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Poster Session A

PA - 01

NONLINEAR DYNAMIC MODELING OF EPILEPSY SUFFERERS AFTER HEAD INJURY

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One of the major roles of electroencephalography (EEG) is as an aid to diagnose epilepsy. Abnormal patterns such as spikes, sharp waves and, spikes and wave complexes can be seen. The type of activity and the area of the brain that is recorded from will assist the physician in prescribing the correct medication for that type of epilepsy. Patients with epilepsy that cannot be controlled by medication will often have surgery in order to remove the damaged tissue. The EEG plays an important role in localizing this tissue. EEG signal represents overall electrical activity of the brain, the methods of nonlinear dynamic and deterministic chaos theory may be used to analyze pathological changes in the brain. The goal of this research is therefore to identify the location of the epileptic foci. In this research, we proposed to integrate these two models; chaotic which has been developed by Iasenidas (Iasemedis et al. 1988) with Fuzzy Topographic Topological Mapping, FTTM (T. Ahmad et al. 2000) in order to model the brainstorm of epilepsy sufferers. The value of this model is both theoretical and practical: it has theoretical value in providing a framework that unifies the great diversity of EEG observations; it has practical value, since it helps to relate clinical EEG recordings more closely to disturbances in brain physiology, and it enhances the quality of information obtained from the data.

PA - 02

CROSS SECTIONAL STUDY OF AETIOLOGY, CLINICAL AND ELECTROENCEPHALOGRAPHIC CORRELATION OF RURAL NORTH EAST MALAYSIAN PATIENTS WITH INTRACTABLE EPILEPSY: IS THERE A HIGH INCIDENCE OF TRAUMATIC HEAD INJURY CAUSING EPILEPSY?

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The incidence of mild, moderate and severe head injuries are higher among younger patients in rural North east Coast Malaysia and associated with motorcycle injuries. A cross sectional study of 44 EEG reports and MRI images of patients with clinical diagnosis of epilepsy of various aetiologies was performed from January 2000 for a period of 24 months. Patients were classified based on the International Classification of Epileptic Seizure proposed by the International League Against Epilepsy (ILAE). EEG reports were reviewed for any localized or generalised abnormality, epileptiform changes, slow background activity and wave frequency. Statistical analysis was performed using Chi-square test. Twenty-six patients were male and 18 were female. Mean age was 20.7 years (range from 5 to 62 year old). Nineteen patients or 43.2% had generalised seizure (12 tonic-clonic, 5 tonic, 1 myoclonic and 1 absence), 22 patients or 50% had partial seizure (5 simple partial, 10 complex partial and 7 partial seizure with secondarily generalisation) and 3 patients or 6.8% presented with unclassified seizure. EEG was abnormal in 30 patients (20 generalised abnormality and 10 localised abnormality). MRI was abnormal in 17 patients (38.6%) and the abnormalities observed were cerebral atrophy (5 patients), hippocampal sclerosis (4 patients), infarct/gliosis (3 patients), cortical dysgenesis (2 patients) and tumour (2 patients). Out of these 17 patients with abnormal MRI, 14 had abnormal EEG. There was no significant correlation between the localisation abnormal EEG and abnormal MRI ($p > 0.05$) as well as neurotrauma. Abnormal MRI was more commonly observed in patients with generalised seizure and sim-

Case	Age	Fracture	Sinus thrombosis	ICH	Heparinisation
1.	42	Rt.occipital,Rt.basal	Rt.sigmoid	SAH, SDH	yes
2.	14	Lt.occipital,Lt.basal	Lt.transverse	SAH, EDH	yes
3.	25	Lt.occipital,Lt.basal	Lt.sigmoid	SAH,contusion	no
4.	50	Lt.occipital	Lt.transverse	SDH,contusion	no
5.	45	Lt.occipital	Lt.transverse	EDH,contusion	no
6.	62	Lt.occipital	Lt.transverse	SAH, EDH	no
7.	15	Rt.occipital,Rt.basal	Rt.sigmoid	SDH,EDH	no

ple partial seizure as compared to those with unclassified seizures ($p < 0.05$). None of the patients with intractable epilepsy had trauma as an aetiology. Intractable epilepsy is not associated with head injuries in our series of patients.

PA - 07

LONG-TERM HEART RATE VARIABILITY CHANGES FOLLOWING TRAUMATIC BRAIN INJURY: A PILOT STUDY

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Introduction: Heart Rate Variability (HRV) represents the interplay between sympathetic and parasympathetic influences on the heart and has been suggested as a sensitive measure of medullary integrity. Abnormalities of HRV have been reported in a number of disease states, including severe traumatic brain injury (TBI) in the acute post-injury period.

Purpose: This pilot study sought to examine the post-acute HRV of survivors of extremely severe TBI and age-matched controls. Secondary analyses compared group differences for patients with different levels of function and examined the natural history of HRV changes in low level patients over time.

Methods: Resting ECG data was collected and processed for HRV. Injury characteristics were obtained from the files of TBI survivors.

Results: Data was obtained from 16 TBI patients and 12 age-matched controls (mean age 32.1 years for both groups). The mean heart rate of the TBI group (mean 77 days post trauma) was 89.0 bpm versus 69.5 for controls ($p = 0.001$). Spectral HRV analysis showed lower total power in the TBI group but a greater low to high frequency ratio (LF:HF of 3.72 and 1.63 respectively, $p < 0.05$). High and low functioning patients at the time of admission showed LF:HF ratios of 2.39 and 4.95 respectively (non-significant). Four low level patients re-tested a mean of 400 days post trauma showed a significant change in mean LF:HF ratio to 3.48 ($p = 0.001$).

Conclusions: Group differences were observed between the TBI and control groups that persisted well into the sub-acute period. The HRV parameters of low level patients appeared to change over time.

PA - 11

DIAGNOSIS OF POSTTRAUMATIC VENOUS SINUS THROMBOSIS WITH CT VENOGRAPHY

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Background: Traumatic sinus thrombosis, potentially life-threatening condition, is thought to be uncommon but may be underdiagnosed using standard imaging techniques

Patients and Methods: We report seven cases of traumatic sinus thrombosis after closed head injury. Their CT all revealed occipital skull fracture, 4 of them revealed basal skull fracture. We evaluated the clinical courses of all patients with CT venography every week after injury.

Results: T hey showed thrombosis of the sigmoid or transverse sinuses by initial CT venography without specific symptoms. They all revealed recanalisation of the sinuses after 2 weeks regardless of heparinisation. The clinical course of all patients was uneventful.

ICH: intracranial hemorrhage,SAH:subarachchnoid hemorrhage, SDH:subdural hemorrhage EDH: epidural hemorrhage.

Conclusion: Early diagnosis of cerebral sinus thrombosis is difficult because of nonspecific and variable clinical presentations, while untreated thrombus progression may be fatal due to venous congestion and infarction. CT venography is a reliable, less invasive and helpful method to depict and follow up the venous sinus thrombosis.

PA - 12
SURGICAL TREATMENT FOR CEREBRAL CONTUSION: EVALUATION OF 182 PATIENTS REGISTERED IN THE JAPAN NEUROTRAUMA DATA BANK
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Surgical indications for traumatic intracranial hematoma, such as acute epidural or subdural hematoma, are definite, whereas that for cerebral contusion remains unclear. In this study, in order to elucidate therapeutic effects of decompression surgery for cerebral contusion, we analyzed data from the Japan Neurotrauma Data Bank (JNTDB) in which total 1002 cases of severe TBI were registered (1998–2000). [Results] In the JNTDB, 182 patients (18%) were entered as the cases of cerebral contusion. Internal and/or external decompression were performed in 61 cases (34%) (Group D), and conservative therapies were chosen in 121 cases (66%) (Group C). There was a tendency that the conservative therapies were selected in younger patients, and decompression surgery in elder patients, especially in 40–60 year-old. The Glasgow Outcome Scale (GOS) was significantly poorer in Group C (GOS = VS/D: 58% in Group C, 31% in Group D). One of the reason for the poorer outcome seemed to be lower levels of initial Glasgow Coma Scale (GCS) in Group C than that in Group D (GCS $< / =$ 8: 87%, and 58%, respectively). However, subgroup analysis within the same GCS levels also showed poorer GOS in Group C. For example, in patients with initial GCS$> / =$ 9, poor outcome (GOS = VS/D) was 56% in Group C, and 28% in Group D. In 29 patients who "talk & deteriorate", 64% died in Group C, whereas 22% in Group D. [Conclusion] In JNTDB, therapeutic decision making was left to each facilities, and was not randomized. Therefore, the results of this study dose not directly confirm the superiority of surgical therapy for cerebral contusion. However, it is certain that the patients who underwent conservative therapy showed poor outcome. The higher mortality rate in patients with good GCS indicates the limitation of conservative therapy for cerebral contusion. In patients who show progressive deterioration of conscious level, decompression surgery should be considered in the early stage.

PA - 13
THE CHEMOKINE FRACTALKINE IN PATIENTS WITH SEVERE TRAUMATIC BRAIN INJURY AND IN A MOUSE MODEL OF CLOSED HEAD INJURY
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The potential role of the chemokine Fractalkine (CX3CL1) in the pathophysiology of traumatic brain injury (TBI) was investigated in patients with head trauma and mice following experimental cortical contusion. In control individuals, soluble (s)Fractalkine was present at low concentrations in CSF (12.6–57.3 pg/ml) but at much higher levels in serum (21,288–74,548 pg/ml). Elevation of sFractalkine in CSF of TBI patients was observed during the whole study period (means: 29.92–535.33 pg/ml), whereas serum levels remained within normal ranges (means: 3,100–59,159 pg/ml). Based on these differences, a possible passage of sFractalkine from blood to CSF was supported by the strong correlation between blood-brain barrier dysfunction (according to the CSF-/serum-albumin quotient) and sFractalkine concentrations in CSF (R = 0.706; $p < 0.01$). In the brain of mice subjected to closed head injury, neither Fractalkine protein nor mRNA were found augmented, however, Fractalkine receptor (CX3CR1) mRNA steadily increased peaking at 1 week post injury ($p < 0.05$, one way ANOVA). This possibly implies the receptor to be the key factor determining the action of constitutively expressed Fractalkine. Altogether, these data suggest that the Fractalkine-CX3CR1 protein system may be involved in the inflammatory response to TBI particularly for the accumulation of leukocytes in the injured parenchyma.

PA - 14
THE ROLE OF S-100ß PROTEIN FOLLOWING TRAUMATIC INJURY
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Glial S-100ß protein is often elevated in serum and CSF following traumatic brain injury, and although increasing levels of S-100ß may indicate poorer outcome after injury, its exact function remains unclear. Various reports have demonstrated that S-100ß can be either neurodegenerative or neuroprotective, making the de-

termination of its potential to heal or harm after injury challenging. We studied the effects of trauma on S-100ß release in mouse hippocampal and cerebellar cultures using a model of stretch-induced injury *in vitro*. In hippocampal cultures, a mild level of injury (31% stretch) increased propidium iodide (PrI) uptake 15 min and 24 hr after injury, indicating membrane damage. There was no reduction in neuronal number, suggesting PrI uptake occurred primarily in glia. Although S-100ß release was not detected 1 hr after injury it was elevated at 24 and 48 hr. Cerebellar cells injured with the same level of stretch demonstrated significant PrI uptake at 15 min and S-100ß release at 1 hr and 24 hr post-injury, which was 10-fold higher than that released from hippocampal cells. When exogenous S-100ß was added to uninjured cerebellar cultures, there was no effect on PrI uptake, but there was a significant reduction of Purkinje neurons. Exogenous S-100ß added to uninjured hippocampal cultures caused no effect on PrI uptake or neuronal counts. However, S-100ß added immediately after injury reduced PrI uptake when measured at 24 hr. Therefore, the amount of S-100ß released from glia, and its effects on neighboring neurons, may vary greatly between different brain regions.

PA - 16
CEREBRAL BLOOD FLOW, CEREBRAL METABOLIC RATE OF OXYGEN OUTCOME IN PATIENTS WITH DIFFUSE AXONAL INJURY
H Tomita, U Ito, O Tone, M Tamaki, M Hara, M Inaji, K Ohno (*Tokyo, Japan*)

The relationship of Cerebral blood flow (CBF), cerebral metabolic rate of oxygen (CMRO2) to clinical outcome was evaluated in 36 patients with diffuse axonal injury (DAI). DAI was clinically diagnosed based on the presence of prolonged coma and CT or MR findings of diffuse brain injury (Gennarelli's criteria). Patients with apparent episodes of severe hypoxia or ischemia were excluded from the study. Global CBF and CMRO2 were measured by the nitrous oxide saturation method within 9 days of head injury (mean 3 days). Clinical outcome was evaluated using the Glasgow Outcome Scale at one year post-injury. The corrected CBF was 60.4$+/-$ 14.7 ml/100 g/min for the good recovery (GR) group (fourteen patients), 47.2 $+/-$ 12.2 ml/100g/min for the moderate disability (MD) group (nine patients) and 42.7 $+/-$ 17.6 ml/100g/min for the poor outcome (PO) group (thirteen patients). The corrected CBF of the PO group was significantly lower than that of the GR group ($p = 0.002$). CMRO2 was 4.43 $+/-$ 1.65 ml/100 g/min for the GR group, 3.44 $+/-$ 0.74 ml/100 g/min for the MD group and 2.53 $+/-$ 0.89 ml/100 g/min for the PO group. The CMRO2 of the PO group was significantly lower than that of the GR group ($p = 0.0003$). Reduced CBF and CMRO2 within 9 days after injury are indicative of poor outcome in patients with DAI.

PA-17
CEREBRAL ISCHEMIA: CEREBROVASCULAR AND NEUROCHEMICAL RESPONSES
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Introduction: the only currently approved treatment for ischemic stroke is clot lysis. Alternative therapies could provide significant improvement in overall outcome from stroke. A complete understanding of the early events following stroke is essential in determining an overall therapeutic regimen. We have measured hypoxanthine, inosine, adenosine, lactate, pyruvate, aspartate, glutamate, and GABA in cerebral extracellular space as well as cortical perfusion in an intraluminal suture model of reversible cerebral ischemia. Methods: Spontaneously hypertensive rats were anesthetized with ketamine/atropine/acepromazine. A microdialysis (MD) probe was inserted into the cerebral cortex in the region supplied by the middle cerebral artery. Ischemia was induced by insertion of the suture and documented by laser Doppler flowmetry. MD samples were collected from 60 minutes pre- to 120 minutes post-occlusion. Results: LDF dropped by 75% immediately in response to suture insertion. Hypoxanthine ($p < 0.004$), inosine ($p < 0.01$), adenosine ($p < 0.04$), lactate ($p < 0.01$), aspartate ($p < 0.01$), glutamate ($p < 0.02$), and GABA ($p < 0.01$) levels increased in response to ischemia. No changes were seen in pyruvate concentrations. Conclusions: Induction of ischemia brings about an early depletion of high energy metabolites and results in a compromised metabolic state. The increases in aspartate, glutamate, and GABA underscore the disruption of cerebral energy/metabolic state during this early period. Return of perfusion during this time frame would seem to carry the potential for formation of free radicals, especially the highly reactive oxygen species. These alterations in amino acid, high energy, and metabolic metabolites should be taken into account when determining both the treatment regimen and the optimal time of reperfusion.

PA - 19
METALLOPROTEINASE-9 EXPRESSION IN HUMAN TRAUMATIC BRAIN INJURY
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We aimed to determine the temporal profile of matrix metalloproteinases-9 (MMP-9) expression and to investigate its relationship with severity and edema formation in patients with traumatic brain injury (TBI). Serial MMP-9 determinations were performed (ELISA, ng/mL) in 21 patients with an acute TBI. Patients were divided into the severe TBI group (initial GCS score < 8) and the mild to moderate TBI group (initial GCS score > 9). Expressions of MMP-9 in brain tissue were examined using gelatin zymography in 4 patients who underwent surgery for contusional brain injury. A total of 71 blood samples were collected from 21 patients (1 to 6 samples in each patient, mean 3.4 samples). Initial plasma MMP-9 levels were 13.3 ± 4.3 ng/mL (range 2–37 ng/mL) in the mild to moderate TBI group ($n = 7$) and 53.3 ± 18.1 ng/mL (range 0–254 ng/mL) in the severe TBI group ($n = 14$) ($p = 0.14$). Three patients with diffuse axonal injury showed low plasma MMP-9 levels. In the severe TBI group, peak MMP-9 was observed at baseline determination (53.3 ± 18.1 ng/mL) and no significant changes were found on follow-up determinations (24 hours: 27.8 ± 9.4 ng/mL and 48–72 hours: 31.9 ± 18.4 ng/mL). The 92-kDa gelatinase (MMP-9) activity was observed in all 4 tissue samples examined. In conclusion, there is a trend to increase plasma MMP-9 levels in patients with severe TBI. MMP-9 is expressed in the brain tissue at early stage after contusional brain injury. These data demonstrate that MMP-9 contributes to the pathophysiology of TBI.

PA - 21
ACTIVATED CASPASE-3 IN HUMAN TRAUMATIC BRAIN INJURY
J Wong, WL Tan, I Ng (*Singapore*)

Introduction: Apoptosis may play a role in cell death in head injury. The activation of caspase-3 has been considered the final common pathway for apoptotic cell death. Many animal studies have suggested that apoptosis occurs after TBI and the injury is directly related to the predominant localization of cleaved caspase-3 in neurons. In this study, we sought to determine if the location of this activated apoptotic caspase in animal models directly reflect or illustrates the phenomenon in human pericontusional tissue.

Methods: Pericontusional tissues were collected from 14 patients during surgical treatment for intracranial hypertension. The tissues were fixed in 4% paraformaldehyde and sectioned using a cryostat and mounted on poly-L-lysine coated slides. The sections were double immunofluorescence labelled with cleaved Caspase-3 and NeuN or GFAP for neuronal or glial cells respectively.

Results: Cleaved caspase-3 was expressed in all 14 tissues collected and expression was found in neurons only. The glial cells did not show evidence of activated caspase-3 staining.

Conclusion: Our results suggest that although cleaved caspase-3 is expressed in neuronal and glial cells of animal TBI models, activated caspase-3 is found only in human neuronal cells. This may be an important consideration in the development of novel pharmaco-therapeutic strategies that specifically target apoptosis.

PA - 22
CASPASE-7: UP-REGULATED AND ACTIVATED IN TRAUMATIC BRAIN INJURED RATS
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The apoptotic executioner protease, caspase-7, is generally believed either not to be present in the brain or if present it has only a minimal impact. We found elevated caspase-7 levels in the staurosporine and thapsigargin treated PC12 rat neuronal cell line, peaking at 8 and 24 hours post-treatment, respectively. We then examined traumatic brain injured (TBI) tissue samples for pro- and processed caspase-7 expression levels in a rat model of controlled cortical impact. Using immunoblots, the caspase-7 proform (35 kDa), the pre-active form (35 kDa) and the large active subunit (18 kDa) levels peaked in the ipsilateral cortex at 3 days, 7 days, and 5 days post-injury, respectively, and in the ipsilateral hippocampus at day 5 for both the pre-active form and the large subunit. Semi-quantitative PCR analysis confirmed elevated caspase-7 mRNA levels in injured rat brains. The moderate-to-severe injury of 1.6 mm compression associates with mRNA levels peaking in our study at day 5 post-injury in the ipsilateral cortex and on day 1 for the ipsilateral hippocampus. Immunohistochemical analysis of day 5 post-injured brain tissue revealed that neurons and astrocytes in both the cortex and the hippocampus expressed elevated levels of caspase-7. These studies are novel and among the first to document that caspase-7

is up-regulated and activated after traumatic brain injury and may contribute to neuropathology. (Supported by DAMD 17-99-1-9565/17-03-1-0066 and NIH R01-NS39091/R01-NS40182).

PA - 23
AMYLOID PRECURSOR PROTEIN AND ACTIVE CASPASE-3 IN HUMAN ACUTE AND CHRONIC COMPRESSIVE MYELOPATHY

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Introduction: This study aimed to analyse immunohistochemically Amyloid Precursor Protein (APP) and Active caspase-3 in post-mortem human specimens in acute and chronic compressive myelopathy.

Studies analysing axonal injury after brain trauma suggest a role for caspase-3 in the cleavage of APP [1]. In addition, caspase-3-mediated cleavage of APP has been found to be associated with the formation of Aβ, a neurotoxic protein thought to contribute to apoptotic cell death in Alzheimer's disease [2]. The current study addressed the hypothesis that APP provides a substrate for the caspase-3 enzyme in human acute and chronic spinal cord compression.

Methods: Spinal cord material from 17 patients with documented SCI was analysed. The spatial distribution of cellular immunoreactivity was qualitatively assessed in injury due to trauma ($n = 5$), iatrogenic event ($n = 1$), Cervical Spondylotic Myelopathy or CSM ($n = 6$) and metastatic tumour ($n = 5$).

Results: Active caspase-3 and APP were present in axons of the white matter and anterior horn cells. Dual-immunolabelling revealed axonal co-localisation of caspase-3 and APP.

Discussion: There is evidence that caspase-3 contributes to the proteolytic cleavage of APP in compressive myelopathy. It is possible that activation of caspase-3 via secondary injury mechanisms may trigger the apoptotic cascade with the subsequent demise of the cell.

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PA - 26
AII-SPECTRIN AND SEVERITY OF INJURY AFTER CONTROLLED CORTICAL IMPACT IN THE IMMATURE RAT BRAIN

JA Pineda, JM Aikman, BE O'Steen, EA Johnson, KK Wang, J Flint, RL Hayes (*Gainesville, USA*)

Our understanding of the mechanisms of damage to the immature brain after traumatic brain injury (TBI) remains quite limited. Proteolytic processing regulated by proteases plays an important role in regulating a wide range of important cellular functions, including cell death (Lopez, 2002). Previous work in our laboratory has demonstrated the role of two important proteases, caspase-3 and calpain in protein breakdown with the formation of distinct breakdown products of a-II-spectrin (SBDP) after TBI in the mature and the immature brain. We recently reported on the association of cerebral spinal fluid (CSF) levels SBDP with injury magnitude and lesion size after traumatic TBI in mature rodents. In the present study, protein analysis was used to immunolabel specific calpain and caspase-3 cleaved breakdown products after two different injury magnitudes in immature (post-natal day 9) rats. Using the controlled cortical impact model (CCI), we studied the formation of protease cleaved SBDP after a 1 mm and 1.3 mm cortical depression. Calpain mediated SBDP (150 kDa) in the ipsilateral cortex and hippocampus were not significantly different between the two injury magnitudes. Caspase-3 mediated SBDP were related to injury magnitude in the ipsilateral hippocampus but not in the ipsilateral cortex when comparing these two injury magnitudes. Current experiments in our laboratory are exploring possible mechanisms for these relationships between SBDP and injury magnitude that are unique to the immature rodent brain. These experiments provide additional insight into the role of both calpains and caspase-3 after TBI in immature rats.

PA - 27

BEHAVIORAL AND HISTOLOGICAL OUTCOME AFTER LATERAL FLUID PERCUSSION INJURY IN MONGOLIAN GERBILS

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Introduction: Traumatic brain injury causes various behavioral and pathological changes, which significantly correlate with prognosis. We investigated the time course of behavioral and histopathological changes after experimental TBI in Mongolian gerbils.

Method: We induced either mild (0.7–0.9 atm) or moderate (1.3–1.6 atm) lateral fluid percussion injury (LFPI) in gerbils. Spontaneous locomotor activity was evaluated by open field test (OFT), asymmetric motor behavior by elevated body swing test (EBST), sensory dysfunction by bilateral asymmetry test and cognitive/memory disturbances by T-maze test (TMT) during 7 days post-trauma. All gerbils were perfused for histopathological examination.

Result: Transient hyperlocomotion was observed in OFT in mild LFPI group with a peak at time of 6hours after LFPI. Animals with moderate LFPI showed persistent and more severe hyperlocomotion with a peak at time of day 3 post-trauma. EBST revealed mild hemiparesis in both groups. TMT revealed working memory disturbances in both groups. Histologically, focal necrosis at the directly injured cortex was found in both groups. Rarefaction of the subcortical white matter was observed in both groups. The white matter change in the moderate LFPI group extended to the whole ipsilateral hemisphere, and the size was significantly larger than the mild LFPI group.

Conclusion: Lateral fluid percussion injury induces various behavioral changes according to the severity of injury. The extent of white matter lesion is an important determinant of behavioral changes after experimental traumatic brain injury.

PA - 30

EXPOSURE TO ENVIRONMENTAL COMPLEXITY COMBINED WITH MULTIMODAL STIMULATION IS ASSOCIATED WITH REDUCED CNS SCAR FORMATION AND REVERSAL OF NEUROMOTOR DYSFUNCTION AFTER TRAUMATIC BRAIN INJURY

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The present study was designed to investigate the effects of enriched environment (EE) combined with multimodal early onset stimulation (MEOS) versus standard housing (SH) without stimulation on neuromotor function and lesion volume after experimental traumatic brain injury (TBI). Sprague-Dawley rats, randomized to the following groups: (i) injured/EE + MEOS; (ii) sham/EE + MEOS; (iii) injured/SH; (iv) sham/SH, underwent moderate fluid-percussion or sham injury. Thereafter EE + MEOS groups were placed into complex EE along with motor, olfactory, auditory and visual stimulation (MEOS); injured and sham SH groups were housed individually without stimulation. Neuromotor function was assessed using a composite neuroscore (NS) test battery at 24 h, 7, and 15 days post injury (DPI). On DPI 15 animals were sacrificed and brains harvested for single coronal section immunocytochemistry for GFAP. Nissl counterstaining allowed demarcation of zones containing reactive astrocytes enabling a quantification of the projection area occupied by GFAP-positive astrocytes; values for total area of all sections and the section thickness served for calculation of the lesion volume in each rat's brain. Neuromotor function was markedly reduced in both injured groups at 24 h post-injury being non-significant. However, injured/EE + MEOS animals performed significantly better when tested for neuromotor function compared to injured/SH animals on DPI 7 ($p < 0{,}05$) and 15 ($p < 0{,}5$). Better neuromotor function in EE + MEOS animals at DPI 15 was associated with significantly smaller lesion volumes compared to SH animals (14.28 $+/-$ 6.83 mm3 vs. 23.46 $+/-$ 4.31 mm^3). This indicates that exposure to EE + MEOS may reverse neurological deficits and reduce CNS scar formation after TBI in rats.

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PA - 31
TRANSGENIC MICE TO STUDY ROLES OF ASTROCYTES AFTER TRAUMATIC BRAIN INJURY
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Astrocytes produce a variety of cytokines and growth factors, and function in the CNS as mediators of immune and inflammatory responses that may contribute to neuroprotection and neurotoxicity; their roles are incompletely understood. Following an injury to the CNS, astrocytes respond by hypertrophy, up regulation of gene expression, and proliferation, a process termed 'reactive astrocytosis'. We are using transgenic mouse models to study the roles of astrocytes following traumatic brain injury (TBI) using a cortical contusion injury (CCI) device. The GFAP-Tk transgenic mouse allows for the selective ablation of reactive astrocytes by administering the pharmacologic agent ganciclovir (GCV) following TBI. In the GFAP-Tk model, transgenic (Tg) and nontransgenic (Ntg) mice were analyzed to determine the degree of tissue degeneration in 7 day survival animals following CCI. At 7 days, brain tissue in which reactive dividing astrocytes have been ablated following injury shows a decrease in ipsilateral cortical volume compared to Ntg, sham, and naive animals. To selectively knock out genes encoding specific molecules synthesized by reactive astrocytes in mice we have used the Cre-loxP system. In the GFAP-Cre-STAT3/loxP transgenic mouse, the STAT3 gene is deleted selectively from astrocytes. STAT3 is a transcription factor thought to play a role in the response to injury in a wide variety of cell types. In this transgenic model, the JAK-STAT signaling pathway is blocked by STAT3 deletion. Our preliminary studies in GFAP-Cre-STAT3/loxP mice show that reactive astrocytosis is attenuated in gray matter following brain injury. These transgenic models allow us to study astrocytes, both reactive and non-reactive, in the context of brain trauma.

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PA - 33
THE NUMERICAL RELATIONSHIP BETWEEN "DARK" NEURONS AND SURVIVING NEURONS FOLLOWING LATERAL FLUID PERCUSSION INJURY IN RATS: THE DIFFERENCE BETWEEN HIPPOCAMPUS AND CORTEX
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Introduction: "Dark" neuron is traditionally known as a typical morphological change of an injured neuron following traumatic brain injury (TBI). The "dark" neuron is considered to undergo necrosis, because the region where "dark" neuron appears coincides with the region where neuronal cell loss occurs. However, it remains unclear whether all "dark" neurons undergo necrosis and result in neuronal cell loss. In this study, to evaluate the relation between appearance of "dark" neurons and subsequent neuronal cell loss, we investigated the time-course of the number of both "dark" neurons and surviving neurons in the hippocampus and the cortex after lateral fluid percussion injury (FPI) in rats.

Methods: Adult male Sprague-Dawley rats ($n = 63$) weighing 300 to 400 g were used. Under general anesthesia, the rats were subjected to lateral fluid percussion injury at a moderate severity. The rats were euthanized and perfused immediately or at 15, 30, 60 min and 6, 24 h after FPI. The brains were embedded in paraffin. Two series of serial coronal sections (5-μm-thick) were taken every 50μ from bregma -2.8 mm to -4.2 mm. One series was stained with Nissl and another series was stained with acid fuchsin. Three adjacent Nissl-stained sections at the level of 3.8 mm posterior to bregma were prepared for cell counting. The specimens were scanned at a magnification of x200 within the identical area, using a microscope equipped with a digital camera system. In the hippocampus, neurons were counted in the pyramidal cell layer of the CA3a to CA3b. In the cortex, neurons were counted in two different regions respectively circumscribed with a rectangle measuring 0.47x 0.35 mm. One was the region apart from gliding contusion and the other was close to the contusion. The mean number of neurons obtained by the three adjacent sections was calculated in each group.

Results: In the hippocampus, "dark" neurons appeared in the CA3 subfield immediately after injury. The number of "dark" neurons reached a maximum level 15 minutes after injury (39.6 ± 31.2 cells), and disappeared after 24 hours of FPI. At the time point

of 24 hours after FPI, the number of surviving neurons (162.3 $\pm$ 27.9 cells) significantly decreased as compared with the number counted by sham operated rats (193.6 $\pm$ 25.7 cells). In the cortex, "dark" neurons emerged as early as in the hippocampus after FPI. The "dark" neurons appeared at the site of injury, especially in the cortical layer. In the cortical region surrounding gliding contusion, the number of surviving neurons after 24 hours of FPI (90.2 $\pm$ 34.8 cells) is reduced as compared with the number counted by sham operated rats (136.7 $\pm$ 12.9 cells). On the other hand, a region apart from contusion, there was no decrease in the number of neurons after 24 hours of FPI (controls: 153.1 $\pm$ 25.8 cells, after 24 hours: 157.2 $\pm$ 23.7 cells), while numerous "dark" neuron appeared in the acute phase.

Conclusion: In the hippocampus, most of "dark" neurons die after trauma. On the other hand, in the cortex apart from gliding contusion "dark" neurons survive 24 hours after trauma.

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ALTERATION OF MEMBRANE PHOSPHOLIPIDS IN THALAMUS FOLLOWING RAT FOCAL ISCHEMIA

M Kubota, M Nakane, JH Son, T Nakagomi, A Tamura, H Hisaki (*Tokyo, Japan*)

Middle cerebral artery occlusion (MCAO) in rats causes infarction in the ipsilateral cortex and caudate-putamen. In this model, morphological change in the ipsilateral thalamus was observed a few weeks after surgery. Such thalamic shrinkage remote from the infarction was considered to be slowly progressive damage. To evaluate early changes of membrane phospholipids in the thalamus, we studied on time-dependent changes of free fatty acid (FFA) levels after MCAO in rats. The male Sprague-Dawley rats were used and left MCAO was performed according to Tamura's method. After various durations of ischemia (1, 3, 6, 12 and 24 hours), animals were treated with microwaves, and then the ipsilateral cerebral cortex and thalamus were dissected for lipid analysis. The total lipids of both samples were extracted by Folch's method. For separation of FFA and phospatidyl ethanolamine (PE), we used Bond Elut (NH_2) column and thin-layer chromatography, respectively. We measured the extent of cell damage in the cortex by the level of PE, and evaluated the remote change in the thalamus by the FFA levels, particularly arachidonic acid (AA) and docosahexaenoic acid (DHA). There was an opposite correlation between the level of PE in the cortex and that of AA or DHA in the thalamus. The levels of AA and DHA in the thalamus increased already at 6 hours after MCAO in some animals. The remote change in the thalamus proved to depend on the extent of the cortical infarction, and to occur much earlier than we have known from histological examination.

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TOPOGRAPHY OF C-FOS IMMUNOREACTIVITY IN AN OVINE HEAD IMPACT MODEL.

JW Finnie, J Manavis, NR Jones, PC Blumbergs (*Adelaide, Australia*)

Cortical neuronal and glial c-fos immunoreactivity has been demonstrated in experimental and human traumatic brain injury (TBI). c-fos is one of the immediate early genes (IEGs) important in signal transduction linking environmental stimuli to the cellular genome. c-fos immunoreactivity was recorded and semi-quantitated using a grid system applied to standard coronal brain sections obtained from six impacted and two control sheep. Glial and neuronal c-fos immunoreactivity was present in the pericontusional (penumbra) region of contusions but absent in the core region (3 of 6 animals). Apart from these focal changes neuronal and glial c-fos immunoreactivity was present in a diffuse distribution (with involvement of up to a maximum of 49% of grid squares) with greater involvement in the cerebrum on the side of the impact. In the cerebellum the Bergmann glia showed prominent c-fos immunoreactivity whereas the Purkinje cells were consistently immunonegative. c-fos immunoreactivity varied in different regions of the brain (focal and diffuse patterns) in this ovine head impact model.

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ATP-DEPENDENT RELEASE OF SYNAPTIC TRANSMISSION FROM ADENOSINE-INDUCED PRESYNAPTIC INHIBITION FOLLOWING OXYGEN-GLUCOSE DEPRIVATIONS IN RAT HIPPOCAMPAL SLICES

Y Park, J Kim, J Kim, T Kwon (*Seoul, Republic of Korea*)

Spontaneous and transient recovery (TR) of synaptic transmission during terminal stage of oxygen-glucose deprivation (OGD) was investigated *in vitro* model. Simultaneous recording of orthodromically evoked pop-

ulation spikes (PS), excitatory postsynaptic potentials (EPSP) and antidromically evoked PS was performed prior to and during exposure of slices to OGD or hypoxic medium. Paired-pulse procedures were also conducted in the experiment. Appearance of TR was highly correlated with both glucose levels and functional status of antidromic PS during hypoxia/OGD. Hypoglycemia and status of moderate functional disturbance of membrane conduction was prone to development of TR. It was not elicited in normoglycemic hypoxia in which, through glycolytic pathway, ATP production to maintain neuronal integrity is not compromised. During TR, presynaptic inhibitory mechanism of paired-pulse facilitation of 2nd fEPSP slope was reversed but recurrent inhibitory mechanism of paired-pulse inhibition of 2nd orthodromic PS was restored. Neither attenuation of Ca++ release from intracellular store (heparin, dantrolene, and BAPTA-AM) nor protein kinase inhibition can suppress TR. Three pharmacological interventions (Na+/K+ ATPase inhibitor, Ca++-ATPase inhibitor, high [K+]e) to compromise neuronal conduction and eventually decay antidromic PS in normoglycemic hypoxic condition without depletion of ATP failed to reproduce TR. However, It was well reproduced after infusion of iodoacetic acid, an inhibitor of glycolytic production of ATP. These results suggest that presynaptic inhibition induced by increase of endogenous adenosine following ATP-deletion in OGD is released by further depletion of ATP.

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POST TBI HYPOXIA EXACERBATES NEUROLOGICAL DEFICIT AND IS REFLECTED BY INCREASED CSF PROTEIN MARKERS OF TISSUE DAMAGE

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Background: Hypoxia that occurs following traumatic brain injury (TBI) is known to exacerbate the extent of secondary brain damage contributing to additional neurological disability and mortality.

Aim: 1. To determine the effect of hypoxia following traumatic axonal injury in the rat on the brain content of the proteins; neuronal specific enolase (NSE), S100b; and glial fibrillary acidic protein (GFAP) and on neurological deficit. 2. To determine concentrations of NSE, S100b; and GFAP in patients with severe TBI with or without hypoxia.

Methods: Traumatic axonal injury (TAI) was induced on male adult SD rats using the acceleration/deceleration impact injury model. Rats were then either ventilated with 33%O_2 (normoxia) or 10%O_2 (hypoxia) for 30 min before being allowed to recover. Rats underwent sensorimotor tests before being killed at 4, 12 & 24 h after injury. The brain content of NSE, S100b; and GFAP was measured by ELISA. In parallel, CSF samples from patients with severe TBI were collected daily and analysed for total protein content and concentrations of NSE, S100b; and GFAP.

Results: Rats that underwent hypoxia with or without TAI were moderately hypoxic after 30 min of 10% O_2 (SaO_2 40–50%). The combination of TAI and hypoxia resulted in a greater sensorimotor deficit, as compared to rats with TAI or hypoxia alone. However, no change in the brain content of NSE, S100b; or GFAP was observed at 4, 12 or 24 h after injury. In contrast, preliminary data indicate that patients with post-TBI hypoxia ($SaO_2 < 90\%$) have a tendency for higher total protein concentration in their CSF. Furthermore, higher NSE, S100b and GFAP concentrations were also detected in human CSF after TBI and hypoxia.

Conclusion: Hypoxia may exacerbate neurological deficit following TBI by increasing neurodegeneration and possibly aggravating blood-brain barrier dysfunction, which is reflected by concentrations of specific proteins in the CSF.

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PHYSIOLOGIC LIMITS OF TOLERABLE HYPOXIA AND HEMORRHAGE IN THE SETTING OF TRAUMATIC BRAIN INJURY

E Lee, R Bauman, AJ Williams, J Blanchard, GSF Ling, FC Tortella, ML Rolli (*Silver Spring, USA*)

Traumatic brain injury and hemorrhagic shock are the leading causes of combat-related casualties. While it is well established that severe levels of hypoxia and hemorrhage significantly worsen outcome in traumatic brain injury, the relationship of less severe insults has not been studied. This work attempts to identify the limits to which the injured brain can tolerate secondary hemorrhage and hypoxia during the first hour after injury. Experimental rats were anesthetized, intubated, and ventilated. A right parasagittal burr hole was used to deliver a moderate (2.75–3.00 atm) fluid percussion injury (FPI). After FPI, rats were subjected to one of three treatments: hypoxia, hemorrhage or neither hy-

poxia nor hemorrhage (controls). Three levels of hypoxia ($pO_2 = 100$, 80, 60 mmHg) and five levels of hemorrhage (10, 15, 20, 25, and 30% total blood volume) were examined. Blood pressure, temperature, respirations, peak inspiratory pressure, arterial blood gases and hematocrit were monitored before FPI and every 15 min for 1 hour after injury. Animals were sacrificed at 72 h and their brains histopathologically studied (H&E). Subjects with mild levels of hemorrhage (10–15% total blood volume) had mild reductions in brain injury volume. More severe hemorrhage resulted in increases in brain injury volume. Mild levels of hypoxia ($pO_2 = 100$ and 80 mmHg) did not significantly alter brain injury volume while a pO_2 of 60 mmHg dramatically increased rat mortality during the first hour after brain injury.

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PENETRATING BRAIN INJURY IN THE RAT: IV PERSISTENCE OF CORTICAL SPREADING DEPRESSION

JA Hartings, AJ Williams, RA Bauman, JB Long, FC Tortella (*Silver Spring, USA*)

Spreading depression (SD) is a principal mechanism of pathogenesis and penumbral deterioration in focal cerebral ischemia and has been postulated to play a similar role in traumatic brain injury (TBI). Here we characterize the incidence of SD in a new rat model of penetrating brain injury (PBI) and compare SD results and pathology to experimental contusive brain injury (CBI). Epidural Ag/AgCl electrodes were implanted over the injured hemisphere for continuous 72 hr monitoring of DC potential in freely behaving animals. PBI was induced by inserting an inflatable metal probe into the right hemisphere and then rapidly inflating the probe ($<$ 10 msec) to 10% or 15% total hemispheric volume, mimicking the temporary cavity caused by energy dissipation and yaw of a penetrating bullet round. CBI was induced by lateral fluid percussion at 2.0–2.7 atm. With 10% PBI, SD began $<$ 2 hr post-injury and consisted of a mean 14 $\pm$ 7 SDs occurring through 72 hr. With 15% PBI, 2/5 rats exhibited 'SD status' with continuous SD at 5–11 events/hr for $\sim$ 7 hr and a mean 68 events within 48 hr; the remaining rats exhibited profiles similar to 10% injury. In CBI, SD was first observed 3–12 hr post-injury and consisted of a mean 6.4 $\pm$ 6.8 events recurring through 72 hr. PBI pathology included extensive intracranial hemorrhage and prominent lesion formation in the frontal cortex and striatum. By comparison, cellular injury and hemorrhage was much less extensive in CBI. Results suggest that SD is highly relevant to acute and delayed pathology in multiple types of TBI, particularly following severe focal insults.

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PENETRATING BRAIN INJURY IN THE RAT: V. CHARACTERISATION OF ELECTROENC–EPHALOGRAPHIC ABNORMALITIES

FC Tortella, AJ Williams, X Chun, M Lu, JA Hartings (*Silver Spring, USA*)

Electroencephalographic (EEG) recordings provide a means to assess brain function and monitor for 'subclinical' seizures in traumatic brain injury patients. Here we characterize the cortical EEG pathology associated with a new model of penetrating brain injury (PBI) in the rat. Screw electrodes were implanted epidurally over both hemispheres and continuous recordings were made for 72 hr post-PBI in freely behaving animals. PBI was induced by frontal insertion of an inflatable metal probe into the right hemisphere followed by rapid inflation ($<$ 10 msec) of the probe to 15% total hemispheric volume, mimicking the temporary cavity caused by energy dissipation of a penetrating bullet round. PBI induced an immediate decrease in high frequency (20–50 Hz) EEG power and an enhancement of delta (0.5–4.0 Hz) activity in the injured hemisphere. Delta waves occurred in the form of arrhythmic polymorphic delta activity and isolated sharp and slow waves. In addition, per iodic lateralized epileptiform discharges (PLEDs) occurred in 2/8 rats and also consisted of sharp/slow waves on a depressed background. Pathologic delta waveforms were significantly attenuated by 72 hr post-injury. In one animal, unilateral seizures were observed beginning 8 hr post-injury and consisted of recurrent spike-and-wave or polyspike-and-wave discharges at variable 1–3 Hz frequencies. Seizures persisted $\sim$ 2 hr and thus constituted a 'status epilepticus' condition. These data hold promise for the clinical relevance of the rat PBI model in replicating features of electrophysiological dysfunction following clinical brain lesions, and suggest the EEG as an informative endpoint in evaluating recovery and neuroprotection treatment efficacies in this model.

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HYPERCARBIA PROVIDES NEUROPROTECTION IN FLUID PERCUSSION INJURED RATS

ML Rolli, E Lee, AJ Williams, J Blanchard, GSF Ling, FC Tortella (*Maryland, USA*)

Traumatic brain injury is a leading cause of death and disability among young adults (18–45 years old) and a leading combat casualty. On the battlefield prehospital resuscitation resources are especially limited. This project examines the effects of hypercarbia on brain injury following fluid percussion injury in the rat. Anesthetized rats underwent tracheostomy, arterial cannulation, and moderate cerebral fluid percussion injury (2.75–3.00 atm). For one hour following fluid percussion injury the animal's $PaCO_2$ was altered by adjusting minute ventilation, and maintained at a predetermined level for one hour. Each group was controlled for temperature and pO_2. The rats were euthanized and perfused at 72 hours and their brains harvested for histopathology (H&E). Brain injury areas were demarcated and total volumes interpolated. Mean injury volumes ($\pm$ SD) were $42.39 + 12.17\ mm^3$, $25.27 + 12.62\ mm^3$, $24.45 + 8.32\ mm^3$, $16.46 \pm 21.73\ mm^3$, in animals with mean pCO_2 values of < 40 (normal controls), 45, 55, and 60 mmHg, respectively. Statistical analysis revealed significant differences ($P < 0.05$) in comparing brain injury volume of hypercarbic animals to normocarbic controls. In conclusion, rats sustaining a moderate fluid percussion injury have a significant decrease in injury volume when treated with one-hour of post-traumatic hypercarbia in the setting of normal oxygenation compared to rats with normal pCO_2 values. These findings warrant further investigation into the mechanisms behind the neuroprotective effects of post-traumatic hypercarbia and the pathophysiological sequelae of its use in the setting of traumatic brain injury.

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PERFLUOROCARBONS IMPROVE BRAIN OXYGENATION AND REDUCE ISCHEMIC DAMAGE IN ACUTE SUBDURAL HEMATOMA IN THE RAT

TH Kwon, D Sun, WP Daugherty, BD Spiess, R Bullock (*Seoul, Korea; Virginia USA*)

Objective. This study was conducted to investigate the effect of perfluorocarbons (PFCs) in improving brain oxygenation and reducing ischemic brain damage in an acute subdural hematoma (ASDH) model in rats.

Methods. Animals were allocated to four groups: 1) control, with ASDH induction; 2) 30-PFC group, with ASDH induction and PFC infusion; 3) 100-O_2 group, with ASDH induction and 100% O_2 administration; and 4) 100-PFC group, with ASDH induction and PFC plus 100% O_2 treatment. Ten minutes after ASDH induction, a single dose of PFC (2.7 g/kg) was infused, 100% O_2 was administered simultaneously in hyperoxic groups. Four hours after ASDH induction, half of the rats were perfused for volumetric study to assess the extent of ischemic brain damage. The brain was dissected; coronal sections were cut and stained. Ischemic areas were measured in the eight predetermined stereotactic planes. Another half of the rats were used to measure brain tissue oxygen tension ($ptiO_2$) using a Licox CC1.r probe.

Results. The volume of ischemic damage were as follows: $162.4 \pm 7.6\ mm^3$ in control, $165.3 \pm 11.3\ mm^3$ in 30-PFC group, $153.4 \pm 17.3\ mm^3$ in 100 O_2 group, and $95.9 \pm 12.8\ mm^3$ in 100-PFC group (41% reduction compared to control, $p = 0.002$). The baseline $ptiO_2$ values were around 20 mmHg, and after ASDH induction, it rapidly dropped to 1–2 mmHg and remained. Treatments with either PFC or hyperoxia improved $ptiO_2$, with final values of 5.14 mmHg and 7.02 mmHg respectively. PFC with hyperoxia improved $ptiO_2$ the most, with final $ptiO_2$ of 15.16 mmHg.

Conclusions. The current study demonstrates that PFC infusion along with hyperoxia can significantly improve brain oxygenation and reduce ischemic brain damage in ASDH.

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INHALED NITRIC OXIDE INCREASES CEREBRAL BLOOD FLOW AND REDUCES ICP AFTER EXPERIMENTAL TBI IN MICE

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Ischemia is one of the major mechanisms leading to secondary brain damage after TBI. Direct treatment is not available yet. We hypothesise that inhaled NO (iNO) reaches the brain parenchyma, reduces pericontusional ischemia and secondary brain damage after TBI by a novel, yet undescribed mechanism.

C57/Bl6 mice were anaesthetised, intubated, and mechanically ventilated. CBF was recorded over both hemispheres by laser Doppler fluxmetry, ICP by a microcatheter implanted into the right forebrain. CCI was performed over the right parietal cortex. 10 min after

TBI animals received 50 ppm iNO and ICP, contra- and ipsilateral CBF were recorded for 90 min. In naive animals iNO did not influence the recorded parameters. After TBI iNO reduced ICP from 29.4+/− 10.8 mmHg in controls ($n = 7$, 80 min; mean+/− SD) to 12.8 +/− 6.4 mmHg (− 55%; $p < 0.01$). In controls CBF decreased to 46 +/− 31% of baseline 90 min after trauma. In iNO treated mice CBF remained at baseline levels (93 +/− 40%; $p < 0.04$). In the contralateral hemisphere of controls CBF (representing global cerebral perfusion) declined to 31 +/− 30% 90 min after TBI while iNO prevented this CBF decrease almost completely (91 +/− 26%; $p < 0.01$). Our current experiments demonstrate that, otherwise as previously expected, inhaled NO does not only act on pulmonary vessels, but also on the cerebral microcirculation. There it is released from its carrier in the blood stream, haemoglobin, by a pO_2-dependent mechanism thereby increasing blood flow selectively in ischemic brain tissue. By this mode of action iNO prevents post-traumatic ischemia in tissue areas at risk and reduces secondary brain damage and pathological ICP increase.

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ATTENUATED ACTIVATION OF NF-KAPPAB IS ASSOCIATED WITH ENHANCED RECOVERY AFTER TRAUMATIC BRAIN INJURY IN MICE

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Transcription factor NF-kappaB is activated in various neuropathological conditions, thereby affecting cellular death and survival. We previously reported that 1–8 days after closed head injury (CHI) NF-kappaB is activated (5-12-fold). Attenuation of NF-kappaB by melatonin improved outcome (Beni et al. FASEB J 2004). In the present study we confirm that inhibition of delayed NF-kappaB activation improves recovery in two other cases of neuroprotection that are under investigation in our laboratory: 1) neuroprotection by the endocannabinoid 2-arachidonoyl-glycerol (2-AG) at 24h after CHI and 2) unexpecteded better neurobehavioral recovery of superoxide-dismutase deficient (SOD1(−/−)) mice at 7-14d after CHI. We also demonstrate the role of CB1 in 2-AG-induced neuroprotection. Materials and Methods: CB1(−/−) or SOD1(−/−) mice, along with their respective WT controls were subjected to CHI using a weight-drop device. 2-AG (5 mg/kg) was injected 1h after injury to WT and CB1(−/−) mice and they were sacrificed 24h later. SOD1(−/−) mice and their WT controls were sacrificed 14d after CHI. Nuclear extracts from the site of injury were analyzed for NF-kappaB transactivation by EMSA. Results: CHI-induced 3–4 fold increase of NF-kappaB transactivation at 24h after CHI in the CB1(−/−) and WT mice, yet, 2-AG abolished it only in the WT mice ($p < 0.001$), in which improved motor function and reduced edema ($p < 0.01$) were also noted. SOD1(−/−) mice demonstrated significantly better neurological scores and failed to induce NF-kappaB transactivation, which was robust in WT mice ($p < 0.005$). These results suggest that delayed NF-kappaB activation after CHI triggers death-promoting signals and its inhibition leads to better outcome.

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POTENTIAL CYTOPROTECTIVE EFFECT OF POSTTRAUMATIC VEGF EXPRESSION IN THE RAT BRAIN.

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Angiogenesis following traumatic brain injuries (TBI) may be of importance for post-traumatic reparative processes but also for the development of secondary edema and inflammation. Vascular Endothelial Growth Factor (VEGF) is a major regulator of endothelial cell proliferation, angiogenesis and vascular permeability and therefore of potential interest in post-traumatic events in CNS. We did therefore study the expression of VEGF and the VEGF receptors in experimental brain contusions in the rat and could show that VEGF mRNA had a maximal expression at about 4–6 days after trauma. No peak expression could be shown for the VEGF receptors that were expressed from 1 to 6 days after injury. Since the peak VEGF expression did correlate in time and space with previously reported secondary edema and inflammation in the same injury model we performed experiments where the VEGF receptor 2 was blocked with the specific VEGFR2 blocker SU5416. Injury development was evaluated with TUNEL, Fluoro-Jade and by measurements of S100beta serum levels. Preliminary results show that S100beta was elevated early after injury in SU5416 treated animals compared to control. I the later phase no differences could be shown. These findings indicate that early VEGF expression might be of importance for cell protection after injury. In conclusion we show that TBI lead to an upregulation of VEGF and the VEGF receptors in and around the lesion

and that this upregulation might actually be cytoprotective. The data provide important knowledge about the importance of posttraumatic VEGF expression and do points towards new potential targets for therapeutic interventions after TBI.

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A SUBSTANCE P ANTAGONIST INCREASES AQUAPORIN-4 EXPRESSION AND REDUCES OEDEMA AFTER TRAUMATIC BRAIN INJURY

C Howard, J Donkin, M Ghabriel, P Blumbergs, R Vink (*Adelaide, Australia*)

Traumatic brain injury (TBI) is known to result in cerebral oedema although the mechanisms associated with its formation are unclear. Recent studies have suggested that the aquaporin-4 (AQP-4) water channel may play a role in the formation and resolution of oedema [1]. Our own studies have shown that the substance P antagonist n-acetyl-tryptophan (NAT) attenuates oedema formation after TBI. Accordingly, the present study examined the effects on NAT on AQP-4 expression following TBI. Male Sprague-Dawley rats were injured by impact-acceleration injury and administered 4.93 mg/kg NAT or equal volume saline at 30 min post-trauma. Animals were then killed at 5 h after injury and AQP-4 immunoreactivity around randomly selected cortical blood vessels visualized using electron microscopy. In vehicle treated animals, AQP-4 expression was significantly decreased at 5 h after injury compared with shams. In contrast, animals treated with NAT showed a profound upregulation of AQP-4 channels. This upregulation in AQP-4 coincided with an attenuation of oedema. We conclude that NAT attenuates oedema formation after TBI by increasing AQP-4 expression.

Reference

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PA - 58
AQUAPORINS IN THE CENTRAL NERVOUS SYSTEM

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Aquaporins are a recently characterised class of water channel proteins that are widely distributed in nature. There is evidence that they play a major role in water movements in the normal state and in various disease states, including cerebral oedema. In the normal rat brain, aquaporin 4 (AQP4) is reportedly the most prevalent aquaporin and is located predominantly on astrocytic perivascular endfeet, as well as on ependymal cells. AQP9 has also been reported on astrocytic processes and cell bodies in the normal mouse brain, although only in ependymal cells and tanycytes in the normal rat brain. Until now, AQP1 has been reported exclusively on the luminal surface of choroid plexus epithelial cells in the rat brain. We have recently conducted some preliminary immunohistochemical studies in the normal rat brain, using antigen retrieval techniques, and have observed AQP1 immunoreactivity not only in choroid epithelium, but also in the meninges, ependyma, occasionally on astrocytic perivascular endfeet, and in some cells and fibres whose identity requires confirmation. AQP4 immunoreactivity was restricted to astrocytic perivascular endfeet, as previously reported. There is evidence that aquaporins, particularly AQP4 in rodents and AQP1 in rodents and humans, play an important role in brain water transport in both vasogenic and cellular cerebral oedema, and may exhibit up- or down-regulation. The previously unreported finding of AQP1 in the rat brain will form the basis of further studies of the role of AQP1 in cerebral oedema.

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INHIBITION OF NEUROGENIC INFLAMMATION ATTENUATES THE INFLAMMATORY RESPONSE FOLLOWING TRAUMATIC BRAIN INJURY IN RATS

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A profound inflammatory response, characterised by the release of cytokines, including interleukin-6 (IL-6), is initiated following traumatic brain injury (TBI). We have shown that neurogenic inflammation plays a role in the development of cerebral oedema following TBI [1]. The present study examines whether inhibi-

tion of neurogenic inflammation, using either capsaicin or an NK1 antagonist, will influence the post-traumatic inflammatory response. TBI was induced in rats using the weight drop model. One group of animals were pre-treated with capsaicin 14 days prior to injury or sham-injury, whilst controls received capsaicin vehicle. A second group received an NK1 antagonist (N-acetyl tryptophan; 1:mol/kg) 30 min after injury or sham injury, whilst controls received drug vehicle. Brain tissues and serum samples were collected 6 hours after injury. Serum levels of IL-6 were determined by ELISA, whilst IL-6 mRNA expression in brain was determined by RT-PCR. In sham-injured animals constitutive IL-6 mRNA expression was observed, whilst serum IL-6 was non-detectable. Following TBI, non-treated animals showed elevated levels of IL-6 mRNA expression, whilst 30% of animals exhibited moderate serum IL-6 levels. However, in those animals treated with capsaicin or an NK1 antagonist, no increase in IL-6 mRNA expression was observed following injury. Similarly, serum IL-6 was not detectable. In conclusion, inhibiting neurogenic inflammation, either through pre-treatment with capsaicin, or post-injury treatment with an NK1 antagonist, significantly attenuates the inflammatory reaction associated with TBI.

Reference

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CORTICAL PERIVASCULAR AXONAL INJURY AND SUBSTANCE P IMMUNOREACTIVITY IN HUMAN TRAUMATIC BRAIN INJURY.

A Zacest, R Vink, J Manavis, PC Blumbergs (*Adelaide, Australia*)

The mechanisms associated with the development of cerebral oedema following traumatic brain injury (TBI) remain unclear. Recent experimental evidence suggests that neuropeptides, and in particular substance P (SP), are released following TBI and may play a significant role in the aetiology of cerebral oedema and the development of neurological deficits [1]. Whether SP may play a role in clinical TBI remains unknown. In the present study, changes in the immunoreactivity of SP were examined in cortical contusions and cerebral cortex underlying subdural haematomas in 29 post-mortem cases of human fatal TBI. Compared to 10 post-mortem cases with normal neuropathologic examinations (controls), increased SP immunoreactivity was observed in all TBI cases in perivascular axons and some pyramidal neurones and astrocytes early following injury. Perivascular axonal injury was observed by amyloid precursor protein (APP) immunostaining in the cortex of 11/13 trauma cases and could be important in deafferentation of the cortical microvasculature following injury. Co-localisation of SP and APP in perivascular cortical nerve fibres suggests perivascular axonal injury could be an important mechanism of release of this neuropeptide. The reduction in perivascular and neuronal SP immunoreactivity over time observed in hypoxic and oedematous human cortex suggests that hypoxia-ischaemia may also contribute to its release. The abundant innervation of the human cerebral microvasculature with SP fibres and terminals, together with the changes observed following TBI in perivascular axons, cortical neurones and astrocytes, suggest a potentially important role for substance P in neurogenic inflammation following human TBI.

Reference

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PA - 61
BLOOD-BRAIN BARRIER ULTRASTRUCTURAL CHANGES IN IMPACT ACCELERATION HEAD TRAUMA

M N Ghabriel, C Zhu, A Q Imran, P C Blumbergs, P L Reilly (*Adelaide, Australia*)

Brain swelling and increased intracranial pressure are critical problems in closed head trauma. In a rat impact acceleration model of diffuse traumatic brain injury oedema appears to be the principal factor in brain swelling[1]. Previous studies of this model have addressed later effects on the blood-brain barrier (BBB), and in the present study we aimed to investigate BBB ultrastructural changes in the short-term, 1–12 h postinjury. Isoflurane-anaesthetised adult Sprague-Dawley male rats (380–450 g) were injured under ethical approval. Severe and moderate injury was induced by a 450 g weight, dropped from 2 m or 1.5 m respectively. At 1, 3, 6 and 12 h after trauma animals were perfused

with 4% paraformaldehyde and 2% glutaraldehyde fixative and the brains processed for electron microscopy. A continuum of changes was seen at 1 h, 3 h and 6 h. Endothelial cells of brain vessels showed numerous cytoplasmic vesicles and large vacuoles, luminal membrane projections and microvilli, and opened tight junctions at 1, 3 and 6 h. At 6 h, swelling of astrocytic end-feet and a slit-like perivascular space appeared, and the astrocytic swelling became maximal at 12 h, but to the contrary endothelial cells now appeared normal. In conclusion, in this model ultrastructural changes occur in endothelial cells 1-6 h postinjury, while astrocytic (cytotoxic) swelling becomes increasingly apparent from 6–12 h.

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ALTERATIONS IN TIGHT JUNCTION PROTEINS FOLLOWING BLOOD-BRAIN BARRIER BREAKDOWN IN BRAIN INJURY
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The tight junctions between cerebral endothelial cells limit the passage of substances from the blood into the brain and in the reverse direction and are an essential structural component of the blood-brain barrier (BBB). Recent studies of noncerebral epithelial cells demonstrate that tight junctions are formed of the three integral proteins – claudins, occludin and junctional adhesion molecule and several accessory proteins that are necessary for structural support. The importance of occludin, claudins-3 and 5 and zonula occludens (ZO)-1 in maintenance of cerebral endothelial tight junction integrity was assessed by studies of the expression of these proteins in the rat cortical cold-injury model over a period of 12 hrs to 6 days post-injury by western blotting and single labeling immunohistochemisty. Western blot analysis showed a significant decrease in signal for these proteins at 2 and 4 days post-injury during the period when BBB breakdown is known to occur. Single labeling demonstrated loss of immunoreactivity for these proteins in lesion vessels at 2 and 4 days. In order to determine if the vascular segments showing loss of immunoreactivity for these proteins were also segments with BBB breakdown, dual labeling for these proteins and fibronectin was done. These results were analysed by laser scanning confocal microscopy which demonstrated that only lesion vessels showing BBB breakdown to fibronectin showed loss of occludin, and claudin-3 while there was reduced localization of claudin-5. These studies underlie the importance of these endothelial tight junction proteins in maintenance of the BBB in steady states.

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MEASUREMENT OF PRIMARY & SECONDARY DAMAGE FOLLOWING FOCAL TRAUMATIC BRAIN INJURY
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Brain damage following head injury involves initial impact damage (primary damage) & subsequent secondary damage in which the damaged area progressively enlarges. One factor promoting secondary damage may be leakage of proteins from disrupted vessels. In adult mice with standardised head injuries, primary lesion volumes, regions of cellular damage and plasma protein spread were calculated from area measurements in 5 μm serial sections 50 μm apart through entire brains. All experiments were conducted with approval of local Ethics Committees and in accordance with NH&MRC guidelines. Primary lesion size varied from 0.19 mm^3 to 1.96 mm^3. Plasma proteins shortly after injury occupied much larger volumes (17.30 mm^3 to 19.47 mm^3) with a significant positive correlation between volume of plasma proteins and primary lesion size. There was no significant difference between 24 h post-injury compared to $<$ 20 min post-injury. Plasma protein-containing volume was not symmetrical around the primary lesion volume and there was poor correlation between areas of plasma protein and areas of primary lesion when measured in individual sections from single brains or sections from many brains. Total volume of secondary cellular damage identified using Nissl stain also correlated with the primary lesion size. The finding of no change in plasma protein distribution between the time of injury and 24h later, suggests evidence indicating significant blood-brain barrier disruption following head injury needs re-evaluation. In addition, the effectiveness of any potential treatment can be better assessed if total volume of secondary damage is compared to total volume of primary damage.

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BLOOD-SPINAL CORD BARRIER PERMEABILITY FOLLOWING INJURY IN NEONATAL MONODELPHIS DOMESTICA

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Spinal cord injury (SCI) compromises blood-spinal cord barrier function with infiltration of blood constituents into perivascular spaces. All experiments were conducted following NH&MRC guidelines and with approval of University of Melbourne, Animal Experimentation Ethics Committee. At postnatal day (P) 7–8 or 14–15, Monodelphis domestica pups were anaesthetized with halothane while attached to anaesthetized mother, given complete thoracic spinal cord transection, and allowed to recover for up to 2 days. Each pup was given an intraperitoneal injection of chicken-egg albumin (Sigma®, 750 μg/g animal in saline) or biotin dextran amine (3000 MW, Molecular Probes, 700 μg/g animal in saline) 1 h or 30 min prior to collection respectively. Pups were killed at intervals following SCI, blood and CSF samples taken, and tissue fixed by immersion in Bouin's fixative. There was cellular uptake of exogenous and endogenous protein, and of biotin immediately following injury with co-localization of all three markers. There was a substantial decrease in cellular staining for exogenous albumin from 2–3 hours post injury, and by three hours no intact labelled cells were detected. The number of cells labelled positively for endogenous protein remained unchanged from 0–3 hours, but these cells became pyknotic by 3 hours. At 24–48 hours post-injury, only monocytic cells were positive for plasma proteins. The period for which the blood-spinal cord barrier is compromised may provide a "window" for use of pharmacological agents not normally able to cross the barrier.

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EFFECT OF NEUROPROTECTIVE AGENTS AGAINST COMBINED MECHANICAL AND ISCHEMIC INJURY IN VITRO.

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Traumatic brain injury leads to cell damage by direct mechanical disruption of the brain and by secondary insults such as ischemia. We utilized an *in vitro* model of stretch-induced injury to investigate the effects of mechanical and combined mechanical/ischemic insults to cultured mouse cortical cells. Stretch injury alone increased uptake of the dye, propidium iodide (PrI), 15 min after injury, suggesting cellular membrane damage. PrI uptake was dependent on the magnitude of stretch and decreased with time post-injury up to 48 hr. Stretch injury also caused a significant reduction in the amount of MAP2-positive neurons. Exposure of cultures to ischemic conditions for 24 hr following stretch produced a level of PrI uptake similar to stretch injury alone, however it was maintained through 48 hr post-injury. In addition, the combined insult paradigm caused a much greater reduction in neurons compared to stretch alone. Next, we tested the effects of the potential neuroprotective agents, 7-nitroindazole (7-NINA), lubeluzole, and superoxide dismutase (SOD) on injured cells. Post-treatment (15 min after stretch) with these agents provided no protection against combined insults, measured by the amount of MAP2-positive neurons. Pre-treatment with lubeluzole (100 nM) and SOD provided modest protection against stretch injury alone, while pre-treatment with 7-NINA (1 μM and 10 μM) and SOD provided modest protection in the combined insult paradigm. These results suggest that neurodegenerative mechanisms caused by primary mechanical damage and secondary ischemia to cortical neurons are cumulative and complex, and may not be amenable to treatment with the pharmacotherapy used in this study.

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THE INFLUENCE OF EXPERIMENTAL SPINAL CORD INJURY ON CARBAMAZEPINE PHARMACOKINETICS

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The purpose of the present work was to study whether spinal cord injury (SCI) alters carbamazepine pharmacokinetic. Male Albino rabbits were subjected to SCI at the T8 level by knife severance method and received a single oral dose of carbamazepine dose (20 mg/kg) 24 hours after injury. Pharmacokinetic parameters including maximal concentration (C max), time to reach maximal concentration (T max), half-life, and the area under the concentration against time curve (AUC) were determined at selected time over a period of 96 hours. C max appeared at 2.8 hours (T max) after administration in sham-lesioned control group with a

concentration of 2.3 μg/ml, whereas in SCI group it appeared at 4.4. hours with a concentration of 2.7 μg/ml. In SCI group, AUC and half-life were increased from 29.1 μg/ml.hr to 38.7 μgr/ml.hr and from 7.7 hours to 14.1 hours compared with sham-lesioned control group, respectively. Statistical analysis of data showed that SCI didn't induce significant changes in carbamazepine pharmacokinetics. It seems that SCI-induced pharmacokinetic alternations are complex and dependent on the sum of SCI effects on absorption, distribution and elimination. Systemic studies on SCI-induced alternations are required to provide information leading to a rational dosing regimen of carbamazepine design for SCI patients.

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CHARACTERIZATION OF THE FUNCTIONAL, MOLECULAR AND CELLULAR CHANGES IN SPINAL CORD AXONS IN THE ADULT LONG EVANS SHAKER DYSMYELINATION MUTANT RAT: IMPLICATIONS FOR THE ENHANCED UNDERSTANDING OF DEMYELINATING NEUROLOGICAL DISORDERS

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Long Evans shaker (LES) rats have a spontaneous mutation of the gene encoding myelin basic protein resulting in severe dysmyelination of the central nervous system (CNS). To date, the functional and molecular changes in CNS white matter in the LES rat are not well understood. Understanding of these issues in the LES mutant could provide critical insights for neurological disorders characterized by demyelination or dysmyelination. In this study, we used *in vivo* somatosensory evoked potential (SSEP) and *in vitro* sucrose gap electrophysiology in isolated dorsal columns, confocal immunohistochemistry, western blotting, and real time PCR to examine the electrophysiological, molecular and cellular changes in spinal cord white matter in LES rats. Confocal immunohistochemistry revealed that dysmyelination is associated with dispersed labeling of both Kv1.1 and Kv1.2 K+ channel subunits along the length of LES spinal cord axons. Western blotting and real- time PCR provided evidence that the dispersed localization of Kv1.1 and Kv1.2 subunits is not associated with changes in protein and mRNA expression. *In vitro* sucrose gap and *in vivo* SSEP electrophysiology revealed significant changes in compound action potentials (CAPs) in LES spinal cord compared to the wild type rats, including; attenuation of amplitude and conduction velocity, high frequency conduction failure, a shift in the stimulus response curve, and enhanced sensitivity to K+ channel blockers 4-aminopyridine and dendrotoxin-I. Our results suggest in LES rats clarify some of the key molecular, cellular and functional consequences of dysmyelination and the effects on myelin-axon interactions.

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HISTOPATHOLOGICAL AND BEHAVIORAL CHARACTERIZATION OF A NOVEL, GRADED CERVICAL CONTUSION INJURY MODEL

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Contusive trauma to the cervical spinal cord is one of the most prevalent injury types in spinal cord injured (SCI) patients, accounting for 28% of all injuries. In contrast, the use of experimental cervical contusion injury models in research is limited to only a few studies. When one takes into account that both (1) the different ways of injurying the spinal cord (compression, contusion, transection) induce very different processes of tissue damage and (2) the architecture of the spinal cord is not uniform, the need arises then to use a model that is more clinically applicable to human SCI. Therefore, in the current study we have developed a rat model of contusive, cervical SCI using the Electronic Spinal Cord Injury Device (ESCID) developed at Ohio State University (OSU) to induce injury by spinal cord displacement. The contusion injury was performed at cervical level C5, using the circular flap tip of the impactor (made of methylmethacralate, 4 mm diameter) to transduce a force of "3 Kdyn (as indicated by the force transducer). This results in slight dimpling of the dura and provides a consistent starting point from which displacement was measured. We used the device to perform mild, moderate and severe injuries (0.80, 0.95 and 1.1 mm displacements, respectively) with a single, brief displacement of $<$ 20 msec upon the exposed dorsal surface of the C5 cervical spinal cord. Characterization of the model involved the analysis of the temporal histopathological progression of the injury over 9 weeks using chemical stains for white and gray matter integrity and immunohistochemistry to examine cellular changes and physiological responses within the injured spinal cord. Accompanying the histological analysis was a comprehensive determination of the

behavioral functionality of the animals using a battery of motor tests (BBB score, hanging/climbing/gripping tests for upper body strength, inverted plane, gridwalk and footprint analysis). Presentation of this characterized, novel model is hoped to enable and encourage future use of this paradigm in the design of therapeutic strategies for human SCI (Supported by The Miami Project, The Buoniconti Fund and NINDS).

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EVENING PRIMROSE OIL INCREASED RATE OF FUNCTIONAL MUSCLE RECOVERY AFTER NERVE CRUSH INJURY IN THE RAT

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This study investigated the effects of treatment with Evening Primrose Oil (EPO) on the rate of muscle recovery after a Sciatic nerve crush injury in the rat. Adult Sprague-Dawley rats were divided into three groups: (a) Control 1 nerve-crushed group, $n = 8$ (b) Control 2 nerve-crushed group, $n = 8$ and (c) Experimental nerve-crushed group (fed with 6000 mg/day EPO), $n = 12$. All rats were observed from the day of nerve crush until they gained full recovery of the hindlimb except for the Control 2 rats which were observed until the Experimental group achieved full recovery. Recovery was assessed using the toe-spreading reflex with full recovery indicated by Degree 4. It was found that there is a significant reduction ($p < 0.001$, Student's t test) of 10 days in the onset of recovery in the EPO-treated group compared to Control 1. EPO treatment significantly reduced the time taken for full muscle recovery by 14.25 days compared to Control 1 ($p < 0.001$, Student's t test). It was also found that at the time the rats of the Experimental group had fully recovered, the Control 2 rats were still at a mean score of Degree 1. EPO treatment also reduced the duration of muscle recovery (Degree 1-Degree 4) by 4 days compared to Control 1 and this reduction is significant ($p < 0.001$, Student's t test). The above results indicate that 6000 mg/day EPO caused a significant increase in the rate of functional muscle recovery after a nerve crush injury in the rat.