Cellular/Molecular

A Role for the GIRK3 Subunit in Methamphetamine-Induced Attenuation of GABA$_B$ Receptor-Activated GIRK Currents in VTA Dopamine Neurons

Michaelanne B. Munoz,[2,3] Claire L. Padgett,[2] Robert Rifkin,[1] Miho Terunuma,[5] Kevin Wickman,[6] Candice Contet,[4] Stephen J. Moss,[5] and Paul A. Slesinger[1,2,3]

[1]Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, New York 10029, [2]The Salk Institute for Biological Studies, La Jolla, California 92037, [3]Department of Biology, University of California–San Diego, La Jolla, California 92037, [4]The Scripps Research Institute, La Jolla, California 92037, [5]Department of Neuroscience, Tufts University School of Medicine, Boston, Massachusetts 02155, and [6]Department of Pharmacology, University of Minnesota, Minneapolis, Minnesota 55455

Repeated exposure to psychostimulants induces locomotor sensitization and leads to persistent changes in the circuitry of the mesocorticolimbic dopamine (DA) system. G-protein-gated inwardly rectifying potassium (GIRK; also known as Kir3) channels mediate a slow IPSC and control the excitability of DA neurons. Repeated 5 d exposure to psychostimulants decreases the size of the GABA$_B$ receptor (GABA$_B$R)-activated GIRK currents ($I_{Baclofen}$) in ventral tegmental area (VTA) DA neurons of mice, but the mechanism underlying this plasticity is poorly understood. Here, we show that methamphetamine-dependent attenuation of GABA$_B$R-GIRK currents in VTA DA neurons required activation of both D$_1$R-like and D$_2$R-like receptors. The methamphetamine-dependent decrease in GABA$_B$R-GIRK currents in VTA DA neurons did not depend on a mechanism of dephosphorylation of the GABA$_B$ R2 subunit found previously for other neurons in the reward pathway. Rather, the presence of the GIRK3 subunit appeared critical for the methamphetamine-dependent decrease of GABA$_B$R-GIRK current in VTA DA neurons. Together, these results highlight different regulatory mechanisms in the learning-evoked changes that occur in the VTA with repeated exposure to psychostimulants.

Key words: addiction; PDZ; plasticity; potassium channel; psychostimulants; reward

---

Significance Statement

Exposure to addictive drugs such as psychostimulants produces persistent adaptations in inhibitory circuits within the mesolimbic dopamine system, suggesting that addictive behaviors are encoded by changes in the reward neural circuitry. One form of neuroadaptation that occurs with repeated exposure to psychostimulants is a decrease in slow inhibition, mediated by a GABA$_B$ receptor and a potassium channel. Here, we examine the subcellular mechanism that links psychostimulant exposure with changes in slow inhibition and reveal that one type of potassium channel subunit is important for mediating the effect of repeated psychostimulant exposure. Dissecting out the components of drug-dependent plasticity and uncovering novel protein targets in the reward circuit may lead to the development of new therapeutics for treating addiction.

Introduction

Drug addiction is a psychiatric disorder characterized by continued use of drug despite severe adverse consequences (Robinson and Berridge, 2003; Koob and Volkow, 2010). Addiction is thought to involve, in part, long-term adaptations in the mesocorticolimbic dopamine (DA) circuits that occur in response to repeated drug use, promoting a risk of relapse after discontinuing use (Lüscher and Malenka, 2011). In rodents, repeated administration of many drugs of abuse produces locomotor sensitization, a progressive increase in the locomotor-stimulating effects of these drugs, a phenomenon that is context dependent and persists for months after the last drug exposure (Vanderschuren and

Kalivas, 2000). Locomotor sensitization has been used as a behavioral read-out of an underlying neural sensitization in addiction circuits (Robinson and Berridge, 2003).

The development of locomotor sensitization requires increased activity in the mesocorticolimbic DA circuit, particularly in the ventral tegmental area (VTA) (Robinson and Berridge, 2003). Several studies have demonstrated that exposure to psychostimulants alters both excitatory and inhibitory inputs to VTA DA neurons (Lüscher and Malenka, 2011). For example, single or multiple exposure to cocaine enhances glutamatergic signaling in the VTA (Ungless et al., 2001; Saal et al., 2003; Chen et al., 2008). Multiple exposure to cocaine also reduces the amplitude of fast GABA_A-evoked IPSCs on VTA DA neurons, promoting LTP induction (Liu et al., 2005) while enhancing IPCSs onto VTA GABA neurons (Bocklisch et al., 2013). Together, these changes promote an increase in activity of VTA DA neurons in drug-exposed animals. Another important pathway that controls the output of the VTA involves the G-protein-gated inwardly rectifying potassium (GIRK, also referred to as Kir3) channels. Activation of GIRK channels produces a slow IPSC that hyperpolarizes the neuron, thereby reducing excitability and inhibiting the firing of DA neurons (Lacey et al., 1988; Seutin et al., 1994; Beckstead et al., 2004; Cruz et al., 2004; Munoz and Slesinger, 2014). Stimulation of GABA_B, DA D_2-like or κ opioid receptors has been reported to activate GIRK channels in VTA DA neurons, wheres GABA_B and μ opioid receptors couple to GIRK channels in VTA GABA neurons (Cameron et al., 1997; Margolis et al., 2003; Beckstead et al., 2004; Cruz et al., 2004; Kotecki et al., 2015).

Recent work with rodents has established that psychostimulant exposure alters slow inhibition mediated by GABA_B receptors and GIRK channels. For example, acute exposure to a psychostimulant such as methamphetamine (METH) or cocaine reduces baclofen-activated GIRK currents ($I_{Baclofen}$) in VTA GABA neurons (Padgett et al., 2012). Multiple injections of cocaine dampen $I_{Baclofen}$ in VTA DA neurons (Arora et al., 2011). Recently, Sharpe et al. (2015) reported that self-administration of METH also diminishes $I_{Baclofen}$ in VTA DA neurons, indicating that both contingent (i.e., self-administration) and noncontingent (i.e., experimenter injections) drug exposure leads to a reduction in GIRK signaling in the VTA. This attenuation in $I_{Baclofen}$ leads to changes in the excitability of VTA DA neurons and is hypothesized to contribute to circuit-level changes in DA signaling involved in addiction. Consistent with a role for GABA_B receptors (GABA_BRs) in the reward pathway, systemic or intra-VTA administration of a GABA_BR agonist is sufficient to prevent the acquisition of locomotor sensitization and self-administration of psychostimulants (Shoaib et al., 1998; Brebner et al., 1999; Lhuillier et al., 2007; Backes and Hemby, 2008).

To develop new therapeutics for treating addiction, it is essential to dissect the components of drug-dependent plasticity in the brain. In VTA GABA neurons (Padgett et al., 2012) and in mPFC pyramidal neurons (Hearing et al., 2013), the reduction in $I_{Baclofen}$ can be explained by a decrease in the surface expression of GABA_BRs, a process that depends on dephosphorylation of a C-terminal serine (S783) in the GABA_BR2 subunit via a PP2A phosphatase (Padgett et al., 2012). Repeated injections of cocaine or self-administration of METH weaken GABA_BR-GIRK signaling in VTA DA neurons (Arora et al., 2011; Sharpe et al., 2015). The subcellular mechanism underlying the reduction in $I_{Baclofen}$ in VTA DA neurons with psychostimulants, however, is poorly understood. In the current study, we investigated whether dephosphorylation of the GABA_BR2-S783 subunit is involved in the METH-dependent reduction of $I_{Baclofen}$ in VTA DA neurons. We discovered that the GIRK3

subunit, and not the dephosphorylation of GABA_BR2-S783, promotes the METH-dependent decrease in $I_{Baclofen}$.

Materials and Methods

*Mouse husbandry.* The following mouse lines were used, Pitx3 $^{+/-}$-GFP (Zhao et al., 2004), GIRK3 knock-out (KO) (Torrecilla et al., 2002), GABA_BR2-S783A knock-in (KI) (Terunuma et al., 2014), TH-Cre (Friedman et al., 2014), and littermate controls. TH-Cre mice were injected with AAV DIO-YFP (Munoz and Slesinger, 2014) to label VTA DA neurons for the control experiment shown in Figure 2F. All mice either originated in C57BL/6 or were backcrossed multiple generations into the C57BL/6 background. Mice were housed together in groups of two to five and separated by sex. All procedures were performed in the light phase of the circadian cycle using Institutional Animal Care and Use Committee-approved protocols for animal handling at the Salk Institute and at the Icahn School of Medicine at Mount Sinai. Animals were housed under constant temperature and humidity on a 12 h light/dark cycle (light 6:00–18:00) with food and water available *ad libitum*. Tail biopsies were collected from animals at weaning (>postnatal day 21) and prepared for PCR genotyping, as described previously (Torrecilla et al., 2002; Zhao et al., 2004; Friedman et al., 2014; Terunuma et al., 2014).

Drug administration. Mice [postnatal day 30 (P30)–P50 male and female] were given intraperitoneal injections of METH (methamphetamine hydrochloride; Sigma-Aldrich) at 2 mg/kg in 0.9% saline or an equivalent volume of 0.9% saline. For antagonist experiments, the D_1R antagonist SCH39166 (0.3 mg/kg, i.p.) or the D_2R antagonist eticlopride (0.1 mg/kg, i.p.) was coinjected with METH (2 mg/kg). For home cage injections, animals were weighed, injected, and returned to the home cage. For novel environment experiments, animals were transported to a different room, weighed, injected, placed in new cage with cage mates for 1 h, and then returned to home cage. The novel environment consisted of a different plastic cage (Innovive) that contained soft bedding and brightly colored paper along the outside. All animals had full access to food and water during drug administration in either home or novel environment cages and injections took place during the light cycle between 10:00 and 16:00.

Electrophysiology. Mice were killed with isoflurane and horizontal or coronal slices from midbrain (200 μm) were prepared in ice-cold artificial CSF (ACSF) containing the following (in mM): NaCl 119, KCl 2.5, MgCl_2 1.3, CaCl_2 2.5, NaH_2PO_4 1.0, NaHCO_3 26.2, and glucose 11, pH 7.2, bubbled with 95% O_2 and 5% CO_2. Slices were equilibrated for ~42 min at 33°C in ACSF supplemented with the following (in mM): L-ascorbic acid 0.4, Na pyruvate 2, myo-inositol 3, and then transferred to a recording chamber equipped with constant perfusion of ACSF (2 ml/min) (Cruz et al., 2004).

Neurons were visualized by infrared illumination on an Olympus fluorescent scope (BX51WI) and whole-cell patch-clamp recordings were made from neurons in the VTA, identified as the region medial to the medial terminal nucleus of the accessory optical tract (Cruz et al., 2004; Labouèbe et al., 2007; Munoz and Slesinger, 2014). DA neurons were identified by presence of a large hyperpolarization-activated current (I_h), large capacitance (30–50 pF), and slow spontaneous firing (1–3 Hz). In some experiments, DA neurons were identified by GFP expression in Pitx3 $^{+/-}$-GFP mice (Zhao et al., 2004), as indicated. No significant differences in firing, capacitance or cell size were observed in DA neurons from Pitx3 $^{+/-}$-GFP mice (Labouèbe et al., 2007). GABA neurons were identified by the absence of I_h, a small capacitance (<20 pF), and a fast spontaneous firing rate (5–10 Hz) (Cruz et al., 2004).

The internal solution contained the following (in mM): K-gluconate 140, NaCl 4, MgCl_2 2, EGTA 1.1, HEPES 5, Na_2ATP 2, Na creatine phosphate 5, and Na_3GTP 0.6, pH 7.3 with KOH. Whole-cell voltage-clamp recordings were used to measure GIRK currents. For agonist-induced currents, changes in holding currents ($V_m = -35$ mV with junction potential -15.7 mV; $V_{hold} = -50$ mV) in response to bath application of a saturating dose of baclofen (300 μM) or quinpirole (30 μM) were measured every 10 s. Currents were amplified (Molecular Devices Axopatch 200B), filtered at 1 kHz, and digitized at 5 kHz (Molecular Devices Digidata 1320). Data were acquired and analyzed with Molecular Devices Clampex 9.0 software. I_h current was monitored through a series
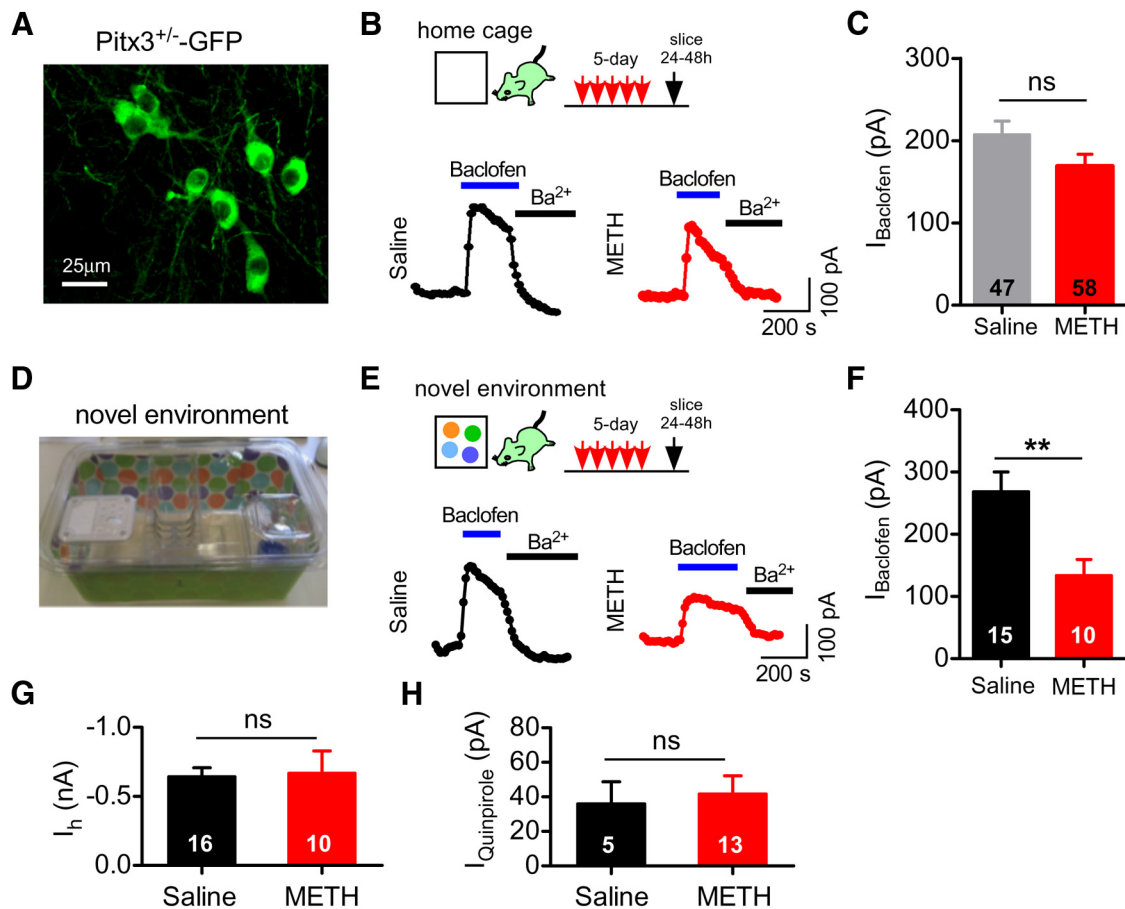
Figure 1. Attenuation of $I_{Baclofen}$ in VTA DA neurons after 5 d METH injections in a novel environment. **A**, Live fluorescent image of GFP$^+$ DA neurons in the VTA of a Pitx3$^{+/-}$-GFP mouse. **B**, Examples of maximal (300 μM) $I_{Baclofen}$ recorded from VTA DA neurons 24 – 48 h after 5 d of intraperitoneal injections of either saline (0.9%) or METH (2 mg/kg in 0.9% saline) in the home cage. Application of 1 mM BaCl$_2$ inhibits $I_{Baclofen}$. **C**, Mean $I_{Baclofen}$ recorded 24 – 48 h after 5 d saline or 5 d METH injected in the home cage (ns, $p > 0.05$ Student's t test). **D**, Photograph of the novel cage environment with different bedding and brightly colored paper. **E**, Examples of $I_{Baclofen}$ recorded from VTA DA neurons 24 – 48 h after 5 d injections of saline or METH in a novel environment. **F**, Mean $I_{Baclofen}$ in VTA DA neurons 24 – 48 h after 5 d injections of METH or saline in a novel environment (**$p < 0.01$, Student's t test). **G**, No difference in mean I_h between 5 d saline- or METH-injected mice. **H**, No change in mean $I_{Quinpirole}$ (30 μM) with 5 d METH compared with 5 d saline in a novel environment.

of hyperpolarizing 200 ms voltage steps to −120 mV. Series resistance (R_s) was monitored throughout the experiment and recordings were excluded from analysis if the R_s varied by >20%. To calculate spike number, the number of spikes elicited by a 500 ms current injection step was measured. Baclofen inhibition was calculated by measuring the spike number in the presence of baclofen divided by the spike number in the absence of baclofen plus the spike number in the presence of baclofen. GIRK basal currents were inhibited with bath-applied 1 mM BaCl$_2$ in ACSF. For the GTPγS experiment, guanosine 5′-O-[gamma-thio]triphosphate (GTPγS; Sigma-Aldrich) at 0.1 μM was substituted for Na$_3$GTP in the intracellular solution.

Statistical analysis. Data were analyzed using Prism 5.0 software. All reported values are mean ± SEM. One or two-way ANOVA with Bonferroni *post hoc* test was used for multiple comparisons; Student's unpaired t test was used for groups of two, as indicated. Statistical significance was defined as $p < 0.05$.

Results

Context pairing enhances METH-dependent decrease in $I_{Baclofen}$

To investigate the mechanism underlying the psychostimulant-dependent decrease in $I_{Baclofen}$ in VTA DA neurons (Arora et al., 2011; Sharpe et al., 2015), we examined the effect of repeated METH injections on the maximally activated $I_{Baclofen}$ in VTA DA neurons. Male and female mice (P30–P50) were injected once daily with 2 mg/kg METH intraperitoneally for 5 d in the home

cage and then brain slices containing the VTA were prepared 24–48 h after the fifth injection for whole-cell patch-clamp electrophysiology. DA neurons were identified based on the presence of an I_h current of ~600 pA at −120 mV (Cruz et al., 2004; Labouèbe et al., 2007; Padgett et al., 2012). VTA DA neurons are heterogeneous, with the I_h-expressing VTA DA neurons primarily projecting to the NAc (Lammel et al., 2008). In addition to I_h, we used Pitx3$^{+/-}$-GFP mice, in which GFP is expressed in DA neurons (Zhao et al., 2004), to further confirm the identification of VTA DA neurons (Labouèbe et al., 2007; Padgett et al., 2012; Bocklisch et al., 2013) (Fig. 1A). Bath application of a maximal concentration of (±)-baclofen (300 μM) (Cruz et al., 2004) elicited an outward current that peaked within ~30 s and decayed with time, as described previously (Cruz et al., 2004). This outward current was largely absent in mice lacking GIRK2 subunits, indicating that it is GIRK dependent (Labouèbe et al., 2007). $I_{Baclofen}$ was inhibited by bath application of Ba^{2+}(Fig. 1B), as expected for an inwardly rectifying K$^+$ channel (Lüscher and Slesinger, 2010). Surprisingly, $I_{Baclofen}$ in VTA DA neurons of 5 d METH mice did not reach statistical significance from $I_{Baclofen}$ in 5 d saline mice (Fig. 1C, $p > 0.05$, Student's t test).

Although sensitization of the locomotor-stimulant effects of psychostimulants can occur with repeated administration in the home cage, it is more robust when the drug is administered in a separate, novel environ-
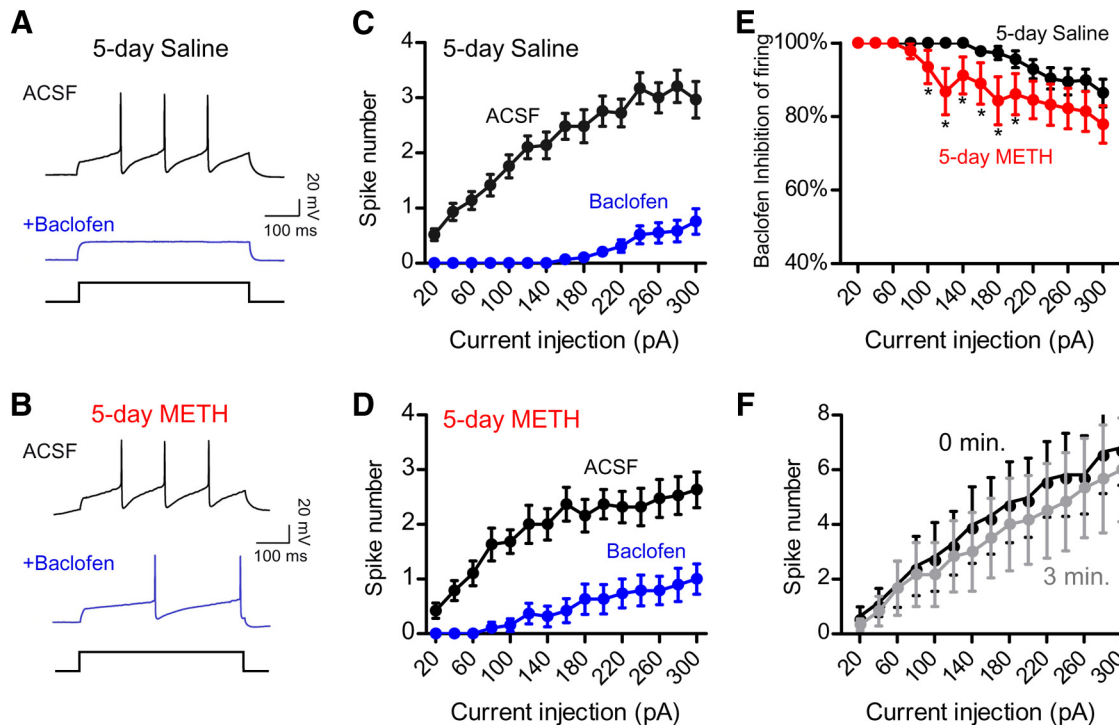
Figure 2. Baclofen-induced inhibition of firing is reduced in VTA DA neurons after 5 d METH injections. *A*, *B*, Current-clamp recordings showing firing induced with a 200 pA current injection before (black trace) and after (blue trace) addition of 300 μM baclofen. Recordings are from a GFP$^+$ VTA DA neuron in a Pitx3-GFP$^{+/-}$ mouse injected with 5 d saline (0.9%) (*A*) or 5 d METH (2 mg/kg) (*B*) in a novel environment. Input–output plots show the increase in spike number with increasing amplitude current injections for 5 d saline- (*C*) or 5 d METH (*D*)-injected mice before (black) and after baclofen (blue) exposure. *E*, Percentage baclofen-dependent inhibition of firing for VTA DA neurons from 5 d saline and 5 d METH groups (*$p < 0.05$ Student's unpaired one-tailed t test, $n = 17$–29). *F*, Input–output plot for VTA DA neuron firing in control mice (TH-Cre, $n = 6$) showing no decrement in spiking over 3 min of recording.
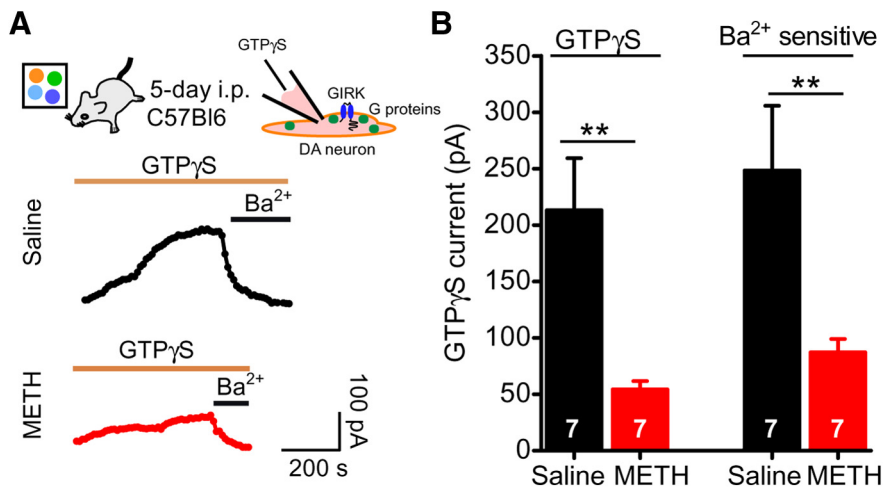

Figure 3. Effect of 5 d METH injections on the direct G-protein activation of GIRK channels in VTA DA neurons. *A*, Examples of outward currents induced by 100 μM GTPγS in the recording pipette from mice injected for 5 d saline or 5 d METH in a novel environment. Outward currents are inhibited with extracellular barium (Ba^{2+}), consistent with G-protein activation of GIRK channels. *B*, Bar graph showing significantly smaller mean GTPγS-evoked and Ba^{2+}-sensitive currents in 5 d METH-injected mice compared with 5 d saline (**$p < 0.01$ Student's unpaired t test).

ment (Badiani and Robinson, 2004). We therefore investigated whether altering the environment (i.e., context) for the METH injection affected the drug-dependent modulation of $I_{Baclofen}$. To enhance the novelty cues, we used a new cage that was wrapped in brightly colored wallpaper and contained different flooring material (Fig. 1D). Mice were also injected in a room separate from the home cage. With this novel cage environment, 5 d METH administration triggered a significant decrease

in $I_{Baclofen}$ in VTA DA neurons (Fig. 1 E,F). Conversely, the amplitude of the I_h current was unaffected by METH administration (Fig. 1G).

In addition to GABA$_B$R, stimulation of DA D$_2$-like receptors (D$_2$Rs) in VTA DA neurons also activates GIRK channels (Beckstead et al., 2004; Cruz et al., 2004), providing a mechanism for auto-inhibition after DA release (Beckstead et al., 2004). Bath application of a maximal concentration of the D$_2$R agonist quinpirole (30 μM) elicited an outward GIRK current, $I_{Quinpirole}$ (Cruz et al., 2004; Arora et al., 2011). In contrast to $I_{Baclofen}$, $I_{Quinpirole}$ in the 5 d METH group was not statistically different from that in the 5 d saline treatment group (Fig. 1H).

We next investigated whether the decrease in $I_{Baclofen}$ was sufficient to alter the GABA$_B$R-dependent inhibition of firing. In current-clamp mode, a series of increasing amplitude current injections elicited one or more spikes, which were suppressed by exposure to baclofen (300 μM) (Fig. 2A,C). In saline-treated mice, VTA DA neurons typically required ~180 pA of current injection to induce firing in the presence of baclofen (300 μM). In VTA DA neurons from METH-treated mice, however, smaller current injections (e.g., ~80 pA) were sufficient to induce firing in the presence of baclofen (Fig. 2A,D), suggesting that there was less inhibition

A

B
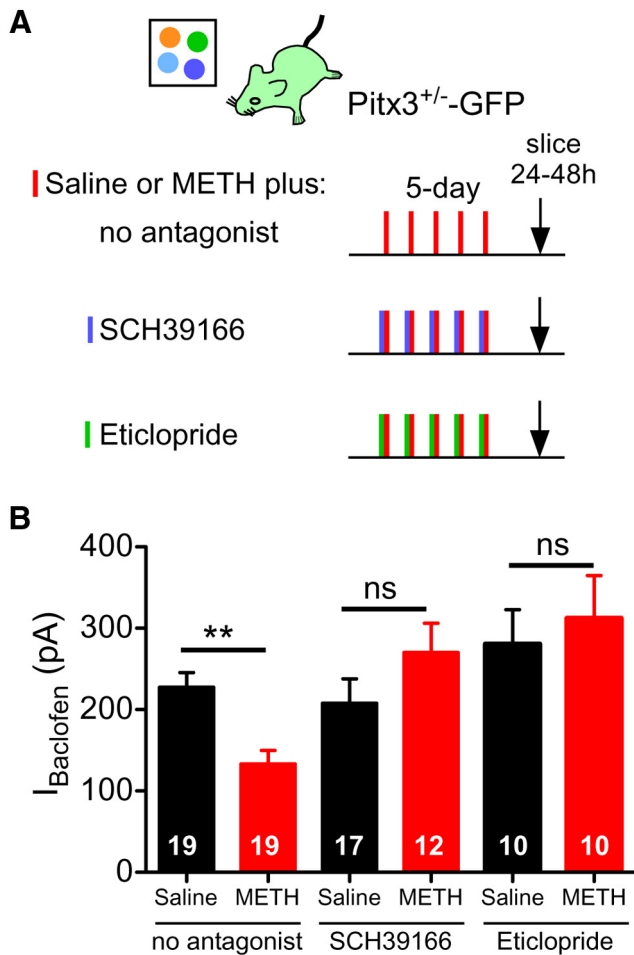
Figure 4. METH-induced decrease in $I_{Baclofen}$ requires activation of D_1-like and D_2-like receptors. **A**, Protocols for examining the effect of D_1R-like or D_2R-like receptor antagonists coinjected with METH or saline in Pitx3$^{+/-}$-GFP mice. **B**, Mean $I_{Baclofen}$ (300 μM) is reduced in VTA DA neurons 24 – 48 h after 5 d injections of METH (2 mg/kg) compared with saline (0.9%). Coinjection of METH with the D_1R-like antagonist SCH39166 (0.3 mg/kg) or METH with the D_2R-like antagonist eticlopride (0.1 mg/kg) prevents METH-dependent decrease in $I_{Baclofen}$ (**$p < 0.01$ two-way ANOVA with Bonferroni *post hoc* test.).

with baclofen. To examine this more closely, we calculated the percentage inhibition of firing with baclofen and determined that there was a significant decrease in baclofen-dependent inhibition of VTA DA neuron firing in 5 d METH-treated mice (Fig. 2E). For control, we examined the input–output relationship for VTA DA neurons in control mice (TH-Cre, $n = 6$) and found no decrement in spiking over 3 min of recording (Fig. 2F). Together, these results suggest that there is a reduction in GABA_BR-dependent inhibition of firing after 5 d of METH treatment.

A decrease in $I_{Baclofen}$ after 5 d of METH treatment could result from reduced surface expression of GIRK channels. To investigate this possibility, we interrogated indirectly the levels of GIRK channels on the plasma membrane by examining the effect of direct G-protein activation of GIRK channels. To accomplish this, we included in the intracellular solution the nonhydrolyzable GTP analog GTPγS (0.1 μM), which constitutively activates endogenous G-proteins in the absence of receptor activation (Logothetis et al., 1987). In these experiments, GTPγS activates G-proteins slowly upon formation of the whole-cell recording, with a rate of activation that takes minutes in the absence of receptor stimulation. In the 5 d saline injection control mice, GTPγS induced an outward current in VTA DA neurons that was

subsequently inhibited by bath application of 1 mM Ba^{2+} (Ba^{2+}-sensitive current; Fig. 3A). We measured maximal activation with GTPγS by subtracting the initial basal current from the peak GTPγS-activated current. In the 5 d METH group, the GTPγS-activated current in VTA DA neurons was significantly smaller than in the 5 d saline group (Fig. 3B). We also analyzed the amplitude of Ba^{2+}-inhibited current following GTPγS-dependent activation and found that this current was also significantly reduced in the 5 d METH group. Together, these results suggest that 5 d of METH exposure decreases the expression of GIRK channels on the plasma membrane, similar to the effect of 5 d of cocaine treatment (Arora et al., 2011).

METH-induced decrease in $I_{Baclofen}$ requires D_1R-like and D_2R-like DA receptor activation

Psychostimulants are powerful enhancers of DA signaling, elevating extracellular DA by blocking reuptake through the DA transporter or inducing the release of DA (Sulzer, 2011). DA stimulates two classes of DA receptors, D_1R-like and D_2R-like (Yager et al., 2015). Activation of both D_1R-like and D_2R-like receptors is involved in the locomotor and sensitization response to psychostimulants (Kalivas and Stewart, 1991; Xu et al., 1994; Kelly et al., 2008; Hikida et al., 2010). We therefore investigated the requirement of both D_1R-like and D_2R-like signaling in the METH-dependent attenuation of GABA_B-GIRK currents in VTA DA neurons. In Pitx3$^{+/-}$-GFP mice, coadministration of the D_1R antagonist SCH39166 (0.3 mg/kg, i.p.) with METH (2 mg/kg) for 5 d in a novel environment completely abrogated the decrease in $I_{Baclofen}$ (Fig. 4A,B). Maximally activated $I_{Baclofen}$ was indistinguishable from saline-injected mice (Fig. 4B). Similarly, coadministration of the D_2R-like antagonist eticlopride (0.1 mg/kg, i.p.) with METH (2 mg/kg) for 5 d also prevented the decrease in $I_{Baclofen}$ (Fig. 4B). These results indicate that METH-dependent attenuation of $I_{Baclofen}$ in VTA DA neurons requires activation of both D_1- and D_2-like receptors.

Subcellular mechanism underlying METH-dependent attenuation of $I_{Baclofen}$ in VTA DA neurons

In VTA GABA neurons (Padgett et al., 2012) and mPFC pyramidal neurons (Hearing et al., 2013), psychostimulant treatment can lead to internalization of GABA_B receptors through dephosphorylation of serine-783 (S783) on the GABA_BR2 subunit. Terunuma et al. (2014) demonstrated that neurons derived from a KI mouse engineered with the GABA_BR2-S783A mutant are insensitive to modulation by PP2A dephosphorylation. We used the GABA_BR2-S783A KI mouse to probe the mechanism underlying the METH-dependent decrease in $I_{Baclofen}$ in VTA DA neurons. For control, we first confirmed that GABA_BR2-S783A KI mice are resistant to the PP2A-dependent decrease in GABA_B receptor surface expression. We examined the effect of a 1 d of METH treatment (Fig. 5A); that is, 24 h after a single 2 mg/kg injection, on $I_{Baclofen}$ in VTA GABA neurons. This attenuation of $I_{Baclofen}$ involves dephosphorylation of S783 on the GABA_B R2 subunit (Padgett et al., 2012). GABA neurons were identified by the absence of I_h, a small capacitance (<20 pF) and a fast spontaneous firing rate (5–10 Hz) (Cruz et al., 2004). As expected, the 1 d METH treatment did not significantly decrease $I_{Baclofen}$ in VTA GABA neurons of GABA_BR2 S783A mice, in contrast to the decrease in littermate controls (Fig. 5B).

We next investigated the effect of 5 d METH injections on $I_{Baclofen}$ in VTA DA neurons from GABA_BR2-S783A KI mice (Fig. 5C). Surprisingly, the 5 d METH treatment in a novel environment decreased $I_{Baclofen}$ in VTA DA neurons of GABA_BR2-S783A
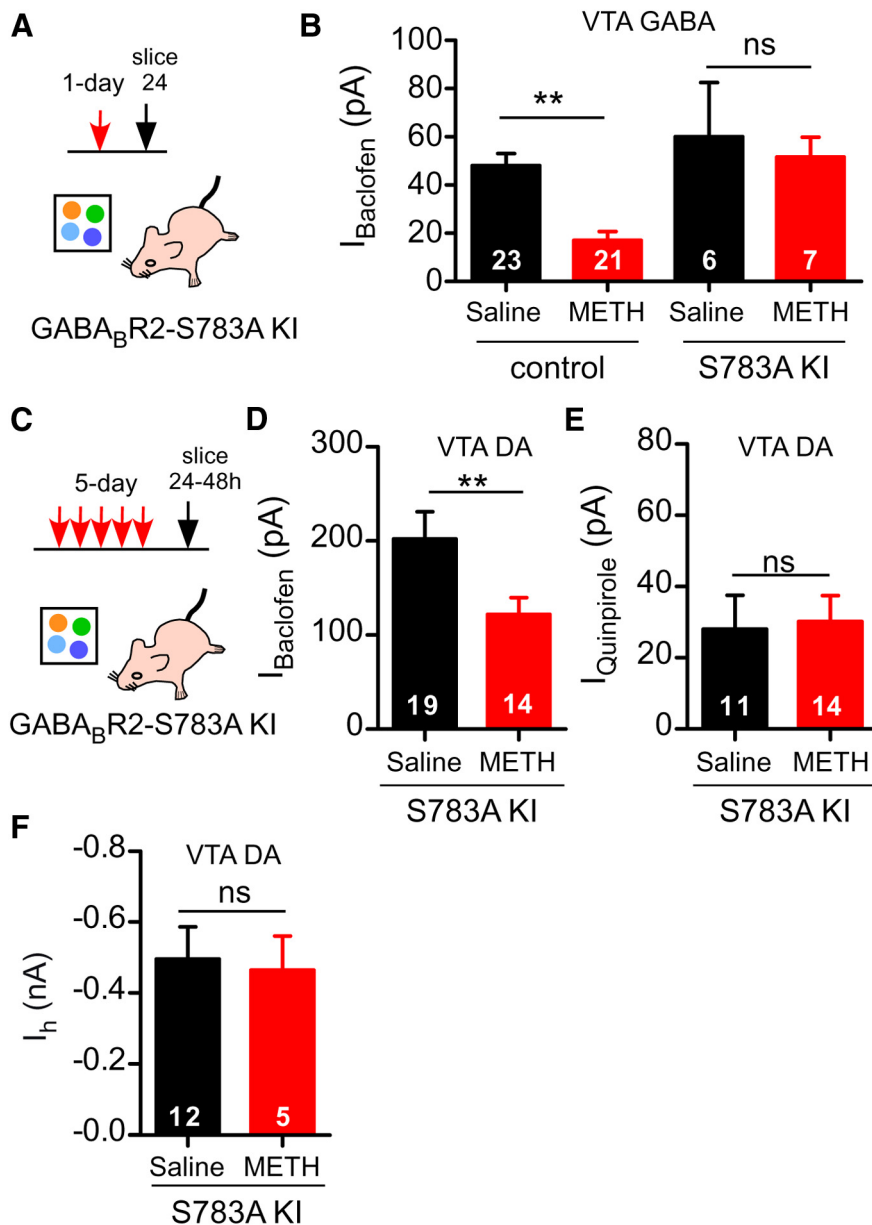
Figure 5. Lack of role for dephosphorylation of GABA$_B$R2–S783 in METH-dependent decrease in $I_{Baclofen}$ in VTA DA neurons. **A**, Protocol for VTA GABA neurons. Single injection of METH in GABA$_B$R2–S783A KI mice. **B**, Mean $I_{Baclofen}$ (100 μM) in VTA GABA neurons is reduced 24 h after a single injection of METH (2 mg/kg, $n = 21$) compared with saline (0.9%, $n = 23$) (Padgett et al., 2012) in littermate controls. **p < 0.01 two-way ANOVA with Bonferroni post hoc test. In GABA$_B$R2–S783A KI mice, there was no decrement in $I_{Baclofen}$ after 1 d METH ($n = 6$ saline, $n = 7$ METH). **C**, Protocol for VTA DA neurons after 5 d injections of METH or saline in GABA$_B$R2–S783A KI mice. **D**, Mean $I_{Baclofen}$ (300 μM) is significantly reduced (24 – 48 h) in VTA DA neurons of GABA$_B$R2–S783A KI receiving 5 d METH (2 mg/kg, $n = 14$) compared with 5 d saline (0.9%, $n = 19$). **p < 0.05 unpaired t test. **E**, Mean $I_{Quinpirole}$ (30 μM) in VTA DA neurons from GABA$_B$R2–S783A KI mice with 5 d METH or 5 d saline treatments. **F**, Mean I_h in VTA DA neurons from GABA$_B$R2–S783A KI mice with 5 d METH or 5 d saline treatments.

KI mice, similar to that in control Pitx3 $^{+/-}$-GFP mice (Figs. 1F, 5D). Similarly, $I_{Quinpirole}$ in GABA$_B$R2-S783A KI mice was not significantly different between the 5 d saline and the 5 d METH groups (Fig. 5E). Further, there was no significant difference in the I_h current of the 5 d METH or 5 d saline GABA$_B$R2-S783A KI mice (Fig. 5F). The finding that the METH treatment reduces $I_{Baclofen}$ in VTA DA neurons, but not in VTA GABA neurons of GABA$_B$R2-S783A KI mice, suggests that the regulation of GABA$_B$ receptors and GIRK channels in VTA DA neurons with psychostimulants involves a subcellular mechanism that is different from the PP2A mechanism in VTA GABA neurons.

Because dephosphorylation of the GABA$_B$R did not appear to be involved, we next investigated the role of the GIRK3 subunit. In contrast to VTA GABA neurons that express GIRK1, GIRK2c, and GIRK3 subunits, VTA DA neurons express only GIRK2c and GIRK3 subunits (Cruz et al., 2004). GIRK2c/GIRK3 channels also have unique properties, including reduced G$\beta\gamma$ sensitivity (Jelacic et al., 2000), reduced coupling efficiency with GABA$_B$Rs (Cruz et al., 2004), and the presence of a PDZ-binding motif that associates with SNX27 (Lunn et al., 2007; Munoz and Slesinger, 2014). In addition, the GIRK3 subunit regulates plasma membrane expression of GIRK channels in cell lines (Ma et al., 2002) and in neurons (Lalive et al., 2014; Munoz and Slesinger, 2014). To study the role of the GIRK3 subunit, we examined the effect of 5 d METH injections in mice lacking the GIRK3 subunit (i.e., GIRK3 KO mice) (Torrecilla et al., 2002; Morgan et al., 2003). In contrast to Pitx3 $^{+/-}$-GFP (Fig. 1F) or GABA$_B$R2-S783A KI (Fig. 5D) mice, 5 d METH injections in a novel environment did not attenuate $I_{Baclofen}$ in VTA DA neurons of GIRK3 KO mice (Fig. 6B). I_h in VTA DA neurons of GIRK3 KO mice was indistinguishable between 5 d saline and 5 d METH (Fig. 6C). Therefore, the GIRK3 subunit, but not dephosphorylation of S783 in GABA$_B$R2, appears to be important for the 5 d METH-dependent decease in $I_{Baclofen}$.

Discussion

Repeated exposure to drugs of abuse induces changes in the neurocircuitry of the reward pathway that underlie addiction (Koob and Volkow, 2010; Lüscher and Malenka, 2011). In the current study, we demonstrate a significant attenuation of $I_{Baclofen}$ in VTA DA neurons 24 – 48 h after repeated 5 d METH injections. This decrease in $I_{Baclofen}$ was enhanced when METH injections were delivered in a novel environment. Similarly, Arora et al. (2011) found that 5 d of cocaine injections outside of the home cage also produced a smaller $I_{Baclofen}$ in VTA DA neurons (Arora et al., 2011). Consistent with a context-dependent effect, sensitization of the locomotor-stimulant effects of psychostimulants is more robust when the drug is administered in a novel environment away from the home cage (Badiani and Robinson, 2004). Further, high-novelty-seeking rodents acquire sensitization and self-administration more quickly to psychostimulants (Hooks et al., 1991; Blanchard et al., 2009). A direct VTA DA projection to the hippocampus is postulated to be important for regulating the long-term memory associated with novel events; these hippocampal pyramidal neurons project back to the VTA indirectly via the nucleus accum-

bens (NAc) and ventral pallidum (Lisman and Grace, 2005; Kafkas and Montaldi, 2015). Our results with the novel environment suggest that hippocampal-mediated inputs into the VTA could play an important role in modulating GABA$_B$R-GIRK signaling in VTA DA neurons.

Previous studies demonstrated that repeated exposure to cocaine (Arora et al., 2011) or self-administration of METH (Sharpe et al., 2015) reduces $I_{Baclofen}$ in VTA DA neurons, similar to our results with 5 d METH in a novel environment. However, the mechanism underlying the attenuation was not fully elucidated in those studies. In mPFC pyramidal neurons (Hearing et al., 2013) and in VTA GABA neurons (Padgett et al., 2012), cocaine and METH exposure decrease $I_{Baclofen}$ through dephosphorylation of S783 in the GABA$_B$R2 subunit. The phosphorylation and dephosphorylation of S783 controls the postendocytic sorting of the GABA$_B$R1/R2 heterodimer (Terunuma et al., 2010). In cortical and hippocampal neurons, NMDAR-dependent attenuation of GABA$_B$R signaling occurs through PP2a-dependent dephosphorylation of S783, which can be abrogated by mutating S783 to alanine (S783A) in the GABA$_B$R2 subunit (Terunuma et al., 2014). Consistent with that mechanism, we found that GABA$_B$R2-S783A KI mice were resistant to the 1 d METH-dependent decrease in $I_{Baclofen}$ in VTA GABA neurons. In VTA DA neurons of GABA$_B$R2-S783A KI mice, however, 5 d METH continued to decrease $I_{Baclofen}$. Conversely, in mice lacking the GIRK3 subunit, 5 d METH did not trigger a decrease in $I_{Baclofen}$, in contrast to the ~50% decrease observed in METH-treated Pitx3$^{+/-}$-GFP or GABA$_B$R2-S783A mice. These results suggest the subcellular mechanisms underlying the METH-dependent modulation of $I_{Baclofen}$ are different for VTA DA and VTA GABA neurons.

How could the GIRK3 subunit in VTA DA neurons mediate the METH-dependent decrease in $I_{Baclofen}$? A simple explanation for a decrease in $I_{Baclofen}$ is a shift in the EC$_{50}$ for baclofen activation. In fact, the EC$_{50}$ for baclofen is shifted to lower concentrations in GIRK3 KO mice (Labouèbe et al., 2007). However, all measurements of $I_{Baclofen}$ in the current study were made with a saturating concentration of baclofen. Another possibility is that the GIRK3 subunit contributes to a change in trafficking of GIRK channels. GIRK3 possesses a strong lysosomal targeting sequence, for example, YWSI, and, when in complex with other GIRK subunits, GIRK3 reduces maximal currents (Ma et al., 2002). Therefore, trafficking of GIRK channels could be impaired in VTA DA neurons of GIRK3 KO mice, making them resistant to the METH-dependent decrease in $I_{Baclofen}$. The reduction in GTPγS-stimulated currents in VTA DA neurons of 5 d METH-treated mice is consistent with a defect in GIRK trafficking. Further supporting a role for GIRK3, the C-terminal domain of GIRK3 was implicated in activity-dependent potentiation of $I_{Baclofen}$ in VTA DA neurons (Lalive et al., 2014). Recently, Sharpe et al. (2015) demonstrated that chelating intracellular Ca^{2+} in VTA DA neurons enabled the recovery of $I_{Baclofen}$ in rats self-
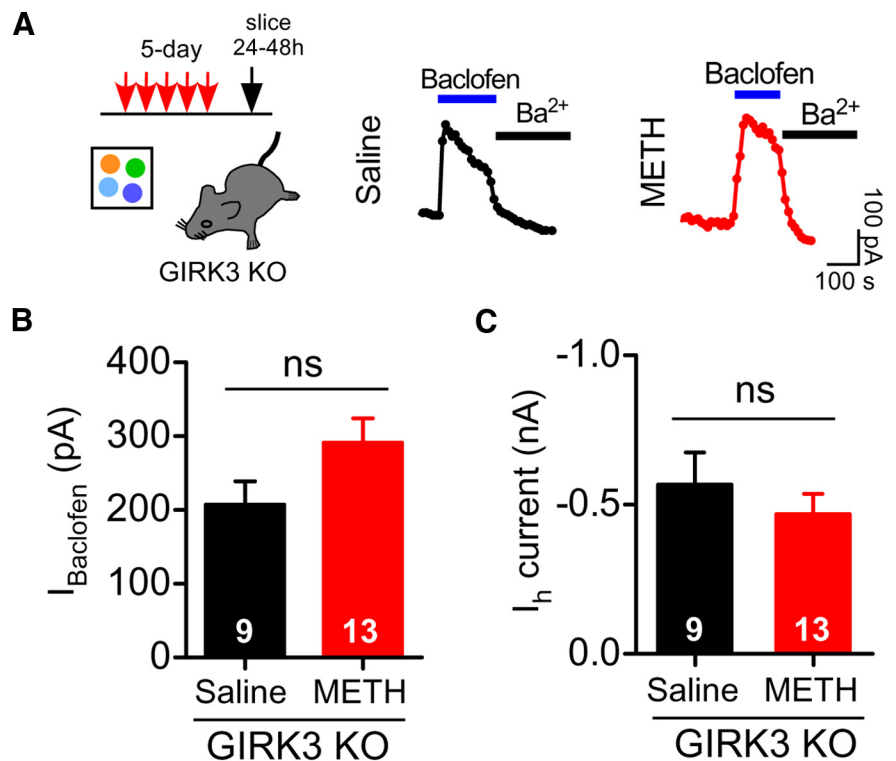
Figure 6. Requirement for the GIRK3 subunit in METH-dependent decrease in $I_{Baclofen}$. **A,** Protocol shown for GIRK3 KO mice: 5 d injections of METH or saline. Examples of $I_{Baclofen}$ (300 μM) recorded from VTA DA neurons 24 – 48 h after 5 d intraperitoneal injections of saline (0.9%) or METH (2 mg/kg) in GIRK3 KO mice. **B,** No significant decrement in mean $I_{Baclofen}$ in VTA DA neurons from GIRK3 KO mice receiving 5 d METH injection ($n = 13$) compared with 5 d saline injection mice ($n = 9$) (ns, $p > 0.05$ unpaired t test). **C,** I_h in VTA DA neurons after 5 d METH or 5 d saline in GIRK3 KO mice.

administering METH. It will be interesting to see whether changes in intracellular Ca^{2+} somehow affect GIRK3-dependent trafficking after drug exposure, especially considering that activity-dependent potentiation of $I_{Baclofen}$ requires NMDAR activation (Lalive et al., 2014).

Both GIRK2c and GIRK3 subunits possess a C-terminal PDZ-binding motif, which has been shown to interact with the PDZ domain of sorting nexin 27 (Lunn et al., 2007). SNX27 is a cytoplasmic trafficking protein that is localized to early endosomes and regulates $I_{Baclofen}$ in VTA DA neurons via a PDZ domain interaction (Cullen, 2008; Munoz and Slesinger, 2014). Further, a splice variant of SNX27, SNX27b, is upregulated in rodents after a 5 d METH treatment (Kajii et al., 2003). Together, these studies suggest that psychostimulant exposure may promote an interaction of SNX27 with the GIRK3 subunit, leading to reduced surface expression in VTA DA neurons and a smaller $I_{Baclofen}$. Future experiments will need to determine whether a decrease in GIRK channel surface expression arises from an increase in channel degradation or a decrease in recycling back to the membrane. Supporting a modulatory role for the GIRK3 subunit, mice lacking the GIRK3 exhibit altered alcohol binge drinking (Herman et al., 2015), exhibit less severe withdrawal from alcohol and other sedatives (Kozell et al., 2009), and self-administer less cocaine than littermate controls (Morgan et al., 2003).

Psychostimulants increase extracellular DA by blocking reuptake or enhancing DA release (Sulzer, 2011), leading to activation of both D$_1$R-like and D$_2$R-like receptors on distinct populations of medium spiny neurons in the dorsal striatum and NAc (Yager et al., 2015). We found that coinjection of an antagonist for either D$_1$R-like (SCH39166) or D$_2$R-like (eticlopride)

DA receptors prevented the 5 d METH-induced decrease in $I_{Baclofen}$ in VTA DA neurons. Similar results were found with METH-dependent decrease in $I_{Baclofen}$ in VTA GABA neurons (Padgett et al., 2012). Arora et al. (2011) reported that an antagonist for D_2R-like, but not D_1R-like, activation prevented acute cocaine-dependent attenuation of $I_{Baclofen}$ in VTA DA neurons. These differences in the involvement of D_1R-like versus D_2R-like receptors could depend on the time course of treatment (i.e., acute vs 5 d). In addition, METH and cocaine differ in their pharmacokinetics in that the actions of METH last longer than those of cocaine (Sulzer, 2011). Activation of D_1R-like receptors is required for the locomotor response to psychostimulants (Xu et al., 1994; Kelly et al., 2008; Yager et al., 2015). However, both D_1R-like and D_2R-like receptors act in concert to influence acquisition of METH sensitization (Kelly et al., 2008) and, when impaired, attenuate psychostimulant-dependent sensitization (Hikida et al., 2010). Additional studies are needed to determine how stimulation of D_1R-like and D_2R-like pathways induces a change in GIRK channel expression in VTA DA neurons.

In contrast to the decrease in $I_{Baclofen}$, we did not observe a METH-induced decrease in $I_{Quinpirole}$, suggesting differential regulation of D_2R- and GABA$_B$R-coupled GIRK channels. One possibility is that GIRK2 homotetramers associate with D_2Rs, whereas GIRK2c/GIRK3 heterotetramers associate with GABA$_B$Rs. However, Lacey et al. (1988) proposed that stimulation of D_2R and GABA$_B$R in substantia nigra DA neurons converges on the same population of GIRK channels. Further, in previous studies, repeated exposure to cocaine or METH attenuated both GABA$_B$R and D_2R-activated GIRK currents (Arora et al., 2011; Sharpe et al., 2015). It will be important to determine whether these differences are due to the psychostimulant exposure conditions or heterogeneity of DA neurons in the VTA.

What impact does attenuation of $I_{Baclofen}$ in VTA DA neurons have on the reward pathway? A reduction of $I_{Baclofen}$ would be expected to increase the excitability of VTA DA neurons. For example, mice lacking the $\beta3$ GABA$_A$ subunit in DA neurons have reduced inhibitory GABA$_A$ currents, which increases DA release in the NAc and enhances acquisition of appetitive learning (Parker et al., 2011). Repeated cocaine exposure downregulates GABA$_A$R signaling, leading to enhanced LTP of excitatory synapses (Liu et al., 2005). For GIRK channels, mice lacking SNX27 in VTA DA neurons have a smaller $I_{Baclofen}$ and an enhanced locomotor response to cocaine (Munoz and Slesinger, 2014), suggesting hyperexcitability of VTA DA neurons. In parallel with changes of inhibitory inputs into VTA DA neurons, psychostimulant exposure also affects excitatory synaptic transmission (Lüscher and Malenka, 2011). Single or multiple exposure to drugs of abuse induces neuroadaptations that prime for compulsive behavior (Lüscher and Malenka, 2011). For example, a single exposure to cocaine produces plasticity changes at excitatory synapses in VTA DA neurons (Ungless et al., 2001; Borgland et al., 2004). Repeated administration leads to potentiation in excitatory transmission and is sufficient to induce downstream plasticity in the NAc (Zhang et al., 1997; Borgland et al., 2004; Bellone and Lüscher, 2006). The psychostimulant-dependent attenuation of inhibition may synergize with enhancement of excitatory transmission, driving dopaminergic activity in the VTA and changing the motivation for drug-seeking behaviors (Lüscher and Malenka, 2011).

References

Arora D, Hearing M, Haluk DM, Mirkovic K, Fajardo-Serrano A, Wessendorf MW, Watanabe M, Luján R, Wickman K (2011) Acute cocaine exposure weakens GABA(B) receptor-dependent G-protein-gated inwardly rectifying K+ signaling in dopamine neurons of the ventral tegmental area. J Neurosci 31:12251–12257. CrossRef Medline

Backes EN, Hemby SE (2008) Contribution of ventral tegmental GABA receptors to cocaine self-administration in rats. Neurochem Res 33:459–467. CrossRef Medline

Badiani A, Robinson TE (2004) Drug-induced neurobehavioral plasticity: the role of environmental context. Behav Pharmacol 15:327–339. CrossRef Medline

Beckstead MJ, Grandy DK, Wickman K, Williams JT (2004) Vesicular dopamine release elicits an inhibitory postsynaptic current in midbrain dopamine neurons. Neuron 42:939–946. CrossRef Medline

Bellone C, Lüscher C (2006) Cocaine triggered AMPA receptor redistribution is reversed in vivo by mGluR-dependent long-term depression. Nat Neurosci 9:636–641. CrossRef Medline

Blanchard MM, Mendelsohn D, Stamp JA (2009) The HR/LR model: Further evidence as an animal model of sensation seeking. Neurosci Biobehav Rev 33:1145–1154. CrossRef Medline

Bocklisch C, Pascoli V, Wong JC, House DR, Yvon C, de Roo M, Tan KR, Lüscher C (2013) Cocaine disinhibits dopamine neurons by potentiation of GABA transmission in the ventral tegmental area. Science 341:1521–1525. CrossRef Medline

Borgland SL, Malenka RC, Bonci A (2004) Acute and chronic cocaine-induced potentiation of synaptic strength in the ventral tegmental area: electrophysiological and behavioral correlates in individual rats. J Neurosci 24:7482–7490. CrossRef Medline

Brebner K, Froestl W, Andrews M, Phelan R, Roberts DC (1999) The GABA(B) agonist CGP 44532 decreases cocaine self-administration in rats: demonstration using a progressive ratio and a discrete trials procedure. Neuropharmacology 38:1797–1804. CrossRef Medline

Cameron DL, Wessendorf MW, Williams JT (1997) A subset of ventral tegmental area neurons is inhibited by dopamine, 5-hydroxytryptamine and opioids. Neuroscience 77:155–166. CrossRef Medline

Chen BT, Bowers MS, Martin M, Hopf FW, Guillory AM, Carelli RM, Chou JK, Bonci A (2008) Cocaine but not natural reward self-administration nor passive cocaine infusion produces persistent LTP in the VTA. Neuron 59:288–297. CrossRef Medline

Cruz HG, Ivanova T, Lunn ML, Stoffel M, Slesinger PA, Lüscher C (2004) Bi-directional effects of GABA$_B$ receptor agonists on the mesolimbic dopamine system. Nat Neurosci 7:153–159. CrossRef Medline

Cullen PJ (2008) Endosomal sorting and signalling: an emerging role for sorting nexins. Nat Rev Mol Cell Biol 9:574–582. CrossRef Medline

Friedman AK, Walsh JJ, Juarez B, Ku SM, Chaudhury D, Wang J, Li X, Dietz DM, Pan N, Vialou VF, Neve RL, Yue Z, Han MH (2014) Enhancing depression mechanisms in midbrain dopamine neurons achieves homeostatic resilience. Science 344:313–319. CrossRef Medline

Hearing M, Kotecki L, Marron Fernandez de Velasco E, Fajardo-Serrano A, Chung HJ, Luján R, Wickman K (2013) Repeated cocaine weakens GABA(B)-Girk signaling in layer 5/6 pyramidal neurons in the prelimbic cortex. Neuron 80:159–170. CrossRef Medline

Herman MA, Sidhu H, Stouffer DG, Kreifeldt M, Le D, Cates-Gatto C, Munoz MB, Roberts AJ, Parsons LH, Roberto M, Wickman K, Slesinger PA, Contet C (2015) GIRK3 gates activation of the mesolimbic dopaminergic pathway by ethanol. Proc Natl Acad Sci U S A 112:7091–7096. CrossRef Medline

Hikida T, Kimura K, Wada N, Funabiki K, Nakanishi S (2010) Distinct roles of synaptic transmission in direct and indirect striatal pathways to reward and aversive behavior. Neuron 66:896–907. CrossRef Medline

Hooks MS, Jones GH, Smith AD, Neill DB, Justice JB Jr (1991) Individual differences in locomotor activity and sensitization. Pharmacol Biochem Behav 38:467–470. CrossRef Medline

Jelacic TM, Kennedy ME, Wickman K, Clapham DE (2000) Functional and biochemical evidence for G-protein-gated inwardly rectifying K$^+$ (GIRK) channels composed of GIRK2 and GIRK3. J Biol Chem 275:36211–36216. CrossRef Medline

Kafkas A, Montaldi D (2015) Striatal and midbrain connectivity with the hippocampus selectively boosts memory for contextual novelty. Hippocampus 25:1262–1273. CrossRef Medline

Kajii Y, Muraoka S, Hiraoka S, Fujiyama K, Umino A, Nishikawa T (2003) A developmentally regulated and psychostimulant-inducible novel rat gene mrt1 encoding PDZ-PX proteins isolated in the neocortex. Mol Psychiatry 8:434–444. CrossRef Medline

Kalivas PW, Stewart J (1991) Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res Brain Res Rev 16:223–244. CrossRef Medline

Kelly MA, Low MJ, Rubinstein M, Phillips TJ (2008) Role of dopamine D1-like receptors in methamphetamine locomotor responses of D2 receptor knockout mice. Genes Brain Behav 7:568–577. CrossRef Medline

Koob GF, Volkow ND (2010) Neurocircuitry of addiction. Neuropsychopharmacology 35:217–238. CrossRef Medline

Kotecki L, Hearing M, McCall NM, Marron Fernandez de Velasco E, Pravetoni M, Arora D, Victoria NC, Munoz MB, Xia Z, Slesinger PA, Weaver CD, Wickman K (2015) GIRK channels modulate opioid-induced motor activity in a cell type- and subunit-dependent manner. J Neurosci 35:7131–7142. CrossRef Medline

Kozell LB, Walter NA, Milner LC, Wickman K, Buck KJ (2009) Mapping a barbiturate withdrawal locus to a 0.44 Mb interval and analysis of a novel null mutant identify a role for Kcnj9 (GIRK3) in withdrawal from pentobarbital, zolpidem, and ethanol. J Neurosci 29:11662–11673. CrossRef Medline

Labouèbe G, Lomazzi M, Cruz HG, Creton C, Luján R, Li M, Yanagawa Y, Obata K, Watanabe M, Wickman K, Boyer SB, Slesinger PA, Lüscher C (2007) RGS2 modulates coupling between GABA_B receptors and GIRK channels in dopamine neurons of the ventral tegmental area. Nat Neurosci 10:1559–1568. CrossRef Medline

Lacey MG, Mercuri NB, North RA (1988) On the potassium conductance increase activated by GABA_B and dopamine D_2 receptor in rat substantia nigra neurones. J Physiol 401:437–453. CrossRef Medline

Lalive AL, Munoz MB, Bellone C, Slesinger PA, Lüscher C, Tan KR (2014) Firing modes of dopamine neurons drive bidirectional GIRK channel plasticity. J Neurosci 34:5107–5114. CrossRef Medline

Lammel S, Hetzel A, Häckel O, Jones I, Liss B, Roeper J (2008) Unique properties of mesoprefrontal neurons within a dual mesocorticolimbic dopamine system. Neuron 57:760–773. CrossRef Medline

Lhuillier L, Mombereau C, Cryan JF, Kaupmann K (2007) GABA(B) receptor-positive modulation decreases selective molecular and behavioral effects of cocaine. Neuropsychopharmacology 32:388–398. CrossRef Medline

Lisman JE, Grace AA (2005) The hippocampal-VTA loop: controlling the entry of information into long-term memory. Neuron 46:703–713. CrossRef Medline

Liu QS, Pu L, Poo MM (2005) Repeated cocaine exposure in vivo facilitates LTP induction in midbrain dopamine neurons. Nature 437:1027–1031. CrossRef Medline

Logothetis DE, Kurachi Y, Galper J, Neer EJ, Clapham DE (1987) The βγ subunits of GTP-binding proteins activate the muscarinic K^+ channel in heart. Nature 325:321–326. CrossRef Medline

Lunn ML, Nassirpour R, Arrabit C, Tan J, McLeod I, Arias CM, Sawchenko PE, Yates JR 3rd, Slesinger PA (2007) A unique sorting nexin regulates trafficking of potassium channels via a PDZ domain interaction. Nat Neurosci 10:1249–1259. CrossRef Medline

Lüscher C, Malenka RC (2011) Drug-evoked synaptic plasticity in addiction: from molecular changes to circuit remodeling. Neuron 69:650–663. CrossRef Medline

Lüscher C, Slesinger PA (2010) Emerging roles for G protein-gated inwardly rectifying potassium (GIRK) channels in health and disease. Nat Rev Neurosci 11:301–315. CrossRef Medline

Ma D, Zerangue N, Raab-Graham K, Fried SR, Jan YN, Jan LY (2002) Diverse trafficking patterns due to multiple traffic motifs in G protein-activated inwardly rectifying potassium channels from brain and heart. Neuron 33:715–729. CrossRef Medline

Margolis EB, Hjelmstad GO, Bonci A, Fields HL (2003) Kappa-opioid agonists directly inhibit midbrain dopaminergic neurons. J Neurosci 23:9981–9986. Medline

Morgan AD, Carroll ME, Loth AK, Stoffel M, Wickman K (2003) Decreased cocaine self-administration in Kir3 potassium channel subunit knockout mice. Neuropsychopharmacology 28:932–938. Medline

Munoz MB, Slesinger PA (2014) Sorting nexin 27 regulation of G protein-gated inwardly rectifying K+ channels attenuates in vivo cocaine response. Neuron 82:659–669. CrossRef Medline

Padgett CL, Lalive AL, Tan KR, Terunuma M, Munoz MB, Pangalos MN, Martínez-Hernández J, Watanabe M, Moss SJ, Luján R, Lüscher C, Slesinger PA (2012) Methamphetamine-evoked depression of GABA (B) receptor signaling in GABA neurons of the VTA. Neuron 73:978–989. CrossRef Medline

Parker JG, Wanat MJ, Soden ME, Ahmad K, Zweifel LS, Bamford NS, Palmiter RD (2011) Attenuating GABA(A) receptor signaling in dopamine neurons selectively enhances reward learning and alters risk preference in mice. J Neurosci 31:17103–17112. CrossRef Medline

Robinson TE, Berridge KC (2003) Addiction. Annu Rev Psychol 54:25–53. CrossRef Medline

Saal D, Dong Y, Bonci A, Malenka RC (2003) Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. Neuron 37:577–582. CrossRef Medline

Seutin V, Johnson SW, North RA (1994) Effect of dopamine and baclofen on N-methyl-D-aspartate-induced burst firing in rat ventral tegmental neurons. Neuroscience 58:201–206. CrossRef Medline

Sharpe AL, Varela E, Bettinger L, Beckstead MJ (2015) Methamphetamine self-administration in mice decreases GIRK channel-mediated currents in midbrain dopamine neurons. Int J Neuropsychopharmacol 18:pii: pyu073. CrossRef Medline

Shoaib M, Swanner LS, Beyer CE, Goldberg SR, Schindler CW (1998) The GABAB agonist baclofen modifies cocaine self-administration in rats. Behav Pharmacol 9:195–206. Medline

Sulzer D (2011) How addictive drugs disrupt presynaptic dopamine neurotransmission. Neuron 69:628–649. CrossRef Medline

Terunuma M, Vargas KJ, Wilkins ME, Ramírez OA, Jaureguiberry-Bravo M, Pangalos MN, Smart TG, Moss SJ, Couve A (2010) Prolonged activation of NMDA receptors promotes dephosphorylation and alters postendocytic sorting of GABAB receptors. Proc Natl Acad Sci U S A 107:13918–13923. CrossRef Medline

Terunuma M, Revilla-Sanchez R, Quadros IM, Deng Q, Deeb TZ, Lumb M, Sicinski P, Haydon PG, Pangalos MN, Moss SJ (2014) Postsynaptic GABAB receptor activity regulates excitatory neuronal architecture and spatial memory. J Neurosci 34:804–816. CrossRef Medline

Torrecilla M, Marker CL, Cintora SC, Stoffel M, Williams JT, Wickman K (2002) G-protein-gated potassium channels containing Kir3.2 and Kir3.3 subunits mediate the acute inhibitory effects of opioids on locus ceruleus neurons. J Neurosci 22:4328–4334. Medline

Ungless MA, Whistler JL, Malenka RC, Bonci A (2001) Single cocaine exposure in vivo induces long-term potentiation in dopamine neurons. Nature 411:583–587. CrossRef Medline

Vanderschuren LJ, Kalivas PW (2000) Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. Psychopharmacology (Berl) 151:99–120. CrossRef Medline

Xu M, Hu XT, Cooper DC, Moratalla R, Graybiel AM, White FJ, Tonegawa S (1994) Elimination of cocaine-induced hyperactivity and dopamine-mediated neurophysiological effects in dopamine D1 receptor mutant mice. Cell 79:945–955. CrossRef Medline

Yager LM, Garcia AF, Wunsch AM, Ferguson SM (2015) The ins and outs of the striatum: Role in drug addiction. Neuroscience 301:529–541. CrossRef Medline

Zhang XF, Hu XT, White FJ, Wolf ME (1997) Increased responsiveness of ventral tegmental area dopamine neurons to glutamate after repeated administration of cocaine or amphetamine is transient and selectively involves AMPA receptors. J Pharmacol Exp Ther 281:699–706. Medline

Zhao S, Maxwell S, Jimenez-Beristain A, Vives J, Kuehner E, Zhao J, O'Brien C, de Felipe C, Semina E, Li M (2004) Generation of embryonic stem cells and transgenic mice expressing green fluorescence protein in midbrain dopaminergic neurons. Eur J Neurosci 19:1133–1140. CrossRef Medline