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Plasticity of L-Type Ca^{2+} Channels After Cocaine Withdrawal

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Abstract

Increased reactivity of certain frontal cortical brain regions to cocaine re-exposure or drug-associated cues in cocaine-abstinent human addicts is linked to drug craving. Similarly, in rats tested after withdrawal from repeated cocaine exposure, cocaine or other strong excitatory stimuli produce greater activation of pyramidal neurons in the medial prefrontal cortex (mPFC). Our recent findings indicate that the increased mPFC neuronal activation depends primarily upon enhanced voltage-sensitive Ca^{2+} influx, most likely through high-voltage activated (HVA) L-type Ca^{2+} channels, but the mechanism underlying the enhanced Ca^{2+} currents is unknown. In this study, we used a protein crosslinking assay to show that repeated cocaine injections, resulting in behavioral sensitization, increased total protein levels and cell surface expression of HVA- $\text{Ca}_v1.2$ L-type channels in pyramidal neurons in deep layers of the mPFC. These changes in $\text{Ca}_v1.2$ L-channels were time-dependent and subtype-specific (i.e., differed from those observed for $\text{Ca}_v1.3$ L-channels). Furthermore, we found enhanced PKA activity in the mPFC of cocaine-sensitized rats that persisted for 21 days after withdrawal. PKA phosphorylation of L-channels increases their activity, so Ca^{2+} currents after cocaine withdrawal could be enhanced as a result of both increased activity and number of HVA- $\text{Ca}_v1.2$ L-channels on the cell surface. By increasing the supra-firing threshold excitability of mPFC pyramidal neurons, excessive upregulation of HVA L-channel activity and number may contribute to the cortical hyper-responsiveness that enhances vulnerability to cocaine craving and relapse. More generally, our results are the first to demonstrate that repeated cocaine exposure alters the membrane trafficking of a voltage-sensitive ion channel.

Introduction

The prefrontal cortex (PFC) plays a critical role in regulating motivated behaviors and drug addiction (Kalivas and Volkow, 2005; Castner and Williams, 2007; Everitt et al., 2007). Brain imaging studies in human cocaine addicts reveal that neuronal activity in certain frontal cortical regions is dramatically decreased during cocaine abstinence, but increased by re-exposure to psychostimulants or stimulant-associated cues (a.k.a. hypo- and hyper-activity, respectively) (Goldstein and Volkow, 2002). PFC hyperactivity in response to drug-associated stimuli may be magnified by basal hypoactivity. Similarly, rats tested after repeated cocaine exposure exhibit hypoactivity of the PFC during withdrawal, but increased activity when re-exposed to cocaine/cues (Febo et al., 2005; Rebec and Sun, 2005; Sun and Rebec, 2006).

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Although the mechanisms underlying PFC hyperactivity are unclear, enhanced Ca^{2+} channel function is a strong candidate. We have demonstrated that pyramidal neurons recorded from the medial PFC (mPFC) of cocaine-sensitized rats after 3 or 21 days of withdrawal exhibited significantly increased firing (Na^+ -dependent action potentials), prolonged Ca^{2+} plateau potentials, and enhanced Ca^{2+} currents (I_{Ca}) (Nasif et al., 2005a,b). Furthermore, selective blockade of L-type Ca^{2+} channels in these cells reduced the prolonged duration of evoked Ca^{2+} spikes to levels comparable to those observed in saline-pretreated control rats (Nasif et al., 2005a).

These results implicated enhancement of I_{Ca} via L-channels in the increased supra-firing threshold excitability of mPFC pyramidal cells associated with cocaine sensitization. However, whether the enhanced I_{Ca} resulted from increases in the activity, total expression, or cell surface targeting of L-channels was unknown. Furthermore, the L-channel subtype affected by cocaine was not determined. The major subtypes of L-channels in neurons are categorized as HVA- $\text{Ca}_v1.2 \alpha_{1C}$ and $\text{Ca}_v1.3 \alpha_{1D}$ L-channels, which activate at more hyperpolarized membrane potential levels like the T-type Ca^{2+} channels (Lipscombe, 2002). Because each subtype of L-channel plays a distinct role in regulating membrane excitability and other neuronal functions, determining their relative contribution in cocaine-sensitized rats is critical for understanding the functional implications of enhanced I_{Ca} for mPFC pyramidal neurons.

In the present study, we determined total levels of $\text{Ca}_v1.2 \alpha_{1C}$ and $\text{Ca}_v1.3 \alpha_{1D}$ L-channels in mPFC tissue from cocaine-sensitized rats after 3 or 21 days of withdrawal. We also used a protein crosslinking assay to quantify intracellular and cell surface L-channel levels. Finally, we measured PKA activity in the mPFC of cocaine-sensitized rats because PKA phosphorylation activates L-channels (De Jongh et al., 1996).

Materials and Methods

Animals and behavioral studies

Young male Sprague-Dawley rats (4-5 weeks of age) were acclimated to the vivarium for 3 days before any treatment. They were randomly divided into two groups that received saline (~0.1 ml) or cocaine (15 mg/kg, i.p.) once-daily for 5 consecutive days. Rats were withdrawn in home cages for 3 days (3d/w, short-term) or 21 days (21d/w, long-term) and then used for behavioral, electrophysiological, and biochemical assessments. In behavioral tests, rats with a 3d/w were individually placed in photobeam test cages (San Diego Instruments; San Diego, CA; see Boudreau and Wolf, 2005 for details) and given a challenge dose of cocaine (7.5 mg/kg, i.p.). Ambulation counts, a measure of horizontal locomotor activity, were monitored for 1 hour. Rats used for biochemical experiments were killed on the last day of withdrawal.

PKA assay

The mPFC and motor cortex tissues from pretreated rats were dissected and lysed in hypotonic buffer [10mM HEPES, pH 7.9, 1.5 mM MgCl_2 , 10 mM KCl, 1 mM DTT, 25 μM (-)-p-bromotetramisole oxalate, 5 μM cantharidin, 5 μM microcystin-LF, 5 μM cyclosporin A], and then supplemented with Complete Protease Inhibitor tablets (Roche Diagnostics). Activity of cyclic AMP-dependent protein kinase (PKA) in 2 μg of each sample was determined by the PepTag PKA assay (Promega). The positive controls contained 10ng of purified PKA catalytic subunit, whereas negative controls contained no PKA. The PepTag assay used the Leu-Arg-Arg-Ala-Ser-Leu-Gly (Kemptide) peptide substrate tagged with a UV-fluorescent dye. PKA activity was measured based on the amount of phosphorylated

substrate migrating toward the anode. Quantification was performed by calculating luminence intensity using TotalLab software (Nonlinear Dynamics).

Protein crosslinking assay

Bis-sulfosuccinimidyl-suberate (BS³) is a protein crosslinking reagent that does not penetrate membranes and therefore crosslinks cell surface but not intracellular proteins. Crosslinked surface proteins form high molecular weight aggregates that can be distinguished from unmodified intracellular proteins by SDS-PAGE and Western blotting. We have previously shown that BS³ crosslinking can be used to detect redistribution of proteins between intracellular and surface compartments following *in vivo* treatments (Boudreau and Wolf, 2005). The mPFC tissues from saline or cocaine-pretreated rats were dissected on ice from 1 mm thick coronal sections corresponding to the region 3.2–2.2 mm anterior to Bregma. Dissected tissues were chopped on a tissue chopper to increase surface area exposed to BS³ and placed into ice-cold aCSF [(in mM): 20 HEPES, 147 NaCl, 2.7 KCl, 1 MgCl₂, 1.2 CaCl₂, 10 glucose] that was then spiked with BS³ (final concentration: 2mM). Samples were incubated at 4°C for 30 min with gentle agitation. Crosslinking was terminated by quenching the reaction with 100 mM glycine for 10 min. Tissues were pelleted by brief centrifugation and resuspended in ice-cold lysis buffer containing protease and phosphatase inhibitors [25 mM HEPES, pH 7.4, 500 mM NaCl, 2 mM EDTA, 1 mM DTT, 0.1% Tween-20, 1 mM PMSF, 20 mM NaF, 25μM (-) *p*-bromotetramisole oxalate, 5 μM cantharidin, 5 nM cirocystin-LF, 5 μM cyclosporin A and Complete Mini Protease Inhibitor Tablets]. Samples were sonicated (5 sec) and stored at -80°C. Total protein content was determined with a modified Lowry assay.

Immunoblotting

Samples (20–30μg total protein) were loaded and electrophoresed on 4–12% Tris-HCl gels, and transferred onto PVDF membranes for immunoblotting. Membranes were blocked for 1 hour at room temperature with 1% normal goat serum and 5% nonfat dry milk in TBS-T (Tris-Buffered Saline with 0.05% Tween-20) and incubated with primary antibody overnight at 4°C. The following primary antibodies recognizing Ca²⁺ channel α1 subunits were utilized (all from Chemicon; Temecula, CA): L-channel subtype-selective antibodies (anti-Ca_v1.2 α_{1C}, 1:6000 and anti-Ca_v1.3 α_{1D}, 1:500), and antibodies to other channel types [P/Q-type (Ca_v2.1), N-type (Ca_v2.2), R-type (Ca_v2.3), and T-type (Ca_v3); all 1:500]. Membranes were washed extensively in TBS-T, incubated with HRP-conjugated secondary antibody, and washed again in TBS-T. Immunoreactive bands were visualized with enhanced chemiluminescence (ECL) detection solutions and ECL Hyperfilm. Films were scanned with a Scanjet 5400c and calibrated to a scanned step wedge of known optical densities. BS³-crosslinked surface proteins of high molecular weight and unmodified intracellular proteins of normal molecular weight were analyzed using Phoretix TotalLab software (Nonlinear Dynamics; Durham, NC). Values for surface, intracellular and total levels were normalized to total protein in the lane (determined by Ponceau S staining). We used two different approaches to measure total α1 subunit levels in mPFC or motor cortex of each rat. First, we summed intracellular and surface values obtained from analysis of crosslinked tissue. Second, we analyzed non-crosslinked tissue from each rat (tissue from one side of the brain was not crosslinked). The latter value was used in all figures, but similar results were obtained by summing surface and intracellular bands of crosslinked samples. Data from cocaine-pretreated rats were normalized to saline control values.

Statistics

For all experiments, results from saline- and cocaine-pretreated rats were compared using either a Student's *t*-test (paired or unpaired) or repeated measures ANOVA (rmANOVA). Significant differences between saline- and cocaine-pretreated groups were expressed as

* $p < 0.05$ or ** $p < 0.01$. The numbers inserted in the bars of some graphs indicate N (number of rats).

Results

Repeated cocaine administration induces behavioral sensitization

We showed previously that I_{Ca} via L-channels was significantly enhanced in mPFC pyramidal cells of cocaine-sensitized rats after 3 or 21 days of withdrawal (Nasif et al., 2005a,b). Here we tested the contribution of altered expression, activation and/or trafficking of $Ca_v1.2 \alpha_{1C}$ and $Ca_v1.3 \alpha_{1D}$ L-channels to this effect. Rats were pretreated with the same cocaine sensitization regimen used in our previous studies (Nasif et al., 2005a,b). Fig. 1 shows that repeated cocaine exposure significantly increased the locomotor responsiveness to cocaine challenge (repeated measures ANOVA, $F_{(1,23)} = 6.24$, $p < 0.05$), verifying that rats in the present study were sensitized to cocaine.

Increased PKA activity in the mPFC of cocaine-sensitized rats

PKA-induced phosphorylation facilitates L-channel activity and consequently increases I_{Ca} through the channel (De Jongh et al., 1996). To determine if this contributes to the increased I_{Ca} in cocaine-sensitized rats, we measured PKA activity in mPFC and motor cortex (a control region) of saline- or cocaine-pretreated rats (see Fig. 2A for schematic of dissections). PKA activity was significantly increased in the mPFC after both a 3-day and 21-day cocaine withdrawal (unpaired Student's t -test, both $p < 0.05$) (Fig. 2B), but was unchanged in the motor cortex (unpaired t -test, $p > 0.05$) (Fig. 2C). These findings support the hypothesis that the increased PKA activity contributes to enhanced I_{Ca} in mPFC pyramidal cells after cocaine withdrawal (Nasif et al., 2005a,b).

Different alterations in $Ca_v1.2 \alpha_{1C}$ and $Ca_v1.3 \alpha_{1D}$ L-channel expression and distribution after repeated cocaine exposure and withdrawal

We used Western blotting to examine protein levels of voltage-sensitive Ca^{2+} channels in the rat mPFC. Consistent with our earlier results showing enhanced I_{Ca} through the L-channels in mPFC pyramidal cells of cocaine-withdrawn rats (Nasif et al., 2005a), we found a significant increase in total protein levels of the $Ca_v1.2$ L-channel α_1 subunit (the "pore"-forming and ligand-binding protein) in the mPFC after a 3-day withdrawal (unpaired Student's t -test, * $p < 0.05$) (Fig. 3B). However, no changes were found in the α_1 subunit levels of T-, N-, P/Q- and R-type Ca^{2+} channels in the mPFC of cocaine-withdrawn rats (data not shown) (all $p > 0.05$). Together, these results indicate a *subtype-specific* effect of chronic cocaine exposure on Ca^{2+} channel expression.

To determine whether the increased L-type Ca^{2+} channels were expressed on the cell surface, and thus part of the functional pool, we used a BS³ protein crosslinking assay to evaluate possible changes in L-channel trafficking in the mPFC after withdrawal from repeated cocaine exposure. This assay, which enables quantification of surface-expressed proteins after *in vivo* manipulations based on their selective modification with a protein crosslinking reagent that does not cross membranes (Boudreau and Wolf, 2005; Boudreau et al., 2007; Conrad et al., 2008; see Methods for more details), was adapted from an approach used to quantify glutamate receptor surface expression in dissociated cells and brain slices (e.g., Hall et al., 1997; Grosshans et al., 2002). Subtype selective antibodies that distinguish the $Ca_v1.2 \alpha_{1C}$ and $Ca_v1.3 \alpha_{1D}$ subunits of the L-channel were used. Control experiments demonstrated that both the surface and intracellular bands were eliminated by preabsorption of the antibodies with their cognate peptides (Fig. 2A) and that optical density of both bands increased proportionately when increasing amounts of protein were loaded (data not shown). We found that a 3-day withdrawal from repeated cocaine exposure increased the total and

intracellular (unpaired *t*-test, $p < 0.01$), but not surface ($p > 0.05$), levels of $\text{Ca}_v1.2 \alpha_{1C}$ subunits in the mPFC (Fig. 3B). However, after a 21-day withdrawal, their surface expression was significantly increased ($p < 0.05$) (Fig. 3C). In contrast, there were no changes in the total, surface or intracellular $\text{Ca}_v1.3 \alpha_{1D}$ subunit levels in cocaine-pre-exposed rats after a 3-day withdrawal (all $p > 0.05$) (Fig. 3D). However, the total and intracellular $\text{Ca}_v1.3 \alpha_{1D}$ subunit levels were increased significantly in the cocaine-exposed mPFC after a 21-day withdrawal ($p < 0.01$, $p < 0.05$, respectively), and there was a trend towards increased surface expression as well (Fig. 3E). These results show that repeated cocaine exposure affects both $\text{Ca}_v1.2 \alpha_{1C}$ and $\text{Ca}_v1.3 \alpha_{1D}$ L-channels but exerts more rapid effects on $\text{Ca}_v1.2$ L-channels.

Discussion

Our results demonstrate that withdrawal from repeated cocaine exposure is associated with increased total expression and cell surface expression of HVA- $\text{Ca}_v1.2 \alpha_{1C}$ subunits in the mPFC of cocaine-sensitized rats. A more slowly-developing increase in total expression of $\text{Ca}_v1.3 \alpha_{1D}$ subunits was also observed, along with a trend towards increased surface expression. Repeated cocaine treatment did not alter L-type Ca^{2+} channel expression in motor cortex, nor did it alter other Ca^{2+} channel subtypes (T-, N-, P/Q- and R-type) in the mPFC. Thus, cocaine sensitization was associated with time-dependent, region-specific, and subtype-specific adaptations in L-type Ca^{2+} channel function. Though L-channel trafficking has been studied previously *in vitro* (Jarvis and Zamponi, 2007), this is, to our knowledge, the first report of a change in the surface expression of L-channels or any other voltage-gated ion channel after cocaine withdrawal or any other *in vivo* manipulation.

Both $\text{Ca}_v1.2 \alpha_{1C}$ and $\text{Ca}_v1.3 \alpha_{1D}$ L-channels are abundant in cortical neurons (Lipscombe, 2002). Due to their distinct properties, the two subtypes of L-type Ca^{2+} channels function independently to regulate membrane potentials (V_m) of neurons at different levels. The HVA- $\text{Ca}_v1.2$ L-channels, which are activated at more depolarized ("high-voltage") V_m (e.g., beginning at ~ -40 mV and achieving maximal levels around -20 to -10 mV) and have a long open time, play an important role in generation and shaping of Na^+ -dependent action potentials. Therefore, they are mainly implicated in regulation of the *suprathreshold* excitability of neurons. In contrast, $\text{Ca}_v1.3$ L-channels, which are activated at very hyperpolarized levels (-70 to -60 mV), similar to the low-voltage activated T-type Ca^{2+} channels, and inactivated before HVA-L channels achieve their maximal activation, play a unique role in regulating both *subthreshold* and *suprathreshold* excitability in response to V_m changes.

A major finding of the present study is that repeated cocaine exposure and withdrawal resulted in increased protein levels of HVA- $\text{Ca}_v1.2$ L-channels in mPFC pyramidal neurons. Total and intracellular levels were increased on withdrawal day 3, whereas total and cell surface levels were increased on withdrawal day 21, perhaps reflecting an increase in total protein levels accompanied by a more gradual shift towards cell surface targeting. Either increased synthesis or increased protein stability could explain our findings. Increased expression of HVA- $\text{Ca}_v1.2$ L-channels provides a mechanism to account for our previous electrophysiological results showing enhanced Ca^{2+} influx preferentially via L-channels in mPFC pyramidal neurons of cocaine-sensitized, withdrawn rats (Nasif et al., 2005a). By increasing the intrinsic excitability of pyramidal cells in the mPFC, particularly when they respond to strong excitatory stimuli, upregulation of HVA- $\text{Ca}_v1.2$ L-channels may explain the augmented excitatory response of mPFC pyramidal neurons in cocaine-sensitized rats to membrane depolarization (Nasif et al., 2005a,b). It may also explain the augmented PFC neuronal bursting response to cocaine infusion during self-administration sessions in cocaine-experienced rats (Sun and Rebec, 2006), although it is interesting that non-contingent (intracerebroventricular) cocaine injection produced greater activation in the

mPFC of cocaine-naïve than cocaine-sensitized rats in a functional MRI study (Febo et al., 2005). More importantly, it is possible that similar cellular changes underlie human imaging results demonstrating hyper-responsiveness of certain frontal cortical regions to cocaine-related stimuli in cocaine users compared to control subjects (Grant et al., 1996; Maas et al., 1998; Childress et al., 1999; Garavan et al., 2000; Wexler et al., 2001). The function of upregulated HVA-Ca_v1.2 L-channels may be further enhanced by the observed increase in PKA activity (see below).

Effects of chronic cocaine exposure and withdrawal on Ca_v1.3 L-channels were more delayed. There was no change in protein levels on withdrawal day 3, but total and intracellular levels increased on withdrawal day 21 (cell surface levels increased by about the same magnitude but this effect did not attain statistical significance due to higher variability). Because Ca_v1.3 L-channels regulate V_m at more hyperpolarized levels, the cocaine-induced upregulation of these channels might be expected to enhance subthreshold excitability and consequentially basal activity of mPFC pyramidal cells. This may appear to be at odds with results of imaging studies in human cocaine addicts and cocaine-experienced primates demonstrating basal PFC hypoactivity during cocaine abstinence (Volkow et al., 1992; Goldstein and Volkow, 2002; Porrino et al., 2007), as well as electrophysiological results in rats showing that chronic cocaine self-administration decreased basal PFC neuronal activity (Sun and Rebec, 2006) and that cocaine sensitization was associated with enhanced inhibitory responses of mPFC pyramidal cells to membrane hyperpolarization (Nasif et al., 2005b). To explain the discrepancy, we hypothesize that the delayed upregulation of Ca_v1.3 L-channels may be a compensatory (but apparently insufficient) response to reduced subthreshold excitability of mPFC pyramidal cells, which serves to dampen the cocaine withdrawal-induced decrease in basal mPFC activity. Supporting an important role for Ca_v1.3 L-channels in promoting excitability, deletion of these channels suppresses glutamate-drive upstate transitions and reduces firing in mouse striatal neurons (Olson et al., 2005). Interestingly, depletion of DA reduces spine density on striatal neurons through a mechanism involving dysregulation of Ca_v1.3 L-channel function (Day et al., 2006). Whether and how the changes in Ca_v1.3 L-channels affect subthreshold synaptic function and basal activity of mPFC pyramidal neurons in cocaine-sensitized rats remains to be determined.

Another important finding in this study is that cocaine sensitization was associated with increased PKA activity in the mPFC, but not in motor cortex, at both 3- and 21-day withdrawal times. This confirms and extends our previous report of increased PKA activity in the mPFC of cocaine-sensitized rats after 3 days of withdrawal (Dong et al., 2005). Increased PKA enzyme activity in the nucleus accumbens has been demonstrated during the first week of withdrawal from repeated cocaine exposure but was no longer evident on withdrawal day 21 (Terwiliger et al., 1991; Hope et al., 2005), although enhanced phosphorylation of some PKA substrates persists on withdrawal day 21 (Boudreau et al., submitted) perhaps due to changes in phosphatase activity or substrate compartmentalization. As PKA phosphorylation contributes to adaptations in Na⁺, K⁺, and Ca²⁺ channel activity in cocaine-sensitized rats, enduring upregulation of PKA activity in the mPFC could lead to persistent dysregulation of these channels in the mesocorticolimbic DA system, which in turn may contribute to cocaine addiction (Hu, 2007).

More specifically, our PKA findings in the mPFC help to explain the observation that enhanced Ca²⁺ influx in mPFC pyramidal neurons in response to strong excitatory stimuli is detected after short-term (3-day) and long-term (21-day) cocaine withdrawal (Nasif et al., 2005a), whereas increased surface expression of HVA-Ca_v1.2 L-channels was only evident at the long-term (21-day) withdrawal time (present results). We suggest that the enhancement of stimulus-evoked Ca²⁺ currents in mPFC pyramidal neurons during early

withdrawal relies predominantly on increased activity of HVA-Ca_v1.2 L-channels due to PKA phosphorylation of the channel (De Jongh et al., 1996). At later withdrawal times, however, the increased Ca²⁺ currents may reflect both increased HVA L-channel activity and an increased number of L-channels expressed on the surface of mPFC pyramidal neurons. However, it should be emphasized that more work is required to demonstrate that the cocaine-induced increase in PKA activity results in PKA-mediated enhancement of L-channel phosphorylation and function. Furthermore, given that the magnitude of ionic current across a specific ion channel within a certain period of time depends upon activation (e.g., the open probability and the open time after it is activated) as well as inactivation of the channel, further investigations should focus on how these properties of HVA-Ca_v1.2 L-channels are altered by repeated cocaine exposure.

Aside from our present and previous results (Dong et al., 2005), no other studies have directly measured PKA activity in the mPFC after withdrawal from cocaine exposure. Two other studies have measured the phosphorylation state of specific PKA substrates in the mPFC after withdrawal from a sensitizing cocaine regimen (Scheggi et al., 2007) or cocaine self-administration (Edwards et al., 2007), but these results are difficult to compare to ours because of differences in regimens and because the phosphorylation state of a particular substrate depends on multiple variables in addition to PKA activity, including phosphatase activity, cross-talk between signaling pathways, and substrate compartmentalization.

In conclusion, based upon our previous findings and novel findings from the present study, we propose that the increased responsiveness of mPFC pyramidal neurons in sensitized rats to re-exposure to cocaine or cocaine-associated cues is attributable, at least in part, to enhancement of L-type Ca²⁺ channel function, especially the HVA-Ca_v1.2 L-channel, due to increases in its phosphorylation and surface expression. By abnormally increasing the responsiveness of mPFC pyramidal neurons to excitatory inputs, excessive L-channel upregulation would enhance glutamatergic output from mPFC pyramidal neurons to the midbrain, the origin of dopamine projections, and to mesolimbic regions (e.g., the nucleus accumbens) critical for addiction. Combined with reduced inhibitory GABAergic feedback from the nucleus accumbens after cocaine withdrawal (Zhang et al., 1998; Hu et al., 2004; 2005), enhanced activation of the mPFC and its targets would amplify the dopamine and glutamate signaling that together promote cocaine sensitization, as well as cocaine-seeking behavior (Wolf et al., 2004; Kalivas and Hu, 2006).

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Figure 1.

Repeated cocaine administration induces behavioral sensitization. A challenge injection of cocaine (COC; 15 mg/kg) elicited a significantly greater locomotor response (measured as ambulation counts) in COC-pretreated compared to SAL-pretreated rats after a 3-day withdrawal (rmANOVA, $p < 0.05$).

Figure 2.

Repeated cocaine treatment increases PKA activity in the rat mPFC. **A.** Shaded areas indicate brain regions used for tissue harvesting. **B.** PKA activity (reflected by phosphorylation of kemptide) was significantly enhanced in the cocaine-exposed mPFC after a 3-day or 21-day withdrawal ($*p < 0.05$). **C.** PKA activity in the motor cortex did not differ between SAL- and COC-pretreated rats (all $p > 0.05$). *p*-Kemptide indicates phosphorylated Kemptide (see Materials and Methods for details).

Figure 3.

Repeated cocaine administration produces a time-dependent increase in protein levels and/or surface expression of Ca_v1.2 α_{1C} and Ca_v1.3 α_{1D} L-type Ca²⁺ channels in the mPFC. Unmodified (intracellular) forms of these channels were detected as bands of ~210 and ~250 kDa, respectively, consistent with many prior studies (Ca_v1.2: Davare et al., 2001; Haller et al., 2008; Tippens et al., 2008; Ca_v1.3: Qu et al., 2005; Kim et al., 2007; Yu et al., 2008). **A.** Preabsorption of antibodies with their respective peptide antigens eliminated specific staining assessed at exposure times equivalent to those utilized below. **B-C.** Western blots of non-crosslinked (N) and BS³-crosslinked (X) tissues. Repeated cocaine significantly increased total and intracellular levels of HVA-Ca_v1.2 α_{1C} subunits in mPFC after a 3-day withdrawal (***p*<0.01). Total and surface expression of Ca_v1.2 α_{1C} subunit increased after a 21-day withdrawal (**p*<0.05). **D-E.** Expression of Ca_v1.3 α_{1D} subunits in mPFC was unchanged after a 3-day withdrawal from cocaine (*p*>0.05), while delayed increases in total and intracellular levels occurred after a 21-day withdrawal (**p*<0.05, ***p*<0.01).