

# Repeated Amphetamine Administration Decreases D<sub>1</sub> Dopamine Receptor-Mediated Inhibition of Voltage-Gated Sodium Currents in the Prefrontal Cortex

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Adaptations in dopamine (DA) transmission in the prefrontal cortex (PFC) are thought to be critical to the development and persistence of drug addiction. Our previous findings showed that medial PFC (mPFC) neurons in rats treated repeatedly with amphetamine exhibit a decreased inhibitory response to iontophoretically applied DA, demonstrating altered DA receptor transmission. To determine the role postsynaptic DA D<sub>1</sub> receptors play in this effect, we used whole-cell patch-clamp recordings of acutely dissociated pyramidal mPFC neurons and inhibition of transient voltage-sensitive sodium current ( $I_{NaT}$ ) as a measure of D<sub>1</sub> receptor function. After 3 d of withdrawal, neurons recorded from amphetamine-treated rats (5 mg/kg for 5 d) demonstrated a significant decrease in whole-cell  $I_{NaT}$  density and in the ability of D<sub>1</sub> receptor stimulation to inhibit  $I_{NaT}$ . Application of a protein kinase A (PKA) inhibitor blocked the ability of D<sub>1</sub> receptor activation to inhibit  $I_{NaT}$  and increased the current density of both groups to similar values. These results suggest that repeated amphetamine exposure results in subsensitivity of the  $I_{NaT}$  to D<sub>1</sub> receptor-mediated inhibition because of a possible increase in basal PKA activity. This adaptation may contribute to perseverative behaviors in animals that self-administer psychostimulants as well as compromised PFC-dependent behaviors in human addicts.

**Key words:** dopamine; sensitization; addiction; impulsivity; inhibitory control; drug abuse

## Introduction

Considerable addiction research has focused on the ability of repeated drug exposure to enhance the incentive motivational qualities of drugs and their conditioned stimuli (Robinson and Berridge, 2003; Di Chiara et al., 2004). More recently, it has been recognized that a failure of inhibitory control, leading to impulsivity and compulsive stereotyped behaviors, is another major factor contributing to the development of addiction (Tiffany and Carter, 1998; Jentsch and Taylor, 1999; de Wit and Richards, 2004; Volkow and Li, 2004). A disruption in medial prefrontal cortex (mPFC) function is thought to be involved in the impaired behavioral inhibition seen in some psychiatric disorders, such as attention deficit hyperactive disorder and schizophrenia (Fuster, 1999), and may also contribute to the development of the perseverative behavioral characteristics seen in human drug addicts and animal models of addiction (Jentsch and Taylor, 1999; Spinella, 2003; Volkow and Li, 2004).

The goal of this study was to further investigate the role of mPFC dysfunction in addiction using behavioral sensitization, an animal model for the intensification of drug craving in human

addicts (Robinson and Berridge, 2003). Behavioral sensitization is defined as the augmentation of behavioral responses to psychostimulants during and after their repeated administration. The mPFC plays a critical role in behavioral sensitization (Wolf, 1998; Vanderschuren and Kalivas, 2000) as well as in reinstatement of drug-seeking behavior after extinction of self-administration (Kalivas and McFarland, 2003). We reported previously that responsiveness of mPFC neurons (prelimbic/infralimbic layers V–VI) to the inhibitory effects of iontophoretic dopamine (DA) is reduced in rats treated repeatedly with amphetamine and withdrawn for 3 d (Peterson et al., 2000). To determine whether this effect was, in part, mediated via postsynaptic DA D<sub>1</sub> receptors, the present study used whole-cell patch-clamp recordings of acutely dissociated mPFC neurons and examined inhibition of transient voltage-sensitive sodium currents ( $I_{NaT}$ ) as a measure of D<sub>1</sub> receptor activation efficacy.  $I_{NaT}$  plays a critical role in neuronal excitability and can also be used to study changes in intrinsic membrane properties (Li et al., 1992). Because recent studies suggest that there exists an optimal level of D<sub>1</sub> receptor stimulation for proper frontal cortex function in normal animals (Williams and Goldman-Rakic, 1995; Zahrt et al., 1997; Dreher and Burnod, 2002), we hypothesized that disruption of D<sub>1</sub> receptor-mediated signaling after repeated drug exposure could contribute to altered PFC-dependent behaviors associated with loss of control over drug-seeking and drug-taking behavior.

## Materials and Methods

**Drug treatment.** After a 3 d acclimation period, 4- to 5-week-old male Sprague Dawley rats (100–150 g body weight) were assigned randomly to

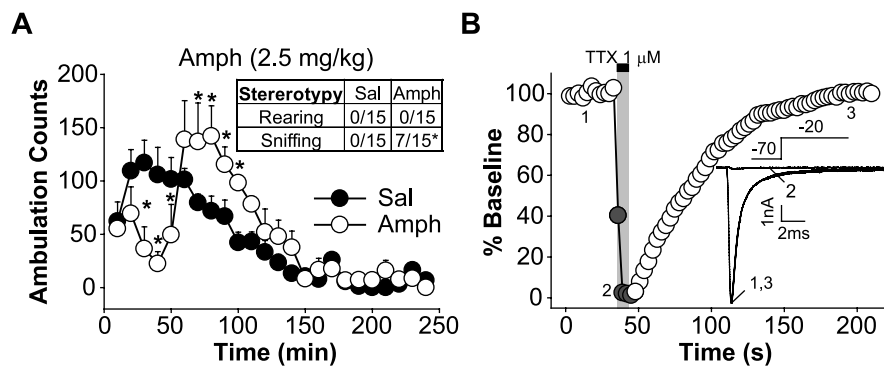
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**Figure 1.** Repeated administration of amphetamine produces behavioral sensitization in 4- to 5-week-old rats. **A**, On the third day of withdrawal, amphetamine (Amph) rats demonstrated sensitized stereotyped behaviors (during the first hour of the test) and post-stereotypy locomotor hyperactivity (during the second hour of the test) compared with saline (Sal) control rats ( $*p < 0.05$ ; Duncan's *post hoc*) in response to an amphetamine challenge injection. Inset, Stereotyped behavior during the first hour of behavioral testing. See Materials and Methods for scoring criteria (Fisher's exact probability test;  $*p < 0.05$ ). **B**, Application of TTX rapidly and reversibly blocks peak inward  $I_{NaT}$  of pyramidal mPFC neurons. A plot of the percentage of baseline of peak  $I_{NaT}$  evoked by a step in HP from  $-70$  to  $-20$  mV as function of time is shown. Inset, Representative traces of peak  $Na^+$  current before, during, and after TTX application.

two groups that received saline (1.0 ml/kg, i.p.) or amphetamine sulfate (5.0 mg/kg, i.p.) once daily for 5 consecutive days. Behavioral tests and electrophysiology recordings were performed 3 d after the final injection. This amphetamine regimen was used in our previous study of DA and glutamate transmission in the mPFC (Peterson et al., 2000). A different challenge dose (2.5 mg/kg free base, equivalent to 3.4 mg/kg amphetamine) and route of administration (subcutaneous) was used because this drug challenge enables excellent behavioral discrimination between sensitized and naive rats (Wolf et al., 1995).

**Behavioral assessments.** Activity was measured using photobeam frames (San Diego Instruments, San Diego, CA). Each frame was  $50 \times 30$  cm, with three photocells (separated by 10.5 cm) located lengthwise, 3.5 cm above the floor. A standard polyethylene rat cage (identical to home cages) was set inside each frame, and one rat was placed in each cage. Ambulation counts, which register after breaking of consecutive photobeams, were used as a measure of horizontal locomotor activity. To assess stereotypy, each rat was observed for 1 min of each 20 min interval during the first hour after amphetamine injection. Intervals were scored, using criteria described previously (Wolf et al., 1995), for the presence of two behaviors characteristic of amphetamine sensitization: (1) continuous in-place rearing and (2) nose-down, in-place stereotyped sniffing.

**Electrophysiology.** Dissociated cell preparation and patch-clamp recordings were conducted as reported previously (Dong et al., 2005). Briefly, rats were anesthetized and decapitated rapidly. Brains were removed quickly, blocked, and sliced into coronal slices ( $400 \mu\text{m}$ ) containing the mPFC. Slices were incubated for 1–6 h at room temperature in a  $\text{NaHCO}_3$ -buffered Earle's balanced salt solution bubbled with 95%  $\text{O}_2/5\%$   $\text{CO}_2$ . The deep layers (V–VI) of the mPFC were isolated and digested in HEPES-buffered HBSS (Sigma, St. Louis, MO) containing protease type XIV (1–2 mg/ml; Sigma) at  $35^\circ\text{C}$  for 25–30 min. The tissue was then mechanically dissociated with a graded series of fire-polished Pasteur pipettes. The cell suspension was transferred to the recording chamber. Cells were allowed to settle for 5–10 min before starting a continuous background flow of  $\text{PO}_4$ -free HEPES-buffered solution containing the following (in mM): 140 NaCl, 23 glucose, 13 HEPES, 2 KCl, 2  $\text{MgCl}_2$ , and 1  $\text{CaCl}_2$  (pH 7.2, 300–310 mOsm/L). Sodium current was isolated using the following solutions (in mM): (1) internal: 120 *N*-methyl-glucamine, 35 HEPES, 20 CsCl, 1 NaCl, 4  $\text{MgCl}_2$ , 12 phosphocreatine, 2  $\text{Na}_2\text{ATP}$ , 0.2 NaGTP, and 0.1 leupeptin (pH 7.2 with  $\text{H}_2\text{SO}_4$ , 265–270 mOsm/L); (2) external: 30 NaCl, 110 choline chloride, 1  $\text{CaCl}_2$ , 1  $\text{MgCl}_2$ , 0.4  $\text{CdCl}_2$ , 5 CsCl, 10 glucose, and 10 HEPES (pH 7.4, 300–305 mOsm/L).  $\text{Na}^+$  current isolating solutions and pharmacological solutions were applied by a gravity-fed sewer pipe system. The array of application capillaries (0.78 mm inner diameter) was positioned a few hun-

dred micrometers away from the cell under study, and solution changes were made by altering the position of the array with a rotating head rapid solution changer (Biologic, Grenoble, France). Complete solution changes were achieved within  $<1$  s. Recordings were obtained with a Molecular Devices (Union City, CA) Axopatch 200A patch-clamp amplifier and controlled and monitored with a Dell pentium personal computer running pClamp8 (version 8.1) with a diData 1200 interface (Molecular Devices). Electrode resistances were 1–2.5  $\text{M}\Omega$  in bath. After seal rupture, series resistance was compensated and monitored periodically. Recordings were discarded when series resistance exceeded  $15 \text{M}\Omega$ . Capacitative and leak currents were subtracted using a P/N technique. Data were low-pass filtered and digitized. All experiments were performed at room temperature ( $22$ – $24^\circ\text{C}$ ).

**Statistics.** Data are presented as mean  $\pm$  SEM. See graphs for statistical measures. All data were collected and analyzed without knowledge of the treatment that the animals had received.

**Drugs.** (+)-Amphetamine sulfate was obtained from the National Institute on Drug Abuse (Rockville, MD). All drug doses refer to salt weights unless noted otherwise. All other drugs and the protein kinase A (PKA) inhibitor fragment 14-24 trifluoroacetate salt were obtained from Sigma.

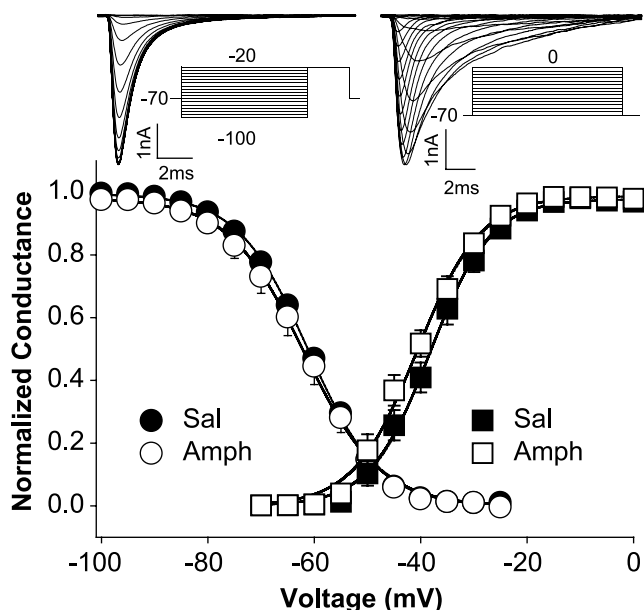
## Results

### Four- to 5-week-old rats pretreated with amphetamine demonstrate behavioral sensitization

Rats 4–5 weeks old are an ideal age for the dissociated cell preparation, but it is unclear whether they develop behavioral sensitization to the amphetamine regimen used in these experiments (Tirelli et al., 2003). Therefore, we first determined whether behavioral sensitization to amphetamine could be demonstrated in 4- to 5-week-old rats using the same injection protocol and housing arrangements that we planned to use for electrophysiology experiments. Animals were administered daily injections of saline (1 ml/kg) or amphetamine (5 mg/kg) intraperitoneally for 5 d, followed by a 3 d withdrawal period. When both groups of rats were challenged with amphetamine (3.4 mg/kg, s.c.; see Materials and Methods), the amphetamine group exhibited a significant increase in stereotyped behavior and post-stereotypy locomotor hyperactivity compared with saline control rats [repeated-measures ANOVA, group  $\times$  time effect: saline ( $n = 5$ ), amphetamine ( $n = 5$ ),  $F_{(23,184)} = 3.96$ ,  $p < 0.00001$ ] (Fig. 1A). These results are similar to those obtained in adult rats (Wolf et al., 1995), verifying that 4- to 5-week-old rats manifest behavioral sensitization in response to repeated amphetamine treatment.

### $I_{NaT}$ density is reduced in amphetamine-pretreated mPFC neurons

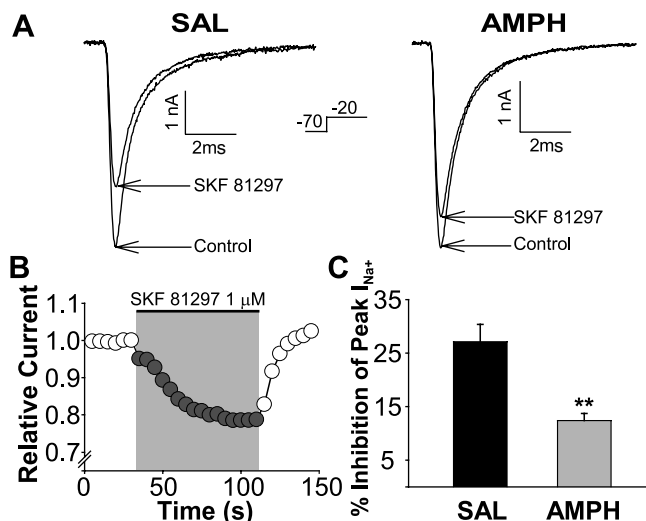
For electrophysiology experiments, rats received the same injection protocol as above but did not receive an amphetamine challenge injection. On the third day of withdrawal, deep-layer (layers V–VI) mPFC neurons were acutely dissociated and prepared for recording. Only mPFC neurons with a characteristic pyramidal shape with one apical dendrite and truncated or no basilar processes were studied. Inward currents elicited by stepping to  $-20$  mV from a holding potential (HP) of  $-70$  mV [approximate resting membrane potential *in vitro* (Nasif et al., 2005)] in a sodium current isolating extracellular recording solution were rap-



**Figure 2.** Repeated amphetamine treatment does not affect steady-state activation or inactivation of mPFC pyramidal neurons. A plot of peak conductance normalized to maximum conductance as a function of test pulse voltage (■, activation) and peak  $I_{NaT}$  normalized to maximum peak  $I_{NaT}$  as function of prepulse voltage (●, inactivation) with representative current traces of  $I_{NaT}$  and voltage protocols (activation: HP,  $-70$  mV; test pulse, 20 ms from  $-65$  to  $0$  mV; increments, 5 mV; intertrial interval, 5 s; inactivation: HP,  $-70$  mV; prepulse, 120 ms from  $-100$  to  $-20$  mV; increments, 5 mV; test pulse, 20 mV for 20 ms; intertrial interval, 5 s) is shown. There was no significant difference between groups. Sal, Saline; Amph, amphetamine.

idly blocked by perfusion of the Na<sup>+</sup> channel blocker tetrodotoxin (TTX; 1  $\mu$ M) (Fig. 1B).

Whole-cell  $I_{NaT}$  density (peak  $I_{NaT}$ /capacitance) and voltage dependence of steady-state activation and inactivation were studied to determine whether intrinsic membrane properties had been affected by repeated amphetamine administration. Whole-cell  $I_{NaT}$  density was significantly reduced [13.6%; ANOVA:  $F_{(1,78)} = 4.074$ ,  $p < 0.05$ ] in mPFC neurons obtained from amphetamine rats ( $336.14 \pm 19.3$  pA/pF;  $n = 36$ ) compared with neurons taken from saline controls ( $389.01 \pm 17.9$  pA/pF;  $n = 44$ ) (see Fig. 4). Voltage-dependent, steady-state half-maximal activation or inactivation ( $V_{1/2}$ ) and slope factor ( $k$ ) values were obtained individually for each neuron tested for steady-state activation or inactivation and compared using ANOVA. There was no significant difference between groups in the kinetics or voltage dependence of steady-state activation [saline ( $n = 11$ ):  $V_{1/2} = -38.6 \pm 3.9$  mV,  $k = 5.65 \pm 2.0$  mV; amphetamine ( $n = 10$ ):  $V_{1/2} = -41.4 \pm 3.3$  mV,  $k = 6.3 \pm 2.6$  mV;  $V_{1/2}$ :  $F_{(1,19)} = 3.31$ ,  $p = 0.085$ ;  $k$ :  $F_{(1,19)} = 0.37$ ,  $p = 0.552$ ] or inactivation [saline ( $n = 16$ ):  $V_{1/2} = -60.9 \pm 3.2$  mV,  $k = 6.60 \pm 0.5$  mV; amphetamine ( $n = 10$ ):  $V_{1/2} = -61.9 \pm 5.3$  mV,  $k = 6.98 \pm 1.4$  mV;  $V_{1/2}$ :  $F_{(1,24)} = 0.366$ ,  $p = 0.551$ ;  $k$ :  $F_{(1,24)} = 0.936$ ,  $p = 0.343$ ] (Fig. 2). Activation of the protein kinase A (PKA) pathway produces effects on peak  $I_{NaT}$  and the voltage dependence of steady-state activation and inactivation of  $I_{NaT}$  similar to those observed in the present study of amphetamine-treated rats (Li et al., 1992), suggesting that the PKA pathway may be involved in the reduced  $I_{NaT}$  density produced by repeated amphetamine administration.



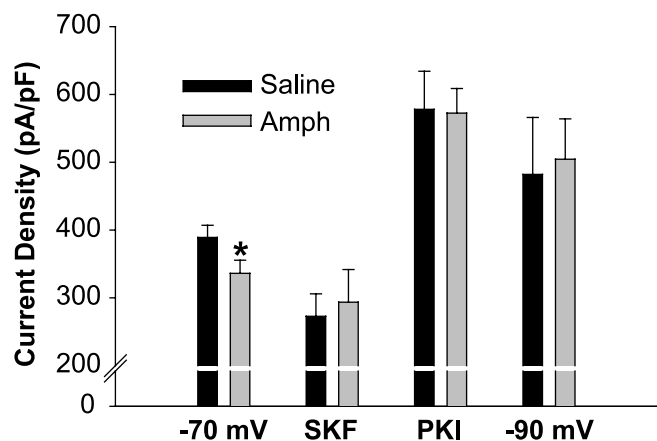
**Figure 3.** Repeated amphetamine treatment reduces the magnitude of the inhibitory effect of D<sub>1</sub> receptor stimulation on the  $I_{NaT}$ . **A**, Representative traces of  $I_{NaT}$  elicited by a change in voltage command ( $-70$  to  $-20$  mV), before and during application of the D<sub>1</sub> receptor full agonist SKF 81297 onto neurons from saline- and amphetamine-pretreated animals. **B**, Plot of relative peak  $I_{NaT}$  current as a function of time. Application of SKF 81297 reversibly suppressed peak  $I_{NaT}$  (21.4%; example taken from a saline control rat demonstrating a drug effect time course). **C**, Bar graph summary of the percentage of inhibition of peak  $I_{NaT}$  by SKF 81297 application onto neurons from saline- and amphetamine-pretreated animals (\*\* $p < 0.001$ ). Error bars indicate SE. SAL, Saline; AMPH, amphetamine.

#### D<sub>1</sub> receptor-mediated suppression of $I_{NaT}$ in mPFC neurons is reduced in animals pretreated with amphetamine

Maurice et al. (2001) showed that activation of PKA, either via G-protein-coupled receptors positively coupled to adenylyl cyclase (e.g., D<sub>1</sub> receptors) or directly using cAMP analogs, inhibits  $I_{NaT}$  in mPFC neurons. To test the functional efficacy of D<sub>1</sub> receptors in mPFC neurons, application of the D<sub>1</sub> receptor full agonist 6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SKF; SKF 81297) (1  $\mu$ M) was used to reversibly decrease peak  $I_{NaT}$  (Fig. 3B). An average peak suppression of  $27.1 \pm 3.2\%$  was observed in saline-treated rats (Fig. 3C). Coapplication of the D<sub>1</sub> receptor antagonist SCH 23390 (7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine; 1  $\mu$ M) blocked the SKF-induced inhibition of  $I_{NaT}$  (average peak suppression,  $6.25 \pm 1.3\%$ ;  $n = 4$ ; data not shown). In amphetamine-treated rats, SKF was only about half as effective ( $\sim 46\%$ ; ANOVA:  $F_{(1,25)} = 14.82$ ;  $p < 0.001$ ) at suppressing the  $I_{NaT}$  (average peak suppression,  $12.4\% \pm 1.3\%$ ) (Fig. 3C), indicating that D<sub>1</sub> receptor efficacy is reduced by repeated amphetamine administration. The number of neurons responding to D<sub>1</sub> receptor stimulation did not differ between groups [ $n = 12$  of 15 neurons responded (73.3%) in the amphetamine group, and  $n = 15$  of 19 neurons responded (77.8%) in the saline group].

#### The PKA inhibitor PKI and a more hyperpolarized HP increased $I_{NaT}$ density in both groups to similar values

Because D<sub>1</sub> receptor stimulation inhibits  $I_{NaT}$  in naive rats via PKA, we hypothesized that increased basal activation of the PKA pathway in mPFC neurons from amphetamine-treated rats could account for our observations of reduced  $I_{NaT}$  density and reduced D<sub>1</sub> receptor efficacy. First, we confirmed that D<sub>1</sub> receptor activation was inhibiting  $I_{NaT}$  via PKA by applying SKF 81297 in the presence of a PKA inhibitor (PKI). The addition of PKI (10 U/ml) in the recording pipette completely blocked the SKF-induced suppression of  $I_{NaT}$  (average peak suppression,  $2.37 \pm 2.1\%$ ;



**Figure 4.** Repeated amphetamine (Amph) treatment reduces the  $I_{\text{NaT}}$  density in mPFC pyramidal neurons; however, PKI or a  $-90$  mV HP increases  $I_{\text{NaT}}$  density to similar values.  $I_{\text{NaT}}$  density is significantly reduced ( $*p < 0.05$ ) in mPFC pyramidal neurons taken from rats pretreated with amphetamine compared with neurons taken from saline-pretreated controls when holding neurons at  $-70$  mV.  $I_{\text{NaT}}$  density in the presence of SKF 81297 (SKF) is shown for representation of raw data values (as opposed to the percentage of inhibition shown in Fig. 3C) and is not significantly different (saline,  $272.73 \pm 33.1$  pA/pF; amphetamine,  $293.58 \pm 48$  pA/pF; ANOVA:  $F_{(1,25)} = 0.136$ ,  $p = 0.716$ ).  $I_{\text{NaT}}$  density of mPFC neurons is increased to a similar level in rats treated with amphetamine or saline in the presence of PKI or when neurons were held at  $-90$  mV. Error bars indicate SE.

$n = 5$ ; data not shown). We then looked at the effect of PKI on  $I_{\text{NaT}}$  density. PKI increased  $I_{\text{NaT}}$  density to similar values (ANOVA:  $F_{(1,39)} = 0.0012$ ;  $p = 0.972$ ) in pyramidal neurons taken from both amphetamine-treated ( $560 \pm 36.7$  pA/pF;  $n = 22$ ) and saline-treated ( $558 \pm 48.4$  pA/pF;  $n = 19$ ) animals (Fig. 4), indicating that the  $I_{\text{NaT}}$  capacity of mPFC neurons taken from both treatment groups is similar in the absence of PKA activity.

A key aspect of PKA modulation of  $I_{\text{NaT}}$  is its voltage dependence (Cantrell et al., 1999; Carr et al., 2003). Cantrell et al. (1999) demonstrated in hippocampal neurons and Carr et al. (2003) demonstrated in mPFC neurons that PKA-induced inhibition of  $I_{\text{NaT}}$  is reduced when holding neurons at more hyperpolarized potentials (e.g.,  $-90$  mV). We therefore elicited  $I_{\text{NaT}}$  by stepping to  $-20$  mV from a more hyperpolarized HP of  $-90$  mV.  $I_{\text{NaT}}$  density was increased to similar values (ANOVA:  $F_{(1,23)} = 0.0459$ ;  $p = 0.832$ ) in pyramidal neurons taken from both amphetamine-treated ( $504 \pm 59.5$  pA/pF;  $n = 12$ ) and saline-treated ( $482 \pm 83.7$  pA/pF;  $n = 13$ ) animals when using this protocol (Fig. 4). Taken together, these data suggest that the basal activity of the PKA pathway in amphetamine-treated animals is increased and may, in part, be responsible for the reduced ability of D<sub>1</sub> receptor activation to inhibit  $I_{\text{NaT}}$ .

## Discussion

Repeated exposure to drugs of abuse causes many neuroadaptations within the mesocorticolimbic system (Wolf, 1998; Vanderschuren and Kalivas, 2000). The current experiments were designed to characterize changes in D<sub>1</sub> receptor function in the mPFC after repeated amphetamine administration. An optimal level of D<sub>1</sub> receptor function in the mPFC is critical to the successful execution of different PFC-dependent behaviors, such as behavioral inhibition and working memory, and disruptions in D<sub>1</sub> receptor signaling have profound effects on these behaviors (Williams and Goldman-Rakic, 1995; Zahrt et al., 1997; Goldman-Rakic et al., 2000; Dreher and Burnod, 2002). In addition, it is hypothesized that altered D<sub>1</sub> receptor signaling is a major contributing factor to aberrant behavioral inhibition in

human drug addicts (Jentsch and Taylor, 1999). Our *in vivo* recordings demonstrated that the iontophoretic application of DA was less efficacious at inhibiting the firing rate of mPFC neurons in rats tested after discontinuing repeated amphetamine injections compared with controls (Peterson et al., 2000), possibly contributing to the impaired behavioral inhibition seen in amphetamine-treated rats (Peterson et al., 2003).

The current experiments demonstrate a reduction in whole-cell  $I_{\text{NaT}}$  density in mPFC neurons from amphetamine-sensitized rats, most likely because of increased activation of the PKA pathway. When comparing the  $I_{\text{NaT}}$  densities at baseline (HP,  $-70$  mV), during SKF 81297 application, in the presence of PKI, and at a  $-90$  mV HP, it is only the baseline  $I_{\text{NaT}}$  density of mPFC neurons taken from amphetamine rats that is significantly different (reduced) compared with controls (Fig. 4). This suggests an increase in tonic PKA activity in the mPFC of amphetamine-sensitized rats, resulting in a tonic decrease in the open probability of voltage-sensitive sodium channels at activating potentials (Li et al., 1992), possibly because of increased entry of the channels into a slow inactivated-like state (Carr et al., 2003). Similarly, Dong et al. (2005) demonstrated that, after repeated cocaine administration, basal PKA activity is increased in mPFC neurons, which results in a decrease in the current density of voltage-gated potassium ( $K^+$ ) currents. It is significant that we found the same results in rats treated repeatedly with amphetamine because, despite similar initial effects of cocaine and amphetamine on extracellular DA levels, the identification of common downstream effects of repeated cocaine or amphetamine on the function of DA-receptive neurons has been a surprisingly elusive goal (Tzschenke, 2001). Activation of the PKA pathway has also been reported in other brain regions, such as the nucleus accumbens, after discontinuing repeated stimulant exposure (Nestler, 2001; Hope et al., 2005).

Because Bonhomme et al. (1995) found no difference in D<sub>1</sub> or D<sub>2</sub> receptor binding in the mPFC at 1 or 15 d withdrawal from a repeated amphetamine regimen similar to ours, a change in D<sub>1</sub> receptor expression is unlikely to be responsible for the reduced ability of SKF 81297 to inhibit the  $I_{\text{NaT}}$  in mPFC neurons from amphetamine rats. Rather, our results indicate that SKF 81297 is less efficacious, because the PKA pathway is already activated in the mPFC of amphetamine-sensitized rats.

Although an increase in basal PKA activity might suggest a decrease in membrane excitability attributable to reduced  $I_{\text{NaT}}$ , the effects of DA receptor activation on mPFC pyramidal neurons are very complex (Seamans and Yang, 2004). Under some conditions, D<sub>1</sub> receptor-mediated PKA activation can actually augment responses to glutamate agonists (Wang and O'Donnell, 2001; Tseng and O'Donnell, 2004; Sun et al., 2005). Moreover, as mentioned above, mPFC neurons recorded after repeated cocaine administration show an increase in PKA activity that decreases voltage-gated potassium channel current density, resulting in increased membrane excitability (Dong et al., 2005; Nasif et al., 2005). In this context, an increase in basal PKA activity would help explain results of our previous *in vivo* recording studies, in which we found that the iontophoretic application of glutamate onto mPFC neurons was more efficacious at increasing firing rates in amphetamine rats compared with control rats (Peterson et al., 2000). However, this may be an oversimplification of this complex subject.

In summary, these results demonstrate that  $I_{\text{NaT}}$  and its modulation by D<sub>1</sub> receptors in mPFC neurons are reduced after repeated amphetamine administration. These reductions are most likely attributable to an increase in basal activity of the PKA path-

way, which could possibly result in an increase in mPFC excitability. These findings clarify mechanisms underlying our previous *in vivo* observations (Peterson et al., 2000) and support the hypothesis that increased excitatory output of mPFC projection neurons after psychostimulant treatment contributes to an increased firing rate of ventral tegmental area DA neurons, probably through polysynaptic pathways (Wolf, 2002), which in turn is critical for initiation of behavioral sensitization (Henry et al., 1998; You et al., 1998). The observed changes in mPFC neuronal function may contribute to the disruption in some mPFC-dependent behaviors seen in human drug addictions, such as impaired behavioral inhibition (Jentsch and Taylor, 1999).

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