Supplementary Material

Pathological Markers of Alzheimer's Disease and Related Dementia in the Rhesus Macaque Amygdala

Immunohistochemistry protocol

The immunohistochemistry used a well-established protocol [1,2]. Free- floating sections (30 μm) of five different levels of the monkey amygdala with entorhinal cortex were washed (4 times, 15 min each) in 0.02 M potassium-phosphate-buffered saline (KPBS). Endogenous peroxidase was blocked with 0.6% H_2O_2 solution in KPBS for 30 min, followed by another wash (4 times, 15 min each) then a blocking of secondary antibody non-specific sites with 2% normal goat serum (NGS, Jackson Immunoresearch Laboratories, West Grove, PA, USA) in 0.4% Triton X-100 for 1 h. After blocking, sections were incubated either with anti-pTau mouse monoclonal antibody (ATB, BioLegend, San Diego, CA, USA) diluted 1:500 or with the purified anti-TDP-43 rabbit polyclonal antibody (cat# 10782-2-AP, Proteintech, Rosemont, IL, USA) diluted 1:1200, both in 2% NGS for 48 h at 4°C. After KPBS washes, sections were incubated respectively either with the Biotin-SP AffiniPure Goat Anti-mouse or Anti-rabbit IgG (H+L, Jackson Immunoresearch Laboratories) diluted 1:200 in 0.4% Triton X-100 for 1 h. After KPBS washes, sections were incubated for 1 h with avidin-biotin-HRP complex (ABC Standard Kit PK-4000, Vector Laboratories, Burlingame, CA, USA) diluted 1:100 in 0.4% Triton X-100. After KPBS washes, the chromogenic detection was attained by incubating the sections for 2 min with ImmPACT DAB Substrate for Peroxidase (Vector Laboratories), diluted according to the manufacturer's instructions. Sections were mounted on Superfrost Plus slides, dried overnight in a desiccator, dehydrated using a graded series of ethanol and xylenes, and coverslipped with DPX mounting medium (Electron Microscopy Sciences, Hatfield, PA, USA).

Paraffin-embedded human amygdala sections (10 μm) were obtained from the OHSU Pathology Core. The subsequent immunohistochemistry was similar to that reported for the rhesus macaque except that the paraffin wax was first removed, using xylenes and alcohol, and the sections were subjected to an antigen retrieval procedure (Tris EDTA, pH 9.0). Importantly, antigen retrieval pretreatment did not alter the quality of immunohistochemical staining of macaque amygdala sections, and so was omitted from the main study, which did not involve paraffin-embedded sections.

REFERENCES

[1] Centeno ML, Sanchez RL, Cameron JL, Bethea C (2007) Hypothalamic gonadotrophin-releasing hormone expression in female monkeys with different sensitivity to stress. *J Neuroendocrinol* **19,** 594–604.

[2] Centeno ML, Sanchez RL, Reddy AP, Cameron JL, Bethea CL (2007) Corticotropin-releasing hormone and pro-opiomelanocortin gene expression in female monkeys with differences in sensitivity to stress. *Neuroendocrinology* **86,** 277–288.

Antibodies used in for the Western blot study

Target Protein	Target ID	Source	Catalogue#	Dilution
TAR DNA binding protein 43	TDP43	Proteintech, Rosemont, IL	10782-2-AP	1/1000
Glyceraldehyde 3-phosphate dehydrogenase	GAPDH	Abeam, Boston, MA	Ab8245	1/10000
Syntaxin	STX1A	SIGMA, St Louis, MO	S1172	1/1000
NK2 homeobox 1	NKX2.1	Donated by Toshimasa Onaya		1/1000
Histone H3	H3	SIGMA	06-755	1/1000
Vimentin	VIM	Proteintech	10366-1-AP	1/1000