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Repeated Cocaine Administration Alters the Electrophysiological Properties of Prefrontal Cortical Neurons

H. Trantham, K. K. Szumlinski, K. Mcfarland, P. W. Kalivas, and A. Lavin*

Department of Physiology and Neuroscience, Medical University of South Carolina, 173 Ashley Avenue, 403 BSB Building, Charleston, SC 29425, USA

Abstract

Recently it has become clear that some of the symptoms of addiction such as relapse to drug-taking behavior arise, in part, from a dysfunction in cognitive and emotional processing. This realization has promoted investigations into the physiology and pathophysiology of forebrain circuits that are both innervated by dopamine and play an important role in cognitive processing, including the prefrontal cortex. In order to study long-term neuroadaptations occurring in the prefrontal cortex of the rat as a consequence of psychostimulant administration, cocaine was repeatedly administered in either a contingent or a non-contingent manner. At least 2 weeks following the last cocaine injection, *in vivo* intracellular recordings were made from neurons located in the deep layers of the prefrontal cortex. Repeated cocaine administration abolished the presence of membrane bistability normally present in neurons located in the limbic prefrontal cortex.

These results indicate that repeated exposure to cocaine produces enduring changes in the basal activity of neurons in the prefrontal cortex that may contribute to previously identify cognitive and emotional dysfunctions in cocaine addicts.

Keywords

prefrontal cortex; cocaine; dopamine; bistability; neuroadaptations

Addictive psychomotor stimulant drugs exert pharmacological actions on limbic, cognitive and motor circuits that subserve adaptive behavioral processes, and drug-induced neuroadaptations within these circuits are central themes in neurobiological hypotheses of drug addiction (Pierce and Kalivas, 1997; Wolf, 1998; Jentsch and Taylor, 1999; Berke and Hyman, 2000). There is *in vivo* evidence for altered metabolic activity within these structures in psychostimulant addicts (Grant et al., 1996; Childress et al., 1999; Volkow and Fowler, 2000). Like-wise, neuropsychological impairments typical of frontostriatal dysfunction have been demonstrated in stimulant abusers (Jentsch and Taylor, 1999; Rogers et al., 1999; Ornstein et al., 2000; Grant et al., 2000).

In this context, neuroadaptations in dopamine (DA) transmission in the prefrontal cortex (PFC) are an enduring consequence of repeated cocaine administration in experimental

*Corresponding author. Tel.: +1-843-792-6799; fax: +1-843-792-4423.

animals (Sorg et al., 1997). DA modulates the electrophysiological activity of pyramidal cells projecting from the PFC to the ventral striatum (Yang and Seamans, 1996), and cocaine-induced alterations in corticostriatal pyramidal neurons have been proposed to be important in behavioral sensitization and drug-seeking behavior (Pierce and Kalivas, 1997; Jentsch and Taylor, 1999; Nestler, 2001). Although data on electrophysiological changes in PFC neurons resulting from cocaine exposure are sparse, the existing data indicate that chronic cocaine may affect the physiological properties of prefrontal cortical neurons in a manner that depends upon dopaminergic dysfunction as well as on neuroadaptations intrinsic to cortical neurons. In the present report, it was hypothesized that pretreatment with repeated cocaine would alter the basal electrophysiological characteristics of pyramidal neurons located in the PFC.

Experimental Procedures

Animal preparation

All the animals were handled in accordance with the procedures outlined in the *Guide for the Care and Use of Laboratory Animals* published by the USPHS, and the Medical University of South Carolina Animal Care and Use Committees approved the specific protocol. Subjects were male Sprague–Dawley rats (Harlan), weighing 200–250 g at the start of the experiment. Animals were housed in pairs in a temperature-controlled colony room on a 12-h light/dark cycle (lights on at 07.00 h), and food and water were available *ad libitum*. Animals were allowed to acclimatize to the colony room for 3–5 days following their arrival, and then were handled for 2–5 min daily for 2–3 days. All behavioral testing was conducted during the light portion of the diurnal cycle, beginning at 12.00 h. Cocaine hydrochloride was donated generously by NIDA (Baltimore, MD, USA). Cocaine was dissolved in 0.9% sterile saline and saline served as the vehicle for control injections (1 ml/kg).

In groups of rats where the drug was delivered by the investigator, subjects were injected daily for 7 days with cocaine (non-contingent group). On the first and last day of drug administration, the rats were injected with 15 mg/kg i.p. of cocaine and motor activity was monitored in a photocell apparatus (Omni-tech, Columbus, OH, USA) as described elsewhere (Pierce et al., 1998). On the intervening days the animals were injected with 30 mg/kg i.p. of cocaine in the home cage. On behavioral testing days, the animals were placed into the photocell cages for 1 h, after which time they were removed from the photocell cages, injected with cocaine and returned to the cages for an additional 2 h. Consistent with previous studies (Pierce et al., 1996), this cocaine treatment produced locomotor sensitivity. In the present study, the total distance traveled over the first 30 min after cocaine injection was significantly augmented when comparing the first day of cocaine treatment to a cocaine challenge (15 mg/kg i.p.) given 21 days after the last daily cocaine injection (Day 1 = 10, 474 ± 1.055 cm; Day 28 = 15, 575 ± 1.549 cm; using a paired Student's *t*-test, $t = 237$, $P < 0.032$). Electrophysiological experiments were conducted between 2 and 4 weeks following the cocaine challenge injection given on Day 28.

In another group, five animals were surgically implanted with jugular catheters and trained to self-administer cocaine (contingent group) to criteria as described by Cornish et al. (2001). In brief, rats were trained on a fixed ratio schedule (FR-1 schedule) of i.v. cocaine

self-administration (0.25 mg/infusion). Rats were allowed to self-administer cocaine for 2 h/day until there was $\pm 10\%$ variance in the number of infusions over three daily trials (requiring 7–15 days). The average dose of cocaine over the last three trials ranged from 10 to 30 mg/kg/trial. Electrophysiological experiments were conducted between 2 and 4 weeks following the last cocaine administration.

The rats were anesthetized with chloral hydrate (400 mg/kg i.p.; Sigma, St. Louis, MO, USA), mounted in a stereotaxic apparatus (Narishige, Japan), and fitted with a lateral vein catheter for subsequent anesthetic or drug administration. The body temperature was maintained at $37\pm0.5^{\circ}\text{C}$ by a thermostatically controlled heating pad (Harvard Apparatus, Holliston, MA, USA). An incision was made in the scalp, the skull exposed, and a burr hole drilled overlying the PFC (coordinates from bregma: anteroposterior (AP): +3.2 mm, lateral (L): 0.6 mm, dorsoventral (DV): 3.0–5.0 mm), the ventral tegmental (VTA) area (coordinates from bregma: AP: –4.9 mm, L: 0.5 mm, DV: 7.7 mm) or the mediodorsal thalamus (MD) (coordinates from bregma: AP: –3.1 mm, L: 0.5 mm, DV: 5–6.5 mm. DV measurements were made from the brain surface; Paxinos and Watson, 1998).

Intracellular recording

Intracellular microelectrodes were pulled from 1.0 mm outer diameter Omegadot tubing (WPI, New Haven, CT, USA) using a Flaming-Brown P-80/PC microelectrode puller (Sutter Instrument Company, Novato, CA, USA). The electrodes were filled with 3.0 M potassium acetate (electrode resistance = 55–90 M Ω *in situ*). Impalements were defined as stable if the resting membrane potential (RMP) was at least –55 mV and the action potential amplitude was at least 50 mV. The amplitude of the spike was measured from the RMP to the peak of the action potential. A headstage amplifier connected to a preamplifier (NEURODATA IR-283; Cygnus Technology, Delaware Water Gap, PA, USA) amplified signals. Current was injected across a bridge circuit, with electrode potentials and current injection amplitude monitored on an oscilloscope (BKA Precision Instruments, Placentia, CA, USA) using a Microstar board (Microstar Labs, Bellevue, WA, USA) as an interface to a PC computer. Recorded data were stored on the hard drive of the computer for subsequent off-line analysis. Analysis was performed using a custom-designed software program (Neuroscope). Input resistance, firing pattern, firing frequency, and membrane voltage oscillations were examined for each cell recorded. In addition, in some rats electrical stimulation was delivered into the MD or the VTA in order to identify cortical cells by antidromic stimulation of thalamic or VTA afferents.

Histology and statistics

At the end of the experiments the animals were given an overdose of chloral hydrate and perfused transcardially with saline followed by 10% buffered formalin. The brain was removed and placed in a solution of 15% sucrose at 4°C. Coronal slices 60 μm thick were cut using a freezing microtome and collected in phosphate buffer (1.0 M, pH 7.4). The slices were stained with Cresyl Violet to aid in the localization of the electrode track and the stimulating electrode. The basic electrophysiological properties of neurons recorded in the PFC of control or cocaine-treated animals were compared using a one-tailed, two-sample unequal-variance Student's *t*-test. All the data compared exhibited normal distributions. The

Fisher exact test was used for statistical comparison of bistability. The probability level for statistical significance was set at $P < 0.05$, all the results are presented as mean $\pm$ S.D. The presence of a bistable membrane potential (MP) was defined by rapid transitions of the MP occurring with a rise of 5 mV/ms or greater, and with depolarizations maintained for a minimum of 100 ms (O'Donnell and Grace, 1995). The bistability consisted of large-amplitude, spontaneous shifts in MP with the shifts occurring between two stable potentials separated by 6–19 mV. Furthermore, time histograms of the MP for individual cells were constructed. In each case, cells classified as bistable exhibited a bimodal distribution in the MP, and values clustered around each of the two modes could be fitted to respective normal curves with an r^2 of at least 0.95 (Fig. 1) (O'Donnell and Grace, 1995; Lewis and O'Donnell, 2000).

Results

A total of 32 cells in 25 rats were recorded in the deep layers of the PFC (prelimbic and infralimbic cortex). In 14 control animals, 15 cells were recorded, and 17 neurons were recorded in 11 animals pretreated with cocaine. Given that there were no statistically significant differences between contingent and non-contingent groups, the results were pooled together.

Using electrical stimulation of cortical afferents, 60% of the cortical cells recorded were identified as corticothalamic neurons and 7% as cortico-VTA cells. When the cells recorded in the PFC were separated according to their projection sites (VTA or MD) no significant differences were found in their basic electrophysiological properties, and thus all the cells were pooled in one group.

Consistent with previous studies (Steriade et al., 1993; Lewis and O'Donnell, 2000), 6/15 (40%) cells in the control group exhibited a bistable MP. The bistability consisted of a depolarized state with an average MP of -60.3 ± 9.9 mV and a hyperpolarized state with an average MP of -72.8 ± 8.8 mV. There was a significant difference in the time spent by the cells in the Up or Down state (369.3 ± 144.8 ms vs. 1368 ± 1605 ms, respectively; $P < 0.0003$, $t = -3.77$). These data indicate that the PFC bistable neurons are resting at hyperpolarized potentials and are interrupted by a barrage of synaptic inputs that bring the membrane to depolarized values.

In contrast, in the cocaine-treated animals none of the 17 neurons recorded in the PFC exhibited a bistable pattern ($P < 0.005$ versus control; Fisher exact test, Fig. 1). In fact, the cells recorded in the cocaine-treated animals exhibited a MP that was intermediate between the MP recorded during the Up and Down states in the control animals (Table 1). Moreover, there was a significant increase in the average values of the input resistance in the PFC neurons recorded from animals pretreated with cocaine (41.5 ± 22.5 M Ω in bistable control cells vs. 73.3 ± 27.3 M Ω in cocaine-pretreated animals, $P < 0.02$; Table 1). The increase in input resistance may indicate that repeated treatment with cocaine is blocking one or several major synaptic currents to pyramidal cells in the PFC. It is tempting to speculate that the synaptic currents blocked by the cocaine pretreatment are the currents underlying the switch to the Up state.

In the cocaine pretreated animals, the values of the spike threshold, the spike amplitude and the firing frequency were similar to the control cells that exhibited non-bistable patterns (Table 1).

Discussion

The most striking finding from the present report is the lack of membrane bistability in PFC neurons in rats withdrawn from repeated cocaine treatment. Consistent with previous reports using *in vivo* intracellular recordings in the PFC (Steriade et al., 1993; Lewis and O'Donnell, 2000), nearly 50% of pyramidal cells recorded from control animals exhibited bistability. The depolarized phase or Up state of the membrane polarization has a long duration (up to 800 ms) and the shift to the Up state is thought to arise from synchronous excitatory inputs that promote the capacity of the cell to generate action potentials (Steriade et al., 1993; Lewis and O'Donnell, 2000). The fact that not a single neuron recorded from cocaine-pretreated animals spontaneously entered the Up state indicates a defect in cortical integration of synaptic inputs. Importantly, all of the effects of repeated cocaine administration were found more than 2 weeks after the final injection, indicating that these are long-lasting consequences of cocaine administration.

Although the mechanisms underlying the lack of bistability in PFC neurons following cocaine treatment are not clear, there are data indicating that following short withdrawal times, spiny neurons in the nucleus accumbens exhibit a reduction in basal whole-cell Na^+ conductances (Zhang et al., 1998). According to Cowan and Wilson (1994), the MP shifts in corticostriatal neurons are governed by changes in the amount and effectiveness of excitatory synaptic inputs. Thus, a cocaine-induced reduction in Na^+ conductances in cortical neurons would contribute to a net decrease in the effectiveness of excitatory synaptic inputs and therefore a decrease in the ability of the cell to shift to the Up state.

Another possible mechanism underlying the lack of membrane bistability in cortical neurons recorded from cocaine-pretreated rats may involve changes in DA transmission on the PFC. It has been shown that DA contributes to the maintenance of the Up states in cortical neurons via D1-mediated mechanisms (Lewis and O'Donnell, 2000). Moreover, previous studies have shown long-lasting changes in prefrontal DA transmission following repeated cocaine administration, including a reduction in evoked DA release (Sorg et al., 1997) and DA content (Masserano et al., 1994; Hedou et al., 2000). Thus, the reduction in evoked DA release in the PFC of cocaine-pretreated animals could jeopardize the maintenance of the Up state and contribute to the loss of bistability after repeated cocaine treatment.

However, the presence of enduring cocaine-induced alterations in the electrophysiological properties of pyramidal cells in the PFC is consistent with previous data, demonstrating a role for corticostriatal glutamate transmission in addicted behaviors such as behavioral sensitization and drug-seeking behavior. The fact that basal levels of extra and intracellular glutamate are reduced in the nucleus accumbens following repeated cocaine is consistent with a lack of bistability and reduced action potential generation in cortical pyramidal cells (Pierce et al., 1996; Keys et al., 1998; Bell et al., 2000).

Conclusion

The present data indicate that long-term neuroadaptations are produced in PFC pyramidal cells by repeated cocaine treatment. The abolition of membrane bistability following repeated cocaine administration is consistent with reported hypofrontality in psychostimulant addicts (Jentsch and Taylor, 1999; Rogers et al., 1999; Ornstein et al., 2000; Grant et al., 2000). Thus, it appears that up to 3 weeks after withdrawal from repeated cocaine, synchrony and/or integration of glutamatergic inputs to PFC is severely disrupted and this condition could contribute to hypofrontality in cocaine-withdrawn subjects under resting conditions.

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Abbreviations

AP	anteroposterior
DA	dopamine
DV	dorsoventral
FR-1 schedule	fixed ratio schedule
L	lateral
MD	mediodorsal thalamus
MP	membrane potential

PFC	prefrontal cortex
RMP	resting membrane potential
VTA	ventral tegmental area

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Fig. 1.

Approximately 50% of the PFC pyramidal neurons recorded intracellularly *in vivo* in control anesthetized animals exhibit a bistable MP. (a) A representative trace from a cortical neuron showing transitions between a depolarized (-64.5 mV) and a hyperpolarized state (-84.5 mV). During the depolarized state action potential firing could occur. (b) The two polarized states exhibited by the neurons can be fitted to two normal functions with high coefficients of determination (r^2). (c) A representative trace from a non-bistable cortical neuron recorded in a control animal. The average resting MP of this cell was -64.8 mV. (d) The corresponding MP histogram shows a unimodal distribution with an average of -62 mV. (e) In rats treated with repeated administration of cocaine, none of the cortical cells recorded exhibited bistability. A trace from a cortical neuron recorded from a cocaine-treated animal. Membrane bistability was absent. The average RMP was -67 mV. (f) The MP histogram shows a unimodal distribution with an average of -65 mV.

Table 1
Basic electrophysiological characteristics of PFC neurons

	Control (<i>n</i> = 9)	Control bistable (<i>n</i> =6)	Cocaine (<i>n</i> =17)
Resting MP (mV)	-66.7 ±7.3 (58.4–81.9)		-70.0± 10.5 (51.2–85.6)
MP Up state (mV)		-57.4 ±6.0 (51.6–64.5)	
MP Down state (mV)		-76.0 ±4.7 (70.5–83.1)	
Duration Up state (ms)		369.3 ± 1144.8 (100–792)	
Duration Down state (ms)		1368 ± 1605^a (155–4751)	
Spike threshold (mV)	-49.1 ±6.5 (49.8–60.2)	-54.1 ±2.4 (51.4–58.3)	-52.7 ±4.4 (44.2–61.4)
Spike amplitude (mV)	55.2 ±7.3 (49–65.4)	61.1 ±8.7 (53.4–70.6)	50.9 ±6.8 (49.3–68.3)
Input resistance (MΩ)	61.3 ±22.0 (30–89)	41.5 ±22.5 (21–79)	73.3 ±27.3 (37.3–129) ^b
Firing frequency (Hz)	4.6 ±8.9 (0–27.8)	7.0 ±7.4 (1.6–20.9)	3.6 ±5.4 (0–24.1)
Bistability		6/15 (40%)	0/17 (0.0%)^c

^aStudent's *t*-test, *P* < 0.0003, comparing with duration of the Up state.

^bStudent's *t*-test, *P* < 0.02, comparing with control bistable.

^cFisher exact test, *P* < 0.05, comparing with control bistable.

Data are shown as mean±S.D