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Selective Ablation of GIRK Channels in Dopamine Neurons Alters Behavioral Effects of Cocaine in Mice

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The increase in dopamine (DA) neurotransmission stimulated by *in vivo* cocaine exposure is tempered by G protein-dependent inhibitory feedback mechanisms in DA neurons of the ventral tegmental area (VTA). G protein-gated inwardly rectifying K^+ (GIRK/Kir3) channels mediate the direct inhibitory effect of $GABA_B$ receptor ($GABA_BR$) and D_2 DA receptor (D_2R) activation in VTA DA neurons. Here we examined the effect of the DA neuron-specific loss of GIRK channels on D_2R-dependent regulation of VTA DA neuron excitability and on cocaine-induced, reward-related behaviors. Selective ablation of *Girk2* in DA neurons did not alter the baseline excitability of VTA DA neurons but significantly reduced the magnitude of D_2R-dependent inhibitory somatodendritic currents and blunted the impact of D_2R activation on spontaneous activity and neuronal excitability. Mice lacking GIRK channels in DA neurons exhibited increased locomotor activation in response to acute cocaine administration and an altered locomotor sensitization profile, as well as increased responding for and intake of cocaine in an intravenous self-administration test. These mice, however, showed unaltered cocaine-induced conditioned place preference. Collectively, our data suggest that feedback inhibition to VTA DA neurons, mediated by GIRK channel activation, tempers the locomotor stimulatory effect of cocaine while also modulating the reinforcing effect of cocaine in an operant-based self-administration task.
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INTRODUCTION

Dopamine (DA) neurons of the ventral tegmental area (VTA) are an integral part of the mesocorticolimbic system, a network of brain regions involved in reward-related behavior. Most drugs of abuse share the ability to increase extracellular levels of DA within this circuit (Nestler, 2005). Cocaine enhances DA neurotransmission by inhibiting transporters that remove DA from the extracellular space, allowing levels of DA to rise in downstream targets of DA neurons (Di Chiara and Imperato, 1988). Elevated DA signaling triggered by cocaine is implicated in behavioral effects, including locomotor stimulation and sensitization, and conditioned place preference (CPP; Pierce and Kalivas, 1997; Zweifel *et al*, 2008).

In addition to enhancing DA levels in downstream targets of VTA DA neurons, cocaine also increases DA within the VTA (Groves *et al*, 1975; Beart *et al*, 1979). The cocaine-induced increase in VTA DA levels activates autoreceptors (D_2R) that, together with $GABA_BR$-dependent feedback (Waddington and Cross, 1978; Wolf *et al*, 1978), temper VTA DA neuron excitability (Einhorn *et al*, 1988). Pharmacological blockade or genetic suppression of G protein-dependent inhibitory feedback pathways in midbrain DA neurons alters behavioral effects of cocaine, including locomotor activation and self-administration (Steketee and Kalivas, 1991; Bello *et al*, 2011; Holroyd *et al*, 2015; de Jong *et al*, 2015). Moreover, inhibitory G protein signaling mediated by $GABA_BR$ and D_2R is decreased following cocaine administration (Ackerman and White, 1990; Kushner and Unterwald, 2001; Arora *et al*, 2011), highlighting the reciprocal relationship between cocaine and inhibitory G protein signaling in DA neurons.

The direct inhibitory influence of $GABA_BR$ and D_2R activation on VTA DA neurons is mediated primarily by activation of G protein-gated inwardly rectifying K^+ (GIRK/Kir3) channels found in the somatodendritic compartment (Beckstead *et al*, 2004; Cruz *et al*, 2004). Although GIRK1/GIRK2 heterotetramers are considered to be the prototypical neuronal GIRK channel (Lujan *et al*, 2014), VTA DA neurons express a GIRK2/GIRK3 heteromer (Cruz *et al*, 2004). *Girk2* ablation eliminates all GIRK channel activity in VTA DA neurons (Beckstead *et al*, 2004; Cruz *et al*, 2004).

Multiple lines of evidence suggest that GIRK channels modulate DA-dependent behaviors. Constitutive $Girk2^{-/-}$ mice are hyperactive, a phenotype normalized by D_1 DA receptor (D_1R) blockade (Blednov *et al*, 2002). Moreover, $Girk2^{-/-}$ mice exhibit enhanced locomotor activation in

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response to morphine and cocaine (Arora *et al*, 2010; Kotecki *et al*, 2015). The many phenotypes and adaptations associated with global *Girk2* ablation, however, confound interpretation of these data (Lujan *et al*, 2014). For example, VTA DA neurons, NAc medium spiny neurons (MSNs), and layer 5/6 medial prefrontal cortex (mPFC) pyramidal neurons from *Girk2*$^{-/-}$ mice exhibit elevated AMPA receptor-mediated neurotransmission (Arora *et al*, 2010; Hearing *et al*, 2013). The recent availability of mice lacking GIRK2 in DA neurons permits a more precise evaluation of the role of GIRK-dependent signaling in modulating DA neuron excitability and cocaine-induced behaviors (Kotecki *et al*, 2015). Here we report that GIRK channel ablation in DA neurons reduces the autoreceptor-mediated inhibition of VTA DA neurons and alters behavioral sensitivity to cocaine.

MATERIALS AND METHODS

Animals

All studies were approved by the Institutional Animal Care and Use Committees at the University of Minnesota and University of Texas Health Science Center, San Antonio. The generation of *Girk2*$^{flox/flox}$, DATCre(+/−):*Girk2*$^{flox/flox}$, and Pitx3-eGFP(+)/DATCre(+/−):*Girk2*$^{flox/flox}$ mice was described previously (Kotecki *et al*, 2015). DATCre (B6.SJL-Slc6a3$^{tm1.1(cre)Bkmn}$) and Drd1a-tdTomato (B6.Cg-Tg(Drd1a-tdTomato)6Calak/J) lines were purchased from The Jackson Laboratory (Bar Harbor, ME), and the Drd2-eGFP strain (Tg (Drd2-EGFP)S118Gsat) was obtained from the Mutant Mouse Regional Resource Center. Mice were maintained on a 12 h light/dark cycle (lights on at 0700 hours), with food and water available *ad libitum*.

Drugs

Quinpirole, sulpiride, tetrodotoxin (TTX), and picrotoxin were purchased from Sigma (St Louis, MO). Cocaine hydrochloride was purchased from Sigma or provided by the National Institute on Drug Abuse drug supply program (RTI International, Research Triangle Park, NC).

Slice Electrophysiology

Horizontal slices (225 μm) of the mouse VTA (5–7 weeks) were prepared as described (Kotecki *et al*, 2015). Neurons medial to the medial terminal nucleus of the accessory optic tract, and identified via GFP expression driven by the *Pitx3* promoter, were targeted for analysis. DA neurons in the most medial aspect of the VTA were avoided as they were reported to exhibit low GIRK2 and D_2R expression (Lammel *et al*, 2008). Whole-cell data were acquired using a Multiclamp 700 A amplifier and the pCLAMPv.9.2 software (Molecular Devices; Sunnyvale, CA). I_h amplitude was assessed using a 1-s voltage ramp (−60 to −120 mV). Somatodendritic currents ($V_{hold} = -60$ mV) were measured in the presence of TTX (0.5 μM). Spontaneous activity was measured in current-clamp mode ($I = 0$) for 1 min. Neurons exhibiting no or irregular spontaneous activities were not evaluated. Rheobase and current/spike relationships were measured by injecting currents from −60 to 220 pA, increasing in 20 pA increments (1 s/step). Rheobase was defined as the minimum current evoking one or more action potentials. To assess the effect of $D_{2/3}R$ activation on excitability, neurons were voltage-clamped ($V_{hold} = -60$ mV) while quinpirole was applied to the bath. At the peak of the quinpirole response, spontaneous activity and excitability were reassessed. All command potentials factored in a junction potential of −15 mV. Series and membrane resistances were tracked throughout the experiment. If series resistance was high (>20 MΩ) or unstable (>20% variation), the experiment was excluded from analysis.

Locomotor Activity

Cocaine-induced activity was assessed in open field activity chambers (Med-Associates, St Albans, VT), as described (Pravetoni and Wickman, 2008). Subjects (7–10 weeks) were acclimated over 3 days, during which the animals were handled and exposed to i.p. injection (saline) and the open field. Distance traveled during the 60-min period following saline injection on the last acclimation day was taken as baseline activity. For acute cocaine-induced activity studies, subjects were given 1 of the 3 cocaine doses (3, 15, or 30 mg/kg i.p.). For cocaine sensitization, subjects received cocaine (15 mg/kg i.p.) for 5 days, followed by cocaine challenge (15 mg/kg i.p.) 10–11 days later.

Conditioned Place Preference

CPP testing was performed in two-compartment chambers (Med Associates) housed within sound-attenuating cubicles. One cohort was evaluated with 0 (saline) or 15 mg/kg cocaine using a three conditioning session design (Mirkovic *et al*, 2012). A second cohort was evaluated using a modified design involving lower cocaine doses (0.5 and 3 mg/kg) (Wydeven *et al*, 2014). Side preference in this study was evaluated twice, after the second and fourth cocaine conditioning sessions. CPP was calculated as the difference in time spent in drug- and saline-paired chamber during the posttest.

Self-Administration

Male mice (15–20 weeks) were group-housed (3–5/cage) on a 12/12 h reverse light–dark cycle (lights off at 0900 hours), with *ad libitum* access to food and water throughout the study. Following jugular catheterization, mice were housed individually and allowed ⩾7 days to recover. Operant sessions (2 h) were conducted as described (Sharpe *et al*, 2014), with minor modifications. During training, responses in the correct nose poke hole were rewarded on a fixed ratio 1 (FR1) schedule of reinforcement for infusions 1–5, an FR2 for infusions 6–8, and FR3 thereafter. Upon completing the response requirement, a green stimulus light in the correct nose poke hole was turned off, a 30-s time out was initiated, and cocaine was delivered (0.5 mg/kg/infusion over 2 s), accompanied by a sound stimulus of 2 kHz. After the timeout, the correct nose poke hole was re-illuminated. Although responding in both holes was recorded during the timeout, no responses were reinforced. Self-administration was considered acquired when infusion number was ⩾8 in two consecutive sessions and the number of nose pokes in the correct hole represented 70% of the total. Mice then advanced to 7 days of training on an FR3 schedule

(0.5 mg/kg/infusion) to ensure stable responding prior to the dose-response assessment. For the dose–response study, cocaine dose per infusion was increased daily (0.03, 0.1, 0.3, 1.0, and 3.0 mg/kg/infusion). Self-administration across this dose range was evaluated over three rounds, with the first round considered training. The average of data for each subject over the last two rounds was used for analysis. Although all doses were calculated based on a typical weight of a young adult mouse (28 g), final intake values were corrected for actual bodyweight. Catheters were flushed before and after sessions to assess patency. Two $GIRK2_{DA}$WT and four $GIRK2_{DA}$KO subjects were excluded from the study owing to inconsistent self-administration or failed catheter patency.

Statistical Analysis

Data are presented throughout as the mean ± SEM. Statistical analyses were performed using Prism (GraphPad Software, La Jolla, CA) and SigmaPlot (Systat Software, San Jose, CA). With the exception of mEPSC and self-administration studies, which included males only, all studies involved balanced groups of male and female mice. Data were analyzed first for effects of sex and genotype using two-way ANOVA when those were the only variables. For studies with additional variables (eg, multiple testing days), the interaction of sex and genotype was assessed using two-way ANOVAs at each level; the effect of sex within each genotype was determined using a repeated-measures two-way ANOVA. When an effect of sex or a sex interaction was observed, data from each sex were analyzed separately. When sex differences were not observed, male and female data were pooled. Pairwise comparisons were performed using Student's *t* or Mann–Whitney *U* tests, or Bonferroni *post-hoc* test, as appropriate. Differences were considered significant if $P<0.05$.

RESULTS

$D_{2/3}$R-Dependent Signaling and Excitability in VTA DA Neurons from $GIRK2_{DA}$KO Mice

We recently demonstrated that VTA DA neurons in DATCre(+):*Girk2*$^{flox/flox}$ mice ($GIRK2_{DA}$KO mice) exhibited diminished inhibitory somatodendritic current responses to $GABA_B$R activation (Kotecki *et al*, 2015). To test whether autoreceptor-mediated signaling in VTA DA neurons from $GIRK2_{DA}$KO mice was similarly blunted, we measured currents evoked by a saturating concentration (20 μM) of the $D_{2/3}$R agonist quinpirole. $GIRK2_{DA}$KO mice were crossed with mice expressing GFP under the control of a DA neuron-specific promoter (Pitx3) to permit targeted characterization of VTA DA neurons. VTA DA neurons from DATCre(−):*Girk2*$^{flox/flox}$ mice ($GIRK2_{DA}$WT mice) exhibited outward currents that were reversed by the $D_{2/3}$R antagonist sulpiride (5 μM; Figure 1a). Although DA neurons from $GIRK2_{DA}$KO mice also showed quinpirole-induced responses, amplitudes were smaller than their wild-type counterparts (Figure 1a and b; Mann–Whitney $U=85.5$, $^{***}P<0.001$). There was no effect of genotype on other properties of VTA DA neurons (Supplementary Table S1).

We next measured spontaneous activity and rheobase in the absence or presence of quinpirole (20 μM). At baseline, VTA DA neurons from $GIRK2_{DA}$WT and $GIRK2_{DA}$KO mice exhibited no difference in spontaneous activity (Figure 2a and b), rheobase (Figure 2c and d), current–spike relationship (Figure 2e), or other properties (Supplementary Table S2). In the presence of quinpirole, spontaneous activity of VTA DA neurons from $GIRK2_{DA}$WT mice was completely eliminated (16/16 neurons; Figure 2a and b). In contrast, quinpirole eliminated spontaneous activity in only 6/12 neurons $GIRK2_{DA}$KO VTA DA neurons, with the remainder showing an incomplete suppression of activity (Mann–Whitney $U=48.0$, $^{**}P<0.01$). Quinpirole markedly decreased excitability (increased rheobase) in $GIRK2_{DA}$WT VTA DA neurons but had less of an effect on $GIRK2_{DA}$KO VTA DA neurons (Figure 2c and d; Mann–Whitney $U=17.5$, $^{***}P<0.001$). Finally, the current/spike relationship was more prominently suppressed by quinpirole in VTA DA neurons from $GIRK2_{DA}$WT as compared with $GIRK2_{DA}$KO mice (Figure 2e; $F_{14,336}=24.5$, $P<0.001$, current × genotype interaction). These data indicate that, while loss of GIRK-dependent signaling does not impact baseline excitability of VTA DA neurons, it does dampen the inhibitory influence of autoreceptor-dependent signaling on these neurons.

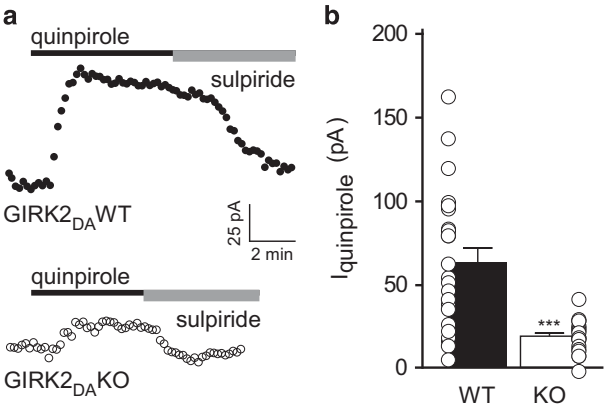

Figure 1 $D_{2/3}$R-dependent somatodendritic currents in VTA DA neurons from $GIRK2_{DA}$WT and $GIRK2_{DA}$KO mice. (a) Representative currents evoked by quinpirole (20 μM) and reversed by sulpiride (5 μM) in VTA DA neurons from $GIRK2_{DA}$WT and $GIRK2_{DA}$KO mice. Currents were recorded with TTX (0.5 μM) in the bath to prevent synaptic activity. (b) Summary of quinpirole-induced currents in VTA DA neurons from $GIRK2_{DA}$WT ($n=23$) and $GIRK2_{DA}$KO ($n=19$) mice. Circles represent individual data points. $^{***}P<0.001$ *vs* $GIRK2_{DA}$WT.

Excitatory Neurotransmission in the NAc of $GIRK2_{DA}$KO Mice

The amplitude and frequency of miniature excitatory postsynaptic currents (mEPSCs) were elevated in MSNs of the nucleus accumbens (NAc) shell from constitutive *Girk2*$^{-/-}$ mice, observations paralleling an increase in AMPA receptor levels at excitatory synapses in these neurons (Arora *et al*, 2010). To discern whether this adaptation is driven by loss of GIRK-dependent signaling in VTA DA neurons, we compared mEPSCs in NAc core and shell MSNs from $GIRK2_{DA}$WT and $GIRK2_{DA}$KO mice. To facilitate the targeted evaluation of neuron subpopulations in the NAc, we crossed $GIRK2_{DA}$KO mice with transgenic mice expressing fluorescent proteins in D_1R-expressing (Drd1a-tdTomato) or D_2R-expressing (Drd2-GFP) MSNs. No genotype difference in mEPSC amplitude or frequency was observed

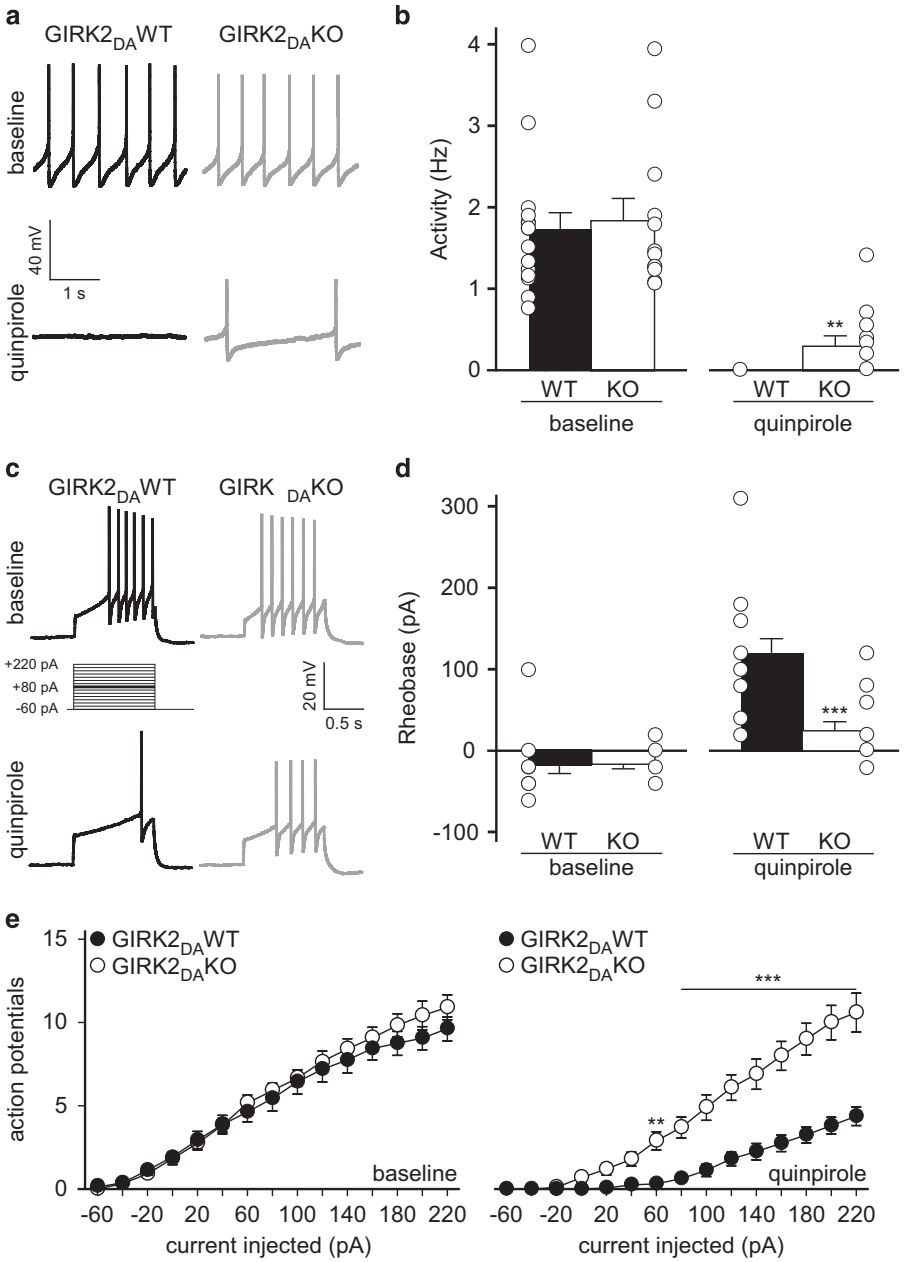

Figure 2 Excitability of GIRK2$_{DA}$WT and GIRK2$_{DA}$KO VTA DA neurons in the absence and presence of quinpirole. (a) Representative traces showing spontaneous activity in GIRK2$_{DA}$WT and GIRK2$_{DA}$KO VTA DA neurons at baseline and in the presence of quinpirole (20 μM). (b) Summary of spontaneous activity in GIRK2$_{DA}$WT ($n=16$) and GIRK2$_{DA}$KO ($n=12$) VTA DA neurons. (c) Typical responses of GIRK2$_{DA}$WT and GIRK2$_{DA}$KO VTA DA neurons to depolarizing current injection (80 pA), at baseline, and in the presence of quinpirole (20 μM). (d) Rheobase summary for GIRK2$_{DA}$WT ($n=16$) and GIRK2$_{DA}$KO ($n=12$) VTA DA neurons at baseline and in the presence of quinpirole (20 μM). (e) Plots showing the number of action potentials elicited by a 1-s current injection (−60 to 220 pA) in GIRK2$_{DA}$WT ($n=16$) and GIRK2$_{DA}$KO ($n=12$) VTA DA neurons at baseline and in the presence of quinpirole (20 μM). $^{**}P<0.01$ and $^{***}P<0.001$ *vs* GIRK2$_{DA}$WT (within current step).

in D_1R- or D_2R-expressing MSNs in the NAc core or shell (Supplementary Figure S1). Thus the increased excitatory neurotransmission observed in NAc MSNs in *Girk2*$^{-/-}$ mice is driven by loss of GIRK2 in a non-DA neuron population(s).

Cocaine-Induced Locomotor Activity in GIRK2$_{DA}$KO Mice

We next evaluated GIRK2$_{DA}$WT and GIRK2$_{DA}$KO mice in an open field activity test (Figure 3). We observed a significant effect of sex ($F_{1,99}=7.7$, $P<0.01$) and genotype ($F_{1,99}=15.0$, $P<0.001$) on baseline activity (saline day 2, S_2), with GIRK2$_{DA}$KO mice exhibiting a small but significantly higher level of activity than GIRK2$_{DA}$WT mice, and females showing higher activity than males. Significant main effects of genotype (male $F_{1,42}=30.8$, $P<0.001$; female $F_{1,49}=8.2$, $P<0.01$) and dose (male $F_{2,42}=74.0$, $P<0.001$; female $F_{2,49}=43.8$, $P<0.001$), as well as genotype × dose interactions (male $F_{2,42}=4.6$, $P<0.05$; female $F_{2,49}=6.7$, $P<0.01$), were observed in both sexes. Notably, mice of both genotypes

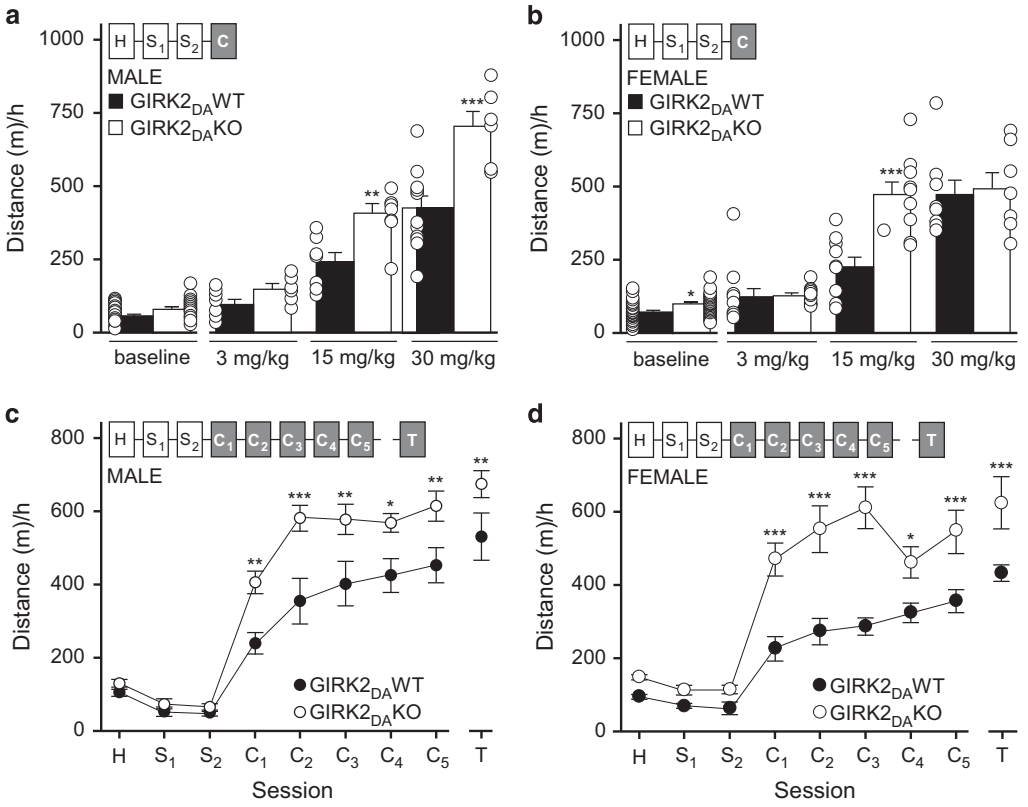

Figure 3 Cocaine-induced locomotor activity and sensitization in GIRK2$_{DA}$WT and GIRK2$_{DA}$KO mice. Distance traveled (in m) by GIRK2$_{DA}$WT and GIRK2$_{DA}$KO mice in an open field test. The test included a handling day (H), followed by 2 days of saline injections (S_1 and S_2), and then a final day with injection of 3, 15, or 30 mg/kg cocaine. Distance traveled on S_2 was taken as baseline activity. (a) Baseline ($n=20$–28 per genotype) and acute cocaine-induced locomotor activity ($n=6$–12 per genotype and dose) in male GIRK2$_{DA}$WT and GIRK2$_{DA}$KO mice. $^{**}P<0.01$ and $^{***}P$ 0.001 *vs* GIRK2$_{DA}$WT (within dose). (b) Baseline ($n=25$–30 per genotype) and acute cocaine-induced locomotor activity ($n=7$–11 per genotype and dose) in female GIRK2$_{DA}$WT and GIRK2$_{DA}$KO mice. Symbols: $^{***}P<0.001$ *vs* GIRK2$_{DA}$WT (within dose). (c) Total distance traveled by male GIRK2$_{DA}$WT ($n=8$) and GIRK2$_{DA}$KO ($n=8$) mice during the locomotor sensitization test. The repeated dosing protocol included a handling day (H) followed by 2 days of saline injections (S_1 and S_2), and then 5 days of cocaine injections (C_{1-5}; 15 mg/kg); mice were challenged again with 15 mg/kg cocaine test (T) 10–11 days after the fifth cocaine injection. Symbols: $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ *vs* GIRK2$_{DA}$WT (within injection). (d) Distance traveled by female GIRK2$_{DA}$WT ($n=11$) and GIRK2$_{DA}$KO ($n=9$) mice during the locomotor sensitization test. $^{*}P<0.05$ and $^{***}P<0.001$ *vs* GIRK2$_{DA}$WT (within injection).

and sexes increased locomotor activity in response to cocaine, with the 15 mg/kg dose revealing elevated responses in male (Figure 3a) and female (Figure 3b) GIRK2$_{DA}$KO mice. Interestingly, there was no difference between female GIRK2$_{DA}$WT and GIRK2$_{DA}$KO at the 30 mg/kg dose. No difference in baseline activity or the locomotor-stimulatory effect of 15 mg/kg cocaine was observed between DATCre(+) and DATCre(−) littermates (Supplementary Figure S2), arguing that activity differences between GIRK2$_{DA}$WT and GIRK2$_{DA}$KO mice are attributable to the loss of GIRK channels from DA neurons.

Repeated cocaine leads to locomotor sensitization, the enhanced response to subsequent cocaine exposures that persists after prolonged withdrawal (Robinson and Berridge, 2001). To test whether locomotor sensitization differed between GIRK2$_{DA}$WT and GIRK2$_{DA}$KO mice, we used a repeated dosing procedure involving 5 days of cocaine injections (15 mg/kg) and a cocaine challenge test. We observed a significant interaction of sex and injection number in GIRK2$_{DA}$WT mice ($F_{8,136}=2.5$; $P<0.05$), and thus male (Figure 3c) and female (Figure 3d) subjects were analyzed separately. Significant main effects of genotype (male $F_{1,112}=10.6$, $P<0.01$; female $F_{1,144}=20.2$, $P<0.001$) and injection number (male $F_{8,112}=110.2$, $P<0.001$; female $F_{8,144}=89.0$, $P<0.001$), as well as genotype × injection number interactions (male $F_{8,112}=3.8$, $P<0.001$; female $F_{8,144}=8.3$, $P<0.001$), were observed for male and female subjects. Male and female GIRK2$_{DA}$KO mice exhibited enhanced locomotor activity relative to GIRK2$_{DA}$WT counterparts in response to cocaine on all days (Figure 3c and d). Moreover, all groups exhibited significantly greater locomotor activity on the challenge test relative to activity measured after the initial cocaine exposure (not shown). Notably, while the activity of GIRK2$_{DA}$WT mice increased with each cocaine injection, the activity of GIRK2$_{DA}$KO mice plateaued between the first and third injections. These findings suggest that GIRK2$_{DA}$KO mice exhibit a 'presensitized' phenotype similar to that induced by genetic suppression of GIRK-dependent signaling in the mPFC (Hearing *et al*, 2013).

Cocaine-Induced CPP in GIRK2$_{DA}$KO Mice

We next assessed cocaine reward in GIRK2$_{DA}$KO mice using a CPP test. As male and female GIRK2$_{DA}$KO mice exhibited increased locomotor-stimulatory effect of 15 mg/kg

cocaine relative to GIRK2$_{DA}$WT counterparts, we used this dose with three drug/side pairings. A separate group of mice was conditioned with saline (0 mg/kg). Surprisingly, cocaine CPP was indistinguishable in GIRK2$_{DA}$WT and GIRK2$_{DA}$KO mice (Figure 4a). Previous CPP studies have shown a shallow or non-existent dose–response relationship for cocaine in the 4–12 mg/kg dose range in mice and a significant effect of conditioning session number on CPP magnitude (Brabant *et al*, 2005). Thus we also evaluated CPP utilizing lower cocaine doses (0.5 or 3 mg/kg) and after both two and four conditioning sessions. Again, no significant difference in cocaine CPP was detected between genotypes (Figure 4b and c).

Cocaine Self-Administration in GIRK2$_{DA}$KO Mice

Finally, we asked whether intravenous cocaine self-administration was impacted by the loss of GIRK channels in DA neurons. No difference between GIRK2$_{DA}$WT and GIRK2$_{DA}$KO mice was observed with respect to acquisition of self-administration (Figure 5a–c), defined as earning eight infusions during two consecutive sessions, with nose pokes at the correct hole accounting for >70% of the total (Supplementary Figure S3A and B). After meeting acquisition criteria, mice were transitioned to an FR3 schedule of reinforcement (0.5 mg/kg/infusion), during which GIRK2$_{DA}$WT (14.3 ±1.2) and GIRK2$_{DA}$KO (12.8 ± 0.8; $t_{138} = 1.1$, $P = 0.29$) earned comparable infusions per session.

After completing baseline FR3 training, self-administration behavior as a function of cocaine dose was assessed using an FR3 schedule of reinforcement. Although patterns of responding were comparable within and across subjects at the various cocaine doses (Supplementary Figure S4), an interaction between genotype and dose with respect to infusions earned was observed ($F_{4,72} = 3.0$, $P < 0.05$); GIRK2$_{DA}$KO mice earned more infusions of cocaine than GIRK2$_{DA}$WT controls, most evident during sessions involving the lowest three cocaine doses (Figure 5d). Importantly, a strong preference for responding in the correct nose poke hole was observed both GIRK2$_{DA}$WT and GIRK2$_{DA}$KO throughout the dose–response study (Supplementary Figure S3C and D). Furthermore, a main effect of genotype was detected for total cocaine intake ($F_{1,72} = 6.1$, $P < 0.05$), with higher levels observed for GIRK2$_{DA}$KO mice relative to littermate controls (Figure 5e).

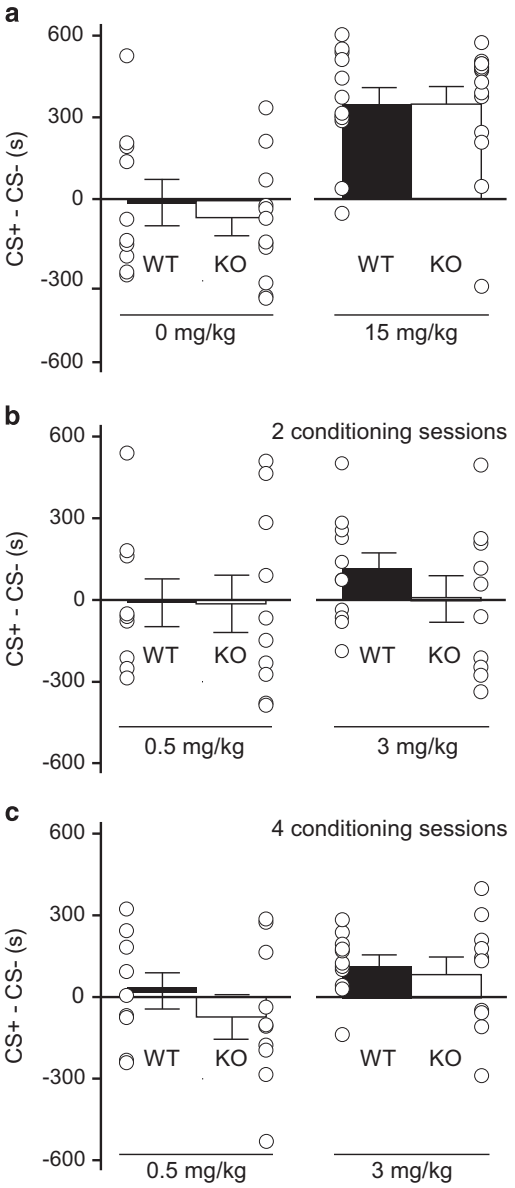

Figure 4 Cocaine-induced CPP in GIRK2$_{DA}$WT and GIRK2$_{DA}$KO mice. (a) Cocaine-induced CPP in GIRK2$_{DA}$WT ($n = 10$–12 per dose) and GIRK2$_{DA}$KO ($n = 12$–14 per dose) mice, measured as the difference in time spent in the drug (CS+) and saline (CS−) paired sides on test day. (b) Cocaine-induced CPP in a separate cohort of GIRK2$_{DA}$WT ($n = 9$–11 per dose) and GIRK2$_{DA}$KO ($n = 10$ per dose) mice, measured after two drug conditioning sessions. (c) Cocaine-induced CPP in the same mice as in panel (b), measured after four drug conditioning sessions.

DISCUSSION

We reported previously that GABA$_B$R-GIRK signaling was diminished in VTA DA neurons, but not in VTA GABA neurons, from GIRK2$_{DA}$KO mice (Kotecki *et al*, 2015). Here we show that autoreceptor-mediated signaling is also diminished in GIRK2$_{DA}$KO mice, while baseline measures of neuronal excitability are unchanged. Thus the GIRK2$_{DA}$-KO mouse is a selective model of decreased inhibitory feedback (both GABA$_B$R and autoreceptor mediated) to DA neurons. Behavioral analysis of these mice revealed the critical influence of inhibitory feedback to DA neurons in both non-contingent (locomotor activity) and response-contingent (self-administration) cocaine-related behaviors.

GIRK-Dependent Inhibitory Feedback and Cocaine-Induced Locomotor Activity

GIRK2$_{DA}$KO mice, similar to constitutive *Girk2*$^{-/-}$ mice (Arora *et al*, 2010), are more sensitive to the locomotor-stimulatory effect of cocaine. This unconditioned behavioral response to cocaine is DA dependent and tempered by GABA$_B$R- and autoreceptor-mediated inhibitory feedback to VTA DA neurons. Indeed, cocaine-induced locomotor activity was enhanced by intra-VTA infusion of pertussis toxin (Steketee and Kalivas, 1991), which inhibits the G proteins that mediate GABA$_B$R and autoreceptor-dependent

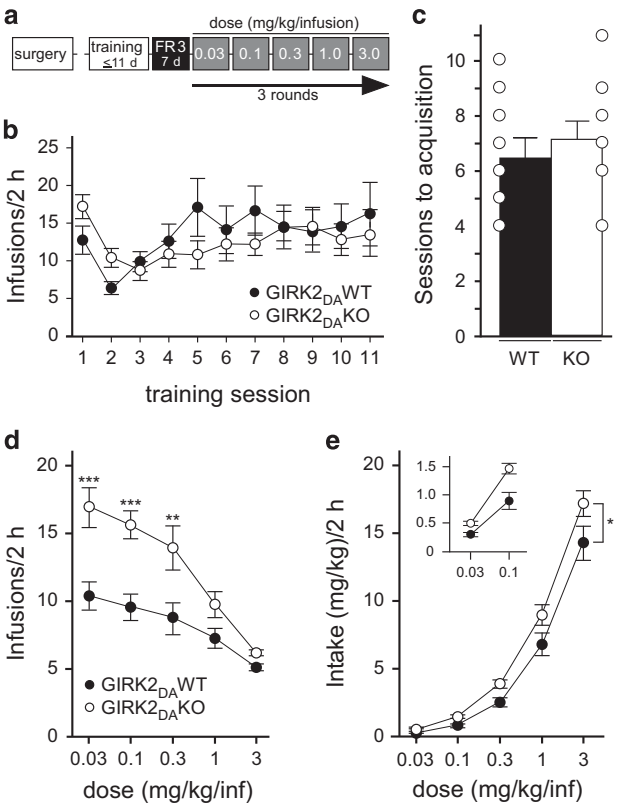

Figure 5 Cocaine self-administration in male $GIRK2_{DA}WT$ and $GIRK2_{DA}KO$ mice. (a) Depiction of the self-administration procedure, beginning with jugular catheterization surgery and a ⩾7-day recovery period. Acquisition of cocaine self-administration was achieved in daily 2-h sessions where the response requirement was raised from an FR1 to an FR3 (0.5 mg/kg/infusion). $GIRK2_{DA}WT$ ($n=9$) and $GIRK2_{DA}KO$ ($n=11$) meeting acquisition criteria were maintained at an FR3 schedule for 7 days (baseline) prior to assessing intake and infusions as a function of cocaine dose. (b) The number of infusions earned during the first 11 days of cocaine self-administration (training). (c) Number of training sessions needed to meet acquisition criteria ($t_{18}=0.65$, $P=0.54$). (d) Number of infusions, with FR3 schedule of reinforcement, earned as a function of unit dose of cocaine (0.03–3.0 mg/kg/infusion). $^{**}P<0.01$ and $^{***}P<0.001$ *vs* $GIRK2_{DA}WT$ (within dose). (e) Cocaine intake as a function of unit dose, with the two lowest doses expanded in the inset. $^{*}P<0.05$ (main effect of genotype).

signaling. In addition, intra-VTA infusion of the $GABA_BR$ agonist baclofen or the $D_{2/3}R$-antagonist sulpiride blocked and potentiated, respectively, the locomotor-stimulatory effect of cocaine (Steketee and Kalivas, 1991; Chen and Reith, 1994). In terms of cocaine-induced locomotor activity, $GIRK2_{DA}KO$ mice behave comparably to mice lacking D_2R in DA neurons (Bello *et al*, 2011). RNAi-dependent suppression of D_2R in VTA in rats also yielded enhanced cocaine-induced locomotor activity (de Jong *et al*, 2015). As GIRK2-containing channels mediate most of the direct inhibitory effect of $GABA_BR$ and autoreceptor activation on VTA DA neurons (Beckstead *et al*, 2004; Cruz *et al*, 2004), these behavioral insights highlight the key influence of the GIRK component of the inhibitory G protein-mediated feedback pathways on acute cocaine-induced locomotor stimulation. Our data further show that while GIRK-dependent signaling in DA neurons is not required for the development of locomotor sensitization, they do provide an opposing influence on this addiction-related phenomenon.

GIRK-Dependent Inhibitory Feedback and Cocaine Reinforcement

Although available evidence shows that inhibitory G protein-dependent feedback to VTA DA neurons modulates the reinforcing effect of cocaine, the nature of this influence differs across studies. For example, we show here that $GIRK2_{DA}KO$ mice display normal acquisition of cocaine-induced self-administration but an overall higher intake of cocaine and, specifically, enhanced responding when presented with low cocaine doses per infusion. Constitutive $Girk2^{-/-}$ mice were also able to acquire cocaine self-administration but displayed decreased responding at lower cocaine doses (Morgan *et al*, 2003). The divergent phenotypes of constitutive $Girk2^{-/-}$ and $GIRK2_{DA}KO$ mice are likely attributable to widespread deficits in GIRK-dependent signaling and alterations in both excitatory and inhibitory ionotropic neurotransmission in the former model (Lujan *et al*, 2014). Interestingly, $GIRK2_{DA}WT$ and $GIRK2_{DA}KO$ mice exhibited different responding for cocaine at a dose (0.3 mg/kg/infusion) comparable to that used during training, wherein no genotype difference was detected. This may reflect an impact of the order of cocaine dose presentation on self-administration behavior or a manifestation of differential adaptations triggered by cumulative cocaine exposure between the groups.

$GIRK2_{DA}KO$ mice also differ from other genetic models of autoreceptor ablation in terms of cocaine reinforcement. For example, the RNAi-mediated suppression of D_2R in the rat VTA enhanced motivation to work for cocaine as assessed using progressive ratio scheduling, whereas not altering acquisition of cocaine self-administration or fixed ratio responding (de Jong *et al*, 2015). Moreover, mice lacking D_2R in DA neurons acquired cocaine self-administration more quickly than control subjects, while other parameters, including intake, motivation, and sensitivity, were normal (Holroyd *et al*, 2015). Interestingly, these mice also exhibited enhanced cocaine CPP at low doses (Bello *et al*, 2011), whereas we detected no CPP phenotype in $GIRK2_{DA}KO$ mice. Apparent behavioral discrepancies across these genetic models could reflect procedural and/or species differences or point to the overlapping but distinct impact of the genetic lesions. With respect to the latter point, it is noteworthy that $GIRK2_{DA}KO$ mice exhibit deficits in both $GABA_BR$- and autoreceptor-mediated inhibitory feedback to DA neurons. $GABA_BR$ activation in the VTA decreases cocaine self-administration in rats (Brebner *et al*, 2000; Backes and Hemby, 2008), suggesting that $GABA_BR$-GIRK signaling in VTA DA neurons may also influence sensitivity to cocaine in a response-contingent procedure. In addition, whereas GIRK channels contribute to the somatodendritic inhibitory impact of autoreceptor activation on VTA DA neurons, D_2R are also expressed on axon terminals of DA neurons in terminal regions where they can modulate neurotransmitter release (Sesack *et al*, 1994). Finally, the residual quinpirole-induced current seen in VTA DA neurons from $GIRK2_{DA}KO$ mice shows that the inhibitory influence of autoreceptor activation on these neurons is mediated by multiple effectors.

Notably, our self-administration studies involving $GIRK2_{DA}KO$ mice did not attempt to dissociate motivation to gain access to cocaine from intake, and thus we cannot speak of the potential impact of GIRK ablation in DA neurons on

motivation separate from intake. Furthermore, it is important to note that increased levels of responding may reflect a decreased sensitivity to the reinforcing effect of the drug. Given that $GIRK2_{DA}KO$ mice exhibit increased sensitivity to the locomotor-stimulatory effect of cocaine, however, our current working hypothesis is that $GIRK2_{DA}$-KO mice are more sensitive to cocaine.

The Unique GIRK Channel in VTA DA Neurons

The VTA DA neuron GIRK channel (GIRK2/GIRK3) is unique, as most neuronal GIRK channels contain GIRK1 (Cruz *et al*, 2004; Koyrakh *et al*, 2005; Labouebe *et al*, 2007). Interestingly, *Girk2* ablation yields a complete loss of VTA DA neuron GIRK channel activity (Beckstead *et al*, 2004; Cruz *et al*, 2004), whereas *Girk3* ablation yields increased sensitivity of the residual channel (GIRK2 homomer) to receptor activation (Labouebe *et al*, 2007; Lunn *et al*, 2007). The opposing contributions of GIRK2 and GIRK3 are also sensed at the behavioral level, where loss of GIRK2 in DA neurons correlates with increased locomotor effects of cocaine and morphine, while constitutive *Girk3* ablation correlates with decreased locomotor-stimulatory effect of morphine (Arora *et al*, 2010; Kotecki *et al*, 2015). The GIRK channel in VTA DA neurons undergoes activity-dependent bidirectional modulation; burst firing increases GIRK-dependent signaling in VTA DA neurons, whereas tonic firing suppresses channel activity (Lalive *et al*, 2014). The latter phenomenon is reminiscent of the cocaine-induced suppression of GIRK-dependent signaling in VTA DA neurons, attributable to a subcellular redistribution of GIRK2-containing channels (Arora *et al*, 2011). The plasticity of GIRK channels provides a plausible explanation for how experience could impact the sensitivity of the mesocorticolimbic system to subsequent experiences, drug or otherwise.

CONCLUDING REMARKS

The unique subunit composition of the VTA DA neuron GIRK channel, and its importance in modulating sensitivity to cocaine and other drugs of abuse, suggests that direct modulation of this target could be useful for treating aspects of addiction. Indeed, $GABA_BR$ activation reduced self-administration and addiction-related behaviors linked to cocaine and other drugs of abuse in rodents (Brebner *et al*, 2000; Xi and Stein, 2000; Ranaldi and Poeggel, 2002; Leite-Morris *et al*, 2004; Liang *et al*, 2006; Filip *et al*, 2007). As most GIRK channels contain GIRK1, a GIRK2/GIRK3-selective activator should selectively enhance GIRK-dependent signaling in VTA DA neurons. Although GIRK2/GIRK3 channel activators have not yet been reported, the development of potent modulators of GIRK1-containing GIRK channels suggests that other channel subtype-selective modulators will be forthcoming (Kaufmann *et al*, 2013; Wydeven *et al*, 2014).

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