Repeated cocaine exposure increases fast-spiking interneuron excitability in the rat medial prefrontal cortex

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Campanac E, Hoffman DA. Repeated cocaine exposure increases fast-spiking interneuron excitability in the rat medial prefrontal cortex. J Neurophysiol 109: 2781–2792, 2013. First published March 13, 2013; doi:10.1152/jn.00596.2012.—The medial prefrontal cortex plays a key role in cocaine addiction. However, how chronic cocaine exposure affects cortical networks remains unclear. Most studies have focused on layer 5 pyramidal neurons (the circuit output), while the response of local GABAergic interneurons to cocaine remains poorly understood. Here, we recorded from fast-spiking interneurons (FS-IN) after repeated cocaine exposure and found altered membrane excitability. After cocaine withdrawal, FS-IN showed an increase in the number of spikes evoked by positive current injection, increased input resistance, and decreased hyperpolarization-activated current. We also observed a reduction in miniature excitatory postsynaptic currents, whereas miniature inhibitory postsynaptic current activity was unaffected. We show that, in animals with cocaine history, dopamine receptor D2 activation is less effective in increasing FS-IN intrinsic excitability. Interestingly, these alterations are only observed 1 wk or more after the last cocaine exposure. This suggests that the dampening of D2-receptor-mediated response may be a compensatory mechanism to rein down the excitability of FS-IN.

ADICTIVE DRUGS LIKE COCAINE act on the mesocorticolicmbic
dopamine (DA) system. The latter, also termed “reward system,” consists mainly of the ventral tegmental area (VTA) and its targets, the nucleus accumbens and prefrontal cortex (PFC). The PFC is involved in high cognitive processes, such as attention, working memory, and executive function, and also in several neurological disorders, including drug addiction (Goldstein and Volkow 2011; Tzschentke 2001). The PFC is known to be involved in the induction of the primary behavioral drug effects (e.g., increased locomotion, conditioned place preference, self-administration), as well as in drug-craving and relapse (Tzschentke 2001). For instance, direct electrical stimulation of the PFC in animals implanted with a stimulating electrode lead to rewarding effects (Mora 1978; Mora and Myers 1977; Phillips and Fibiger 1978; Robertson et al. 1982; Rolls and Cooper 1973) and enhanced the reward value induced by cocaine (Corbett 1991; McGregor et al. 1992; Moody and Frank 1990). Some studies also report that lesion of the PFC prevents cocaine-induced behavioral sensitization and associated neuroadaptations (Li et al. 1999; Pierce et al. 1998; Tzschentke and Schmidt 1998a, 1998b). In addition, there is evidence showing that a disruption in medial PFC (mPFC) neurotransmission systems, including dopaminergic, but also glutamatergic, GABAergic, acetylcholinergic, noradrenergic, serotoninergic, and peptidergic systems, may affect the development of psychostimulant-induced behavioral sensitization (Steketee 2003).

More specifically, psychostimulant effects of cocaine are mainly attributed to inhibition of the DA transporter that results in an increase of dopaminergic transmission (Sulzer 2011). Chronic drug exposure to psychostimulants like cocaine leads to adaptations in synaptic transmission and cellular intrinsic excitability within the reward circuit that may underlie compulsive drug consumption and relapse even after long periods of abstinence (Hu 2007; Hyman et al. 2006; Luscher and Malenka 2011). In the PFC, these modifications have been mostly characterized in the pyramidal neurons from the deep layer that constitutes the output of the cortical circuit (Ben-Shahar et al. 2009; Ford et al. 2009; Huang et al. 2007; Lu et al. 2009, 2010; McFarland et al. 2003; Nasif et al. 2005a; Steketee 2003). However, mesocortical dopaminergic fibers innervate both pyramidal neurons (Goldman Rakic et al. 1989; Verney et al. 1990) and GABAergic interneurons (IN) (Benes et al. 1993; Sesack et al. 1995). GABAergic IN are very heterogeneous with highly variable intrinsic firing rates (Markram et al. 2004) and degree of recruitment in cerebral rhythms and synaptic kinetics (Gupta et al. 2000). Different subtypes are defined according to immunohistochemical, morphological, and electrophysiological criteria (Ascoli et al. 2008; Cauil et al. 1997; Kawaguchi and Kondo 2002; Klausberger and Somogyi 2008; Markram et al. 2004). PARvalbumin (PV)-positive fast-spiking INs (FS-IN), which constitute one of the major subtypes of IN in cortical layer 5 (Markram et al. 2004; Rudy et al. 2010), synapse close to the soma of pyramidal neurons and other local IN (Kawaguchi and Kubota 1997, 1998).

In drug-naive animals, DA modulates IN activity (Gorelova et al. 2002; Tseng and O’Donnell 2007) and GABAergic synaptic transmission onto pyramidal neurons in the PFC (Gao et al. 2003; Seamans et al. 2001; Trantham-Davidson et al. 2004). Repeated cocaine exposure alters this DA modulation, probably due to a significant decrease in D3 receptors (D3R) expression and/or less effective receptors (Bowers et al. 2004; Briand et al. 2008; Kroener and Lavin 2010). However, little is known about how psychostimulant withdrawal affects GABAergic IN. We report here that both intrinsic excitability and basal synaptic transmission (spontaneous, action-potential independent transmission) are affected in FS-IN after long-term cocaine withdrawal, whereas no change is observed after...
short-term withdrawal. The increase of intrinsic excitability is associated with a decrease in the hyperpolarization-activated current ($I_{h}$) and basal excitatory synaptic transmission.

**Materials and Methods**

**Animals and Treatment**

All animal procedures were conducted in accordance with US National Institutes of Health guidelines, as approved by the National Institute of Child Health and Human Development Animal Care and Use committee. Male Sprague-Dawley rats (3 wk old) received repeated administration of cocaine (15 mg·kg$^{-1}$·day$^{-1}$ ip) or isosvolemic saline (0.9%) for 5 consecutive days, followed by 3–6 days (short-term withdrawal) or 10–13 days (long-term withdrawal) of abstinence prior to the experiment. Before each injection, animals were habituated for 10–15 min and handled approximately daily until the day of experiment.

**Slice Preparation and Electrophysiology**

Saline- or cocaine-treated rats were decapitated under chloral hydrate anesthesia (400 mg/kg ip), and the brain was immediately excised and immersed in ice-cold solution containing the following (in mM): 90 sucrose, 80 NaCl, 1.3 KCl, 1 NaH$_2$PO$_4$, 2 CaCl$_2$, 1 MgCl$_2$, 10 mM glucose, 3 mM pyruvic acid; pH 7.2–7.3; 310 mOsm/l. Coronal slices (300 μm) containing the mPFC were cut with a vibrotome (Leica VT1200S or DSK Microslicer DTK-1000). After recovery, incubation for ~15 min at 33°C was followed by ~45 min at 22°C in artificial cerebrospinal fluid (in mM): 125 K-gluconate, 20 KCl, 10 phosphocreatine (pH 7.2, 290 mOsm). For all currents, Kir, K$_{leak}$, or, K$_{ir}$ currents were isolated by subtracting traces obtained in ZD7288 from control traces. For analysis of activation curves, the tail current amplitudes ($I_{fast}$) were normalized to the maximal value ($I_{fast\, max}$) evoked by the command step at ~120 mV and plotted against the corresponding command step. The resulting data points were fit with a Boltzmann equation.

**Drugs**

All chemicals were purchased from Tocris (Ballwin, MO), Abcam (Cambridge, MA), or Sigma Chemical (St. Louis, MO).

**Data Acquisition and Analysis**

Electrophysiological recordings were obtained using a multiclamp 700B amplifier and PClamp 10 (Molecular, Devices, Sunnyvale, CA). Data were analyzed using Microsoft Excel, Minianalysis (Synaptosoft, Decatur, GA), and/or IGOR Pro (WaveMetrics, Lake Oswego, CA).
OR). Pooled data are presented as either mean ± SE or box plots, and statistical analyses were performed using Mann-Whitney U-test or the Wilcoxon test for paired data. P values are reported in the text or Figs. 2–7 with values < 0.05 considered as significant (*P < 0.05, **P < 0.01, ***P < 0.001).

Immunochemistry and Confocal Imaging

From acute slices. For morphological identification of FS-IN, neurons were filled with biocytin (0.2–0.4%). Additionally, the expression of the Ca2⁺-binding protein PV, a molecular marker of FS-IN, was also examined (Fig. 1A). After recording, slices were fixed with 4% paraformaldehyde in phosphate-buffered solution (PBS; 0.1 M, pH 7.3) overnight at 4°C. Following wash with PBS, slices were permeabilized and blocked with 0.3% Triton X-100, 5% goat serum, and 1% bovine serum albumin in PBS (blocking solution) for 1 h at 22°C. The blocking solution was also used to dilute primary and secondary antibodies. After incubation overnight at 4°C with a primary monoclonal antibody against PV (P3088, Sigma, St. Louis, MO, 1:1000) and streptavidin-Alexa 488 conjugated (Invitrogen, Carlsbad, CA, 1:1000), slices were washed in PBS and incubated 2 h with secondary antibody (AlexaFluor 546, 1:500; Invitrogen) and streptavidin-Alexa 488 (1:1,000). Slices were coverslipped with Mowiol and imaged. Slices were imaged using a LMS 510 confocal microscope (Carl Zeiss Microimaging, Thornwood, NY) with a ×40 oil objective. PV + FS-IN were selected and manually traced. D2R analysis was based on measuring the fluorescence along the cells’ perimeter. First PV + FS-IN perimeter was manually traced for maximum accuracy (on the PV immunostaining image), and then intensity of D2R was measured (on the D2R immunostaining image). Intensity was expressed in arbitrary units of fluorescence per micrometer. For the quantification, 8–10 PV + FS-IN, from 3 different slices, were used (3 saline and 3 cocaine animals).

All image processing was performed using Image-J (National Institutes of Health, Bethesda, MD) and Photoshop 10 (Adobe Systems, Mountain View, CA).

RESULTS

To determine whether chronic exposure to cocaine leads to electrophysiological changes in FS-IN, juvenile male rats were injected during 5 consecutive days with 15 mg/kg of cocaine or saline, and FS-IN were recorded in layer 5 of the prefrontal and infralimbic PFC after short- (3–6 days) or long-term (10–13 days) withdrawal (Fig. 2A).

Repeated Administration of Cocaine Increase FS-IN Excitability

The passive and active membrane properties of mPFC FS-IN were studied after short- (3–6 days) or long-term withdrawal...
Polarized after long-term withdrawal (Table 1). Neither did we observe a change in FS-IN input resistance (Fig. 2D). However, after long-term withdrawal, there was a significant leftward-shift in the input-output curve, indicating an increase in intrinsic excitability (Fig. 2C). Spike firing was increased in cocaine-injected animals, and the rheobase, defined as the minimal current to evoke a single spike, was significantly smaller (saline 258.60 ± 13.41 pA vs. cocaine 169.63 ± 13.46 pA, P < 0.001) (Table 1). First spike parameters such as onset time, threshold, rise time, afterhyperpolarization, and width were measured both at rheobase and 15-Hz firing. No significant changes in any of these parameters were observed after short- (data not shown) or long-term withdrawal (Table 1). Passive membrane properties measured by the input resistance at −100 pA were also affected by cocaine. Input resistance was significantly enhanced in cocaine animals after long-term withdrawal (Fig. 2D, Table 1). Resting membrane potential remained unaffected, although the cells were slightly more depolarized after long-term withdrawal (Table 1).

**Cocaine Alters I_h After Long-term Withdrawal**

Change in neuronal intrinsic excitability results from modification in neuronal intrinsic excitability (Spitzer 1999). Previous studies showed that, in layer 5 pyramidal neurons from cocaine-withdrawn animals, Na⁺ and Ca²⁺ spikes are increased (Dong et al. 2005; Ford et al. 2009; Nasif et al. 2005a) due to decreased voltage-gated K⁺ and K_ir currents (Dong et al. 2005; Nasif et al. 2005b). In addition, it was previously reported that DA increases FS-IN excitability by reducing different K⁺ currents, such as K_leak, K_ir, and K_m (Gorelova et al. 2002). We isolated and measured the major ionic currents expressed in FS-IN using voltage-clamp protocols and pharmacological manipulations. We found no difference in K⁺ current properties between groups (Fig. 3, A–C). Neither did we find changes in Ca²⁺ currents (Fig. 3D). The maximum current density recorded from all of these current exhibited no difference between the saline and cocaine groups (K_leak: saline 79.07 ± 18.53 pA/pF vs. cocaine 93.02 ± 22.86 pA/pF, P > 0.05; K_h + K_ir: saline −47.7 ± 3.61 pA/pF vs. cocaine −48.8 ± 2.77 pA/pF, P > 0.05; K_m: saline −21.84 ± 2.34 pA/pF vs. cocaine −23.23 ± 1.79 pA/pF, P > 0.05; Fig. 4A). We did, however, observe a significant decrease in the I_h after cocaine (saline −11.64 ± 0.86 pA/pF vs. cocaine −8.74 ± 1.13 pA/pF, P < 0.05; Fig. 4, A and B). No shift in the activation curve was noted (Fig. 4C and Table 2), and I_h kinetics were not affected in cocaine animals (Fig. 4D). These results suggest that I_h reduction is due to a decrease in the maximal conductance.

To confirm if the role of I_h on FS-IN intrinsic excitability and 2) its implication in increased excitability after long-term cocaine withdrawal, we measured the effect of I_h blockade, using its specific blocker ZD7288, on FS-IN membrane properties and input-output function in saline and cocaine animals after long-term withdrawal. We found that current/voltage and input/output curves are significantly affected by I_h blockade in saline animals. Suppression of I_h enhanced the voltage response to a given current step (Fig. 5A) and increased the number of spikes evoked by supralinear depolarizing current step (Fig. 5C). In contrast, there is no significant effect of I_h blockade in cocaine animals (Fig. 5, B and D). Our results

**Table 1. Passive and active membrane properties of FS-IN in layer 5 mPFC neurons from saline- and cocaine-treated rats after long-term withdrawal**

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Saline</th>
<th>Cocaine</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>19</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Passive membrane properties</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>RMP, mV</td>
<td>−78.03 ± 0.98</td>
<td>−75.76 ± 0.91</td>
<td>ns</td>
</tr>
<tr>
<td>Input resistance, MΩ</td>
<td>103.58 ± 4.65</td>
<td>131.25 ± 8.29</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Active membrane properties</td>
<td></td>
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<tr>
<td>Rheobase, pA</td>
<td>258.60 ± 13.41</td>
<td>169.63 ± 13.46</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>First spike parameters @ rheobase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset, ms</td>
<td>206.72 ± 51.79</td>
<td>258.78 ± 49.98</td>
<td>ns</td>
</tr>
<tr>
<td>Threshold, mV</td>
<td>−40.73 ± 1.07</td>
<td>−41.28 ± 3.36</td>
<td>ns</td>
</tr>
<tr>
<td>Rise time 10–90%, ms</td>
<td>0.29 ± 0.01</td>
<td>0.33 ± 0.04</td>
<td>ns</td>
</tr>
<tr>
<td>Maximum, mV</td>
<td>9.86 ± 1.38</td>
<td>13.01 ± 1.56</td>
<td>ns</td>
</tr>
<tr>
<td>AHP amplitude, mV</td>
<td>−21.72 ± 1.03</td>
<td>−19.79 ± 0.88</td>
<td>ns</td>
</tr>
<tr>
<td>Width, ms</td>
<td>0.56 ± 0.02</td>
<td>0.54 ± 0.01</td>
<td>ns</td>
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<tr>
<td>First spike parameters @ 15-Hz firing</td>
<td></td>
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<tr>
<td>Onset, ms</td>
<td>42.19 ± 16.87</td>
<td>70.02 ± 29.59</td>
<td>ns</td>
</tr>
<tr>
<td>Threshold, mV</td>
<td>−44.64 ± 1.35</td>
<td>−46.84 ± 1.44</td>
<td>ns</td>
</tr>
<tr>
<td>Rise time 10–90%, ms</td>
<td>0.34 ± 0.04</td>
<td>0.34 ± 0.03</td>
<td>ns</td>
</tr>
<tr>
<td>Maximum, mV</td>
<td>9.05 ± 1.75</td>
<td>11.16 ± 1.65</td>
<td>ns</td>
</tr>
<tr>
<td>AHP amplitude, mV</td>
<td>−15.78 ± 1.24</td>
<td>−16.32 ± 1.61</td>
<td>ns</td>
</tr>
<tr>
<td>Width, ms</td>
<td>0.57 ± 0.02</td>
<td>0.55 ± 0.00</td>
<td>ns</td>
</tr>
<tr>
<td>Accommodation index</td>
<td>13.08 ± 4.01</td>
<td>5.36 ± 1.34</td>
<td>ns</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of neurons (saline, n = 19, 8 rats; cocaine, n = 18, 7 rats). RMP, resting membrane potential; AHP, afterhyperpolarization. Parameters of the first spike were measured during step current injections corresponding at the rheobase or when neurons fired at approximately 15 Hz (i.e., 12–18 action potentials during the 1-s current step). Accommodation is defined by (t_spike n − t_spike 1)/(t_spike n − t_spike n−1), where t is time. *Significant difference. ns, Not significant.
therefore indicate that \( I_h \) plays an important role in FS-IN intrinsic excitability and is altered in cocaine animals after long-term withdrawal.

Cocaine Effects on Basal Synaptic Transmission After Long-term Withdrawal

In the mPFC, cocaine is known to induce many changes in neurotransmission (Steketee 2003), and synaptic background activity has been shown to influence the neuronal input/output function (Destexhe and Pare 1999; Wolfart et al. 2005). Therefore, we examined potential effects of cocaine on basal synaptic transmission by recording mEPSCs from FS-IN from saline- and cocaine-injected rats (Fig. 6). The amplitude of mEPSCs was decreased in cocaine animals compared with saline-treated animals, indicating that \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptor properties and/or expression were affected by cocaine exposure (saline 16.99 ± 0.92 pA vs. cocaine 14.36 ± 0.70 pA, \( P < 0.05 \)) (Fig. 6, A and B). Interestingly, we found more small mEPSCs (<10 pA, \( P < 0.05 \)) and fewer large mEPSCs (>80 pA, \( P < 0.05 \)) in cocaine-injected animals (Fig. 6B, right). The frequency of mEPSCs was also affected by cocaine exposure with the number of events per second being significantly decreased in cocaine animals (saline 8.74 ± 0.85 Hz vs. cocaine 6.81 ± 1.17 Hz, \( P < 0.05 \)) (Fig. 6C).

FS-IN receive also local inhibitory inputs, we also measured inhibitory activity. We found no change in either mIPSC amplitude or frequency (amplitude: saline 13.33 ± 1.16 pA vs. cocaine 13.49 ±1.57, \( P > 0.05 \); frequency: saline 3.39 ± 0.35 vs. cocaine 3.39 ± 0.36, \( P > 0.05 \)) (Fig. 6, D and F).

Altered D\(_2R\) Intrinsic Excitability Modulation After Long-term Cocaine Withdrawal

Consistent with previous studies, bath application of the D\(_2\)R agonist quinpirole increased spike firing evoked by a positive step of current in FS-IN after long-term withdrawal (Kroener and Lavin 2010; Tseng and O’Donnell 2007). During short-term withdrawal, no difference was observed in the enhancement of spike numbers after 5 min quinpirole between saline and cocaine groups (saline 179 ± 4% vs. cocaine 184 ± 4%, \( P > 0.05 \)) (Fig. 7, A and B). However, after long-term withdrawal, cocaine-injected animals were found to be less sensitive to quinpirole application (saline 261 ± 6% vs. cocaine 197 ± 5%, \( P < 0.05 \)) (Fig. 7, C and D). These data suggest that, as for our observed changes in intrinsic excitability (Fig. 2, Table 1), changes in D\(_2\)R activity occurred only after a long withdrawal period.

It was previously shown that D\(_2\)R expression and/or activity is decreased in mPFC after cocaine exposure (Briand et al. 2008). However, in this study, mPFC was analyzed in its...
entirety. Therefore, we examined D2R expression after long-term withdrawal specifically in layer 5 FS-IN with double immunohistochemistry labeling for D2R and PV, a specific cell maker for FS-IN. As expected D2R labeling was mostly found at the cell periphery (Vincent et al. 1995) (Fig. 7E). After long-term withdrawal, D2R expression in FS-IN+ and PV+ neurons from cocaine-injected animals was decreased compared with saline-injected animals (saline 131 ± 7 vs. cocaine 105 ± 5, P < 0.01) (Fig. 7F). This observation is consistent with our previous results showing that intrinsic excitability modulation induced by D2R agonist application is less effective in cocaine animals compared with saline animals (Fig. 7, C and D).

We next tested the effect of D2R activation on Ih, which we showed here to be crucial in FS-IN intrinsic excitability. We measured Ih in control conditions and after quinpirole application after long-term cocaine withdrawal. In both saline and cocaine-treated animals, quinpirole induced a significant reduction in H-current density (Fig. 7G). However, this reduction is significantly larger in saline compared with cocaine animals (Fig. 7H). Taken together, our data suggest that, after long-term cocaine withdrawal, the ability of D2R to control FS-IN activity and H-current are reduced due to a decrease in D2R expression.

DISCUSSION

In this study, we have examined the excitability of FS-IN in the mPFC after a period of short-term or long-term cocaine withdrawal. We found that modifications in FS-IN occurred only after long-term withdrawal. Indeed, no significant changes are observed during short-term withdrawal. We showed that 5 days of

<table>
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<tr>
<th>Pretreatment</th>
<th>Saline</th>
<th>Cocaine</th>
<th>P Value</th>
</tr>
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<tbody>
<tr>
<td>V1/2, mV</td>
<td>−77.19 ± 1.03</td>
<td>−78.80 ± 1.32</td>
<td>ns</td>
</tr>
<tr>
<td>Slope factor</td>
<td>14.04 ± 1.02</td>
<td>15.26 ± 1.35</td>
<td>ns</td>
</tr>
<tr>
<td>τ Activation @ −120 mV, ms</td>
<td>36.62 ± 6.41</td>
<td>47.23 ± 4.84</td>
<td>ns</td>
</tr>
<tr>
<td>Ô inactivation @ −5 mV, ms</td>
<td>26.21 ± 4.05</td>
<td>29.02 ± 4.30</td>
<td>ns</td>
</tr>
<tr>
<td>Ih density at −110 mV, pA/pF</td>
<td>−8.35 ± 0.58</td>
<td>−6.08 ± 0.75</td>
<td>&lt;0.05*</td>
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</table>

Values are means ± SE. V1/2, half-activation voltage; τ, time constant; Ih, hyperpolarization-activated current. *Significant difference.
repeated cocaine exposure followed by 10–13 days of withdrawal facilitated spike firing, reduced $I_{h}$ current, decreased mEPSC activity, and reduced D_{2}R expression in FS-IN.

**FS-IN Intrinsic Plasticity Induced By Cocaine Exposure**

Withdrawal from repeated cocaine exposure is associated with altered membrane properties of FS-IN in the mPFC. It has been previously shown that chronic cocaine increased mPFC intrinsic excitability of L5 pyramidal neurons by modulating K^+ and Ca^{2+} channel properties (Dong et al. 2005; Ford et al. 2009; Nasif et al. 2005a, 2005b). This increased intrinsic excitability has also been attributed to a decrease in GABAergic inhibition by reducing the surface expression of GABA_{A} receptors in juvenile cocaine-treated animals or after in utero cocaine injection (Huang et al. 2007; Lu et al. 2009, 2010). Unlike pyramidal neurons that exhibit these cocaine-induced electrophysiological adaptations after only 3 days of withdrawal, we observed changes in FS-IN appeared after several days of cocaine withdrawal (requiring more than 6 days). This increase in FS-IN excitability is consistent with the reported increase in extracellular concentration of GABA and GABAergic transmission in the mPFC during cocaine withdrawal, as previously described (Jayaram and Steketee 2005).

**Mechanisms of FS-IN Increased Intrinsic Excitability After Cocaine Exposure**

We observed cocaine-induced changes in FS-IN firing, along with a decrease in $I_{h}$. Pharmacological $I_{h}$ blockade significantly affected current/voltage and input/output curves in saline animals, while there was no significant effect of $I_{h}$ blockage in cocaine animals (Fig. 5, B and D). Our results, therefore, indicate that $I_{h}$ plays an important role in FS-IN intrinsic excitability and is altered in cocaine animals after long-term withdrawal. Although we did not observe significant changes in any other ionic currents measured (Figs. 3 and 4A), the discrepancy between the magnitude of firing frequency change after long-term cocaine withdrawal (Fig. 2C) and that found after $I_{h}$ block (Fig. 3C) may indicate that other unresolved factors are involved.

In FS-IN, $I_{h}$ controls the resting membrane potential and the input resistance, and therefore shapes the dendritic integration and the input-output function (Aponte et al. 2006). $I_{h}$ also plays...
an important role in rhythmogenesis (Aponte et al. 2006). Recent studies showed that regulation of \( I_h \) might contribute to drug sensitization neuroadaptations. For instance, an increase in VTA DA neuron excitability is observed in association with a downregulation of \( I_h \) after withdrawal from repeated ethanol (but see also Bandyopadhyay and Hablitz 2007; Hopf et al. 2007; Okamoto et al. 2006) or cocaine exposure (Arencibia-Albite et al. 2012). Our observed reduction in \( I_h \) amplitude is caused by a reduced number of H-channels at the cell membrane, as we found no change in its activation curve or kinetic properties.

It has been previously shown that stimulation of D2R in VTA neurons produces a cAMP-independent decrease in the maximal conductance of HCN currents without changing the activation voltage dependence (Jiang et al. 1993). This mechanism is relatively unlikely here, since \( I_h \) downregulation was associated with decreased D2R expression. One possible explanation of our results would be via the activation of protein kinase C. It has been previously shown that inhibition of \( I_h \) by neuromodulators involves protein kinase C (Cathala and Paupardin-Tritsch 1997). Regulation by other kinases, like protein kinase A or calcium/calmodulin kinase II is also possible, since H-channels have a putative site for phosphorylation (Santoro et al. 1997; Zong et al. 2005) and D1/D2R pathways involve many different kinases (Beaulieu and Gainetdinov 2011). It has been previously reported that DA through D1R and/or D2R affects K\(^+\) currents (Dong et al. 2004, 2005; Gorelova et al. 2002). For instance, activation of D2R suppresses a Cs-sensitive \( K_w \) and a resting \( K_{\text{leak}} \) in FS-IN (Gorelova et al. 2002). However, we did not find any change in K\(^+\) currents after cocaine exposure in this study.

Chronic cocaine is well known to induce changes in glutamatergic neurotransmission within the mesocorticolimbic system (Bowers et al. 2010). Here, we reported a decrease in mEPSC amplitude in cocaine-treated animals that might be the consequence of a downregulation of AMPA receptors (AMPARs) at the FS-IN cell surface. A decrease in the number of AMPARs is consistent with the increased input resistance that we observed. It has also been shown that DA regulates AMPAR surface and synaptic expression in pyramidal neurons of the PFC (Sun et al. 2005). The reduction in mEPSC frequency could also reflect a presynaptic effect from the L2–3

Fig. 6. Amplitude and frequency of miniature excitatory postsynaptic currents (mEPSCs) and miniatures inhibitory postsynaptic currents (mIPSCs) in FS-IN following long-term cocaine withdrawal. A: average mEPSC (scale bars: 5 ms/10 pA) and sample traces of mEPSCs (scale bars: 10 s/10 pA) recorded in saline (black) or cocaine-injected (gray) rats. Graph is shown for pooled data values and graph plotting cumulative probability of mEPSC amplitude (B) and frequency (C) (saline, \( n = 25, 4 \) rats; cocaine, \( n = 26, 5 \) rats). D: average mIPSC (scale bars: 5 ms/10 pA) and sample traces of mIPSCs (scale bars: 10 s/10 pA) recorded in saline (black) or cocaine-injected (gray) rats. Graph is shown for pooled data values and graph plotting cumulative probability of mIPSC amplitude (E) and frequency (F) (saline, \( n = 20, 3 \) rats; cocaine, \( n = 19, 3 \) rats). Significant difference: \(* P < 0.05, ** P < 0.01.\)
or L5 pyramidal neurons, the two main glutamatergic input into FS-IN. Bidirectional changes such as increased excitability vs. reduced synaptic input might result from homeostatic compensation mechanisms.

**Altered DA Modulation After Cocaine Exposure**

DA controls cell excitability and synaptic transmission in the PFC (Seamans and Yang 2004), and, as a result, DA also regulates local network activity (Bandyopadhyay and Hablitz 2007). DA receptors thus represent an important subject of interest in drug addiction research (Di Chiara and Bassareo 2007; Le Foll et al. 2009; Nicola et al. 2000). For instance, D₂R activation plays an important role in cocaine sensitization. Intracortical D₃R agonist injection in the mPFC blocked the initiation and blunted the expression of cocaine-induced behavioral and neurochemical sensitization (Seamans and Yang 2004). As with L5 pyramidal neurons, FS-IN express both D₁ and D₂ classes of DA receptors (Gaspar et al. 1995; Vincent et al. 1995, 1993). D₁-class DA receptors (D₁R and D₅R) activate the Gₛ family of G proteins to stimulate cAMP production by adenylate cyclase, whereas D₂-class DA receptors (D₂R, D₃R, and D₄R) couple to Gᵢ/o family of G proteins and thus induce inhibition of adenylate cyclase. In neurons, through its Gᵢ/o subunits, D₂-class DA receptors are known to directly

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**Fig. 7.** FS-IN spike-firing modulation by D₂ receptors (DR2) after short- and long-term cocaine withdrawal. Representative traces show the effects of quinpirole (10 μM for 5 min) on FS-IN spike-firing in saline- and cocaine-treated animals following short- (A) or long-term withdrawal (C). Time course is shown of normalized spike number during quinpirole bath application after short- (B: saline, n = 13, 7 rats; cocaine, n = 12, 5 rats) or long-term withdrawal (D: saline, n = 9, 5 rats; cocaine, n = 8, 6 rats). Note that quinpirole is less effective in cocaine animals after long-term withdrawal. E: representative images for DR2 and parvalbumin immunostaining for saline- and cocaine-injected animals after long-term withdrawal (green, DR2; red, parvalbumin; yellow, overlapping signals). Insets, higher magnification of soma pointed by arrows. Scale bar: 50 μm, 10 μm inset. F: quantification of DR2 expression in parvalbumin⁺ cells (saline, n = 85, 3 rats; cocaine, n = 84, 3 rats). G: examples of whole-cell traces of Iₕ and current density graph from saline (black) or cocaine-pretreated (gray) animals after long-term withdrawal in control (filled circle) or after quinpirole (10 μM) application (open circle) (saline, n = 14, 4 rats; cocaine, n = 15, 4 rats). H: quantification of H-current reduction. Significant difference: *P < 0.05, **P < 0.01, ***P < 0.001.
regulate L- and N-type calcium channels, as well as some K⁺ channels (Beaulieu and Gainetdinov 2011). It is difficult to predict the effect of DA on neurons, as the final effect will depend on the dynamic interaction between DA receptors. For instance, a previous study showed that DA concentration is a critical determinant for cortico inhibition. Low DA concentration activated D₁R, whereas a high concentration activated both D₁ and D₂R (Trantham-Davidson et al. 2004).

An interesting finding in our study is the altered DA modulation in cocaine-treated rats. Our data confirmed that, after long-term withdrawal, FS-IN D₂R-mediated increase of current-evoked firing is reduced (Kroener and Lavin 2010). Like changes in spike firing and input resistance, changes in D₂R modulation are only observed after long-term withdrawal, suggesting that D₂Rs are responsible for these changes in excitability. Accordingly, while we found the D₂R agonist quinpirole to reduce Iᵣ in both saline- and cocaine-treated animals after long-term withdrawal, the effect was significantly greater in saline conditions (Fig. 5, G and H).

Physiological and Functional Relevance of Altered Inhibition


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Author Contributions

Author contributions: E.C. and D.A.H. conception and design of research; E.C. performed experiments; E.C. analyzed data; E.C. interpreted results of experiments; E.C. prepared figures; E.C. drafted manuscript; E.C. and D.A.H. edited and revised manuscript; E.C. and D.A.H. approved final version of manuscript.

References


Pierce RC, Reeder DC, Hicks J, Morgan ZR, Kalivas PW. Ibotenic acid lesions of the dorsal prefrontal cortex disrupt the expression of behavioral sensitization to cocaine. *Neuroscience* 82: 1103–1114, 1998.


Rolls ET, Cooper SJ. Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. *Dev Neurobiol* 71: 45–61, 2011.


