Developmental Disruption of Medial Prefrontal Cortical Gamma-Aminobutyric Acidergic Function by Non-Contingent Cocaine Exposure during Early Adolescence

Supplemental Information

Supplemental Methods and Materials

Cytochrome Oxidase Histochemistry and Densitometry

Rats were deeply anesthetized with 8% chloral hydrate, the brains quickly removed, fixed in a 0.1M phosphate buffer (PB 0.1M) solution containing 4% paraformaldehyde (PFA) for 2 hours and stored in 30% sucrose (in 0.1M PB) for 3 days. Serial coronal sections 50 µm thick were collected using a freezing stage (PFS-30MP controller, Physitemp Instruments, Clifton, NJ) on a sliding microtome (HM430, Thermo Scientific Microm, FL). Sections were then mounted on Superfrost Plus slides (VWR, Batavia, IL), dried under the hood for 20 min and incubated for 90 minutes at 37°C in 0.1M PB (pH 7.42) containing 0.50 g/L of 3,3-diaminobenzidine, 0.33 g/L of horse heart cytochrome c, 44 g/L of sucrose and 0.2 g/L of catalase (hydrogen peroxidase oxidoreductase). After incubation (90 min), slides were rinsed, dehydrated and coverslipped. The levels of cytochrome oxidase staining in the frontal cortex (2.7 to 1.2 mm rostral to bregma; (1)) were determined by means of densitometry using a slide scanner (Coolscan V, Nikon, Japan).

Medial Prefrontal Cortex (mPFC) Local Field Potentials Evoked by Ventral Hippocampal Stimulation In Vivo

Rats were deeply anesthetized with 8% chloral hydrate (400 mg/kg i.p.), placed in a stereotaxic apparatus (ASI instruments, MI), and maintained at 37-38°C (TCAT-2LV controller, Physitemp Instruments, Clifton, NJ). The local anesthetic lidocaine (2% lidocaine hydrochloride with 1:100,000 epinephrine, Cooke-Waite, Atlanta, GA) was applied subcutaneously ~5 min before any skin incision was made. Fifteen minutes after the rat was placed in the stereotaxic frame, a steady supplementary anesthesia level (300-400 µL of 8% chloral hydrate per hour) was delivered using a syringe minipump (BASi Baby Bee Syringe Drives, CA) attached to an i.p. cannula (26-gauge butterfly needle). The pattern of cortical electroencephalogram activity was used throughout the recording session to monitor the level of anesthesia as described elsewhere (2). The coordinates for the mPFC recordings were: 3.2 to 2.7 mm anterior from

bregma, 0.8 mm lateral from the midline, 4.2 mm below the brain surface (3). Prefrontal local field potential recordings were conducted using a concentric bipolar electrode (SNE-100x 50 mm; Rhodes Medical Instruments Inc., Summerland, CA), amplified (Cygnus Technology Inc., Delaware Water Gap, PA), filtered (bandwidth 1-100 Hz) and digitized (Digidata 1440A, Molecular Devices, Sunnyvale, CA) at a sampling rate of 10 kHz. A second concentric bipolar electrode (NE-100x 50 mm) was used to stimulate the ventral hippocampus (5.8 mm posterior from bregma, 5.2 mm lateral from the midline, 4.5 mm below the brain surface) (3). Ventral hippocampal stimulation was driven by a computer-controlled pulse generator Master 8 Stimulator (AMPI, Jerusalem, Israel).

All recordings were conducted following a previously reported protocol (3). Briefly, single and trains of square pulses of 300 μs duration were delivered every 15 s, typically at 0.75 mA stimulating intensity. For each set of train stimulation, 10 pulses of evoked field potential responses were delivered at 10, 20 and 40 Hz. These frequencies were chosen because they are suitable for assessing changes in mPFC network responses resulting from a developmental disruption of local GABAergic transmission (3). The pattern of the evoked response at a given frequency was estimated by averaging the amplitude (from the onset to the peak amplitude) of each of the evoked field potentials obtained from 10-15 sweeps of train stimulation and normalized to the first evoked response. Finally, changes in prefrontal local field potential responses following ventral hippocampal high-frequency stimulation (50 pulses at 100 Hz/15 s x 4) were examined to determine whether a history of early adolescent cocaine exposure is sufficient to alter plasticity within the hippocampal-mPFC pathway. It is well known that changes in synaptic plasticity within the ventral hippocampal-prefrontal pathway can be assessed in vivo by means of local field potentials (4).

Histological Confirmation of the Electrode Placements

Rats were anesthetized with 8% chloral hydrate (in 0.9% NaCl), transcardially perfused with cold saline (150 ml) followed by 200 ml of 4% PFA in PB 0.1M. The brains were quickly removed and fixed in 4% PFA for 24 hours before they were stored in 30% sucrose (in 0.1M PB). Coronal sections 50 μm thick were obtained from the mPFC and the ventral hippocampus. Sections were first mounted on Superfrost Plus slides (VWR, Batavia, IL) and treated with formol ethanol, before undergoing dehydration, treatment with xylene, rehydration, and staining with Cresyl violet. Slides were then washed and dehydrated, coverslipped with Permount (Fisher Scientific, Pittsburgh, PA), and examined under the microscope.

Table S1. Cytochrome Oxidase I (CO-I) histochemistry

CO-I histochemistry (ROD)

B: 2.7	IL/PL	OFC	CG	MC	SS
early adolescent saline	0.296 +/- 0.008	0.302 +/- 0.008	0.295 +/- 0.006	0.299 +/- 0.007	0.305 +/- 0.005
early adolescent cocaine	0.357 +/- 0.008	0.343 +/- 0.009	0.323 +/- 0.013	0.331 +/- 0.010	0.337 +/- 0.010
adult saline	0.294 +/- 0.005	0.290 +/- 0.009	0.304 +/- 0.006	0.301 +/- 0.004	0.312 +/- 0.005
adult cocaine	0.275 +/- 0.008	0.258 +/- 0.007	0.280 +/- 0.013	0.257 +/- 0.011	0.274 +/- 0.013

B: 1.7	CG	MC	SS
early adolescent saline	0.349 +/- 0.011	0.339 +/- 0.011	0.340 +/- 0.010
early adolescent cocaine	0.353 +/- 0.013	0.329 +/- 0.010	0.334 +/- 0.010
adult saline	0.358 +/- 0.006	0.340 +/- 0.007	0.338 +/- 0.004
adult cocaine	0.337 +/- 0.015	0.309 +/- 0.016	0.322 +/- 0.020

B: 1.2	CG	MC	SS
early adolescent saline	0.370 +/- 0.009	0.345 +/- 0.010	0.341 +/- 0.011
early adolescent cocaine	0.370 +/- 0.013	0.342 +/- 0.011	0.325 +/- 0.008
adult saline	0.365 +/- 0.007	0.350 +/- 0.008	0.342 +/- 0.009
adult cocaine	0.331 +/- 0.014	0.299 +/- 0.015	0.304 +/- 0.016

Changes in CO-I staining (ROD: relative optical density) in early adolescent saline: 10 (rats); early adolescent cocaine: 12; adult saline: 8; adult cocaine: 12.

B, bregma; CG, cingulate cortex; IL, infralimbic; MC, motor cortex; OFC, orbitofrontal cortex; PL, prelimbic; SS, somatosensory cortex.

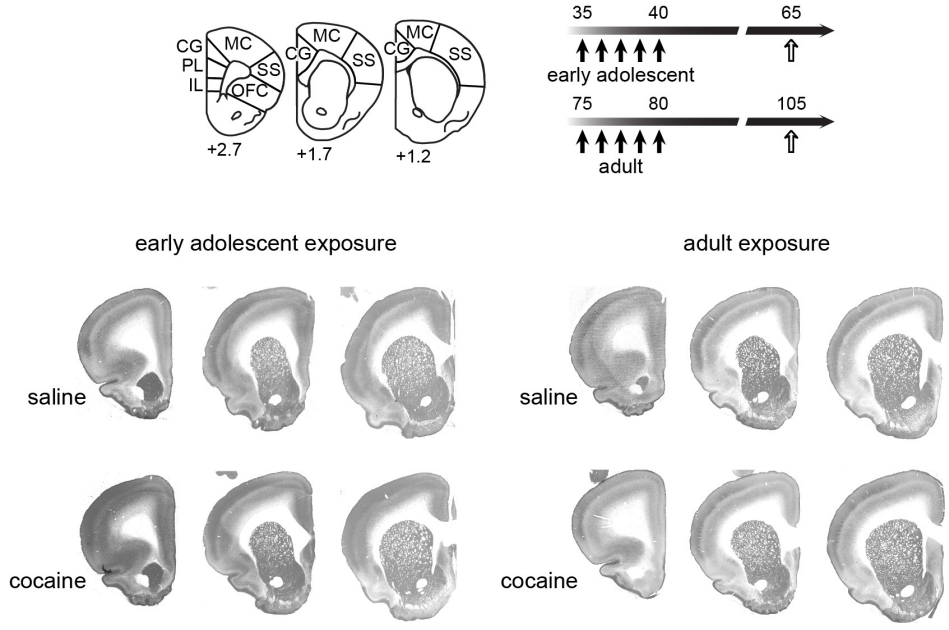

Figure S1. Representative coronal sections of CO-I staining. See Table S1, above, for abbreviations.

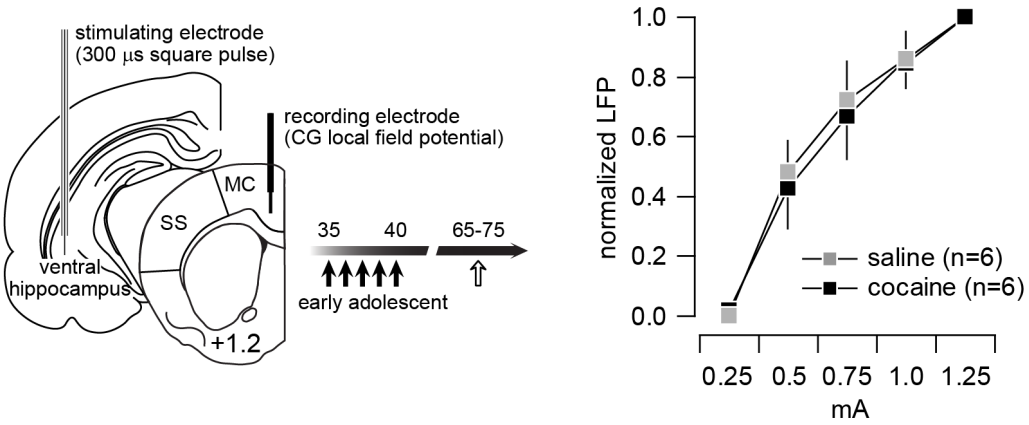

Figure S2. Saline-and cocaine-treated animals exhibited similar pattern of ventral hippocampal-evoked local field potential (LPF) responses in the cingulate cortex (CG; bregma 1.2), a cortical region where the cytochrome oxidase staining remained unchanged following early adolescent cocaine exposure. MC, motor cortex; SS, somatosensory cortex.

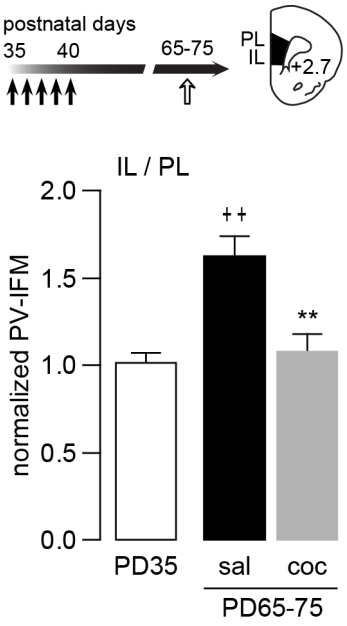

Figure S3. Developmental disruption of parvalbumin (PV) immunoreactivity in the medial prefrontal cortex (PFC; IL, infralimbic; PL, prelimbic) by early adolescent exposure to cocaine (from postnatal days -PD- 35 to 40). Analysis of PV immunoreactivity in the medial PFC by mean fluorescent intensity (IFM). Here, a gray matter region control (i.e., layer I) was used to correct the background signal instead of the white matter (Figure 9). Similar to the results obtained using the white matter as control, we found that relative to the PD35 age group (n = 9), a significant increase in PV immunoreactivity can be detected in the adult medial PFC of saline (sal) treated rats (n = 6). In contrast, PV immunoreactivity in the early adolescent cocaine-exposed group (coc, n = 8) resembles that observed in the juvenile medial PFC (**p < .005 vs. saline; ++p < .005 vs. PD35, Tukey post-hoc test after significant analysis of variance).

Supplemental References

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