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Extended access to cocaine self-administration results in reduced glutamate function within the medial prefrontal cortex

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Abstract

Previous studies have shown that brief access to cocaine yields an increase in D2 receptor binding in the medial prefrontal cortex (mPFC), but that extended access to cocaine results in normalized binding of D2 receptors (i.e. the D2 binding returned to control levels). Extended access conditions have also been shown to produce increased expression of the NR2 subunit of the NMDA receptor in the mPFC. These results implicate disrupted glutamate and dopamine function within this area. Therefore, in the present study, we monitored glutamate and dopamine content within the mPFC during, or 24 hrs after, cocaine self-administration, in animals that experienced various amounts of exposure to the drug. Naïve subjects showed decreased glutamate, and increased dopamine, levels within the mPFC during cocaine self-administration. Exposure to 7 lhr daily cocaine self-administration sessions did not alter the response to self-administered cocaine, but resulted in decreased basal dopamine levels. While exposure to 17 lhr sessions also resulted in reduced basal dopamine levels, these animals showed increased dopaminergic, but completely diminished glutamatergic, response to self-administered cocaine. Finally, exposure to 17 cocaine self-administration sessions, the last ten of which being 6h sessions, resulted in diminished glutamatergic response to self-administered cocaine and reduced basal glutamate levels within the mPFC, while normalizing (i.e. causing a return to control levels) both the dopaminergic response to self-administered cocaine as well as basal dopamine levels within this area. These data demonstrate directly that the transition to escalated cocaine use involves progressive changes in dopamine and glutamate function within the mPFC.

Keywords

cocaine; dopamine; glutamate; microdialysis; no-net-flux; self-administration

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Authors contribution

OMB was responsible for the study concept and design, supervised and contributed to the acquisition of data, analyzed the data and drafted the manuscript. KKS was also responsible for the study design, was responsible for analysis of microdialysis samples using the HPLC, and provided critical revision of the manuscript for important intellectual content. AC, EG, KLP, JD, NB, NMR, ANN, AS, KP, GAC and NW contributed to the acquisition of animal data. KDL constructed all the probes and helped with the microdialysis sample collection. AC provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved final version for publication.

Introduction

Cocaine addiction develops after prolonged and excessive use of the drug and is characterized by loss of control over drug use expressed as escalation of drug intake and continued use, and relapse to use, in spite of adverse consequences (APA, 2000; Gawin, 1991). The reinforcement efficacy of a drug is a critical predictor of its abuse liability (Foltin and Fischman, 1991; Schuster, 1975), and cocaine's strong reinforcement is dependent on the drug's ability to block the dopamine transporter within areas of the mesocorticolimbic dopamine pathway (Ritz et al., 1987, 1988; Volkow et al., 1997). Still, cocaine's reinforcement cannot fully account for the development of addiction, and the transition to cocaine addiction involves functional aberrations within the system critical for cocaine's initial reinforcement - one of the most documented of these changes is decreased function within ventromedial PFC (vmPFC; Bolla et al., 2003; Franklin et al., 2002; Lim et al., 2002; Volkow et al., 1992).

The vmPFC is critically involved in impulsivity, or the inhibition thereof, pertaining specifically to the ability to delay gratification, to inhibit pre-potent responses, or to adapt one's response according to changed stimulus-response contingencies (Clark, Cools, & Robbins, 2004; Clarke et al., 2004; Damasio, 1996; Konishi et al., 1998; Rolls et al., 1994). Thus, reduced vmPFC function in cocaine addicts stimulated the hypothesis that the inability of cocaine addicts to inhibit drug-taking behavior stems, at least in part, from deterioration in the function of this brain area, resulting in reduced inhibitory control over behavior (Jentsch and Taylor, 1999; Kelley, Schochet, & Landry, 2004; Volkow et al., 2010). Consistent with this notion certain specific aspects of impulsive behavior observed upon vmPFC damage are also exhibited by cocaine addicts (Bechara, Dolan, & Hindes, 2002; Bechara and Damasio, 2002; Coffey et al., 2003; Grant, Contoreggi, & London, 2000). However, since all these studies are correlational in nature, it is not clear whether the reduced vmPFC function observed in cocaine addicts is the cause for, or the result of, prolonged and excessive consumption of cocaine, nor is the mechanism by which such changes occur clear. In order to determine the aberrations resulting specifically from prolonged and excessive consumption of cocaine and the mechanism by which such aberrations occur, one needs an animal model for prolonged and excessive drug use.

Intravenous drug self-administration in animals is viewed as an especially strong animal model of human drug addiction (Foltin and Fischman, 1991; Gawin, 1991; O'Brien and Gardner, 2005; Sanchis-Segura and Spanagel, 2006). Of particular relevance to processes mediating the transitions from recreational drug use to addiction are self-administration protocols that allow prolonged daily access to the drug. One such protocol (Ahmed and Koob, 1998) includes a group of rats given extended daily access (i.e. 6h) to IV self-administered cocaine that show escalated cocaine intake over days, and another group of animals given brief daily (1h) access to the drug and exhibiting stable drug-intake over days. Using this model, we have demonstrated that, within the rat vmPFC (i.e., anterior cingulate + prelimbic cortices, corresponding functionally to Brodmann's areas 25 and 32; Uylings & VanEden, 1990), extended access to cocaine results in a “normalized” c-Fos response to cocaine and “normalized” D2 receptor binding (compared to the brief access group), but increased expression of Homer1b/c, NR2b, and NR2a at 1,14 and 60 days withdrawal, respectively (Ben-Shahar et al., 2004; 2007; 2009). Such changes may be indicative of changes in dopamine and glutamate function within these brain areas. To test this hypothesis directly, in the present study both traditional and no-net-flux microdialysis approaches were used to monitor dopamine and glutamate levels within the mPFC during basal conditions, as well as during cocaine self-administration, after differential exposure to self-administered cocaine.

Materials and Methods

Subjects

The subjects (n=80) were male albino Sprague-Dawley rats weighing 275–325 g at the beginning of each experiment and obtained from Charles River Laboratories (Hollister, CA). Rats were paired-housed in a temperature- and humidity- controlled colony room at 22°C (70% humidity) under a reversed 12-h light cycle (lights on at 20:00). Rats had *ad libitum* access to food and water, except during food training – a period of up to one week. The experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Surgeries

Rats underwent catheter and stereotaxic surgeries as described before (Ben-Shahar et al., 2004; Zaraya et al., 2011). Briefly, rats were implanted with a chronic silastic catheter into the right jugular vein under ketamine/xylazine (56.25 and *7.5,* respectively, mg/kg IM), or isoflurane gas (4% for induction; 2.0–2.5% for maintenance) anesthesia. Flunixin meglumine, a nonopiate analgesic (2 mg/kg SC) was provided to treat postsurgical pain. Catheter patency was maintained by daily flushing with 0.1 ml of sterile heparin/timentin/saline (60 IU/ml and 100 mg/ml, respectively) solution. Immediately following the catheter implantation and while they were still under anesthesia, rats were also implanted bilaterally with stainless steel guide cannulae (20 gauge; Plastics One). Guide cannulae were aimed above the mPFC (from Bregma: AP + 2.7, DV – 3.0 and ML ± 0.75 flat skull implanted at 6° angle from vertical; Paxinos & Watson, 1998), and secured to the skull. Obdurators were inserted into the cannulae to protect the exposed end from contamination.

Procedure

Food training and self-administration procedures utilized 15 standard operant chambers (Med Associates), and occurred during the dark phase of the light/dark cycle at the same time each day, as described previously (Ben-Shahar et al., 2009). Briefly, rats were initially trained to lever-press for a 45 mg food pellet on a fixed-ratio (FR) 1 schedule. Surgeries were performed 1–2 days later, and 7 days after surgeries, microdialysis and/or self-administration sessions began. Self-administration sessions were initiated by the extension of the levers into the operant chambers and terminated by their withdrawal. Each right-lever press resulted in an infusion (0.1 ml) administered over 4 sec, of either saline or cocaine hydrochloride (0.25 mg; which is about 0.67 mg/kg for our average rat weighing 375 grams), which was accompanied by the illumination of the right cue light for 20 sec. During the presentation of the cue light, additional right-lever presses were recorded but had no scheduled consequences. Throughout the session, responses at the left lever were recorded but had no programmed effects.

In vivo microdialysis—Dialysis probes (24 gauge, 23 mm in length) were constructed as previously described (e.g., Zayara et al. 2011). Probes were inserted unilaterally into the mPFC, then connected to a liquid swivel and perfused with the microdialysis buffer (NaCl, 147 mM, CaC12, 1.2 mM, KC1, 2.7 mM, MgC12, 1.2 mM, Na2HP04, 0.5 mM; adjusted to pH=7.4) at a rate of 2.0 μl/min. Sample collection began three hours later, which is a sufficient time to allow baseline neurotransmitter levels to stablize (See Figures 2 & 3). Dialysate was collected in 20-min fractions into vials containing 10 μl of preservative. For the no-net-flux experiments, glutamate or dopamine was dissolved in the microdialysis buffer in concentrations of 2.5, 5 and 10 nM. Following 1 hr of baseline sample collection, glutamate or dopamine concentrations were perfused through the probe via a liquid switch (CMA Microdialysis, Acton MA) for 60-min intervals each. In order to minimize the total number of subjects needed, each subject underwent two microdialysis sessions: the first

conducted unilaterally using the right (in approximately half the subjects) or left hemisphere (in the remaining subjects), and the second using the opposite hemisphere. All aspects of this procedure were previously used and described by us and others (e.g. Qu et al., 2008; Zaraya et al., 2011).

HPLC with electrochemical detection—The procedures for sequential detection of dopamine and glutamate in the dialysate using high-pressure liquid chromatography (HPLC) were identical to those recently described (Zayara et al., 2011). Briefly, dopamine was separated using a MD-150 × 3.2 column and detected using an ESA 5014B analytical cell with two electrodes (E1, -150 mV; E2, +220 mV). Glutamate was separated using a CAPCELL PAK C18 MG column (5 cm;) and detected using an ESA 5011A analytical cell with two electrodes (El, +150 mV; E2, +550 mV), following precolumn derivatization with *o*-phthalaldehyde using the autosampler. To analyze for glutamate or dopamine content, the peak height in each sample was compared with the appropriate external standard curve (i.e. for glutamate or for dopamine) and quantified using ESA Coularray for Windows software.

Experiment 1: Measuring glutamate and dopamine content in the mPFC during self-administration—Rats were assigned to either a "brief access" or an "extended access" group. Microdialysis was done during the 1st, 8th, or 17th, session of self-administration for the brief access group, and during the 8th (first 6h) and 17th (last 6h) session for the extended access group. On microdialysis days, subjects were connected to the self-administration boxes and to the microdialysis probes 4 hrs before the daily session began. Sample collection began at the start of the forth hour, which served for baseline measurements of dopamine and glutamate concentrations, and continued throughout the self-administration session. Thus, we measured cocaine-induced changes in the mPFC in drug naïve animals (i.e. 1st day/hour of self-administration), in cocaine-experienced animals (i.e. 8th session/hour, or 17th session/hour), in response to the first cocaine binge (i.e. first 6h session), and after repeated excessive use of cocaine (i.e. last 6h (10th) session; see also Figure 1A).

Experiment 2: Measuring basal glutamate and dopamine concentration in the mPFC following various exposures to cocaine—The procedures for this experiment were identical to the first one except that: 1. Microdialysis was conducted outside of the self-administration environment. 2. Following the first hour of baseline sampling, 3 concentrations (as described above) of dopamine or glutamate were infused into the mPFC for an hour each while samples were collected every 20 min. Still, measurements were taken from the same groups as in experiment 1: in drug naïve animals (i.e. 24 hrs before the 1st self-administration session), in cocaine experienced animals (i.e. 24 hrs after the 7th or 17th brief access session), in response to the first cocaine binge (i.e. 24 hrs after the first 6h session), and after repeated excessive use of cocaine (i.e. 24 hrs after the last (10th) 6h session; see also Figure 1A & 1B).

To verify cannulae placement, at the end of each of experiment, rats were deeply anesthetized with sodium pentobarbital (l00mg/kg IP), perfused with 60 ml saline followed by 60 ml of formalin, and their brains removed. Samples of the PFC were stained using cresyl violet. Only animals with tracks within the mPFC were included in the analysis.

Statistical analysis

Differences in response rates during self-administration were analyzed by One Way ANOVA's. Differences in glutamate or dopamine content during self-administration were analyzed by mixed-factorial ANOVAs, followed by Simple Effects analyses. As the experiments described are very difficult technically, though an attempt was made to test

each rat during two sessions of self-administration, we anticipated that we will be successful in recording neurotransmitter content over only one self-administration session in many of the subjects and therefore that the composition of subjects for each of the microdialysis sessions will be different. We therefore designed these experiments as between-subjects, rather than within-subjects, comparisons. Differences in basal glutamate or dopamine concentration were analyzed using One-Way ANOVA's, followed by Tukey post-hoc tests.

Results

Self-Administration Behavior

During the 1st session of cocaine self-administration, brief access subjects took 34.33 ± 2 infusions, while during the 7th or 8th session they administered 19 ± 1.46 infusions. This reduction was significant ($F(l,50)=32.368$, $p<0.001$). This reduction in intake of cocaine over eight self-administration sessions seems to contrast with our previous reports of stable cocaine self-administration in brief access subjects (Ben-Shahar et al., 2004; 2007; 2009), when behavior is measured between the 8th and 17th sessions (i.e., after animals have had the opportunity to adjust and stabilize their preferred daily intake). In the present report, we wished to compare the naïve to the limited-access state; thus, we are assaying for neurochemistry and behavior on the very first day of self-administration, when subjects typically infuse greater amounts of cocaine, than later during self-administration training. Indeed an examination of the behavior of rats exposed to 17 brief-access sessions revealed that these subjects administered 35.45 ± 5.5 infusions on the 1st session, 16 ± 1.5 on the 8th session, and 17 ± 2 on the last session of self-administration; thus, these subjects also showed a significant reduction from the 1st to 8th session ($F(l,10)=15.359$, $p=0.003$), but no further change in intake across the subsequent 9 self-administration sessions ($F(l,10)=0.185$, $p=0.676$).

As observed previously, subjects escalated their cocaine intake over the 10 sessions of extended access to cocaine. This was evident by a significant increase in intake of cocaine between the first hour of the first 6h session (17.4 ± 2.1 infusions), and the first hour of the last 6h session (22.8 ± 1.36 infusions; $F(l,34)=5.064$, $p=0.031$). Similarly there was a significant increase in cocaine self-administration observed during the entirety of the first 6h session (85.35 ± 6.7 infusions) and during the entirety of the last 6h session (110.2 ± 8.5 infusions; $F(l,30)=10.1$, $p= 0.003$).

Experiment 1: glutamate and dopamine concentration in the mPFC during self-administration

1a. 17 sessions of cocaine self-administration result in diminished glutamatergic responsiveness to cocaine self-administration: Baseline, and cocaine-induced changes in, levels of glutamate within the mPFC during the 1st, 8th and 17th sessions of brief-access cocaine self-administration, as well as during the first hour of the first and last 6h sessions, are illustrated in Figure 2. As the cocaine experience of animals during the 8th 1h session is equivalent to that of animals during the first hour of their first 6h session (i.e. 8th self-administration session), these data were combined. As presented in Figure 2A, significant group differences in basal mPFC glutamate levels were observed ($F(3,43)=26.689$, $p< 0.001$). A 2 (Drug: baseline vs. SA) * 3 (Time: 20 min blocks) * 4 (Session:1st vs. 8th vs. 17th 1hr vs. last 6h which is also 17th session of self-administration) ANOVA analyzing these data revealed an effect for Drug ($F(l,40)= 19.994$, $p<0.001$), an effect for Session ($F(3,40)=33.523$, $p<0.001$), and a significant interaction of Drug * Session ($F(3,40)=5.287$, $p=0.004$). Simple effects analysis, conducted to see in which session cocaine induced a significant change in extracellular glutamate levels, revealed that during the 1st and 8th sessions, cocaine self-administration resulted in a significant decrease in mPFC glutamate

(F(l,8)=6.524 p=0.034, and F(l,12)=29.878, p<0.001, respectively). In contrast, during the 17th lh session, as well as the first hour of the last extended access session (their 17th self-administration session), cocaine self-administration failed to affect glutamate levels, relative to baseline, within the mPFC. To better illustrate these group differences in the glutamate response to self-administered cocaine, the results are also represented as percent of the average baseline values for each group (Figure 2 Panel B). For the sake of consistency, the rest of the data depicting glutamate and dopamine content within the mPFC during self-administration will be graphed as both raw and percent of baseline data.

Cocaine-induced changes in extracellular glutamate levels within the mPFC during the entirety of the first and last extended access sessions are illustrated in Figure 3. Average baseline extracellular glutamate levels were significantly different between the first and the last extended access session (F(1,18)= 7.824, p=0.012). A Session:* Drug * Time ANOVA conducted on these data (Figure 3 Panel A), yielded an effect for Drug (F(6,102)=4. 259, p=0.001), an interaction for Drug * Session (F(6,102)=3.702, p=0.002), and an interaction for Drug * Session * Time (F(12,204)=2.076, p=0.02). A simple effect analysis then revealed an effect for Drug only (F(6,48)=3.987, p=0.003) over the first extended access session. Further analysis revealed significant reduction in glutamate levels within the mPFC over the first (p=0.014), second (p=0.039), third (0.032), and fourth (p=0.044), but not fifth (p=0.953) or sixth (p=0.802), hours of the first extended access session. No effect for Drug was found for the last extended access session (p=0.747). Together, these data demonstrate that a history of excessive cocaine intake blunts the capacity of self-administered cocaine to reduce mPFC extracellular levels of glutamate.

1b. Cocaine self-administration results in increased extracellular dopamine within the mPFC: Figure 4 illustrates the cocaine-induced changes in extracellular DA levels within the mPFC during the 1st 1h session, the 8th 1h session/the first hour of the first extended access session, the 17th 1h session, and the first hour of the last extended access session (i.e. 17th session) of cocaine self-administration. As was done for the analysis of glutamate, the dopamine data for animals during the 8th brief access session was combined with that for animals during the first hour of their first 6h session because the cocaine experience of these animals was equivalent. The mean baseline levels of extracellular dopamine did not differ between sessions and thus the data for each session was normalized to the mean of its baseline values for further analyses, as is most commonly done in the literature. This also allowed for the statistical confirmation of our claim that after three hours acclimation to the probe, baseline neurotransmitter levels were stable. Thus, a Session * Time ANOVA conducted on the raw baseline data (Figure 4 Panel A) yielded no effect for Time (p=0.555), and no interaction between Time and Session (p=0.21). Next, a Session * Drug * Time ANOVA conducted on the percent baseline data (Figure 4 Panel B) revealed an effect for Drug only (F(l,36)=18.161, p<0.001). Thus, cocaine self-administration resulted in increased extracellular dopamine levels within the mPFC (with average percent increases as follows: 1-1h – 123 ± 15; 8-lh – 129.5 ± 16; 17-lh – 164.5 ± 20; Last6h – 139 ± 19), regardless of previous cocaine experience.

Dopamine content during the first and last extended access sessions is shown in Figure 5. As no significant difference in average baseline dopamine levels were observed between sessions, here too the data for each session was normalized to the mean of its baseline values for further analyses. A Session * Drug * Time ANOVA conducted on the percent baseline data (Figure 5, panel B) revealed no effect for Drug (p=0.258), nor were there session differences (p=0.175), or an interaction between drug and session (0.296). However, an analysis of total area under the curve revealed significant increase in DA levels within the mPFC during the first (One tailed one sample T-test t(6)=2.104, p=0.04), but not the last,

extended access session. That is, 10 sessions of extended access to cocaine diminished the ability of cocaine to increase DA levels over the entire six-hour session.

Finally, it is important to note that on microdialysis days there was no correlation between the amount of cocaine self-administered by each individual animal and the percent of change from baseline in neurotransmitter levels observed during cocaine self-administration (for glutamate R=-0.033, p=0.833; for dopamine R=-0.143, p=0.407). This suggests that there was no direct correlation between the amount of cocaine self-administered on any individual day by any individual animal and its' microdialysis results, rather the relationship was between the overall previous exposure to cocaine and extracellular neurotransmitter levels.

Experiment 2: basal dopamine and glutamate content in the mPFC is decreased following brief and extended access to cocaine self-administration, respectively, when assayed using quantitative microdialysis approaches— Measures of basal neurotransmitter levels collected using conventional *in vivo* microdialysis procedures are influenced by probe recovery (Parsons & Justice 1994) and thus do not necessarily hold when quantitative microdialysis approaches are employed (e.g., Kapasova and Szumlinski, 2008). By virtue of our experimental design (Figure 1), the different groups of rats were tested in separate cohorts, raising the possibility that inter-cohort differences in probe construction and/or the HPLC system employed for neurochemical analysis may have contributed to the observed group differences in baseline glutamate levels (Figures 2 and 3). Thus, we conducted no net-flux microdialysis procedures to quantify the effects of a history of brief vs. extended access to cocaine upon basal mPFC glutamate and dopamine levels.

Ten sessions of extended access to cocaine self-administration resulted in significant reduction of mPFC basal glutamate concentration (x-intercept: $F(4,45)=3.244$, $p=0.021$; Tukey: $p=0.023$; Figure 6), without any change in glutamate clearance (i.e. slope). While inspection of Figure 6 also suggested that 1 session of extended access (First6h) or 17 sessions of brief access (17-1h) to cocaine self-administration reduced basal extracellular glutamate content, relative to the naïve state, these reductions did not reach significance ($p=0.189$ and 0.536, respectively). Finally, a significant negative correlation ($R^2=-0.419$, $p=0.017$) was found between the total cocaine intake and basal glutamate levels in individuals subjects across groups. These data confirm that a history of excessive cocaine intake reduces basal extracellular mPFC glutamate concentration.

Relative to the cocaine-naïve condition, brief access to cocaine self-administration resulted in decreased basal dopamine concentration within the mPFC (x-intercept), without an effect on dopamine clearance (i.e. slope; Figure 7). In contrast, mPFC glutamate levels did not differ between subjects with a history of 7 brief access sessions followed by 10 extended access sessions (i.e. Last6h) and cocaine-naïve controls. Thus, an ANOVA yielded a significant effect for Session ($F(4,36)=14.5$, $p<0.001$) and Tukey post hoc tests revealed significant reductions in basal dopamine concentration from the drug-naïve state to that of subjects experiencing 7 days of brief access (i.e. 7-1h $p<0.001$), 17 days of brief access (i.e. 17-lh $p<0.001$), and subjects with a history of 7 brief access sessions plus 1 extended access session (i.e. First6h $p<0.001$). These data indicate that while a history of cocaine intake under brief access conditions reduces basal mPFC dopamine concentration, this effect normalizes with excessive cocaine intake.

Discussion

To the best of our knowledge, we are the first to monitor changes in basal and cocaine-induced changes in dopamine and glutamate levels within the mPFC resulting from stable vs. escalated cocaine self-administration. We found that cocaine self-administration

produces a moderate increase in extracellular dopamine levels within the mPFC of cocaine-naïve, brief access, or extended access, subjects, with a tendency for brief access subjects exposed to 17 sessions to show higher increases in dopamine content. However, in extended access subjects such increase in dopamine levels was maintained over the first, but not last, extended access session. In addition, a history of stable, moderate cocaine intake under brief access conditions resulted in decreased mPFC basal extracellular dopamine content, an effect that was lost after additional cocaine self-administration under extended access conditions. In parallel, cocaine self-administration lowered extracellular glutamate levels in the mPFC of naïve and brief access subjects previously exposed to 7 self-administration sessions, but this response was lost by the 17th session of self-administration in both the brief and the extended access conditions. Finally, extended access to cocaine decreased basal extracellular glutamate content within the mPFC. Thus, cocaine self-administration significantly altered both dopamine and glutamate function within the mPFC, and the nature of these alterations was dependent on the duration (number of days/sessions) of cocaine access and the amount of drug intake.

Repeated access to cocaine under extended, but not brief, access conditions significantly altered the capacity of self-administered cocaine to elevate dopamine content. In addition, a significant reduction in basal dopamine content was observed within the mPFC of brief access subjects, and this reduction was lost after extended access to the drug. Still, the moderate changes in dopamine during cocaine self-administration, did not correlate with the changes observed in basal dopamine concentration. This suggests that changes in basal concentration of mPFC dopamine observed after various exposures to cocaine self-administration were not driven by cocaine-induced dopamine release in this brain area. A possible mechanism for the observed decrease in basal dopamine content in the brief access subjects is the observation that repeated administration of cocaine using a “sensitization protocol” resulted in increased clearance of dopamine within the mPFC (Meiergerd, Schenk, & Sorg, 1997); however, we failed to detect signficant group differences in dopamine clearance in this study of self-administering animals. The “normalization” of basal dopamine content within the mPFC of the extended access subjects might have resulted from additional changes in glutamate function, as glutamate can facilitate (via NMDA receptors) or inhibit (via AMPA receptors) dopamine release in this brain area (Takahata & Moghaddam, 1998).

We have reported previously that a history of moderate cocaine intake under brief-access conditions produces a “sensitization-like” behavioral effect (Ben-Shahar et al., 2004). Activation of mPFC D1 receptors was shown to block (Sorg, Li, & Wu, 2001), whereas depletion of mPFC DA was shown to facilitate (Beyer & Steketee, 1999), behavioral sensitization induced by non-contingent cocaine administration. Thus, it is possible that the reduction in basal mPFC dopamine content produced by a history of moderate cocaine intake contributed to their sensitized behavioral response. In contrast, a history excessive cocaine intake failed to influence basal mPFC dopamine content in the present study, suggesting a “normalization” of mPFC dopamine concentration. Interestingly, and consistent with the notion that basal mPFC dopamine may play an important role for the behavioral sensitizing consequences of cocaine experience, animals with extended access to cocaine also failed to exhibit a “sensitization-like” behavioral effect following their excessive drug experience.

Cocaine self-administration resulted in decreased glutamate content within the mPFC in naïve subjects as well as in subjects that previously experienced 7 brief access sessions. Since cocaine does not directly interact with glutamate receptors or transporters, and since the decrease in glutamate levels paralleled increased dopamine levels within the mPFC of these subjects, one putative cause for the decrease in glutamate observed during cocaine

self-administration is cocaine-induced increase in extracellular dopamine. This hypothesis is consistent with other reports showing that within the mPFC, dopamine depresses glutamate release via activation of Dl receptors (Abekawa et al., 2000; Mair & Kauer, 2007; but see Porras, Sanz, & Mora, 1997).

In contrast to the effect observed on the 1st and 8th brief access session, cocaine self-administration failed to alter glutamate content within the mPFC on the 17th session, in either brief or extended access subjects. These data for self-administered cocaine contrast with other reports of a rise in glutamate content in response to IP cocaine injection (Lominac et al., 2005; Williams and Steketee, 2004). Opposite effects of self-administered vs. experimenter-administered cocaine have also been observed in the nucleus accumbens where cocaine self-administration inhibited cell firing (Chang et al., 1994; Peoples et al., 2004), but experimenter-administered cocaine increased cell firing (Rebec, 2006). Such discrepancies reiterate the important role played by not only the route of drug administration, but also non-pharmacological factors, in the direction of drug-induced neuroadaptations within mesocorticolimbic structures and has important implications for our understanding of addiction neurobiology.

It may be argued that the lack of an effect of self-administered cocaine upon glutamate in the animals tested during their last extended access session may be attributable to a floor effect, as for some reason likely related to probe performance and/or HPLC sensitivity, the glutamate recovery of this group was very low. However, the issue of a glutamate floor effect clearly does not apply to the 17-day brief access animals and extended access animals exhibited, if anything, a slight increase in glutamate levels during cocaine self-administration. The fact that self-administered cocaine failed to influence extracellular glutamate levels from baseline levels in animals with a 17-day history of brief or extended cocaine access strengthens the conclusion that with increased daily exposure to cocaine, glutamatergic tolerance to the drug develops. As dopamine inhibits glutamate release within the mPFC via D1 receptors (Abekawa et al., 2000; Mair & Kauer, 2007), it could be that exposure to 16 sessions of cocaine self-administration, whether these sessions were brief or extended, results in reduced function of D1 receptors within the mPFC. However, it is clear that the blunted glutamate response to self-administered cocaine alone cannot account for the transition to escalated cocaine use, as this change is also observed in brief access subjects exhibiting very stable cocaine use. This also raises the intriguing possibility that after 17 sessions brief access, subjects are further along the neurochemical continuum of the transition to escalated cocaine use as compared to their state at the 8th session, but this hypothesis requires further study.

In addition to blunting the capacity of self-administered cocaine to lower mPFC glutamate levels, a history of extended access to cocaine also significantly lowered basal glutamate content, relative to the naïve state and *this* neurochemical adaptation was not observed in the corresponding 17-day brief-access group. As reduced basal glutamate content is predicted to decrease stimulation of NMDA receptors within the mPFC, this observation is consistent with the findings of Allen, Dykstra, & Carelli (2007) showing that continuous blockade of NMDA receptors facilitated the escalation of cocaine self-administration in extended access subjects. One of the main sources for glutamate projections to the mPFC is the contralateral mPFC (Uylings et al., 2003). Reduced basal activation of pyramidal projection cells within the mPFC in each hemisphere could cause reduced basal extracellular glutamate levels within each contralateral hemisphere. Thus, the reduction in basal glutamate concentration observed in extended access subjects could result from lowered function of the mPFC expressed as hypoactivity of glutamate projection cells. This hypothesis is consistent with reports showing that reversible or irreversible inactivation of the mPFC facilitates cocaine self-administration, increases breakpoints for cocaine self-administration in a progressive

ratio paradigm, and facilitates reinstatement of cocaine seeking behavior (Grakalic et al., 2010; Peters et al., 2008; Weissenborn, Robbins, & Everitt, 1997; but see Koya et al., 2009). This hypothesis is also consistent with the numerous reports on decreased function within the mPFC of human cocaine addicts (Bolla et al., 2003; Franklin et al., 2002; Lim et al., 2002; Volkow et al., 1992).

To summarize, during the 1st and 8th 1h sessions, cocaine self-administration reduced glutamate content within the mPFC. Additional exposure to cocaine self-administration under brief or extended access conditions resulted in the abolition of this glutamate response to cocaine self-administration. Our data are therefore consistent with the notion that a decrease in glutamate content within the mPFC during cocaine self-administration, combined with both decreased basal glutamate concentration and “normalized” basal dopamine concentration, is important for stable use of the drug. Moreover, the decrease in basal glutamate concentration within the mPFC is consistent with the notion that excessive cocaine self-administration results in decreased function of the mPFC, which then leads to compulsive cocaine-use.

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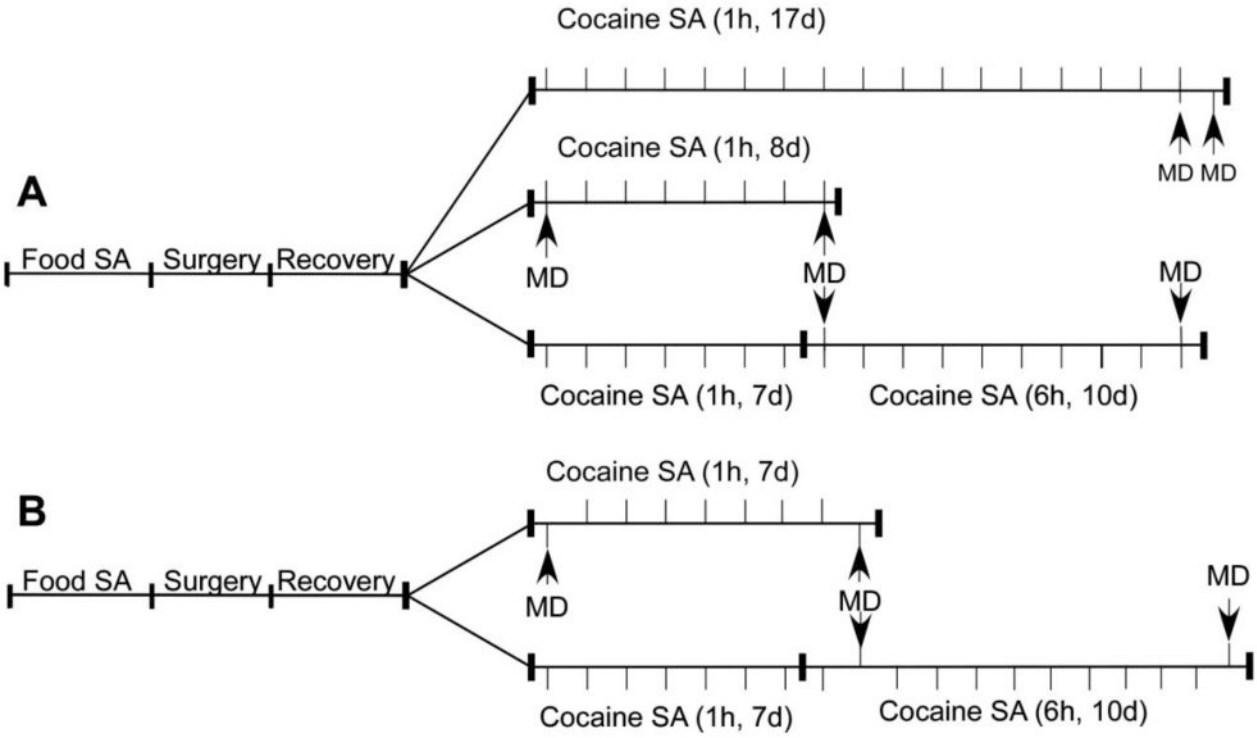

Figure 1.
Outline of the experimental schedules: Exp. 1 – Panel A; Exp. 2 – Panel B (with the no-net-flux for the 17 1hr group illustrated at the upper part of Panel A).

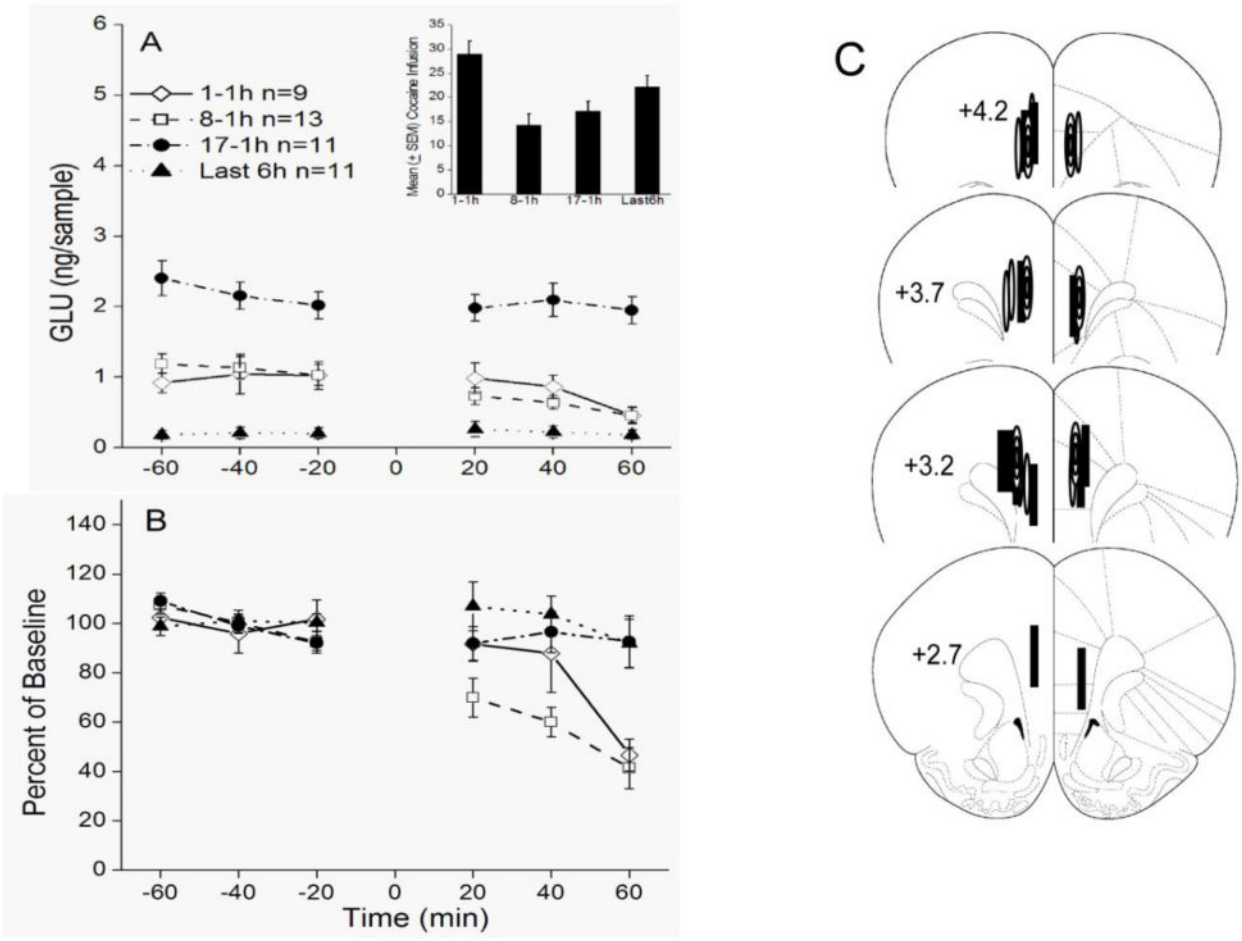

Figure 2.

Cocaine-induced changes in glutamate content within the mPFC during the 1st, 8th, and 17th 1h cocaine self-administration session, and the first hour of the last (i.e. 10th) 6h session. Panel A – Raw extracellular glutamate content. Inset showing average number of cocaine infusions in subjects, from the four experimental groups, from which measurements of glutamate content were taken; Panel B – Data from A expressed as a percent change from the average baseline values; Panel C – probe location: filled bars = extended access, open ellipses = brief access. Cocaine self-administration started at the 0 time point. Cocaine self-administration induced significant reduction in glutamate content over the 1st and 8th h of self-administration.

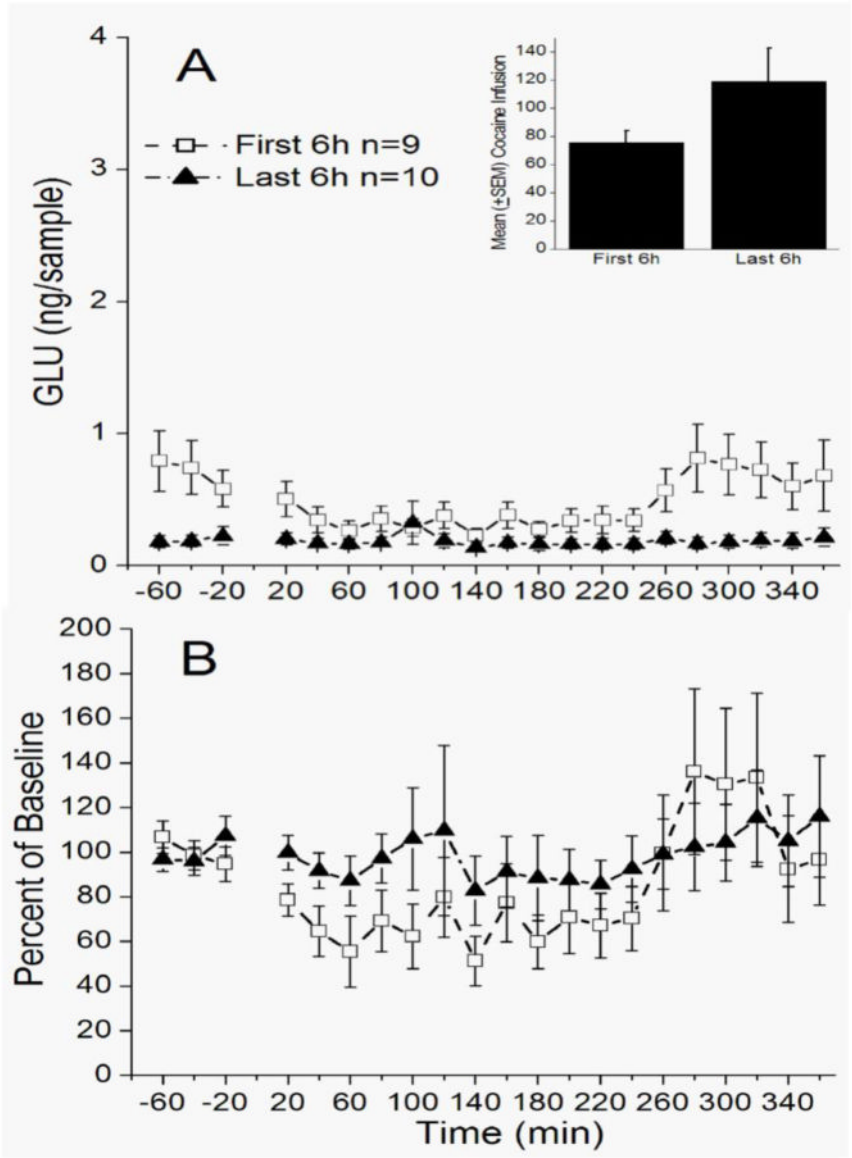

Figure 3.

Cocaine-induced changes in glutamate content within the mPFC over the first and last 6h session. Panel A – Changes in extracellular glutamate content resulting from cocaine self-administration (starting at the 0 time point). Inset showing average number of cocaine infusions in subjects, from the two 6h groups, from which measurements of glutamate content were taken; Panel B – Data from A expressed as a percent change from the average baseline values. Cocaine self-administration induced significant reduction in glutamate content over the first 4 hours of the first extended access session.

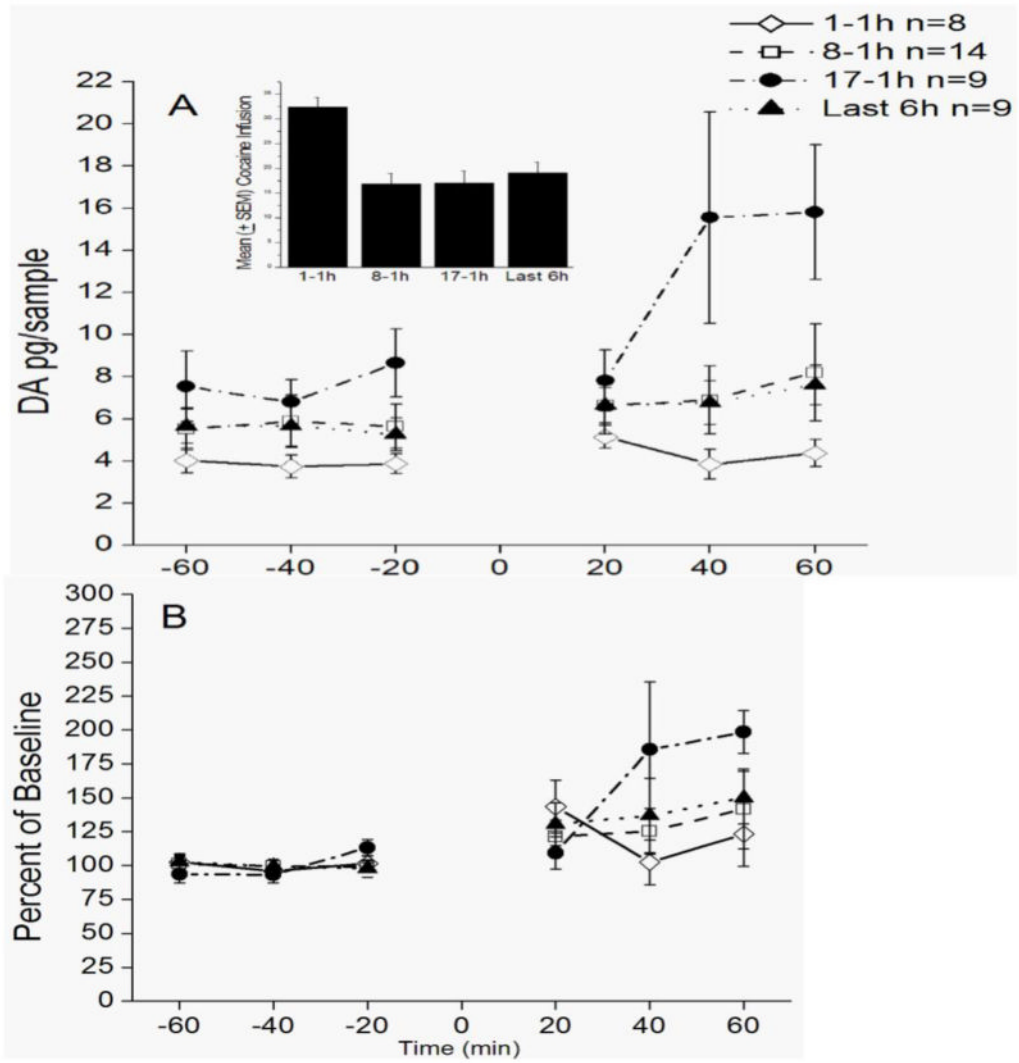

Figure 4.

Cocaine-induced rise in dopamine content within the mPFC during the 1st, 8th, and 17th lh cocaine self-administration session, and the first hour of the last (i.e. 10th) 6h session. Panel A – Changes in extracellular dopamine content resulting from cocaine self-administration (starting at the 0 time point). Inset showing average number of cocaine infusions in subjects, from the four experimental groups, from which measurements of glutamate content were taken; Panel B – Data from A expressed as a percent change from the average baseline values. Cocaine self-administration started at the 0 time point.

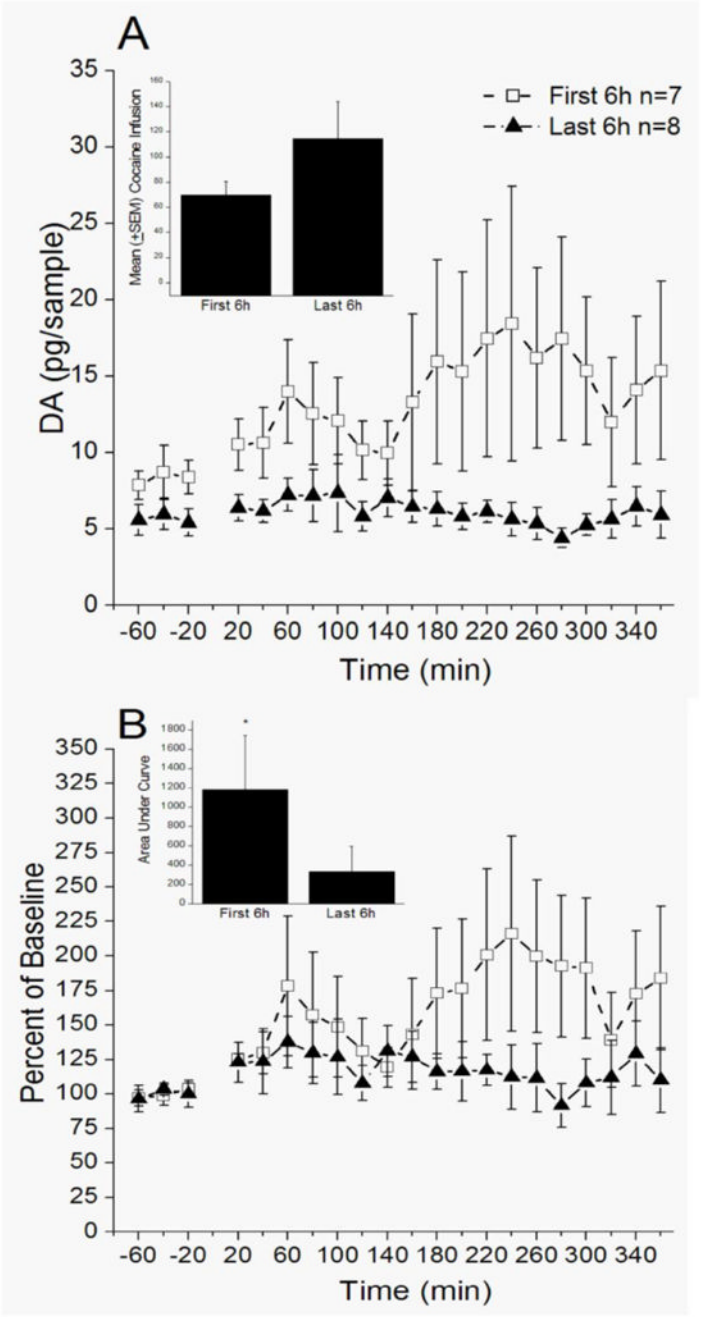

Figure 5.

Cocaine-induced rise in dopamine within the mPFC over the first and last 6h session. Panel A – Changes in extracellular dopamine content resulting from cocaine self-administration (starting at the 0 time point). Inset showing average number of cocaine infusions in subjects, from the two 6h groups, from which measurements of glutamate content were taken; Panel B – Data from A expressed as a percent change from the average baseline values. Cocaine self-administration started at the 0 time point. Inset showing average area under the curve for the two 6h groups (* represents significant difference from 0, or significant increase in DA content p=0.04).

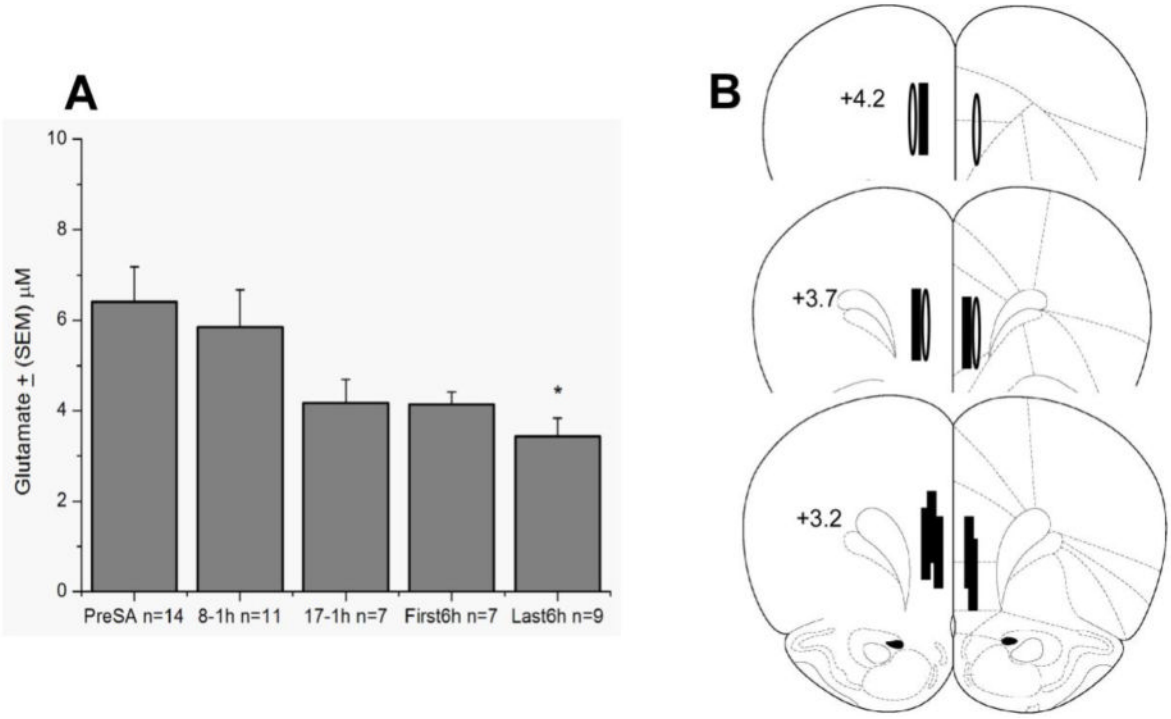

Figure 6.

Basal concentration of glutamate (expressed as μM of glutamate at the point of nonet-flux-Y axis) within the mPFC 24 hrs before the first session of cocaine self-administration (PreSA), or 24 hrs after the seventh or seventeenth day of brief access (8-lh and 17-1h, respectively), the first day of extended access (First6h), and the last day of extended access (Last6h). Basal glutamate levels were significantly lower after 10 days of extended access to cocaine. * Represent significant difference from the PreSA group p=0.021. Panel B – probe location: filled bars = extended access, open ellipses = brief access.

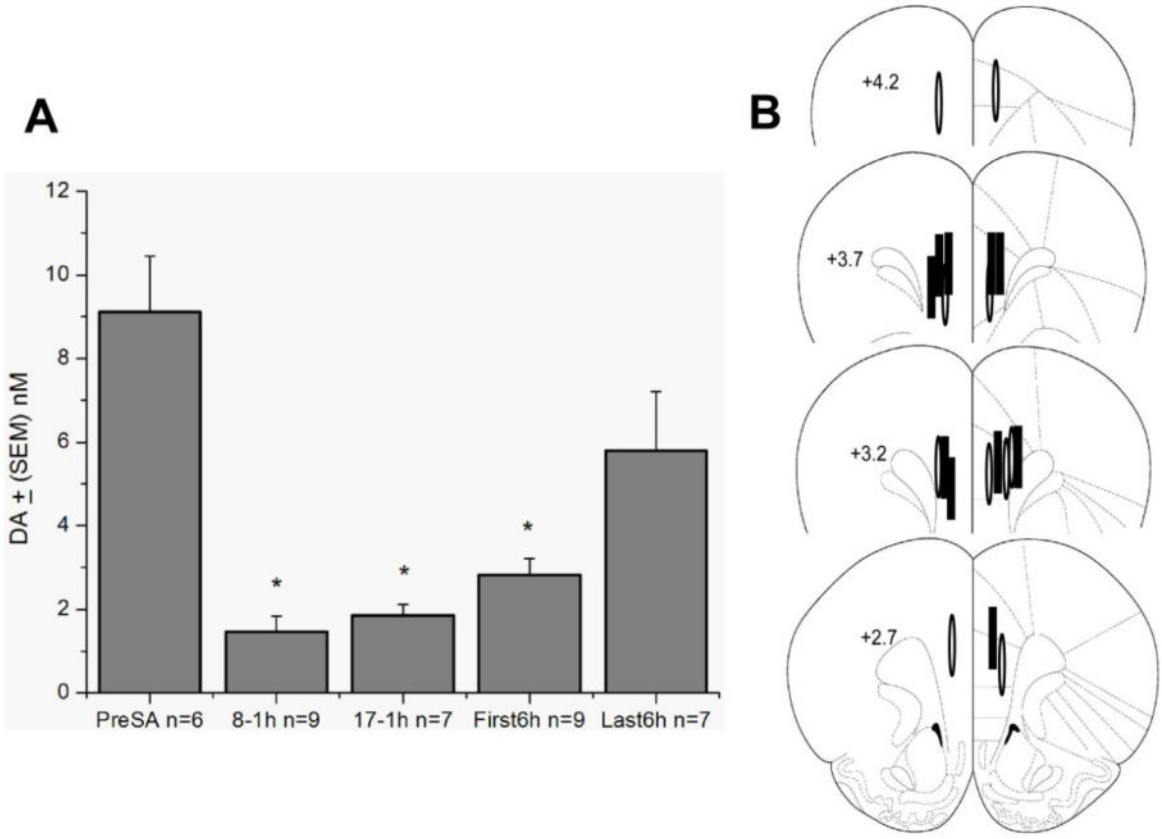

Figure 7.

Basal concentration of dopamine (expressed as μM of Dopamine at the point of nonet-flux-Y axis) within the mPFC 24 hrs before the first session of cocaine self-administration (PreSA), or 24 hrs after the seventh day or seventeenth day of brief access (8-lh and 17-lh, respectively), the first day of extended access (First6h), and the last day of extended access (Last6h). Basal dopamine levels were significantly lower after 7 or 17 days of brief, or 1 day of extended, access to cocaine. * Represent significant difference from the PreSA group $p<0.001$. Panel B – probe location: filled bars = extended access, open ellipses = brief access.