Repeated Cocaine Self-Administration Alters Processing of Cocaine-Related Information in Rat Prefrontal Cortex

WenLin Sun and George V. Rebec
Program in Neuroscience, Department of Psychological and Brain Sciences, Indiana University, Bloomington, Indiana 47405-7007

One of the core symptoms of cocaine addiction is compulsive drug-seeking behavior. Although the precise neural substrates are unknown, it has been hypothesized that this behavior involves cocaine-induced hypofunction of the prefrontal cortex (PFC) or “hypofrontality.” To test this hypothesis, PFC neuronal activity was monitored in rats during 3 weeks of cocaine self-administration (SA). Rats were trained to press a lever to self-administer cocaine in daily 2 h sessions. Responding was reinforced contingent on a modified fixed-ratio 5 schedule of reinforcement. In the first SA session, the overall firing rate and burst rate were significantly decreased after cocaine infusions relative to the period immediately before the session. These effects disappeared after ≥10 d of drug SA and were replaced by a significant increase in burst duration and firing rate within a burst. Notably, however, the level of basal activity before the first drug infusion of each SA session decreased significantly after multiple weeks of cocaine exposure. Collectively, these data support the view that although repeated sessions of cocaine SA decrease basal PFC activity, increased burst-related firing in response to cocaine infusions suggests that processing of cocaine-related information is enhanced and may contribute to increased control by cocaine over cocaine-seeking behavior.

Key words: cocaine; prefrontal cortex; electrophysiology; self-administration; addiction; neuroplasticity

Introduction

Relapse is one of the most challenging issues in treatment of cocaine addiction. Understanding its neurobiological mechanisms is a critical step toward development of better anti-addiction therapies. A recent theory proposes that drug-induced hypofunction of the prefrontal cortex (PFC) may be critically involved in compulsive drug-seeking and drug-taking behavior (Jentsch and Taylor, 1999). It is well known that the PFC plays an important role in control of behavior by regulating the function of subcortical motor structures such as the basal ganglia (Roberts and Wallis, 2000). For example, lesions to monkey PFC impair reversal learning of object or spatial discrimination tasks (Dias et al., 1996). Such effects are thought to reflect an inability to inhibit responding toward the stimuli previously paired with a reward (Roberts and Wallis, 2000). Chronic exposure to cocaine mimics these lesion effects. Cocaine-treated rats, for example, show persistent responding to the stimulus previously associated with cocaine and do not easily shift their responding to the stimuli currently associated with reward (Jentsch et al., 2002). This biased and persistent responding is reminiscent of the focused and persistent drug-seeking behavior observed in drug addicts. Indeed, recent imaging studies reveal that the metabolic activity of the PFC in drug addicts is lower compared with normal controls (Volkow et al., 2003). Interestingly, exposure of addicts to environmental stimuli previously associated with the drug experience activates the PFC, which is correlated with self-reported drug craving (Childress et al., 1999). Rats self-administering cocaine also show cue-related activation of PFC neurons (Rebec and Sun, 2005). It has been suggested that lower basal PFC activity may amplify the PFC response to drugs and drug conditioned stimuli (CSs) and therefore allow these stimuli to exert greater control over behavior.

Imaging studies, however, cannot determine whether “hypofrontality” is a condition induced by drugs of abuse or exists as a pre-addiction condition. To determine whether chronic exposure to cocaine can induce hypofunction of the PFC, we used chronically implanted microwire electrodes to record neuronal activity in rat PFC during a 3 week period of cocaine self-administration (SA). It also was of interest to investigate whether chronic exposure to cocaine increases PFC neuronal responding to cocaine, because such increases may underlie enhanced processing of cocaine-related information in the PFC. Our results show that although chronic cocaine SA indeed decreased basal PFC neuronal activity, PFC neuronal responding to cocaine was increased as evidenced by increased burst duration and firing rate within a burst, suggesting that processing cocaine-related information is enhanced in rats with a history of cocaine SA.

Materials and Methods

Subjects. Male Sprague Dawley rats (350 – 400 g) were used. One week before operant training, rats were placed on a restricted diet to reach 85% of free-feeding weight. After training, ad libitum access to food was available for 1 week before and after surgery. Food restriction was then reinstated to maintain 85–90% of free-feeding weight throughout.
Needle (27 ga). The electrodes were implanted bilaterally in the PFC at an
angle (25°) from the vertical to shield electric noise. A fluid pump with adjustable
speeds (PHM-100VS; Med Associates, East Fairfield, VT) was placed outside the
cubic. A modified electrical commutator/liquid swivel assembly (Plastics One, Roanoke, VA) was mounted on the ceiling of the cubic to allow uninterrupted flow of electrical signals and intravenous
drug solution.

Operant training. Rats (n = 9) first learned to press the lever reinforced by
sucrose solution (10%, v/v) under a fixed-ratio 1 (FR1) schedule in a
cubicle to allow uninterrupted flow of electrical signals and intravenous
drug solution.

Operant chambers. Two locally constructed Plexiglas operant cham-
bers, equipped with one Plexiglas lever and two cue lights mounted on a
side wall, were used. A fluid well was installed below the lever. A house
light was installed on the other side wall. An audio speaker was located
outside the chamber. Each chamber was housed inside a grounded gal-
vanized cubic box to shield electric noise. A fluid pump with adjustable
speeds (PHM-100VS; Med Associates, East Fairfield, VT) was placed outside the
cube. A modified electrical commutator/liquid swivel assembly (Plastics One, Roanoke, VA) was mounted on the ceiling of the cubic to allow uninterrupted flow of electrical signals and intravenous
drug solution.

Surgical procedures. Animals received ketamine (80 mg/kg, i.p.) and
xylazine (10 mg/kg, i.p.) for anesthesia. Ketamine supplements (10 mg/
0.1 ml) were given as needed throughout surgery. A catheter made from
PE10 and PE50 tubing was inserted into the right jugular vein as de-
scribed previously (Sun and Rebec, 2003). After catheterization, rats were
fixed in a stereotaxic frame for implantation of microwire electrodes as
described previously (Rebec and Sun, 2005). Each electrode assembly
consisted of eight 25-μm-diameter Fromvar-insulated stainless steel mi-
crowires (California FineWire Company, Grover Beach, CA) arranged in
cylindrical bundles and threaded through a stainless steel hypodermic
needle (27 ga). The electrodes were implanted bilaterally in the PFC at an
gle of 10° away from the midline. The coordinates for the PFC were as
follows: +3.0 mm anteroposterior, ±0.6 mm mediolateral, and −3.0
mm dorsoventral, relative to the bregma, midline, and skull surface,
respectively. Four additional holes were drilled for stainless steel support
dents. Dental acrylic held the screws and electrodes in place. During
recovery, animals received daily 0.1 ml of gentamicin (10 mg/ml, i.v.) for
1 week to minimize infection.

SA training. We used a modified FR5 schedule in which the first re-
response was reinforced by an infusion of 0.125 mg of cocaine in a volume
of 0.05 ml over 1 s. Compound stimuli (cue light and tone) were paired with
cocaine infusions and lasted 4 s, followed by a 16 s timeout signaled
by illumination of the house light. During the cocaine infusion and time-
out (total 20 s), responding was recorded but had no programmed con-
sequences. After the first cocaine infusion, subsequent responses were
reinforced by a train of at least three spikes having an
interspike interval of 5 ms, or 60 infusions were delivered, whichever occurred first. Catheter
patency was evaluated as necessary by infusing 0.1 ml of Brevital (1%);
loss of muscle tone within 5 s after infusion indicated a patent catheter.

Electrophysiological recording. A headplate attached to a flexible wire
cable was plugged into the electrode assembly, and the fluid tubing was
connected to the guide cannula installed on the rat’s head during surgery.
The headcase contained 16 miniature, unity-gain, and field-effect transis-
tors. The cable was connected to the commutator/swivel assembly. Elec-
trical signals from the microwires were passed through a pre-amplifier and fed into the SIG board of the Multichannel Acquisition Processor System (MAPS) (Plexon, Dallas, TX) in which the signals were further amplified, filtered (0.3–10 kHz), and digitized (40 kHz analog-to-digital conversion). Spike sorting was performed by the digital signal processor board on the MAPS controlled by the Sort Client program (Plexon) in
real time. The first step was to set a voltage threshold for each channel. Typically, the threshold was set to reach a minimum signal-to-noise ratio
>2:5:1. A template algorithm was used to isolate units (Rebec and Sun,
2005). All of the waveforms that matched the templates were saved for
additional off-line spike sorting (Plexon), which was used to refine the
discrimination of the units. Isochronous units was verified by post hoc
analysis of interspike intervals (ISIs) (Gulley et al., 2004). The interspike-
interval histograms of single units must exhibit a clearly recognizable refractory period (>1 ms). When all units were isolated (∼30 min,
baseline activity was recorded for 1 min, after which the session started.
Recording lasted the entire session. Spike waveforms and time stamps
were saved on the computer.

The events occurring in the operant chamber including lever press,
onset of the stimuli, and fluid pumps were recorded and controlled by a
program (Med Associates) running on a Pentium computer. These
events were sent to the digital inputs on the MAPS for time stamping
through a transistor–transistor logic board controlled by the program.

Histology. After the experiments, rats were anesthetized in preparation
for subsequent histological analysis. A 10 s, 20 μA positive current was
passed across all electrodes to mark recording sites. Rats were then per-
fused transcardially with PBS (0.9%), followed by 4% paraformalde-
hyde/2% potassium ferrocyanide, which reacts with iron deposits to pro-
duce Prussian blue spots at the electrode tip. All electrodes were marked
to verify that they were within the target area; individual recording sites,
however, did not have enough spatial resolution to be identified reliably.
To ensure that all marked wires were identified, tissue sections (60 μm
thickness) were analyzed up to 2 mm anterior and posterior to the elec-
trode entry point.

Statistics. Data from sessions 1, 10–12, and 19–21 were used for sta-
tistical analysis. The firing rate and burst rate were calculated as spikes per
second and bursts per minute, respectively. Baseline data were based on a
1 min period immediately before the session. The firing rate in response to
self-administered cocaine was based on a 10 s time window starting from
10 s after each cocaine infusion and averaged across all infusions in a
session. This time window was selected because the rats showed a
sudden increase in locomotion and a burst of face washing during this
period, which collectively suggest the onset of direct pharmacological
effects of cocaine. Because we previously showed that PFC neurons are
phasically activated by cocaine CSs with a latency of ∼500 ms and a
duration of ∼700 ms (Rebec and Sun, 2005), analyses that include the
entire timeout period would be confounded by neuronal responding to
CSs. In addition, PFC neurons do not respond to onset of the house light
in the presence of the CSs paired with cocaine infusions (Rebec and Sun,
2005). Given these considerations, we believe that any change in neuro-
nal activity during the 10 s time window is attributable to the effects of
cocaine rather than those of the CSs or house light. We also analyzed
burst firing by assessing the burst rate, the rate within a burst, and burst
duration. Bursts were defined as a train of at least three spikes having an
80 ms maximum onset ISI and a 160 ms minimum offset ISI. There is not
a universal standard for defining bursts, and different investigators have
used different ISIs as cutoff points to define burst activity recorded from
neurons in the PFC and hippocampus neurons (Legendy et al., 1985;
Quirk et al., 1999; Laviolette et al., 2005). To determine whether analyses
with different cut-off ISIs produce different statistical results, we analyzed
the data using our parameters and the cutoff ISI 45 ms proposed by
Grace’s group (Laviolette et al., 2005). No differences were found, except
that the analysis with our parameters revealed a significant decrease in
burst rate by cocaine in the first cocaine SA session. Thus, all results were
reported based on our parameters. Because all of these electrophysiological
variables did not follow Gaussian distribution (D’Agostino and Pear-
son omnibus normality test, p < 0.01), a nonparametric Kruskal–Wallis
test was used to compare their median rather than mean. Differences in
neuronal activity across sessions were compared with Dunn’s test. Dif-
fences between baseline activity and activity after cocaine infusions
within each session were tested with a Wilcoxon signed-rank test. In
contrast, the amount of cocaine infused in different sessions passed the
normality test, and therefore a repeated one-way ANOVA was used to
compare cocaine intake. The significance level was set at α = 0.05.

Results
The positions of our microwire electrodes are shown schemati-
cally in Figure 1. All recording sites were located within area 1 of
the anterior cingulate and dorsal prelimbic cortex.

The amount of cocaine infused during sessions 1, 10–12, and
19–21 was 2.1 ± 0.5, 4.0 ± 0.4, and 2.6 ± 0.5 mg/session, respec-
tively. Because two rats did not reach the third week of training
because of catheter failure, the ANOVA was based on the remain-

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Our results demonstrate that PFC basal activity was significantly decreased after multiple weeks of cocaine SA training. In addition, the drug produced different effects on PFC activity in cocaine-naive and cocaine-experienced rats. Thus, during the

Discussion

Next, we investigated the effects of chronic cocaine SA on basal burst activity of PFC neurons. As shown in Figure 2, the median basal burst rate (50th percentile range) in sessions 10–12 and 19–21 was 1.0 (0.0–8.0) and 1.0 (0.0–8.0) bursts/min in both cases, which was significantly lower than the session 1 rate of 8.0 (2.0–18.5) bursts/min (Kruskal–Wallis test, p < 0.0001). No differences were found between sessions 10–12 and 19–21. Cocaine significantly decreased the burst rate in the first SA session (Wilcoxon signed-rank test, p < 0.01) but not in the other sessions.

We then studied the effects of chronic cocaine SA on burst strength, measured as the rate within a burst and burst duration. As shown in Figure 3, the median (50th percentile) basal rates within a burst in sessions 10–12 and 19–21 were 15.0 (0.0–21.5) and 15.0 (0.0–20.2) spikes/s, respectively, which were significantly lower compared with session 1 (Kruskal–Wallis test, p < 0.0001). There were no significant differences between sessions 10–12 and 19–21. Cocaine did not significantly decrease the rate within a burst during the first SA session but significantly increased it in sessions 10–12 and 19–21 (Wilcoxon signed-rank test, p < 0.01 and p < 0.001, respectively).

The effect of cocaine on burst duration is summarized in Table 1. Again, basal burst duration was significantly decreased in sessions 10–12 and 19–21 relative to session 1 (Kruskal–Wallis test, p < 0.001), and there was no significant difference between sessions 10–12 and 19–21. Moreover, cocaine did not decrease burst duration during the first SA session but significantly increased it in sessions 10–12 and 19–21 (Wilcoxon signed-rank test, p < 0.01 and p < 0.05, respectively).

Discussion

ing seven rats. Although there was an increase in the amount of cocaine infused during sessions 10–12, this increase was not significant (F(1, 12) = 3.88; p > 0.05).

Nine rats were recorded in session 1 and sessions 10–12 and yielded 132 and 95 units, respectively. Seven rats were recorded in sessions 19–21 and yielded 79 units. As shown in Figure 1, the median (50th percentile range) basal firing rate of PFC neurons recorded in sessions 1, 10–12, and 19–21 were 2.2 (0.9–3.8) spikes/s, 1.1 (0.5–2.6) spikes/s, and 1.0 (0.5–2.4) spikes/s, respectively. The basal firing rates during sessions 10–12 and 19–21 were significantly decreased compared with session 1 (Kruskal–Wallis test, p < 0.001). There were no significant differences between sessions 10–12 and 19–21. In addition, we investigated whether these neurons show changes in responding to cocaine. We found that the firing rate was significantly decreased after cocaine infusions during the first SA session (Wilcoxon signed-rank test, p < 0.05) but not during the other sessions.

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Discussion

Our results demonstrate that PFC basal activity was significantly decreased after multiple weeks of cocaine SA training. In addition, the drug produced different effects on PFC activity in cocaine-naive and cocaine-experienced rats. Thus, during the
first cocaine SA session, rats responded to cocaine with decreased firing and burst activity, but these effects disappeared after ~2 and 3 weeks of cocaine SA. PFC neurons also showed increased burst strength in response to cocaine as evidenced by the increased firing rate within a burst and burst duration in cocaine-experienced but not cocaine-naive rats. Together, these data support the idea that although chronic cocaine exposure decreases PFC function, processing of cocaine-related information is enhanced. Such enhancement may contribute to increased control by cocaine over cocaine-seeking behavior.

One alternative explanation for increased burst strength is that cocaine may normalize PFC activity rather than increase processing of cocaine-related information. For example, basal burst strength was significantly decreased in sessions 19–21 compared with session 1, but cocaine appeared to bring basal burst strength back to the session 1 level. If nonspecific normalizing effects are responsible for this reversal, cocaine should also normalize the firing rate and burst rate. The fact that we did not observe such effects argues against this explanation.

The resting membrane potential of PFC pyramidal neurons alternates between an “up” state, when these neurons fire, and a “down” state, when they do not (Peters et al., 2000). In anesthetized rats, acute cocaine causes depolarization, eliminates the oscillation between down and up states, and decreases the firing rate of these neurons (Trantham-Davidson and Lavin, 2004). It is possible that elimination of membrane oscillation prevents PFC neurons from reaching the up state and thus is responsible for the decrease in firing rate. This interpretation is consistent with our results that self-administered cocaine decreased the firing rate in cocaine-naive rats. Similarly, chronic cocaine also depolarizes PFC neurons and eliminates the membrane oscillation; these effects, moreover, are still apparent 2 weeks after the last administration of cocaine (Trantham et al., 2002). The long-lasting membrane effects may explain the decreased basal firing rate observed in the current experiments.

Although cocaine decreases PFC activity in cocaine-naive rats, such inhibitory effects are no longer apparent in cocaine-experienced rats. In fact, in these rats, PFC neurons showed increased burst strength in response to cocaine. It has been demonstrated that repeated exposure to cocaine decreases inwardly rectifying K⁺ currents and increases voltage-sensitive Ca²⁺ currents in response to depolarizing stimuli (Dong et al., 2005; Nasif et al., 2005). These changes could contribute to enhanced responsiveness of PFC neurons to psychostimulants. One potential mechanism underlying the effects of cocaine on K⁺ channels involves the cAMP/protein kinase A (PKA) pathway (Dong et al., 2005). PKA can phosphorylate K⁺ channels and consequently decrease K⁺ currents (Dong and White, 2003). Repeated exposure to cocaine upregulates the cAMP/PKA pathway in PFC neurons (Dong et al., 2005). The upregulated PKA may increase the phosphorylation of K⁺ channels and, consequently, decrease K⁺ currents. Both D₁- and D₂-like dopamine (DA) receptors regulate the cAMP/PKA pathway. Activation of D₁-like receptors increases activity of the cAMP/PKA pathway, whereas activation of D₂-like receptors decreases it (Stoof and Kebabian, 1981). Because cocaine increases extracellular DA levels by blocking DA uptake, it may either increase or decrease PKA activity depending on which receptor subtype is predominantly activated. There is evidence that chronic cocaine may preferentially increase signaling through D₁-like receptors. For example, AGS3, a member of the activator-of-G-protein-signaling family, is upregulated in rat PFC by repeated exposure to cocaine (Bowers et al., 2004). This protein disrupts the interaction between Giα and Gpγ subunits to decrease signal transduction related to Giα. Because D₁-like receptors are associated with Giα, upregulation of PFC AGS3 will decrease D₂-like receptor-mediated signaling and therefore preferentially increase D₁-like receptor-mediated signaling. An increase in D₁-like receptor-mediated signaling may contribute to upregulation of cAMP/PKA, a decrease in K⁺ currents, and thus increased responsiveness of PFC neurons to cocaine.

DA plays a critical role in sustaining activation of primate dorsolateral PFC neurons during a delayed-response task (Sawaguchi, 2001); this persistent activation has been proposed as a neuronal mechanism involved in working memory (Goldman-Rakic, 1995). The activity-sustaining effects of DA may be related to its effects on the membrane potential of PFC neurons. For example, burst stimulation of the ventral tegmental area induces up states of PFC neurons, and duration of the up states seems to be dependent on activation of D₁-like receptors (Lewis and O’Donnell, 2000). Thus, in cocaine-experienced rats, increased D₁-like receptor signaling could increase residence time of the up state of PFC neurons. This increased up state could explain the increased burst duration induced by self-administered cocaine in cocaine-experienced rats.

There is evidence that increased signaling through D₁-like receptors in the PFC is critically involved in cocaine-seeking behavior. For example, overexpression of ASG3 increases cocaine-
seeking behavior, whereas inhibition of ASG3 upregulation with antisense oligonucleotides microinfused into the PFC blocks reinstatement of cocaine-seeking behavior (Bowers et al., 2004). We recently demonstrated that blockade of D₁-like receptors in the PFC dose-dependently blocks cocaine-primed reinstatement of cocaine-seeking behavior (Sun and Rebec, 2005). Together, these data indicate that increased D₁-like receptor-mediated signaling in the PFC after chronic exposure to cocaine may be an important mechanism underlying compulsive cocaine-seeking behavior.

In summary, our results provide direct evidence that chronic exposure to cocaine decreases basal activity in the PFC. Processing cocaine-related information, however, seems to be preferentially increased as evidenced by increased burst strength in response to self-administered cocaine. The combination of lower basal activity and enhanced burst strength may bias the PFC to process information related to cocaine and thereby contribute to focused and persistent cocaine-related behavior.

References


