with good outcome. PAV is more sensitive and specific than the Glasgow Coma Score.

P207. A new bed-side monitoring in severely head-injured patients – online intracranial compliance

W. N. Schoening, K. L. Kiening, W. R. Lanksch, A. W. Unterberg (Berlin, D)

A new monitoring technology provides online assessment of intracranial compliance (ICC). Goals of our research were: (1) ICC (critical threshold:<0.5 ml/mmHg) in episodes of pathological elevated intracranial pressure (ICP) (> 20 mmHg) and reduced cerebral oxygenation (brain tissue PO2 (PtiO2)<10 mmHg). (2) The average of ICC in distinct age

classes. (3) Dependency between ICC and outcome. (4) Calculation of 'time-of-good-data-quality' (TGDQ) of ICC. Up to now 7 patients with severe closed head injury (GCS<9) were included. Data assessment was performed using a multimodal cerebral monitoring system (sampling rate 1/min) providing 830 hours of data. TGDQ [%] was calculated as artefactfree time [min] x 100 [%] / total monitoring time [min]. Outcome was determined 6 months posttrauma (Glasgow Outcome Score). (1) 43 episodes of pathologically elevated ICP and 39 of critical ICC were found. 17 episodes revealed overlapping periods while in 9 of these ICC anticipated ICP in reaching pathological values. Low PtiO2 was never related to high ICP or low ICC. (2) ICC was found to be significantly different in each age class. At an ICP of 20 mmHg, average ICC of children (n=2) was 0.9, of middle-aged adults (n=3) 0.7 and of elderly patients (n=2) 0.6 ml/mmHg. (3) Adverse outcome was indicated best by high ICP followed by low ICC. (4) TGDQ in ICC was 72% compared to 95% in ICP and 98% in PtiO2. High ICP was superior to ICC and PtiO2 in indicating adverse outcome. The different average of ICC in each age class points to the need of an age-dependent ICC interpretation.

P208. Towards autoregulation-oriented therapy

M. Czosnyka, P. Smielewski, S.K. Piechnik, J.D. Pickard (Cambridge, UK)

Factors determining outcome following head injury are still poorly identified. Traditionally, admission GCS, age, brain imaging were listed as strong predictors of outcome, but inclusion of other factors as ICP, CPP, pre-defined secondary insults, brain oxygentaion, etc., were problematic. Over 400 sedated and ventilated head injured patients were studied in 1992-1999. All had continuous ICP and ABP monitoring with an on-line assessment of cerebrovascular pressure reactivity using ICP waveform analysis or response of ICP to spontaneous changes in arterial pressure (ABP). 200 from theses patients were examined day-by-day using bedside Transcranial Doppler to assess cerebral autoregulation. Rigorous CPP-oriented therapy using active treatment of ICP>20 mm Hg and ABP support to keep CPP>70 mm Hg was introduced in 1994-1995. However, there was no evidence of significant improvement in outcome (mortality was around 25% and remained unchanged, rate of favourable outcome improved from 47% to 57%, although insignificantly: p>0.05). After 1994 outcome became independent on CPP, however its dependence on ICP remained significant (p<0.05). In multivariate model of outcome GCS and age were always included, but CPP not (with p>0.05). Significance of vascular reactivity were as high as GCS (p<0.003). Significance of raised ICP was lower (p<0.01). High ICP (>25 mm Hg) with deranged linear relationship between pulse amplitude and mean ICP occurred to be strongly associated with fatal outcome (p<0.00001). TCD- and ICP/ABP derived indices of autoregulation was on average worse in patients with unfavourable outcome (p<0.00001). These differences were more significant during two first days following injury. In conclusion, a success in treatment of low CPP failed to improve mortality rate significantly. Our results suggest that derangement of vascular reactivity is an important factor determining bad outcome. Therefore autoregulation-oriented therapy, guided by continuous monitoring of cerebrovascular reactivity, can be postulated as a next step towards improvement in neuro-intensive care.

P209. The mechanisms of fever after traumatic brain injury – early fever is a function of elevated cytokines, glutamate and vasospasm

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Background: The impact of fever after brain injury is well established in animal studies, but the mechanisms of fever are not well understood.
Purpose: To determine the causes of post fever after traumatic brain injury.
Methods: Serial hourly measurements of core body temperature were undertaken in 89 patients with severe traumatic brain injury. The cause of fever, infection or non-infectious was determined. Concurrent serial studies of transcranial doppler ultrasound were made in all patients. In a subgroup of patients extracellular concentrations of glutamate, lactate and cytokines were measured using cerebral microdialysis. Standard cultures, chest radiographs and microbiologcial studies were performed for all episodes of fever. CT lesion type and location was assessed to determine if hypothalamic injury or intraventricular (IVH)blood results in fever.
Results: 88% of patients had fever during the 14 post injury days (PID). Infectious causes were pneumonia (83%), bacteremia (5%), line sepsis (5%), sinusitis (3%) and other causes (3%). Fever that occurred during PID 0-4 resulted from infection in only 36% of cases, compared to 94% of cases on pID 5-14 (p<0.01). During PID 0-4, fever was associated with elevated levels of TNFa (15 +/- 11 pg/ml), glutamate (25 +/- 15 uM) and lactate (1.7 +/- 0.6 mmol). Temperature tended to decrease at the onset of vasospasm, only to increase in the next 24 hours (p<0.03). Contusional brain injury was associated with a greater fever burden and higher TNFa levels during PID 0-4.
Conclusion: (1) Fever is common after traumatic brain injury. (2) Early fever is non-infectious and is associated with elevated levels of TNFa and

glutamate. (3) Contusional brain injury results in early fever rather than hypothalamic injury or IVH. (4) Dynamic temperature changes occur with vasospasm after traumatic brain injury.

P210. Validation of secondary insult data comparison of methods

M. Bos, I. Piper, L. Dunn (Glasgow, UK)

Head injury research involving the detection and analysis of secondary insults during the acute phase of patient management is an increasingly common method used in many neuro-intensive care units. Manual methods for removal of artifactual data is time consuming and a limiting step in any research study or clinical audit. This study compares: a) Manual artifact detection, b) Automatic computer based artifact detection and c) No artifact detection to determine the degree of error caused in the calculation of secondary insults by different validation methods. As part of a current clinical research protocol studying the influence of the ApoE genotype upon patient outcome, minute by minute physiological data monitored during the acute management of head injured patients was collected and archived. Twenty-five patients physiological data were prospectively collected and analysed for the presence of secondary insults using the Edinburgh Browser secondary insult Software tool. Heart rate, blood pressure, intracranial pressure and pulse oximetry data were manually scanned for artifact according to a pre-defined protocol. In addition, data was automatically scanned for artifact, with fixed threshold and rate of change algorithms, using in-house designed software. Across all tested insult types, the mean difference in the detection of invalid data, between any of the three methods of validation ranged from –1.68% to 2.14% of the calculated invalid monitoring time. This translates into an error of between –2.06% to 3.1% of the calculated insult duration in minutes. ANOVA showed no significant difference between any of the methods in the duration of detected secondary insults. We conclude, that using our data collection methodology for selected data types, time consuming manual validation of patient data is not necessary.

P211. The use of Receiver Operator Characteristic Curves to examine the threshold levels of cerebral perfusion pressure and intracranial pressure in relation to outcome in a series of patients with severe head injury

I.R. Chambers, L. Treadwell, A.D. Mendelow (Newcastle upon Tyne, UK)

Introduction: The use of intracranial pressure and cerebral perfusion pressure measurements is widespread in the management of severely head injured patients. However there is little published evidence establishing whether or not there are threshold levels of these parameters in the determination of outcome.
Methods: We obtained data from a total of 291 severely head injured patients (207 over the age of 16 years). Outcome was categorised into favourable outcome (good recovery or moderate disability) and unfavourable outcome (severely disabled, vegetative or dead) using the Glasgow Outcome Scale and patients were also classified according to Marshall. Rolling averages over one hour periods for ICP and CPP were calculated and the maximum ICP reading (defined as ICPM) and the minimum CPP (CPPm) used to calculate the sensitivity and specificity over a range of values.
Results: For both adults and children CPP appeared to be a better predictor of outcome in comparison to ICP. A threshold of CPPm of 55mmHg and ICPM of 35mmHg appear to be the best predictor in adults. For children the levels appear to be 43-45mmHg for CPPm and 35mmHg for ICPM. Higher levels of CPPm seem important in adults with mass lesions.
Discussion: Thresholds of 45mmHg for children and 55mmHg for adults are lower CPP values than previous predictions and may be clinically important, especially in children in whom a lower blood pressure level is normal. The data also suggests that CPP management at higher levels may be more important in adults with mass lesions. The accuracy of these thresholds could be improved if a larger series of patients could be observed.

P212. Diagnosis of brain hyperthermia in head injury patients

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Since 1987 works of Busto at al. have demonstrated that small increases in Brain Temperature could increase ischemic injuries, what was proven by Mellegard in 1990.1992 and 1994, through a micro-sensor placed in the distal extremity of a catheter which could survey safe and trustful Brain Temperature associated to Intracranial Pressure data, showing that increases of two degree centigrades above the basal intracranial temperature (6,4ºc), can promote damages to the nervous cell, worsening tecidual ischemia in human beings. Once Mellergard has set the difference between body and brain temperatures, becoming thus necessary to monitor the Intracranial Temperature, it's being proved that Head Trauma patients prognosis not only worsens when associated to Hyperthermia, but also that promising results are being achieved with the use of mild systemic hypothermia in the handling of severe Head Trauma. In this way the use of brain

temperature monitouring in becoming a new and important method of control and treatment of the patient with severe head injury. This work has the purpose of showing the management of the intraventricular brain temperature in severe Head Injury patients with or without cerebrospinal fluid infection based on specific treatment for both temperature and infection.

Poster Session 5

Cell injury, genomic involvement and transplantation in SCI

P213. Transferrin and ferritin in the human spinal cord following traumatic injury

B. Koszyca, J. Manavis, R. Cornish, P.C Blumbergs (Adelaide, AUS)

Although iron is a vital factor in normal central nervous system function, it must be strictly controlled to prevent excessive amounts of free iron which lead to the production of free radicals. Traumatic spinal cord injury (TSCI) leads to cell damage and haemorrhage, both of which may liberate large amounts of iron. This immunocytochemical study examined the response in TSCI of the iron transport protein transferrin (Tf) and the iron storage protein ferritin (Ft). It was proposed that TSCI would result in increased numbers of Ft positive cells because of the role of Ft as a safe store for excess iron, whilst the number of Tf positive cells would decrease as a protective measure to prevent iron uptake. The spinal cords of 12 individuals who had died at intervals ranging from 5 hours to 26 days following TSCI resulting in para- or quadraplegia and 2 spinal cords with no TSCI were examined according to a standard protocol. The number of Tf and Ft positive cells using a monoclonal antibody to Tf and a polyclonal antibody to Ft, were semiquantitated by counting the number of cells within 5 high power fields (x 400 magnification) in 6 defined sectors of the spinal cord at the level of maximal injury and the segments immediately proximal and distal to the lesion. The severity of the focal cord injury was assessed as the percentage area of necrosis of the total cord area at the site of maximal injury (focal area injury score FAIS). There were significantly fewer Tf positive cells in the study group compared to the controls ($p=0.01$). The mean number of Tf positive cells was significantly lower in the lesion compared to the proximal and distal segments ($p=0.03$). The number of Ft positive cells was increased compared to controls ($p=0.03$) and this increase was most marked after 6 days. There was a significant correlation between the number of Ft positive cells and the severity of injury as measured by the FAIS ($r=0.846$, $p=0.0005$).

P214. Operative treatment of post-traumatic syringomyelia

A.V. Komarevsky, A.A. Suphyanov, R.V. Kibort (Irkutsk, RUS)

Objectives: The aim of this investigations is an evaluation of the results of surgical treatment of the patients with the posttraumatic syringomyelia after the transplantation of the fetal neural tissue and nimodipine administration.
Methods: We operated 17 patients in the late period of spinal cord injury. They were 14 men and 3 women (the age of 7-45). 14 patients had cavities within the spinal cord. 3 patients had myelomalacias of spinal cord. They were divided into two groups: the first group patients were undergone the transplantation of the fetal neural tissue (10 patients); the second group received above that nimodipine therapy (7 patients). The neurotransplantations was performed by two ways: the fetal neural tissue was injected into spinal cord cavities in the case of posttraumatic syringomyelia; wile in the case of the transplantation of fetal neural tissue was performed into the defects of the spinal cord and it's distal part. Therapy by nimodipine was according to the scheme: 10 ml/h for 3 hours for 8 – 10 days.
Results: The neurological status data of the patients were analyzed before the surgery and 3 years later. The results of the treatment were satisfactory in all patients. The 7 patients from group 1 improved their motoring function, in the 5 patients from group 2; sensitive disorder decreased in 8 cases (1 group) and in the 6 cases – group 2. The positive dynamics of neurological disorders in the patients of group 2 was marked to the end of first postoperative month.
Conclusion: The results of the investigations confirm sensible use of fetal neural tissue transplantation in the case of posttraumatic syringomyelia. The use of pharmacological neuroprotection in the postoperative period improves the results of operation.

P215. Spinal cord injury alters fos expression in the primary and secondary somatosensory cortices of the rat

H.N. Allbutt, K.A. Keay, P.J. Siddall (Sydney, AUS)

Traumatic injury to the CNS results in dramatic changes in the levels of inflammatory mediators and cytokines, and the upregulation and release of many neurotransmitters. Such changes are thought to underly abnormal neural processing subsequent to injury. For example, spinal cord injury (SCI) results in both sensory and motor changes, which are undoubtedly due to these processes. SCI in the rat evokes increased expression of NADPH-d in ventral horn neurons and a decrease in superficial dorsal horn neurons in spinal segments at, and adjacent to

the injury level. SCI evokes also widespread decreases in the expression of Fos, suggesting a decrease in neuronal activity, throughout the entire spinal cord. In this series of experiments we investigated whether SCI altered Fos expression in somatosensory cortex (SI & SII), a major recipient of ascending spinal information. In halothane-anesthetised Wistar rats, a weight was dropped onto the surface of the L4 spinal segment. The rats survived for seven days prior to tissue fixation and histological examination. Comparisons were made with tissue from unoperated rats. Both NADPH-d and Fos expression were investigated in serial sections of SI & II. Fos expression was decreased in SI and II of injured rats, however, there were no differences in NADPH-d staining in SI and SII of injured vs unoperated animals. The results indicate that changes in Fos expression are likely due to changes in activity of corticofugal circuits originating at, but remote from the site of injury, whereas changes in NADPH-d are likely due to local spinal changes. The changes may well underly the sensory dysfunction often accompanying SCI.

P216. The neural toxicity of cNOS compared to iNOS in acute traumatic spinal cord injury of rats

K.S. Kim, D.H. Yoon, Y.S. Kim (Chonju, Chonbuk, Seoul, KOR)

Introduction: One of the main difficulties in the treatment of spinal cord injury is that neural deficit is originated not only from direct physical damage, but also from secondary biochemical change. NO (nitric oxide), which is converted from L-arginine by NOS (nitric oxide synthase), is known as one of the important neural toxic materials. cNOS exist normally in vessels or neural tissues, and iNOS is produced in inflammatory cells or neural cells by trauma or external toxic stimulation. There are many different opinions about the functions of cNOS and iNOS. This study was conducted to know the function of cNOS and iNOS in acute spinal cord injury, particularly early neural protection or neural toxicity of cNOS compared to iNOS.
Methods: L-NAME (NG-nitro-L-arginine methyl ester) was used to inhibit NOS. The New York University (NYU) spinal cord impactor was used to induce moderate injury. Rats were divided into the control group (saline injection only) and the other 3 groups as follows: injection of L-NAME (iv, 60mg/kg) 15 minutes before injury, injection of L-NAME 2 hours (iv, 40 mg/kg) and 5 hours (ip, 40 mg/kg) after injury, injection of L-NAME 24 hours (iv, 40 mg/kg) and 27 hours (ip, 40 mg/kg) after injury. Somatosensory evoked potential (SSEP) was performed 5 days after spinal cord injury (each group, n=5), and 2 weeks after injury (each group, n=10). Basso, Beattie and Bresnahan (BBB) behavior test was performed 1 day, 4 days, 7 days, 10 days and 14 days after spinal cord injury. Statistical analysis was done by ANOVA test.
Results: In the measurement of SSEP 5days after spinal cord injury, the control group treated with saline only did not appear wave. However, the other groups treated with L-NAME showed waves. In the measurement of SSEP 2 weeks after injury, all of the groups including the control group showed waves. The group pretreated with L-NAME showed more increased amplitude of SSEP (N1=0.0541+/-0.0253, P1=0.1047+/-0.0511) than the control group (N1=0.0122+/-0.0062, P1=0.0164+/-0.0072) with statistical significance ($p<0.05$). The BBB behavior score of the group pretreated with L-NAME showed a significantly higher score ($p<0.05$) than the control group from the 7th day. At 14th day, the mean BBB score of pretreated group (13.9+/- 2.9) was much more increased than the control group (9.4+/-1.6) ($p<0.05$).
Conclusion: Pretreatment of L-NAME can inhibit the cNOS which exist normally in vessels and neural tissues. From the results, we think that neural toxicity of NO, which is made by NOS after spinal cord injury, could be inhibited by NOS inhibitor (L-NAME), and cNOS might contribute to the early secondary spinal cord injury much more than iNOS.

P217. Lentiviral gene transfer and stable transgene expression in the adult rat spinal cord

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Inherited or acquired spinal neurological disease still has numerous uni-dentified factors, however an increasing number of trophic factors are known to be involved. Transplantation and gene transfer in order to correct the dysfunction has become one of the most challenging tasks in gene therapy of the spine. Since mechanism of the spinal cord involve specific targeting and only limited amount of gene products are re-quired gene transfer in the spine is potentially amenable to current gene transfer methods. Direct gene transfer in the spinal target cell however was limited because viral vectors did not obtain stable expression in terminally differentiated cells like neurons, or the vector did not infect cells like oligodendocytes. Lentiviral vectors based on the HIV have shown stable long term expression in a variety of differentiated cells like neurons of the CNS without transgene shut of or immune response up to two years..In these experiments we examine transgene expression via lentiviral vectors delivered to the contused rat spinal cord. A contu-sion lesion was made in adult Fischer rats at the 8th thoracic level using the Ohio State injury device. Three months later we injected replication deficient lentiviral vector

encoding GFP distal to the lesion into the gray matter of the spine. The animals received cortical BDA injections 14 days before sacrifice. Immunohisto-chemistry revealed that a variety of spinal cells were stably expressing the transgene. Cells displaying neuronal morphology were co-expressing NeuN, a marker for termi-nally differentiated neurons, cells morphologically displaying oligoden-drocytes were further characterized by counterstain with APC and RIP. A minority of transgene expressing cells were positive for OX42 and therefore microglia or for GFP and therefore astrocytes. Neuronal fi-bers were filled with the transgene product detectable multiple levels. These data suggest lentiviral vectors may have unique potential for gene delivery of the injured spinal cord, resulting a stable transgene ex-pression in a sufficient number of cells allowing for the direct in vivo delivery of different growth factors in future. (Supported by The Hollfelder Foundation, the German American Academic Council, German Research Foundation, The International Spinal Research Trust and NS 10165-21.)

P218. Death of corticospinal neurons in the neonatal mouse following axotomy – a role for apoptosis and caspase-3

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It has been reported previously that corticospinal neurons (CSN) die in response to axotomy in the neonatal rat. However, the axotomy-induced death of these cells and the mode by which they die has not been shown directly in the mouse. Our present findings demonstrate the death of neonatal mouse CSN axotomized at the low thoracic level (T10). Three pieces of evidence indicate the death of these cells following axotomy results from apoptosis. First, CSN retrogradely labeled with Fast Blue at the time of injury were observed to exhibit apoptotic DNA fragmentation, as detected by TUNEL. Second, bisbenzamide staining demonstrated nuclear morphologies consistent with apoptosis in many neurons within cortical layer V following axotomy. Third, activation of the pro-apoptotic protease, caspase-3, was detected in axotomized CSN by the MF397 antibody specific for the active form of caspase-3. Double-labeling of sections with the MF397 antibody and bisbenzamide revealed cells immunoreactive for active caspase-3 possessed apoptotic nuclei. Together, these results indicate neonatal mouse CSN undergo apoptosis following spinal axotomy, a process that appears to involve caspase-3 activation. We are presently testing the influence of an inhibitor of apoptosis protein, NAIP, on the death of these cells in transgenic mice over-expressing this anti-apoptotic protein. (Supported by the Rick Hansen Institute – Neurotrauma Foundation of British Columbia.)

P219. Migration of olfactory ensheathing glia after transplantation into contused spinal cord

K. liu, W. Huang, W. Young (Piscataway, USA)

Recent studies suggest that transplanted olfactory ensheathing glial (OEG) cells promote regeneration after spinal cord injury. In the olfactory bulb, these cells migrate and accompany growing axons during development. We were interested in the survival, migration rates and preferences of OEG cells in injured spinal cords, as well as their association with regenerating axons. The spinal cords of 12 Long-Evans hooded rats were injured with a 25 mm weight drop contusion (NYU Impactor) at T9-10. We transplanted OEG cells labeled with Hoechst 33342 into the spinal cord immediately after contusion. The cells were obtained from 10-day old Long-Evans hooded rats and cultured for 10 days before transplantation. The spinal cords were examined at 3 days, 1 week, and 2 weeks after injury. The results showed that OEGs survived and migrated rapidly in the spinal cord. At 3 days after transplantation, cells were present 1.5 cm from the injection site, suggesting an initial migration rate of >6 mm per day. By 1 and 2 weeks, many OEG cells were present up to T5 and down to S1 cord. The rats were not immunosuppressed and the cells survived for at least 2 weeks. The OEG cells migrated in both gray and white matter, and in both intact and degenerating tracts; there was no differences in OEG migration in the dorsal or ventral tracts. However, the OEG cells were strongly attracted to the corticospinal tract and the central canal, forming a queue in the corticospinal tract and clustering densely around the central canal. Growing axons were associated with the OEG cells. It is unclear why the OEG cells were not migrating beyond T5 in the proximal cord. One possibility is that factors at the injury site were stimulating the migration and the cells stopped migrating as they moved more than a few cm from the injury site. The central canal and corticospinal tracts are clearly expressing potent factors that attract and retain OEG cells.

P220. Acute inflammatory responses in spinal cord after olfactory ensheathing glial transplantation

H. Huang, W. Huang, W. Young (Piscataway, USA)

Recent studies have suggested that macrophages activated by exposure to peripheral nerve promote axonal regeneneration when transplanted into injured spinal cord. Likewise, several groups have reported that olfactory ensheathing glia (OEG) transplants also

promote spinal axonal regeneration. Injury induces a very strong inþammatory response in the spinal cord, associated with a massive invasion of macrophages by 48 hour and peaking 1-2 weeks after injury. We wondered whether the beneÞcial effects of OEG transplants may be related to macrophage activation. We examined the temporal and spatial distributions of activated macrophages and transplanted OEG cells in injured rats spinal cords. Pentobarbital anesthetized rats were injured with a 25-mm weight drop T9-10 contusion (NYU Impactor) followed by transplantation of labelled (Hoechst 33342) OEG heterografts cultured from 10-day old rats. Suspensions of OEG cells were injected into the impact site. At 3, 7, and 14 days after transplantation, we examined the injured spinal cords for ED-1 positive macrophages and labelled OEG. Some rats were immunosuppressed with cyclosporin and others were not. Briefly, the major findings of the study are as follows. First, although macrophages were seen engulfing dead OEG cells and debris, healthy-appearing OEG cells lived amidst dense congregations of macrophages at the injury site. Second, particularly in cord away from the injury site, OEG cells were often not accompanied by macrophages, suggesting that healthy OEG cells did not stimulate endogenous microglial activation. Third, OEGs but not macrophages were strongly attracted to ependymal cells, forming dense clusters around the central canal. We conclude that healthy OEG cells do not necessary activate microglia or macrophage cells.

P221. Increase in IL-1b up to 6 months after traumatic human spinal cord injury and its association with apoptosis

B. McColl, M.-A. Mackinnon, D.I. Graham (Glasgow, UK)

Secondary Degeneration is a feature of traumatic spinal cord injury (TSCI). Possible mechanisms include cytokine associated inflammation and apoptosis. Paraffin sections of spinal cord for 20 cases of human TSCI with survival 1 hour to 6 months, and 9 controls were stained, H & E, LFB/CV. and immunohistochemically for astrocytes (GFAP), microglia/macrophages (CD68) and IL-1b, and for apoptosis (TUNEL). Increasing amounts of IL-1b and CD68+ immunoreactivity were seen between 1 in 3 weeks (P>0.01) post injury with increased reactivity up to 6 months. IL-1b+ immunoreactivity was seen in reactive microglia/ macrophages – but not in astrocytes. TUNEL staining (P<0.01) paralelled the CD-68+ immunoreactivity. After human TSCI there is an increase in IL-1b immunoreactivity and that a major source are reactive microglia/macrophages. There was a paralell association between CD68+ and TUNEL+ reactivity. Concluded that cytokines in some patients are part of a cascade that triggers apoptosis thereby increasing the amount of damage post injury.

P221A. Localization of the nerve growth factor receptors, TrkA and p75, on normal and injured cultured SCG neurons

I. Kulbatski, C.H. Tator (Toronto, CDN)

Neuronal sensitivity to nerve growth factor (NGF)may be based, in part, on the distribution of the NGF receptors, TrkA and p75. Generally, NGF receptors are highly regulated following injury, undergoing upregulation in motor neurons and downregulation in sensory neurons. Studies on the growth and survival requirements of cultured sympathetic ganglia cells have shown that NGF is only needed at the distal neurites. Sympathetic neurons may exhibit such NGF requirements based on the distribution of TrkA and p75. Moreover, following injury, such requirements may change based on the regulation of TrkA and p75. We are investigating whether the distribution of TrkA and p75 on the normal cultured superior cervical ganglion (SCG) neuron is a reflection of the needs for growth and survival, and if injury alters this distribution. We are testing the hypotheses that the distribution of TrkA and p75 on the uninjured neuron is predominantly on the distal neurite, and that neuritotomy leads to upregulation of TrkA and p75 on the distal neurite. The antibodies MC192 (gift from Dr. P. Barker) and RTA (gift from Dr. L. Reichardt) have been used to immunocytochemically localize p75 and TrkA, respectively. Two visualization methods- DAB (phase microscopy) and texas red(fluorescence microscopy)- are being used. To date, our results show that p75 and TrkA are distributed ubiquitously along the soma and neurites of uninjured SCG cells and that the relative distribution of p75 does not change after injury. In conclusion, the distribution of p75 at a gross level on normal and injured SCG cells is not spatially segregated.

Poster Session 6

Ionic homeostasis and membrane pathology

P222. Level of the neuronal free Ca2+, BBB permeability and ultrastructure in head injury with secondary insults

Z. Fei, X. Zhang (Xi'an, PRC)

Objectives: To study changes of the free Ca2+, BBB permeability and ultrastructure in brain diffuse axonal injury (DAI) with secondary brain insults(SBI).
Methods: Sixty-six SD rats were randomized into control and another five injury groups that were 0.5 h, 2 h, 12 h, 24 h, 48 h post trauma. The animal models

of DAI and DAI with SBI were made as we described before. Fluorescence probe Fluo-3/Am was used to measure free Ca2+ in neurons. Laser scan microscopy was used to detect fluorescence intensity. After the animals were anesthetized, Lanthanum nitrate blending liquid was used for intracardiac perfusion. Under transmission electron microscope (TEM, JEM1200EX), the changes of cerebral ultrastructure and BBB permeability were observed.
Results: The fluorescence intensity is weak in control. Concentration of free Ca2+ in neurons was obviously increased at 30 min after brain injury, reaches a peak at 12h till 24h($p<0.01$), and decreases at 48 h after injury. In DAI alone group, BBB tight conjunction opening and particles of Lanthanum nitrate outside the vessels were found at 30 min after injury, and peaked at 24 h. In DAI with SBI, the damage of ultrastructure and BBB permeability was more severe than that in DAI alone group at the same time interval. The tendency of fluorescence concentration curve was alike basically for both kinds of brain injury. But the intensity of fluorescence in DAI with SBI was higher than that in DAI alone group at the respective time interval ($p<0.05$).
Conclusion: In DAI alone and DAI with SBI, Ca2+ overload and BBB permeability changes act on each other and both of them play important roles in the aggravation of secondary brain damages.

P223. Concurrent initiation of pro- and anti-apoptotic mechanisms following diffuse traumatic brain injury in rats
R. Vink, G.P. Hamlin, S.M. Chapman, I. Cernak (Townsville, AUS)

Neuronal cell death resulting from TBI appears to have two primary forms – necrosis and apoptosis. The cells that die within the first 24 h after the traumatic event are thought to do so primarily by necrosis. The cells that die at much later points (24-72 h) are thought to undergo a process known as apoptosis. It is now clear that apoptotic cell death may continue well after trauma and appears to contribute to the functional neurological deficits that follow from such injuries. While a number of studies have examined apoptotic events in TBI using more focal models of trauma, few studies have characterized apoptosis in a brain injury model that causes a significant degree of diffuse axonal injury. Such characterization is essential from a clinical viewpoint since diffuse axonal injury is a major component of human head injury. The present study examines the expression of active and pro-active caspase-3, and the bax, bcl-2 and bcl-x members of the bcl-2 family, to characterize the temporal profile of apoptosis following severe diffuse traumatic brain injury in rats. Pentobarbital anesthetized male Sprague-Dawley rats were injured using the 2-meter impact-acceleration model of diffuse traumatic brain injury. After injury, diffuse trauma resulted in an increased bax expression followed by induction of caspase-3. The increase in caspase-3 was simultaneous with an increase in anti-apoptotic bcl-2 expression. Bcl-x levels were increased after induction of caspase-3 and the increased levels of bcl-x were sustained to the end of the 5-day observation period. TUNEL positive cells were detected at all time points when caspase-3 levels were increased. No TUNEL positive cells were detected at 7 and 14 days after injury. Increased active caspase-3 levels were significantly correlated with increased bcl-2 levels ($r=0.80$; $p<0.001$) suggesting that the apoptotic cascade after diffuse traumatic brain injury is a carefully controlled cellular homeostatic response.

P224. Study on curative effect of Nimodipine in experimental brain injury
S.-Y. Yang (Tianjin, PRC)

Objectives: To study the curative effect and mechanism of Nimodipine in experimental brain injury.
Methods: During experiment Nimodipine was i.v adminstered, then the methods of the electric microscopy, brain water content quantitation, calcium fluorescent indication, transcranial doppler (TCD) and intracranial pressure (ICP) monitoring were performed to evaluatate the curative effect of Nimodipine to experimental brain injury.
Results: After the treatment of Nimodipine, the level of neuronal cytosolic free calcium was decreased markedly, the pathological damages of brain tissue were lightened and the reduction of spasm of middle cerebral artery (MCA) was found. But the changes of ICP had no remarkable difference between treatment group and trauma group.
Conclusion: Nimodipine exerts its protective properties in brain injury through obstructing the series of pathological reactions induced by neuronal calcium-overload and reducing the spasm of brain vessel to improve brain blood flow.

P225. Magnetic resonance detectable lipid increases after cortical impact
C. Gasparovic, W.M. Brooks, D.M. Feeney, G. Golarai (Albuquerque, USA)

Cell membrane degradation is one of the many consequences of traumatic brain injury (TBI), producing high levels of free fatty acids, neutral lipids, and other lipolysis products. Magnetic resonance spectroscopy (MRS) has demonstrated great potential for the diagnosis of TBI by detecting various metabolic markers of injury. However, the observation of TBI-induced lipids by MRS remains speculative. We report that MRS-visible lipids markedly increase in rat brain

after impact injury. Sensorimotor cortices of adult rats were injured by weight drop (1). 1 and 7 d later, brain slices from the peri-injury region of the parietal cortex, or from a homologous region in uninjured rats, were superfused and examined by short-echo 1H MRS (2). The sampled region was ventral to the contusion cyst and surrounding edematous tissue, as characterized by histology and/or MRI on separate animals. Difference spectra analysis revealed large increases in signals from the methylene (1.28 ppm) and methyl (0.90 ppm) protons of fatty acyl groups (Fig. 2). The ratio of the methylene peak height in the difference spectrum to the creatine (3.04 ppm) peak height in the original spectrum was -0.001±0.101 (n=5) in samples from uninjured rats, 0.322±0.226 (n=5) in samples 1 d after impact, and 0.433±0.257 (n=3) 7 d after impact. The control group mean differed significantly from the injury group means in t-tests ($p<0.05$). In a previous study on this injury model (3), lipid signal increases in the occipital cortex, remote from the contusion site, were small relative to the peri-injury increases of the present study, suggesting a gradient in cellular injury. Together, these results suggest that 1H MRS signals from lipids in traumatically injured brain may be a useful clinical MRS marker of membrane degradation.
References: 1. Golarai G. et al., Behavioral, structural and physiological evidence for epilepsy after traumatic brain injury, submitted (2000). 2. Gasparovic C. et al., Brain Res Protocols, 4: 97-102 (1999). 3. Gasparovic C. et al., Decrease and recovery of N-acetylaspartate/ creatine in rat Brain remote from focal injury, submitted (2000).

P226. Apoptosis after experimental diffuse axonal injury

M. Crowe, D. Graham, T. Gennarelli (Milwaukee, USA; Glasgow, UK)

Apoptosis has been described in numerous animal models of neurological trauma such as ischemia, spinal cord injury and traumatic brain injury. However, no study has described the presence of apoptosis after traumatic brain injury that induced diffuse axonal injury (DAI) in non-human primates. We undertook a retrospective study of six non-human primate brains that were subjected to a single controlled head acceleration. Survival times were 5-8 days post-injury. Non-head-injured non-human primate brains (n=4) from archival materials served as controls. Of the experimental animals, four sustained prolonged coma and pathological evidence of severe DAI. Two animals experienced brief periods of post-traumatic unconsciousness and had minimal DAI. Using paraffin-embedded sections (8 microns thick), we examined cerebral hemispheric white matter for morphological evidence of apoptotic cells using a combination of nuclear dye staining (Hoechst 33342) and a modification of the terminal deoxynucleotidyl-transferase mediated dUTP-biotin nick end-labeling (TUNEL) assay. No cells displaying an apoptotic morphology were visible in the two subjects that showed mild DAI or in any of the control animals. Cellular labeling with the TUNEL assay was visible in 3 of the 4 animals that had severe DAI. In two, TUNEL labeling was confined to a small area of contused cortex and was associated with inflammation resulting from the contusion. In the third subject, extensive cellular labeling with both the TUNEL assay and the Hoechst 33342 nuclear dye was present in the cingulate cortex. While labeled cells demonstrated morphological characteristics consistent with apoptosis (i.e. small, condensed and fragmented nuclei), many also elaborated numerous neuronal-like processes. Despite extensive DAI in the white matter, little-to-no evidence of apoptosis was visible in the white matter and no TUNEL-positive cells were seen in the cortex associated with these white matter changes. This retrospective study indicates that at one week after injury, apoptosis does not appear to play a significant part in the response to DAI in the non-human primate brain.

P227. The effect of lazaroid, U83836E, on Na+/K+ and Ca2+/Mg2+ ATPase activities, blood-brain permeability and edema in traumatized rat brain

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In the present study, we aimed to test the time-course changes of blood- brain barrier (BBB) permeability, tissue water content, synaptosomal Na+/K+ and Ca2+/Mg2+ ATPase activities, and tissue malondialdehyde (MDA) levels as an indicator of lipid peroxide, in the traumatized hemisphere. We also aimed to evaluate the efficacy of lazaroid, U-83836E, on these parameters. In adult rats, open cerebral trauma was induced by dropping a 10 g brass rod from a distance of 7.5 cm onto the right cerebral cortex. Two protocols were followed. In the first, animals were used for determining time course tissue changes of synaptosomal Na+/K+ and Ca2+/Mg2+ ATPase, and MDA levels, in the traumatized hemisphere. A sham-operated group (n=7) was used as a control. The second, third and fourth groups (n=9 in each) were sacrificed at 2, 6m and 24 hours after trauma. A fifth group of animals was treated intraperitoneally with U-83836E at a dose of 10 mg/kg 0.5 h before trauma and decapitated at 24 h. In the second protocol, animals were used for determining changes in BBB permeability and tissue water content. The animals were given Evans blue dye (%2, 5ml/kg) intravenously, which was allowed to circulate for 1 h before the decapitations at the specific times of 2, 6 and 24 h after trauma. Two groups of animals

was treated with U-83836E and sacrificed at 2 and 24 h after trauma. Edema in injured hemisphere was determined by wet weight to dry weight ratios. BBB permeability was measured by spectrophometric quantitation of Evans blue in dried tissue of the injured hemisphere. The loss of Na+/K+ ATPase activity at 2, 6 and 24 h were 36.46 % ($p<0.001$), 37.97 % ($p<0.01$) and 37.78 % ($p<0.01$) of control, respectively, while loss of Ca2+/Mg2+ ATPase activity was 29.41% ($p<0.01$) at 2 h, 16.70 % ($p>0.05$) at 6 h and 42.53% ($p<0.001$) at 24 h after trauma. MDA levels were 122.27 % ($p<0.05$) of control at 2 h, 135.92 % at 6 h and 209.01 at 24 h posttrauma. ATPase returned to close to control levels ($p>0.05$) by the effect of U83836E. U-83836E also inhibited lipid peroxidation significantly ($p<0.001$). Extravasation of Evans Blue into traumatized hemisphere was maximal at 2 h ($p<0.001$), starting to decline at 6 h ($p<0.01$) and back close to control levels at 24 h. Trauma induced an increase in the water content of the injured hemisphere; 0.54 % ($p>0.05$) at 2 h, 1.32 % at 6 h ($p<0.001$) and 2.09 % ($p<0.001$) at 24 h. U-83836E showed no effect on either BBB permeability or tissue water content at 2 h, although at 24 h, while there was no effect on BBB permeability ($p>0.05$), the edema was reduced ($p<0.05$). Our results suggest that BBB damage contributed temporarily to edema development in severe TBI. Hence, edema would seem to be largely of the cytotoxic type in this model. The protective properties of U-83836E, as a brain-penetrable antioxidant, on plasma or organelle membrane integrity, and the amelioration of edema at 24 h, support this theory.

P228. Early expression of glutamate transporters in microglia after controlled cortical impact injury in the rat

F. van Landeghem, J. Stover, W. Brück, A. Unterberg, A. von Deimling (Berlin, D)

The pathological mechanisms underlying secondary neuronal damage following controlled cortical impact injury (CCII) have not been definitely determined. Extracellular glutamate concentration increases after CCII and may result in excitotoxic neuronal death. Active glutamate uptake is essential for normal transmission at glutamatergic synapses terminating the synaptic signal by removing this neurotransmitter from the synaptic cleft. As shown previously, glutamate transporter expression is seriously altered after CCII. Furthermore, a posttraumatic de novo expression in microglia could be observed. After contusion to the left temporoparietal cortex in 35 adult male Sprague-Dawley rats, different posttraumatic survival periods (4, 8, 24, 48, 72 and 168 hours) were investigated. Immunohistochemical examinations were performed using antibodies to GLAST, GLT-1, EAAC1, MHC class I and II, GFAP, Griffonia simplicifolia isolectin B4 and ED1. Immunohisto-chemistry demonstrates a marked reduction in astrocytes expressing glutamate transporters. 8 hours post injury, ramified microglia expressing GLAST, GLT-1 and EAAC1 are detected within hippocampus and perilesional cortex. The number of immunopositive microglia increases in the traumatized hemisphere until reaching a plateau at 24 hours. MHC class I antigen is first detected in scattered glial cells in the traumatized hemisphere 1 hour post injury. Increasing over the examined survival periods, MHC class I expression reaches a plateau at 48 hours. In contrast, MHC class II expression is observed in scattered glial cells of the subcortical white matter 16 hours post injury. At 168 hours, a moderate number of immunopositive cells are seen in the traumatized hemisphere, preferentially with perivascular localisation. In conclusion, our findings suggest that reduced glutamate transporters contribute to secondary posttraumatic injury and indicate an important transient role of microglia in posttraumatic regulation of synaptic transmission.

P229. DNA-bound poly-ADP-ribose polymerase activity during reperfusion after transient forebrain ischemia in gerbils. Effect of 3-aminobenzamide

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Our previous studies indicated that Nitric Oxide (NO) released during reperfusion after transient forebrain ischemia in gerbils was responsible for the degeneration of some population of neurons and that the effect was mediated by activation of free radical formation and lipid peroxidation. Moreover in cerebellum this type of ischemia evoked activation of glutamatergic system stimulating NO dependent signal transmission. The inhibitor of neuronal isoform of NOS 7-nitroindazol had a significant ameliorating effect on biochemical alteration and protective effect against death of selected small population of neurons. Our results suggested that probably other factors next to NO were involved in neurodegeneration following ischemic insult. The molecular targets for free radicals and cytotoxins is DNA damage leading to activation of DNA bound poly(ADP-ribose) polymerase (PARP) which is involved in DNA repair. However excessive activation of PARP can deplete NAD and ATP leading to cell death. Our studies indicated biphasic alteration of PARP activity during reperfusion after 5 min ischemia in gerbils exclusively in hippocampus with no significant changes in brain cortex. Significant stimulation of PARP activity was observed 15 min after ischemia in hippocampus, 2 hours after ischemia activity of PARP was noticably decreased and after 4

days subsequently increased. The inhibitor of PARP, 3-aminobenzamide (Aß) in a dose of 30 mg per kg b.w. injected intravenously protects neuronal cells against death. Transient forebrain ischemia (3 min) caused 76 % cell death, 3-AB administered 10 min before and shortly after ischemia resulted in survival of 58 and 64 % of neurons respectively as compared to control in corresponding CA1 fields of ischemic hippocampus. The effect of inhibitors significantly depends on dose and time of administration. The results indicated that specific inhibitor of PARP applicated after ischemia may provide a benefit in protection of neurons against degeneration evoked by forebrain ischemia and may be useful in treatment of postischemic encephalopathy.

Poster Session 7
Neurotrauma models 2

P230. Upregulation of neuronal amyloid precursor protein (APP) and APP mRNA following magnesium sulphate (MgSO4) therapy in traumatic brain injury

C. Van Den Heuvel, J.W. Finnie, P.C. Blumbergs, J. Manavis, N.R. Jones, P.L. Reilly, R.A. Pereira (Adelaide, AUS)

The aim of this study was to topographically assess and quantitate the effects of post traumatic intravenous MgSO4 on neuronal perikaryal APP antigen and mRNA expression in sheep brains 2 hours after a controlled focal head impact. The percentage brain area with APP immunoreactive neuronal perikarya was 71%, 56%, 27.5% and 5.5% respectively in MgSO4 treated head injured animals, head injured animals without any treatment, MgSO4 treated non-injured animals and non-injured non-treated control sheep. Although there was no statistically significant difference in APP immunoreactive neuronal perikarya in the MgSO4 treated head injured group (mean 71%) compared to the head injured group without any treatment (mean 56%), northern analysis showed that there was a 2.3 fold increase in APP mRNA in the thalamus of treated impacted animals compared to untreated impacted animals ($P<0.005$). However, MgSO4 treated non-impacted control animals also showed a 1.6 fold increase in APP mRNA compared to untreated non-injured controls ($P<0.005$). This study demonstrates for the first time a link between MgSO4 therapy and APP expression. MgSO4 therapy results in upregulation of neuronal APP mRNA and APP expression which is quantitatively greater following a focal head impact.

P231. Characterization of a novel brain trauma model in the mouse

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We describe a novel methodological approach to induce cold lesion in the mouse as a model for human cortical contusion trauma. To validate its reproducibility and reliability, dexamethasone (Dxm) was repeatedly applied to demonstrate possible antiedematous drug effects. Under halothane anesthesia the dura was exposed via trephination. A pre-cooled (-78°C) copper cylinder, 3 mm in diameter, was impressed with a micromanipulator 1 mm onto the dura for 30 s under microscopic control. Body temperature was held constant at 37 °C throughout the procedure. Blood pressure, measured by a modified photosensor-monitored tail-cuff method, and arterial acid-base status were not significantly altered when analyzed before and after cold lesion and prior to sacrifice. However, there was a marginal mixed respiratory and metabolic acidosis. The antiedematous action of Dxm was studied in 4 standard pre- and post-treatment paradigms: 2 x 0.5 mg/kg (II), 2 x 12.5 mg/kg (III) and 4 x 6.25 mg/kg (IV: 3 x pre-, 1 x post-treatment; V: 1 x pre-, 3 x post-treatment). Physiological saline injections served as controls. High doses of Dxm (III-V) significantly attenuated the cold lesion-induced loss of body-weight. Dxm treatment also resulted in a reduction of brain water content (III; $p<0.05$), and brain swelling (IV; $p<0.05$) in the lesioned hemisphere, relative to controls. In conclusion, we characterized a novel cold lesion model in the mouse to mimic traumatic brain injury and the beneficial effect of Dxm treatment on the extent of brain edema.

P232. A culture system for assessment of nerve cell responses to mechanical compression injury

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A culture system was developed for the assessment of effects of mechanical static compression for defined time periods to differentia-ted neuron. The load was achieved with a device consisting of a weight-loaded rod in a guide tube, delivering compression when pushed onto the cells in a marked area (diameter 3.0 mm) on a culture dish. Phase contrast, time lapse video recordings documented the morphological reactions. Confocal fluorescence microscopy were used for assessing cytoskeletal changes and the nerve cell surface marker NCAM. The fraction of dying and of dead cells were revealed by release of LDH, labelling for apoptosis, analysis of DNA fragmen-tation and fluorescence assay for live/dead cells.

Differentiated neurons appeared damaged after 0.8 g loading for 15 sec. The severity of the cell damage increaseced with the compression. Apoptotic cells could be demonstrated in 24 h. Nuclear damage was revealed by propidium iodide and Hoechst 33258 labelling. Neurons, adjacent to the compression zone, displayed increased immediate early gene expression, i.e. of c-jun, and could as well appear apoptotic. Neurofilaments accumulated in the bulbs of the twisted axons. Axons in and at the compression zone displayed bulbs and segmentation already 0.5 h after 2.8 g load for 15 sec. The NCAM expression was not changed within 24 h after * 2.8 g injury for 15 sec. In conclusion, our nerve cell culture model of neuronal compression injury simulated many of the features at the cellular level of the reactions elicited by a trauma to the brain in vivo without being complicated by e.g. secondary hypoxia, oedema formation and accumulation of waste products. It may prove useful for rapid screening of e.g. treatment strategies for CNS trauma.

P233. A new model for intracranial compliance measurements after cortical contusion trauma in the rat

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Introduction: Measurement of intracranial compliance (IC) is believed to offer early detection of changes in intracranial dynamics following traumatic brain injury (TBI). The introduction of the Spiegelberg compliance device has made IC monitoring in patients possible. The aim of this study was to develop a clinically relevant experimental TBI model where IC can be changed and measured with normal ICP maintained.
Methods: Twenty male Sprague-Dawley rats were intubated and mechanically ventilated. Bilateral parietal craniotomies were made. Animals were subjected to right-sided sham trauma or controlled cortical impact by the weight drop method (21 g from 35 cm; 1.5mm compression). Intracranial volume was reduced by gluing rubber film (RF) inside the bone flaps before they were replaced after trauma. ICP measurements were made with an intraventricular catheter in the left ventricle. A micordialysis probe was placed in the trauma region. Six bolus injections of sterile saline (50 microliter) were given in the ventricle with 10 minute intervals. Results from the bolus injections were used for calculation of pressure volume index (PVI). The animals were divided into 4 groups: Gr.1 (sham), Gr.2 (sham + RF), Gr.3 (trauma), Gr.4 (trauma + RF), n=5 / group.
Results: PVI showed a significant reduction related to a layer of RF in both sham and trauma groups (means ± sd for each group): Gr.1 versus Gr. 2 [0,0858+0,0131 ml to 0,0778+0,0098 ml] ($p<0.05$), Gr.3 versus Gr. 4 [0,0913+0,0148 ml to 0,0746+0,0169 ml] ($p<0.05$). ICP was within normal limits in (means ± sd for each group): Gr.1: 11.7+2.3 mmHg, Gr.2: 11.1+2.9 mmHg, Gr.3: 13.8+3.1 mmHg, Gr.4: 11.2+2.4 mmHg. There was no significant difference in PVI and ICP between sham and corresponding trauma groups. Microdialysis samples have not been analysed in this study.
Conclusion: This model allows experimental studies of TBI where IC can be changed and ICP is normal. The model also allows biochemical studies of the extracellular fluid with microdialysis.

P234. Endogenous hormonal neuroprotection following traumatic brain injury in intact females versus male or ovariectomized female rats

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Several investigators have reported a positive effect of hormonal treatment in a variety of injury models. The purpose of this study was to determine whether endogenous hormones circulating within females would provide histopathological protection following traumatic brain injury (TBI) compared to ovariectomized females and male rats. Intubated, anesthetized animals (225-395g) were subjected to a moderate parasagittal fluid percussion (FP) pulse (1.7-2.2 atm). Female rats were injured in either the proestrous (TBI-FP, n=18) or nonproestrous phase of their cycle (TBI-FNP, n=19). Another group of female animals underwent FP injury 10 days following ovariectomy (TBI-OVX, n=10). Male rats also were subject to TBI (TBI-M, n=17). Animals were sacrificed 3 days after TBI for quantitative histopathological analysis. There was a significant overall difference in contusion volume between groups ($p<0.03$). Posthoc analyses showed a significant ($p<0.05$) decrease in contusion volume in both intact female groups compared to males. In addition, TBI-FNP contusion volumes were significantly ($p<0.04$) smaller than the ovariectomized females. There was no significant difference between TBI-M and TBI-OVX animals or between the two intact female groups. These findings indicate that circulating hormones within the intact female rat provide neuropathological protection following FP injury compared to TBI males and ovariectomized females. Furthermore, the time of the cycle at which the female rat is injured does not appear to effect the size of the contusion. Although it is unknown if the protection observed in females is due to the activity of a single or combination of circulating hormones in this model, these data do demonstrate the presence of endogenous hormonal neuroprotection in the female rat following TBI. (Supported by NIH 30291 and Eli Lilly and Company.)

P235. New experimental method of modelling of brain ischemia in rats

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Brain ischemia is actual problem neurosurgery. Purpose of this work was elaboration of new minimal invasive model of brain ischemia which maximally forthcoming to natural conditions of ischemic stroke development The work is carried out on 20 rats, weighing 150-200 g. Brain ischemia was performed by combination of intraluminal occlusion of middle cerebral artery (MCA) with bilateral carotid artery (CA) occlusion. Occluder was introduced through common CA (CCA) and internal CA before MCA at a 17-20 mm depth. All surviving rats were killed 72 hours after the onset of occlusion. The neurologic examinations were performed 1,2 and 3 days after onset of occlusion and neurologic findings were scored on a six-point Longe scale (in our modification). Histological examinations were performed in brain sections stained by hematoxylin-eosin and Nissle. Morphological damage was graded on a six-point scale (Werner C., 1990). In the 6 rats were performed only CCA occlusion without intraluminal occlusion of MCA. All rats exhibited clinical and neurologic deterioration characterised by Horner's syndrome, disturbance of the eye blood supply, sensibility, reflexes, ataxia, tremor, and hemiparesis (neurological scale 5,1 balls (N=14). Histologic examination showed varying degrees of common ischemic damage of the frontoparietal cortex and medial segment of the caudate nucleus (hystological scale 2,2 balls (N=6)). In rats with only CCA occlusion without intraluminal occlusion of MCA neurologic and histologic examination showed no pathologic alterations. Advance of this model is minimal invasiveness, high reproducibility and maximal forthcoming to natural conditions of ischemic stroke development. Occluder's material and method's features prevent brain vessels from damage and cause occlusion in 100 % cases.

P236. A novel approach for cerebral blood volume measurement in traumatic brain injury using magnetic resonance and a contrast agent with long intravascular half life

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MRI may be a useful diagnostic tool to evaluate regional cerebral blood volume (rCBV) in traumatic brain injury (TBI). This is currently done by rapidly injecting Gadolinium-chelates with short intravascular half-life, requiring high temporal resolution. We hypothesise that rCBV mapping using a contrast agent with long intravascular half-life could be done at steady-state, thereby facilitating the diagnostic method. 6 rats were subjected to a moderate to severe (2.5-2.7 atm) lateral fluid percussion injury and then tranported to the MRI-facility. An ultra-small superparamagnetic iron oxide contrast agent was injected as a bolus. The MRI was performed within 7 hours post-trauma using a dynamic T2* weighted gradient echo acquisition. Diffusion weighted imaging was used to guide the placement of the regions of interest. The baseline images before and after bolus administration were averaged separately and the difference between the signal intensities ipsilaterally and contralaterally to the injury was compared. The results show a significant difference in signal drop between the baseline images pre and post bolus passage in the injured vs. normal regions (3.7% vs. 16.3%, $p<0.005$). The exact correlation between the signal change and blood volume requires further investigation.
Conclusion: Even though no quantitative comparison with other methods was done, a reduced rCBV was clearly detected in the injured region. Steady-state rCBV imaging of TBI can be performed using an intravascular contrast agent with long half-life, improving the MRI paradigm for rCBV investigations.

P237. Mechanical, functional and morphological characterization of a rat model of mild / moderate diffuse brain trauma

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Although mild and moderate degrees account for the majority of traumatic brain injuries (TBI), only few studies have focused on these categories in experimental models. We have adapted a model of closed diffuse TBI in rats to low impact-acceleration forces and characterized mechanical reproducibility as well as functional and morphological changes. TBI was performed under volatile anaesthesia by dropping a brass weight (450 g) onto a metal disk fixed on the skull of adult male Sprague-Dawley rats. Animals (n=31) were subjected to injuries of different severity (25, 50, 100, or 150 cm dropping-height; sham-surgery). Impact-acceleration was measured below the head using a quartz accelerometer (sampling rate 500 kHz). Reflexive outcome was assessed in a battery of 9 tests up to 30 minutes after injury. Histo-morphological examination was done after 24 h of survival. Peak acceleration (25 cm: 114 G, 50 cm: 202 G, 100 cm: 323 G, 150 cm: 462 G; mean) strongly correlated with dropping-height ($r=.789$, $p<.001$). A linear correlation was also found between injury severity and neurological deficits for head support ($r=.51$, $p=.004$), escape response ($r=.55$, $p=.002$), and startle response ($r=.68$, $p<.001$). Additionally, significant differences were found between groups for

escape response (sham vs 100 / 150 cm) and startle response (sham vs 100 / 150 cm, 25 vs 150 cm, 50 vs 100 / 150 cm). The presence of superficial haemorrhage increased with trauma severity (Chi2 trend=11.7, p<.001). Since cognitive deficits are described in this model hippocampal damage was assessed by counting acidophilic neurones. No significant differences between groups were apparent in CA1-3, hilus, or dentate gyrus. However, reactive astrocytosis was induced by TBI in the hippocampus as visualised by immunohistochemistry (GFAP). No significant hepatic or pulmonary changes were observed. The rat model of mild and moderate TBI leads to reproducible trauma as quantified by acceleration measurements. However, neurological impairment was detected only after more severe injury, and HE-stains could not differentiate reliably between severities. But a cellular response has to be assumed due to glial changes. More subtle morphological and long-term functional assessment are required for further studying mild TBI in this model, similar to the situation in humans.

P238. Development of a biomechanical model for the prediction of cortical contusions in human head injury

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Since cortical contusions are believed to result directly from cortical impact, the appearance and extent of contusions should be related to the translational movement of the brain within the skull. A simple biomechanical model was developed to calculate translational displacement of the brain during the impact. For a series of bicycle accidents information was collected on the accident circumstances and the contusions seen on CT head scan were analysed. The aim of the study was to examine the predictive value of the model. A series of 44 patients with severe head injury after a bicycle accident, not involving a motorized vehicle, was examined. In 20 cases it was possible to make a good estimation of the mechanical parameters of the event. In 10 of the 20 cases the (maximum) volume of contusions could be estimated. A head model was developed using a one-dimensional spring-damper system with two masses: head and brain. The impact on the head was calculated by modelling body and head as two rigid masses connected by a hinge. The vertical component of the impact at the impact site was used as input to the head model. The maximum relative brain displacement was then calculated. The relation between this parameter and the maximum volume and number of contusions was examined. The (fictive) maximum relative brain displacement ranged between 37 and 95 mm. A positive correlation could be found with the total contusion volume (R2=0.52), with number of contusions (R2=0.25) and with the volume of the coup contusions (R2=0.41). Using physical parameters from real bicycle accidents as input for a one-dimensional spring-damper head model, calculated brain displacement correlated well with the maximum volume of cortical contusions. Though these values of displacement should be considered fictive, they can be regarded as a measure for the severity of the contact between skull and brain. So they can serve as a criterion for prediction of contusions in experimental head injury.

P239. The influence of cerebral perfusion pressure on the development of traumatic brain edema in rabbits

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Objectives: To investigate the influence of cerebral perfusion pressure (CPP) on the development of traumatic brain edema in rabbits injuried by decelaration.
Methods: 30 rabbits were divided into 5 groups: group 1: Control (no head injury); group 2: CPP 90–110 mmHg; group 3: CPP 70–80 mmHg; group 4: CPP 60–70 mmHg; and group 5: CPP 35–45 mmHg, each consisting of 6 rabbits. The severe head in jury of decelaration in group 2 to 5 was produced by application of impact force of 1100±100 N. The CPP control was achieved by regulating blood pressure with vasopressors and vasodilator at 80 minutes postimpact. The intracranial pressure (ICP) during the peroid of 1 to 6 hours postinjury and the water content of brain at 6th hour after in jury were investigated.
Results: The physiological responses of head injury.raised ICP,increase of water content of brain, traumatic subarachnoid hemorrhage,and brain contusion were noted in all of the head-injuried animals,the pathological lesions(contusions) were presented from two to three cerebral lobes.The water content of brain in group 5 (81.19±0.74%) and group 2 (81.35±1.02%) increased more than that in group 3 (80.27±0.48%) and group 4 (80.31±0.70%) (P<0.05), while the ICP in group 2 and group 5 didn't show a statistic higher than that in group 3 and group 4.
Conclusion: there is the least brain edema at 6 hour postinjury in the severely head-injuried rabbits of the CPP maintained between 60 mmHg and 80 mmHg. Both maitaining CPP above 90 mmHg by raising blood pressure and CPP falling to 50 mmHg or less promote tranmatic brain edema. This finding imply that it is suitable for us maitaining CPP around the low end (60–80 mmHg) of the normal CPP of 70–100 mmHg in early period of severe head injury of human.

Key words: Traumatic Brain Injury, Cerebral Perfusion Pressure, Brain Edema, Intracranial Pressure, Rabbits.

P240. Upregulation of ICAM-1 and sensory motor dysfunction are independent of neutrophil accumulation after experimental traumatic axonal injury in rats

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Diffuse axonal injury is present in most of the patients with severe traumatic brain injury and has been associated with bad outcome. In the present study we investigated whether expression of ICAM-1 occurs after experimental traumatic axonal injury (TAI) and is paralleled by neutrophil accumulation as well as neurological dysfunction. 44 adult rats were subjected to moderate acceleration-impact brain injury. Animals were sacrificed at ten different time points from 1 hour up to 14 days after trauma and their brains were analyzed immunohistochemically using a neutrophil-specific Ab RP-3 and a monoclonal anti ICAM-1 Ab, respectively. Expression of ICAM-1 was quantified by counting ICAM-1 positive vessels per visible field. For evaluating the neurological deficit various sensorimotor tests were applied at days 1, 3, 7, 14, 21, 28 on 12 traumatized and 12 sham operated animals. An increase of ICAM-1 positive vessels ranging from 2- to 4-fold was observed in frontal and basal cortex, corpus callosum, thalamus and caudate putamen during the first 24 hours after trauma compared to controls. Maximal intensity of staining up to 10-fold was detected at 4 days after trauma and by 1 week ICAM-1 expression decreased in all brain regions to normal levels. No neutrophil infiltration could be detected. In all neurological tests, a significant difference between traumatized and sham operated animals was found from day 1 after trauma (grasping reflex of the hindpaws: $p<0.001$, vibrissae-evoked forelimb placing: $p=0.002$, lateral stepping: $p=0.037$). While the traumatized animals showed full recovery of function at vibrissae-evoked forelimb placing and lateral stepping by 21 days post-lesion, the deficit at grasping reflex of the hindpaws remained impaired during the whole study period. These results indicate that upregulation of ICAM-1 and sensorimotor dysfunction after TAI are independent of neutrophil accumulation.

P241. Correlation between neurological severity score and T2- weighted MRI in head injured mice

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Objectives: 1.To develop a simple neurological severity score (NSS) in head-injured mice, for quantification of damage and prediction of prognosis. 2. To obtain T2-weighted MR images, in-vivo, and to correlate the radiological damage with NSS.
Methods: 45 head-injured male sabra mice, weighing 35-45g, underwent head-injury by a weight-drop device, mimicking the “focal head-injury” in humans. A 10 point NSS method, that included tasks reflecting motor function, balance and alertness, was developed. NSS at 1h (NSS1) post-injury (PI) was determined, and mice were divided to 3 groups: Group A (n=18) for neurological evaluation, by repeated NSS measurements, at 24 h, 48 h, 7 and 14 days PI. Recovery was calculated individually for each mouse by subtracting NSS at any time point from NSS1. Group B (n=16) underwent T2-weighted MRI studies within 24h after CHI. The extent of radiological abnormalities, based on their size and location, was correlated with NSS1. Group C (n=11) was sacrificed after determining NSS1, to measure the extent of anatomical cortical damage (trauma intensity) in relation to NSS1.
Results: NSS1 was predictive of both mortality and morbidity. Mean NSS at 1h and 14 days after injury (6.381.34 and 2.441.01, respectively) correlated with $R=0.62$, $p<0.05$. All mice with NSS1 of 9-10 points died within 48h. NSS1 also correlated with size of pathological signals on T2-weighted MR images ($R=0.8$, $p<0.001$). Posterior and medial sites of radiological damage were associated with worse neurological performance. NSS1 also correlated with anatomical macroscopic cortical injury ($R=0.86$, $p<0.05$). MR images demonstrated high rate of post-traumatic hydrocephalus, and in some mice detected a pattern of diffuse axonal injury.
Conclusion: NSS is a reliable tool for evaluation of neurological damage in head-injured mice. Even though more complex MR studies may be used, a combination of NSS and T2-weighted images may be sufficient and useful in determining severity of head-injury, and in evaluating recovery along time. Such a setup, enabling repeated in-vivo evaluation of the head-injured mouse, may be important in studies conducted with transgeneic mice and testing of novel neuroprotective treatments.

Poster Session 8

Hypothermia, stem cells and transplant-ation

P242. Neural cell transplantation in post-traumatic coma treatment

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The cell suspension of fetal neural tissues were grafted subarachnoidally via lumbar puncture to 26 patients with severe brain contusion (5 to 8 scale points according to the Glasgow scale of comas) on 18-29 days after trauma immediately following achievement of restoration of self-dependent respiration, normalization of system blood circulation and regression of brain edema and intracranial hypertension. The reaction for the transplantation in some patients was a transient hyperthermia up to 40*, which resolved in the following 4-8 h without any intervention. In 19 cases coming out of coma (GSC) was registered on 3 to 7 days after the transplantation. On 12-18 days all of those patients had the consciousness in a degree sufficient to be without assistance. No systemic complications were established in any case. The life quality of these patients followed up for 3 years, was quite satisfactory. Most of them came back to their previous professional activity. The observed effects of neural cell transplantation on coma patients are proposed to be mainly due to stimulation of brain nervous centers by products of grafted cells.

P243. Effect of selective brain cooling by intravascular perfusion of cold crystalloid solution on cold brain injury in dogs

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Objectives: Selective brain cooling is expected to have more cerebroprotective effects owing to no temperature limit. We have developed an extracorporeal selective brain cooling system by intravascular perfusion of cold crystalloid solution and evaluated its effects on traumatic brain injuries, comparing the extracellular concentration of glutamate and lactate in cooled and non-cooled dogs.
Methods: Microdialysis probes were implanted into the bilateral parietal cortices of beagle dogs. A cold brain injury was made in the right parietal cortex near the microdialysis probe after intravenous administration of Evans-blue (20mg/kg). Thereafter, cold Ringer's solution was infused into the right vertebral artery after ligating 3 other neck arteries to the brain in the cooling group (n=9). Brain temperature was maintained at about 25°C for 1 hour. Hemodiluted blood was dialyzed and rewarmed. In 7 non-cooled dogs, 3 neck arteries, except for one vertebral artery, were ligated for 1 hour after injury. Four hours after injury, the range of extravasation of Evans-blue was examined.
Results: The extracellular concentration of glutamate in the non-cooling group increased significantly in the injured hemisphere compared to the normal hemisphere ($P<0.03$). On the other hand, those in the cooling group did not increase bilaterally. The extracellular concentrations of lactate in the cooling group increased gradually during cooling. The extravasation of Evans-blue was significantly smaller in the cooling group than in the non-cooling group ($P<0.01$).
Conclusion: Selective brain cooling with cold crystalloid solution suppresses the excitatory neurotransmitter and reduces posttraumatic brain edema, although mild hypoxemia was anticipated during cooling.

P244. Effects of mild hypothermia on glutamate neurotoxicity in vivo – a study using retrograde microdialysis

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The effects of mild hypothermia on glutamate neurotoxicity were studied. Glutamate-induced cortical lesions were produced in hypothermic (32 degrees centigrade) and normothermic (37 degrees centigrade) rats by perfusion of a 0.5 mol/l (M) glutamate solution via a microdialysis probe. Histological changes were evaluated by hematoxylin-eosin (H-E) staining, and the volume of the lesion was quantified at 7 days. We also examined the effect of hypothermia on the diffusion of exogenously delivered material into the extracellular space using autoradiography of the perfused glutamate solution containing 14C-labeled sucrose. The volume of damage was decreased by hypothermia, irrespective of whether it was induced before or after glutamate perfusion. The volume of 14C diffusion was also decreased by hypothermia. Histological changes in normo- and hypothermic animals were studied using monoclonal antibodies against GFAP (for astrocytes) and ED1 (for macrophages) 0, 1, 3 and 5 days after glutamate perfusion. TUNEL staining was used for evaluation of apoptosis. In normothermic animals, macrophage proliferation was observed from 1 day after glutamate perfusion and had become prominent at 5 days. In hypothermic animals, macrophage proliferation was apparently decreased and delayed. GFAP-positive cells were observed in the ipsilateral cortex subjected to glutamate perfusion from 3 days. TUNEL-positive cells were observed at 1 day in normothermic animals and at 3 days in hypothermic animals. These results provide evidence that mild hypothermia decreases glutamate neurotoxicity. The change in glutamate diffusion in the extracellular space may be one mechanism by which mild hypothermia exerts its protective effect. Although the sequential histological changes in the two groups were basically the same, the present results suggest that mild hypothermia delays macrophage proliferation and apoptosis.

P245. Deleterious effect of hypothermia on the PaO2/FiO2 ratio of patients after traumatic brain injury

J.L. Nates, E.R. Miller, D. Springer, G.L. Clifton (Houston, USA)

Introduction: Moderate therapeutic hypothermia (33 °C) is considered safe and beneficial in the management of traumatic brain injury (TBI). Specific analyses of the effects of hypothermia on other body systems are limited.
Materials and methods: The National Acute Brain Injury Study: Hypothermia (NABIS:H) was a randomized multi-center study with 392 patients in two groups, standard management at normothermia (N) or standard management at hypothermia (H) for 48 hours. Patients age 16-65 were enrolled with a Glasgow Coma Score<8. Abbreviated Injury Scores (AIS>4) were used to exclude patients with significant multiple trauma. All patients were managed for at least 72 hours on the Roto-Rest Kinetic Treatment Table (KCI, Inc., San Antonio, TX). There was no difference in Glasgow Outcome Score at six months.
A subgroup of 40 patients from one center in NABIS:H was reviewed (H 19, N 21). Age, sex, diagnosis, injury severity score (ISS), fractional inspired oxygen concentration (FiO2), positive end expiratory pressure (PEEP), respiratory rate, blood gases, end-tidal carbon dioxide, PaO2/FiO2 ratio (P/F ratio), and complications were examined. Data were analyzed using an unpaired t-test, $p<0.05$ was considered significant.
Results: In this subgroup, the groups were well matched for age (H 27.3±12, N 30.4±12; NS), admission GCS (H 5.5, N 5.3; NS), Injury Severity Score (H 27.8±9, N 30.8±7; NS), although there were more males in the hypothermia group (H 84%, N 57%). There were no patients with ARDS, but pneumonia was common (H 73%, N 71%; NS). No statistically significant differences were found in PaO2, VT, pH, PaCO2. End tidal CO2 was notably lower in the hypothermia group on all four days ($p<0.05$). Differences were found for FiO2 (H 0.49, N 0.35; $p<0.03$) and PEEP (H 7 cmH2O, N 5 cmH2O; $p<0.04$) on Day 4. The P/F ratio was significantly decreased in hypothermia patients on Days 3 and 4 (Day 3: H 261, N 350; $p<0.002$; Day 4: H 229, N 363; $p<0.0000$).
Conclusion: Hypothermia was associated with a significant ventilation/perfusion mismatch, as indicated by the notable and significant decrease in P/F ratios during the first 96 hours after injury. However, no differences in respiratory morbidity were detected. Possible explanations include atelectasis, bronchorrhea, or fluid shifts.

P246. Chronic hypothermia induced protection of rat hippocampal CA1 neurons in diffuse traumatic brain injury coupled with hypoxia and hypotension

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Background: Previous studies have demonstrated that hypothermia is neuroprotective for cortical neurons 24-hours after diffuse TBI with secondary insults. The aim of this study was therefore to assess the influence of hypothermia on neuronal death chronically over 7-days post-injury.
Methods: 48 adult male Sprague-Dawley rats (340-375 g) were divided into four experimental groups: (i) Sham injury (ii) Impact Acceleration injury (450g/2m) (D-TBI) (iii) D-TBI coupled with 10 mins hypoxia and hypotension (D-TBI+HH) (iii) D-TBI+HH treated with hypothermia at 30°C for 60 min with rewarming over 2 hours. At designated time points (24h, 48h, 72h and 7 days), animals were sacrificed and neuronal damage assessed in the supraventricular cortex and CA1 region of the hippocampus based on pre-defined criteria.
Results: Secondary insults increased neuronal damage compared to trauma at all time-points ($p<0.05$). In the CA1 region secondary insults caused a biphasic pattern of neuronal death which peaked at 24 h (6.607 ± 4.303/hpf) and 7 days (10.38 ± 3.88/hpf). Hypothermia did not influence cell death in the supraventricular cortex at 48hrs, 72hrs, or 7 days, but did however provide nearly complete protection up to 72 hrs in the CA1 region, with neuronal damage equal to trauma alone ($p<0.05$). A small secondary increase in cell death occurred at 7 days in the treated group which was however significantly less than the untreated group (3.44 ± 1.29/hpf, $p<0.01$).
Conclusion: This study confirms that hypothermic neuroprotection following TBI with second insult is regionally specific. Neuroprotection in the cortex is short-lived, whereas protection in the CA1 region persists up to 7 days. Treatment immediately post-trauma protecting against secondary neuronal death provides supportive evidence that early tissue events program delayed cell death. These results have important implications for the therapeutic use of hypothermia.

P247. Effect of hypothermia with cold injury in mice using micro-dialysis monitoring system

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Objectives: The present study investigates the effect of hypothermia using real time micro-dialysis monitoring system.

Methods: Animals underwent 20 sec of cold injury by application of a metal probe cooled with liquid nitrogen. Hypothermia condition was 30°C for 6 h. Five groups were studied (n=5 in each group): hypothermia initiated immediately after injury; 2 h after injury; 4 h after injury; Mannitol injected at 2h following injury; and combined Mannitol injected (0.5g/kg) at 2h and hypothermia initiated 4h following injury. Cerebral extracellular glutamate were monitored during experiment. Twenty four hours after injury, histological changes in the peripheral cortex area of lesion (TUNEL staining) and water content in injured hemisphere were studied.
Results: Hypothermia initiated immediately, 2 h after injury and combined therapy had significant protective effects on neuronal damage and brain edema in 24 h after insult (both $p<0.05$ vs. no treatment). However, hypothermia initiated 4 h after injury failed to protect the neuronal damage and brain edema ($p<0.05$ vs. normal control). An increase of glutamate was observed about 30 min after injury. Hypothermia initiated immediately after injury group significantly suppressed glutamate release ($p<0.05$).
Conclusion: Immediate induced hypothermia has effect of suppression of glutamate release. However, delayed induced hypothermia might suppress the events following glutamate release in cold injury when treatment was applied within 2 h after the onset of injury.

P248. Cell based therapies offer exciting potential for attenuating injury and facilitating repair of the central nervous system
C. Watts, J.D. Pickard, S.B. Dunnett (Cambridge, Cardiff, UK)

To date there are no clinical trials of cell transplantation in patients with traumatic brain injury. Clinical transplantation programs in patients with Parkinson's and Huntington's diseases are based on extensive experimental data. Many of the principles involved in cell transplantation in these model systems are relevant to models of traumatic brain injury and may facilitate the development of clinically applicable strategies for CNS repair. Transplant-mediated recovery of function in rodent and primate models of basal ganglia degeneration (Huntington's disease) is dependent on reconstruction of neural circuits. Successful functional outcome is influenced by the choice of donor tissue, its preparation and delivery and the host environment. Using an excitotoxic model of basal ganglia degeneration in the rat, which spares fibers of passage, we have compared histological integration and functional efficacy of primary embryonic striatal tissue from different donor ages prepared as cell suspensions or tissue pieces. The tissue was implanted stereotactically one week after an excitotoxic insult. Survival and integration of striatal neurons was correlated with efficacy in a retrieval task. The selection of appropriate embryological material of a gestational age consistent with recognised patterns of neurogenesis of basal ganglia neuropil is a primary factor in the functional integration of cell implants in the injured brain. Immediate early gene activity in grafted cells in response to a physiological stimulus suggests graft-host connections may be functional. These findings are relevant to clinical application of these techniques in patients.

P249. Transplantation of human neural progenitor cells into hippocampal lesions following ischemic and traumatic brain injury in the rat
M. Koshinaga, Y. Katayama, M. Fukushima, T. Suma (Tokyo, J)

It has been demonstrated the EGF and FGF responsive neural progenitor cells can differentiate into neurons and glial cells. In the present study, in order to examine the feasibility of neural progenitor cells for reconstruction of the damaged neural circuits, we transplanted into the adult rat hippocampus following ischemic or traumatic injury, and examined for their survival and the differentiation in the host brain. Transient forebrain ischemia was induced by occlusion of the common carotid arteries together with exsanguination. The human fetus-derived neural progenitor cells were transplanted into the ischemic lesions in the hippocampus at 7 days following ischemia under immunosuppressive condition. In another experiment, the neural progenitor cells were transplanted into the corpus callosum following the stub wound injury in the hippocampus. In both experiments, the animals were sacrificed 7-14 days following transplantation. It appeared that the human derived neural progenitor cells could survive even in the rat brain under immunosuppressive condition. Most of the cell originated from the progenitor cells showed glial marker immunoreactivities, such as vimentin or glial fibrillary acidic protein (GFAP), and only small number of the cells showed immunoreactivity of neuronal marker, such as calbindin, parvalbumin, or MAP2, however they did not remain and replace the ischemically damaged pyramidal cell layer by newly produced neurons following forebrain ischemia. In contrast, transplanted cells easily migrated to stub wound lesion via the corpus callosum. The direction of the cell migration seemed to be influenced by the environment surrounding the cells. These findings indicated that in order to utilize the neural progenitor or stem cells as a donor cell for transplantation therapy, further studies will be needed to clarify the environmental cues for the replacement of lost neurons as well as the promotion of differentiation into the neurons in vivo.

P250. Effects of hypothermia on ATPase following brain trauma in the rats

H. Huang, D. Zhi, L. Zhang, Z. Wangyin (Tianjin, PRC)

Objectives: The present study was conducted to investigate the effects of hypothermia on the content of Na+-K+-ATPase.Mg++-ATPase and Ca++-ATPase in traumatic rats.
Methods: 60 Wistar rats were divided randomly into three groups: the non-traumatic control group (NTG), room temperature traumatic group (RTG) and hypothermia treatment group (HTG).The brain trauma was induced on the left cerebrum by the free settling mothod. The third group were treated with whole body cooling (head temperature 30°) for 1 hour,then rewarming by heating. The cerebrum tissue of three groups in 3 hours, 1 days, 3 days, 5 days, 7 days were obtained and ATPase activity of tissue homogenate were measured. Comparison of date was made by T test and the Analysis of Variables (ANOVA).
Results: (1) Compared with the control group, the activities of Na+-K+-ATPase in RTG and HTG stepwisely increased after 3 hours trauma, then markedly decreased in following days ($p<0.01$). This activities of HTG in 3 days were much higher than that of RTG (HTG 2.567 ±0.283/3 day; RTG 1.607 ±0.136/3 day; NTG 3.864 ±0.322; Unit: U/mgProtein/h); (2) Mg++-ATPase: The activities in both RTG and HTG were significantly decreased after 24 hours compared with NTG.The speed of decenting in HTG in 1 day and 3 days were much lower than that in RTG (NTG 4.015 ±1.226; RTG 1.342 ±0.352/1 day; 1.065 ±0.204/3 day; HTG 2.353 ±0.516/1 day; 2.16 ±0.418/3 day); (3) Ca++-ATPase:The activities of Ca++-ATPase in RTG was markedly decreased in 1 day compared with NTG (RTG 0.791 ±0.372 versus NTG 3.739 ±1.336). The activities of that in HTG were kept in normal until 3 days, which still much higher than that of RTG conclutions: 1. There was an irritable reaction of cerebrum tissue in the early brain trauma on Na+-K+-ATPase.Hypothermia had little effect on sodium pump; 2. Hypothermia could evidently increase the activities of Ca++-ATPase and Mg++-ATPase compared the room temperature traumatic group and had a significant regulation on calcium pump. 3. The sodium and calcium pump can be kept in stabe situation at lower activities after hypothermia. Hypothermia treatment could delay the injury time of cerebrum calcium pump and decreased the influx of calcium and cerebral edema.

Poster Session 9
Clinical trials and outcome measures

P251. Emergency management of craniocerebral firearm wounds

X. Zhang, Z. Fei, L. Fu (Xi'an, PRC)

Objectives: To improve the outcome of treatment in patients with craniocerebral firearm wound.
Methods: Prospectively and retrospectively reviewed a series of 93 patients presented to the Xi-Jing Hospital of Fourth Military Medical University with a diagnosis of craniocerebral firearm wound during a period of 29 years from July 1970 to July 1999. All the patients had acute craniocerebral firearm wound. Of these, it consisted of 81 males (87.1%) and 12 females (12.9%) ranging from 3 months to 58 years in age (median 24.6 years). The lesion included 16 tangential wounds, 58 tubular wounds and 19 through-and-through wounds. The cases were urgent and in serious and unstable condition. All the patients underwent surgical intervention and aggressive perioperative management in the neurosurgical intensive care, including resuscitative protocols.
Results: After emergency treatment and operation, 9 cases died (9.7%). Follow-up studies at three months postoperative showed that 56 cases (66.7%) had made good recovery. Rates of moderate disability, severe disability or vegetative state in this series were 19.0%, 10.7% and 3.6% respectively. Long term follow up studies (median 5.5 years) found that 42 (50.0%) were capable to resume their occupation.
Conclusion: Craniocerebral firearm wounds are often severe, needing urgent treatment for the patients. Timely, proper and thorough initial debridement are crucial for avoiding rapid neurological deterioration.

P252. Difficulties in implementing a standardized transfer policy in severe head injuries in Greece. A prospective appraisal of 220 patients

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Objectives: To examine the most important pre-hospital/hospital parameters in relation to the 6-month outcome in patients with severe head injury
Design: January 1998-April 2000. Prospectively recorded: type of injury, secondary episodes, admission GCS/pupils, CT classification, ICP monitoring, type of management. The 6-month outcome was assessed by an independent institute. Entry criteria: age>14 yrs, GCS<=8
Results: 220 consecutive patients. 76 % male. Mean age 43 years. 96% were intubated (59 % at first hospital). Pre-resuscitation hypoxia and hypotension was respectively as follows: (a) definite 7% and 7% (b) suspect 20% and 4% (c) no data 65% and 72%. GCS distribution (3-8) 8:8%; 7:34%; 6:17.6%; 5:12%; 4:18%; 3:10%. Marshall CT classification (I-VI) I:3%;

II:26%; III:31%; IV:1%; V: 29%; VI:10%. ITU admission 65%. ICP monitoring 19%. Evacuation of a hematoma 29%. Favorable 6-month outcome 24 % (GOS: I:61%; II:4%; III:11%; IV:7%; V:17%). The six-month outcome does not apply to the last 52 patients (after October 1999) the mortality remains stable
Conclusion: The complex geography of Greece (mountainous terrain, 75 inhabitated islands), combined with non-standardized record-keeping and incomplete national guidelines for transportation/initial management of the severely head-injured, are the main causes for the higher than the currently expected mortality/morbidity. The introduction of (a) national guidelines (b) data forms (c) progressive improvements in the motor network and (d) provision of all-weather ambulance helicopters, are expected to improve the outcome.

P253. The complex relationship between cognitive impairment and health related quality of life after closed head injury
B.O. Hütter, M. Weinzierl, J.M. Gilsbach (Aachen, D)

The assessment of health-related quality of life (HRQOL) in brain-damaged patients becomes increasingly important in clinical research and practice. However, not much is known about the relationship beween measures of HRQOL and the neuropsychological test performance. A series of 87 patients with an age mean of 37,8 years (25 females and 62 males) were studied one to two years after head injury by means of the Aachen Life Quality Inventory (ALQI) and neuropsychological tests including attention, figural and verbal short- and long-term memory, aphasia, concentration and spatial cognition). According to the Aachen Coma Scale (ACS), 13% of the patients had sustained a severe, 28% a moderate and 59% a mild head injury. Proxy-ratings could be obtained of 71 life partners of the patients. In the case of self-rated HRQOL, the statistically significant correlations were modest and ranged between r=0,26 (p<0,01) and r=0,46 (p<0,001). Functions of attention and memory had the closest relationship to HRQOL. The associations between the neuropsychological test performance and HRQOL as rated by the proxies were consistently weaker. Analyses of variance showed significant (p<0,05, respectively) effects of the degree of neuropsychological impairment. Multivariate analyses revealed figural short- and long-term memory and the simple reaction time as the most important predictors of the overall HRQOL sharing 27% of common variance while the psychosocial aspect of HRQOL was explained by the capacity of the figural long- and verbal short-term memory and finally the simple reaction time sharing 24% of common variance. The associations between HRQOL and the neuropsychological test performance are at best modest. The complex relationship between HRQOL and cognitive impairment should be explored in further studies investigating patients with focal brain damage.

P254. Early factors in outcome and outcome prediction for multiple trauma patients with severe head injury
L. Song, T. Xu, B.Y. Rong, H.L. Tian, J. Hu, B.X. Gu (Shanghai, PRC)

Objectives: To analyze the early factors in the neurological outcome of severe head injury in multiple traumatic patients.
Methods: 134 consecutive multiple traumatic patients with severe head Injury (Injury Severe Score (ISS) >16, Mean 26.8; Glasgow Coma Scale (GCS) Score <8, Mean 6.1) were treated at the department of Neurosurgery in our hospital between Jan. 1990 and Dec. 1999. All early clinic factors with easy observation in relation to the neurological outcome of the patients were studied, and the overall outcome was assessed at discharge using the Glasgow Outcome Scale.
Results: The functional recovery (good outcome or moderate disabillity) rate was 50.7%, with a mortality rate of 40.3% (the mortality rate of severe head injury of the same period in our department was 31.9%). The data revealed that the patients with lower admitted GCS score (<5), fixed dilated pupils, accompanied shock (systolic blood preassure <12 kpa), and accelerated respiration (the rate of per minute >25) have a higher mortality rate (p<0.01). In the meantime, other significant factors associated with marked increased morbidity and mortality were higher age (>60 years old), lower partial pressure of blood oxygen (PaO2 <8Kpa) and presence of acute diffuse brain swelling on computerized tomography (CT) scan (obliterated basal cistern or small bilateral ventricles, and subarachnoid hemorrhage). (P<0.05).
Conclusion: Based on this study, there were 4 early most important factors for poor outcome prediction in multiple traumatic patients with severe head injury. They were the GCS sore, pupillary size and light reaction, respiration rate and blood pressure on admission. Compared with severe neurotrauma, the multiple traumatic patients with severe head injury have a much poorer outcome, which correlates with early poor perfusion with blood and hypoxemia as well as the severity extent of primary and secondary brain injury.

P255. The importance of decompressive craniotomy in severe head injuries
P. Bartels, T. Haas, A. Gräwe, U. Meier (Berlin, D)

Neurosurgical therapy after traumatic brain injury aims to minimize secondary brain damage. Beside intensive conservative care with its pharmacological opportunities optimized for ICP and ptO2 in case of severe raised intracraniell pressure surgical interventions have to be considered. Apart from removal of space occupying intracranial hemorrhagies or reduction of cerebrospinal fluid volume by implanting an external ventricular catheter the creation of additional space by decompressive craniectomy is a last possibility. Since the opening of our hospital in September 1997 we treated 211 patients for severe head taruma until februaryy 2000. 28 of them undergone a decompressive craniectomy. The outcome after decompression depends on clinical status at admission, age and additional major injuries. According to the results of Gaab et al. the indications for a decompressive craniectomy are discussed. Taking our results into account patients until a biological age around 50 years profit from decompressive operations in case of conservativly uncontrolable generalized brain edema. With additional major injuries the age-border is to set with 30 years.

P256. Mental therapy in neurologic rehabilitation of arm function in sensory motor disorders
R. Miltner, U. Simon, V. Hoemberg, J. Netz (Düsseldorf, D)

Motor behavior is centrally represented and therefore accessible for specific cognitive processes like motor imagery. PET-studies and functional MRI-studies of the last few years have given evidence that the activation of cortical structures during imagination of movements are nearly the same as during the actual performance of the same movement. A two-phase cognitive therapy model was developed. The first phase intended to reestablish a cognitive representation of the movement to be trained by a complex multi-sensory feedback, the second phase consisted of a mental training supported by a video display. We investigated a grasping movement to a glass with the paretic arm in 23 patients after stroke in a chronic state (mean duration=44,5 months) and 14 patients after severe head injury (mean=36,8 months after accident). The cognitive therapy had a duration of 4 weeks, with daily sessions of about 20 minutes. Results showed a significant functional improvement of the grasping movement and a decrease of muscle tone in the paretic arm, surface sensation and selective force of hand and finger were improved as a trend only. The results of this study support the hypothesis that cognitive therapy is a valuable therapeutic measure in the therapy of patients with central paresis even in a chronic state.

P257. Frontotemporal brain lacerations
I. Poeata (Iasi, RO)

54 patients with clinical and images/surgical features corresponded with old concept of frontotemporal brain laceration are analized in order to better define this controversial entity according with the following concepts: 1. sulcal neurosurgery (silvian fissure and insula are large borders between frontal and temporal lobe); 2. diversity of brain parenchyma primary injury: contusions, laceration, diffuse axonal injury; 3. advances in neuroimaging. Conforming to these criteria we make distinction between 3 groups of patients: In the first group of 34 patients the images and/or surgical appearances were corresponding with a double (bifocal) distinct lesion one frontal and another temporal usualy in the vicinity of sphenoid arrest. In this group 24 patients was comatous (Glasgow coma score 8 or less). 30 cases presented with an acute subdural hematoma associated. Mortality rate was 91.5 for comatous patients and 30 for the patients with Glasgow coma score more then 8. In the second group of 17 frontotemporal laceration cases there was not cleare landmark between a frontal and a separeted temporal brain lesion. At silvian fisure level the laceration seems to be continuos involving both temporopolar and frontotempotal surface brain regions. In this group 12 patients was comatous. All patients presented subdural hematoma, mortality rate vas 100 for comatous and 60 for noncomatous patients. 3 cases presented a deep true frontotemporal (frontoinsulo-temporal) laceration-hemoragic contusion end only 1 cases presented with acute subdural hematoma associated. All patients were comatous and mortality rate were 1000. Mechanism of a deep brain laceration – hemoragic contusion in a sulcus (silvian fissure) seems to be different with impact contusion or shearing forces. The vicinity of silvian vessels, paralimbic areas and basal ganglia rise a lot of problems concerning local vasospasm, systemic neurovegetativ instability and comatous- neuropsihic disturbances. The true frontotemporal brain laceration (frontoinsulotemporal) is a distinct entity then the double focal frontal and temporal brain separated or joints laceration. The cases are rare, with a particular etiopatogenic mechanism and neurophiziologic involvement. The prognosis is pour with current therapy.

P258. Classification of traumatic subarachnoid hemorrhage by outcome study
M. Yamaguchi, H. Ando, T. Yamada (Kobe, Mihara, J)

If the traumatic subarachnoid hemorrhage (t-SAH) was identified in the initial CT scan, the prognosis

varies from a fatal condition to a very mild one. So, we tried to classify t-SAH by the outcome.
Methods: We interviewed 45 cases of t-SAH. They were injured during on duty work or on the way to and from their official places for work. They had right to be evaluated for compensation by labor law in Japan. No litigant was included. All of them reached plateau phase of recovery. Eight massive bleeding cases with or without brain contusion manifested on CT were classified as the severe group. Cases with small amount of blood were divided into 2 groups based on the length of loss of consciousness (LOC): the mild group (8 cases) had LOC less than 6 hours, and 29 cases with the diffuse axonal injury (DAI by Gennarelli's classification) had longer period of LOC (over 6 hours), even if their bleeding was very small amount.
Results: Mean length of LOC in mild group was 2 hours and 4 cases represented good recovery (GR) of Glasgow Outcome Scale (GOS). Rate of resuming work was 62.5 %. In DAI group mean of LOC was 20 days excluding 2 cases of no correct record of LOC. GR on GOS was 48.3 %, and 55.2 % of the group could resume work. In severe group, the mean duration of LOC was 28.5 days and 12.5 % of the group could resume work.
Discussion: Since only survivors were studied in our report, the outcome might be calculated better than whole injured population. We would like to report that the prognosis varies in relation to the clinical condition, especially to the length of LOC. We would propose that 6 hours of LOC is a very important point to predict the prognosis of t-SAH.

P259. Dexanabinol in severe head trauma – results of phase II escalating dose clinical trial
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Introduction: Dexanabinol is a novel synthetic, non-psychotropic cannabinoid-derived neuroprotective agent.The purpose of the current trial was to establish the safety of escalating doses of Dexanabinol (3 doses, 48-200mg) in severe head injury.
Methods: The double blind, placebo controlled, randomized, escalating dose study commenced in 10/1996 and ended 1/2000. One hundred and one patients with severe head injury (GCS 4-8) were enrolled in 6 Israeli Neurotrauma centers following approval of the protocol by the IRBs and the Israeli Ministry of Health. The drug or placebo (vehicle) was adminstered as a single intravenous dose within six hours of injury. Hemodynamic parameters, ICP and CPP were continuously monitored over the first 3 days in the ICU. Adverse medical events, blood chemistry and hematology were evaluated over a six months follow up period. GOAT, GOS and DRS were evaluated at 10days and 1,3 and six months postinjury.
Results: The demographics of the patients were those characteristic of the severe head trauma population. Randomization was successful in The first two dose groups. In the third (200mg) cohort, the drug group had significantly more severe, high-risk patients. Dexanabinol was found to be safe and well tolerated over the dose range tested. Dexanabinol had no effect on blood pressure. There were no severe or moderate adverse events attributable to the drug. Elevations of ICP above 25mmHg were substantially inhibited in all three Dexanabinol treated groups. There was no significant difference in 6 months neurological outcome between the
drug treated groups and placebo, although a trend towards an increase in good neurological outcome was evident in the first two groups. Detailed analyses directed at dose selection are ongoing.
Discussion and conclusion: Dexanabinol was found to be safe and well tolerated in severe head injury and to improve ICP/CPP management. A Pivotal clinical trial will commence late 2000.

P260. A systematically developed instrument for the assessment of health-related quality of life in multiple injured patients – the Polytrauma Outcome (POLO) Chart
N. Pirente, B. Bouillon, B. Schäfer, E. Neugebauer, AG "Polytrauma" der DGU (Cologne, D)

Even years after having sustained multiple injuries patients often suffer from its sequelae. These comprise restrictions in physical function, but also pain, social and psychological impairments. Although the Meran Consensus Conference in 1990 defined the contents of Quality of Life (QoL) measures in surgery, still no instrument is available for the valid assessment of all relevant QoL domains in multiple injured patients. Within this study we systematically developed a modular instrument for the assessment of health relevant QoL. Within three phases (phase I: generation of items, phase II: item reduction, phase III: pre-testing in 70 multiple injured and control patients) a questionnaire of 59 items was developed, which measures all relevant trauma-related aspects of QoL. Together with the Glascow Outcome Scale (GOS), the EUROQOL and the SF-36, the newly developed instrument builds the Polytrauma Outcome Chart (POLO -Chart) which will be used as "Sheet E" for outcome assessment within the "Trauma registry" of the German Society for Trauma Surgery. In phase IV, POLO Chart will finally be validated in five trauma centres (Celle, Essen, Hanover, Cologne und Munich). (Supported by DFG (NE 385/5-1).)

P261. Serum glucose levels and outcome after severe head injury

A. Rovlias, E. Konstandinidis, K. Kouzelis, M. Papadopoulos, G. Makrygiannakis, S. Kotsou (Ymittos, Athens, GR)

To investigate the relationship between early post-traumatic serum glucose levels and neurological outcome after head injury, we prospectively studied the clinical course of 267 head-injured patients (Glasgow Coma Scale scores, 3-12), who all were treated surgically for evacuation of an intracranial haematoma and/or placement of an intraventricular intracranial pressure monitoring catheter. Patients with severe head injury had significantly higher serum glucose levels than did those with moderate injury. Patients who subsequently had a bad prognosis had significantly higher glucose levels than did those with a better prognosis. Among the severely head-injured patients, a plasma glucose level greater than 200 mg% was associated with a worse outcome, and a significant relationship was found between postoperative serum glucose levels, pupillary reaction, and maximum intracranial pressure. Multivariate analysis showed that postoperative glucose levels were an independent predictor of outcome. We conclude that early hyperglycemia is a frequent component of the stress response to head injury, a significant indicator of its severity, and a reliable early predictor of neurological outcome.

Poster Session 10
Immunology, vascular mechanisms and imaging in SCI

P262. The complement depletion reduces macrophage / microglia activation and lesion formation in experimental spinal cord injury

T. Kumagai, S. Shimizu, T. Watanabe, M. Ogata, T. Nakahara, T. Yamamoto (Fukushima, J)

To investigate the role of serum complement in spinal cord injury (SCI), we compared macrophages / microglia activation in the vicinity of injury and the lesion size between untreated and serum complement-depleted Wistar rats, using intravenously administered cobra venom factor (CVF). The animals were sacrificed at 72 hrs (group 1) and 1 week (group 2) after their spinal cord had been compressed by an aneurysm clip. Macrophages / microglia activation induced by complement was quantitated as to the number and cell surface area of macrophages / microglia immunostained with ED-1 antibody by an image-analyzer (only group 1). The lesion size was quantitated as to astrocyte-free area (AFA) not immunostained with anti-GFAP antibody and Luxol Fast Blue (LFB) negative area (group 1 and 2). Serum complement was below the detectable level 24hrs after CVF injection. Complement depletion significantly reduced both the number and the cell surface area of ED-1 positive cells (number: $p<0.001$, area: $p<0.01$). AFA and LFB negative area were smaller in complement-depleted rats than in control rats at two time points. Especially, in group 2, we recognized statistically significant reduction of the measured areas by both methods (AFA: $p<0.01$, LFB negative area: $p<0.01$). We conclude that serum complement depletion reduces the macrophages / microglia activation and the lesion size in SCI.

P263. Activation of peripheral blood monocytes (pbmcs) by gram-positive (G+) bacterial walls does not require the presence of wall attached teichoic acids

P.A. Majcherczyk, D. Heumann, M.P. Glauser, P. Moreillon (Lausanne, CH)

Introduction: Gram+ walls are complex insoluble structures that trigger cytokine-release (e.g., TNF) from PBMCs. In most studies, the Gram+ walls used to stimulate PBMCs contain both the peptidoglycan (Pg) framework plus its covalently-linked teichoic acids (TA). However, TA make up to 60% of the Gram+ wall. Moreover, the composition of TA varies between different bacteria. Therefore, the role of TA in PBMC-stimulation by Gram+ walls must be determined.

Objectives and methods: to study the role of TA in PBMC-activation by Staphylococcus aureus and Streptococcus pneumoniae walls. These bacteria contain similar amino sugars and amino acids in their Pg, but have different TA, made of polyribitol phosphate and choline-polyribitol phosphate respectively. S. aureus walls were purified, stripped or not of their TA with hydrofluoric acid, and tested for their ability to induce TNF-release from PBMCs. S. pneumoniae walls were purified, solubilized with the pneumococcal amidase, and fragments with (TA+) or without TA (TA-) attached were separated by affinity chromatography before testing with PBMCs.

Results and conclusion: The table shows the minimum amounts of stimulant (in mg/ml) inducing a >10-fold increase in TNF production by PBMCs above levels induced with medium alone:

Controls		S. aureus (insoluble)		S. pneumoniae (soluble)	
No wall	LPS	TA+	TA-	TA+	TA-
No activity	0.0001	0.1-1	0.1-1	0.1-1	0.1-1

In S. aureus, stripping TA from the purified wall did not affect their TNF-releasing activity. In S. pneumoniae, insoluble and amidase-solubilized walls had the same stimulating activity (data not shown). Moreover, separation of TA+ and TA- soluble fragments did not reveal a role for TA in TNF-release. Thus, TA were not necessary for PBMC-stimulation by these walls.

This supports recent studies (J. Biol. Chem. 1999; 274:12537-43) indicating that the Gram+ wall-stimulating activity was concentrated in specific Pg fragments.

P264. Peripheral blood monouclear cells are activated by streptococcus pneumoniae cell wall components in the absence of choline and n-acetylglucosamine residues

P.A. Majcherczyk, E. Rubli, M.P. Glauser, P. Moreillon (Lausanne, CH)

Background: The peptidoglycan (PG) of Gram+ bacteria is a complex macromolecule known to trigger release of cytokines (e.g., TNF) from peripheral blood monocytes (PBMCs). However, it requires 100-1000x more Gram+ PG than Gram- lipopolysaccharide (LPS) to release the same amounts of cytokines from target cells. This suggests that either the Gram+ PG is poorly active, or that only part of this structure is required for PBMC-activation. Indeed, we recently described the purification and structural analysis of pneumococcal peptidoglycan stem peptides that carried either non-inflammatory activity, or were very inflammatory when exposed to PBMCs.
Purpose and methods: To test whether these stem peptides, were solely accountable for the inflammatory activity, or whether additional wall components not detected by MS were responsible, radiolabelled cell walls were prepared from Strep. pneumoniae grown in the presence of either [^{3}H] lysine, [^{3}H] choline or [^{3}H] N-acetlyglucosamine (GlcNac), resulting in the labelling of peptides, teichoic acids or sugar components. The resulting labelled walls were digested with the major pneumoccocal wall hydrolase-amidase, and/or muramidase. Solubilized walls were separated by reverse-phase HPLC and the radioactivity present in each fraction measured by liquid scintillation counting.
Summary of results: The lysine labelled wall components detected by UV absorbance gave a chromatogram that correlated to that obtained using radioactivity detection. 83% of the radioactivity loaded was recovered. In contrast the chromatograms obtained for both the choline and the GlcNac labelled walls did not correlate when measured by these methods. In both cases peak radioactivity was detected when UV absorbance was minimum and did not correspond to any of the previously identified structures. Moreover, only 2.8% (choline) and 30% (GlcNac) of total material loaded was recovered, suggesting that fractions containing these components are complex and not resolved in the conditions used.
Conclusions: The absence of radiolabelled GlcNac and choline in the previously identified inactive and active wall components suggests that these structures are indeed simple and free of both sugars and teichoic acids. Thus, PBMCs can be activated by specific Strep. pneumoniae wall components that neither contain sugars nor teichoic acids.

P265. The timing of macrophage invasion and cytokine expression in contused rat spinal cords

A. Altscher, H. Y. Huang, J. J. Liu, K. Liu, W. C. Huang, R. Hart, W. Young (Piscataway, USA)

Recent studies suggest that activated macrophages promote tissue repair and regeneration when transplanted into injured spinal cords. Activated macrophages are known to produce pro-inflammatory cytokines. We therefore examined the time course of macrophage invasion and cytokine expression in contused rat spinal cords. Rats were injured with a 10 gm rod dropped 25 mm onto the T9-10 cord. The experiments examined injured and untreated rats (control) with injured rats transplanted with olfactory ensheathing glia (OEG) at 1, 2, 7, and 14 days after injury. The experiments yielded surprising results. Using an RNAase Protection Assay (RPA) kit, we had earlier shown a rapid increase of IL-1a, IL-1b, IL-6, and TNF-a mRNA in the spinal cord, peaking at 6 hours after injury. Our current results indicate that all four of these cytokines approached pre-injury levels by 24 hours and were at or below baseline levels at 48 hours. At 48 hours, immunohistological staining of the spinal cords revealed large numbers of ED-1 positive macrophages present at the injury site. Thus, our results indicate no overlap in the time course of cytokine expression and macrophage invasion of injured spinal cords. When cytokine mRNA levels were highest, there were few or no macrophages present at the injury site. When macrophages were present at 48 hours after injury, the spinal cords show little or no mRNA for either cytokines or neurotrophins. This suggests strongly that something in the injured spinal cord must be shutting down cytokine expression by macrophages in the spinal cord. The cause of this shut-down is not clear. A potential candidate is TGF-beta which is expressed early in injured spinal cords.

P266. Relationship of cytokines and growth factor expression in contused rat spinal cords

E. W. Su, J. J. Liu, M. Brus-Ramer, S. Phrombaset, W. C. Huang, R. Hart, W. Young (Piscataway, USA)

We recently showed that contusion injury of the rat spinal cord causes a rapid and large rise in mRNA for four pro-inþammatory cytokines: IL-1a, IL-1b, IL-6, and TNF-a. We used an RNAase Protection Assay (RPA, Pharmingen) to measure cytokine mRNA levels in rat spinal cords at 6 hours after a standardized 25-mm weight drop contusion. In the same tissue

samples, mRNA levels for several growth factors also rose to high levels by six hours, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF), ciliary derived neurotrophin factor (CNTF), neurotrophin-3 (NT-3), and neurotropin-4 (NT-4). Neurotrophin mRNA levels correlated highly with the rise in cytokine mRNA levels. The glucocorticoid methylprednisolone (30 mg/kg bolus at 10 minutes after injury) almost completely prevented the rise in IL-1b mRNA levels and partially reduced IL-1a, IL-6, and TNF-a mRNA levels; it also reduced NGF, GDNF, BDNF, and CNTF levels by about 50%. On the other hand, intravenous administration of the monosialic ganglioside GM1 (30 mg/kg i.v. at 10 minutes after injury) significantly increased IL-1a and IL-1b levels and also neurotrophin levels. Inflammation has long been speculated to promote axonal growth in the central nervous system. Because two disparate drugs both seemed to alter cytokines and growth factor expression in the same direction, we hypothesized that cytokines are tightly coupled to growth factor expression in injured spinal cord. To test this hypothesis, we assessed the effects of three other treatments that may affect cytokine and neurotrophin expression in injured spinal cord: cyclosporin (10 mg/kg i.p.), pregnenolone (10 µM solution given intrathecally), and acetaminophen (100 mg/kg p.o. before injury). Cyclosporin (a calcineurin inhibitor and immunosuppressant) significantly reduced IL-1a and IL-1b levels and also generally reduced growth factor mRNA levels. However, pregenolone (a neurosteroid precursor to progesterone) significantly reduced IL-1b and IL-6 but increased TNF-a levels while increasing growth factor mRNA levels. Acetaminophen (an anti-inflammatory drug that inhibits CNS prostanglandin synthesis) significantly increased IL-1a but decreased IL-1b without significantly changing growth factor expression. These data indicate that a wide variety of drugs can have complex effects on interleukin and TNF-a expression in injured spinal cords, as well as growth factors. We conclude that mRNA levels of interleukins and growth factors are not necessarily tightly coupled in injured spinal cords. TNF-a appears to be the only cytokine consistently correlated with growth factor expression.

P267. Effect of monosialic ganglioside and methylprednisolone on cytokine and neurotrophin expression in contused rat spinal cord

M. Brus-Ramer, S. Phrombaset, L. Peng, W. C. Huang, R. Hart, W. Young (Piscataway, New York, USA)

Methylprednisolone (MP) and monosialic ganglioside (GM1) are two drugs that have been reported to improve functional recovery in spinal cord injury. MP is believed to act during the Þrst few hours after injury while GM1 is intended to stimulate growth and recovery during 6-12 weeks after injury. In 1994, Constantini & Young reported that the combination of MP and GM1 shortly after injury seemed to antagonize the neuroprotective effects of MP in a rat spinal cord injury model. Brus-Ramer, et al. (1999) recently reported that injury produced a rapid and large increase of IL-1a, IL-1b, IL-6, and TNF-a, as well as NGF, BDNF, NT-3, GDNF, and CNTF in rat spinal cords. MP remarkably and selectively reduced the injury-induced rise in IL-1b and partly reduced IL-1a, IL-6, and TNF-a mRNA expression in contused spinal cords. It also reduced all the growth factors by about 50%. We therefore assessed the effects of no treatment, MP (30 mg/kg), GM1 (30 mg/kg), and MP+GM1 on cytokine and neurotrophin expression in contused spinal cords. A total of 20 rats (n=4 per treatment group) received 25 mm weight drop contusions producing severe spinal cord injuries. At 6 hours after contusion, the spinal cords were rapidly removed and frozen. Pharmingen RNAase Protection Assay (RPA) kits were used to measure cytokine and neurotrophin mRNA from five 5-mm samples of cord: Imp (Impact site), P1 (proximal adjacent to the impact), P2 (proximal adjacent to P1), D1 (distal adjacent to the impact), and D2 (distal adjacent to D1). The results conÞrmed our previous study showing that MP (30 mg/kg bolus at 5 minutes after injury) blunted both cytokine and neutrophin expression in the spinal cord. In contrast, the GM1 doubled IL-1a and increased by 20-30% IL-1b, IL-6, NGF, and GDNF expression. These results suggest that GM1 is pro-inþammatory while MP is anti-inþammatory. Cytokine and neurotrophin mRNA levels in the MP+GM1 treated group did not differ signiÞcantly from MP treatment, suggesting that MP completely masked the GM1 effects at 6 hours after injury. MP and GM1 had opposite effects on both cytokines and neurotrophins. This is the first report of MP and GM1 effects on cytokine and neurotrophin mRNA levels in spinal cord injury.

P268. Demonstration of MRI serial intramedullary changes of acute cervical cord injury

M. Akino, H. Saito, Y. Iwasaki, K. Hida, I. Koyanagi (Sapporo, J)

MRI is very informative to determine the injured site and the degree of spinal cord injury noninvasivelly. Serial MRI observation of intramedullary changes of spinal cord injury in human being show precise pathological process after injury and information about practical treatment of spinal cord injury patients. 4 cases of cervical cord injury were seriously studied by 1.5T MRI in acute stage. 3 cases received anterior decompression and fusion in acute state. Initial MRI

clearly demonstrated injured sites. Serial MRI showed hemorrhagic changes, cord swelling and Wallerian degeneration. One case showed posttraumatic syringomyelia 6 months later. Sequential pathological changes of spinal cord injury were well demonstrated by Serial MRI. These findings are thought clinically worthwhile to the treatment of spinal cord injury.

P269. Role of complement activation in acute SCI
A. Anderson, W. Young, C. Cotman (Irvine, Piscataway, USA)

Previous studies have examined the cellular inflammatory response and regulation of inflammatory cytokines following spinal cord injury (SCI). In the present study, we tested the hypothesis that the complement cascade may also play a role in inflammation following SCI. Complement can be activated via either the classical or alternative pathways; these pathways in turn converge on the terminal pathway and lead to membrane attack complex (MAC) formation. We examined the pathways, cellular localization, timecourse, and degree of complement activation in rat spinal cord following mild, moderate, and severe contusion-induced SCI at 1, 7, and 42 day post injury time points (n=45, 18 mild, 9 moderate, and 18 severe). Classical (C1q and C4), alternative (Factor B) and terminal (C5b-9) complement pathways were strongly activated within 1 day of SCI. Cellular localization of complement proteins was predominantly found in neurons and oligodendrocytes, cell types which are vulnerable to degeneration, and was not generally observed in infiltrating inflammatory or glial cells. Surprisingly, immunoreactivity for complement proteins was also evident 6 weeks after injury, and complement activation was observed to propagate as far away as 20 mm rostral to the site of injury. Additionally, we observed axonal staining by C1q and Factor B, suggesting a potential role for the complement cascade in demyelination or axonal degeneration. In summary, these data support the hypothesis that complement activation may play an important role in spinal cord injury. However, while these experiments define the activation of complement after contusion injury to the rat spinal cord, the cellular regulation of complement proteins remains to be defined and the role of complement components and pathways in degenerative and regenerative events remains to be established. Nonetheless, these findings suggest that experimental assessment of the effect of complement inhibition on functional recovery following acute SCI may provide insight into therapeutic strategies.

P270. The pathogenic factors promoting development of neuropathic conditions with traumatic spinal cord injury patients
O. Dulub (Minsk, WEI)

The purpose of research is to study peculiarities of occurence and progress of neuropathic conditions of spine and extremities with traumatic cord injury patients. We have studied 737 cases of consecutive patients with different periods of traumatic spinal cord injuries and varying degree of expressiveness. We have evaluated the available X-ray, myelography, scintigraphy, computer X-ay tomography, magnetic resonanceimage and electromyography data. All patients have undergone treatment at rehabilitation department of Belarusian Institute of Traumatology and Orthopedics in Minsk. Eleven patients had neuropathic spinal arthropathy, osteolysis or pseudoinfection condition of spine, three patients had marked neuropathic lesions of extremeties distal parts, and 27 had heterotopic ossification of extremities or spine. The complicated progress of disease required changes in tactics of tratment, surgery of spine and extremities. Correlation was established between development and severalty of neuropathic conditions in spineand extremities with gravity of spinal cord elements damage, pseudarthrosis, after anterior or posterior fusion, progress of post-traumatic spinal deformity, instability, presence of polytrauma in anamnesis, long inflammatory conditions, peculiarities of neuralgic restoration at primary paraplegia of the patients

Poster Session 11
Immunology and genomic involvement

P271. Changes in cortical metabolism after closed head injury in human apolipoprotein-E transgenic mice
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Apolipoprotein E (apoE) is expressed in humans as three common isoforms. ApoE4 is a major genetic risk factor of Alzheimer's disease (AD) which is characterized by hypometabolic brain. Closed head injury (CHI) is associated with increased risk for AD, and the effects of CHI and apoE4 in AD are synergistic. This study was designed to analyze regional cortical metabolism in wild-type (WT), apoE deficient (KO) and apoE4 mice and to determine how trauma may affect glucose and lactate content in their brain. Human apoE4 transgenic mice were generated on an apoE-deficient C57BL/6J background utilizing human apoE4 transgenic constructs. WT, KO and

apoE4 mice were subjected to CHI as described previously. At 4 and 24 h after sham surgery or CHI, bioluminescence imaging of glucose and lactate was performed. Quantification was achieved by computer-assisted densitometry bilaterally in the cortex. I) Basal glucose in KO sham-mice was higher than in apoE4-sham (p=0.04). No difference was found between WT and the other groups. II) At 4 h after CHI glucose has declined in the KO mice (p=0.027) but not in the WT or the apoE4. Glucose remained low at 24 h in the KO (p=0.043), and a trend for decline was found in the WT (ns). III) Lactate in all sham groups was similar. At 4h an increase in lactate was observed only in the apoE4 mice (p=0.035). At 24 h, a trend towards increased levels was observed in the KO, whereas a significant decrease was observed in the WT (p=0.022). Low basal glucose level in apoE4 mice in combination with a rapid lactate increase may correlate well with our previous findings that apoE4 transgenic mice are highly susceptible to CHI. The differential effect of CHI on glucose metabolism in the apoE4, KO and WT mice calls for further investigation into the role of impaired energy metabolism after CHI, as a risk for development of AD.

P272. The effects of ethanol on heat shock protein expression following traumatic brain injury

S.A. Dutcher, J. Pilitsis, B.D. Underwood, Walker, D.B. Michael, (Detroit, USA)

The Traumatic Brain Injury (TBI) National Database Management Center reports that 52% of TBI victims have have positive blood ethanol (ETOH)levels at injury. ETOH influences Heat Shock Protein(HSP) expression (Calabrese et al, Free Radic. Biol. Med. 24: 1998).Our group has previously reported changes in expression of HSP following TBI (Dutcher et al.; J Neurotrauma 15:6 1998). Hypothesis: The presence of ETOH effects HSP biosynthesis following TBI.
Methods: Human brain tissue was collected during craniotomy, in accordance with hospital and federal regulations (45CFR46), and processed by Northern and Western analysis for HSP expression using non-TBI tissue for comparison. Blood ETOH levels were determined at the time of hospitalization by standard methods. Statistical analysis used studentized Tukey & ANOVA.
Results: Preliminary results would suggest that ETOH intoxication increases the expression of Heat Shock Proteins following human TBI.
Conclusion: These preliminary results support the hypothesis that ETOH influences HSP gene expression following TBI. Study of post TBI and ischemic gene alteration has led to molecular treatment strategies (e.g. bFGF therapy). This study suggests the effect of ETOH intoxication on post TBI gene expression should be considered in the design of such treatment. (Supported by WSU-SOM, The L.M.Thomas, M.D. Fund, and NIH Grant NS30550 (P.D.W.).)

P273. Neuroprotection after closed head injury in transgenic mice over-expressing IL-1 receptor antagonist

I. Yatsiv, S.M. Beni, E. Beit-Yannai, K. Iverfeld, S. Andell-Jonsson, E. Shohami (Jerusalem, IL; Stockholm, S)

Various cytokines (TNF, IL-1, IL-6) are over produced and released in the brain hours after closed head injury (CHI). These increased levels were shown to be harmful, mainly at the early post-injury period. The endogenous IL-1 receptor antagonist (IL-1ra) is also upregulated under stress conditions and was shown to provide neuroprotection in an ischemic brain injury model. We studied the effects of high IL-1ra levels in transgenic mice (TG +/+ or +/-) undergoing CHI. Clinical status was evaluated by the Neurological Severity Score (NSS), which tests reflexes, behavior and motor functions. A point is given for failing to perform a task. A baseline NSS was performed and animals were subjected to CHI of similar severity (comparable 1-hour NSS) and followed for 48h and 30 d. Edema was evaluated as an increase in tissue water-content at 48 h post CHI in the contused hemispheres. Similar response was found in both TG +/+ and +/- mice. Baseline NSS in TG mice (+/+) indicated minor motor deficits compared to wild type (WT) mice (p=0.056). After CHI, clinical recovery at 24 h (difference between NSS at 24 h to baseline) was facilitated in these mice (n=14) as compared to controls (n=15) (p=0.0129). This trend sustained throughout the 30 d follow-up. TG mice (+/+) (n=4) had less edema than controls (n=4) in the traumatized hemispheres 80.69±1.17 vs 81.42±0.87%, respectively, reflecting 30% decrease in the accumulation of water in the contused hemisphere in TG mice. The present study supports the detrimental role that IL-1 might play in the pathophysiology of the early stages after CHI. It should be noted that chronic overexpression of IL-1ra seems to impair motor function thus timing of IL-1 inhibition after trauma might be crucial.

P274. Increased level of a multifunctional protein, clusterin, after experimental neurotrauma

R. Erber, T. Wahle, M. Herten, R. Witzgall, F. Fiedler (Mannheim, D)

Objectives: The secreted glycoprotein, clusterin, consists of two different subunits in its native form. Clusterin is considered a multifunctional protein associated with apoptotic cell death, protein folding, or complement activation. In the brain expression of

clusterin has been shown to be upregulated under different pathophysiological conditions such as ischemia. Therefore, we have studied the presence of clusterin and its changes over time after traumatic brain injury (TBI) induced by cold lesion of the cortex.
Methods: In anesthetized male Wistar rats a right-sided craniotomy was performed over the parietal cortex. Brain trauma was induced by applying a precooled (-68°C) copper cylinder (diameter, 4 mm) to the exposed dura for 30 seconds. After recovery times of 12, 24, 36, 48, 72, and 120 hours animals were re-anesthetized and killed. The brain was removed and the tissue covered by the trauma dissected and homogenized. Western blot analysis was performed using a polyclonal rabbit antiserum which detects both subunits of rat clusterin.
Results: After cold lesion only small variations in total protein content were noted along with a marked increase of clusterin content. Two bands of approximately 33 and 40 kDa molecular weight could be detected. Increase of clusterin content was first observed at 24 hours reaching a peak between 48 and 72 hours. Maximum value was approximately 10fold higher for both the 40 kDa and 33 kDa bands compared to sham-operated animals. Even at 120 hours after lesion clusterin content was still higher than in sham animals.
Conclusion: Increase of brain tissue clusterin following cold lesion favors a role of this protein in the pathophysiological sequelae after TBI. The time course of expression may reflect glial cell activation and suggests a possible involvement in repair mechanisms.

P275. Gene therapy strategy for the treatment of TBI

K. Yang, L. Zou, R. Hayes, X. Yuan, H. Zhou (Houston, USA)

Traumatic brain injury (TBI) is characterized by forebrain cholinergic neuronal loss and cognitive deficits. These consequences can be counteracted by nerve growth factor (NGF) through continuous intraventricular infusion in animal models. However, direct gene transfer in treating TBI is not fully explored. Our laboratory has systemically studied the potential of in vivo gene transfer for treatment of TBI. We have first tested non-viral vector/cationic liposome-mediated NGF gene transfer in rodent TBI models, and found that liposome-mediated NGF gene transfer could attenuate TBI-induced cholinergic neuronal loss. Although cationic liposome is nontoxic and convenient for use, the gene transfer efficiency is not as high as viral vector. The following study, we found that first generation adenoviral vector (fgAdv) exhibited potent transgene expression in rat brains. However, strong host immunological and toxic responses induced by fgAdv leads to gradually decrease of the transgene expression. We next studied the new generation help-dependent (gutless) adenoviral vector (hdAdv). These novel constructs have deletion of almost the entire viral genome, thereby avoiding any possibility of viral gene expression. Our experiments demonstrated that hdAdv elicited much less immune response as evidenced by reduced numbers of infiltrating macrophage and T cells than fgAdv. Furthermore, significantly prolonged transgene expression was found following hdAdv-mediated gene transfer in rat brain for at lease 66 days. We conclude: 1) Cationic liposomes have low toxicity and mediate short term transgene expression, which may provide a useful therapy for short term treatment of TBI. 2) The fgAdv has highest initial transgene expression level but disadvantaged by strong immunogenecity and toxicity; 3) hdAdv has higher gene transfer efficiency, longer duration and less toxic than fgAdv, which may provide a long term transgene expression for treatment of TBI
(Supported by NIH grants RO1-NS35502-02.)

P276. Differential gene expression analysis after traumatic brain injury by subtraction/suppression hybridization

C. Glenn (Gainesville, USA)

In an attempt to identify genes which show changes in expression following traumatic brain injury (TBI), we performed subtraction/suppression hybridization (SSH) on mRNA samples isolated from the cortex of rat sham controls and animals receiving a cortical contusion six hours post surgery or injury, respectively. Using control cDNA as driver and injury cDNA as tester, expression products specific to the injury model are enriched and therefore represent genes which are expressed either exclusively or at a much higher transcriptional rate compared to the control (driver). Sequence analysis of approximately 80 cDNAs and subsequent BLAST searches of clones from the SSH library revealed some expected and some unexpected gene products. The most "hits" were for ribosomal RNAs (13 hits; V01270.1) and cytochrome c subunits encoded on the mitochondrial genome (6 hits; M27315.1). In total, our analysis revealed cDNAs corresponding to 35 gene products, 9 of which had no significant homology to sequences in GenBank, and therefore may represent novel genes. We are in the process of determining the full-length sequences of clones of interest and intend to perform real-time PCR analysis to confirm increased expression levels in the TBI model. Our initial experience with SSH in the rat TBI model indicate that a specific and limited number of gene products can be detected by this differential gene expression analysis technique. We are in the process of performing

functional studies with a limited number of these genes and will generate antibodies for western blot and immunohistochemical analysis for both in-vitro and in-vivo studies. (Supported by The State of Florida Brain and Spinal Cord Injury Rehabilition Trust Fund, NIH R01-NS 21458, NIH F32-NS 10584, and Navy Research Grant N0014-97-1-1064.)

P277. The role of ICAM-1, P-selectin up-regulation in vascular endothelium and leukotriene B4 production in the migration of leucocytes into traumatized rat brain

R. Durmaz, E. Dundar, O. Colak, A. Arslantas, O. Alatas, E. Tel (Eskisehir, TR)

Purpose: The aim of this study was to test the interactions between the expression intercellular adezion molecules-1 (ICAM-1, CD54), P-selectin (CD62P) of vascular endothelial cells and leukocyte (HIS 48) subset infiltration, as well as myeloperoxidase (MPO) activity and leukotriene B4 levels in traumatically injured brain tissue. The efficacy of 5-lipooxygenase enzyme inhibitor AA-861 on MPO activity and LTB4 synthesis in tissue were also studied.
Methods: In adult Spraque-Dawley rats, traumatic brain injury (TBI) was induced by use of a modification of the Feeney weight-drop method; a 50 gm. cm force was applied to exposed dura over the right parietal cortex. Two protocols were used. In the first, animals were divided into six groups (each; n=9, except the sham-operated group; n=7). Following trauma, animals were decapitated at the specific times 0.5, 4, 24, 48 and 72 h in order to evaluate time-course tissue changes of MPO activity and LTB4. One group of rats (n=9) was treated intraperitoneally with AA-861 at a dose of 60 mg/kg, 0.5 h before the trauma and sacrificed at 24 h after trauma. In the second protocols, animals were used for immunocytochemical staining with CD54, CD62P, HIS48 and CD 11a antibodies. Animals were sacrificed at the specific times of 0.5, 4, 24, 48, and 72 h after trauma. ICAM-1 and P-selectin expression was quantified by counting the number of immuno-positive vessels in 10 high-power (X 200) field. The intensity of immunoreactivity for granulocytes and CD11a-positive leukocytes were estimated semiquantitatively.
Results: When compared to the control levels (200 pg/ g wet weight), a significant increase in LTB4 level in the injured hemisphere was observed at 4 h posttrauma ($p<0.001$) and remained significantly elevated at 24, 48 and 72 h ($p<0.001$). A significant increase in MPO activity was observed at 24 h (0.436 units/ g brain, $p<0.01$) which reached maximal levels at 48 h (0.601 units/g brain) after trauma ($p<0.001$). AA-861 inhibited LTB4 synthesis and MPO activity significantly. The increase in the number of ICAM-1 positive vessels within 0.5 and 4 h posttrauma was not significant. Endothelial expression of ICAM-1 further increased at 24 h ($p<0.001$) and remained significantly high at 48 and 72 h posttrauma ($p<0.001$). Similarly, the number of P-selectin positive vessels at 0.5 and 4 h after trauma was not significant, but reached a maximal number at 24 h ($p<0.001$) and remained increased at 48 and 72 h ($p<0.001$) postrauma. Granulocytes were seen in the perivascular area and parenchyma at 24 h ($p<0.5$), appearing mostly in the parenchyma at 48 h ($p<0.01$) after trauma ($p<0.01$). CD11a-positive leukocytes were seen in the parenchyma at 24, 48 and 72 h posttrauma.
Conclusion: This data suggests that there is an interaction between leukocyte accumulation and upregulation of ICAM-1, P-selectin in vascular endothelium and also LTB4 production. AA-861 may be useful in the treatment of inflammation in TBI.

P278. Cytokines and the euthyroid sick syndrome after traumatic brain injury

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Traumatic brain injury (TBI) induces an immediate and long lasting release of cytokines into blood and cerebrospinal fluid (CSF). Cytokines are not only inflammatory mediators but are also involved in posttraumatic metabolic changes. Thyroid dysfunction is common in severe diseases and has been related to serum levels of several cytokines. In this study we investigated the presence of euthyroid sick syndrom (ESS) in TBI patients in correlation with cytokine levels in CSF and serum. TBI patients (n=20) were monitored for up to 3 weeks. GOS was assessed 6 months after injury. Cytokine concentrations were measured in CSF and serum (IL-1, IL-6, IL-10, TNF-alpha, TGF-beta; ELISA) daily and thyroid function was evaluated in serum (T3, FT3, T4, FT4, TSH; chemiluminescence). A possible interdependence was investigated statistically using maximum / minimum values from each patient. ESS was found in 18 patients with T3 levels below normal range, and 7 cases showed low T4 values, too. IL-6 and IL-10 were elevated in almost all injured, whereas serum concentrations of TGF-beta and TNF-alpha remained within the normal ranges in half the TBI patients (increased in CSF – IL-6: 20, IL-10: 19, TGF-beta: 19, TNF-alpha: 14; increased in serum – IL-6: 20, IL-10: 18, TGF-beta: 8, TNF-alpha: 10). Measurements for IL-1 gave no reliable data. Multiple regression analysis of thyroid hormons on cytokines revealed only two significant adjusted R squared (T4 vs. TGF-beta in serum: $R2=.357$, $p=.003$; T3 vs. log IL-6 in CSF: $R2=.228$, $p=.019$). Unpaired t-test did not

disclose any differences between patients with a favorable (GOS 4-5) or with an unfavorable (GOS 1-3) outcome, neither for thyroid hormons nor for cytokines. ESS is a frequent sequel of severe TBI, as is also the marked release of pro- and antiinflammatory cytokines into CSF and blood. Although experimental results and data from patients with systemic injury (e.g. surgery) suggest a causal association of cytokinaemia with the ESS, such a correlation was not evident in this study. The functional significance of increased levels of TGF-beta in serum an of IL-6 in CSF can only be assumed. These findings may be due to several reasons: Unlike systemic diseases not directly afflicting the CNS, TBI induces an inflammatory response intrathecally. Thus, TBI can affect thyroid function in a complex way at all levels, i.e. hypothalamus and pituitary as well as thyroid gland. Furthermore, intensive care treatment includes administration of fentanyl, dopamine and metoclopramide, compounds known to influence thyroid function. Therefore, interactions suggested from previous work may be blunted after TBI in the clinical situation. And, – the sample size might have hampered some findings, thus demanding further investigations on the exact role of cytokines in the development of ESS after TBI.

P279. Does the frequency of brain injury after-effects depend on the number of endured injuries?

V.I. Gorbunov, I.V. Gannushkina, D.K. Sybaev (Utyanovsk, RUS)

Some authors have mentioned, that the secondary Brain Injury (BI) is more severe than the first one. Brain immunologic "privileges" lead to autoagressive reactions in the secondary BI, stimulating the development of aftereffects and consequences. But there is no information of their expressed dependency on BI frequency. Clinico-immunologic examination of 49 patients at the age of 15-50 with secondary minor BI in acute period (29 patients- 2 BI in anamnesis, 12-3 BI and 8-4 BI) and 30 patients -in distant one have been carried out. The dynamics of cell state (T-, B-ymphocytes, T-helpers, T-supressors, phagocytic activity of leucocytes), humoral immunity (immunoglobulin maintenance – Ig M, G, A), neurosensibilization indices (inhibition reaction of leucocytes' migration with cerebral antigen, anticerebral antibodies level and circulate immunal complexes) have been studied. On the third week of traumatic period against a background for tendency to immunologic indices normalization, in 57% of patients it was marked a strong splash of all immunity status characteristics (48,3% after the 2nd BI, 75% after 3rd BI, and 100% after the 4th BI in anamnesis), especially reflecting specific neurosensibilization, that was correlated with clinical deterioration of patients state. In distant period in 28 patients from 30 (93%) after secondary BI different consequences (vegetative dysfunctions in 16 patients, arachnoiditis in 8 patients, spasmatic syndrom in 4 patients) were revealed. Moreover only 7 patients (24,1%) were after 2 BI, 6 (50%) after 3 BI and 7(87,5%) after 4 BI in anamnesis. Thus, autoagressive brain lesion leads to the development of distant consequences. Frequency of their beginning depends on the number of BI in anamnesis.

P280. Temporal profile of CSF glutamate, IL-6, and TNF-α in relation to traumatic brain edema and contusion in rats

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Traumatic brain injury is associated with release of the excitotoxin glutamate and production of pro-inflammatory cytokines IL-6 and TNF-a. In vitro, the cytokines IL-6 and TNF-a have been shown to influence glutamate-mediated excitotoxicity. To investigate similar dependencies under in vivo conditions, the relationship between CSF glutamate, IL-6, and TNF-a and tissue damage were determined following controlled cortical impact injury (CCII) in 42 rats. At different time-points following CCII (8, 24, and 48 hours), rats were sacrificed to assess brain edema, contusion volume, and CSF glutamate, IL-6, and TNF-a concentrations. Differences in results (mean ± SEM) are rated significant at $p < 0.05$. Compared to non-traumatized rats CSF glutamate, IL-6 and TNF-a levels were significantly elevated by 8 hours after CCII. Parallel to increased brain swelling (24 h: 7.5 ± 1.0%; 48 h: 9.3 ± 0.9%) and contusion volume (24 h: 47.0 ± 9.7 mm3; 48 h: 46.2 ± 8.3 mm3) CSF glutamate was significantly elevated over time, reaching highest levels by 48 hours (33 ± 4 mM). IL-6 and TNF-a showed a different pattern as maximum values were reached at 24 hours (42 ± 7 and 4.7 ± 1 pg/ ml) followed by significantly reduced concentrations at 48 hours (16 ± 3 and 1.7 ± 0.4 pg/ml). The contribution of IL-6 and TNF-a to evolving tissue damage seems limited to an early phase following CCII. IL-6 and TNF-a appear to influence glutamate-mediated excitotoxicity as maximally increased IL-6 and TNF-a levels precede elevated CSF glutamate concentrations.

Poster Session 12

Intensive care, rehabilitation and instrumentation in SCI

P281. Surgical management of acute lumbar fractures by porous nickelide-titanium (Ni-Ti) explants

A. Yepiphantsev, V. Karpenko, V. Martynenkov (Novokuznetsk, RUS)

Between 1994 and 2000, 44 patients with complicated injuries of 1umbar spine were operated on at the Novokuznetsk Neurosurgical Clinic. In 34 patients the level L1-L2 was the most frequently affected. Complete spinal cord conduction impairment was detected in 6 patients, and in the rest patients there was incomplete conduction impairment. All patients in acute period of trauma underwent vertebrectomy, deformation correction, and interbody fusion by the application of porous Ni-Ti explants. In 15 patients in addition to interbody fusion the posterior fixation (transpedicle) was performed. The patients were mobilized following 5–7 days after surgery. Removal of the explant was neccessary in 1 patient due to wound suppuration. Spinal bearing function has improved in all our patients. The results (Frankel H.L. et al.) were graded as follows: "excellent" In 11 patients, "good" In 25 patients, and 8 patients remained unchanged. The anterior stabilization technique with porous Ni-Ti has provided good long term stability of traumatic lumbar spinal lesions avoiding the necessity of bone grafting and having very few complications.

P282. Relationship between pain and neuropsychological functioning – a comparison of whiplash patients and patients with chronic cervical rheumatic disease

G. Di Stefano, B.P. Radanov (Bern, CH)

Objectives and methods: To investigate the influence of cervical pain on neuropsychological functioning, we administered several tests of attention and verbal learning and memory to 112 patients after sustaining a whiplash injury a few days earlier and to 34 patients with chronic cervical rheumatic disease. Both syndrome groups are characterized by similar subjective complaints. By comparing the neuropsychological performance of whiplash patients and patients with chronic rheumatic disease, we aimed to distinguish the effect of a sudden acceleration of the neck and head from the effect of a cervical pain on neuropsychological performance. We hypothesized that whiplash injuries do not cause any damage to the brain and therefore neuropsychological deficits should be attributed to the effect of secondary factors, such as pain. If this is true, patients with similar symptoms, but without traumatic injuries, such as patients with chronic, cervical rheumatic disease, should manifest similar neuropsychological test results.
Results: The results show similar patterns of attention and verbal learning and memory in the groups of whiplash patients and the patients with chronic cervical rheumatic disease.
Conclusion: We conclude that neuropsychological impairment after whiplash injury may be the sequel of cervical pain.

P283. Iatrogenic nerve root injury and continuous electromyography during cervical spine surgery

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Background and rationale: Peripheral nerve root injury may inadvertently occur during cervical spine surgery. Continuous electromyography (EMG) from selected muscles may be useful for detecting motor nerve root irritation but its role during cervical spine surgery and its relationship to neurological outcome is not clearly understood.
Methods: Intraoperative EMG recordings were obtained from monopolar needle electrodes placed 5 – 7 centimeters apart within muscles supplied by nerve roots most likely at risk during surgery. The EMG was continuously displayed on the computer screen and simultaneously heard on an audio monitor using a 2 or 4 channel EMG machine. Surgeons were informed of EMG discharges. EMG discharges were classified as either unsustained, or sustained greater than 2 seconds. Pre- and post-operative neurological examinations were performed by an individual blind to the EMG results.
Results: Fifty-three patients were included in the study, and 45 of those had pre-operative neurological deficits (23 myelopathy, 17 radiculopathy, 4 myelopathy and radiculopathy). Twelve patients had cervical spine surgery by a posterior approach, and 41 by an anterior one. Neurological deficits related to iatrogenic nerve root injury were identified in 6 patients (11%), and all 6 were predicted by “sustained” EMG discharges. Sixteen patients had EMG discharges that were not associated with new neurological deficits, but 6 of those had only unsustained discharges. For all patients with EMG discharges, the cumulative duration of EMG discharges was longer in those who suffered iatrogenic nerve root injury than those who did not ($p=0.05$). All patients without EMG discharges, or with unsustained bursts of EMG, did not have new neurological deficits related to iatrogenic nerve root injury.

Conclusion: Continuous EMG monitoring is a simple, real-time technique that is useful for alerting the surgeon to risky surgical maneuvers that put cervical nerve roots at risk. Iatrogenic cervical nerve root injury was associated with "sustained" EMG discharges. Although it is difficult to prove that the continuous EMG monitoring improved neurological outcome, the high false positive rate (30%) may actually be related to improved outcome after the surgeon was warned of initial nerve root irritation.

P284. Anterior approach for facet interlocking of the cervical spine

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Objects: Surgical management of facet interlocking of the cervical spine is still controversial. To demonstrate the efficacy of the anterior approach, we retrospectively analyzed our experience of 13 patients with facet interlocking.
Methods: There were 13 patients (10 male and 3 female) with facet interlocking, who ranged in age from 19 to 73 years (mean 48.3 years). Six patients were injured in a fall, three were in motor vehicle accidents, two in sports accidents, and two were injured accompanied with head trauma. The level of facet interlocking was C3/4 in one, C4/5 in five, C5/6 in two, and C6/7 in four patients. Eight of thirteen patients presented with unilateral (right side in all cases) and five patients with bilateral cervical facet interlocking. The initial neurological state by Frankel grade was as follows; A: 3cases, B: 4cases, C: 2cases, D: 3cases and E: 1case. All patients underwent plain radiorography, computerized tomography (including three-dimentional imaging), and magnetic resonance imaging evaluation of the cervical spine on admission. The reduction procedures included a direct skull traction in four, manual traction under fluoroscopy in three, manual traction under general anesthesia in one, and intraoperative traction in five patients. All patients underwent decompression and stabilization of the dislocated spine via anterior approach using a iliac bone graft and titanium anterior cervical plate implant within ten days (mean 1.6 days). There were no surgery-related complications.
Conclusion: The anterior approach for cervical facet interlocking allows reduction, decompression and stabilization of the dislocated spine in one stage operation. We stress the safety and efficacy of the anterior approach in the management of facet interlocking of the cervical spine.

P285. Our experience with spinal instrumentation in spinal cord injured patients

I. Poeata, C. Popescu, Z. Faiyad (Iasi, RO)

Spinal instrumentation diversity rapidly enlarged in the last decade. The useful of different devices for a better spine stabilisation and sometime reduction with minimal rate of complications is a current problem. In a series of 37 consecutive cases of spinal instrumentation in spinal cord injured patients we have 27 dorsolombar fractures treated with posterior plates and transpedicular screws, 5 cases of lower cervical spine fracture-dislocation with anterior interbody bone graft and plate fixation and 5 cases of odontoid fracture-dislocation with occipito-atlanto-axoidian bone graft and wiring. Alternatively methods used in the same period were: traction-reduction and external plaster imobilisation, anterior cervical interbody fusion without plate fixation. We work in a mixed team: neurosurgeon (first surgeon for decompression) and orthopedist (first surgeon for screwing). Open reduction was the first choice in 31 cases, the second decision after the failure of extension method in 3 cases. In the remainder 3 cases the goal of the surgery was decompression and stabilisation in an already reduced dislocation. The two-month control shows neurologic status improvement in 32 cases. Complete neurologic lesion did not improve. Transitory immediate postoperative neurological worsening occurred in 2 cases. Exterior plaster imobilisation was applied in 6 cases when the internal fixation was apreciated not perfect for the reabilitation period movements. Infectious complication occurred in one case and was cure with drainage and antibiotics without plate removal. The modern concept of implants in spine fracture-dislocation internal stabilisation gives the advantage of immediate mobilisation and avoids secondary deformity, dislocations and spine instability syndrome. Type of dislocation, neurological status, age and associated conditions, failure of external reduction are criteria in surgical decision. The methods have a low rate of complications.

P286. The treatment of acute spinal cord injury by oxygenating perftoran

C. Musalatov, P. Katunyan, D. Dzukaev, D. Merenkov, A. Garkavi (Moscow, RUS)

As was shown experimentally on rats before, the early decompression, intravenous (i/v) infusion of Perftoran (Pf) and its local subarachnoidal perfusion around SC limit the ischemia and secondary damage of neural structures following SCI. Pf is perfluorocarbon suspension, capable of carrying O2 and CO2 gases. Pf has important for neurological disorders phosphlipase A2 inhibiting ability too. Of the 30 patients, the most with Th11-L1 traumatic compression (18 control by saline/macrodex solution (SMS) and 12 by Pf treated) were investigated and decompressed during 6-96 h after acute SCI. All the

patients were either paraplegically or paraparetically injured, but without rupture of medulla. The SMS was perfused into subarachnoidal space of the damaged region of the cord to 18 control patients for 30 min by slowly flow. In Pf care, 400 ml Pf was injected i/v before SC decompression and subarachnoidal perfusion of 60-80 ml oxygenated Pf into injured region was performed for 30 min. The i/v injections of Pf were repeated during the next few days. The oxymetric data shown increase oxygen saturation in neighboring SC tissue and density of cord vessel's network. After control care only 5 patients more or less began to walk 5-7 ws later. Out the 12 patients under Pf care 7 began to walk. These findings indicate to the protective role of Pf treatment in SCI. It may be assumed, that the temporary extravasal SC oxygenation breaks off vascular spasm and restores medullar microcirculation, that results in repair axonal conduction. Also the flow of Pf particles perhaps more actively, than SMS capture and evacuate metabolites like eicosanoids and proteases. Authors appreciate SAT MUTUA de Sabadell for financial support in this investigation.

P287. Gatism treated with neuroanastomosis
S.C. Zhang, J. Zhao (Shanghai, PRC)

To solve Gatism (loss of control of bowel and bladder due to paraplegia). The authors advised a method for anastamosing an intercostals nerve to the pudendal nerve. The transplanted nerve was conjoined to the pudendal nerve using vascularied nerve brighing. The procedure was performed on 20 cadavers prior to use in humans. The procedure has been done on 6 patients. During emergency surgery, this patient's spinal cord was found to be completely destroyed. Electromyogram (EMG) confirmed loss of the nervi erigens. Six months after this severe injury, the above anastamosis was performed, joining the pudendal nerve with an intercostals nerve using a 16-21cm piece of the nervi suralis along with the saphena parva. One-three years follow-up, perineal sensation returned on the patient's right side and EMG showed a conductive potential of 30 msec. For this measurement, the intercostals nerve was stimulated even though the action potential of the rectal and urethral spincters was not marked. This patient can now sense imminent excretal contamination and, prior to bowel evacuation, can ask for help. The benefit of this procedure for Gatism in paraplegic patients seems clear, provided the patient is below the age of 40 and it has been proved that the spinal cord injury is irreversible.

Poster Session 13
Experimental therapy and management 1

P288. Application of bradykinin B2 receptor antagonist is protective after brain injury, while antagonism of B1 receptors has no effect
C. Görlach, T. Hortobágyi, S. Hortobágyi, Z. Benyó, J. Relton, E. Whalley, M. Wahl (Munich, D; Budapest, H; Cambridge, USA)

The aim of the present study was to measure the therapeutic effects of bradykinin (BK) antagonists on lesion volume and brain swelling induced by cold injury in the parietal cortex of rat and mouse, respectively. Cold lesion was induced by application of a precooled (-78°C) copper cylinder (diameter 3 mm) to the intact dura of rat and mouse for 6 s and 30 s, respectively. The brains were removed 24 h after the injury and lesion volume was determined by the triphenyltetrazolium-chloride method in rats. In the mouse brain swelling was expressed as percentage increase in weight of the injured hemisphere which is compared to the contralateral side. After a subcutaneus priming dose of 18 mg/kg a 1 h pre- and 24 h posttreatment using osmotic minipumps (300 ng/kg*min) was applied (Hoe 140, B 9858). The dose of Hoe 140 significantly reduced hypotension induced by exogenous BK, but without changing resting blood pressure. Vehicle (saline) treated animals were taken as control. Hoe 140, a BK receptor 2 antagonist, revealed a 19 % reduction of lesion volume ($p<0.05$) in the rat (see figure) and a 14 % diminution of brain swelling ($p<0.05$) in the mouse. In contrast, the BK receptor 1 antagonist, B 9858, had no effect on lesion volume compared to sham treated rats (see figure). When B 9858 was given in combination with Hoe 140 a significant reduction in lesion volume was seen which was equivalent to and not different from that seen with Hoe 140 alone in the rat. We conclude that brain injury after cold lesion is partially mediated by BK and can be successfully treated with B2 antagonists. (Supported by BMBF and DAAD-MÖB.)

P289. Microsurgical omental transplantation (MOT) for neuropsychological rehabilitation following brain injury
A.L. Krivoshapkin, V.V. Fonin, I.I. Volkova (Novosibirsk, RUS)

In keeping with the literature our own experience is that the MOT is an effective procedure for focal brain lesions. In the present study twenty five patients with severe neuropsychological deficits and seizures (n=18) underwent MOT in 6-72 (20±4) months after brain injury. Following craniotomy a vascularized omental flap was applied to the brain lesion, with end-to-end anastomoses between scalp and

gastroepiploic vessels. Pre- and postoperative clinical, magnetic resonance imaging (MRI) and computerized tomography (CT) data were evaluated. Glasgow outcome scale (GOS) was used in determining patient disability. The follow-up was up to 82 (46±4) months. All cases displayed preoperative focal lesions determined by both MRI and CT. Nine patients had moderate and 16 showed severe disability before surgery. Following surgery 22 patients improved GOS. Nine patients demonstrated good recovery, 13 had moderate and only 3 still showed severe disability. In 15 epileptic patients surgery produced a cure. The inter-ictal interval was extended in the remaining three. If the difference of brain lesion volume detected by MRI and CT was greater than 20% the patients had obviously benefited by MOT. We conclude that MOT is an effective procedure to improve the outcome in severe head injury.

P290. New way of protection of neurons in a experimental model brain ischemia in rats

G.Z. Sufianova, L.A. Usov, A.A. Suphyanov, A.G. Schapkin, L.Yu. Raevskaya, S.S. Golubev (Irkutsk, RUS)

Purpose of this work was study protective action of A1- adenosine agonist Cyclopentyladenosine (CPA). The work is carried out on 24 rats, weighing 150-200 g.. Brain ischemia was performed by combination of intraluminal occlusion of middle cerebral artery (MCA) with bilateral carotid artery (CA) occlusion. Occluder was introduced through common CA (CCA) and internal CA before MCA at a 17-20 mm depth. All surviving rats were killed 72 hours after the onset of occlusion. The neurologic examinations were performed 1,2 and 3 days after onset of occlusion and neurologic findings were scored on a six-point Longe scale (in our modification). Histological examinations were performed in brain sections stained by hematoxylin-eosin and Nissle. Morphological damage was graded on a six-point scale (Werner C., 1990). In the 10 rats CPA were introduced into the lateral cerebral ventricle in the dose 25 mkg/kg 60 min before occlusion. Data were analysed using at Student's test. All rats exhibited clinical and neurologic deterioration characterised by Horner's syndrome, disturbance of the eye blood supply, sensibility, reflexes, ataxia, tremor, and hemiparesis. Histologic examination showed varying degrees of common ischemic damage of the frontoparietal cortex and medial segment of the caudate nucleus. Intracerebroventricular injection of CPA reduced neurological and hystological disturbance comparative to rats with ischemia (neurological scale 3,4 (N=10) and 5,1 (N=14) (P< 0,05); hystological scale 1,2 (N=6) and 2,2 (N=6) (P<0,1)). CPA has protect action from incomplete brain ischemia in the rats.

P291. Influence of decompressive craniectomy on outcome in patients with severe head injury

M. Schütze, D. Woischneck, R. Firsching (Magdeburg, D)

Introduction: The conservative treatment of severe head injuries is based on a few principles. Resondly, the indication for decompressive craniectomy is increasingly performed., even if the indication for the operation is controversial. This study analyses the influence of decompressive craniectomies on outcome.
Patients and methods: In the recent 2.5 years, 45 patients with severe head injury were treated by decompressive craniectomy. All were comatose at the time of operation.
Results: Mortality was 51%. 9% of the patients remained in a persistant vegetative state. An unfavourable outcome was associated with increased age, duration of coma, and coma grade prior to operation. In singular cases, patients above 60 years of age, coma duration of more than 6 hours or bilaterally fixed pupills survived with a good or moderate outcome.
Discussion: Age, coma duration and coma grade are no exclusion criterias for decompressive craniectomies. Persistant vegetative state in most cases seems to develope independently from intracranial hypertension. It therefore is probably not prevented by decompression, but on the other hand its frequency will not increase by decompressive craniectomy. In comatose patients with clinical signs of the onset of herniation, wide responsless pupills, extensor response, a liberal indication for decompressive craniectomy appears justified.

P292. Decompressive craniectomy to treat intracranial hypertension – ICP, CPP and neurological outcome

T. Bardt, G.-H Schneider, W. R. Lanksch, A. Unterberg (Berlin, D)

Objectives: In the management of in severely head injured patients, decompressive craniectomy is often the final option to treat otherwise uncontrollable intracranial hypertension. Aim of this study was to investigate to investigate the effect of decompressive craniectomy (DC) on intracranial pressure (ICP), cerebral perfusion pressure (CPP) and neurological outcome.
Methods: 70 patients with severe head injury and DC were included. Treatment was performed according to the AANS guidelines. In all patients, a DC was performed when intracranial hypertension could not be controlled by sedation, head elevation, mannitol, moderate hyperventilation, and barbiturate coma.. Initial Glasgow Coma Score (GCS) was 7,3 (± 3,4),

74,2% were male, age was 42,5 (± 20,4) years. Mean time from injury to DC was 3,4 days. Outcome was determined according to the Glasgow Outcome Scale (GOS).
Results: Mean ICP was 39,1 (± 9,4) mmHg 1 hour before DC. Mean CPP was 58,6 (± 11,1) mmHg which was maintained at this level though by infusion of catecholamines. 1 hour following DC, mean ICP was 10,2 (± 7,7) mmHg, and mean CPP was 74,5 (± 11,6) mmHg. Catecholamines were required in lower doses. 12 hours following DC ICP was 25,9 (± 9,8) mmHg and CPP was 67,1 (± 18,1) mmHg. In-hospital mortality was 25 %. 6 months after trauma from the ICU 33,3 % of the patients had died, 20,8 % had an unfavourable outcome (GOS 2+3) and 45,8 % had achieved a favourable outcome (GOS 4+5).
Conclusion: Decompressive craniectomy was highly effective to lower ICP, to improve CPP, and to diminish therapy intensity. Therefor, it is suggested to treat otherwise uncontrollable intracranial hypertension. A significant number of such patients finally achieved a favourable neurological outcome.

P293. Extensive craniotomy in recurrent chronic subdural hematoma treatment
I. Poeata, M. Rusu, R. Sorete, Z. Faiyad, C. Aldescu (Iasi, RO)

Recurrent subdural hematoma is a challenge therapeutic problem. By 130 operated chronic subdural hematomas cases we head 11 recurrences after limited craniotomy and subdural speace dreanage. In all cases brain collapse was involved. In all cases therapeutic attitudes consists of repeated surgery with craniotomy enlargement and subdural dreanage. All cases improved after repeated surgery but 5 cases worse again after subdural dreanage removing. In this cases a haemicraniectomy was perform. 8 cases was cured and 5 was secondary submitted to an acrylic plate cranioplasty. 3 cases died: 2 by irreducible brain collapse phenomenon and 1 by infectious complications. Enlarged craniotomy in chronic subdural hematoma is an useful therapeutic alternative. Reduction of intracranian volume concerning an atrophic and collapsed brain is the mechanism of cure. Failure arises because of infectious complications or in comatous patients.

P294. Tacrolimus (FK506) suppresses IL-6 and TNF-a in cerebrospinal fluid without significantly reducing brain edema following controlled cortical impact injury in rats
J. Stover, B. Schöning, O. Sakowitz, C. Woiciechowsky, A. Unterberg (Berlin, D)

Disturbance of calcium homeostasis contributes to tissue damage and energetic impairment following traumatic brain injury (TBI). Calcium-mediated activation of calcineurin results in production of damaging NO and oxygen radicals. Inhibition of calcineurin by the immunosuppressant tacrolimus reduces structural and functional damage following ischemia. Time- and dose-dependent short-term anti-edematous effects of tacrolimus (FK506) were investigated in 51 rats after TBI. Following controlled cortical impact injury (CCII) tacrolimus (1 or 3 mg/kg body weight) was administered via single intraperitoneal injection at 5, 30 minutes, or 4 hours after CCII. Control rats received physiological saline. Water content of traumatized and non-traumatized hemispheres as well as CSF levels of mediators reflecting tissue damage (pro-inflammatory cytokines IL-6, TNF-a, excitotoxin glutamate, and ATP-degradation product hypoxanthine) were determined 24 hours after trauma. While CSF IL-6 and TNF-a levels were completely suppressed by tacrolimus at all time points and both concentrations, CSF glutamate and hypoxanthine as well as edema formation were only marginally influenced. Significant reduction of cerebral water content was confined to the non-traumatized hemispheres. In addition, higher dosage of tacrolimus failed to exert significant anti-edematous effects on the traumatized hemispheres. Under the present study design, potency of tacrolimus in reducing edema formation following CCII seems limited. However, its immunosuppressive effects could be of value in influencing the posttraumatic inflammatory response known to aggravate tissue damage.

P295. Unilateral or bilateral craniectomy in severely head-injured patients
M. Olivecrona, S. Naredi, L-O.D. Koskinen (Umea, S)

Objectives: To evaluate the effect on ICP and outcome of patients with severe head injury treated according to the “Lund-concept” and in whom unilateral or bilateral craniectomies were performed in order to control the ICP.
Methods: A prospective study of 31 consecutive patients with severe head injury admitted to the Umeå University Hospital during 1998. The patients were treated according to a standardised protocol based on the “Lund-concept”. In patients where the ICP not was brought under control by other measures, pharmacological, surgical (removing mass lesions) or ventricular drainage, unilateral or bilateral craniectomies were performed. Inclusion criteria were: age 16-70yrs, GCS =8 at time of intubation. Follow up was done after >9 months.
Results: In 11 of the 31 patients 16 craniectomies were performed, 6 unilateral and 5 bilaterally. The mean GCS at admission in the craniectomised group was 6,3 compared with 6,5 in the other group. The

craniectomy significantly lowered the ICP. The outcome for the patients were favourable (GOS 5-4) in 6, 54,5% (GOS 5 n=6), and unfavourable (GOS 3-1) in 5, 45,5% (GOS 3 N=4, GOS 2 n=1) patients. This can be compared with the patients without craniectomy where the resluts were favourable in 80%(n=16), unfavourable in 15% (n=3) and dead in 5% (n=1). There were no surgical or other complications related to the craniectomies.
Conclusion: The craniectomy seems to be beneficial for the severely head injured patients in whom the ICP can not be brought under control with other means, producing good outcome. One can assume that these patients would have had a worse outcome without craniectomy.

P296. Thioctic acid action on ischemic neurotrauma in rats
B. Nickel, L.S. Godlevsky (Frankfurt am Main, D; Odessa, UKR)

The aim of the studies was to investigate behavioral and EEG data in postischemic period under condition of different thioctic acid dosages. Ischemia was produced via both common arterii corotici compression. Coordinated locomotor activity was abolished as well as pain threshold increased during after ischemic trauma. EEG revealed the prevalence of delta activity in frontal cortex, presence of spikes and sharp waves in ventral hippocampus and amygdala. Ischemia performed after thioctic acid (10.0-50.0 mg/kg, i.p.) dose-dependency caused protection from loosing of posture, coordinaton and increasing of pain threshold. Delta activity in frontal cortex and epileptiform discharges in limbic structures were prevented in dose-depended fashion as well. In case of greatest dosage of pharmacon administration 7 out of 12 animals were alive during 24 h of observation while in sham- treated group all rats were dead in 12 h from the moment of compression. In this group histological investigations revealed diminished size of ischemic trauma.

P297. Cerebral circulatory pressure index (CCPI) as a proposed guideline of treatment of severe head injury (SHI) patients
J. Mierzwa, J. Wronski, H. Juniewicz, J. Ferber, A. Weiser, R. Zaluski (Wroclaw, PL)

CCPI is the quotient of Cerebral Perfusion Pressure (CPP) and Intracranial Pressure (ICP). CCPI has been analysed in 94 SHI patients treated within 1994÷99, in whom ICP and CPP have been monitored simultaneously. Distinct differences have been stated in patients considering outcome evaluated in Glasgow Outcome Scale (GOS), especially in patients who survived or died. All patients in whom the CCPI values were over 3.0 had good prognosis and those who had CCPI values below 1.5, died. The patients with CCPI between 3.0 and 2.0 needed intensive CPP oriented treatment aimed at decreasing ICP, improving Mean Arterial Blood Pressure (MABP), or both. The authors' clinical analysis shows that CCPI can advise not only necessity and direction of treatment but also may have the prognostic value.

P298. Hippocampal prostaglandin changes following TBI – treatment with COX2 inhibitors
R.J. Meagher, R.K. Narayan (Philadelphia, USA)

It is now clear that traumatic brain injury (TBI) results in the release of vasoactive factors and cytokines that initiate a central inflammatory response. In rat TBI models and in humans, prostaglandin levels rise acutely following injury. The cyclooxygenase enzymes (COX1 and COX2) catalyze the first step in the formation of prostaglandins from arachidonic acid, producing free radicals in the process. Free radicals damage cell membranes and may endanger cellular metabolism. We have measured an acute rise and chronic elevations of COX2 in rat cortex and hippocampus after lateral cortical impact (LCI) TBI. COX2 inhibitors have been shown to limit postinjury prostaglandin production, decrease infarct size after temporary focal ischemia, and suppress hypermetabolism. Two anti-COX2 treatments have been shown to improve functional recovery in our rat model of TBI. DFU [5,5-dimethyl-3(3-flourophenyl)-4(4-methylsulphonyl) phenyl-2(5H)-furanone] (Merck-Frosst) is a third generation, highly specific COX2 enzyme inhibitor. DFU treatment (1mg/kg, ip., BID, starting 10 min before injury and continuing for 3 days, n=15) markedly improved neurological performance at 3d postinjury, compared to vehicle-treated littermates. DFU improved performance on a complex coordination task (Beam Walk) by 75% ($p<0.05$, Scheffe). In another study, we used a nonandrogenic dehydroepiandrosterone analog (DHEF). In cell cultures, DHEF prevented interleukin-1 mediated COX2 induction. DHEF treatment (25mg/kg ip., 3d, n=24) improved complex coordination 75%, neurological reflexes (neuroscore) improved 20%, and declarative memory (Morris water maze) 50%, compared to injured vehicle treated littermates ($p<0.05$, Scheffe). The current study characterizes the pattern of prostaglandin levels after TBI, with or without these drugs, to confirm the association between behavioral improvement and attenuation of COX2 activity. We measured prostaglandins, prostacyclin, and thromboxane. In addition, the presence of spontaneously formed arachidonic acid metabolites may indicate the oxidative state of the tissue. In vivo microdialysis was conducted with a 1.3mm, 150µm diameter probe stereotactically placed

in the hippocampal CA3 region, ipsilateral and contralateral to injury. Dialysates were collected on ice in 30 min fractions (1µL/min) for 3h prior to LCI TBI to the left somatosensory cortex (3mm depth, 4m/s, 100ms), and for varying times afterward. In a second, complementary technique, micropunches were dissected from 300µm frozen sections of hippocampal CA3 regions, and the tissue was immediately sonicated in ice cold ethanol. An aliquot of clarified supernatant was taken for protein determination and the eicosanoids were extracted using reverse phase column chromatography. EIA kits (Cayman Chemical) were used to determine relative levels of prostaglandins. Changing patterns of prostaglandin levels were detected following injury and with pharmacological interventions.

BMBF-Poster Session 14

BMBF-Research Consortium "Neuro-traumatology and neuropsychological rehabilitation" (sponsored by the German Federal Ministry of Education and Research)

P299. Volumetry of the urinary bladder with implantable ultrasound sensors in paraplegic patients

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Basic scientific investigations have demonstrated that the pressure intensification of the detrusor depends on its predistension. This means that the maximum detrusor contraction achieved is related to the actual volume of the urinary bladder. It was further observed that the strength of detrusor contraction declines immediately after overdistention of the urinary bladder. Most paraplegic patients with a sacral anterior root stimulator in accordance with Brindley, rigid stimulation protocols and a missing sensory feedback system, e.g. volume assessment, do not allow adequate adaptation of the stimulation parameters to the bladder volume. For this reason, residual urine is detected in a number of patients and self catheterism can not always be completely avoided. A bladder stimulator with a flexible and self-adjustable stimulation protocol can pocess information on the actual filling state of the bladder that leads to optimum stimulation and subsequent effective and almost physiological voiding. An additionally integrated volume assessment device would further allow paraplegic patients to stimulate at optimal bladder volume. The presented study investigates the reliability of specially developed implantable ultrasound sensors as a sensoric system for continuous monitoring of the bladder volume. In six anaesthetised pigs two ultrasound sensors, one transmitter and one receiver, were implanted on the bladder wall at different locations (latero-lateral, dorsal-ventral, rostral-caudal). After closing the abdominal wall, the bladder was filled with 9% NaCl solution in consecutive 50 ml steps up to a maximum of 250 ml. After each filling the running time of the ultrasound signal was registered by the oscilloscope. In all experiments reproducible results and a high correlation of the measured running times with bladder volume were observed. The latero-lateral configuration of the sensors seemed to be most confidential. This configuration generally revealed a very slight mean variation of the measured signal running time for a defined bladder volume. Depending on bladder volume, a constant increase of the mean signal running time was registered starting from 21.9 µs with an empty bladder to 45.0 µs with a bladder volume of 250 ml. With the dorso-lateral sensor configuration an association between the measured signal running time and bladder volume was found in the majority of the trials. There were no statistically relevant differences between the latero-lateral configuration and the dorso-lateral sensor configuration. No distinct association with the bladder volume was found with the caudo-rostral configuration of the ultrasonic sensors. The mean variation of the measured values was comparatively greater as the standard deviation showed. The presented study indicates that bladder volume assessment with implantable ultrasound sensors is possible with minimal technical prerequisites. This promising technique for continuous bladder volumetry could play an important role in the development of an intelligent and autoadaptive neurostimulator of the urinary bladder in paraplegic patients.

P300. Functional reorganization of cortical connectivity in the early stage of traumatic brain injury

P. Stude, M. Ahrenkiel, D. Osenberg, W. Ischebeck, D. Stolke, M. Keidel (Essen, Hattingen, D)

Traumatic brain injuries lead to disturbances of the neuronal network and they are compensated by changes in the functional strength of coupling between neuronal populations of different brain areas and by a reorganisation of the temporal pattern of cortical interregional coupling. We investigated the spatio-temporal pattern of neuronal coupling between the ´active´ motorcortex or the frontal cortex contralateral to the movement and all other cortical areas in relation to a simple voluntary movement (self paced, brisk abductions of the right index finger). In 13 patients (37 ± 14,7 yrs., 47 ± 17 days after traumatic brain injury) and in 20 normal subjects (27,2 ± 5,7

yrs.) the electroencephalogram was recorded from 30 electrodes according to the 10-20 system. In relation to movement onset absolute and relative changes in coherence, as a measure of the functional interregional coupling strength, were analysed. Recordings of identical channel-pairs under a controlled resting condition served as reference. The significance of the difference between both conditions was tested by a multiple t-Test. Analysing the cohort of healthy subjects, we observed movement related increases of coherence in the alpha-, beta1- and beta2-band between the ´active´ motor cortex and ipsilateral frontal regions followed by coupling of both homologous motor cortices. First results of patients with traumatic brain lesions in the early stage showed a decrease in the absolute interregional coupling strength between the contralateral ´active´ motor cortex and the contralateral frontal cortex. However, a wider dynamic range of the movement related increase of the contralateral fronto-central coupling strength was observed. The movement related contralateral fronto-centroparietal and fronto-parietal coupling was also found to be increased. The observed specific increase of the (lefthemispherical) interregional fronto-central and senso-motor connectivity sufggests an augmented cortical control of motor preparation an execution in brain injured patients. Since different brain areas were coupled in a defined spatio-temporal pattern to preperate, execute and evaluate movements, the results support the idea that neuronal populations form a specific movement-related neuronal network implicating connectivity encoding of voluntary movements. Forming novel (task-specific) patterns of cortical connectivity seems to be a basic principle of functional reorganisation or compensation of neuronal network disturbances caused by a traumatic brain lesion. (Supported by German Ministry of Education and Research, BMBF# 01K09807/2.)

P302. The role of prehospital hypotension and respiratory dysfunction – results of an epidemiological study

M. Raum, B. Bouillon, B. Buchheister, R. Lefering (Cologne, D)

Beside the primary injury the outcome after severe brain trauma is influenced by the magnitude of the secondary injury. Chesnut suggested that prehospital hypotension and hypoxemia increased significantly the mortality rate. We therefore conducted a study to reevaluate the role of prehospital hypotension and respiratory dysfunction in a german urban region using the Cologne Traumatic Data Bank. All 90.000 prehospital emergencies that were cared for by emergency physicians in Cologne from 1990 until 1996 were reviewed for identification of severe brain trauma. Their clinical course was documented using standard charts and patients were included if they had their accident within the city of Cologne and fullfilled the final inclusion criteria of GCS<=8 or AISHead>=3. 518 eligible patients were identified of whom 453 had complete datasets (follow-up 87%) of whom 214 had complete data sets for evaluation of prehospital second hit phenomena. Univariate statistical analysis was performed for all relevant variables. Mortality rates for presence or absence of hypotension (RRsys<90 mmHg) and/or respiratory dysfunction were calculated. The overall mortality rate was 50%, 58% of deaths occurring within the prehospital setting. Hospital mortality increased from 20% if hypotension or respiratory dysfunction was absent to 40% if respiratory dysfunction, to 69% if hypotension and 75% if both occurred. Secondary brain injury caused by prehospital hypotension or prehospital respiratory dysfunction contributed significantly to the overall mortality rate.

P303. In search of the optimal time point for long-bone fracture surgery in multiple trauma patients with brain injury – where is the evidence?

D. Rixen, B. Bouillon, S. Sauerland, G. Grass, E. Neugebauer (Cologne, D)

The optimal time point for definitive long-bone fracture treatment in multiple trauma patients with brain injury remains controversial. Two differing therapeutic concepts exist: a) the primary-definitive osteosynthesis and b) the temporary osteosynthesis with secondary-definitive surgery. This controversy between "earl" and "delayed" fracture treatment has remained in discussion since 30 years. Thus, it was the aim of this study to systematically analyse the literature with the "evidence based medicine" approach. From 36 papers listed in MEDLINE to this subject (from the years 1964-1999; with n=18 to n=1582 documented patients) only eight studies had a prospective or randomised study design. The majority of papers relied on retrospective clinical data. The primary endpoint was "lethality". Secondary endpoints were: complication rate (from malunion rate to incidence of sepsis or multiple organ failure), ICU – and hospital stay and cardiopulmonary parameters. Only a few authors justified there therapeutic recommendations by prospectively documented biochemical data. Not a single paper focused on the patients future quality of life as a criterion for decision making. Seven authors were in favour of a "delayed" long-bone fracture treatment, while fifteen papers recommended the "early" treatment. Six authers were undecided. Furthermore several authors stressed that contraindication for certain surgical procedures is given in patient subgroups (chest and/or brain injury). In summary, the results from literature are

contradictory and don't lead to a general conclusions. As this is a clinically relevant, often raised critical question it is necessary to perform further prospective studies with adequate study designs.

P304. Nitric oxide in the rat cortex underneath a traumatic brain lesion

M. Stoffel, M. Rinecker, N. Plesnila, J. Eriskat, A. Baethmann (Munich, D)

Objectives: The secondary growth of a cortical necrosis occurring within 24 h after trauma is most likely part of the secondary brain damage and therefore mediator driven. This study sought to analyze the cerebral concentration of nitric oxide (NO), a potential mediator of the secondary necrosis growth by measuring its oxidation-products nitrate and nitrite in the extracellular fluid of the brain.
Materials and methods: A microdialysis probe was stereotactically implanted into the parietal cortex of male SD-rats (n=10), app. 2 mm underneath the brain surface in an angle of 50°. 24 h later, the right parietal cortex was exposed by trephination and a highly standardized cortical necrosis was induced just above the dialysis probe by cold injury (I). Animals of the sham group (II) were treated identically apart from trauma induction. Dialysate was collected until 24 h after trauma in hourly intervals and analyzed for nitrate/nitrite by HPLC-EC.
Results: Under baseline conditions, the dialysate concentration of nitrate was 1.88 ± 0.78 µM in group II and 2.28 ± 0.62 µM in group I. The nitrate concentrations in animals of both groups did not show significant alterations within the observation period compared to baseline values. Furthermore, there were no significant differences between the groups at the individual study time points. The baseline concentration of nitrite in the depth of the cortex was 5.00 ± 1.50 µM in sham-operted and 3.25 ± 1.32 µM in trauma animals. In accordance to nitrate, the nitrite concentrations did neither show significant alterations over time nor between the groups.
Summary and conclusion: This study failed to demonstrate significant changes of nitrate/nitrite in the extracellular space adjacent to a cortical necrosis until 24 h after trauma. This is in contrast to immunohistochemical results showing a dense iNOS-staining around the cortical necrosis beginning 6 h after trauma. Accordingly, it is surmised that glial uptake mechanisms stay intact around the necrosis. Short lasting – however potentially toxic – increases of NO in the synaptic cleft might stay hidden for microdialysis. (Supported by the BMBF-Verbund „Neurotrauma" München, FKZ: 01 KO 94026.)

P305. Disordered comprehension in patients with TBI

A. Geigenberger, S. Lamberts, S. Karch, W. Ziegler (Munich, Würzburg, D)

Introduction: Patients with traumatic brain injury (TBI) may often present communication problems although they are not aphasic in the sense of standardized aphasia assessment batteries. Prominent in their communication behaviour are disturbances of the pragmatic and paralinguistic aspects of language use and the processing of complex verbal material. It is supposed that these deficits result from cognitive and behavioural impairments and that they have a considerable influence on the rehabilitation outcome. Nevertheless, clinical approaches in the assessment of non-aphasic communication disorders are still lacking. The aim of the present study was to investigate disabilities in different aspects of language comprehension and to look for their clinical relevance.
Methods: Following a parallel-processing model of language comprehension we used three experimental tasks to examine the role of prosody in the communication deficits of TBI patients. Experiment 1: Monitoring of conversational prosody. A reaction time paradigm was used to test the patients' ability of inferring information relevant for the control of turn-taking behaviour. Experiment 2: Comprehension of emotional prosody. Patients were required to rate the valence of test phrases spoken in different emotional expressions. Experiment 3: Comprehension of emphatic accent. A focus-recognition task was used to examine the comprehension of prosody in the context of a specific linguistic function. In order to validate the results of these experiments we applied a new clinical assessment tool and a questionnaire: Screening: Spoken language comprehension. A new screening test was developed for the clinical assessment of language comprehension at the discourse level. The test includes 10 short stretches of discourse, focussing on macrostructural aspects of language comprehension such as inferencing, comprehension of metaphors, and creation of situation-models. Questionnaire: Everyday communication. In a questionnaire for patients and their relatives we asked for deficits in everyday communication.
Patients: Experimental group: Non-aphasic TBI patients undergoing rehabilitation of cognitive and motor deficits (N=30). Control groups: Patients with focal cerebrovascular lesions (N=50). Normal controls: N=30.
Results: The three experiments revealed a clear pattern of left vs. right hemisphere involvement in the patients with focal cerebrovascular lesions. A subgroup of the TBI patients showed a pattern compatible with the right hemisphere group. This

result could be validated by the clinical screening test. It is hypothesized that in these patients the parallel processing of verbal and prosodic information was affected to an extent that discourse processing was critically impaired. Unlike aphasics, traumatic patients were often unaware of their deficits in everyday communication, although their relatives reported on problems relating to psychosocial aspects of language use.

P306. Chronic bilateral sacral neuromodulation in patients with bladder dysfunction

P. Braun, K. Jünemann, C. Seif, P. Alken (Mannheim, D)

Introduction and objectives: Sacral root neuromodulation can be a beneficial treatment option in patients suffering from therapy-resistent detrusor instability or detrusor hypocontractility. The implantable neuromodulation system as described by Tanagho and Schmidt enables unilateral sacral nerve stimulation. The electrode is inserted unilaterally into the sacral canal via the sacral foramen (S3). Reports have been made on sacral neuromodulation failures of up to 50% in patients undergoing this procedure. We preferred bilateral electrode implantation in order to achieve better effectivity of the chronic sacral neuromodulation.
Materials and methods: After assessment of the beneficial effect by means of PNE test, 20 patients (14 with detrusor instability, 6 with hypocontractile detrusor) underwent tailored laminectomy for bilateral electrode placement. Minimally invasive laminectomy was performed. The electrodes were bilaterally positioned. Laminectomy allows optimum electrode placement and fixation.
Results: In the patients with detrusor instability the incontinence episodes were reduced from 7.2 to 1 per day and the bladder capacity improved from 280 to 350 ml. In patients with hypocontractile detrusor, the initial residual urine level of 350 ml (180 to 468) dropped to 58 ml (38 to 79). Maximum detrusor pressure during micturition rose from initially 12 cmH2O (8 to 15) to 34 cmH2O (28 to 45). The average followup period was 13.5 months. There was no sign of deterioration in the effect of modulation in any of the patients.
Conclusion: Bilateral electrode implantation results in optimal neuromodulation in either hyper- or hypocontractile detrusors.

P307. Enhanced neurogenesis after brain trauma in rats characterized by expression of ßIII-tubulin

H. Braun, K. Schäfer, V. Höllt l (Magdeburg, D)

There is an accumulating evidence for the existence of neuronal stem cells (NSC) in the adult brain of mammalians including humans. NSC which are involved in neurogenesis in the adult brain are localised in the subventricular zone of the ventricle and in the subgranular cell layer of the hippocampal dentate gyrus. Using an antibody against ßIII-tubulin-a specific marker for newborn neuronal cells- we analysed neurogenesis in rat brain in response to a contusion trauma. The trauma resulted in a primary focus in the cortex and a secondary injury in the ipsilateral hippocampus. Cell death occurred in many regions of the hippocampus (CA1, CA3, CA4, dentate gyrus) with CA3 as the most vulnerable area. Cell loss was most pronounced two days after injury and became significantly less at seven days following the trauma. This partial regeneration appears to be associated with neurogenesis. Thus, on day three an increased number of ßIII-tubulin positive cells was observed in the subgranular zone of the dentate gyrus which migrate to the CA4 and the internal hilar regions. In addition, cells expressing ßIII-tubulin appeared in the CA1-region near the subiculum. Additionally 7 days after trauma there is a proliferation of the subepithelial layer detectable, which delineates the third ventricle. A subpopulation of these proliferating cells expresses ßIII-tubulin and migrates into the hippocampus. From the ependym ßIII-positive cells also migrate to the thalamus, where clustered populations of ßIII-tubulin expressing cells are located. Finally, ßIII-tubulin positive cells were detected in the cingulate cortex and a drastic increased number of those cells migrate to the focus of the lesion. These findings strongly indicate that neurogenesis is involved in the partial regeneration of neuronal cell loss following brain injury.

P308. Stability of visual field enlargement following computer-based training In patients with hemianopia

B. Sabel, U. Bunzenthal, E. Kasten (Magdeburg, D)

Partial blindness after brain injury has been considered non-treatable for several years. In two independent clinical trials we showed that a computer-based visual restitution training (VRT) increased the size of the intact visual field [Nature med., 1998, Vol. 4, 1083]. Patients with post-chiasmatic brain injury (n=19) or with optic nerve injury (n=19) received either VRT or fixation training (placebo) one hour daily for 150 hrs. We now report a follow-up study in which 31 patients of the original 38 patients were again assessed (i.e. 16 out of the treatment groups and 15 out of the placebo groups) to determine if the visual field enlargement was stable even after a 6 months training-free interval. Follow-up assessment was made with high-resolution perimetry (HRP) and with the Tübinger Automatic Perimeter. At follow-up, patients showed only a minor loss of visual functions

which they had regained due to the restitution training (0.8% decrease). In the Placebo-groups which originally received fixation training we also noted no change (+0.3%). Thus, visual field enlargement after visual restitution training is stable, which indicates that patients are using there newly gained visual functions in everyday life. (Supported by the Government of Saxonia-Anhalt (Germany), Kuratorium ZNS and Deutsche Forschungsgemeinschaft (DFG).)

P309. Long-term neuropsychological outcome after traumatic brain injury (TBI) in children: Effects of severity and age at injuy on cognitive development, neuropsychological functions and psychosocial adjustment

B. Benz, A. Ritz, S. Kiesow (Bremen, D)

Introduction: Survivors of childhood TBI experience a variety of neuropsychological seque-lae which can act as risk factors for ongoing development. The situation of this group in late adolescence or early adulthood is of specific interest for clinical neuropsychology.
Subjects and methods: Subjects age 16 to 23 years at follow-up, who sustained traumatic brain lesions at age 2 ½ -14 and subsequently had received rehabilitation treatment or experts' evaluation, are reevaluated after at least 3 years post trauma. The ongoing retrospective follow-up study* includes a comprehensive neuropsychological examination. A semi-structured interview and questionnaires answered by subjects and their parents assess long-term posttraumatic emotional adaptation and social integration.
Results: For the current sample of 106 patients, more than 60 % with severe TBI, mean follow-up interval is 9 (± 3) years post trauma. Highly significant effects of trauma severity and age at trauma on subsequent cognitive development and neuropsychological level of functioning can be established. Trauma severity alone affects speed of information processing ($p<.0001$) and visual-spatial performance ($p<.007$). Additional age-at-trauma effects emerge for attentional functions ($p<.04$), memory ($p<.005$) and verbal competence ($p<.0002$). Age at trauma is related to executive skills ($p<.012$), while there is no effect of trauma severity. Patients with scores <1 SD below the reference mean in the composite scores pertaining to executive functions (WCST, COWA, RFFT, CVLT/Semantic Clusters, TMT-B/A) are more severely impaired on various neuropsychological measures and show both lower and more discrepant IQ scores (WAIS-R V-IQ/P-IQ: 73/62 vs. 94/91); earlier injury results in overall lower levels of intellectual and neuropsychological functioning. Whereas there is a long-term positive trend for total-group results from posttraumatic assessment to longterm follow-up, subgroups exhibit different patterns of neuropsychological recovery and subsequent intellectual development. In some cases, repeated assessment reveals late-emerging cognitive deficits in relation to the reference group. Group means for Attentional deficit, Depression/Anxiety and Social Problems as measured by the CBCL and YSR problem scales are elevated in our clinical sample, compared to a recent representative German study. 17 % of the trauma patients exhibit clinically relevant long-term psychosocial problems, with significant corrrelations to some neuropsychological measures.

P310. Restitution of the cat target-reaching and food-taking movements after lesion of the dorsolateral funiculus in the cervical spinal cord

A. Boczek-Funcke, J. Schattschneider, M. Illert (Kiel, D)

In an animal model basic aspects of the potential of the CNS for the restitution of complex motor functions after brain lesions were investigated. The cat with a C5-lesion of the dorsolat-eral funiculus (DLF) is well suited for that purpose. In qualitative experiments Alstermark et al. 1981 (Exp. Brain Res. 42:299-318) showed that in the target-reaching (TR) and food-taking (FT) paradigm (Gorska & Sybirska, 1976) FT is very much dependent on the segmental input from the corticospinal tract to forelimb motoneurones, whereas TR was virtually unaf-fected. We have investigated this postulate and compared postlesional data (observation pe-riod after C5-DLF lesion up to 200 days) with controls, using different methods of movement analysis (optoelectronic system, x-ray cinematography). Acute deficits: In the acute period both TR and FT were affected. During the first days the performance rate (successful/unsuccessful reaching trials) was decreased; the movement time was prolonged; the spatial velocity was decreased; the location of the mean spatial trajectory was unchanged, but the standard deviations of the trajectories enlarged. These acute deficits seemed to be more pronounced when the DLF-lesions were larger. The lesions did not affect the angular excursions of the proximal joints, significant effects were confined to the joints within the paw. The "aperture of the paw" (extension of interphalangeal and/or metacarpo-phanlangeal joints) was distinctly decreased which did not allow the animal to properly grasp the morsel of food. Recovery: Most of the parameters showed a recovery. Recovery was seen in the performance rate, movement time and spatial velocity. This occurred within the first 20-30 days, and seemed to correlate with the size of the DLF-lesion. The recovery of the kinematic features of the FT movement was different in the cats. In one cat the angular changes leading to a de-crease of the "aperture of the paw" recovered completely. In other

animals the acute kinematic deficits did not recover at all, although there was an increase in the performance rate of the behaviour. In general the time-course of the different parameters was different (i.e. recovery did not occur in parallel).
Summary: The quantitative movement analysis in the C5-DLF lesioned cat allows a precise description of the restitution of motor functions which can be supplemented by a structure-function correlated investigation. The results allow to differentiate unspecific and specific components in the recovery process and suggest that the kinematic functions of the FT move-ment are tightly linked to the segmental inflow from the corticospinal tract onto the forelimb motoneurones. (Supported by BMBF Neurotrauma: FKZ 01KO9512.)

P311. Arm ability training for stroke and traumatic brain injury patients with mild arm paresis. A single-blind, randomized, controlled trial
T. Platz, T. Winter, N. Müller, C. Pinkowski, C. Eickhoff, K.-H. Mauritz (Berlin, D)

Objectives: 1. Efficacy of the Arm Ability Training, and 2. of additional knowledge of result among traumatic brain injury (TBI) and stroke patients with mild central arm paresis.
Design: single blind, randomized control trial.
Setting: Inpatient rehabilitation center.
Patients: Consecutive sample of 74 patients with mild central arm paresis after stroke or TBI; 60 patients completed the study, 45 stroke and 15 TBI patients; 37 patients received a 1-year follow-up.
Intervention: Daily Arm Ability Training with (N=20) or without (N=20) knowledge of result, or no Ability Training (N=20) during a 3 week intervention period.
Main outcome measures: Summary time scores of the "TEMPA", a test of upper extremity function with ADL-like activities (focal disability) and kinematic analysis of aimed movements.
Results: Superior improvement for those who received the Ability Training as compared to controls: mean improvement of time needed to perform 1. all TEMPA tasks 41.4 vs. 12.8 sec (p=0.0012), 2. unilateral TEMPA tasks 16.5 vs. 4.2 sec (p=0.0036), and 3. ballistic component of aimed movements 96 vs. 20 msec (p=0.0115). Knowledge of result did not substantially modify these effects. A functional benefit was still present at 1 year follow-up.
Conclusion: The Arm Ability Training improves focal disability among stroke and TBI patients with mild central arm paresis. (Supported by BMBF grant 01K095168.)

Poster Session 15

Experimental therapy and regeneration in SCI 1

P312. Neural recovery following delayed x-irradiation of the injured spinal cord
G. Li, H. Onyiuke, S.-Y. Ho, R. Gionfriddo, S. Locher, R. Dowsett (Hartford, Farmington, USA)

Objectives: Prior studies have shown structural and functional recovery following spinal cord injury in various models. Specifically, structural recovery after unilaterally transected adult rat spinal cord followed by x-irradiation has been demonstrated. However, the relationship between structural and functional recovery following blunt/contusional spinal cord injury has not been previously evaluated. This study was designed to test the hypothesis that delayed x-irradiation can enhance the functional and structural recovery of the injured mammalian spinal cord.
Methods: 70 adult Sprague-Dawley rats were randomly divided into two groups of 35 rats each. The control group (group 1), sustained a one minute clip compression (30g weighted) injury of the spinal cord at the T1-T2 level, without x-irradiation. The experimental group (group 2), in addition received x-irradiation 14 days post injury. Neurological function was assessed by the modified Tarlov method, inclined plane and open field movement techniques. These tests were performed in a blinded fashion at days 3, 7, 14, 21, 28, 35 and 42 post irradiation. The morphology/integrity of the pyramidal tract was determined by sector axonal count, facilitated by using Holmes staining.
Results: A total of 24 rats in the control group and 26 rats in the experimental group were analyzed. Statistically significant difference was achieved in the open field movement group from day 35($p<0.05$), and the inclined plane technique from day 28($p<0.001$), but not in the pain withdrawal (Tarlov) group. The mean axonal count difference also met statistical significance ($p<0.05$).
Conclusion: These findings indicate that delayed x-irradiation following spinal cord injury enhances functional recovery by improving and restoring structural integrity (axonal re-innervation), in the mammalian spinal cord.

P313. Neural repair of spinal cord injuries in neonatal rats – relationship between the grade of restored function and the extent of regenerated neural connections
T. Hase, S. Kawaguchi, T. Nishio (Kyoto, J)

Spinal cord injuries are repairable in a neonatal period by replacement of spinal cord segments (Nature 367, '94). To investigate the relationship between the grade

of restored function and the extent of regenerated neural connections a model of neural repair of spinal cord injuries in neonatal rats was quantitatively studied morphologically and behaviorally. On postnatal day 2, 1.5-2mm of the lower thoracic spinal cord was resected and embryonic homologous structures were transplanted into the vacancy. Four weeks after surgery, locomotor performance of the rats was quantified using the BBB open field locomotor scale (Exp Neurol 139, '96). One week after the behavioral study, 2% Fast Blue solution was injected into the lumbar enlargement and retrogradely labeled neurons were counted in the cerebral sensorimotor cortex, red nucleus, vestibular nucleus, and raphe nuclei. In the rats whose BBB scale was no more than 11, no neuron was labeled in the cerebral cortex and brainstem nuclei. In the rats that acquired incomplete hind-forelimb coordination (BBB scale, 12-14), labeled neurons were recognized in the vestibular and raphe nuclei but not in the red nucleus and cerebral cortex. In the rats that acquired consistent hind-forelimb coordination (BBB scale, 15 or 16), neurons were labeled in all the brainstem nuclei but not in the cerebral cortex. In the rats that achieved excellent performance (BBB scale, 17-20), labeled neurons existed in all the brainstem nuclei and also in the cerebral cortex although the number was small. Evidently, the more neurons were labeled in the more rostral brain structure, the better was the locomotor performance. In conclusion, replacement of spinal cord segments made it possible to reconstruct functionally significant neural connections; the grade of restored function was intimately related to the extent of regenerated neural connections.

P314. Retrograde and anterograde labeling of spinal cord tracts in the same animal as applied to spinal cord repair

E.C. Tsai, G. Schwartz, C.H. Tator (Toronto, CDN)

Background: It has been difficult to examine both anterograde and retrograde labeling of spinal cord tracts in the same animal. Recently, spinal cord repair strategies have produced widely disparate results in animals subjected to the same repair strategy. One possible reason is that anterograde and retrograde labeling has involved different animals. To circumvent this problem, we have developed a method of labeling spinal cord tracts in anterograde and retrograde directions in the same animal.
Methods: DiI, a lipophilic dye, was applied unilaterally to the sensorimotor cortex and gelfoam soaked in Fluoro-Gold (FG) was placed in the spinal cord at T13/L1 of adult rats. Other tracer combinations using DiI with DiA, DiO, Rhodamine Green dextran, or fluoroscein dextran were also investigated. Animals with transected spinal cords at T8/9 and repaired by bridging, peripheral nerve grafts or spinal cord reanastamosis were also assessed. Control animals had complete cord transection and no repair.
Results: In the same, normal animal, DiI labeled the corticospinal tract, and FG labeled the cortical and brainstem nuclei of several spinal cord tracts. Control animals with complete spinal cord transection showed no diffusion of tracer across the transection site. Results in animals with repair are pending.
Conclusion: Thus, DiI and Fluoro-Gold are useful for demonstrating simultaneous anterograde and retrograde labeling of spinal cord tracts in the same animal. This tracing strategy may also be useful for demonstrating axonal regeneration in spinal cord repair strategies.

P315. A simple intraspinal infusion system with minimal impact on locomotor function

F. Hamers, S.E.J. Duis, I.M. Pans, A.J. Lankhorst, W.H. Gispen (Utrecht, NL)

In the treatment of experimental spinal cord injury localized application of drugs is sometimes used. Several methods exist, ranging from infusion through a fixed cannula or implantation of drug loaded collagen gels to implantation of genetically modified cells or injection with viral vectors. Infusion through a fixed cannula is simplest, but often leads to serious damage and functional deficits because of the great mobility of the spinal cord within the spinal canal. Here we describe a spinal cannulation technique that causes only minor and temporary functional deficits. Essentially, three vertebrae are fused together by repairing a laminectomy site with a polyamide/nylon shield fixed to the adjacent vertebrae by stainless steel screws. This setup severely limits the flexibility of the spinal column and thus movements of the cord relative to a cannula fixed within the shield. Use of the system in uninjured animals led to only a transient small decrease in locomotor function as assessed by the BBB score. In contusion injured animals a slight delay of recovery was observed 7 days after lesioning and implantation compared with unimplanted animals. However, from 14 days onward locomotor function was similar in both groups. We conclude that this system provides a nice entry route for studying functional effects of intraspinally administered drugs.

P316. Involvement of the cell adhesion molecules L1 and N-CAM in endogenous tissue repair following penetrating injuries of the rat spinal cord

T. Hermanns, R.G. Gieling, E.A.J. Joosten, A.B. Schmitt, P. Leprince, J. Noth, G.A. Brook (Aachen, D; Utrecht, NL; Lieges, B)

It is widely accepted that the devastating consequences of spinal cord injury are due to the failure of lesioned central nervous system axons to regenerate. The current study of the spontaneous tissue repair processes following dorsal hemisection of the adult rat spinal cord demonstrates a phase of rapid and substantial nerve fibre ingrowth into the lesion which was derived largely from both rostral and caudal spinal tissues. The response was characterised by increasing numbers of axons traversing the clearly defined interface between the lesion and the adjacent intact spinal cord, beginning by 5 days post operation (p.o.). Having penetrated the lesion, axons became associated with a framework of low affinity nerve growth factor receptor (NGFr)-positive non-neuronal cells (Schwann cells and leptomeningeal cells). Surprisingly few of these axons were derived from dorsal root ganglion neurons. At the longest survival time (56 days p.o.), there was a marked shift in the overall orientation of fibres from a largely rostro-caudal to dorso-ventral axis. Attempts to identify which recognition molecules may be important for these re-organisational processess during attempted tissue repair demonstrated the widespread and intense expression of the cell adhesion molecules (CAM) L1 and N-CAM. Double immunofluorescence suggested that both Schwann cells and leptomeningeal cells contributed to the pattern of CAM expression associated with the cellular framework within the lesion. (Supported by the BMBF (Neurotrauma NRW, Teilprojekt II-4).)

P317. Protective action of α-agonists in a model of spinal cord injury

G.Z. Sufianova, A.A. Suphyanov, L.A. Usov, A.G. Schapkin, L.Yu. Raevskaya, S.S. Golubev, Y. Perelomov, T.P. Morozova (Irkutsk, RUS)

The purpose of work was study of protective action of adenosine A1-agonists (adenosine and CPA) on model of spinal cord injury.
Materials and methods: The work is carried out at 27 rats, weighting 150-200 g. The model of spinal cord injury was created by compression damage and based on Tator CH technique. A laminectomy of Th12 was made. The spinal cord was injured with 50 g metallic cylinder, applied over the exposed dura for 1 min. In control 1 of series was investigated 9 rats. In 2 of series the spinal cord of 6 rats was injured with above described technique. In 3 of series of rats (N=6) was studied the adenosine influence of the area of neuronal loss. Adenosine was injection subcutaneal in the dose 300 mg/kg. In 4 of series was studied the CPA influence on area of neuronal loss. One hour before injury rats receive*ƒ* a single intracutaneal dose of CPA 2.5 mg/kg. The estimation of neuroprotective effects of preparations was based on degree of neural impairments (4 point neurologic scale) in 1, 3 and 7 day after spinal cord injury; on SEMG and morphological investigations in 7 days after spinal cord injury. SEMG was carried out with use of needle electrode stimulation of ischiadic nerve with measurement of M-response and H-reflex. The injured segment of spinal cord was fixed in 96% spirit. Was studied section of spinal cord stained by hematoxylin-eosine.Data analised using Student`s-test.
Results: The animals(2 of series) developed paraplegia, anesthesia and pelvis disturbances in all term of supervision (the degree of neural impairments was 4 points). The results of SEMG: the average characteristics of M-response threshold (T)= 3.55 mV, latent period (LP)=10,92 msec. The amplitude (A)=5.17; duration (D)=5; the H-reflex was not marked. This data significantly differed from normal (accordingly M-response are: 1.58, 5.3, 11.78, 6.5; and H-reflex : T=5.12, LP=17.0, A=7.0, D=5.2). In 3 of series the degree of neurological disturbances by 7 days was a little smaller rather 2 of series, but without statistical difference (accordingly 3.5 and 4 score). The clinical symptoms was confirmed by the data SEMG. The characteristics of M-rasponse are : T=4.02, LP=8.83, A=5.17, D=5.67. Insignificant positive dynamic however, was marked H-reflex on the background of adenosine administration (T=14.6, LP=25.83, A=4.17, D=4.5), but can siderably decreased. The characteristics of M-rasponse are : T=2.25, LP=7.67, A=9.3, D=6.0; The characteristics of H-reflex are : T=11.27, LP=26.67, A=5.17, D=5.0.The morphological research has only oedema of gray and white matter, chromatolis and loss of dendrites of separate neurons. The results of the present study suggest that A-agonists (adenosine and CPA) provide significant neuroprotective effect at spinal cord injury. Selective A1-agonists (CPA) has more expressed protective action.

P318. Axonal regrowth and functional locomotor recovery after spinal cord transection and transient immunological myelin suppression

J.K. Dayer, C. Cantu, J.D. Steeves (Vancouver, CDN)

Failure of axonal regeneration in the adult mammalian spinal cord has been ascribed to the presence of reactive astrocytes, inhibitors associated with myelin, and a lack of an appropriate neuronal cell body response to initiate axonal growth. Previously we have described axonal regrowth after a partial injury due to the removal of myelin-associated inhibitors. Transient demyelination is achieved by the infusion of serum complement along with an antibody specific to a myelin surface epitope, i.e. galactocerebroside (GalC). Removal of myelin in the region of the lesion occurs within 7d; after therapeutic intervention for 14 days, 35d post injury and recovery ~30% of injured

rubrospinal fibers have re-grown and taken up retrograde tracer (Fluorogold). We now describe the effects of immunological demyelination after a complete spinal cord injury (transection). Animals received a complete lesion of the spinal cord at the 9th thoracic vertebra level of the cord. The lesion gap was filled with fibrin glue, +/- trophic factors (e.g. FGF-1). A cannula was then implanted intraspinally to a depth of 3mm, and held in place by means of a micro-screw and dental cement. A 14d (0.5 micro L per hour) Alzet Osmotic Mini-pump was attached to the cannula. Contents of the pump include either (a) complement alone, (b) complement + anti-GalC or (c) complement + anti GalC + FGF-1. Thus, we have assessed the therapeutic potential of combined immunological demyelination and trophic factor administration after spinal transection by anatomical (retrograde and anterograde labeling) and behavioral measures (BBB Open Field Locomotor Scale). Treatment with complement alone or complement + anti-GalC, resulted in a sustained improvement in locomotor ability (BBB score of 3.8 vs. 2.1 after 6 weeks recovery). Addition of FGF-1 to the fibrin glue (in combination with immunological demyelination) resulted in an increase in BBB score (3.5 vs. 2.5 after 3 weeks recovery). Addition of FGF-1 directly to the osmotic mini-pump along with the demyelination reagents resulted in further functional improvement (increase to 5.5 on the BBB scale). This data suggests that for a severe, i.e. complete spinal lesion addition of FGF-1 to an immune stimulated CNS (e.g. demyelinated) promotes an improvement in functional locomotor recovery greater than immunological demyelination treatment alone. (Supported by a BC Neurotrauma Initiative Grant and Salary support to JKD.)

P319. Inhibition of collagen scar formation in lesioned rat spinal cord

S. Hermanns, F. Lausberg, H.W. Müller (Duesseldorf, D)

Traumatic injury of central nervous system (CNS) axons generally leads to deposition of extracellular matrix in the lesion scar. Basement membrane is a predominant structure to the lesion scar and is mainly composed of collagen type IV and laminin which act as a scaffold for associated proteins that are discussed to be inhibitory for axonal regeneration (Stichel et al., 1999a). We have previously shown that basement membrane deposition following transection of the postcommissural fornix in the adult rat is a major impediment for axon growth and contributes significantly to the failure of CNS axons to regenerate in vivo (Stichel et al., 1999b). The present study was performed to reduce basement membrane deposition in a trauma lesion model of the adult rat spinal cord. The dorsal corticospinal tract (CST) was transected at midthoracic level using a Scouten wire knife. Immediately after transection we applied either the iron chelator bipyridine (DPY) an inhibitor of prolyl-4-hydroxylase (PH) or a combination of the more potent inhibitor BPY-DCA and 8-Br-cAMP. The latter treatment should simultaneously inhibit the biosynthesis of collagen and reduce the proliferation rate of collagen-synthesizing fibroblasts that invade the lesion. After different timepoints the corticospinal tracts were anterogradely traced by BDA pressure injections into the sensorimotor cortex. Animals were allowed to survive for up to three months before they were sacrificed by perfusion. Spinal cord tissue was embedded in paraffin, cut and the spatial relationships between CST axons and the lesion scar were examined. Our results demonstrate that the combination treatment of BPY-DCA plus 8-Br-cAMP, but not DPY alone, successfully suppressed deposition of collagenous basement membrane in the spinal cord lesion. The effects of the anti-collagen treatment on axon regeneration will be discussed. (Supported by the Deutsche Forschungsgemeinschaft, SFB 194/B5.)

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Poster Session 16
Vascular mechanisms and metabolism 1

P320. The effects of hyperbaric oxygen on cerebral metabolism and intracranial pressure in severely brain-injured patients

S. Rockswold, G. Rockswold, J. Vargo, R. Sutton, T. Bergman (Minneapolis, USA)

Hyperbaric oxygen (HBO) has been shown to reduce mortality by 50% in a prospective randomized trial of severely brain-injured patients at our institution. The purpose of this study was to determine the effects of HBO on cerebral blood flow (CBF), cerebral metabolism, and intracranial pressure (ICP) and to determine the optimal HBO treatment paradigm. HBO treatments (100% O2, 1.5 atmospheres absolute) were given to 37 patients (mean Glasgow Coma Scale score 5.8) for 60 minutes every 24 hours for up to 7 treatments. CBF (nitrous-oxide method), arteriovenous difference of oxygen (AVDO2), cerebral

metabolic rate of oxygen (CMRO2), ventricular cerebrospinal fluid (CSF) lactate, and ICP values were obtained 1 hour pre-dive and 1 and 6 hours post-dive. The patients were categorized by reduced, normal, and raised pre-dive CBF (n=22, 46, and 44 dives), based on Obrist's classification system. Data were analyzed with repeated measures ANOVA. CBF and CMRO2 were raised 1 and 6 hours post-dive in dives beginning with reduced CBF ($p<0.05$). CBF and CMRO2 were raised at 1 hour ($p<0.05$) but fell by 6 hours post-dive in dives beginning with normal CBF. CBF was reduced at 1 and 6 hours post-dive ($p<0.05$), but CMRO2 was unchanged in dives beginning with raised CBF. AVDO2 remained constant from pre- to post-dive in all patients. CSF lactate levels were decreased 1 and 6 hours post-dive, regardless of CBF category ($p<0.05$). Pre-dive ICP values > 15 mmHg were decreased 1 and 6 hours post-dive ($p<0.05$). The effects of each HBO treatment did not last until the next dive. The increased CMRO2 and decreased CSF lactate levels after treatment indicate HBO may improve aerobic metabolism in severely brain-injured patients. HBO seems to normalize coupling of CBF and CMRO2. This is the first study to show a prolonged effect of HBO treatment on post-dive CBF and cerebral metabolism. Our data suggests shorter, more frequent dives may optimize HBO treatment.

P321. Complement depletion does not lessen the infarct size in ischemia-reperfusion model in the rat

S. Shimizu, T. Kumagai, M. Ogata, T. Yamamoto (Fukushima, J)

In experimental spinal cord injury, our studies have shown that microglia/macrophage activation is suppressed in the vicinity of lesion formation and beneficial effects of complement system and thus microglial activation may influence the cerebral ischemic process. In an attempt to evaluate the effect of complement depletion in cerebral ischemia, we have studied the rat ischemia model with and without complement depletion. Sprague-Dawlay male rats given a purified cobra venom factor 24 hours prior to unilateral occlusion of the carotis-MCA artery. After 24hrs, serum complement level was under detectable level and the low level lasted 72hrs or more. The intracarotid thread model was utilized for induction of ischemia under halothane anesthesia. Cannulation was kept for 60 minutes and then removed with the common carotid having been occluted. The animals were allowed to survive for 72 hrs, the removed brain was sliced and stained with TTC. Volumetry was performed with computer-assisted image analyzer. The infarct size varied considerably even among the control group. The comparison of two groups(each 10 rats) did not reach the statistically significant difference with regard to the infarct size in this ischemia-reperfusion model. There appear to be several reasons. The blood-brain barrier is not immediately compromised in cebral ischemia, as opposed to traumatic spinal cord injury and thus serum complement may not be activated promptly. The infarct produced in our experiment might have been too profound to find any major difference in infarct size. In fact, soluble CR1 was shown (Science 285,595-599,1999)to be effective in reducing infact size. Thus, more work needs to be done by comparing the infarct size and pathology in less stringent ischemia-reperfusion in order to attest our hypothesis.

P322. Alterations of glucose metabolism and ATP production after fluid percussion injury in rats

N. Marklund, S. Sihver, B. Långström, M. Bergström, G. Ronquist, L. Hillered (Uppsala, S)

Changes in cerebral blood flow and glucose metabolism are commonly seen after both clinical and experimental traumatic brain injury (TBI) and may contribute to the secondary injury process. [18F]Fluoro-2-deoxyglucose (FDG) is frequently used to estimate local cerebral glucose metabolism (lCGU). FDG is trapped early after its uptake into the cell and thus provides information on glycolysis but not oxidative metabolism. Here, we compared the early (10-20 min) and late (12 h) time-course of FDG and 14C-labeled glucose in the hippocampus and parietal cortices bilaterally after fluid percussion injury (FPI) in rats. Furthermore, FDG uptake and cortical ATP levels were measured at 40 min and 12 hours after the injury.
Materials and methods: Pentobarbital (60 mg/kg i.p.) anesthetized rats were subjected to a moderate (2.5-2.6 atm) lateral FPI. 15 MBq of FDG and 0.2 MBq of 14C glucose was injected 3 min prior to FPI followed by decapitation at 10 or 20 min after injection. In addition, FDG uptake alone was assessed at 40 min or 12 hours. In separate animals, brains were rapidly frozen and cortical samples were dissected out and ATP levels were subsequently determined by HPLC.
Results: There was a significantly early (10-40 min) increase in FDG uptake in ipsilateral cortex and hippocampus compared to the contralateral side. In contrast, there was no relative ipsilateral increase in 14C glucose at 10 and 20 min after injury. At 12 hours, a hypometabolic state was seen with decreased ipsilateral uptake of both FDG and 14C glucose. This corresponded to normal ATP levels at 40 min that were significantly decreased at 12 hours.
Conclusion: Our data show that TBI causes an early increase of glycolysis sufficient to maintain ATP production. However, there was a decreased glucose uptake with a corresponding decrease in ATP

production at 12 hours. This suggests a delayed disturbance of oxidative metabolism, perhaps allowing time for therapeutic intervention.

P323. Uncoupling of blood flow and metabolism following cerebral contusion in the rat

H.K. Richards, S. Simac, J.D. Pickard (Cambridge, UK)

PET scans of head-injured patients often show discrete areas of high blood flow or metabolism. However, such scans are usually performed some time after the initial ictus. We have investigated cerebral blood flow and metabolism using quantitative autoradiographic methods 2 hours following controlled head injury in an animal model. Experiments were performed on 18 anaesthetized, ventilated (1.5% halothane in 2:1 Nitrous oxide/oxygen) Sprague-Dawley rats weighing 300-330gm. A burr hole was made over the left parietal cortex, and all animals received a piston impact on the intact dura (2mm diameter, 2.0 m/s, 2mm depth). All animals remained anaesthetized and ventilated for a further 2h, after which quantitative autoradiography was used to determine either 1) Local cerebral blood flow (LCBF) using 14C-iodoantipyrine; 2) Local cerebral glucose utilization (LCGU) using 14C-deoxyglucose or 3) Local cerebral glucose content (LCGC) using 14C-methylglucose. LCBF, LCGU and LCGC were measured in 5 regions, adjacent to the contusion and values were then normalized on the contralateral cortex. There was no significant difference between normalized LCBF, LCGU or LCGC in ipsilateral cortex nor in the ischaemic core of the contusion. However there were marked changes in the patterns observed in the boundary (within 1mm of the contusion). In all six rats used for LCGU measurement there were discrete areas of high metabolism, whereas in all six rats used for LCBF measurement, flow was universally depressed in the boundary zone. Of the six rats used for LCGC determination, in only one was there a discrete area of high signal. We conclude that there are discrete areas of uncoupling of cerebral blood flow and metabolism following head injury within 2 hours of cerebral contusion in the rat which cannot be explained by changes in cerebral glucose content.

P324. Neuronal damage in pericontusional edema zone

H. Kushi, T. Saito, N. Hayashi (Tokyo, J)

Objectives: We previously reported that the cerebral blood flow (CBF) and cerebral blood volume (CBV) in pericontusional edema zone (PEZ) were very low levels. In this report, we investigate the constructive mechanism of the PEZ from a molecular biology and pathological vantage point.

Methods: We examined 5 obstructive head injury patients who all had undergone surgical removal of hematoma. Patient age fell in the 52 ± 27 years range, presented a GCS ranging from 5 to 9 when admitted to our institution. Internal decompression performed 72 hours after admission. Both the hematoma and PEZ were surgically removed, and the latter was reserved for subsequent pathological analysis. Additionally, cerebrospinal recordings of IL-6, IL-8 and IL-10 were made at baseline (admission), at 24 hours, and at 72 hours.

Results: Pathological analysis had revealed a high concentration of neutrophils within the PEZ vessels, a portion of which leaked beyond this region.

	Admission	24 Hours	72 Hours
IL-6 (pg/ml)	550 ± 950	4350 ± 1679	878000 ± 512000
IL-8	715 ± 320	804 ± 420	24900 ± 10500
IL-10	15 ± 6	4.0 ± 0.5	5.0 ± 0.1

Conclusion: The IL-6 and IL-8 were abnormally high from early on, and continued to climb as the PEZ expanded further. However, it became clear that in head injury, IL-10 does not inhibit the inflammatory response. The inflammatory response is intimately linked to the occurence and expansion of PEZ, and the corresponding drop in CBF and CBV in this region is believed to result from the vascular closure triggered by neutrophils that were in turn stimulated by the inflammatory cytokines.

P325. Neuronal activity determined by quantitative EEG and cortical microdialysis is increased in brain-injured rats

O.W. Sakowitz, A.W. Unterberg, J.F. Stover (Berlin, D)

Increased glutamate release linked to sustained neuronal activation results in posttraumatic tissue damage. Thus, further increase in neuronal activity and e.c. glutamate should be avoided. The aims of the present study were to 1) determine changes in synaptic activity using quantitative EEG and microdialysis and 2) estimate the impact of neuronal activity on e.c. glutamate following controlled cortical impact injury (CCII) in 20 rats. EEG was recorded before, directly after, 4 and 24 hours following CCII. E.c. glutamate was analyzed 4 and 24 hours after CCII with a microdialysis catheter positioned within the contused cortex. Functional changes in synaptic activity and e.c. glutamate were investigated by modulating inhaled isoflurane concentration. Differences in results (mean ± SD) was significant at $p< 0.05$. Using the same isoflurane concentration (1.8 vol%) EEG power was significantly depressed directly after trauma compared to pre-trauma levels (13 ± 6 vs. 6 ± 2 microV2), followed by a significant increase by 4 hours (32 ± 14 microV2) persisting up to 24 hours after trauma (22 ± 14 microV2). Modulating

isoflurane resulted in concentration-dependent changes in EEG power. Nevertheless, this responsiveness was significantly reduced at all time points following CCII compares to pre-trauma reactivity. Overall, EEG power correlated significantly with pathologically elevated e.c. glutamate levels (-100 microM) (n= 58; R2= 0.54). 1) Posttraumatic disturbance of the excitatory pathway is reflected by increased synaptic activity, decreased responsiveness to increasing isoflurane, and elevated e.c. glutamate. 2) Neuronal activity needs to be considered for the interpretation of e.c. glutamate levels.

P326. The evaluation of biochemical disorders in patients with head injury by mathematical modelling

V.V. Zotov, G.M. Yakhnenko, N.V. Guzhovskaya, T.S. Bondar (Kiev, UKR)

The urgency of investigated problem is caused by necessity of dynamic control of efficiency conducted treatment of patients with head injury and first of all-forecasting and prevention of ishemical violation in brain, that is formidable forerunner of developing power-generating insufficiency owing of the oppression of Krebs cycle. The present research is based on study of the complex biochemical parameters of blood by 87 patients with head brain injury of various degree of gravity: potassium, sodium of plasma and erythrocitites; general, free and connected water; haematocrit, acid-main balance, protein and protein fractions, sugar, me-dium molecular peptides, haemoglobin, erythrocities and their sorption ability. The computer-statistical analysis of actual material is conducted, are deduced the clinical-biochemical correlations. Is shown, that prevailing biochemical violations are expressed acidotic infringements of parity of oxygen and carbonic acid pressure, degree which is defined of gravity of head injury and character of current of after-injury period. This infringement of parity of protein fractions and sulphidril groups, ionic relations in plasma and erythrocities, increase the contents of medium molecular peptides and sorption erythrocities ability as parameters of endogenic intoxication. Is conducted the mathematical simulation of data, constructed index of gravity, enabling to floor the additional criterias of forecast of clinical current of head injury and valuation of efficiency of conducted treatment.

P327. Continuous monitoring of local cerebral blood flow by laser Doppler flowmetry in the traumatic rats and effects of acupuncture

H. Huang, L. Zhang, D. Zhi, L. Wang (Tianjin, PRC)

Objectives: Continuous monitoring of local cerebral blood flow (ICBF) was carried out using laser Doppler flowmetry in the traumatic rats and the change of cerebral blood flow with the treatment of acupuncture was studied.
Methods: 15 Wistar rats were divided randomly into normal group, traumatic group and acupuncture group. The model of brain trauma was induced on the left cerebrum by the free setting method. ICBF of right cerebrum dura were monitored for 1 hour during 1,3,5,7 days before and after injury. Acupuncture "renzhong" and "neiguan" of rats for 1minute.
Results: 1. ICBF was increased 49% and 18% after injury for 1 minute and 2 minute, respectively. Its were remarkably decline down after 20 minutes of injury. ICBF was increased 25% after acupuncture of "renzhong". ICBF was kept in the normal range and markly increased after 40 minutes of injury in the acupuncture group.2. ICBF of the third day was the highest in each group and the acupuncture group was the highest in the third day.
Conclusion: 1. ICBF of the traumatic group was tentatively increased after injury and not stable during the 7 days. 2. The ICBF can be remarkably increased and maintained the higher level after acupuncture of "renzhong".it's hint that the function of brain tissue can be recovered quickly with the treatment of acupuncture as early as possibly after head injury.

Poster Session 17

Experimental therapy and management 2

P328. Conservative treatment of traumatic intracranial hematomas

A. Lantukh, V. Banashkevich, S. Gulaeva, A. Korobtsov (Vladivostok, RUS)

The analysis of clinical, first and repeated CT data and also catamnesis of 64 patients with solitary traumatic intracranial hematomas, which were subject the conservative treatment has been done. There are 37 cases of inracerebral hematomas (IH), 17 epidural hematomas (EH) and 10 cases of subdural hematomas (SH). The following indication have been developed for conservative treatment of different types of hematomas: 1. The consciousness level of patients is not lower than 11-12 points at Glasgo Coma Scale. 2. The dimension of IH are maximum diameter for CT no more than 4 cm; EH dimension – maximum axial dimension no more than 6 cm and thickness of EH no more than 1 cm; the volume of SH no more than 30 ml.

P329. Hypopituitarism after traumatic brain injury – a preliminary report

D. Kelly, IT. Gaw Gonzalo, P. Cohan, N. Berman, R. Swerdloff, C. Wang, (Los Angeles, Torrance, USA)

Recognizing pituitary insufficiencies after TBI may be important given that hypopituitarism-related neurobehavioral problems are typically alleviated by hormone replacement. This prospective study sought to determine the rate and risk factors of pituitary dysfunction after TBI in patients at least 3 months post-trauma. Patients underwent dynamic anterior pituitary function and posterior pituitary function testing. Results were compared to those of 18 age, sex and body mass index-matched volunteers. The patients included 18 men and 4 women, (mean age 28 years, initial GCS 3-15). Eight patients (36.4%) had a subnormal response in at least one hormonal axis: 4 were growth hormone (GH) deficient; 5 had an inadequate gonadotroph response (4 men, all with normal testosterone levels, and one woman with low estradiol); 1 had both GH and thyrotroph deficiency and 1 had GH deficiency and borderline cortisol deficiency. At the time of injury, all 8 patients with pituitary dysfunction had a GCS<11, and compared to the 14 patients without dysfunction, were more likely to have diffuse swelling on CT (8/8 vs 7/14, $p<.05$), and sustained a hypotensive or hypoxic insult (7/8 vs 6/14, $p=.07$). A good recovery (GOS 5) was achieved in 1 of 8 hormonally insufficient patients compared to 9 of 14 patients without insufficiency ($p<.05$). Of the 12 patients in the cohort who did not achieve a GOS of 4 or 5, 58.3% had hypopituitarism. From this preliminary study, some degree of hypopituitarism appears to occur in approximately 40% of patients with an initial GCS <13, with GH and LH/FSH deficiencies being most common. More severe injuries and secondary insults are likely risk factors for hypopituitarism. Post-acute pituitary function testing may be warranted in most moderate and severe TBI patients, particularly those with brain swelling and those sustaining hypotensive or hypoxic insults. The neurobehavioral benefits of GH replacement after TBI warrant further study.

P330. Guidelines implementation in the management of severe head injury in Latvia

I. Aksiks, E. Valeinis, R. Sverzickis, D. Kurme (Riga, LET)

Since November, 1998 Neurosurgery Clinic of P. Stradins Hospital is involved in the collaboration project with Brain Trauma Foundation (BTF), previous Aitken Neuroscience Center, New York, USA. Patients with severe brain trauma are managed observing evidence based scientific Guidelines developed by BTF and approved by the USA and World Neurosurgical Societies. 100 patients with GCS 3-8 were entered in the Internet database (52 patients with GCS 3-5, 48 with GCS 6-8). Since starting intracranial pressure (ICP) monitoring in May, 1999 it was monitored in 62 patients out of 76 (82%) and postoperative treatment was based on maintaining ICP below 25 mm Hg and cerebral perfusion pressure (CPP) above 70 mm Hg. Of the 70 patients who have been followed for 6 months after trauma 33 (47%) had good outcome, 4 (6%) – fair, 33 (47%) died. In admittance GCS 6-8 group good outcome was observed in 29 patients (74%), fair in 2 patients (5%), 8 patients (21%) died. Poor outcome was closely related to late hospitalisation, poor pre-hospital treatment and deep coma (GCS 3-5) in admittance. ICP>30 mm Hg and CPP<60 mm Hg were predictors of poor outcome. Better outcome in severe brain injury patients can be achieved by appropriate treatment in the pre-hospital stage, rapid transportation directly to the Neurotrauma centre, immediate hematoma evacuation, intracranial pressure monitoring. Treatment must be based on correcting intracranial hypertension and maintaining sufficient cerebral perfusion pressure. Guidelines' implementation is very effective in improving patient outcome after severe brain trauma and they should be accepted in other Latvian neurotrauma hospitals.

P332. L-arginine and SOD ameliorate cerebral ischemia after controlled cortical impact injury

L. Cherian, C. Robertson (Houston, USA)

Severe controlled cortical impact injury (CCII) in rats causes a marked reduction in cerebral blood flow (CBF) at the impact site. This study examined the effects of L-arginine, superoxide dismutase (SOD) and D-arginine on these abnormal cerebral hemodynamics after severe injury. Fasted Long Evans rats were anesthetised with isoflurane and subjected to severe (5 m/sec, 3 mm deformation) CCII. Rats received L-arginine or D-arginine, both 300mg/kg 5 min after injury or SOD 24,000 units/kg pre-injury +1600 units /kg/min for 15 min after injury. Control rats received saline 5 min after injury. CBF, using laser Doppler, and nitric oxide (NO) using a NO electrode, was measured at the impact site for 2hr after injury. Rats treated with saline or D-arginine exhibited a 29% reduction in CBF and the NO concentration was decreased by 18± 4.3 nM. L-arginine administration returned CBF and NO to near normal levels and pre-injury SOD administration prevented the decrease in CBF and NO during the 2hr period. The SOD studies suggest that free radical production contribute to the decrease in NO after CCII. The L- and D-arginine studies suggest that the mechanism of L-arginine's effect on CBF is by increasing levels of NO.

P333. Feracryl – new perspective in endoscopic neurosurgery

A.A. Suphyanov, A.G. Schapkin, G.Z. Sufianova, L.Yu. Raevskaya, S.S. Golubev (Irkutsk, RUS)

General toxicity of feracryl is very low, but neurotoxicity is unknown. This point restrict using of feracryl in neurosurgery. Purpose of this research was to investigate possible neurotoxicity of 1% solution of feracryl in the intracranial experimental rats surgery. We used 52 rats with 180-250 g. weight. We used feracryl performed by Irkutsk "PUSK" factory. In the first group of rats (N=10) we performed cranial trepanation (size 4x8 mm) and linear encephalotomy in the fronto-parietal region of right hemisphere with following application of hemostatic sponge wetted by 1% solution of feracryl on the injured surface of brain. In the rats of control group (N=10) we maked application of hemostatic sponge wetted by physiological solution. In the second group (N=6) we performed cranial trepanation (size 5x5 mm) and maked cavity by partial aspiration of frontal lobe of right hemisphere (0,15-0,20 ml) with following intracavitary implantation of hemostatic sponge (23-25 mg) wetted by 1% solution of feracryl. In control group (N=6) performed intracavitary implantation hemostatic sponge wetted by physiological solution. Third group of rats (N=10) included intracerebroventricular injection of 0,03 ml of 0,1 % feracryl solution. In control group (N=10) introduced 0,03 ml of physiological solution. Neurological disturbance evaluation maked during first 150 min and following 5 days. After 120 hours brain removed and fixed in 96 % alcohol. Microscopic sections stained by hematoxylin-eosine. During all time of observation neurological disturbances in the first and second groups of rats were same with control rats and conformed to the localisation and levels of brain damage. Rats from third group were without the neurological damage. Histological investigations of rats brain operated with feracryl revealed same change of neurones and pathways with control animals. Absent of specific neurological and histophatological changes in the operated with feracryl animals and high level of hemostatic activity determine the perspective of feracryl in neurosurgery.

P334. PCO2 monitoring during an acute craniotomy

J. Ferber, H. Juniewicz, R. Pieniek, E. Glogowska, J. Wronski (Wroclaw, PL)

Clinical data suggest that cerebral blood flow (CBF) can be abnormally low within the first four to eight hours after SHI (1). An aggressive hyperventilation can additionally worse CBF and provoke cerebral ischemia (2). Therefore an accurate PCO2 monitoring in SHI patients (pts) is necessary. PetCO2 failed to reflect PaCO2 in SHI pts treated in NICU (3). Up to now, the validity of PetCO2 monitoring in estimating PaCO2 during an acute posttraumatic craniotomy has not been studied. Forty five adult SHI pts operated on because an acute intracranial posttraumatic haematoma within 8 hours after head trauma entered the study. The standard anaesthetic protocol included N2O/O2, fentanyl and pancuronium bromide anaesthesia, and mechanical ventilation with respiratory rate 10 ÷ 12 bpm and tidal volume in mL = body weight (kg) x 10–100. After obtaining a stable PetCO2 arterial blood sample was taken for PaCO2 measurement and DPCO2 = PaCO2 – PetCO2 was calculated. DPCO2 ranged from -9 to 20 mm Hg (5 ± 6, mean ± SD). DPCO2 within 2 ÷ 6 mm Hg occurred in 17 (37 %) pts only. A negative DPCO2 was stated in 20 % of pts. No relationships between DPCO2 and pts age, mean arterial pressure and heart rate were found. DPCO2 was higher in normocapneic than in hyperventilated pts. We can conclude that during an acute craniotomy in SHI pts, PetCO2 does not reflect accurately PaCO2 and the monitoring of adequacy of ventilation should be based on repeated or continuous measurements of an arterial PCO2.

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P335. Noradrenaline increases cortical perfusion and tissue oxygen but fails to reduce traumatic brain edema in rats

J.F. Stover, O.W. Sakowitz, S.-N. Kroppenstedt, P. Mayr, A.W. Unterberg (Berlin, D)

Following traumatic brain injury noradrenaline (NA) is applied to increase cerebral perfusion. Beneficial effects of increased perfusion could be off-set by NA-mediated vasoconstriction and edema formation. Effects of NA on cortical perfusion, brain tissue oxygen, CSF glutamate and hypoxanthine, and brain edema were investigated at 4 and 24 hours after controlled cortical impact injury (CCII) in 30 rats. At 4 or 24 hours after CCII, NA was continuously infused (90 min) increasing MABP to 120 mmHg while controls received NaCl (MABP 90 mmHg). Cortical perfusion and brain tissue oxygen were measured before, during, and after infusion. At the end of the study period (8 or 28 hours), CSF glutamate and hypoxanthine, and brain swelling were determined. Differences of results (mean ± SEM) are significant at $p < 0.05$. At 4 hours cortical perfusion was significantly reduced by 50% reaching pre-trauma levels again at 24 hours. Infusion of NA significantly increased cortical perfusion and brain tissue oxygen by 60 and 58%, respectively at 4 and 24 hours after CCII.

Pathologically elevated CSF glutamate and hypoxanthine remained uninfluenced by NA at 8 and 28 hours. At 8 hours, brain swelling showed a trend to higher values under NA (7.7 ± 0.8 vs. 5.8 ± 1.0%). At 28 hours, brain swelling was similar under NA and NaCl (10.4 ± 0.9 vs. 9.6 ± 1.8%). At MABP of 120 mmHg NA does not seem to induce vasoconstriction as cortical perfusion and brain tissue oxygen are not reduced and CSF glutamate and hypoxanthine levels are not increased. Nevertheless, persisting excitotoxicity and energetic impairment could explain the tendency towards increased brain edema at 8 hours after trauma.

P336. The investigation of meksidol injection influence on neurological disturbances in rats after 12-min cardiac arrest

N.A. Gorenkova, I.V. Nazarenko, A.V. Volkov (Moscow, RUS)

The aim of present study was to determine if the meksidol – the new original nootropic substance with antioxidant activity – could influence on the mortality level and recovery of the neurological deficit on the early postischemic stage. The cardiac arrest type cerebral ischemia was induced by compression of intrathoracic main vessels with special hook under the ether anaestesia. The resuscitation of animals was started 12 min after onset of ischemia and included approximately 1-2 min of cardiac massage and 30 min of artificial ventilation with respirator. Meksidol at 50 mg/kg i.s. was injected with onset of resuscitation. Meksidol at single injection was shown to be able to improve significantly parameters of rat´s vital functions recovery and to accelerate the neurological recovery. The data of three main physiological parameters suggest that the meksidol in the single injection markedly accelerate appearance of breathing and corneal reflexes after the end of resuscitation. But there was not significant influence on the recovery of core´s functions. The disappearance of neurological deficit after cardiac arrest was observed in ischemic rats on the 7th day and on the 5th day in meksidol group. Moreover single injection of meksidol resulted in significantly lower level of neurological deficit at the first day. As for mortality level it wasn´t revealed any major influence after meksidol injection. Thus meksidol seems to be possessed of compensatory effects on the neurological disturbances in postischemic period after cardiac arrest. The data obtained suggest that meksidol could be the very perspective base for therapy practise in early postischemic recovery period.

P337. Changes and surgical treatment of cysts septi pellucidi (C.S.P) following brain trauma

C. Jiayi, L. Mezha, G. Guoliang (Wuchang Wuhan, PRC)

Cysts septi pellueidi (C.S.P) is more rarely, we reported 20 cases of changes of Expanding C.S.P following brain trauma in recent 12 years. These cysts are small, incidence decline with increasing age, and they are generally of no clinical significance. Such cysts obviously expand are believed to be of some clinical significance, as they may interfere with the internal drainage of c.s.f, thus causing hydrocephalus distinguished between: – asymptomatic cysts, – symptomatic cysts. Consecutive CT seans showed the expanding cysts was discovered because a pathologic chamges after brain trauma. The method of treatment included streotactic introduction of catheter as an internal shunt. trans cranial burr hole subarchnoid space introduction of catheter as an external shunt. Treatment of these cysts were satisfactory.

Poster Session 18

Epidemiology, mild and moderate brain injury 1

P338. Traumatic coma – a modified scale (Unitary Coma Scale)

St.M. Iencean (Iasi, RO)

A number of 210 head-injuried patients in coma without surgical indication and without other trauma was studied. Cerebral CT showed no brain compression lesions, but diffuse cortical hemorrhages, cerebral edema etc. We apply the WFNS coma scale, GCS, Glasgow-Liege scale and a personal modified coma scale. Glasgow-Liege coma scale made a correct prediction of outcome in 65% cases for 1-12 years old and for over 54 years old group patients. The modified scale – a unitary scale has an accurate prediction in traumatic coma in over 85% cases. This scale is : *A. phonal response to pain:* – moans and grumbling, 3 points; – incomprehensible vocalism, 2 p; – no response, 1 p; *B. motor response to pain:* – localizes, 5 p; – withdraws from pain, 4 p; – abnormal flexion, 3 p; – extension, 2 p; – flaccidity, 1 p; *C. vegetative status was estimated:* – normal respiration, 3 p; – abnormal rspiratory patterns, but normal pulse, 2 p; – abnormal respiration,unstable pulse and blood pressure, 1 p; *D. brain stem reflexes:* – frontal orbicular response, 7 p; – oculovestibular response, 6 p; – pupils reactive to light, 5 p; – normal deglutition, 4 p; – pharingeal deglutition, 3 p; – oculoheart response, 2 p; – no reflexe present, 1 p. This modified scale, named Unitary Coma Scale has

the advantages: – as a theoretical pattern it includes the first neurological and vegetative principles; – the concordance with the outcomes is very accurately (over 85-90% in all cases); – this UCS is in accordance to WFNS scale and includes the modified elements of GCS,Glasgow-Liege scale and vegetative elements; – is easy to use and maintains a common language. I hope the utilization of UCS will become better and will establish its true importance.

P339. The significance of early prognostic factors for the neurobehavioral outcome in patients after closed head injury

B.O. Hütter, M. Weinzierl, J.M. Gilsbach (Aachen, D)

The knowledge about early predictors of the late quality of life and persistent cognitive impairments after traumatic head injury is sparse as of yet. The present study was performed in order to identify the prognostic significance of parameters assessed early after the trauma. A consecutive series of 76 patients was examined one to two years after head injury by the Aachen Life Quality Inventory (ALQI) and a comprehensive neuropsychological examination. According to the Aachen Coma Scale (ACS), 16% of the patients had sustained a severe, 32% a moderate and 52% a mild head injury. The following parameters of the acute course were employed: ACS, Glasgow Coma Score (GCS), duration of amnesia and of coma, increased intracranial pressure, presence of a traumatic subarachnoid hemorrhage (tSAH), presence and location of cerebral contusions. The rating of the pupil reaction exhibited the largest correlations with impairments of the overall life quality, restrictions in the areas of activation and cognitive capacity sharing up to 20% of common variance. The pupil reaction score showed the strongest correlations with simple reaction time (r=-.53), reaction time of divided attention (r=-.49) and reaction time in a Go/NoGo task (r=-.49) ($p<0.01$, respectively). The duration of coma predicted quality of life and the degree of physical impairment, but showed no relationship to cognitive impairments. The presence and severity of a tSAH exhibited a grave prognostic significance. Multivariate analyses revealed as the most important prognostic factors tSAH, disturbances of the pupil reaction and the acute state of consciousness (ACS). The present findings suggest that several parameters of the acute course can significantly predict the later degree of neurobehavioral impairment after traumatic head injury. Beyond well established variables, the rating of the pupil reaction proved as an additional important predictor for the neurobehavioral impairments after head injury.

P340. Multiple injured with head injury versus isolated head injury – differences and predictors

U. Lehmann, M. Lorenz (Hannover, D)

Intention of this study was to analyse the difference between severe head injuries (SHI) and multiple injured with severe head injury (MI-SHI)
Patients and methods: All patients (pat.) (age>14 years) admitted to Hannover Medical School within 4 hours after accident with an initial Glasgow Coma Scale (GCS)<9 or a decrease <9 during the clinical course and/or a pathological finding in the first CT from 1.2.1996 to 31.7.1997 were included. As multiple injuries were regarded any additional injury with 3 or more points in the abbreviated injury score. Last examination 12 months after injury. Parameters at scene: bloodpressure (BP), O2-saturation (SO2), GCS, pupil-reaction, intubation, amount of infusion. Measurement every hour during day 0: BP, pH, base excess, SO2, ventilation (FiO2, frequency and volume), hemoglobin, bloodcount PTT, Quick, central venous and intracranial pressure (ICP); kind and amount of all drugs and fluids. Statistical analysis performed using ANOVA.
Results: Included 83 pat. (76% male; 15-73 years of age) 41 pat. with SHI and 42 MI-SHI. No difference were found for rescue time, heart rate (HR), SO2 and GCS. Motor-GCS better in the MI-SHI group ($p=0.048$). Emergency room: Higher HR and lower BP was observed in MI-SHI patients ($p=0.004$). Regarding pat. with a BP<90 mmHg at scene 38% of SHI and 55% MI-SHI died. When it was impossible to increase BP above 90mmHg in the emergency-room 55% MI-SHI and 75% SHI died ($p=0.02$). There were no differences in bloodgases or chemical parameters. ICU: BP in MI-SHI group was always lower despite cathecholamines. MI-SHI survivors had a lower ICP than SHI pat. Critical CPP at day 11.
Outcome: Head injury is main reason for death: 69,2% SHI and 57, 1% of MI-SHI. Glasgow Outcome Scale (GOS) 1-3: MI-SHI 41,8% versus 57,1% of SHI, GOS 4+5: SHI 42,9% and Mi-SHI 58,2%.
Conclusion: Despite identical rescue, diagnostical and therapeutical regimes, the results in MI-SHT were poor in comparison to isolated SHI.

P341. The best materials and timing for dural, skin and bone reconstruction in patients with war head injuries

M. Jovanovic, S. Ivanovic, B. Nestorovic, M. Rakic (Belgrade, YU)

During the five years period (1990-1994) more than 290 patients were treated for war head injuries at Neurosurgical department of Emergency Center, Clinical Center of Serbia in Belgrade. Besides other problems related to direct lesions of brain tissue, we

have noticed some specific problems with other tissue defects and its importance in further complications. In this study we have tried to find the best methods and materials for dural, bone and skin reconstruction. Summarizing our experience we can conclude that for dural reconstruction the best materials are aloplastic (autotransplantation of periosteum or fascia lata) resulting in less complications and better results. In small skin defects we used local skin flaps, for bigger skin defects, in minority of cases we had to mobilize and rotate skin flap with further free skin transpantates sec Tirsch to cover the secondary skin defect. To prevent infection: both dural and skin reconstruction has to be done as soon as possible but for bone defects we suggest the delayed surgery for the same reason. Only big bone defects were treated surgically (heteroplastic material: Palacos was used), after 6-12 months, in cases with no signs of infection. In correlation with other materials, methods and timing of surgery this strategy gave better results.

P342. Head injury management in relation to changes in the availability of CT scanning services – a national study 1994-1998

L.T. Dunn, D. Beard, J. Henry (Glasgow, Edinburgh, UK)

Background and objectives: The availability of 24 hour CT scanning services outwith neurosurgical centres in Scotland has increased over the last decade. Early scanning of appropriately selected patients is assumed to improve management and outcome. This study has assessed the effect of changes in the provision of CT scanning services on head injury management in Scotland.
Design: Retrospective review of data from a national trauma audit database over a 4 year period.
Methods: The Scottish Trauma Audit Group prospectively collects detailed information on trauma admissions in Scotland and currently includes more than 90% of Scottish trauma admissions. Head injury has been defined as an Abbreviated Injury Score (AIS) for the head of 3 or greater. Data on all head injuries included in the database between 1994 and 1998 (2637 in total) has been reviewed. Location of and time to first CT scan, time to theatre and mortality has been extracted.
Results: In 1994 36% of patients had their first CT scan at the hospital of their initial admission. In 1998 this figure had risen to 49%. 6% of patients were transferred to a regional neursosurgical unit (NSU) for CT in 1994 compared to 8% in 1998. In 1994 24% did not have a CT scan compared to 15% in 1998. In the remainder the location of the scan was not recorded. The median time to CT from arrival at hospital was 111 minutes in 1994 and 90 minutes in 1998 for patients having their scans at their initial admitting hospital. For patients transferred to a NSU the median times were 155 and 146 minutes respectively for 1994 and 1998. The median time to theatre for patients with head and multiple injuries was 3 hours in 1994 and 5 hours in 1998. For isolated head injuries the median times were 5 and 8 hours respectively. Overall mortality in 1994 was 25% and in 1998 22%.
Conclusion: Over the period of the study more patients underwent CT scanning at their hospital of initial admission and had their scan sooner after injury. This has not been associated with any consistent change in time to theatre or mortality.

P343. Scale evaluation in brain injury patients – discordant cases and remarks

I. Poeata (Iasi, RO)

Failure in precise knowledge of coma mechanisms phenomenon and reticulate system recording possibilities give rise to a lot of coma scales. Glasgow coma scale is mostly use. In 200 severe brain injury patients we analyze difficulties with this evaluation method. Main problems was etilic-traumatic coma: 8%, seizure with brain injury in epileptic patients: 4%, motor score in thetraplegic-hemiplegic patients: 60%, verbal score in aphasic patients: 25%. Cautions mast be taken in brain injury patients evaluation with Glasgow coma scale. Associated conditions: alcohol abuse, seizures, stroke, worse the comatous statement in brain injured patients. Neurologic deficits (thetraplegia, hemiplegia, aphasia) rise difficulties in motor and verbal score evaluation. When comparing different series of brain injured patients correction factors have to be discussed concerning with protocol evaluation.

P344. Epidemiology of head injury in bicycle accidents without motor vehicle – analysis of 42 cases

B. Depreitere, S. Maene, J. Goffin, C. Plets, J. Vander Sloten, R. Van Audekercke, G. Van der Perre (Leuven, B)

It is not well documented whether there are specific biomechanical characteristics of head trauma in bicycle accidents. We present a retrospective analysis of 42 victims of bicycle accidents in which no motor vehicle was involved. The aim of the study is to recognize biomechanical patterns in this subset of accidents. In the past 10 years 89 victims of bicycle accidents were admitted to our neurosurgical department for operation or ICP-monitoring. A questionnaire was sent to all patients and their records and CT head scans were analysed. 44 patients suffered a fall without involvement of a motor vehicle. 42 could be analysed. The site of impact was equally distributed over the frontal, low parietal and

temporal regions, occipital impact being less frequent. Skull fractures were present in 93% of cases, extradural haematomas in 38%, acute subdural haematomas (ASDH) in 38%, cortical contusions in 76%, subcortical intracerebral haematomas (ICH) in 24%. Diffuse axonal injury was not diagnosed. No deep ICH's were found. Age correlated strongly with the presence of ASDH and with the number and total volume of contusions and the evolution of a contusion to ICH. Disruption of bridging veins was documented as the cause of ASDH in two cases. All ASDH's were accompanied by underlying contusions. Extradural haematomas were most frequently situated over the temporal and parietal lobe, had an overlying fracture in 94% and were more frequent in the younger population. Impact site influenced the appearance of extradural haematomas and ASDH. Cycling velocity was only significantly correlated with the appearance of contusions. We conclude that in this subset of accidents head injury is predominantly caused by impact on the skull and the brain and that acceleration only plays a minor role. Age is an important factor in determining nature and extent of lesions. Cycling velocity seems less important.

P345. The prognostic significance of midbrain lesions revealed by MR imaging and electrophysiologic investigation in severe head injury

C. Wedekind, R. Fischbach, M. Lippert-Grüner, M. Ebel, N. Klug (Cologne, Münster, D)

The occurrence of a midbrain dysfunction is known as an in-dicator of a severe course in head injured patients. To inve-stigate the prognostic signi-ficance of a midbrain lesion re-vealed by electro-physiologic testing (mas-seter reflex) and/or by MR imaging, a total of 49 cases suffering from severe head injury (Glasgow Coma Score (GCS)<8) were sepa-rated into 2 groups: one with radiologic and/or electrophy-siologic evidence of mid-brain lesion (n=29) and the other one without these findings (n=20). The latter 20 cases were mat-ched with 20 sub-jects of the further group accor-ding to GCS, sex, and age. Outcome was assessed 6 to 24 months after injury by means of the Glasgow Out-come Score (GOS). T-test for matched pairs revealed a signi-ficantly worse outcome for the patients with midbrain lesion (mean GOS=2.7 vs. 3.8 for the group without midbrain le-sion). The outcome was equally influenced irre-spec-tive of the diagnostic tool: Mean GOS was 3 vs. 3.8 ($p=0.005$; t-test for independent samples) for electrophysiologically detected mid-brain lesions and 2.6 vs. 3.8 ($p<0.001$) for midbrain lesions revealed by MR imaging. It is concluded that the presence of electrophysiologically detected and/or MR imaged lesions of the midbrain strongly indicate a severe course and major disability in severe head injury.

P346. Classification of brain injury based on magnetic resonance imaging

R. Firsching, D. Woischneck, S. Klein, S. Reissberg (Magdeburg, D)

Objectives: Magnetic resonance imaging is undoubtedly less practicable in the acute emergency after head injury than computerized tomography, but it is more precise in depicting local lesions of brain tissue. As it is rarely performed in the acute stage, there is hardly any data available on MRI in comatose patients.
Patients and methods: In 100 patients comatose after head injury early magnetic resonance imaging was performed after an initial computerized tomography scan while the patient was still on ventilation. The location and extent of the lesions identified with magnetic resonance imaging was related with the duration of coma and outcome.
Results: Four types of lesions were distinguished, which had a distinctly different prognosis arranged according to their significance/mortality: I. Lesions restricted to the hemispheres only. II. Unilateral lesions of the brain stem at any level. III. Bilateral lesions of the mesencephalon. IV. Bilateral pontine lesions. Patients in grade I had a mortality of 9%, in grade IV all patients died. This correlation was highly significant.
Conclusion: As mortality was closely and significantly related with outcome, the above classification is considered more relevant than previous attempts to classify head injury according to computerized tomography.

P347. Teleconsulting system in neurotrauma emergency cases

Z. Czernicki, M. Mierzejewski, M. Glowacki, J. Bogucki (Warsaw, PL)

Objectives: The goal was to optimalize the treatment of head injured patients treated in hospitals far away from neurosurgical center.
Methods: Teleconsulting system is based on CT images transmission.CT images are transmited directly from the peripheral CT scanners to the PC in the Departament of Neurosurgery using standard 56kbps modems and regular telephone lines. System runs with network software MultiView (EMED,Inc.).Standard CT examination consist of 18 images is transmitted in about 5 minutes.
Results: Applied system improved decission making process concerning qualification of the head injured patients for neurosurgical intervention and consecuitevely for transportation.Until today above

600 transmissions has been performed. About 40% of the consulted patients were treated conservatively in peripheral hospitals.Those qualified for neurosurgical intervention were transported to the Department of Neurosurgery Polish Academy of Sciences in Warsaw.
Conclusion: System enables: 1. Avoidance of unnecessary transportation of head injured patients who could be treated conservatively in peripheral hospitals. 2. Monitoring of conservative treatment performed in peripheral hospitals (repeated neurosurgical consultation). 3. Treatment costs reducing.

P348. Traumatic versus spontaneous intracerebral hemorrhage – a comparative study
M.S. Siddique, H.F. Fernandes, L. Treadwell, J. Barnes, B.A. Gregson, A.D. Mendelow (Newcastle-upon-Tyne, UK)

We present a comparison of the clinical characteristics, treatment and outcome of traumatic and spontaneous intracerebral haemorrhage (TICH vs. SICH). This is an observational study based on detailed prospectively collected data on 441 cases of SICH (1993-1999) and 90 cases of TICH (1987-99). The median age of patients with TICH (51) was lower than for SICH patients (65). Of the SICH group, 24% had a favourable outcome at 6 months [favourable outcome (FO) = Good Recovery + Moderate Disability on the Glasgow Outcome Scale (GOS); Unfavourable outcome (UO) = Death + Vegetative State + Severe Disability]. For the TICH group, FO at 6 months was much higher at 67%. The median Glasgow Coma Score (GCS) at presentation was significantly higher in patients with FO in comparison with patients with UO in both the SICH (15 vs. 12) and the TICH (14 vs. 7) groups. Patients with a FO were also significantly younger in both the groups: median age 60 vs. 67 for UO in SICH and 43 vs. 56 in TICH. In the SICH group, 16% of the patients had their haematoma evacuated. The median age of these patients was 60 years with a GCS of 11, compared with a median age of 66 years and median GCS of 13 for those who did not undergo surgery. In the TICH group, 33% of the patients had their haematoma evacuated. The median age of these patients was 50 years, median GCS 11, compared with a median age of 52 years and median GCS of 13 for those who did not have surgery. These groups are non-randomised and therefore evaluation of the effects of clot evacuation is not possible. Nonetheless, there was no statistically significant difference in the outcome between the operated and the non-operated patients in either the SICH or the TICH groups. These data demonstrate, however, that age and presenting GCS are strong predictors of outcome for both types of haemorrhage. For the SICH group younger patients were more likely to have surgery, but for the TICH group, neither age nor neurological status determined surgical treatment. This confirms that indications for surgical intervention are not well defined. The international STICH (Surgical Trial in Intracerebral Haemorrhage) trial may resolve the dilemma for SICH. A similar trial is needed for TICH.

Poster Session 19
Intensive care and neuromonitoring 2

P349. NSE and S100 in serum as markers for the severity and prognosis of traumatic brain injury (TBI)
G. Pelinka, H. Pelinka, W. Mauritz, E. Tögel, H. Redl (Wien, AT)

Objectives: to investigate the course of neuronal markers, neuron specific enolase (NSE) and S100 following TBI in serum and their potential use as prognostic markers in TBI.
Patients and methods: 3 patient groups were studied: isolated TBI (TBI, n=16), TBI plus multiple trauma (TBIMT,n=15) and multiple trauma without TBI (MT,n= 5). Blood was drawn upon arrival in the emergency room (ER), immediately after ICU admission and daily during the first 2 weeks in the ICU.
Results: Patients were separated into survivors and non-survivors and their data were classified into 3 periods, ER, admission to ICU (ICU1), last value in ICU (ICU2).
Conclusion: Upon admission to the ER, all patients show significantly elevated neuronal markers, which are not directly proportional to the extent of TBI. Following a subsequent drop in all patients, lethal outcome is then preceeded by a second peak, which requires further investigation as a potential prognostic marker.

P350. Decompressive craniectomy in post-traumatic brain swelling
N. Barrientos, J. Silva, E. Terra (Santiago, RCH)

Objectives: To assess the effectiveness of this procedure in controlling ICP due to posttraumatic brain swelling
Methods: A random sample of 50 consecutive patients with severe head injury admitted to our emergency department underwent decompressive craniectomy with dural expansion to control massive postraumatic brain swelling. This sample includes a wide range of ages (15 to 66 years) and multiple trauma. Surgery was performed according to the following parameters: Glasgow Coma Scale (GCS) score equal or lower than 8; elevated ICP refractory to conventional medical treatment such as hyperventilation with

normocapnia, osmotic diuretics and even barbiturate coma; rapid worsening of GCS associated to CT scans showing massive brain swelling, midline shift, cisternal obliteration and impending herniation, with or without acute collections.
Results: According to the Glasgow Outcome Scale good results were achieved in nearly half of the cases (42%). Old age,multiple trauma, acute subdural hematoma, rapid increase in ICP, low CGS were all bad predictive factors and explain the high mortality rate (46%) in the sample. The larger percentage of deaths ocurred within the first 24 hours. Early surgery was performed within 24 hours in 60% of the cases and within 72 hours in 84% of them. Mean ICP readings before decompression amounted to 33.9 mm Hg and after surgery they decreased to 15.7 mm Hg.
Conclusion: Early decompressive craniectomy was effective in controlling elevated ICP due to massive refractory brain swelling. These encouraging results may broaden the future therapeutic scope in these cases.

P351. Study on short latency somatosensory evoked potentials and brainstem auditory evoked potentials in patients with head trauma
C. Liu, R. Wu, H. An (Guiyang, PRC)

The short latency somatosensory evoked potentials (SSEPs) and brainstem evoked potenetials(BAEPs) in 115 patients with head trauma were analysed by a new method. The results revealed that the abnormal rates of the SSEPs were 94.8%. The pathological bases resulting in it were the direct and/or indirect lesion on the somatosensory pathway. The SSEPs recorded six months after the trauma indicated that the electric activities of neurons in some of the patients were still abnormal although they recovered well. The patients complicated with traumatic lesion of acoustic nerve, blood clots in external ear canal and pre- existing hearing loss had abnormal BAEPs that could not be used in diagnosting brainstem lesion. The patients without these complications in whom BAEPs were abnormal had an unvavourable outcome. The SSEPs and BAEPs simultaneously recorded could aid localiation of the injury in the brain and enable discovery of the fact that the rigidity after decortication or decerebration is a result of severe injury of the cerebral hemisphere.

P352. Long-term monitoring in neurological rehabilitation
M. Lippert-Grüner, D. Terhaag (Cologne, D)

Early brain-injury rehabilitation has rarely made use of conti-nuous long-term monitoring. However, is is in this early phase of treatment, that the uninterrupted recording of the vegetative parameters in particular, which often show irregularities, is necesary for a speedy application of complex rehabilitative care. We developed a multimodal monitoring programe, which was implemented in the earliest phase of brain-injury rehabilitati-on. Until now, we have examined 30 patients after severe brain-injury, who were either unconscious or showed severely limited avareness (GCS < or = 8). The results indicate that the recording of neurophysiological parameters allows targeted monitoring of the patients during the course of the day. This offers greater therapeutic security and leads to the early recognition of potential instabilities. In addition, looking for "therapeutic windows", i.e. phases of improved awareness during the course of the day, brings about ptimal therapy scheduling and therapy division, and facilitates the recovery of a day-and-night rhythm.

P353. Concurrent monitoring of local cerebral electrophysiology, metabolism, and pH in severely head-injured patients
T. Reeves, T. Clausen, M. Reinert, R. Bullock (Richmond, USA)

Cerebral microdialysis has provided novel information on ion fluxes, transmitter release, and metabolism after human traumatic brain injury. Furthermore, monitoring of brain tissue oxygen tension (ptiO2), carbon dioxide (ptiCO2) tension and pH has evolved as a promising tool in the intensive care management of these patients. We have developed a technique to combine these microdialysis and physiological measurements with electrophysiological monitoring by attaching an extracellular recording electrode to the microdialysis probe, to detect neuronal spike discharges, field potentials, and local EEG within the cortex. Two-hour samples of electrophysiological data were acquired in 8 severely head injured patients, and the activity of 10 isolated single units was selected for detailed analysis. Observed neuronal discharge rates were aberrantly low (<0.5 spikes/sec). In three patients pathological synchronized activity was indicated by aberrant field potentials, or pronounced rhythmicities in serial correlograms. The EEG was dominated by delta frequency activity (mode=3.1 Hz). In all patients, one or more of the following tissue parameters were measured concurrently with the electrophysiology: [glucose]o, [lactate]o, [glutamate]o, [K+]o, ptiO2, pCO2, or pH. These results demonstrate the feasibility of electrophysiological recording concurrently with the monitoring of diverse physiological substrates in a focal brain region following severe head injury. A future goal of this research will be to determine to what extent the recorded neural activity correlates with the other tissue measurements, and to examine the prognostic

value of the electrophysiological parameters. (Supported by NS 12587.)

P354. Therapy of malignant intracranial hypertension by controlled lumbar cerebrospinal fluid drainage

P. Vajkoczy, E. Münch, C. Bauhuf, M. Quintel, P. Schmiedek (Mannheim, D)

Objectives: Lumbar drainage of cerebrospinal fluid (CSF) in intracranial hypertension is currently considered contraindicated due to potential transtentorial or tonsillar herniation. The objective of this study was to assess the efficacy and safety of controled lumbar CSF drainage for the treatment of increased intracranial pressure (ICP) and to evaluate side effects of this therapeutic modality in adult patients suffering from refractory intracranial hypertension.
Methods: 23 patients (age 36 ± 17 yrs) with severe traumatic brain injury (TBI) (n=12) or vasospasm after subarachnoid hemorrhage (SAH) (n=11) were analysed prospectively. Patients were considered for controled lumbar CSF drainage if they suffered persistent intracranial hypertension (ICP>25 mmHg for more than 15 minutes) and failed to respond to high intensity treatment (including barbiturate coma, decompressive craniectomy, etc.). Lumbar CSF drainage was contraindicated in patients with tight basal cisterns. After institution of the lumbar drain, aspiration of 5 to 20 ml CSF was performed and controled continuous CSF drainage was maintained. ICP and cerebral perfusion pressure (CPP) were documented before and after bolus-aspiration of CSF. CCT scans taken on admission, before and after institution of the lumbar drainage and before discharge from the ICU were analyzed to determine the visibility of the lateral ventricles and the basal cisterns. The Glasgow Outcome Scale (GOS) was evaluated 6 months after injury.
Results: Lumbar drainage was installed 4.8 ± 3.0 days after TBI/SAH and the average period of drainage was 6.6 ± 5.1 days. Upon bolus CSF aspiration, all patients showed an abrupt and lasting decrease of ICP (-17.4 ± 11.4 mmHg) and increase of CPP (+14.3 ± 10.9 mmHg). Visibility of lateral ventricles and basal cisterns did not change. Despite initial malignant intracranial hypertension that was refractory to high-intensity treatment, ten patients (44%) showed a favorable outcome (GOS 4-5), four patients (17%) survived with a severe permanent neurological deficit (GOS 3), one patient (4%) remained in a persistent vegetative state (GOS 2) and eight patients (35%) died (GOS 1). Two patients showed a transient unilaterally dilated and fixed pupil at 6 and 8 hours after lumbar drainage, respectively, which rapidly reversed after discontinuation of CSF drainage. In one patient CSF leakage sustained for 2 days after withdrawl of the catheter. There was no instance of epidural- or subarachnoid hemorrhage and no infection that could be related to the lumbar catheter. In five patients (21.7%) the lumbar catheter needed replacement because of obstruction (n=3) or dislocation (n=2).
Conclusion: Controled lumbar CSF drainage represents an highly efficient strategy to significantly reduce therapy-resistant intracranial hypertension. The risk of transtentorial or tonsillar herniation can be minimized by considering lumbar drainage only in the presence of discernible basal cisterns.

P355. The time course of brain interstitial glycerol activity in severely head injured patients

K. Koenig, E. Rickels, H.E. Heissler (Hannover, D)

Introduction: Interstitial glycerol may be a potential marker for posttraumatic membrane phospholipid (MPL)breakdown. Glycerol, an end product of MPL degradation, was measured in brain interstitial fluid following traumatic brain injury using microdialysis methodology.
Patients and methods: In eight patients (GCS 3-10, age 14-58 years, 6 males, 2 females) matching criteria for neuromonitoring microdialysis (MD) were performed. MD samples were collected hourly and analysed for glucose, lactate, pyruvate (alternatively glutamate), and glycerol. The MD catheter was placed next to the lesion. Biochemical data was completed by merging blood pressure, intracranial pressure and oxygenation data.
Results: In seven out of eight patients the time course of glycerol traces showed an increase in activity after insertion of the MD catheter, reaching peak activities (up to tenfold) about 25 hours (range 17-30 hours) later. This pattern was found regardless of the time elapsed since admission. Glycerol levels decayed differently but generally tended to fall. In the individual case subsequent increases in glycerol activity were observed. One patient showed a "paradox" glycerol response.
Conclusion: Interstitial glycerol elevations after catheter insertion have been found in patients as well as in laboratory animals and were exclusively attributed to the preceding trauma. We found an amazing uniform pattern in glycerol responses always after MD catheter insertion at markedly different times after trauma. We therefore doubt these glycerol levels to be specific for the trauma event but assume an artefact.

P356. Clinical features and dynamics in cerebral circulatory metabolism of acute subdural hematoma caused by boxing injury

T. Saito, H. Kushi, N. Hayashi (Tokyo, J)

Objectives: We investigated the clinical features and changes in cerebral circulatory metabolism of head injury due to boxing.
Methods: The subjects were 10 professional boxers admitted to our institution for emergency operation. Their initial GCS scores were 4 to 15 and their age in 17 to 27 years. We studied their changes in consciousness after injury, pupil abnormalities, CT findings, and time lapse from pupil abnormalities until operation, and outcome (GOS score at 6 months). In six of these patients, we measured CBF, CBV, and CMRO2 using stable Xe-CT and dynamic CT.
Results: 5 of 10 patients showed lucid interval within 15 minutes. 9 patients had pupil abnormalities (anisocoria 6, bil. mydriasis 3). CT scan showed all patients had unilateral acute subdural hematoma (ASDH). Time lapse from pupil abnormalities until operation were 85 to 205 minutes. The operation found all patients suffered from ASDH without cerebral contusion. The outcome of 10 patients were; good recovery 4, moderate disability 2, severe disability 1, and dead 3. In the favorable outcome group, CBF changed from hypo- to hyperperfusion, CBV changed within almost normal range, and CMRO2 decreased to 50% of normal value. On the other hand, in the poor outcome group, CBF showed hypoperfusion, CBV climbed above normal range, and CMRO2 decreased to below 30% of normal value.
Conclusion: We found that head injury due to boxing was ASDH without cerebral contusion caused by the collapse of the bridging vein. The changes in acute phase cerebral circulatory metabolism were found using stable Xe-CT and dynamic CT.

P357. Clinical comparison of ICP readings obtained from the new Spiegelberg intraparenchymal pressure transducer with ventricular pressure

I. Chambers, A. Mendelow, M. Siddique, K. Banister (Newcastle upon Tyne, UK)

Objectives: The clinical evaluation of new monitoring devices is essential to ensure their accuracy, suitability and robustness before their introduction into general use. This study has evaluated the accuracy of a new parenchymal ICP monitoring device over extended periods of monitoring in the clinical environment.
Methods: Eleven patients in whom there was a clinical need for the insertion of an external ventricular drain also had a Spiegelberg parenchymal transducer also inserted on the ipsilateral side. Paired of readings were taken every hour until the ventricular drain was no longer required for clinical management. Prior to each reading the external drain was closed and the system allowed to equilibrate. A transducer connected to the external drain, sited at the level of the external auditory meatus provided measurement of the ventricular pressure. The zero and calibration of the external transducer were checked twice daily. Altman-Bland plots were created for all the paired readings and descriptive statistics used to evaluate the measurements.
Results: A total of 843 hourly readings were obtained (median recording 76 hours, range 40–111 hours). No Spiegelberg transducers failed during the monitoring period and no incidence of infection due to their insertion was noted. One patient developed a small intracerebral haemorrhage 40 hours after insertion of the device. The values obtained from the Spiegelberg transducer closely matched the ventricular pressure and this was maintained over extended periods of monitoring. A high percentage (78.5%) of the readings were within ±5 mmHg, and 95.9% were within ±10 mmHg. The mean difference between the two readings was less than 0.1 mmHg (EVD – Spiegelberg) with a standard deviation of 4.9 (95% confidence interval 0.3).
Conclusion: The Spiegelberg parenchymal transducer provides an accurate measurement of intracranial pressure when compared to ventricular pressure over extensive periods of monitoring. The transducer was found to be robust in the clinical environment.

P358. Quantitative pupillary measurements in head injury

T.A. Gennarelli, L.F. Marshall (Milwaukee, San Diego, USA)

In order to determine the utility of quantitative measurements of pupillary function in head injured patients, sequential recordings were made in two university hospital neurosurgical ICUs. Pupillary sizes were quantified by a hand held computerized pupillometer (NeuroOptics) at the bedside. This instrument measured pupillary size before and after a constant intensity brief light stimulus as well as the latency to the onset of pupillary constriction, the constriction velocity and two dilitation velocities. When ICP was measured, we compared these quantitative pupillary observations to the magnitude of ICP. In this abstract we report the relationships between ICP and pupillary contractility that were observed. These early observations showed that when ICP was elevated to more than 20mmHg, the latency to pupillary constriction was longer and the constriction velocity was lower than normal. We conclude that precise measurements of pupillary function can increase the precision of the neurological examination and that the pupillary quantification may be an indicator of elevated ICP.

Poster Session 20

Experimental therapy and regeneration in SCI 2

P359. A new cocktail of bone morphogenetic proteins promotes functional motor recovery in a model of traumatic spinal cord injury

D. A. Braguglia, P. Bittmann (Winterthur, CH)

At present no effective treatments are available to treat traumatic or pathological SCI. In the present study we investigated the properties of a mixture of growth factors and morphogens isolated from bovine long bones ("bone proteins" or "BP"). In vitro BP enhances PC12 neurite extension and differentiation, promotes survival of embryonic chick ciliary ganglion and rescues embryonic telencephalic neurons from glutamate induced toxicity. In vivo, we used a standardized model of rat spinal cord injury where parameters could be set to induce, using an inflatable balloon, either a fully reversible paraplegia or to have animals which permanently remain in the paraplegic state. Initially, parameters are fixed to induced a fully reversible paraplegia within 18±1 (n=6) days. After administration of BP (7.0 microgr/kg for 7 days) in the fourth ventricle, animals improved their performance and regained alternate stepping behavior within 10±1 days (n=6). The neuroregenerative effect of BP was also visible at the cellular level (decrease in the lesion's induced astroglial cellular density) and by the decrease of the cystic cavity associated with the contusion. A model in which the animals would stay permanently paralyzed would even more closely match the situation encountered in the emergency rooms. Parameters of inflation were adapted to induce an irreversible paraplegia. Animals in the vehicle group arrested their progression at the hindlimbs flexion stage whereas administration of BP (7.0 microgr/kg for 10 days) was able to almost fully restore the motor functions within 13±2 days (n=6). Neuroregeneration was monitored by measuring the cystic cavity (3 fold smaller) and the decrease in the astroglial cellular density upon injury (to a level comparable to that of healthy animals). Due to its combined action on critical steps involved in both neuronal cell death and regrowth, BP shows promise for new strategies against neurotrauma.

P360. Neuroprotective effects of glutamine synthetase in hypoxic injury of neonatal rat spinal cord

M. Matsumoto, K. Paul, M. Imielinski, Y.S. Yoon, P. Ceraulo, W. Huang, W. Young (Piscataway, USA)

Glutamine synthetase (GS) catalyzes the conversion of glutamate to glutamine. Recently, sheep GS has been reported to be neuroprotective when applied to the injured embryonic retina of the chick. We examined the effects of sheep and bacterial GS treatments on axonal depression in a neonatal rat hemicord preparation subjected to hypoxia. Compound action potentials from the corticospinal tract were activated by supramaximal constant current electrical stimuli and recorded with glass micropipette electrodes. The hypoxia was produced by superfusing with anoxic Ringer's solution (bubbled by 95% N2 and 5% CO2) for 30 min followed by cessation of superfusion for 60 or 120 min. The atmosphere of the chamber was maintained at 95% N2 and 5% CO2 during the hypoxic period. The hypoxia reduced response amplitudes that continued through the following 90 min of re-oxygenation. Treatment was applied 30 min after the start of the 30 min hypoxic period. Superfusion was stopped during this period, and resumed during re-oxygenation. Sheep (10μM) and bacterial (10μM, 1μM) GS significantly improved recovery of response amplitudes, and sheep GS (1μM) also tended to improve the recovery. The results suggest that sheep and bacterial GS have neuroprotective effect in hyoxic injury of spinal cord. Proposed mechanisms of their neuroprotective effect including increased glutamate conversion to glutamine, inhibition of GABA release, ammonium clearance and blockade of GABAA receptors will be discussed.

P361. Spatio-temporal pattern of the re-expression of the putative inhibitory molecule, keratan sulphate proteoglycan, following experimental rat spinal cord injury

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Keratan sulphate proteoglycan (KSPG) is developmentally regulated and is reported to be a barrier molecule, directing axonal growth during the formation of the central nervous system. It is possible that the re-expression of this putative repulsive molecule following spinal cord injury (SCI) may contribute to the prevention of axonal regeneration. In the present study, the spatio- temporal pattern of KSPG following SCI has been investigated to assess the possible correlation between KSPG and the failure of axonal regrowth. The compression injury effectively caused a local maceration of the spinal cord with no indication of axonal sparing at the lesion epicentre. By the end of the first week post injury (p.i.), there was a mild, diffuse increase of KSPG in the spinal cord parenchyma close to the lesion interface. Over the ensuing 3 weeks, KSPG expression close to the lesion interface returned to basal levels, while an intense re-expression could be detected within the lesion. The distribution of KSPG immunoreactivity demonstrated both cellular and extracellular matrix-

like patterns. During this period, there was a dramatic increase in the number of low affinity nerve growth factor receptor (NGFr)-positive cells (including Schwann cells) within the lesion. These cells formed an overlapping framework throughout the lesion site and supported substantial neurofilament-positive axonal growth. There was no apparent spatial restriction of axonal growth within the connective tissue scar of the lesion site. Regenerating nerve fibres could be seen in both KSPG-positive and KSPG-negative areas. The lack of any direct correlation between KSPG expression and the inhibition of spontaneous nerve fibre regrowth suggests that (at least) some populations of axons are capable of resisting the putative inhibitory effects of KSPG. (Supported by the BMBF (Neurotrauma – NRW, Teilprojekt II-4).)

P362. Local infusion of an antibody against TAPA/CD81 enhances functional recovery after spinal cord injury in rats

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The response of glial cells to spinal cord injury is a promising target for future treatment strategies. The monoclonal antibody AMP1 was found to alter the stability of astrocyte-astrocyte contact in vitro and to inhibit the proliferation of astrocytes and microglia. Furthermore, the AMP1-antigen (TAPA/CD81) is upregulated after traumatic spinal cord injury. Therefore we investigated whether intralesional infusion of AMP1 could enhance functional recovery after spinal cord injury. Adult rats were subjected to a moderate (12.5 gcm) spinal cord contusion using the NYU impactor device. A steel cannula was implanted at the lesion site and connected to an osmotic minipump. Two different doses of AMP1 and one dose of control IgG were infused for 14 days. Neurological function was assessed weekly on several functional tests for 8 weeks. Starting at 3 weeks post injury, rats treated with a low dose of AMP1 performed significantly better on the BBB locomotor rating scale (1.5-2 points) as compared to control IgG-treated animals. Hindpaw fine motor function, as assessed by BBB-subscores and gridwalk tests, was significantly increased from 2 weeks onward. Treatment with a high dose of AMP1 did not result in differences from IgG control on any of these parameters. Our data indicate that the AMP1 antibody stimulates functional recovery after spinal cord injury in rats, possibly as a result of modulating the inflammatory reaction and/or affecting the formation of the glial scar. Funded by GLAXO-RESEARCH-NL (GLN 95016) and the Spinal Cord Society NL.

P363. The pro-cysteine compound L-2-Oxothiazolidine-4-Carboxylate promotes retention of function following spinal cord trauma

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We tested the hypothesis that minimizing oxidative stress following spinal cord trauma will decrease inflammation thereby minimizing secondary damage and promoting functional retention. To test this we applied a 50 g compression force to T6 spinal cord followed by the administration of either saline or the procysteine compound L-2-oxothiazolidine-4-carboxylate (OTC). Spinal trauma caused oxidative stress that was rapid in onset and widespread as determined by glutathione (GSH) depletion, glutathione reductase inactivation and protein carbonyl formation. OTC administration (12 mmoles/kg initially followed by 4 mmoles/kg maintenance dose) for 5 days greatly reduced oxidative stress that was associated with decreased NFkB activation and decreased pro-inflammatory gene (e.g., iNOS) expression. When examined 6 weeks post-trauma, OTC administration correlated with better preservation of white matter (at T6: less than 1% in saline-treated vs 16% in OTC-treated animals). Better tissue preservation correlated directly with better functional retention, with OTC-treated animals having an angleboard score of 41.6±2.9 and BBB score of 12.9±0.5 versus 32.2±0.8 and 5.3±0.4 respectively in saline-treated animals. More recent work suggests that an OTC dose of 1 mmole/kg gives rise to comparable therapeutic effects. We conclude that decreasing oxidative stress following spinal cord injury through better maintenance of tissue GSH by administration of the cysteine precursor compound OTC effectively decreases the extent of secondary damage allowing better functional retention. (Supported by the Neurotrauma Initiative, Saskatchewan and the Saskatchewan HSURC.)

P364. Effect of acute caffeine treatment on experimental spinal cord injury in rats

K. Al Moutaery, S. Al Deeb (Riyadh, KSA)

Adenosine is an endogenous purine nucleoside which is released in brain during ischemia,epileptic seizure and hypoxic insults.Recent studies suggest the beneficial effect of adenosine in spinal cord injury (SCI). Caffeine is a potent adenosine receptor antagonist which is known to interfere with several neurotransmitters in central nervous system. The aim of the study was to determine the influence of acute caffeine treatment on neurological outcome following compression induced SCI in rats. Male Sprague-Dawley rats weighing 225-250g were anaesthesized

with chloral hydrate and laminectomy was performed at T 11-12 level leaving the dura intact. A compression plate(2.2x5.0mm) loaded with a weight of 35g was placed on the exposed cord for 5 minutes. Animal were divided into seven groups of ten rats each. The animals in Group 1 served as control whereas rats in Group 2 underwent laminectomy alone (sham). The rats in Group 3 to 6 were subjected to SCI as mentioned above. Animals in Groups 4, 5 and 6 also received caffeine in the doses of 25, 50 and 100mg/kg, i.p., respectively in addition to SCI, whereas the rats in Group 7 received caffeine alone at a dose of 100mg/kg body weight. The first dose of caffeine was given 30 minutes after SCI, followed by daily administration of drug for 9 days. Post traumatic neurological recovery was recorded daily for 10 days using a modified Tarlov score, inclined plane test and sensory and vocal score. For biochemical studies, a separate set of animals were sacrificed at 24 hr after SCI and the injured site of spinal cord was analyzed for lipid hydroperoxides, conjugated dienes and vitamin E. Neuropathological changes in the spinal cord were assessed using light microscopy. The rats exposed to SCI alone showed a maximum neurological deficit at 24hr and then a gradual recovery was observed over period of 10 days. The rats treated with caffeine along with SCI showed poor recovery as compared to untreated animals. Our histopathological studies also confirmed that concomitant exposure to caffeine produces a significant deleterious effect on the recovery from SCI.

P365. Regeneration of primary sensory axons through a peripheral nerve graft implanted into the adult rat spinal dorsal column

P. Dam-Hieu, S. Liu, A.S. Bonnard, N. Boisset, G. Said, M. Tadié (Le Kremlin-Bicêtre, F)

To investigate the role of peripheral nerve graft (PNG) in supporting the regrowth of dorsal root ganglia (DRG) axons into the dorsal column of adult rats, we grafted an autologous PNG bridging L3 and L4 left dorsal roots to the left dorsal column (at T12 level) after performing a left dorsal cordotomy at T13 level and rhizotomies of L3 and L4 left dorsal roots (n=12). One year after operation, HRP was retrogradely injected into the spinal cord 3 cm rostrally to the implantation site and HRP-stained neurons were searched in lumbar DRGs. In 4 rats, no HRP-stained neuron was found. But in 8 rats, HRP-stained neurons were found in left L3 and L4 DRGs (range: 2-259; mean: 71 +/- 94; median value: 29.5) and not in L1,L2,L5,L6 DRGs. By contrast, in the group of not repaired animals, which underwent only cordotomy and rhizotomies (n=12), no HRP-stained neuron was found in any animal. Histological examination of PNG showed evidence of axonal regeneration in the 8 positive animals. We can conclude that PNG joining dorsal roots to dorsal column and shunting the CNS-PNS junction may support a limited regeneration of DRG primary sensory axons, at least 3 cm rostrally to the implantation site.

P366. Nerve Degeneration of Lower Extremity After Paraplegia

Z. Shaocheng, X. Xianlun, L. Quanhua, Z. Jie, Y. Jinguo, Y. Baoqing, Z. Xuesong (Shanghai, PRC)

Objectives: To study the nerve degeneration of lower extremity after paraplegia.
Methods: (1) The spine cord of experimental dogs was severed between T11-12. The section of main nerve trunks of lower extremities were studied under microscope after 3-6 weeks; (2) The sural nerve of old paraplegic experimental dogs and obsolete paraplegic patients was taken and stained (Ponder-Kinyoun stain). The nerve trunks were analysed by microscopic cytologic examination.
Conclusion: No obvious degeneration is found in lower extremity nerve if the injury place is higher than T11.

P367. Implanting the Peripheral Nerve tissues to Treat obsolete Injury of Spinal Cord

Z. Shaocheng, P. Yu, Z. Yongjiang, Z. Chuansen (Shanghai, PRC)

Objectives: Supplying nerve fibres, nerve cells and factors included in them to spine cord improves the recovery of the function of the spine cord.
Methods: After releasing the spine cord, incises it vertically at the appropriate length and depth. One's own peripheral nerve (usually is sura nerve), which is removed its epineurium, nearilemma and incised it open and cut off a proportion of nerve fibres with microsurgery technique so that its character and appearance looks like cauda equine, is transplanted multi-striply, vertically inside the spinal cord incised and fastens appropriately to dura mater with 8"0"nylon thread and covered with sacrospinal muscle flap.
Results: At 1/2-3 years follow up post-operation, according to classification of Frankel: 4 cases recovered above 2 grade, 2 cases recovered above 1 grade.
Conclusion: This method can supply good enviroment of nerve growth and function of bridge and plank to segment of injury spine cord, thus can accelerate the recovery of spine cord.

P368. The functional reconstruction of femoral nerve and sacral plexuses in paraplegia with vascularized ulnar nerve transfer

Z. Shaocheng, D. Ruishan, Z. Jie (Shanghai, PRC)

To reconstruct partial peripheral nerve function in complete paraplegia patients with transverse injury of thoracic spinal cord.
Methods: One side of the ulnar nerve was cut off in the carpal level, of which the deep branch of the distal end was anastomosed with the pronator quadraturs muscle branch of anterior interosseous nerve and the superficial branch of the distal ulnar nerve was anastomosed with the superficial branch of radial nerve. The proximal ulnar nerve was dissected from the subcutaneous tunnel to it's originate in the axilla. The separated a vasculartized ulnar nerve was conducted into the lateral thoracic wall tunnel. The thoracodorsal artery and venous were exposed ad anastomosed end-to-end to superior ulnar collateral vessels. The femoral nerve and sacral plexuses were dissected in an ilioinguinal incision. The ulnar nerve was anastamosed with above nerves.
Results: 3 and 5 years follow-up of six patients got basic function recovery of the done nerves. They can hardly walk with sticks support because recovered the quadriceps power. The partial sensation and defecation urinary function were recovered. No significant claw hand was found in the donor site and the sensor in the hand improved to S3 and recovered to the original region.
Conclusion: Vascularized ulnar nerve transfer can reconstruct partial neurological function of complete transverse injury of the thoracic spinal cord.

Poster Session 21
Vascular mechanisms and metabolism 2

P369. The changes of rat blood-brain barrier permeability and expression of heat shock protein 70 after infrasonic damage

X. Zhang (Xi'an, PRC)

Objectives: To study the changes and significance of blood-brain barrier(BBB) permeability and the heat shock protein 70(HSP70) after infrasonic damage to the rats so as to probe into the mechanism of infrasonic biologic effects.
Methods: 40 SD rats were randomized into control, infrasonic damage 1 time, 7 time and 14 time four groups. 8Hz¡¢120dB infrasound was applied to infrasonic damage groups for 2 hours once according to the planned schedule. Lanthanum nitrate blending liquid was used for the brain fixation through intracardiac perfusion. Right frontal and temporal cortex were sampled for immunohistological examination. Under electronic microscope the changes of cerebral ultrastructure, BBB permeability were observed.
Results: With the increase in the times of infrasonic damage, cerebral ultrastructure become more aggravated. 1 and 7 time group shared the same changes of BBB permeability in which tight conjunction opened and Lanthanum nitrate particles passed BBB into the brain tissue. Compared with that of 1 and 7 time group 14 time group has more radical changes in which a great deal of Lanthanum nitrate particles exist in the brain tissue($P<0.05$). While in 1 time group there is a weak positive HSP70 neuronal expression, 7 and 14 time group shared the same changs of strong HSP70 neuronal expression which happened mostly in the pyramidal layer of the cortex.
Conclusion: Infrasound can directly damage the function and structure of cerebral ultrastructure and BBB and in the same degree, the extent of brain damage and positive HSP70 neuronal expression is closely related to the times of infrasonic injury. It is suggested that infrasound play a pivotal role in brain damage which can in turn limit the expression of HSP70 through increasing BBB permeability.

P370. Proteolysis in cerebrospinal fluid of patients with a severe head injury

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The damage of neurons activates proteinases, and their entering from a blood, owing to dysfunction of a blood-brain barrier significantly boosts the loss of neurons in a zone of ischemia. The purpose of research – to assess changes of the contents of proteolytic enzymes and their inhibitors in a cerebrospinal fluid (CSF) in patients with severe head injury (SHI). The proteolytic potential of CSF was studied under the contents of a neutral proteinase (PG / PN). Antiplasmin capacitance was studied by definition of three main inhibitors of PG / PN – a2-antiplasmin (a2-AP), a2-macroglobulin (a2-MG) and a1-antitrypsin (a1-AT). PG/PN, a2-MG, a2-AP, a1-AT were determined in CSF with the help of Enzyme-lynked Immunosorbent Assay (ELISA) and low-voltage rocket immunoelectrophoresis with applying of monoparticular antiserums. The contents of protein in CSF of patients with lumbar osteochondrosis considered to be normal: PG/PN-1.65mg/l, a2-MG - 1.47021 mg/l, a1-AT – 201 mg/l. By ELISA a2-AP was not determined neither in control group, nor in patients with SHI. The research of CSF was conducted in 30 patients with SHI, in the first, third, fifth, seventh and ninth day after injury.

	1 day	3 day	5 day	7 day	9 day
PG/PN	2.88±0.62	2.26±0.44	1.63±0.33	1.47±0.53	1.27±0.33
a2-MG	36.61±7.63	23.28±3.36	15.69±3.34	9.80±2.17	9.15±1.37
a1-AT	0.24±0.08	0.17±0.05	0.15±0.055	0.12±0.036	0.08±0.015

The analysis of proteolytic processes in CSF of patients with SHI has shown, that for this category of patients the concentration of PG/PN is considerably heightened. a2-MG is the main inhibitor, which derivates complexes with proteinases, where its active

center remains free and saves the capability to damage the neurons. The reason of poor activity of a1-AT remains not clear up to the end, inhibitor, which neutralizes PG / PN, derivating with it a complex without protease activity.

P371. Cranioplasty and its influence on postural blood flow, cerebrovascular reserve capacity and cerebral glucose metabolism

P. A. Winkler, W. Stummer, R. Linke, K.G. Krishnan, K. Tatsch (Munich, D)

The indications for cranioplasty after decompressive craniectomy are cosmetic repair and restoration of cerebral protection. The reasons for neurological improvement after cranioplasty remain unclear. Few observations concerning the impact of CSF hydrodynamic and atmospheric pressure were published during the last decades. Relevant data concerning the cerebrovascular reserve capacity and cerebral glucose metabolism before and after cranioplasty are lacking. For further insight the present study was undertaken to investigate the impact of cranioplasty on indices of cerebral blood flow regulation and metabolism. Thirteen patients with extensive craniectomies underwent a meticulous study of blood flow velocities in the middle cerebral artery (MCA) and extracranial internal carotid artery (eICA), as could be assessed by transcranial Doppler (TCD) during postural maneuvers and during stimulation with 1g acetazolamide for the interpretation of cerebrovascular reserve capacity (CR). Twelve patients underwent 18-FDG-PET. These measurements were made before and 7 days after cranioplasty. Cranioplasty improved preoperative differences in MCA flow velocities when comparing the injured side to the noninjured hemisphere. Cranioplasty resolved decreases in eICA flow in the injured hemisphere induced by postural changes, which was a constant finding prior to this procedure. More strikingly, however, the CR, that was severely impaired in both hemispheres, significantly increased after the procedure. Metabolic deficits, that were observed in the injured hemisphere as compared to the non-injured one, were found to improve after reimplantation of the skull bone flap. Cranioplasty appears to markedly affect postural blood flow regulation, cerebrovascular reserve capacity and cerebral glucose metabolism. Early cranioplasty is warranted to facilitate rehabilitation in patients after decompressive craniectomy.

P372. Expression of angiogenic molecules in brain contusions and brain swelling

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To study the modification of astrocytic and vascular compartments in brain contusion and swelling, the expression of separate angiogenic molecules in samples from brain contusions and brain swelling was investigated. Samples of brain contusions and brain swellings were taken at different days from injury accordingly to the day of surgery and immediately frozen at –70°C. The expression of separate angiogenic molecules (FGF-2, VEGF, PDGF-AA, PDGF-BB, integrin alphavBeta3, integrin alphavBeta5) was investigated by immunohistochemistry and Western Blot. Micro vessel count and density were determined counting vessels highlighted by CD34. Samples of normal brain were used as comparison. Our data showed that vessels and astrocytes in brain contusion and brain swelling expressed FGF-2, VEGF, PDGF-AA, PDGF-BB, integrin alphavBeta5. The expression of integrin alphavBeta3 was restricted to vasculature. VEGF expression was stronger around areas of necrosis or hemorrhage. The expression of angiogenic molecules increased in contusion surgically removed after 48 hrs from injury compared to those removed earlier. An increase of microvessel indices (count and density) was also documented in the same samples. The expression of separate angiogenic molecules by astrocytes and vasculature in brain contusions and brain swellings suggests 1) the activation of the glial and vascular compartments, 2) a possible role of the angiogenic process in the development of these entities.

P373. Prevalence, time course and severity of post-traumatic vasospasm of the middle cerebral and basilar arteries – prospective study in 307 cases

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The purpose of this prospective study was to evaluate the prevalence, the severity and the time course of vasospasm after severe head injury in a population of 307 patients after head trauma. 133Xe-Cerebral Blood Flow (CBF) and Transcranial Doppler (TCD) studies were performed during the first 2 weeks after trauma at regular intervals. The following methods for the detection of vasospasm were compared: BA and MCA velocitiy (VMCA) alone, VMCA and Lindegaard ratio, Spasm Index (SI) for MCA and BA. The prevalence of posttraumatic vasospasm was recorded at 33.3%. Basilar vasospasm was found in 19.5% of all patients after TBI. The most common day of onset for vasospasm in the anterior and posterior cerebral circulation was post-injury Day (PID) 2 with a up to 37.14% of positive studies for vasospasm. The highest prevalence was found on PID 9 (37.08-50%). Of all trauma patients, 24.8% showed mild to

moderate vasospasm with VMCA between 120 and 180 cm/sec. Severe vasospasm (VMCA>180 cm/s or with VMCA>120 cm/sec and CBF<30 ml/100g/min) developed 8.5% of patients. The highest MCA velocities were observed on post-injury day 11. The time course of CBF showed similar patterns both subgroups (VMCA>180 cm/sec or VMCA>120 cm/sec and CBF< 30 ml/100g/min) consisting of hypoperfusion, hyperemia and ischemia. We concluded that post-traumatic vasospasm is a significant event for one third of patients after head injury. Despite the majority of patients showed mild to moderate vasospasm, special emphasis must be put on patients who develop severe vasospasm defined by MCA velocities over 180 cm/s or CBF<30 ml/100g/min with TCD VMCA>120 cm/sec.

P374. Effects of endothelin A receptor antagonists on the cerebral microcirculation after global cerebral ischemia in gerbils

E. Hauck, K. Ueda, A. Heimann, O. Kempski (Mainz, D)

Endothelin 1 is produced by the cerebrovascular endothelium. There is evidence that endothelin receptors are activated after cerebral ischemia and are responsible for delayed hypoperfusion. So far only laser Doppler data are available to ascertain this hypothesis (1). We used intravital microscopy in Mongolian gerbils where endothelial leukocyte interactions and the cerebral microvasculature can be studied on the capillary level. Aim of the current study was to use endothelin (ETA) receptor antagonists to examine effects on postischemic microcirculation. Gerbils received a parietal craniectomy with the dura remaining intact. Arterial pressure, temporal muscle temperature and blood gases were monitored. Intravital microscopy was used to examine endothelial leukocyte interactions (rollers/stickers) in postcapillary venules (Rhodamine G), vessel diameters, and capillary red cell velocity. In addition a laser Doppler allowed to compare data thus obtained. After a control phase both common carotid arteries were ligated for 15 min. Animals received either vehicle or the ETA receptor antagonist BQ123 (0.8mg/kg) 10 min after reperfusion. The gerbils were then monitored for 2 hours. Animals which had received BQ123 did not any longer show the typical delayed hypoperfusion phase: after an initial postischemic hyperperfusion laser Doppler flow remained normal (23.6±1.8 LD-units; baseline: 26.7±0.9, n=11) until the end of the experiment. In vehicle treated animals flow was depressed to 14.8±0.98 LD-units from baseline 28.3±1.2 (n=8). Rollers and stickers which had significantly increased after 60 min reperfusion in vehicle treated animals were normal after BQ123. Therefore, we conclude that ETA receptors are involved in postischemic hypoperfusion. ETA receptor antagonists improve flow by affecting arteriolar diameter but also may directly interfere with endothelial leukocyte interaction and thereby improve microcirculatory flow.

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P375. Vasospastic cerebral damage in severe brain trauma

G.Y. Wu, X.Q. Huang (Shantou, PRC)

5 cases of traumatic secondary non-hemorrhagic vasospasticcerebral damage were reported. The examination of CSF were all negative, no RBC found, CT scanning was taken before and after treatment, Transinternal carotid encephalograms were done before and after. The patients frequently have a history of milder decelerated head injury that commonly occur in children, without primary unconsciousness and localizing nervous signs, the secondary unconsciousness is usualy mild or even absent, localizing nervous signs frequently appear as a slight hemiplegia mainly; no subarachnoidal hemorrhage. CT shows low density over distributed area identical to the localizing nervous signs. no skull fracture or hematona and cerebral laceration in all cases. Cerebral angiography showed no shadow of the artery identically with the low density lesion in CT scanning. The mannitol of 20%, cortical hormone, para-carotid sympathetic block, and vasodilatatoria are usually used. 4 cases were completely cured without any sequela, one case alived but with residual cerebral function disorder. At the early stage its pathology is reversible, if the diagnosis and treatment are obtained in time withuot sequela of cerebral function damage, only rarely it develops into an irreversible infarct. Its pathological characteristics and effecf were discussed ang advise fo name it "the traumatic secondary vasospasmotic cerebral damage", and classified theminto the hemorrhagic and non-hemorrhagic groups, and list as a kind of secondary cerebral damage.

P376. The use of microdialysis and a glucose biosensor coupled with fluorescence imaging to characterize the metabolic transients associated with peri-infarct depolarisations

M.G. Boutelle, D.A. Jones, C. Wong, A.J. Strong (London, UK)

Introduction: The transient changes associated with peri-infarct or peri-lesion depolarisations (PIDs) are well characterised in respect of cation, redox and flow transients, but less so in relation to dynamic changes

in glycolysis. The flow-injection assay (FIA) system that we have developed offers the important advantage of considerably higher time resolution (1 minute) than other microdialysis assays in current use. We have tested its capacity to detect and characterise transient metabolic changes associated with PIDs occurring in the ischaemic cerebral cortex, and have compared the results from FIA with those from the continuous output available from an enzyme-based glucose biosensor.
Methods: Experiments were carried out (under specific licence from the Home Office) in adult cats under terminal chloralose anaesthesia. The left cerebral hemisphere was carefully exposed and protected with mineral oil and after baseline observations the middle cerebral artery was occluded for 4 hours. PIDs were detected by fluorescence imaging{}. The microdialysis probe and glucose biosensor were located in the suprasylvian gyrus ("SG", usually core) in 1 study, and in the marginal gyrus ("MG", usually penumbra) in the remaining 4.
Results: In the SG study, glucose dialysate levels in the suprasylvian gyrus were typically 60 mmol.(similar to awake rat striatum and cortex): glucose disappeared from the dialysate from SG (n=1) immediately upon occlusion, and lactate showed a transient rise at occlusion. In the MG, occlusion slightly decreased the basal glucose measured by MD and the biosensor but did not significantly affect lactate levels. Arrival of a PLD event at the glucose biosensor led to a complex biphasic response. Initially, there was a rapid rise in glucose (occasionally preceded by a transient fall) reaching a peak after 1 min: the signal then returned to baseline. There was then a slower rise reaching a maximum typically 5-10 min after PLD arrival. The MD probe could not resolve the rapid glucose rise but clearly showed the later glucose increase. Dialysate lactate increased immediately reaching a peak 4-6 min after the arrival of the PLD, then resolving to control over a similar time course.
Comments and conclusion: There is excellent temporal correlation of optical and biosensor transients, and the FIA has sufficient temporal resolution to detect glucose and related lactate transients linked with ischaemic PIDs.

Poster Session 22

Epidemiology, mild and moderate brain injury 2

P377. Factors predicting outcome following mild traumatic brain injury in adults

J.L. Ponsford, C. Willmott, P. Cameron, R. Nelms, C. Curran, A. Kelly (Melbourne, AUS)

The impact of mild head injury or mild traumatic brain injury (TBI) is variable and determinants of outcome remain poorly understood. This study aimed to establish factors associated with persisting problems 3 months after injury, and evaluate the impact of early assessment and information provision. 136 adults with mild TBI were assessed 1 week and 3 months post-injury, and 129 mild TBI adults at 3 months post-injury only. They were compared with two control groups (n=75 and 67) of patients presenting with minor injuries not involving the head. Participants completed measures of pre-injury psychological adjustment, concurrent life stresses, post-concussional symptoms and tests of attention, speed of information processing and memory. Mild TBI participants seen at 1 week were also given an information booklet outlining symptoms associated with mild TBI and suggested coping strategies. Mild TBI participants reported more symptoms than controls at 1 week, and showed slowed information processing. Initial problems resolved for most participants by 3 months post-injury, but a subgroup (18%) reported significant ongoing problems. They had longer post-traumatic amnesia, but were also more likely to be students, females, have neck/back pain, been injured in a motor vehicle accident and/or have other stresses in their lives. The group who were not seen at 1 week and did not receive the information booklet were somewhat more stressed at 3 months post-injury. It was concluded that the presence of other stressors may reduce the injured person's ability to cope with cognitive slowing and other symptoms. Provision of an information booklet may reduce anxiety and thereby lower the incidence of ongoing problems

P378. Location of the chronic subdural hematoma – role of the gravity and cranial morphology

K.S. Lee, S.M. Yoon, J.W. Doh, H.G. Bae, I.G. Yun (Chonan, KOR)

Chronic subdural hematoma (SDH) frequently originates from subdural hygroma (SDG). The cranial morphology can determine the location of SDG. Since SDG is the precursor of chronic SDH, the shapes of the cranium will act an important role in location of chronic SDH. We tried to test this hypothesis. We re-evaluated the computed tomographic scans or magnetic resonance images of 118 consecutive patients with chronic SDH. We checked symmetry of the cranium and location of the lesion. The cranium was symmetrical in 55 patients (47%) and asymmetrical in 63 patients (53%). Chronic SDH was bilateral in 25 patients (21%) and unilateral in 93 patients (79%). It was more commonly bilateral in symmetrical craniums than in asymmetrical craniums (29.1% vs. 14.3%) (P=0.0496). In 63 patients with asymmetric cranium, the chronic SDH was bilateral in

9 patients, located on the opposite side of the flat side in 38 patients, and located on the same side of the flat side in 17 patients. This unequal distribution was statistically significant (P=0.03). In four patients, the hematoma was originated from the acute SDH located on the same side of the flat side. We could not find any reason in remaining 13 patients. We conclude that chronic SDH originate from SDG usually locates on the opposite to the flat side of the skull. The shape and posture of the cranium can predict the location of chronic SDH, as in the SDG.

P379. The Munich Neurotrauma Scale (MNTS), a new practical scale for assessment of brain-injured patients. Introduction and preliminary report

A.M. Frank, A. Harth, W. Reeker, A.E. Trappe, E. Kochs (Munich, D)

Objectives: Up to now scoring of brain-injured patients is difficult.
Materials and methods: A new grading scale with ordinary scaling of ten parameters was created: 1. eye-opening,2.speech,3.awakeness,4.behaviour of bulbi,5.pupils,6.reaction to pain,7.brain-stem reflexes,8.age,9.other injuries,10.Blood pressure and respiration rate. This scale has been applied to 15 patients, 10 of them intubated and sedated.
Results: The mean values of MNTS, GCS, GLS and RLS85 at different days showed, that MNTS is very sensitive in assessing changes of neurological status,better than the other scales.
Conclusion: MNTS is a new and easy to handle scale for assessment of brain-injured patients.

P380. Predictors of outcome in head injury: a new scaling system

M. Schaan, H. Jaksche (Murnau, D)

In a statistical analysis of 10 years, 554 patients with head trau-ma were analysed on general epidemiological parameters,clinical state, CT-pathology and outcome.There were 376 men and 178 women (67,9 and 32,1%), age distribution was from 3 to 92 years with a median of 41 years. Nearly half of the patients suffered from traffic accidents (47,9%). After collection of the complete data, mortality and morbidity of each clinical and radiological parameter were detemined. According to different mortality and morbidity, a different grading from one to eight points was atta-ched to each parameter and later used for each patient in an additive matter. A statistical significant difference in outcome ($p<0.01$,Mann-Whitney-U-Test) could be registered in steps from 0-10 pts (Group I), 11-20 pts (Group II) and more than 20 points (Group III). patients in group I had a mortality of 8,3%, patients in group II died in 51,2 % and in group III in 80,8%. Patients in group I had a GOS of 4 and 5 in 19,4 and 56,7%, wether group II reached GOS 4 or 5 only in 9,9 and 1,7%. Patients in group III never reached GOS 4 or 5. The presented scaling system allows a predictive value in morta-lity and morbidity for each patient suffering on brain trauma.

P381. Epidemiology as the basis for planning brain injury treatment in an area

F. Servadei, G. Giuliani, V. Antonelli, E. Gardini, L. Salizzato (Cesena, I)

Objectives: Neurosurgical units in Europe are regionalised. Most patients classified as mild head injury, some patients with moderate head injury and even a few patients with a GCS<8 are treated in Hospitals without Neurosurgical Units. Area based protocols are of outmost importance for improving patients outcome. Epidemiological studies of the area can show the efficacy of the applied guidelines.
Methods: The referral area of our Neurosurgical Unit is constituted by a population of approximately 1 million people raising up to 2 million in summer due to tourism. In the area there are 6 large hospitals with ICU and CT scan, all linked (CT computer link) to the central Neurosurgical Unit. A protocol on head injury management containing indication for transfer of patients and of images was applied in 1998. From 1/1/98 to 31/12/98 a prospective data collection on head injuries was established in the whole area, co-ordinated by the Neurosurgical Unit.
Results: A total of 2890 head injury patients were admitted to the hospital care (250/105 pop. / year in relation to residents). Out of 2890 patients, only 328 (11,3%) were admitted to the Neurosurgical Unit. The incidence of positive post-traumatic CT scan was 497 examinations (39/ 105 pop. / year). One hundred and twenty-eight patients were submitted to operation (ICP monitoring excluded), an incidence of 11/105 pop/ year. There were 80 deaths in hospitals, an incidence of 8/105 pop/ year. We examined all the records of the 43/80 patients who died in the area outside the Neurosurgical Unit. No unexpected deaths were identified.
Conclusion: Once established area protocols for head injury management, in the presence of a Neurosurgical teleconsulting system, there is no necessity to increase the number of Neurosurgical Units. Brain injuries occurring in an area of 1 million people can be treated in different hospitals co-ordinated by one Neurosurgical Centre.

P382. Minor head injuries in children

L. Djordjic-Vujotic, V. Antunovic, M. Rakic, M. Kontic, I. Piscevic (Belgrade, YU)

Head injuries in children are more frequent cause of death when compared with adults. The are significant differences in head trauma characteristics in children and adults, mostly seen in mechanism of injury, different reaction to trauma, outcome and sequels. The special problem in children with minor head injuries (MHI) is adequate screening of patients. The difficulties are seen in application of GCS, proper judgment of neurological symptoms and signs, patient's and parent's fear connected to injury, and risks of home observation. In this study we have analyzed patients treated in 2 years period at Emergency center of Belgrade. In that period total number of 3278 patient were hospitalized, and among them 472 children up to 16 years (14%). We have analyzed the mechanisms of injury, dynamic of conscious, local status, neuroradiological image, delayed deterioration with its causes and outcome. As very important conclusion, we want to point at fact, that there is risk of delayed deterioration in 3,8%, and development of complications that required surgery in 1,9%, after MHI in children.

P383. Epidemiology of head trauma in Khorasan province

M. Farajirad, H. Mashhadinejad (Mashhad, IR)

To evaluate the epidemiology of head trauma in Khorasan province, Iran, we studied 8499 patients admitted to Emdad hospital, Mashhad, between september 1994 and september 1995. Out of 8499 patients 6235 (73%) were male and 2264 (27%) were female. Fourty five percent had 15 years old or less. The motor vehicle accidents (51%) and fall (33%) were the most common mechanism of head trauma. In children less than 10 years old fall (57%) was the most common mechanism. Eighty seven percent had mild trauma (GCS=13-15), 8% moderate (GCS=9-12), and 5% severe head trauma (GCS=3-8). The most common mechanism of severe head trauma in both adult and children was motor vehicle accident. Out of 8499 patients 1210 (14%) had skull fracture (10% in mild head trauma versus 49% in severe head trauma). Nineteen percent of patients had trauma in other organs in addition to head. Out of 8499 patients 658 (8%) were operated. The most common causes of operation were depressed fracture and epidural hematoma. Out of 8499 patients 412 (5%) died, most (78%) had coma (GCS=3-8) in admission and the most common mechanism (72%) was the motor vehicle accident. As the motor vehicle accident is the most common mechanism of head trauma the most effective mean of adressing head trauma is through prevention; e.g. seat belt use or respecting the traffic laws. Also improving prehospital care will decrease the morbidity and mortality of head trauma.

P384. Delayed recognition of mild brain damage

A. Hirayama, S. Yamasaki, M. Miyata (Osaka, J)

With the present ethos in the insurance industry, it is sometimes difficult for neurological patients with no immediately observable physical or mental incapacities to obtain a sympathetic response to their claims for treatment and compensation. This study was of a group of 32 patients apparently suffering from routine neurological symptoms. On admission each patient underwent a CT scan and was prescribed appropriate pain-killer medication and discharged a few days later, since no definite neurological signs had been noticed. Nevertheless these patients failed to recover to their previous levels of activity. The age range of the group was from five years old to seventy eight years old. After a six month observation period the patients were given an MRI scan, EEG, Auditory Evoked and Mental Performance tests. Eight patients from the 32, showed abnormal findings and had experienced transient loss of consciousness at injury. The two pediatric cases, disclosed congenital anomalies of arachnoid cyst and cavum septi pellucidi on the CT scan at the time of admission. Auditory evoked potential was adjusted between P1 and P5. A Paroxysmal asymmetrical slow wave was noted on the EEG. There was no cortical atrophy in six adult cases, whose ventricles displayed asymmetrical dilation on the MRI scan. The images of the thalamus and corpus callosum appeared shrunken. This seemed to correspond with below normal results in mental tests and muddled indecisive movements in currying out the accustomed actions of their employment. These results supported that both the white matter and fibrous deep structure of the brain were affected at the time of injury rather than the cortical cell. This indicates possible tearing or rupturing of axon as the result of sheer strain and supports the contention that, even with cases of minor contusion, structural damage may occur which can only be diagnosed by prolonged careful observation, examination, and delayed study.

Poster Session 23
Imaging

P385. Evaluation of 99m-Tc-HMPAO SPECT at the early stage and during a 1-year follow-up of mild head injury

E. Put, A. Jacobs, M. Ingels, A. Bossuyt (Hasselt, Brussels, B)

Objectives: This study was undertaken to determine whether regional cerebral blood flow (rCBF) disturbances detected by single photon emission computed tomography (SPECT) after mild head injury

(MHI) can be useful in the objectivation of postconcussive symptoms and to determine the predictive capacity for clinical outcome during a one year follow-up.
Methods: We prospectively studied 136 MHI patients who underwent an initial 99m-Tc-HMPAO brain SPECT (SPECT0) within 4 weeks after the trauma. Re-evaluations took place after a mean time interval of respectively 3.2 months (T3mo), 6.1 months (T6mo) and 12.2 months (T12mo) postinjury. Simultaneous clinical reassessments (CLIN) were performed.
Results: Initial SPECT: The negative predictive value of SPECT0, given as P(CLIN3mo- / SPECT0-), was 58/63 (92%) and P(CLIN12mo- / SPECT0-) was 100%. In contrast, the positive predictive value of the initial SPECT, given as P(CLIN3mo+ / SPECT0+), was only 32 / 73 (44%). Follow-up SPECT: At all considered times of evaluation a high sensitivity and negative predictive values were found, increasing from respectively 91% and 89% at T3mo to 100% at T6mo and at T12mo. The specificity and positive predictive value remained low at T3mo (61% and 64% respectively) and T6mo (53% and 52% respectively). However, at T12mo the specificity had increased to 85% and the positive predictive value to 83%. A relatively more pronounced persistence of frontal SPECT lesions compared to other cortical localisations was observed.
Conclusion: A normal rCBF SPECT is a reliable tool in the exclusion of significant clinical sequelae after a mild head injury. At 12mo postinjury a SPECT study may contribute to the objectivation of post-traumatic sequelae in patients with postconcussive symptoms. Therefore, rCBF SPECT may play an important role in the judgement on the mild head injury patient's ability to return to work.

P386. Post-traumatic secondary bilateral basal ganglia degeneration
L. Song, T. Xu, B.Y. Rong, W.Q. Cheng (Shanghai, PRC)

Objectives: To verify that clinical and pathological syndrome of degeneration-like of Central Nervous System may be revealed following head injury.
Methods: To report two rare young cases (one male 38 yr., another female 24 yr.) which suffered from secondary bilateral basal ganglia degeneration after traumatic head injury and to analyze their clinical data.
Results: All of following features were revealed: (a) Healthy young adult without family hereditary history of Central Nervous System disease and history of intoxication. (b) Subacute onset following head trauma and no intracerebral lesion finding in first radiological examination (computerized tomography, CT or magnetic resonance imaging, MRI) during admission after trauma. (c) Epilepsy, severe anaemia, cardiac arrest were respectively emerged during their early hospitalization following head trauma (d) Having clinical manifestations of extrapyramidal system malfunction accompanied by abnormal density or signal at bilateral basal ganglia in CT scan or MR imaging. (e) Effective with medication and no change in radiological examination (CT and MRI) in 5 years follow-up.
Conclusion: We suggest that secondary bilateral basal ganglia degeneration is caused by both occlusive bilateral basal ganglia ischemia followed by cerebral circulation disturbance and cerebral hypoxemia after head trauma. The pathogenesis may be the progressive basal ganglia neuron coagulative necrosis. MR imaging is useful in evaluating secondary bilateral basal ganglia degeneration. In clinical practice, this secondary degeneration should not be mistaken for other congenital bilateral basal ganglia lesions such as Wilson´s disease.

P387. The relation between the local inflammatory response after traumatic brain injury and the primary and secondary damage seen on CT-scans
K. Schwerdtfeger, M. Müller, B. Maier, A. Mautes, F. Cortbus, I. Marzi, W.I. Steudel (Homburg-Saar, D)

Introduction: After traumatic brain injury (TBI) the brain shows a marked local inflammatory response with a triphasic time course. An initial peak of the pro-inflammatory cytokines (P1) is followed by a decrease around the third posttraumatic day (P2) and a secondary peak between day 5 and 7. We analysed to which extent this is related to primary and secondary structural damage seen on CT-scans.
Methods: In a prospective clinical study 197 patients either with isolated TBI (GCS<8), multiple trauma (ISS>18) or a combination of TBI and multiple trauma were assessed. In 28 patients the levels and time course of interleukine 6, 8 and 10 in the cerebrospinal fluid (CSF) were analysed. By aggregation phase-specific values were obtained which could be compared with related CT-findings of contusion and oedema.
Results: In individual cases there seems to be some relation between P3-IL6 and oedema formation which, however, could not be proven statistically. All cytokines are related to the initial damage with a prolonged decay of proinflammatory mediators in cases of large contusions.
Conclusion: Our results indicate that cytokine production is a consequence of the initial brain damage. Their role in causing secondary damage remains unclear. (BMBF-Grant 01 KO 9707)

P388. There is a nonlinear relationship between the recovery of neurological function and resolution of reduced metabolism following head injury – an FDG-PET study

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In animal studies, the normalization of the post-traumatic cerebral metabolic rate of glucose (CMRglc) correlates with recovery of neurological deficits. This study sought to determine whether a similar correlation between metabolic and neurological recovery occurs in humans following TBI. 13 TBI patients with mild to severe injury were prospectively studied with two FDG-PET studies: an early PET within 1 month of injury and a follow-up at 1 year. Regions of interest of standard anatomic brain regions were drawn on co-registered parametric CMRglc PET images. Neurological recovery was assessed by the Disability Rating Scale (DRS) determined at hospital discharge and at 1 year. A correlational analysis was made between the change in DRS to the absolute or percent change in CMRglc. 11 of 13 patients demonstrated an increase in the global cortical CMRglc. Acute absolute hyperglycolysis accounted for one of the patients with a drop in CMRglc. All patients experienced neurological improvement to various degrees (median change in DRS was –14, range –2 to –23). The correlation between degree of change in DRS and the change in CMRglc, either globally or regionally, was modest to poor (best r=-0.51, mean r=-0.39). Qualitative analysis of PET regions as a percent of global revealed that the cerebellar hemispheres and the left mesiotemporal lobe showed a reverse relationship with positive r values (range 0.40 to 0.57). The extent of global neurological recovery following TBI is not predicted by the degree of change in regional or global CMRglc. This can partly be explained by an uncoupling of global CMRglc and level of consciousness acutely following TBI due to a complex metabolic dysfunction.

P389. N-acetylaspartate and atrophy predict cognitive outcome following traumatic brain injury in humans – a MR spectroscopy and imaging study

W.M. Brooks, H. Petropoulos, M.A. Barlow, B. Harner, E.J. Bedrick, S.D. Friedman, R.E. Jung, D.C. Weers, C. Gasparovic (Albuquerque, USA)

Prediction of outcome following Traumatic Brain Injury (TBI) is challenging with few good indicators available. Overall brain atrophy following TBI provides some measure of gross injury and is correlated with cognitive performance. However, the changes in brain volume seen after TBI are slow to evolve. We have shown that N-acetylaspartate, a marker of neuronal integrity, measured soon after injury by non-invasive proton magnetic resonance spectroscopy provides a sensitive prediction of cognitive outcome. It was our aim to determine whether measurements of atrophy add to NAA measurements for predicting outcome. Nineteen patients (mean age=32.1 (13.2) years, mean GCS=8.4 (3.4)) were studied at 1.5 months and 6 months after TBI. Cognitive outcome was quantified at 6 months after injury using a battery designed to probe cognitive functions commonly impaired by TBI: attention and information processing speed, verbal and visual memory, perceptual-motor function, frontal "executive" functioning, pre-morbid intelligence, and orientation. Results were converted to a mean cognitive z-score which was used as an outcome measure. Localizing imaging sequences included a T1-weighted fast-SPGR scan (TE=6.9 ms, TR=17.7 ms, flip angle=25, 3mm slices) and a conventional proton density/T2-weighted series (TE=30/100 ms, TR=2800 ms, 4 mm slices). Cerebrospinal fluid (CSF) and total intracranial volume (ICV) were measured by automated k-means segmentation of the T2-weighted images. The CSF/ICV ratio was used as a measure of atrophy. A STEAM pulse sequence, including water suppression, was used to sample 25 x 35 x 21 mm3 voxels (TE=30 ms, TR=2000 ms). Voxels were po□6Ä

P390. Heterogenous mechanisms of edema formation in the acute phase of cerebral contusion – diffusion MRI and ADC mapping study

T. Kawamata, Y. Katayama, N. Aoyama, T. Mori, Y. Murata (Tokyo, J)

Objectives: Cerebral contusion is sometimes associated with a non-hemorrhagic mass effect which progresses rapidly within 24-48 hours post-trauma. In order to determine the mechanisms underlying such a mass effect, we analyzed data obtained from diffusion MRI in the acute phase of cerebral contusion without massive intracerebral hematoma.
Methods: Diffusion imaging and ADC mapping were performed in 25 patients with a relatively benign clinical course, employing 1.5T echo planar MRI. ADC values were expressed as a ratio relative to the values of intact brain areas (contusion/normal brain).
Results: Within 24 hours post-trauma, diffusion images revealed a low intensity core in the central area and a high intensity rim in the peripheral area of contusion. The ADC ratio increased in the central area (1.32±0.32) and decreased in the peripheral area (0.67±0.14). These findings suggest the occurrence of cellular disintegration and tissue homogenization in the central area and cellular swelling in the peripheral area. A crescent-shaped zone of very high ADC ratio (1.64±0.09) was observed at the border between these two areas during the period of 24-48 hours post-

trauma in patients who demonstrated a large low intensity core on diffusion images. This appears to represent edema fluid accumulation within the region of tissue homogenization. Interstitial edema (a high ADC ratio) extending along white matter fibers developed around the peripheral area of contusion at more than 72 hours post-trauma.
Conclusion: It appears that, during the period of 24-48 hours post-trauma, the capacitance of edema fluid accumulation is elevated by cellular disintegration in the central area, whereas the resistance to edema fluid propagation is elevated by cellular swelling in the peripheral area. We suggest that such events facilitate extracellular edema fluid accumulation within contused brain tissue and contribute, together with cellular swelling itself, to the non-hemorrhagic mass effect of cerebral contusion.

P391. Assessing a new CT classification of head injury
E. Teasdale, S. Egeler-Peerdeman, G. Murray (Glasgow, UK)

Introduction: Classification of CT findings in head injury is important in management, prognosis and clinical trial. Although the Trauma Coma Data Bank(TCDB) method is popular its use is not without problems. These difficulties mainly relate to the allocation of patients with a mass lesion. The 25cc volume definition is complex to assess with irregular or elliptical masses and the allocation to Type 5 or 6 is determined by whether or not the lesion is surgically evacuated. Even if a mass of less than 25cc is evacuated, the classification automatically becomes a 5.
Methods: In an attempt to detach the CT appearances from the unpredictability of surgical action we have devised an alternative classification based solely on the CT appearances of the effect of any mass on the individual brain in terms of compartmental shift and CSF dynamics irrespective of its actual volume. In any individual patient the overall effect of any focal lesion will depend upon the pre-traumatic anatomy of the brain. Two authors (ET, SE-P) blinded to the patients outcome independently re-classified the CT scans of all the 924 patients in the SAPHIR study. The patients CT scans had been previously classified for the SAPHIR study by one of the authors (ET) using the TCDB method.
Results: The results were correlated with the known outcome at three months and inter-observer variability assessed and the results compared with the previously applied TCDB classification. The new method appears more objective and can be applied at the time of recruitment into a clinical trial. The results correlated well with patient outcome.

Poster Session 24
Neuropsychology, rehabilitation 1

P392. Revised functional living index – a new method to assess the quality of life in traumatic brain injury patients
Z. Yuanli, W. Chungcheng, Z. Jizong, F. Zhuang (Beijing, PRC)

Objectives: To evaluate the quality of life (QOL) of traumatic brain injury (TBI) patient with a new assessment device – RFLI (Revised functional living index).
Methods: From 1996 to 1997, a total of 245 TBI patients treated at Department of Neurosurgery, Beijing Tiantan Hospital were included in our study. All patients were grouped on their gender, age, Glasgow coma scale (GCS) score and treatment methods respectively. Their Glasgow outcome scale (GOS), Karnofsky patient scale (KPS) and RFLI scores were observed during and after their admission. The RFLP included 20 questions, the score of each question range from 1 to 5 according to the answer judged by the patients' themselves. These questions were designed to analyze the physical, psychological, cognitive and social ability respectively. A full mark of 100 means full recovery with independent ability to living and working. Until Feb. 2000, detailed follow-up results with a mean period of 3-4 years were also got. All these data were analyzed statistically.
Results: The RFLI score not only correlated with GCS, GOS and KPS significantly, but also reflected well the long-term recovery of TBI patients, which document that it is useful in summarizing both the physical, psychosocial and cognitive impairment of TBI patients.
Conclusion: The use of a multidimensional approach that also reflects physical, psychosocial and cognitive aspects proved to be effective in assessing the QOL of TBI patients and in evaluating the different new treatment methods. Key Words: Quality of life Traumatic brain injury

P393. Post-traumatic headache – a symptomatic approach to treatment
G. Di Stefano, B.P. Radanov (Bern, CH)

Objectives: Headache following minor head injury or whiplash is one of the most prominent problems in neurotraumatology. Previous research is inconclusive regarding the symptomatic approach of this type of headache. This may lead to inappropriate treatment strategies because recent advances in therapy of different headache types may be neglected. We tried to fill this research gap.
Study Design and methods: We investigated 112 patients (mean age=39.5±10.5 years, women=66

[59%]) with chronic posttraumatic headache following cranio-cervical acceleration/deceleration trauma after an average of 2.5±1.9 years from trauma. Patients were investigated at the outpatient service of the Depratment of Psychiatry. Headache was analyzed according to its principal localisation, laterality, projection, quality, precipitation or aggravation and possible additional symptoms. For this analysis headache was diagnosed according to the classification of the International Headache Society.
Results: 42 patients (37%) had tension-type headache, 30 (27%) were identified as migraine whereas 20 patients (18%) had cervicogenic headache. An additional 18% of patients suffered from headache which did not fulfill criteria of a particular category. In 104 patients (93%) neck pain was associated in time with headache.
Conclusion: Each of the diagnosed headache types in this study may require specific treatment strategies based upon empirical studies of non-traumatic headache types. For these reasons a detailed analysis of headache following cranio-cervical acceleration/deceleration trauma is necessary.

P394. Functional reorganization after training of alertness

W. Sturm, F. Longoni, S. Weis, C. Holtel, K. Specht, H. Herzog, B. Achten, K. Willmes (Aachen, Jülich, D)

Recent studies have indicated that there is a specific right hemisphere cortical and subcortical network for the control of intrinsic alertness and sustained attention (1). In a study with patients presenting with right hemisphere vascular lesions and deficits of alertness, we aimed at assessing changes in the individual functional networks after an intensive specific computerized alertness training. The training (subprogram "Alertness" of the AIXTENT attention training battery (2)) was administered for 14 sessions of 45 minutes each. Before and after the training both a 15O-Butanol-PET activation and a comprehensive neuropsychological test battery for attention functions (TAP; 3) and neglect (Behavioral Inattention Test (4)) were carried out. We present the results of two patients: one patient, who showed a significant improvement in alertness after the training, and the other one, who did not. After the training, the former showed an at least partial restitution of the right hemisphere functional network especially in the frontal cortex (superior frontal gyrus BA 8, middle frontal gyrus BA 6, inferior frontal gyrus BA 47, middle temporal gyrus BA 38), whereas before the training a considerable left hemisphere activation (postcentral gyrus BA 40) with only small RH foci was present. The latter didn't present with a RH functional restitution (except for a brain stem activation) but there was an increase of LH activation (postcentral gyrus BA 1, BA 4) and bilateral activation in the cerebellum. In the two cases presented, the change of activation pattern after a training of alertness corresponds to behavioral changes. Specifically, it seems that a behavioral improvement can be achieved only if the right frontal structures responsible for the control of alertness in normals are reactivated. On the contrary, activation of left brain areas does not lead to a successful recovery of alertness function.
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P395. Nucleus basalis of Meynert pathology after human head injury

I. Murdoch, D. Graham, J. Nicoll, D. Dewar (Glasgow, UK)

Loss of cholinergic innervation of the cerebral cortex after a head injury is indicated by postmortem deficits of choline acetyltransferase activity. Cholinergic innervation of the cerebral cortex arises predominantly from neurons located in the nucleus basalis of Meynert. The aim of the present study was to determine if nucleus basalis neurons were damaged after a head injury and if the severity of neuronal pathology correlated with the loss of cortical cholinergic innervation. Formalin-fixed tissue was obtained from twelve head-injured patients who had survived for between 4 and 300 hours after injury and 4 controls. Blocks containing the nucleus basalis of Meynert were identified macroscopically using the anterior commissure and optic tract as anatomical landmarks. Paraffin-embedded sections were step-serially cut and stained with haematoxilyn and eosin, combined cresyl violet and luxol fast blue or a monoclonal antibody against choline acetyltransferase. The severity of neuronal damage was semi-quantitatively assessed using a scoring system (0=no damage; 1=0-10%; 2=10-25%; 3=25-50%; 4=50-100% cells damaged). Eight of the 12 head-injured patients had nucleus basalis pathology with scores of : 1, n=3; 2, n=1; 3, n=2; 4, n=2. Three distinct morphological changes were present : ischaemic cell change with or without incrustations; infarction of part or all of the nucleus and evidence of mechanical distortion resulting from compression of the brain tissue and the development of plastic creep in neurons due to internal herniation. Choline acetyltransferase staining of nucleus basalis neurons in head-injured patients was reduced compared to controls. There was a correlation between the severity of neuronal damage in the nucleus basalis and the loss of choline acetyltransferase activity in the

cingulate cortex. The results demonstrate that neurons of the nucleus basalis of Meynert are damaged after a head injury and this may contribute to the loss of cortical cholinergic innervation. If similar pathology occurs in patients who survive their injury it may contribute to impaired cognitive function.

P396. Benton Visual Retention test on the patients with traumatic brain injury

T. Yamada, M. Yamaguchi, K. Seki, H. Ando (Mihara, Kobe, J)

After severe head injury, the mentally impaired survivors at their plateau level of recovery need to be evaluated properly for their compensation. Although WAIS-R is an excellent tool to estimate the patients' intelligence, the execution of this test requires almost 90 min or more. Since some survivors with traumatic brain injury (TBI) frequently complained of lack of concentration and fatigability during performance of any task, easily applicable test is strongly expected. In contrast with WAIS-R, Benton visual retention test (BVRT) can be performed within 10 min. So, we tried to find out whether BVRT can be used as a screening tool before execution of WAIS-R on TBI patients.
Methods: Twenty one male and 6 female survivors (Age: 17-71) were examined. They were composed of 15, 6, and 6 cases after diffuse axonal injury, focal contusion, and others, respectively. BVRT were tested by administration A using form C. On the table of the norm of BVRT in the manual, a correlation between already estimated premorbid IQ and executed BVRT scores is demonstrated. Although the table does not indicate real IQ by the obtained scores of BVRT, we dared to predict IQ inversely by the BVRT scores, as a trial. We called this suspected IQ as B-IQ.
Results: Both IQs estimated by WAIS-R (W-IQ) and B-IQ were over 80 on 6 cases (high B and W). Five patients, however, showed B-IQs below 80 and W-IQs were between 90 and 120 (low B and high W). Other 16 cases had lower IQs below 80 by both methods (low B and W). Group of high B and low W was never identified in our experience.
Discussion: By our result, cases with high B-IQ always showed higher IQ by WAIS-R. No additional WAIS-R may be required. If lower B-IQ was detected, we recommend additional evaluation by WAIS-R. When some patients kept high W-IQ, but showed worse performance on BVRT, they may have trouble of concentration or impaired memory function. Those patients' handicap is evident. Conclusively, we recommend BVRT as a screening test to the rather severely disabled patients, because BVRT needs only 10 min or less for execution.

P397. IMF(R)Therapy – Intention-controlled Myofeedback – an additional method in the rehabilitation of patients with traumatic brain injury

H.D. Bennefeld, R. Blumenstein (Bad Liebenstein, D)

Introduction: In traumatic brain injuries motor deficits, disturbances of sensitivity, speech disorders and loss of intentional focusing of attention occur in consequence of the pathological changes in the CNS. The resulting impairments of mobility and ability to work substantially reduce the quality of life of those affected. Improving this as a main objective of neurological rehabilitation inter alia, it requires the restoration of voluntary functions. It is just as important to treat disorders of attention as it is to treat the pathological sensorimotor functions. We have been using IMF(R) therapy (Intention-controlled myofeedback) successfully in our department of rehabilitation for about two years to treat pareses and cognitive disorders in support of traditional therapies. This is an integrated method of mental training, EMG registration and myofeedback with the support of the electronic system MfT Z2.
Methods: Surface EMG and stimulation electrodes are placed on the muscles in which functional paralysis is to be alleviated. The patient is asked to imagine a targeted movement. The mere idea of movement gives rise to voluntary action potentials in the targeted muscles that are registered by the surface EMG of the apparatus, amplified and – via the stimulation electrodes – relayed to the muscles responsible for the function. The patient perceives a slight muscle tension in the targeted muscles. Since the MfT Z2 works with several channels, more complex movements can be facilitated at the same time, e.g. by abduction of the arm and stretching of the elbow. The effects of IMF(R) therapy go far beyond improvement of motor function. The recovery of lost motor functions requires a large degree of activation of the brain areas involved. IMF(R) therapy can enhance awareness in the course of treatment. The improvement of cognitive functions and speech capacity increases markedly compared to that in patients treated without IMF(R) therapy. One case report as an example: A 53years old male suffered from traumatic brain injury after car accident more than three years ago causing hemiparesis on the left. Following a five weeks rehabilitation program in our clinic there were another seven months using of the MfT Z2 at home up to twice a day. The cognitive and motor dysfunction disappeared tremendously and the patient is able to contiue his previous work. Based on several hopeful case results we begun a study to evaluate this new treatment in patients with traumatic brain injuries.

P398. Acrylic cranioplasty with silicone molding – a simple and inexpensive alternative method in cranioplasty
M. Bierschneider, H. Jaksche (Murnau, D)

Objectives: Acrylic cranioplasty is a common neurosurgical procedure that is performed when the original bone flap becomes infected or is unusable for other reasons.A simple technique to produce a complete copy of the original bone flap from acrylic bone cement using silicone as a mold is described.
Methods: Silicone was used to form a mold of the original bone flap.The mold was sterilized.Methylmethaacrylat was placed inside the mold to produce an exact duplicate of the original bone flap.33 patients underwent cranioplasty with this technique.
Results: A perfect copy of the patients original bone flap was easily produced and excellent cosmetic results were obtained. One cranioplasty had to be removed because of an epidural hematoma.No further complications occured. Operative time was shortened.
Conclusion: This technique is a simple and inexpensive alternative method in reconstruction of cranial defects.

P399. Changes in outcome in survivors of severe head injury and late cognitive sequelae
S. Thornhill, G. Teasdale, G. Murray, K. Millar, J. Nicoll, K. Penny, P. Wilson (Glasgow, UK)

Objectives: To further investigate outcome in 1st year after severe head injury and late cognitive status.
Design: We investigated reported worsening by comparing early (6 month) and late outcome in subjects, who were traced, having survived a severe head injury more than 10 years ago, with age, time since injury and specific cognitive sequelea.
Subjects: 388 patients admitted for neurosurgical care after a severe head injury between 1968 and 1988.
Outcome measures: Glasgow Outcome Scale at 6 months and 18 years post injury, Neurospchological assessment of memory, attention and cognition after a mean of 18 years.
Results: Almost half (46%) of the group reported changes in their overall outcome from early to late assessment, of whom two thirds (69%) had deteriorated. The patient's ability to complete the battery of cognitive tests was strongly related to their current age and GOS at 6 months ($p<0.001$). 6 month GOS was also highly predictive ($p<0.001$) of the patient's actual score on each of the cognitive tests.
Conclusion: There was no evidence of late change in the second decade since injury nor with age at injury or follow up. However 6 months GOS remained highly predictive of late cognitive sequelae. This suggests variations in social factors and support may contribute to change in overall outcome.

P400. The significance of neuropsychological testing in patients with head injury
T.S. Bondar, N.V. Guzhovskaya (Kiev, UKR)

It was investigated 45 patients in clinical course of head injury easy degree's.Patients were devided on the 2 groups to conventional treatment:alongside with conventional with neuropsychological treat-ment. The research was conducted twice:in early period and two months past after traumas.The results were compared with contol group-healthy persons, without phisiological disorders, with IQ not below-115-120%, without blood alcohol. The techniqu updating MMPI, test Gamilton,Beck (on depression),Ljusher-Spylberg,neuropsychological tests on memo-ries,stress attention. The received results have enabled to reveal.The hid-den psyhopatological state without pathological and deviations, ACTS, EEG and biochemical bllod researchs. Specially these infringements depression, state of stress, hidden stress, displayed in remote period of trauma. The results were subjected th mathematic analysis and the series of constants, reflection dependes neuropsycho-logical of deviations from age, variaty of injury, intelligence and social environment were revealed. It permits more precisely to form patients groups for neuropsychological rehabilitation. For the first time by use were neuropsychological techni-que in rehabilitation patients with head injury of easy degree. It was in-creased the efficiency rehabilitation of these patients and preventions or elimination hidden psychopathology that significance in has given favor-able of socialreabilitation forecast.

Poster Session 25
Models of SCI

P401. Improved motor function and neuroprotection after SCI in mice lacking ICAM-1/P-selectin, NOS (inducible) -/- and NOS (neuronal) -/- but not in TNF-α-/- or CAM-1-/-
M. Farooque, J. Isaksson, Y. Olsson (Uppsala, S)

The purpose of this study was to develop a spinal cord injury model in the mouse. Various degrees of extradural compression were used to induce mild, moderate or severe compression injuries. Furthermore, a locomotor rating scale was developed by which the functional outcome of the spinal cord injury can be assessed. MAP2 and luxol staining was used to evaluate damage to grey and white matter respectively. Nitric oxide, tumor necrosis factor alpha,

and adhesion molecules (e.g. ICAM-1 and p-selectin) play an important role in neuronal injury. Therefore we studied the effect of severe spinal cord injury on animals lacking these molecules. Mice lacking ICAM-1/P-selectin (double knock out), NOS inducible and NOS (neuronal) had a better hind limb motor function and less tissue damage compared to wild type controls. On the other hand mice lacking TNF-a or CAM-1 did not differ from wild type injured controls. These studies indicate that mouse can be an interesting model to study the SCI. Futhermore nitric oxide and ICAM-1/P-selectin negatively effect the SCI.

P402. Glutamine synthetase induces spinal seizures in rats

Y.S. Yoon, M. Matsumoto, W. Huang, P. Ceraulo, W. Young (Piscataway, USA)

Glutamine synthetase (GS) is a key enzyme in the regulation of glutamate neurotransmission in the central nervous system. It is responsible for converting glutamate to glutamine, consuming an ATP and NH3 in the process. The neurotransmitter glutamate is neurotoxic when it accumulates in extracellular fluids. GS is also known to protect against neuronal degeneration in injured retinal tissues. We investigated the effects of GS in the spinal cord injury (SCI) model and normal rats. Low (2μM) to high (55μM) dosages of GS were injected in the rat after general anesthesia. Immediately after intrathecal injection into L1-L3 space, rats developed seizure activity in the spinal cord. This seizure initially consists of myoclonic twitches of paravertebral muscles close to the injection site, repeated tonic and clonic contractions and extensions of the hindlimbs (hindlimb seizures) which spread to the forelimbs, and finally rotational axial movements of the body. EMG of paravertebral muscles, forelimbs and hindlimbs showed the spread of muscle activities. 2μM GS caused spinal seizures in rats after SCI and 6μM GS in uninjured anesthetized rats. Denatured GS (70°c, 1hour) also produced spinal seizures although at higher concentrations. We hypothesize that GS may be directly blocking GABA release or receptors in the spinal cord. This is being tested.

P403. New animal model to investigate spinal cord injury caused by aortic occlusion in the rat

G.Z. Sufianova, L.A. Usov, A.A. Suphyanov, S.S. Golubev, L.Yu. Raevskaya, A.G. Schapkin, O.N. Smekalina (Irkutsk, RUS)

Spinal cord ischemia is actual problem of neurosurgery. Purpose of this work was development new, greatly useful to clinic picture of abdominal and thoracic divisions of aorta obliteration model of spinal cord ischemia suitable for using for small laboratory animals. The work is carried out on 22 rats, weighing 100-160 g. Transitory ischemia of lumbar division of spinal cord was created by the complete abdominal aorta and its branches occlusion. For this group of rats (N =10) through both femoral arteries under surface anaesthesia introduced occluders before level of diaphragm (on depth 6-7 refer to). Through left common carotid artery on direction to occluders introduced polymeric substance, capable be fixed on the occluders. Occluders were extracted after 45 minutes. In the second group rats introduced only occluders (N=6). To the third group rats (N=6) introduced only the polymeric substance. During the whole period ischemia each 5 minutes the estimation deep, tactile, temperature and pain sensitivity on 6 ball scales, offered by us. On time of steady degree of reduction of sensitivity was conducted the estimation of neurological deteriorations. Through 48-72 hours the spinal cord was extracted and fixed in 96 % alcohol. Were studied the transverse cuts of spinal cord at a level of lumbar bulge, stained by hematoxylin-eosine. Macroscopic and microscopic investigation revealed ischemic change of all organs of abdominal cavity, retroperitoneal space and lumbar area at modelling ischemia. At rats was noted paraplegia, anaesthesia and atrophia of lumbar area and limbs muscles. Histological examination revealed remarkably constant pattern of ischemic spinal cord damage, characterised by severe ischemic changes in most neurones. In the first group of rats, time of appearance of reduction of reaction for deep, tactile, temperature and pain sensitivity accordingly are 18.5, 15 and 17.5 minutes. In the second group of rats reduction of sensitivity was not noted, ischemic change of internal organs did not note, it were sparingly motor breaches in distal divisions of limbs. In the third group during first day animals died caused by embolisms of branches of aorta. Advance of this model is minimal invasiveness, high reproducibility and maximal forthcoming to clinic of abdominal and thoracic divisions of aorta obliteration and opportunity using for small laboratory animals.

P404. New experimental method of modelling spinal cord ischemia

G.Z. Sufianova, L.A. Usov, A.A. Suphyanov, A.G. Schapkin, O.N. Smekalina, L.Yu. Raevskaya, S.S. Golubev (Irkutsk, RUS)

Acute vascular insufficiency of spinal cord frequently is complication spinal cord injury, during neurosurgical spinal cord operations, vascular operations at aorta and aortagraphy and is actual problem of modern medicine. Purpose of this work was development new model of spinal cord ischemia at laboratory animals. The work is carried out on 6 rats, weighing 300-350 g. Transitory ischemia of lumbar division of spinal cord

was created by the selective aorta branches occlusion. For this group of rats (N =6) through right femoral arteries under surface anaesthesia introduced occluder. Occluder was introducing through aorta up to left common carotid artery. Occluder was extracted after 40 minutes. During the whole period ischemia each 5 minutes the estimation deep, tactile, temperature and pain sensitivity on 6 ball scales, offered by us. On time of steady degree of reduction of sensitivity was conducted the estimation of neurological deteriorations. Through 24 hours the spinal cord was extracted and fixed in 96 % alcohol. Were studied the transverse cuts of spinal cord at a level of lumbar bulge, stained by hematoxylin-eosine. Macroscopic and microscopic investigation revealed ischemic change of all organs of abdominal cavity, retroperitoneal space and lumbar area at modelling ischemia. At rats was noted paraplegia, anaesthesia and atrophia of lumbar area and limbs muscles. Histological examination revealed remarkably constant pattern of ischemic spinal cord damage, characterised by severe ischemic changes in most neurones. Time of appearance of reduction of reaction for deep, tactile, temperature and pain sensitivity accordingly are 14.2, 11.7 and 15.8 minutes. Advance of this model is minimal invasiveness and high reproducibility. This model can be recommended for study of pharmacological means having neuroprotective properties.

P405. Spontaneous recovery following incomplete injury of spinal cord in adult rats – combined functional and MRI study

R. Duvdevani, R. Nossim, A. Levy, Y. Cohen (Ness Zionna, Tel-Aviv, IL)

Our previous studies have shown that a unilateral hemi-crush of the rat spinal cord (SC) caused primarily a paralysis of one hind limb. Functional testing (beam and grid walking and swimming test) revealed an improvement of this paralysis, without any treatment, during the first 6 weeks post trauma. The present study examines the MRI parameters of hemi-crush injury of the rat SC, combined with functional testing and histopathological outcome, during the recovery period. Rats were subjected to hemi-crush SC injury and tested at three time points (4 days, 10 days and 6 weeks post trauma) for their functional performance in 3 tests: simple reflexes, grid walking and swimming. At each time point following testing, rats were sacrificed and their SCs were analyzed by in vitro high-resolution MRI at 8.4T(voxel size of 100x100x2000mm). The MRI protocol included T1, T2 and heavily diffusion weighted high resolution MRI (DWI). DWI data (TR/TE/D/d=1500/60/150/2ms, gradient strength of up to 150 g cm-1) was analyzed using the q-space approach providing MR displacement maps. SCs were then sectioned and stained for myelin and for general histology. Histopathological examination of both stainings has verified the MRI findings: tissue damage of half the spinal cord during the first 10 days following trauma, including necrosis and inflammatory processes, and a normal spinal cord tissue, except for minor inflammatory changes, at 6 weeks post injury. Our data clearly shows that partial injured rat SC recovers spontaneously, both functionally and structurally. Current efforts are directed at examining the mechanism that is responsible for this spontaneous recovery following unilateral hemi-crush.

P406. Acute cervical cord injury associated with ossification of the posterior longitudinal ligament

I. Koyanagi, Y. Iwasaki, K. Hida, H. Imamura, S. Fujimoto, M. Akino (Sapporo, J)

Objectives: Ossification of the posterior longitudinal ligament (OPLL) is one of common spinal pathologies in patients of acute cervical cord injury without fracture or dislocation of the spinal column in Japan. In the present study, we reviewed our experience of the OPLL patients presenting with acute cervical cord injury to clarify clinical features of this type of spinal cord injury.
Materials: Twenty-three patients of acute cervical cord injury associated with cervical OPLL were retrospectively analyzed. There were 21 males and 2 females, aged from 45 to 78 years (mean 62.8 years). Thirteen patients (57%) were injured in a fall on the level ground. Most patients showed incomplete spinal cord injury (Frankel grade; A: 2. B: 1, C: 13, D: 7). The type of OPLL included continuous OPLL in 8 cases, mixed OPLL in 4 cases and segmental OPLL in 11 cases.
Results: Magnetic resonance imaging (MRI) demonstrated that spinal cord injury occurred at the narrowest spinal canal levels due to OPLL in 11 patients. Other 12 patients showed spinal cord injury at the intervertebral disc levels adjacent to the OPLL. Most patients in the latter group had segmental OPLL. The sagittal diameter of the spinal canal was narrowed to 4.1-10 mm at the narrowest spinal canal level due to OPLL. Surgical treatment was performed in 19 patients either by posterior (14 cases) or anterior (5 cases) decompression at 1 to 61 days after trauma. Sixteen out of 23 patients showed improvement of Frankel grade during the follow-up periods of 1 to 3 months.
Conclusion: Acute cervical cord injury occurs at the level of OPLL or adjacent disc levels. MRI is useful to understand mechanisms of spinal cord injury in the OPLL patients. Further investigations are needed to elucidate the role of surgical decompression.

P407. Transient increase in P300-latency by American football training

M. Yamaguchi, H. Ando, K. Seki, Y. Yanagida (Kobe, J)

During routine training of American football, players sometimes feel slight fainting-like sensation or dizziness just after the attacking contact to their heads. This condition may be called as a mild concussion defined by Gennarelli (1984). To study the electrophysiological effects of this condition, changes in latency of event related potentials (P300) induced by impacts to the head were measured.
Methods: The event related potentials were recorded by using auditory stimuli of odd ball paradigm. Ten healthy players of college American football team (mean age: 20) were consented. After recording the control P300, the player in helmet and shoulder protectors hit his head several times to other object person as an attacking style of usual training manner. When he felt slight fainting-like sensation or dizziness, he stopped hitting and recordings of P300 were started within 2 min. The first recording was carried out for 150 sec and then the second one was followed for another 150 sec.
Results and discussion: Mean latency of the control was 319.2 msec (S.D.=16). After the impact, mean latency was 345.6 msec (S.D.=45) in the first period. The difference was significant (P=0.038), when compared with the control by paired t-test. However, the latency reduced to 330.8 msec (S.D.=29), in the second period. Difference was insignificant when compared with the control by paired t-test (P=0.13). Our study demonstrated that the latency of P300 prolonged significantly by head hitting in the routine training of football, but recovered very soon. Thus, unlike punch drunk syndrome, which is thought to be caused by repeated concussive impacts during boxing, our study suggests that the impact to the American football players' head in the helmet is mild and harmless.

P408. A mouse model of graded spinal cord injury – difference between inbred strains

H. Nakamura, O. Yoshino, Y. Abe, T. Kimura (Toyama, J)

Development of genetic engineering enables gene manipulation in animals, especially in mice. Therefore, a mouse model of spinal cord injury (SCI), which was developed recently, provides new ways to examine the effects of specific molecule on neural damage after SCI. However, differences between different inbred strains in SCI have not been fully understood. To elucidate the effects of inbred strains in SCI, three major strains of mice; C57BL6, Balb-C and MRL strains, were examined in locomotor function and histopathological findings using the spinal cord impactor, which enables graded SCI. Experimental groups, 8 weeks of age, were distinguished by the amount of height of the weight. Locomotor function rated by the Basso, Beattie and Bresnahan scale were the best in C57BL6 and the worst in Balb-C. Areas of damaged white and gray matter evaluated at 6 weeks after contusion by light microscopy were the largest in MRL and the smallest in C57BL6. Rcovery of locomotor function was correlated with spared white matter in each strain. C57BL6 mouse showed encapsuled scar within damaged area that is not seen in the other strains. These results reveals the differences of SCI between different inbred strains, which must be taken into consideration to use genetically modified mice for SCI research. The knowledge about genetic background of the inbred strain may provide some clue on pathophysiology of SCI.

P409. New minimal invasiveness model of transitory spinal cord ischemia

G.Z. Sufianova, L.A. Usov, A.A. Suphyanov, A.G. Schapkin, O.N. Smekalina, Yu.G. Schapkin (Irkutsk, RUS)

Development of new minimal invasiveness, universality and high reproducibility models of spinal cord ischemia is actual problem of modern biomedical, pharmacological and others and researches. Purpose of this work was development new universal model of spinal cord ischemia suitable for using for any laboratory conditions. The work is carried out on 5 rats, weight 150-180 g.. Transitory ischemia of lumbar division of spinal cord was created by the intraluminal aorta occlusion. For this group of rats through right femoral arteries under surface anaesthesia introduced synthetic string. The synthetic string was introducing through aorta up to left common carotid artery. On the end of string was formed occluder, which was introduced in aorta. Occluder was extracted after 40 minutes. On time of steady degree of reduction of sensitivity was conducted the estimation of neurological deteriorations. At all rats of experimental group was noted paraplegia and anaesthes. The introduction by control group animal in right common carotid artery of paint solution has shown presence blood flow only in right subclavicular artery. Advance of this model is minimal invasiveness, universality and high reproducibility. This model can be recommended for study of pharmacological means having neuroprotective properties or others biomedical researches.

P410. New universal model of global spinal cord ischemia

G.Z. Sufianova, L.A. Usov, A.A. Suphyanov, A.G. Schapkin, O.N. Smekalina, Yu.G. Schapkin (Irkutsk, RUS)

Purpose of this work was development new universal model of global spinal cord ischemia suitable for using for any laboratory conditions. The work is carried out on 5 rats, weight 150-180 g.. Global acute spinal cord ischemia was created by the abdominal aorta extraction and thoracic aorta occlusion. For this group of rats through right femoral arteries under surface anaesthesia introduced synthetic string. The synthetic string was introducing through aorta up to left common carotid artery. With use of synthetic string and special methods were made abdominal aorta extraction and toracic aorta occlusion. On time of steady degree of reduction of sensitivity was conducted the estimation of neurological deteriorations. At all rats of experimental group was noted paraplegia and anaesthes. Usually animals did not live more than 24 hours. The introduction by control group animal in right common carotid artery of paint solution has shown presence disturbance of aorta branches blood flow. This model in induce acute global ischemia and may therefore be useful in the studing of drags with neuroprotective action or others biomedical researches.

Poster Session 26

Neuropathology, progressive degeneration

P411. Vimentin and bFGF reaction to reactive astrocytes after an experimental cerebral injury

S. Hashimoto, Y. Hayashi, Y. Tamai, T. Itoh, T. Satou (Osakasayama, J)

To clarify reactions of astrocyte to a cerebral injury an immunohitochemical study on vimentin and bFGF was performed.
Methods: Under nembutal anesthesia rat cerebral cortices were traumatized by a pneumatic cortical injury device. From 30min. to 90 days after the injury, vibratome brain sections, 50um in thickness, after the fixation by perfusion of 4% paraformaldehyde were reacted to antibodies against vimentin, bFGF, and GFAP. H-E stain was also examined. Positive reactive astrocyte areas around the trauma were figured by NIH image software. For bFGF, the numbers of positive cells were counted.
Results: Increased numbers of GFAP positive astrocytes were significant around the damaged area 30min. after the injury, and continued to increase for up to 7days. Thereafter positivity became significant on the fibers of the astrocytes. Vimentin and bFGF positive astrocytes were recognized around the injured areas from 3 to 7days. Thick astrocytes fibers demarcated the injury and showed significant positive reaction to vimentin. Positivity of bFGF appeared in the cytoplasm of the astrocytes, and as time passed the positivities pf vimentin and bFGF was reduced. It coincided with the location and timing of the proliferation of the fibers of the reactive astrocyte.
Conclusion: Vimentin and bFGF seem to be related to the beginning of demarcation, by astrocytic fibers, of the damage.

P412. BDNF intraventricular infusion improved open field behavioral abnormalities of rats with impact acceleration injury

E. Kohmura, T. Fujinaka, T. Yuguchi, M. Nishio, M. Nonaka, Y. Nakajima, T. Yoshimine (Suita, J)

Neurotrophic factors have been proposed as a therapeutic treatment for traumatic injury of the central nervous system. The present study determined whether intraventricular administration of brain-derived neurotrophic factor (BDNF) could effect behavioral recovery in rats with diffuse brain injury. Male SD rats (500–550 g BW) received an impact acceleration injury (450 g, 1.5 m). Survival rate just after injury was 80.5% and 50.7% at 2 weeks (n=77). Immediately after injury, they were infused intraventriculary with saline or BDNF (20 micrograms/day) for 14 days with an osmotic mini-pump. Open-field behavioral data (free movement for 90 sec) were collected before and 2, 7, 14 days after the injury. Moved length (Path), number of crossed areas (CA) and latency to go out from the starting circle (Latency) were evaluated and displayed as percentage of before injury. Path of animals of saline group was 42.9+-3.0% at 2 days, 53.5+-30.2% at 1 week and 55.1+-16.6% at 2 weeks. In the group infused BDNF, Path was 80.7+-23.7% at 2 days, 119.7+-80.2% at 1 week and 138.8+-33.7% at 2 weeks. CA and Latency were also improved in BDNF group. Thus intraventricular infusion of BDNF improved behavioral abnormality observed in animals after an impact acceleration injury. Body weight loss and reduced survival rate was considered as adverse effect. Possible mechanisms will be discussed.

P413. Neurofilament compaction and impaired axoplasmic transport can occur within distinct classes of traumatic axonal injury

J.R. Stone, R.H. Singleton, J.T. Povlishock (Richmond, USA)

Traumatic Axonal Injury (TAI) contributes to morbidity and mortality following Traumatic Brain Injury (TBI). Single-label immunocytochemical studies employing

antibodies to neurofilament compaction (NFC), RM014, and antibodies to APP, a marker of impaired axoplasmic transport (AxT), have shown that TAI involves both NFC and disruption of AxT. Although thought that both events occur within the same injured axon, this has not been confirmed. To determine the relationship between NFC and impaired AxT, dual-label immunofluorescence was employed. To compare and contrast specific changes associated with these two markers of TAI, single-label electron microscopy was also used. Rats were subjected to an impact acceleration injury (30 min – 6h survival), and their brains prepared for dual-label immunofluorescence and single-label electron microscopy. APP and RM014 immunoreactivity was consistently localized to two distinct classes of TAI. The first class, which showed only RM014 immunoreactivity, was thin and elongate, sometimes vacuolated, and revealed little progressive change over time. The second class was distinguished by the presence of APP immunoreactivity alone, or in combination with RM014 immunoreactivity. This class of fibers was swollen, and revealed progressive change involving swelling and disconnection over time. Ultrastructural examination of these two classes of TAI revealed NFC and mitochondrial dilation with no signs of pooling organelles in the RM014 single labeled axons. However, the APP single labeled/APP-RM014 dual labeled axons revealed a progressive accumulation of organelles associated with increased axonal swelling over time. In contrast to previous thought, it now appears that NFC and impaired AxT may be independent traumatic axonal events. This finding underscores the complexity of TAI, suggesting the need of multiple immunocytochemical approaches to fully assess the axonal response to TBI. (Supported by NIH grant NS20193.)

P414. Quantitation of cortical dendritic injury using microtubule associated protein (MAP-2) in mild and severe human traumatic brain injury (TBI)

J. Manavis, C. Chong, P. Blumbergs, P. Reilly, N. Jones (Adelaide, AUS)

Denditic pathology associated with TBI may be identified by MAP-2 immunohistochemistry. The aim of this study was to quantitate the dendritic response in the cerebral cortical mantle in mild (GCS 13-15) and severe (GCS 3-5) TBI using MAP-2 immunostaining as a marker for dendritic injury. The loss of cortical MAP-2 immunostaining was quantitated using a computerised image analysis system applied to whole brain coronal sections obtained from 5 cases each of severe (GCS 3-5) and mild (GCS 13-15) head injury and 5 normal controls. The survival times ranged from 1.75 hrs to 8 days for the severe TBI group and 3.75 hrs to 8 days for the mild TBI group. The average cortical MAP-2 loss was 28.35% in severe TBI (rang 11.01 to 43.22%), 2.34% in mild TBI (range 1.3 to 3.12%) and 3.16% in controls (range 1.76 to 4.38%). Total MAP-2 loss in severe TBI was a variable mixture of lossess associated with contusions and hypoxic-ischaemic damage as well as loss in areas which were otherwise histologically normal on staining with H&E. This study shows that there is widespread loss of MAP-2 immunostaining in the cortical mantle following severe head injury which is not apparent in mild head injury.

P415. Quantitative methods for assessment of ischemic damage in axons, oligodendrocytes and neuronal perikarya

V. Valeriani, J. McCulloch, D. Dewar (Glasgow, UK)

Cerebral ischaemia produces damage in both grey and white matter although quantitative histopathological analysis detects damage to neuronal perikarya but not pathology in axons or oligodendrocytes. The aim of this study was to use immunohistochemical markers of axon and oligodendrocyte pathology in conjunction with histology to quantify white and grey matter damage in a rodent model of ischaemia. We compared permanent with transient ischaemia as a proof of concept to detemine the sensitivity of these methods to an intervention strategy. Focal cerebral ischaemia was induced in rats by the intraluminal vascular occlusion technique either permanently (24h) or transiently (2h). Animals were killed at 24h and the brains processed for histology and immunohistochemistry. Sections at 8 predetermined coronal planes were stained with haematoxylin and eosin or double immunostained for amyloid precursor protein (APP), to label damaged axons and for tau protein, to label ischaemic oligodendrocytes. Immunostained sections were captured digitally and the images printed at fixed magnification. The distribution of APP-positive axons was plotted onto the digitised images and quantified by overlaying a counting grid. A total APP score was derived from the sum of grid intersections with APP-positive axons at the 8 planes. The distribution of tau-positive oligodendrocytes was plotted onto scale diagrams of the 8 planes and the volume of tissue containing tau-positive oligodendrocytes computed by image analysis. The distribution of ischaemically damaged neuronal perikarya was determined from haematoxylin and eosin-stained sections and plotted onto scale diagrams of the 8 planes and the volume of damage computed. The hemispheric extent of axonal, oligodendrocyte and neuronal perikaryal damage was significantly reduced in the transient ischaemia compared to the permanent group. This methodological approach for quantifying ischaemic

damage in axons, oligodendrocytes and neuronal perikarya is capable of detecting alterations in grey and white matter pathology with intervention strategies. These methods may also be of utility in other models of brain injury which affect both grey and white matter.

P416. Accumulation of BDNF protein in the locus coeruleus neurons in an impact-acceleration brain injury model

T Fujinaka, E. Kohmura, T. Yuguchi, M. Nishio, T. Yoshimine (Suita, J)

It is known that brain-derived neurotrophic factor (BDNF) regulates the survival and differentiation of the target neurons. And it have been recently reported that BDNF is anterogradely transported and released from the terminals of noradrenergic neurons to regulate synaptic activities. In this study, alteration of BDNF protein in locus coeruleus (LC) neurons was examined in a diffuse brain injury model. Adult male SD rats weighing 500-550 g were anesthetized with chloral hydrate and placed in prone position on a foam bed with spontaneous breathing. Dropping a brass weight (450 g) freely by gravity from a height of 1.5 m onto a metallic helmet fixed to the skull vertex, impact-acceleration brain injury was produced. At 1, 2, 7, 14, 28 and 56 days after injury, two animals each from the experimental and control groups were anesthetized and perfused intracardially with 4% paraformal-dehyde. Serial coronal sections from 8 mm to 11 mm posterior to the bregma including LC were made and processed for immunohistochemistry for BDNF, dopamine-beta-hydroxylase and neurofilament. Immunohistochemistry for anti-68kD-neurofilament showed extensive axonal injury particularly in the brain stem at 1 or 2 days after injury. Neuronal cell bodies in LC were partially positive for BDNF immunoreactivity and faint immunostaining was rarely seen in noradrenergic fibers in the control animals. At 24 hours after injury, cell bodies of LC neurons were strongly positive for BDNF immunoreactivity and significant immunostaining could be seen in swollen axons. At 7, 14 and 28 days after injury, however, BDNF immunostaininng was weak compared with control animals, and restored in 56 days. Increase of BDNF immunoreactivity in the early stage probably indicated the accumulation and stagnancy of BDNF in neuronal cell bodies and axons caused by the impairment of axonal anterograde transport of BDNF due to axonal damage. Decrease of BDNF immunoreactivity in the late stage may have been caused by the dysfunction of neuronal cell bodies or by the active down-regulation. Further studies should be made about the transcriptional level and the change in other regions of the brain.

P417. Brain-Derived Neurotrophic Factor (BDNF) is increased with exercise after fluid-percussion brain injury in the developing rat

G.S. Griesbach, D.A. Hovda, L. Ying, F. Gomez-Pinilla (Los Angeles, USA)

Traumatic brain injury during the first weeks of life is likely to disrupt developmental plasticity resulting in subsequent cognitive impairments. Deficits following injury sustained at a young age may not necessarily be overt, but instead reflect a deviation from optimal development. BDNF plays an important role in use-dependent hippocampal plasticity as well as the promotion of LTP. Given that levels of BDNF mRNA significantly decrease after fluid percussion brain injury (FPI) we determined if this reduction in BDNF could be reversed with exercise (voluntary wheel running) which has been shown to increase levels of BDNF mRNA in caudal cerebral cortex and hippocampus in normal uninjured animals. Consequently, isofluorane-anaesthetized pre-weanling rats underwent lateral FPI or sham injury. The day after surgery animals were individually caged in the following 4 groups: FPI + exercise (n=8), sedentary FPI (n=4), sham + exercise (n=4) and sedentary sham (n=7). After 7 days, rats were sacrificed and hippocampal BDNF mRNA levels were determined using TaqMan EZ RT-PCR. Exercised-sham rats showed a 20% increase in mRNA compared to the sedentary-sham group. The ipsilateral hippocampus of the sedentary-FPI rats had a 30% decrease in BDNF levels compared to the contralateral side and the sedentary-sham group. The exercised-FPI group showed a restoration of hippocampal BDNF mRNA to the sedentary-sham level. These results further support the concept that sustained early in life, traumatic brain injury can compromise mechanisms implicated in neural plasticity. (Supported by: NS30308, NS27544, Lind Lawrence Foundation, NS38978.)

P418. Temporal changes of axonal function in rat corpus callosum following traumatic brain injury

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To study temporal changes of axonal function in rat corpus callosum following traumatic brain injury (TBI), anaesthetized male Sprague-Dawley rats (350-450g) were subjected to moderate midline fluid percussion injury (FPI) just posterior to the bregma. Then brain slices (400mm thick), which included the entire transverse corpus callosum, were obtained from sham control (0 atm) rats and injured (1.8-2.0 atm) rats at 3 hours, 1 day, 3 days and 7 days post-FPI. The compound action potential (CAP) was evoked by electric stimulation and recorded extracellularly in the

corpus callosum. The CAP amplitude was quantified as a peak-to-peak measurement between the negative peak and the positive one preceding it. The results are shown as in the figure. We conclude that TBI attenuates the axonal function in white matter and that axonal function temporally changes in white matter following TBI. (Supported by the Ontario Neurotrauma Foundation.)

P419. Axonal bulb size and survival time after head injury

S.M. Gentleman, M.A. Stephenson, P.D. Leclercq, D.I. Graham (London, Glasgow, UK)

Diffuse axonal injury (DAI) is a near universal pathological feature associated with fatal head injury. The axonal damage usually manifests itself in the form of axonal swellings or retraction bulbs. It has recently been suggested that there is a correlation between axonal bulb size and survival time (up to 85 hours) and that this relationship may be of use in forensic medicine for dating injuries. To test this hypothesis we studied 26 consecutive cases of head injury (survival times 10 hrs-3 weeks, ages 5-79 yrs) with evidence of DAI in coronal sections of the corpus callosum, cut at the level of the lateral geniculate nucleus. Sections were immunostained with an antibody to the amyloid precursor protein (APP) and assessed blind to survival time, using a novel computer image analysis protocol. A number of different bulb parameters were measured including area, max and min diameter, feret diameters and projected volumes. In this preliminary study no correlation was found between any of the bulb measures and survival time. This is perhaps not surprising bearing in mind the heterogeneity of the injuries and the inherent variation in normal axonal size in the corpus callosum. However, there was a significant negative correlation between bulb size and age, the basis of which remains unclear. The numbers used in this study are small and a larger study is already underway but based on these initial results we would suggest that more work needs to be done before such measurements are considered for use in forensic medicine.

P420. Diffuse distant structural changes of brain tissue by head gunshot injuries

S. Kasumova, A. Potapov, L. Likhterman, G. Shahinjan, A. Kravtchouk (Moscow, RUS)

Introduction: The morphological researches of experimental animals` brain after a head gunshot injuries, shown, that simultaneously to the bullet`s passage through a brain, an essential diffuse structural changes of brain tissue on optical or ultra structural level. (1) appear on significant distance from the wound (2). However, at survival of primary brain injury for the period of several hours till several days it is difficultly to differentiate primary degeneration from the secondary (3). The purpose was to specify the time of primary distant brain tissue degeneration occurrence in case of head gunshot injuries.
Materials and methods: we carried out macro – and microscopic studies of 10 brain autopsies (death was immediately after a gunshot), and 6 brain autopsies of patient, who died in period of 9-65 days after injury.
Results: 1) In case of instant death at the through out wounds of the head were made by bullets of caliber of 7,62 mm or 9 mm from close distance (about 1,5-2 meters) there was not revealed significant intracranial hemorrhage, only small local subarachnoid hemorrhage, which was larger in size on the side of an outgoing aperture. There was neither brain edema nor attributes of detoriation. The bullet`s trace passed through the middle brain structure, oral, caudal or lateral brain stem. Small hemorrhages were marked in the area of the wound, which were submitted as unstructured eosinofilic mass with small collection of non-changed red blood cells at microscopic research. Brain stem neurons in the area outside of the bullet trace were found in necrosis and degeneration. Most demonstrative was an axon`s damage, which was marked mainly in conducting ways located perpendicularly to a course of trace-force waves. 2.) There were found widely distributed dystrophic and degenerative changes of brain tissue of both hemispheres and brain stem, slowed down processes of reparation and organization in brain wound in case of survival.
Conclusion: Severe head gunshot injuries is accompanied instantly shown primary diffuse destructive changes of brain tissue. The expression degree of this changes depends on bullet`s ballistic properties.
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Poster Session 27

Epidemiology, mild and moderate brain injury 2

P421. Location of the traumatic subdural hygroma – role of the gravity and cranial morphology

S.M. Yoon, K.S. Lee, J.W. Doh, H.G. Bae, I.G. Yun (Chonan, KOR)

Traumatic subdural hygroma (TSH) is frequently bilateral and locates on the top of the head in a supine position. It suggests that the gravity and cranial posture act a certain role. We tried to test this hypothesis. We re-evaluated the computed tomographic (CT) scans or magnetic resonance (MR) images of 86 consecutive patients with TSH. We checked the symmetry of the cranium, the posture of the head during the radiological examinations, and the location of the lesion. The cranium was symmetrical in 47 patients and asymmetrical in 39 patients. TSH was more commonly bilateral in patients with symmetrical cranium than those with asymmetrical cranium (77% vs 62%). The asymmetrical cranium tended to turn to the flat side. It was more frequently oblique in MR images which has a long scanning time than in CT (29% vs 18%). In 39 asymmetric craniums, TSH was bilateral and symmetrical in 14 cases. In the remaining 25 cases, TSH located opposite to the flat side in 18 cases. In 7 patients with the same side TSHs, four patients had it on the side of atrophy, two had on the opposite side of a mass lesion. We conclude that the gravity and cranial posture can predict the location of TSH. TSH usually occurs at the least pressure in the cranium as a lesion of ex vacuo.

P422. Prediction of recovery from post-traumatic vegetative state with cerebral MRI

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Various clinical and laboratory tests have failed to predict recovery from posttraumatic vegetative state (VS), so we assessed the value of cerebral MRI in prediction of recovery. 80 adult patients in posttraumatic VS had cerebral MRI between 6 and 8 weeks after injury. The patients were assessed at the time of MRI and at 2, 3, 6, 9, and 12 months after the injury using the Glasgow Outcome scale. At 12 months 38 patients had recovered, while 42 patients remained in the VS. The demographic characteristics and causes and severity of injury were similar in patients in persistent VS (PVS) and those who recovered (NPVS). An average of 6.1 different brain areas were injured in patients in PVS compared with 4.6 areas in patients who had NPVS. Patients in PVS revealed a significantly higher frequency of corpus callosum, corona radiata, and dorsolateral brainstem injuries than did patients who recovered. Logistic regression showed that corpus callosum and dorsolateral brainstem injuries were predictive of non-recovery. The adjusted odds ratios for non recovery of patients with a corpus callosum lesion and dorsolateral brainstem injury were 214 (95% CI 14-3213) and 7 (1-43). Clinical characteristics, including GCS, age, and pupillary abnormalities failed to predict recovery. Cerebral MRI findings in the subacute stage after head injury may predict the outcome of PVS. Corpus callosum and dorsolateral brainstem lesions are highly significant in predicting non-recovery.

P423. Intra-observer and inter-observer agreement of the Traumatic Coma Data Bank CT-classification

P.E. Vos, A.C. van Voskuilen, T. Beems, P.F.M. Krabbe, O.J.M. Vogels (Nijmegen, NL)

Assessment of CT scan abnormalities has improved since the Trauma Coma Data Bank (TCDB) classification is used in which the status of the mesencephalic cisterns, the degree of midline shift and the presence of a mass lesion provides a ranking order of the severity of the initial injury (1). The objective of this study was to validate the CT TCDB-classification by determining the interrater and intrarater reliability.

Methods: Patients with severe closed head injury with a Glasgow Coma Score  8 with a CT scan taken within 12 hours were included. Data included age, sex, GCS and the Glasgow Outcome Score (GOS) at 3 and 6 months. All initial CT scans were independently evaluated by four observers (2 neurologists, one neurosurgeon, one medical student), two of them classifying the scans twice at a time-interval of at least two weeks. CT-scans of all patients were classified according to the TCDB-criteria.

Results: Sixty-three patients (36 males, 27 females; age range, 4 to 82 years; mean age, 34  24 years) had a Glasgow Coma Score  8 after resuscitation. The 63 initial CT-scans of all patients were classified by the four raters. CT classification was 6% class I, 27% class II, 30 % class III, 6 % class IV, 25 % were class V and 5 % class VI. Interrater reliability was 0.80 and intrarater reliability was 0.86 (intraclass correlation coefficient). Glasgow outcome scores after 6 months were: 19 dead (30%), 1 vegetative (2%), 5 severely disabled (8%), 17 moderately disabled (27%) and 21 good recovery (33%). Association measures (Somers' D) between CT and GOS scores were statistically significant for all observers

Conclusion: The TCDB classification shows a high intra- and inter-observer agreement in the assessment

of CT scan abnormalities of head injured patients and confirms the predictive power on outcome.
References: 1) Marshall, LF, Marshall, SB, Klauber, MR, et al. 1992 J. Neurotrauma. 9 (S1), S287–92.

P424. Delayed recognition of brain damage
A.Hirayama, S. Yamasaki, M. Miyata (Osaka, J)

With the present ethos in the insurance industry, it is sometimes difficult for neurological patients with no immediately observable physical or mental incapacities to obtain a sympathetic response to their claims for treatment and compensation. This study was of a group of 32 patients apparently suffering from routine neurological symptoms. On admission each patient underwent a CT scan and was prescribed appropriate pain-killer medication and discharged a few days later, since no definite neurological signs had been noticed. Nevertheless these patients failed to recover to their previous levels of activity. The age range of the group was from five years old to seventy eight years old. After a six month observation period the patients were given an MRI scan, EEG, Auditory Evoked and Mental Performance tests. Eight patients from the 32, showed abnormal findings and had experienced transient loss of consciousness at injury. The two pediatric cases, disclosed congenital anomalies of arachnoid cyst and cavum septi pellucidi on the CT scan at the time of admission. Auditory evoked potential was adjusted between P1 and P5. A Paroxysmal asymmetrical slow wave was noted on the EEG. There was no cortical atrophy in six adult cases, whose ventricles displayed asymmetrical dilation on the MRI scan. The images of the thalamus and corpus callosum appeared shrunken. This seemed to correspond with below normal results in mental tests and muddled indecisive movements in currying out the accustomed actions of their employment. These results supported that both the white matter and fibrous deep structure of the brain were affected at the time of injury rather than the cortical cell. This indicates possible tearing or rupturing of axon as the result of sheer strain and supports the contention that, even with cases of minor contusion, structural damage may occur which can only be diagnosed by prolonged careful observation, examination, and delayed study.

P425. The role of early CT scan in severe head injuries
G. Tomei, D. Spagnoli, L. Bello, M. Sinisi, S. Magnoni, A. Colombo (Milan, I)

This study evaluates whether CT scan performed after stabilization is indicative of the clinical evolution of patients with severe brain injuries. 75 consecutive patients were included. Eighty-five per cent were admitted with a GCS score <=8, and 15% with a score > 8. The latter deteriorated within the following 24 hrs. All patients were submitted to continuous ICP, CPP and SjO2 monitoring. Monitoring parameters and clinical course were correlated with the first and the following CT scans. 24 % of patients (18) had a diffuse injury (DI) type I and II, 15 % (11) had a DI type III and IV, 57 % (43) had an evacuated mass lesion (EML) and 4% (3) a non EML. Overall bad outcome (died and vegetative – GOS) rate was 20 %. In the group of DI I and II as well as that with DI III and IV, no further evolution toward an EML was documented. However, 72% of patients with DI I and II and all patients with DI III and IV had an increased ICP (ICP >=20 mm Hg) that required reinforced or extreme medical treatment in 50% and 73% respectively. Moreover, 72% and 81% of the two groups had at least one episode of < CPP (CPP<=55 mm Hg). 95 % of patients with EML were operated on within 24 hrs from trauma, 5% thereafter and 20% required a re-operation within one week due to > ICP. 74 % of these had post-operative > ICP that required reinforced or maximal medical therapy in 53% of the cases; 79% had at least one episode of < CPP. Surgery was able to normalize brain shift and/or the volume of the hemorrhagic mass in 55 % of the cases. Two out 3 patients with non EML had > ICP and one required extreme medical treatment. Patients with no CT signs of > ICP (DI I and II) present a high incidence (72%) of > ICP and < CPP that is not significant different from patients with CT signs of > ICP (DI II and IV). Patients with EML need a strict monitoring because 2o % of them require re-operation within one week from trauma due to > ICP. Extensive monitoring of patients with severe head injury is associated with a reduced bad outcome.

P426. The characteristics of head injuries during the war in former Yugoslavia
M. Rakic, L. Djordjic-Vujotic, M. Jokovic, I. Piscevic, V. Bascarevic (Belgrade, YU)

Introduction: The head injuries that can be seen in wartime have special characteristics. They are caused by firearms, projectiles or explosions. Craniocerebral war injuries are classified as the most severe head injuries and generally are accompanied with big mortality.
Materials: During the four years (1990-1994) more than 300 patients wounded in the civil war in Yugoslavia were operated on for head trauma at the Neurosurgical department of Emergency center, Clinical center of Serbia, Belgrade.
Results: The missiles having different characteristics caused the brain damage related to two parameters: its velocity and its mass. The missile energy that in fact causes the damage highly changes with the

speed of projectiles. The missiles are classified in three groups according to their velocity (I up to 365 m/sec – low, II 365-760 m/sec – moderate, and III over 760 m/sec – high velocity).
Conclusion: In this study we compare effects caused by missiles of similar velocity on experimental model and on brain tissue on clinical material. In our patients the best results were observed in head injuries caused with low velocity missiles, but in cases of high velocity missile out shoot injuries no survivals were seen.

P427. Prognostic value of traumatic intraventricular hemorrhage
M. Jokovic, V. Antunovic, M. Rakic, M. Kontic (Belgrade, YU)

The CT scan revealed intraventricular traumatic hemorrhage is supposed to be the bad prognostic factor. In this study we tried to give the real view to this statement.
Materials and results: Analyzing all patient treated at Emergency Center Belgrade, Neurosurgical department for head injuries in five years period (more than 8400 patients) we found less than 2% (154) having intraventricular hemorrhage with other pathological findings such as focal or global brain concussion, subdural, epidural or inracerebral clots. These patients were in severe condition at admission and had poor prognosis with mortality rate over 90%. In very few cases (0.04%) the minor intraventricular hemorrhage was isolated pathologic finding on CT scan, and surprisingly all these patients were with good outcome.
Conclusion: We can point at the fact than not intraventricular hemorrhage but other associated severe brain lesions predict bad outcome in patient with head injuries. Rare isolated intraventricular hemorrhage have good prognosis.

P428. Subdural hematoma possibly due to strong vibrating force
M. Yamaguchi, H. Ando, T. Yamada, T. Fukumori (Kobe, J)

The authors experienced a case of the acute subdural hematoma (SDH) probably caused exclusively by vibrating force to the head in the construction area. Neither blow impact nor falling accident was identified.
Case report: From 9 a.m. to 4:30 p.m., a healthy 39 year-old male worked to chip off concrete mass with a piecemeal manner in a narrow space under the floor of a house. Since the height of the space was only 50 cm, he must have taken supine position and manage manually a powerful chipping gun powered by compressed air. The machine was supposed to be operated only at standing position when held with worker's arms. At about 3 p.m., he complained of headache and felt nauseated. The next day, he took off of work due to his headache and nausea. The 3rd day, he visited a hospital and a convulsion with transient unconsciousness occurred when he waited for CT scan. He was transferred to a neurosurgical service and a thin subdural hematoma of thickness below 1 cm was found in his left frontotemporal area. He was observed conservatively and discharged after 1 week without any neurologic deficit. His vascular system in the cranium was checked with digital subtraction angiography and no abnormal vessel was noted. He denied any head injury in his past history and no hemorrhagic tendency was reported.
Discussion: As the patient had no blow impact to the head, the tremendous vibrating motion might have damaged his bridging vein. On a supine position, he must have kept his head up all the time. If his head was placed on floor, the strong vibrating force would pass through his cranium to floor. Although shaking baby syndrome was widely accepted in the infant population, the acute subdural hematoma by this cause has not been reported in adult. The authors would propose a possible mechanism of bleeding by handling of chipping gun in the construction area.

P429. Stereotactical phenomena in brain injury biomechanics
C. Obreja (Bagnolet, F)

Traumatic brain injury (TBI) is the consequence of the spatiotemporal pressure variations occurring inside the brain during head traumas. The pressure gradient is responsible for the tissue strains (compression, tensile, shear), the cerebral lesions' distribution and the consequent neurological signs. Beside skull's deformation caused by the contact loading and followed by skull vibrations and/or fractures, current biomechanical theories concern two inertial phenomena: the linear acceleration and the rotational head movements. The first theory explains the superficial brain contusions while the second one better explains the diffuse axonal injury (DAI) and the brain concussion. The stereotactical theory (STT) here exposed is complementary to these two approaches. The skull-brain relative movements, caused by the acceleration phenomena – linear or rotational – and by the skull vibrations, generate secondary pressure waves with curved wave fronts. As the brain tissue is approximately isotrop on concentric plans parallel to the interface skull-brain, the pressure waves concentrically propagate toward the deep cerebral regions. The wave front's spoke and surface progressively decrease and, according to the energy conservation law, the amplitude of the pressure waves progressively increases. If no significant energy consumption process occurs

before, the PG will be maximal in the geometrical center of the involved skull segment. Clinical observations, physical arguments and previously reported experimental findings support the STT. The stereotactical phenomena explain common neurological signs (brain concussion, memory disturbances), cerebral lesions (diffuse axonal and contrecoup injury) and the protective role of the ventricular system. Thus, they could allow better understanding the TBI pathophysiology and related pathological entities like boxers' chronic encephalopathy or even Alzheimer's disease. Further human observational research is needed.

P430. Bilateral chronic subdural hematomas

S. Chun, J. Koh, Y. Lim, T. Kim, W. Leem, B. Rhee (Seoul, KOR)

Objectives : We studied clinical analysis of chronic subdural hematoma(CSDH) in order to find the differences between unilateral and bilateral CSDH in the aspects of clinical characteristics and postoperative recovery and recurrence.
Methods : We analyzed chronic subdural hematomas retrospectively, which were treated to the department of neurosurgery, Kyung-Hee University Hospital from January 1995 to December 1999. In 298 cases, various factors including etiology, clinical presentation, radiologic findings and surgical outcome were compared between unilateral and bilateral CSDH.
Results : Out of 298 cases of CSDH, unilateral CSDH were 253 cases(84.9%) and bilateral CSDH were 45 cases(15.1%). The past history of trauma were noted in 187 cases(74.0%) of unilateral CSDH and 32 cases(71.1%) of bilateral CSDH. The history of alcoholsim were recorded in 82 cases (32.4%) of unilateral CSDH and 14 cases(31.1%) of bilateral CSDH. In unilateral group, prevalence of symptom & signs were headache-72%, hemiparesis-68%, vomiting-38%, impaired consciousness-31% and seizure-5%. In bilateral group, prevalence of symptom & signs were headache-75%, paraparesis -56%, vomiting-32%, impaired consciousness-27%, hemiparesis-18% and seizure-3%. The symptom duration were mean 5.4 and 8.6 weeks in unilateral and bilateral CSDH, respectively. In unilateral CSDH, 239 cases(94.5%) had showed favorable outcome in similar that 41 cases(91.1%) in bilateral CSDH. The recurrence rate was higher in bilateral CSDH (11.1%) than unilateral CSDH (5.5%)
Conclusion: There are a few limited differences between unilateral and bilateral CSDH. In bilateral CSDH, symptom duration was slightly longer than unilateral CSDH and paraparesis had more common symptom than hemiparesis. Also, recurrence rate was higher than unilateral CSDH. (no significance, $p>0.05$). It seems to have a long prediagnostic period and high recurrence rate in bilateral CSDH.

P431. Relationship between mortality and the presence, or not, of prehospital hypotension or hypoxemia, alone or together in head trauma patients

M. Alvarez, J.M. Nava, R.M. Gracia, L. Marruecos, J. Moreno, E. Zavala, A. Bonet, J. Valles (Barcelona, E)

Objectives: To asses, in patients with head trauma, how the presence of hypotension or hypoxia in the prehospital setting, alone or together, can modify the hospital mortality, and know the exact prognostic value of each systemic insult.
Methods: We studied in a prospective way 370 head trauma patients admitted to Intensive Critical Unit (ICU) in seven universitary hospitals in Catalonia between February 1998 and January 1999. We recorded if they presented hypoxia ($PaO2<60$ mmHg), hypotension (systolic blood pressure <90 mmHg) or both before they were admitted to ICU. We analysed the hospital mortality by using a chi-square test or Fisher exact test and odds ratio (OR) with 95% confidence interval (CI).
Results: The patients were male in 74.6% cases, the mean age was 40 years, they presented a mean Glasgow Coma Score (GCS) of 8.6 points and had a 21.6% hospital mortality. Prehospital hypotension or hypoxia were absent in 300 cases who had a 14.9% mortality. In the 32 cases with only hypotension, the mortality was 46.9% with $p<0.001$ (OR 5.4 CI 2.6-11.4) comparing with the group without it. In those with hypoxia alone, 14 patients, the hospital mortality was 42.9% with $p<0.03$ (OR 3.6 CI 1.4-11) comparing with the group without it. If both systemic insults were present, 24 cases, the mortality was 75% with $p<0.001$ (OR 18.4 CI 6.4-55) comparing with the rest of patients. If we studied the cohort of patients with GCS less than 9 points the mortality were affected in a similar level.
Conclusion: The presence of hypotension or hypoxia in head trauma patients in the prehospital setting are a major prognostic index. If they are present together the mortality is strongly increased. The major therapeutic efforts, in order to reduce the hospital mortality, should be addressed in this way, to improve the prehospital care of such patients.

P432. Procedures following minimal traumatic brain injury in ice hockey and other contact sports to prevent second impact syndrome

N. Biasca, R. Agosti, Y. Tegner, H. Battaglia, C. Gerber (Zurich, Luzern, CH)

We observed an alarming increase in the rate of traumatic brain injury (TBI) (previously also termed

'concussions') in professional ice hockey players over the last 15 years despite improved protective head gear. TBIs account for 2 to 14% of all ice hockey injuries. Among our concerns is the occurrence of a second TBI within a short time leading sometimes to disproportional severe sequelae, so called 'second impact syndrome' with permanent neuropsychological impairments or even death due to hemorrhages or brain edema, especially in the very young players. In order to prevent these catastrophic brain injuries we suggest to exclude players temporarily after TBIs based on a novel evaluation plan. Based on the 1997 American Academy of Neurology classification of concussions (mild traumatic brain injuries) we suggest criteria to quickly evaluate and grade injured players using field observations (e.g. duration of loss of consciousness (LOC)) combined with instant neuropsychological and neurological tests on the locker room and neuroimaging as needed. Mild TBIs will be graded I to III and we have established a novel set of actions to be installed according to the grade of mTBI (e.g. return to play the next day if neuropsychological evaluation is normalised within 15 minutes after an injury in case of a grade I or, in case of a grade III with LOC of more than one minute, return to play after 28 symptoms free days at rest and while training). Our suggestions have been accepted for application by the International Ice Hockey Federation (IIHF).

Poster Session 28

Age and gender influence

P433. Low dose aspirin prophylaxis and risk of intracranial hemorrhage in mild and moderate head injury in elderly population – a prospective study

S. Agus, S. Spector, V. Merkin, S. Constantini (Jerusalem, IL)

Objectives: Low dose Aspirin (LDA) is a commonly used medication among the elderly population. The purpose of this study was to investigate whether LDA treatment by elderly patients sustaining a head injury is associated with a higher rate of traumatic intracranial hemorrhage and whether these patient need a different approach in the emergency room (ER).
Methods: 231 patients of 60 years of age and older, which arrived to the ER after moderate (GCS 9-12) and mild (GCS 13-15) head injury were divided into two study groups. One hundred and ten patients who received daily LDA prior to the injury comprised the Aspirin group (AG) and 121 patient that did not receive daily LDA prior to the injury comprised the Control group (CG). There was no significant difference between the groups in age, sex, mechanism of trauma, time from trauma to arrival to the ER and GCS on arrival. The most common cause of injury in both groups was falls (88.2% in the AG and 85.1% in the CG). All patients underwent in the emergency room the same neurological evaluation and a head CT scan.
Results: Any form of traumatic intracranial hemorrhage (TICH) on CT was revealed in 27 patients in the AG (24.5%) and in 31 patients in the CG (25.6%). Surgical treatment was necessary for 5 patients in the AG (4.5%) and 5 patients in the CG (4.1%). Among the patients, who arrived with GCS 15, TICH on CT was not infrequent (in 11.5% patients of the AG and 16.5% of the CG), but surgery was not required in any of them.
Conclusion: There was no significant difference in prevalence and types of TICH between the Aspirin and the control groups. We conclude that LDA does not increase surgically relevant parenchymal bleeding following moderate and minor head injury in the elderly population. Elderly patients with arriving in GCS of 15 usually will not have TICH that will warrant surgical treatment regardless of history of LDA use.

P434. Neuropsychological rehabilitation of children and adolescents after traumatic brain injury – first results of an out-patient program

L. Suhr, P. Melchers, A. Maluck, S. Scholten, B. Schmidt, J. Rybniker, G. Lehmkuhl (Cologne, D)

Rationale: Only a comparatively small share of severely brain injured children and adolescents is admitted for in-patient rehabilitation after the acute phase. Therefore ambulatory concepts for rehabilitation are of mayor importance. In this study a two-stage, multimethodal rehabilitation program has been developed, aiming at the promotion of cognitive functions, the avoidance of psychopathological alterations, and quality of life improvement.
Treatment and methods: 100 children and adolescents in the age of 4 to 16 years with severe traumatic brain injury will be included into a prospective, randomized and controlled study on therapy evaluation. In addition to routine medical attendance, patients of the experimental group are initially treated with a coma stimulation program, which varies depending on the current medical state. The program is related to all available sensoric channels. After regaining a sufficient level of consciousness a neuropsychological rehabilitation program is applied comprising diverse materials and techniques applicable according to the results of differentiating neuropsychological assessment. Interventions address the following main areas: Orientation, perception and motor-integration, alertness and concentration, learning and memory,

organizational processes, reasoning, problem solving and social perception. In the course of remediative therapy, specified psychotherapeutic interventions are integral parts in order to promote the child's coping with the trauma and its sequelea or minimize psychopathological alterations. Parents are closely involved in early and late stages of treatment. Counseling and psychotherapeutic support on the parent's side is also applied, since they have to learn how to deal with the trauma, its sequelea and their own emotional reactions. Main goal of this part is the promotion of psychosocial familial stability.
Results: Since the study is still in progress preliminary results concerning the evaluation of the program will be presented. After including more than 60% of the expected sample, the results of follow-up assessments 6 and 12 months after trauma are available. The data demonstrate treatment effects on intellectual functioning, nonverbal learning abilities, quality of life and psychopathological alterations. (Funded through the research program "Gesundheit 2000" by the German federal government, FKZ 01 KO 9517.)

P435. Kinematic analysis of prehension movements in children after traumatic brain injury
M. Gölge, M. Dreesmann, M. Holzhäuser, J.P. Kuhtz-Buschbeck, A. Boczek-Funcke, J. Pohl, B. Benz, A. Ritz, M. Illert (Kiel, Bremen, D)

In a longitudinal study in children we investigate restitution of motor functions after traumatic brain injury. Children (age=5-15 years), who were admitted to a rehabilitation center, were selected by the grade of brain injury (II°). First date of examination (T0) was defined by the Barthel Index, re-examinations followed after one (T1) and two (T2) months. Prehension movements were analysed with kinematic techniques. Triggered by an acoustic start signal, the children reached and grasped cylindrical target objects with their non-dominant hand. Experimental conditions consisted of small objects (10% of the subject´s maximum finger span) located far away (60% of the subject´s arm length) from the starting position. One block of 10 prehension trials was performed under normal room-lit conditions, and another block was performed in darkness, preventing visual feedback. Reflective markers were attached to the wrist and the fingernails of the thumb and index finger. The movement of these markers were recorded by an infrared optoelectronic motion analyser. The velocity profiles of hand transportation were calculated from the wrist marker, grip formation was described by analysing the distance between the thumb and index finger. The reach-to-grasp movements of humans show stereotypic kinematic features. The velocity profile of the reaching hand is normally approximately bell-shaped and hand velocity increases in parallel with movement amplitude. Patients with traumatic brain injury showed segmented velocity profiles with multiple peaks. In particular, the profile of the hand velocity and the movement amplitude (trajectory) differed from normal values at T0, but conformed to a bell-shaped profile of velocity and trajectory at T2. The maximal trajectory height inproved also to normal values after two month.

P436. Hypertonic saline in pediatric severe head injury – first clinical data
S. Berger, M. Schwarz, R. Huth (Mainz, D)

Introduction: Hypertonic saline has been shown to reduce posttraumatic intracranial hypertension in adult head-injured patients even when mannitol has lost its therapeutic effect. Treatment of therapy-resistant ICP-elevation in head-injured children with hypertonic saline has not been reported yet.
Case report: A 12-year old boy with an admission GCS of 6 and an isolated parietal contusion developed an ICP > 40 mmHg at 12 h post trauma. When mannitol infusions were ineffective to lower ICP, a bolus infusion of 30 ml of 20 % NaCl was given over 30 min via a central line. Hereby, ICP was temporarily reduced (for 2 hrs). When a control CT scan revealed growth of the contusion area and perifocal brain edema as a cause of the ICP-elevation, a decompressing craniotomy was made over the injured hemisphere. Thereafter only hypertonic saline was used to lower ICP when indicated. In comparison to mannitol, NaCl-infusions resulted in higher cerebral perfusion pressure due to maintenance or minimal increase in arterial blood pressure, while blood pressure dropped markedly after mannitol. GCS improved to 14 at 3 days after the introduction of NaCl and the surgical decompression. At 2 weeks after the injury no focal neurological deficits and no functional deficits besides moderately impaired short term memory were present.
Discussion: As previously shown in animal experiments and adult patients, hypertonic saline prooved effective and safe for lowering posttraumatic ICP-elevations in a child. Rebound phenomena limit the therapeutic effect of mannitol on intracranial hypertension after several applications. Since the effect of hypertonic NaCl on ICP is comparable to that of mannitol, an alternating use of both hypertonic solutions might prolong the total time period of efficient hyperosmolar therapy in severe head injury in children and adults.

P437. Motor recovery in children with traumatic brain injury
M. Dreesmann, M. Goelge, J.P. Kuhtz-Buschbeck, J. Pohl, B. Benz, A. Ritz, M. Illert (Kiel, Bremen, D)

As the inherent plasticity of the maturing nervous system allows compensation of CNS functions after traumatic brain injury, the CNS is able to either maintain complex behaviours or even to restitute acquired functions. We investigate restitution of motor functions in children after traumatic brain injury in a longitudinal study. Therefore we selected children by the age (5-15 years) and by the grade of brain injury (II°). Patients were examined four times, first date (T0) was defined by the Barthel Index, further examinations followed after a 1-month (T1), 2- months (T2) and 5-months (T3) intervall. The Glasgow Coma Scale and the Injury Severity Index were chosen to evaluate the trauma's severity. Neurological outcome was measured by the clinical examination, Rappaport Disability Scale and Glasgow Outcome Scale. To evaluate restitution of locomotion and hand function standardized tests and quantitative analysis of movements were combined: Jebsen-Taylor test of hand function, perdue pegboard, gross motor function measurement and the Brinckmann gait analysis. Ten patients underwent all examination dates in the five month intervall so far. They showed relief of clinical symptoms as well as improvement in the standardized motor tests. Though all patients were able to improve their motor ability the degree of motor advance differed between normalisation of function in the mayority of the patients and just slight improvement in few patients.

P438. Early routine evaluation with CT scan in minor head injury: preliminary results of the international study

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Objectives: The main purpose of our multicenter research is: outline standard case data; study/describe normal clinical evolution; facilitate the clinical study of outcome and promote a comparative study of results obtained.
Materials and methods: Our study includes children between 0 and 12 years of age with history of Head Injury. All patients examined includes with a Minor HI: Loss of consciouness, amnesia for the event, and Glasgow Coma Score or Paediatrics Coma Score 13–15. The management of procedure included a routine early CT Scan within 6 hours after injury at the emergency department and discharge after normal CT Scan. Admission only at the following aspect: CT Scan positive, or fracture (High risk Site), social situation, (active issues), clinical deterioration. Finally all patients were examined at the follow-up 3 months after injury.We present our first 2,021 patients evaluated in 13 countries participated to the ISHIP.
Results: Of the total 2,021 patients enrolled in the study, 12 months, 59.4% were boys and 40.6%, girls. The age distribution was a follows: 40.8% 0–2 years; 29.2% 3–6 years; 14% 7–9 and 16% 10–12 years. Most (99.8%) of these patients completed the follow-up. A fall was the mechanism of injury in more than half of the patients (72.4%) followed by road accidents (17.6%). 780 children (38.6%) who required hospitalization. Skull fracture were identified in 19% of cases, and 32.8% of CT scans were pathologic. 81 patients (4%) suffered neurosurgical intervention and the mortality rate was 0.05%.
Conclusion: Our data reveal the high incidence of positive CT scan, more than 60% in two age intervals, 0-2 and 3-6 years. The mechanism doesn't to differentiate the CTs, except that pedestrian and motor vehicle accidents have a higher number of positive CTs. These data needed confirmation at the end of our study and the early routine evaluation with imaging at the emergency department is possibly cost saving in Minor HI.

Poster Session 29
Neuropsychology, rehabilitation 2

P439. Functional outcome after brain injury and polytrauma

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Brain-injured patients suffering additional polytrauma shows a higher rate of mortality and complications in the initial and acute phases of medical treatment. In most cases, it is assumed that a worse outcome is to be expected for patients with additional polytrauma than for tho-se with isolated brain damage. We compared the outcome of two groups of severe brain-injured pa-tients; 38 patients with isolated brain injury, 23 patients with brain in-jury and additional polytrauma. After 6 months the majority of the pa-tients from both groups were able to achieve a high degree of inde-pendence in carrying out the tasks of daily life. After 12 Months there still were no significant differences between the two groups in their need of care and professional reintegration. These results showed that the presence of additional polytrauma had no significant influence on the outcome of the severe-brain-injury pa-tients. If the patients survive the acute phase and are given continuous rehabilitative care from as early a period as the acute phase of the ill-ness up to their professional reintegration, then their prospects are no worse than those of patients with an isolated form of severe brain da-mage.

P440. Recovery of consciousness after severe traumatic brain injury (TBI)

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The objective of this study was to find predictors for recovery of consciousness in TBI patients. We looked for recovery in terms of time to follow commands (Tcomm). Seven rehabilitation centers constructed a database of vegetative and minimally conscious patients to build predictive models of recovery of consciousness. The records included injury history, neuroimaging data, medical complications, pharmacological treatment and weekly Disability Rating Scale (DRS) scores. In this analysis we evaluated the data of a subgroup of TBI patients who did not follow commands at time of enrollment and had a DRS score >15 between 29th day and 112th day post injury (N=66). The dependent variable Tcomm was the time until patients follow verbal commands, either consistently or inconsistently, as measured by the motor subscore of the DRS scale. To assess significance we used chi-square analyses for categorical variables and Spearman correlation for continuous variables. The minimum Tcomm was 35 days, the maximum 229 days and the median 67 days. Time of enrollment, DRS at enrollment as well as one-week-change and two-week-change in DRS correlated with Tcomm ($p<.05$). Initial GCS, complications like elevated ICP, hypoxia or ischemia and evidence of hermiation were not significant, nor was therapy intensity. There was no relationship between type and localisation of lesion and Tcomm apart from bilateral lesions, which showed a negative effect. As far as medication was concerned, only serotonergic drugs had a positive effect on Tcomm ($p<.05$). In conclusion time since injury and DRS at enrollment as well as early DRS changes and administration of serotonergic drugs predicted recovery of consciousness in terms of time to follow commands. Except bilateral lesions clinical variables were no significant predictors. Future controlled studies should investigate the role of pharmacological interventions in the process of recovery of consciousness further.

P441. Subjective impairment, neuropsychological performance, post-traumatic psychological stress and coping in patients after closed head injury

B.O. Hütter, A. van Oosterhout, M. Weinzierl, J.M. Gilsbach (Aachen, D)

A well-known hypothesis proposes that the subjective complaints of patients after head injury result from an unsuccessful coping process. Empirical research showed only weak associations between both variables. In the present study, we investigated the significance of the psychological trauma for the subjective complaints after head injury. The study sample consisted of 61 patients (age mean 40,3 years) including 16 females and 45 males with a mean 17,6 months after head injury. According to the Aachen Coma Scale (ACS), 16% of the patients had sustained a severe, 23% a moderate and 61% a mild head injury. Subjective complaints were assessed by the Aachen Life Quality Inventory (ALQI). Further questionnaires employed were: the Freiburger Fragebogen für Krankheitsverarbeitung (FKV), the Impact of Event Scale (IES) and the Beck Depression Inventory (BDI). In addition, a neuropsychological examination and a psychopathological interview were conducted. According to DSM IV criteria, a posttraumatic stress disorder (PTSD) was present in 20 (33%) patients. Go/NoGo task performance correlated with $r=-0,35$ ($p<0,01$) with the frequency of intrusive thoughts (IES). No relationship between different indicators of physical and psychological trauma severity could be revealed. The severity of the psychological trauma was associated with the degree of subjective impairment, depressive coping and wishful thinking, sharing up to 36% of the common variance. In case of PTSD, the patients showed significantly ($p<0,01$) more depressive coping and complained of more restrictions in all aspects of their daily life (ALQI). A multivariate analysis revealed the presence of PTSD, coping and Go/NoGo task performance as the most important predictors of the frequency of intrusive thoughts (IES) explaining 64% of the variance. The results underline the significance of the psychological trauma after closed head injury for psychosocial adjustment.

P442. Evaluation of handwriting training in patients with writer's cramp

B. Baur, T. Schenk, B. Steidle, N. Mai (Munich, D)

Writer's cramp is mainly characterized by muscle spasms and unusual postures of the upper limb during writing. Most patients show increased stiffness of the involved joints and excessive pressure on pen and desk. Their handwriting is inefficient and also exhausting or even painful. Spontaneous remission of the disorder was not reported in previous long-term studies of writer's cramp. The effects of oral medication or Botox injections often proved unsatisfactory. Applying the method of Mai (1), we performed and evaluated handwriting training in writer's cramp patients. After comprehensive examination of the disorder, training was individually

tailored to each patient. The training typically included muscle relaxation-procedures during the writing process, application of a modified pen grip and adjustment of script according to ergonomic principles. Training started with simple drawing movements and proceeded step by step to standard handwriting. The writing movements of 14 patients have so far been recorded with a digitizing tablet before and after training and again after a follow up period (0.5–4 years). The marked improvement of writing performance observed after training and during follow-up was confirmed by kinematic measures (significantly increased writing speed and reduced pressure). We conclude that the applied training proved successful. Thus, such comprehensive handwriting training should be preferred to other more invasive methods for treatment of writer's cramp.
References: (1) Mai N. & Marquardt C. (1999) Schreibtraining in der neurologischen Rehabilitation. In: EKN-Materialien für die Rehabilitation. Borgmann Publishing.

P443. Junctional DREZ coagulation for treatment of brachial plexus avulsion pain
B. Prestor (Ljubljana, SLO)

In the treatment of intractable deafferentation pain after brachial plexus avulsion, different procedures in the DREZ have proved effective. Spot-like techniques do not include entire DREZ area, and special equipment is mandatory. The aim of this study is to present the junctional DREZ coagulation and clinical results of the new technique. To investigate the pain relief 21 cases with intractable brachial plexus avulsion pain underwent microsurgical junctional DREZ coagulation lesion along entire dorsolateral fissure of involved spinal cord segments using bipolar forceps. The postoperative analgesic effect was judged by the patients (analogues scale, use of analgetics). Pain duration before the procedure was from 5 months to 27 years. All patients described permanent intensive pain and frequent attacs of severe intractable pain. Result was good with more than 50 % pain relief in 19 cases (90.5 %), excellent in 17 cases (80.9 %) with more than 70 % pain relief, and complete pain relief was achieved in 10 cases (47.6 %) out of 21 patients. Follow-up was 10–120 months. Transient sensory neurological disturbances lasted up to 8 weeks were observed in 4 cases (19 %), there was no permanent neurological deficit. We conclude that junctional coagulation DREZ lesion for the treatment of brachial plexus avulsion pain offers long lasting and excellent pain relief. There is no need for special equipment for creating DREZ lesions. The lesions are precisely placed along dorsolateral sulcus only with bipolar electrode so that entire DREZ area and DREZ structures important for deafferentation pain are included.

P444. Computer Assisted Design (CAD), pre cast craniofacial reconstruction using bone source osteoconductive bone substitute material
D.B. Michael, L.P. Caragine, F.C. Vinas, A. Galaniuk, A.B. Rogers, D. Kuldjanov (Detroit, Ann Arbor, Kalamazoo, USA)

We report 2 cases of craniofacial reconstruction using a computer assisted design (CAD), precast implant made of BoneSource Osteoconductive Bone Substitute Material (Stryker Leibinger, Kalamazoo, MI). Patient 1 is a woman treated for post op osteomyelitis of an orbitozygomatic osteotomy flap. Patient 2 required removal of a frontotemporal flap following a shotgun blast to the head. In each case a CT utilizing a three dimensional aquisition format was obtained and the data transmitted to Cyberorthology, Inc. (Ann Arbor, MI). A mold was fashioned using CAD. The implant was case in this mold using BoneSource. The prosthesis was brought to Detroit Receiving Hospital, sterilized using standard autoclave methods and implanted in the patients. Both patients returned home on the third post op day and have done well to date. Details of the operative technique, pre op, immediate post op, three and six month three dimensional CT images will be shown. These initial cases demonstrate the feasibility of craniofacial reconstructive using a pre case, hydroxyapatite bone matrix. This cranioplasty technique results in an immediate cosmetically superior result and should provide for long term integration into the patient's skull. (Supported by the WSU-SOM Fund for Education and Research; The L.M.. Thomas, M.D. Fund; Cyberorthology Inc; and the Stryker Leibinger Corporation.)

Poster Session 30

Clinical trials and outcome measures in SCI

P445 Usefulness of hyperbaric oxygen (HBO) therapy
S. Asamoto, H. Sugiyama, H. Doi, M. Iida, K. Matsumoto, Y. Mochizuki, Y. Ikeda (Tokyo, J)

Purpose: In our department of neurosurgery, hyperbaric oxygen (HBO) therapy has been performed in patients with acute traumatic cervical spinal cord injury with very good results as reported in this paper.
Subjects and methods: In order to obtain the patient□fs unification, 34 cases of hyperextension spinal cord injury without bone damage and previous

history of surgical intervention were selected for this retrospective study. These 34 cases were divided into 2 groups with or without of HBO therapy, HBO and non-HBO groups. The neurological findings at admission and their outcomes were evaluated by means of Neurological Cervical Spine Scale (NCSS) to estimate the rate of improvement1. Then the average improvement rates in individual groups were compared.
Results: The improvement rate ranged from 100% to 27.3% with the mean value of 75.2% in the HBO group, while these values were 100%, 25.0% and 65.1% respectively in the non-HBO group.
Conclusion: In the HBO group as compared with the non-HBO group, the improvement rate was distinctly high, indicating its excellent effectiveness on acute traumatic cervical spinal cord injury.

P446. Clinical outcome of minor trauma in cervical spinal stenosis
K. Cho, P. Huh, C.K. Park, D. Yoo, D. Kim, J. Kang (Seoul, Uijongbu, KOR)

Introduction: The most common cause of the cervical spinal stenosis was the degenerative disease of the cervical spine (including cervical OPLL and cervical spondylotic myelopathy). The influence of minor trauma to the neck on the neurological outcome in patients with cervical spinal stenosis was evaluated retrospectively.
Materials and methods: We treated 147 cases (72 cervical OPLL and 75 cervical spondylotic myelopathy) of cervical spinal stenosis for 5 years. There were 45 (30.6%) minor trauma cases to the cervical spine out of 147 patients. Of these 45 patients, 13 developed myelopathy, 9 showed deterioration of pre-existing myelopathy, and no neurological change was observed in 23 patients. Minor trauma included motor vehicle accident, fall-down, slip down, being struck by an objects and sports activity. The neurological status assessed by Japanese Orthopaedic Association (JOA) score.
Results: Regarding the relationship between the diameter of the residual spinal canal and the neurological outcome in these 45 patients, 16 out of the 18 patients with a narrow spinal spinal canal (< 10 mm) developed neurological deterioration, whereas that occurred in 6 of the 27 patients with a wider spinal canal (> or = l0 mm) ($P<0.05$). Regarding patients who were treated surgically, there were 6 improvement more than 50% JOA Score among 18 in narrow spinal canal group and 22 improvement among 27 in wider spinal canal group ($P<0.05$). The recovery rate was 33.7% in patients with trauma, and 65.7% in those without trauma ($P< 0.05$).
Conclusion: These results indicate that even indirect minor trauma to the neck can cause irreversible changes in the spinal cord if there is marked stenosis of the cervical spinal canal; such patients who are at risk, must be educated.

P447 Acute spinal cord injury – early care and treatment in a multicenter study with gacyclidine
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280 patients with acute spinal cord injury (SCI) were enrolled within 2 hours after their accident. Aged of 18 to 65, with an ASIA motor score from 0 to 15 inclusive at the lower limb the most injured, without experience of episode of low blood pressure for more than 15 minutes. After informed consent, they were randomized into 4 strata : level (cervical (T1 inclusive) or thoracic), severity (complete or incomplete lesion) and assigned to 4 groups of treatment in a double blind manner : placebo, 0.005 mg/kg, 0.01 mg/kg, 0.02 mg/kg of gacyclidine. The treatment was injected IV and repeated once after 4 hours. Gacyclidine is a non competitive NMDA antagonist. When needed the patients underwent surgery for early decompression, recanalization and stabilization of the spinal cord within 6 hours. Follow up was one year. A high rate of patients with complete lesion (72%) contributed to define a very severely injured population with poor prognosis. At one month a trend is observed in an overall analysis ($p=0.08$), with the dose of 0.02 mg/kg identified as the best dose; but not confirmed at one year. The cervical incomplete strata shows positive results of large magnitude with a gain of more than two metameric levels on the motor score compared with placebo at one month. This difference is maintained at one and half metameric level at one year. The safety profile of gacyclidine was good at the different doses. The rapidity of the initial care, the improvement of the care management, the early decompression of the spinal cord, the care coordination could have contributed to make the patients recover quite well in the different strata, when compared with literature data.

P448. Transpedicular approach in lumbar and thoracolumbar burst fracture
K. Cho, P. Huh, C. Park, D. Yoo, D. Kim, J. Kang (Seoul, KOR)

Objectives: There are various surgical strategies for the treatment of lumbar and thoracolumbar burst fractures. Standard laminectomy with manipulation of the spinal cord can cause significant spinal cord injury. Transthoracic and transabdomonal approaches offer direct visualization of the diseased region. However, these surgical strategies also involve

extensive dissection through tissue which is not involved in the disease process. Transthoracic and transabdominal procedures may require long operative times and introduce the possibility of thoracic and/or abdominal complications. We evaluate the availability of transpedicular approach for lumbar and thoracolumbar burst fractures.
Patients and methods: We have performed transpedicular approach for 3 years for lumbar and thoracolumbar burst fractures in 9 patients. There were 4 male and 5 female patients. The mean age is 39 year-old-age (range; 21–61). Bilateral transpedicular routes were used in 3 patients and supplemental laminectomies in 2. The final outcome was measured by Prolo's Functional-Economic Outcome Rating Scale.
Results: Final functional outcomes were; good in 6, moderate in 2, and poor in 1 case. Spinal canal decompression was confirmed with follow up CT scan or MRI. There were three operative complications – two CSF leakages and one delayed wound infection.
Conclusion: The transpedicular approach to lumbar and thoracolumbar burst fractures is an effective procedure which allows adequate decompression of ventral encroachment without further spinal cord injury. Compared to anterior approach, the transpedicular approach has few potential complications such as violation of the pleural space, manipulation of the mediastinal structure, impotence, great vessel injuries, and it enables immediate posterior stabilization, if necessary.

P449. A critical appraisal of the reporting of the NASCIS studies of MPSS in acute spinal cord injury
W.P. Coleman (Annapolis, USA)

From the beginning, the reporting of the results of NASCIS II and NASCIS III has been incomplete, leaving the SCI community to use or avoid using Methylprednisolone (MPSS) in acute Spinal Cord Injury (SCI) on the basis of faith, instead of a publicly developed consensus based on science. NASCIS II was initially reported by NIH announcements, an NIH fax to emergency room physicians, and in the news media. The subsequent report in NEJM used wording that led people to think there was a positive result in the primary efficacy analysis for the entire 487 patient sample. In fact, this analysis was negative -- and the positive result was only for a secondary analysis in the subgroup that received treatment within 8 hours and had, apparently, only 62 patients on MPSS and 67 on placebo. NASCIS II and III show statistical artifacts that call their results into question. In NASCIS II the placebo group treated before 8 hours did poorly, not only when compared to the MPSS group treated before 8 hours but even when compared to the placebo group treated after 8 hours. Thus the positive result may have been due to a weakness in the control group rather than to any strength of MPSS. – In NASCIS III there was a randomization imbalance that allocated a disproportionate number of patients with no motor deficit (and therefore no room to recover) to the 24 MP control group. When this imbalance is controlled for, much of the superiority of the 48 MP group disappears. The NASCIS group's decision to admit minor spinal cord injuries with minimal or no motor deficit not only enables statistical artifacts, it makes it difficult to interpret the results from the population actually sampled. Perhaps one half of the NASCIS III sample may have had at most a minor deficit. We thus do not know whether the results of these studies reflect the severely injured population to which they have been applied. The numbers, tables and figures in the published reports are scant and are inconsistently defined, making it impossible even for professional statisticians to reduplicate the analyses, to guess the effect of changes in assumptions, or to supply the missing parts of the picture. Nonetheless, even nine years after NASCIS II, the primary data have not been made public. Detailed comparison of the reporting of the NASCIS studies with the guidelines of the ICH/FDA and of the Evidence-based Medicine Group show that they have fallen very far short. Despite the lucrative "off label" markets for MPSS in SCI, no FDA indication has been obtained. There has been no public process of validation. These shortcomings have denied physicians the chance to use confidently a drug that many were enthusiastic about and has left them in an intolerably ambiguous position: in their therapeutic choices, in their legal exposure, and in their ability to carry out further research to help their patients.

P450. The GM1 ganglioside acute spinal cord injury (SCI) study II – efficacy and safety results
F.H. Geisler, F.C. Dorsey, G. Grieco, S. Piva, D. Poonian, R. Fiorentini (Chicago, Barrett Park, New York, Rockville, Washington, USA; Abano Terme, I)

Objectives: To determine the efficacy and safety of GM1 in acute SCI after standard methylprednisolone (MPSS) treatment.
Background: In a 37-patient single-center study (NEJM 1991), significantly (p=0.03) more GM1 than placebo patients had marked motor improvement.
Design and methods: This was a double-blind parallel study of placebo vs "low-dose" GM1 vs "high-dose" GM1, stratified by injury level, age and Baseline severity. Marked Recovery was a =2 grade improvement in the post-Baseline modified Benzel vs the Baseline ASIA Impairment Scale (AIS). Other efficacy measures included: the time course of Marked Recovery. ASIA (American Spinal Injury

Association) motor scores, sensory scores levels, and bowel and bladder function.
Results: Efficacy results are given for 330 GM1 100mg and 331 Placebo patients. The percentages of patients with Marked Recovery at Week 26 (principal endpoint) were similar for both groups (32.5% for GM1; 31.2% for Placebo; p=0.741, Cochran-Mantel-Haenszel test). In the time course of Marked Recovery, treatment differences always favored GM1, and were statistically significant at Weeks 8 and 16 (p=0.003 and 0.043, respectively, Cochran-Mantel-Haenszel test). In the time course of Marked Recovery by Severity group, greater proportions of the Severity B and Severity C+D GM1 than Placebo subjects improved in early visits; only the difference for the C+D group (79.2% vs 52.8%) was statistically significant (p=0.0072, Fisher's exact test), at Week 8. In the non-operated group (62 GM1, and 60 Placebo) there was a statistically significant difference in the proportion attaining Marked Recovery by Week 26 in favor of GM1 (51.6% vs 25%, p=0.003. Fisher's exact test).
Conclusion: The GM1 100mg group demonstrated earlier Marked Recovery than did the Placebo group. In Severity group A this effect was modest, and possibly constrained by the limited recovery attainable in these subjects. Greater effects were seen in Severity groups B and C+D. These conclusions are under review by the US FDA and the Canadian Health Authorities.

P451. “Natural" recovery from acute spinal cord injury among placebo patients in the GM1 ganglioside multi-center study: Interating clinical function and statistical analyses in therapeutic trials

G. Grieco, F.H. Geisler, F.C. Dorsey, S. Piva, D. Poonian, R. Fiorentini (New York, Chicago, Barrett Park, Rockville, Washington, USA; Abano Terme, I)

Objectives: Characterize recovery from acute spinal cord injury (ASCI), integrating functional anatomy with standard rating instruments and their clinical and statistical properties, so as to facilitate design and interpretation of clinical trials.
Background: Detailed longitudinal data on evolution of deficits from ASCI have not previously been reported. The 797-patient (346 on placebo) GM1 ASCI trial is the largest controlled treatment human ASCI study, with the most comprehensive database on "natural" recovery in a population given standard care.
Design and methods: The NASCIS II MPSS dosing regimen was started =8 hours post-injury. Follow-ups were at 8, 16, 26, & 52 weeks. Assessments included: AIS (Baseline only), Modified Benzel Classification (MBC; not at Baseline), ASIA Motor Score (AMS; 5 muscles/limb), ASIA Sensory Evaluation (ASE), relative and absolute levels of impaired pin and light touch sensation, and bowel and bladder function. The statistical properties of these assessments for placebo patients were analyzed and contrasted with spinal cord anatomy and function.
Results: The spinal cord, SCI, and recovery from SCI constitute related but distinct anatomical and functional continua. No one assessment provides a continuous measure of global cord function. Motor, sensory, and autonomic functions are separately assessed on scales with radically different clinical and statistical properties. All motor scales suffer from failure to assess the 12-segment "no man's land" of T2-L1. AMS distributions are therefore mostly unanalyzable.
Conclusion: The properties of the ASE and sensory levels of impairment most closely conform to functional anatomy. Motor scales have substantial clinical and statistical limitations.