Supplementary Materials

METHODS

Immunohistochemistry (IHC)

Hippocampal tissues collected from control, cocaine-treated, and heroin-treated monkeys were fixed in 4% formaldehyde for 24 h, washed with distilled water, and dehydrated gradually with a series of 70%−100% ethanol solutions. The tissues were immersed in xylene, embedded in paraffin, and sliced into 3-μm sections. The obtained sections, including sections obtained from paraffin blocks of MA-treated monkeys from our previous study (Choi et al.[7]), were transferred onto slides and the slides were deparaffinized with xylene. The slides were hydrated through washes in graded alcohols and water. For antigen retrieval, the slides were heated at 95°C for 20 min in Dako™ Target Retrieval Solution, pH 6.0 (Dakocytomation, Carpinteria, CA, USA). After cooling for 20 min, the slides were quenched with 3% H_2O_2 for 5 min. The slides were incubated with a rabbit anti-ADAM10 antibody (1:300) (ab1997, Abcam) for 2 h at room temperature. Endogenous peroxidase was blocked using DAKO REAL peroxidase blocking solution (Dakocytomation) for 10 min. The antibody was detected using DAKO EnVision+ for rabbit antibody (K4003, DAKO, Glostrup, Denmark) for 1 h, and the signal was detected with a Dako REAL™ DAB+ Chromogen detection system (Dakocytomation) according to the manufacturer's instructions. The slides were counterstained with hematoxylin.

The slices were scanned using a Panoramic MIDI scanner (3DHISTECH, Ltd., Budapest, Hungary). Three samples from each group and two slides per sample were scanned for density measurements. Digital image analysis of each slide was performed at 200x magnification with Panoramic Viewer and HistoQuant software (3DHISTECH, Ltd.). The expression intensity of ADAM10 in the hippocampal region was then measured as the H-score using the HistoQuant tool in the Panoramic Viewer. The mean H-scores within each group were calculated, statistically analyzed, and presented in a graph.