

RESEARCH ARTICLE

Methamphetamine Self-Administration in Mice Decreases GIRK Channel-Mediated Currents in Midbrain Dopamine Neurons

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Abstract

Background: Methamphetamine is a psychomotor stimulant with abuse liability and a substrate for catecholamine uptake transporters. Acute methamphetamine elevates extracellular dopamine, which in the midbrain can activate D2 autoreceptors to increase a G-protein gated inwardly rectifying potassium (GIRK) conductance that inhibits dopamine neuron firing. These studies examined the neurophysiological consequences of methamphetamine self-administration on GIRK channel-mediated currents in dopaminergic neurons in the substantia nigra and ventral tegmental area.

Methods: Male DBA/2J mice were trained to self-administer intravenous methamphetamine. A dose response was conducted as well as extinction and cue-induced reinstatement. In a second study, after at least 2 weeks of stable self-administration of methamphetamine, electrophysiological brain slice recordings were conducted on dopamine neurons from self-administering and control mice.

Results: In the first experiment, ad libitum-fed, nonfood-trained mice exhibited a significant increase in intake and locomotion following self-administration as the concentration of methamphetamine per infusion was increased (0.0015–0.15 mg/kg/infusion). Mice exhibited extinction in responding and cue-induced reinstatement. In the second experiment, dopamine cells in both the substantia nigra and ventral tegmental area from adult mice with a history of methamphetamine self-administration exhibited significantly smaller D2 and GABA_B receptor-mediated currents compared with control mice, regardless of whether their daily self-administration sessions had been 1 or 4 hours. Interestingly, the effects of methamphetamine self-administration were not present when intracellular calcium was chelated by including BAPTA in the recording pipette.

Conclusions: Our results suggest that methamphetamine self-administration decreases GIRK channel-mediated currents in dopaminergic neurons and that this effect may be calcium dependent.

Keywords: GIRK, electrophysiology, methamphetamine, self-administration, dopamine, mouse

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Introduction

Methamphetamine (METH) is a commonly abused psychomotor stimulant whose reinforcing properties are thought to be mediated by an elevation of extracellular dopamine levels (Volkow et al., 2009). While high doses of METH and related compounds are substrates for vesicular monoamine transporters, low doses enhance extracellular dopamine levels and dopamine neurotransmission by acting as substrates for the plasmalemmal dopamine transporter and possibly by enhancing neurotransmitter release (Sulzer et al., 1993; Branch and Beckstead, 2012; Daberkow et al., 2013). In the substantia nigra and the lateral ventral tegmental area (VTA), extracellular dopamine can inhibit excitability by activating D2-type autoreceptors (Aghajanian and Bunney, 1977; Sesack et al., 1994). Published work supports an inverse relationship between dopamine autoreceptor signaling and psychostimulant use. Subsensitization of somatodendritic D2 autoreceptors in rodents both in vivo and in brain slices has been observed in response to repeated exposure to uptake inhibitors, including cocaine (Henry et al., 1989; Pierce et al., 1995; Marinelli et al., 2003), amphetamine (White and Wang, 1984; Seutin et al., 1991; Wolf et al., 1993), and METH (Yamada et al., 1991). In humans, somatodendritic D2 autoreceptor expression is inversely correlated with impulsive behavior and amphetamine “wanting” (Zald et al., 2008; Buckholtz et al., 2010). Furthermore, mice lacking D2 autoreceptors on dopamine neurons are supersensitive to the rewarding properties of cocaine (Bello et al., 2011). Determining the specific cellular mechanisms responsible for the interaction between psychostimulants and dopamine autoreceptors could be instructive for understanding and preventing drug-seeking behaviors.

Studies in rodent brain slices demonstrate that somatodendritic D2 autoreceptors hyperpolarize mesencephalic dopamine neurons primarily by activating G-protein coupled inwardly rectifying potassium (GIRK) channels (Lacey et al., 1987; Beckstead et al., 2004). In these same neurons, metabotropic GABA_B receptors can also activate a similar and partially overlapping GIRK channel conductance (Lacey et al., 1988; Cruz et al., 2004; Beckstead and Williams, 2007). GABA_B receptor-mediated currents in dopamine neurons can be transiently decreased with noncontingent injections of psychostimulants, possibly through decreased surface expression of GIRK channels (Arora et al., 2011; Padgett et al., 2012). In contrast, GIRK channel-mediated currents are enhanced in dopamine neurons when intracellular calcium is chelated, an effect that exhibits both D2 receptor-specific and nonspecific components (Beckstead and Williams, 2007; Perra et al., 2011). It is not known if calcium-dependent regulation of GIRK channel signaling in dopamine neurons plays a role in the pharmacological effects of psychostimulants or contributes to their self-administration.

Previous studies suggest that the effects of repeated exposure to METH and other psychostimulants on dopamine signaling are often dependent on contingency of drug delivery (Hemby et al., 1997; Stefanski et al., 1999 2002; Paladini et al., 2004). Operant self-administration of intravenous psychostimulants in rodents is commonly used to model human drug use and is taken as evidence of drug reinforcement. Unfortunately, practical concerns (such as jugular vein diameter) make this technique difficult to perform in the mouse, limiting the genetic tools that can be employed to investigate hypotheses. Groups that do perform self-administration studies in rodents often restrict the diet of their experimental animals to enhance motivation and to increase drug intake and efficacy (eg, Carroll et al., 1979; Carroll and Meisch, 1984; de la Garza and Johanson, 1987).

However, we recently reported an effect of chronic, mild food restriction on glutamate and dopamine autoreceptor signaling in dopamine neurons that could confound interpretation of results from self-administration studies using food restriction (Branch et al., 2013). Furthermore, food-training mice on an operant task prior to drug self-administration while using the same stimulus cues may interfere or confound the interpretation of operant responding (Thomsen and Caine, 2011). Thus, to investigate the effect of self-administration of METH on dopamine cell function without the confounding effects of feeding, it is important to develop models of self-administration in ad libitum-fed mice that are not food trained to the operant task.

Here we report and characterize a model of intravenous METH self-administration in DBA/2J mice that were neither food-deprived nor food trained. We show that ad libitum-fed mice will robustly self-administer METH at levels that produce locomotor activation and also exhibit extinction and cue-induced reinstatement of responding. In addition, dopamine neurons in the midbrain of adult mice given access to METH daily for either 1- or 4-hour sessions exhibited a decrease in D2- and GABA_B receptor-mediated currents compared with neurons from drug-naïve mice. This effect was not observed when intracellular calcium was chelated, suggesting that METH self-administration produces a decrease in GIRK channel-mediated currents in dopamine neurons that may be calcium-dependent.

Methods

Animals

Adult male DBA/2J mice (Jackson Labs, Bar Harbor, ME; 8–10 weeks at arrival) were housed in polycarbonate boxes with rodent bedding. Food and water were always available in the home cage, unless otherwise stated. The room was maintained under a reverse light cycle (14:10 with lights off 9:00 AM to 7:00 PM). Mice were initially housed 3 to 5 per cage but were individually housed with Shred-A-Bed (Novalek Inc., Hayward, CA) for nest construction and stress reduction for at least 5 days before surgery. Care of the mice conformed to *The Guide for the Care and Use of Laboratory Animals*, and procedures were approved by the University of Texas Health Science Center Animal Care and Use Committee.

Surgery

Mice were anesthetized with isoflurane (2–3%, 1.5L/min of O₂) for aseptic surgery to place an indwelling catheter in the right jugular vein. Catheters were constructed from micro-renathane tubing (0.025-inch outer diameter, 0.012-inch inner diameter, 4cm long) with a silicone bead 10mm from the tip. The tubing connected to 27-gauge stainless-steel hypodermic tubing bent in a 90° angle and exited through the center of a nylon screw at a dorsal incision between the scapulae. Polyester felt mesh (oval shape approximately 15mm x 8mm; Surgical Mesh, Brookfield, CT) was attached to the base of the screw and was situated subcutaneously at the dorsal exit site. The catheter exit was occluded while the mice were in the home cage by a short piece of heat-fused PE20 tubing to decrease clotting and infection. Postsurgery, mice received subcutaneous injections of ticarcillin and carprofen as needed to prevent infection and alleviate pain. Mice were allowed a minimum of 5 to 7 days after surgery to

recover before daily operant sessions were initiated. Catheters were flushed daily with 0.025 mL of heparinized saline (30 units/mL) beginning 3 days after surgery.

Operant Self-Administration

Mice were not food restricted at any time during the self-administration procedure, although neither water nor food was available in the operant chamber during the daily sessions. Self-administration was conducted in modular mouse operant chambers (Lafayette Instruments) housed inside sound-attenuating cabinets with a fan to mask external noise. Each chamber was equipped with 2 nose poke holes on one wall. One hole was designated active and nose pokes in that hole resulted in METH infusion, while nose pokes in the other hole resulted in no consequence. The active side was counterbalanced across chambers, and a stimulus light illuminated within the active hole signified METH availability. A white stimulus light located between the nose poke holes was illuminated during the infusion of METH and postinfusion time out. Mice ($n=24$) were trained in 5 daily sessions each week. Mice were initially started on a fixed ratio 1 (FR1), where one nose poke in the active hole resulted in an infusion of METH (12 μ L/infusion over 2 seconds, with a 15-second time-out after the infusion). Initial training sessions were 4 hours in length and used 0.1 mg/kg/infusion METH (generally for the first 2 days only). These extended sessions gave the mice an opportunity to explore the novel environment and begin to learn the nose poke operant task. This initial dose of METH ensured that the subject would experience the pharmacology of METH with even small numbers of infusions. During training, the response requirement was increased to FR3 and the session length was decreased to one hour. Approximately 33% of mice that were started for self-administration were not successful at acquiring self-administration because of clogging of their catheter or a lack of reliable performance in the behavioral task. Subjects were considered to have acquired self-administration if the responding in the active nose poke was >80% of total responses with <25% variation in mean intake across days.

Dose-Response Manipulation

Sixteen mice were subjected to dose-response manipulation, which was initiated after no more than 10 self-administration training sessions (see Table 1 for experimental timeline). The concentration of METH per infusion was increased each week (5 sessions per concentration). The manipulation was run in ascending order of dose (0.0015, 0.015, 0.05, and 0.15 mg/kg/infusion based on the assumption of a 28-g mouse) to minimize the possibility of desensitization or development of tolerance. Only 3 to 4 days of self-administration were conducted at 0.15 mg/kg/infusion dose due to the death of 3 mice on day 3 at the highest dose. For statistical comparison, self-administration-related dependent measures (number of infusions and total session intake [mg/kg]) from the last 2 days at each concentration were averaged for analysis (days 2 and 3 at the 0.15 mg/kg/infusion dose were used for statistical analysis for consistency between animals). The catheters were flushed with saline (0.025 mL) before and with heparinized saline (0.025 mL) after each session to ensure catheter patency.

Locomotor Activity

Each day immediately following the self-administration session, a subset of the self-administering mice ($n=7$) was placed in an open field (16 inches x 16 inches, Opto-Varimex, Columbus

Table 1. Timeline for Operant Self-Administration

Experiment days	Dose METH (mg/kg/inf)	Sample size	Training		0.0015 (mg/kg/inf)	0.015 (mg/kg/inf)	0.05 (mg/kg/inf)	0.15 (mg/kg/inf)	Saline Substitution	Extinction	Cue-Induced Reinstatement
			5-10 days (mean of 6)	0.1 (days 1-2)							
			1-5	6-10	11-15	16-19	20-22	23-30	31		
			0.0015	0.015	0.05	0.15	0	0	0		
			$n=16^a$	$n=16^a$	$n=16^a$	$n=16^a$	$n=10$	$n=10$	$n=10$		

Abbreviation: METH, methamphetamine.

^a Subset of subjects ($n=7$) had locomotor activity measured immediately after operant session.

Instruments, Columbus, OH) for 20 minutes to measure total horizontal distance traveled. Data were collected using Opto-Varimex AutoTracker software (version 4.4). Following the session, mice were returned to their home cage.

Saline Substitution, Extinction, and Reinstatement

To examine the effect of removing METH on self-administration behavior, immediately after the dose response study a subset of mice ($n = 10$) was placed in the operant chambers as usual for their daily session with the syringe filled with saline (Table 1). Nose pokes (FR3) produced illumination of the stimulus light previously associated with METH infusion; however, saline was now infused instead of METH. After 3 days of saline substitution, nonreinforced responding was measured in 1-hour daily sessions for 8 days. During the extinction sessions, mice were connected to the intravenous tether, the light in the active nose poke was illuminated, but responding in the active nose poke was not reinforced either with infusion or illumination of the center stimulus light. Mice were allowed 1 hour in the operant chamber, and nose pokes in the active and inactive holes were measured. After 8 days of extinction responding, mice were placed in the chamber, and completion of the FR3 nose poke in the active hole resulted in activation of the stimulus light that had previously signaled METH infusion, but no infusions were given (cue-induced reinstatement).

Electrophysiological Recordings

In a separate experiment, mice were trained to self-administer METH contingently. Typically, the initial 2 days of training sessions were 4 hours in length at the concentration of 0.1 mg METH/kg/infusion. After this initial training, the dose of METH per infusion was decreased to 0.05 mg/kg, and the daily session was shortened to 1 hour as the response requirement was increased to an FR3. The training took 6 to 10 sessions to reach stable intake for the mice in this study. After this time, they were divided into 2 groups. One group continued 1-hour daily sessions (short access, $n=10$), and the other group had the daily session length increased to 4 hours (long access, $n=9$). Mice were maintained on long or short access for 12 to 32 days before they were sacrificed for electrophysiology. Mice who did not acquire self-administration of METH or developed very early catheter problems were used as a control group ($n = 14$). A small number of cells in the low calcium chelation condition (<33%) came from mice that were initially food trained to the nose poke task. For the measures examined in these cells, there appeared to be no difference between previously food-trained and non-food-trained mice (eg, current amplitudes in response to dopamine in the VTA were 118 ± 17 and 121 ± 23 pA, respectively). As we were not attempting to interpret behavioral motivation, the data were combined for analysis. On the day of the experiment, mice were deeply anesthetized and killed by decapitation approximately 24 hours after the end of their final self-administration session. Brains were quickly removed and placed into nearly frozen artificial cerebral spinal fluid (aCSF), which was constantly bubbled with 95% O_2 /5% CO_2 and contained (in mM) 126 NaCl, 2.5 KCl, 1.2 $MgCl_2$, 2.4 $CaCl_2$, 1.2 NaH_2PO_4 , 21.4 $NaHCO_3$, and 11.1 glucose, plus 1.25 kynurenic acid for slicing. The ventral mid-brain was blocked with a razor blade and mounted on a vibrating microtome (Leica Microsystems, Wetzlar, Germany). Horizontal slices (200 μ m thick) containing the substantia nigra and VTA were obtained and placed into 34°C aCSF plus 10 μ M MK-801 for 30 minutes and thereafter maintained at room temperature.

At the time of recording, slices were mounted on an upright microscope (Carl Zeiss, Oberkochen, Germany) and continuously

perfused at a rate of 2 mL/min with aCSF. Patch clamp recordings were conducted using glass microelectrodes (1.8–2.5 M Ω resistance, World Precision Instruments, Sarasota, FL), filled with an internal solution that contained (in mM) 115 K-methylsulfate, 20 NaCl, 1.5 $MgCl_2$, 10 K-4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), 2 ATP, 0.4 GTP, and either 10 BAPTA or 0.025 ethylene glycol-bis(2-aminoethylether)-N, N, N', N'-tetraacetic acid (EGTA), pH 7.35–7.40, 267–272 mOsm/L. Dopamine neurons of the substantia nigra pars compacta and lateral VTA were initially identified by appearance and location in relation to the medial terminal nucleus of the accessory optic tract (MT) and the medial lemniscus (Ford et al., 2006; Ikemoto, 2007). Substantia nigra neurons were located near MT. VTA neurons were located 100 to 200 μ m medial of MT or, in cases where the slice was too dorsal for MT to be visualized, along the medial edge of the medial lemniscus. Spontaneously firing cells were recorded for 10 seconds in cell-attached mode before breaking in, and recordings were terminated if a neuron exhibited an extracellular spike waveform width <1.1 millisecond. After breaking in, cells were voltage clamped at -55 mV to bath ground. A 50-mV hyperpolarizing step was applied for 1 second to test for the HCN channel-mediated current I_H . An iontophoretic electrode (50–100 M Ω) was placed near the neuron, and dopamine (1 M in the pipette) was applied with a pulse of +200 nA for 1 to 5 seconds. Each ejection was manually terminated when the outward current reached a maximal plateau (Branch et al., 2013). Cells were deemed putative dopamine neurons if we observed an immediate outward current in response to dopamine iontophoresis and at least one of the following: an extracellular waveform width >1.1 ms or $I_H \geq 100$ pA. Baclofen (30 μ M) was applied by bath perfusion.

Noncontingent Drug Administration

We also performed an electrophysiological experiment in brain slices from mice that received METH in a noncontingent (experimenter-delivered) manner. Mice ($n = 9$) were divided into 3 groups. Saline controls received daily intraperitoneal injections of saline (0.25 mL) for a minimum of 14 days. The “METH x3” group received 11 days of saline injections followed by 3 days of 2-mg/kg METH injections. The “METH x14” group received 14 days of 2-mg/kg METH injections. Mice were sacrificed for electrophysiology 24 hours after their last injection.

Statistics

Statistical analyses were performed with Graphpad Prism (La Jolla, CA). Data are presented as mean \pm SEM in results and figures unless otherwise stated. One-way repeated-measures analyses of variance (ANOVAs) were used to analyze self-administration and locomotor data for dose-response. Pearson correlation was conducted to determine the relationship between METH intake and total horizontal distance traveled. Saline substitution, extinction, and reinstatement data were analyzed with 1-way repeated-measures ANOVA followed by Tukey's or Dunnett's posthoc test. One-way ANOVAs were used to compare electrophysiological data across groups of mice, followed by Dunnett's test. Electrophysiological data were acquired using Axograph X (www.axograph.com) and LabChart software (AD Instruments, Colorado Springs, CO). For all analyses, α was set a priori at 0.05.

Drugs

METH hydrochloride was a generous gift from the National Institute on Drug Abuse drug supply program (Bethesda, MD).

NaH_2PO_4 was from Calbiochem, K-methylsulfate was from Acros Organics (Geel, Belgium), BAPTA was from Invitrogen (Carlsbad, CA), and isoflurane was from Baxter (Deerfield, IL). All other drugs and reagents were from Sigma-Aldrich (St. Louis, MO).

Results

METH Dose Manipulation

Ad libitum-fed mice were trained to self-administer METH. Responses in the active nose poke comprised >85% of total nose poke responses (data not shown). The average number of reinforcers earned on the last 2 days of training (0.05 mg/kg/infusion) was 14.4 ± 1.8 , and the average days of training were 6. After training, mice had 5 days of self-administration at 4 ascending doses of METH. Increasing the amount of METH delivered per infusion produced a significant main effect on the number of infusions earned per session ($F_{3,63}=5.7$, $P=.002$; Figure 1A) as well as on the amount of METH taken (mg/kg/session, $F_{3,63}=60.0$, $P<.0001$; Figure 1B). An examination of the individual data from the 16 mice tested showed that 10 of the 16 mice exhibited an inverted U-shape (Figure 1C), with 5 mice showing the greatest number of infusions at the highest dose tested (Figure 1D).

Locomotor Activity

In a subset of mice self-administering METH, locomotor activity was measured in an open field immediately after the daily operant session. The horizontal distance traveled in 20 minutes increased significantly as the concentration of METH per infusion increased (main effect of dose: $F_{3,27}=22.2$, $P<.0001$) (Figure 2A). Pearson correlation on the daily intake (each of the last 2 days at each concentration presented) vs the distance traveled showed a significant association between the 2 variables ($r^2=0.90$, $P<.0001$) (Figure 2B). These results demonstrate that ad libitum-fed mice will self-administer behaviorally relevant quantities of METH.

Saline Substitution, Extinction, and Reinstatement

After the dose manipulation, a subset of mice was examined for responding during saline substitution, extinction, and cue-induced reinstatement (5 mice from the dose manipulation were run before we began this experiment, and 1 mouse had a clogged catheter before completion of this part of the study). For statistical analysis, we defined baseline responding as nose pokes on the last day at 0.15 mg/kg/infusion. Analysis of responding throughout the saline substitution, extinction, and reinstatement resulted in a significant effect of day ($F_{12,129}=34.4$, $P<.0001$) (Figure 3A). When saline was substituted for METH in daily sessions, posthoc analysis revealed a significant increase in nose pokes in the active hole on the first day, but not on days 2 or 3 of saline when compared with baseline (Figure 3A). After 3 days of saline substitution, mice were subjected to extinction conditions where nose pokes resulted in no scheduled consequence (no cue lights or infusion). Defining extinction as <30% of baseline nose poke responding (using the last day at 0.15 mg/kg/infusion as baseline), 8 of 10 mice reached extinction within the 8 sessions. Posthoc analysis indicated that nose pokes in the active side were increased from baseline on day 1 of extinction and decreased from baseline on days 2 to 8 of extinction (Figure 3A-B). The average number of sessions to reach extinction criteria was 4.9 ± 0.7 for the mice that reached extinction. After 8 days of extinction conditions, mice underwent cue-induced reinstatement. Responding during reinstatement showed a

marked increase from day 8 of extinction conditions ($503 \pm 138\%$ increase) and no change from baseline ($41 \pm 20\%$ increase).

Brain Slice Electrophysiology

In the substantia nigra and lateral VTA, inhibitory synaptic input through GABA_B and dopamine D2 receptors can powerfully hyperpolarize dopamine neurons through activation of GIRK channels (Lacey et al., 1987 1988; Beckstead et al., 2004; Cruz et al., 2004). Acute administration of dopamine reuptake inhibitors such as METH can also indirectly activate GIRK channels by increasing ambient extracellular concentrations of dopamine (Sulzer et al., 2005; Branch and Beckstead, 2012). We next sought to identify if METH self-administration alters GIRK channel-mediated currents in midbrain dopamine neurons. Average intake of METH on the last 5 days before recording was 1.8 ± 0.3 and 4.4 ± 0.7 mg/kg/session for the short and long access groups, respectively. We examined GIRK currents in neurons located in both the substantia nigra and VTA and conducted the experiments both under control conditions and with intracellular calcium chelated by 10 mM BAPTA in the recording pipette.

Dopamine neurons were voltage clamped at -55 mV, and GIRK channel-mediated currents were determined through iontophoresis of dopamine. In most cells we subsequently examined GABA_B receptor-mediated currents activated by bath perfusion of 30 μM baclofen. Drug exposures were always done in this sequence, because dopamine iontophoresis does not persistently reduce subsequently measured GIRK currents (eg, Branch et al., 2013). Using standard recording conditions, neurons in the VTA of mice with a history of METH self-administration exhibited smaller GIRK channel-mediated currents than neurons from control mice. This effect was observed regardless of whether the GIRK channels were activated by dopamine (1-way ANOVA, $F_{2,30}=8.15$, $P=.0015$; Figure 4A,C) or baclofen (1-way ANOVA, $F_{2,23}=6.55$, $P=.0056$; Figure 4B-C). However, this difference was not observed when calcium was chelated by the inclusion of 10 mM BAPTA in the cell that was being monitored (1-way ANOVAs, $F_{2,27}=0.122$, $P=.89$ and $F_{2,22}=0.254$, $P=.78$ for dopamine and baclofen, respectively). In the substantia nigra, we also observed that dopamine neurons from mice with a history of METH self-administration exhibited smaller GIRK channel-mediated currents (Figure 4D). This was observed whether the GIRK channels were activated by dopamine (1-way ANOVA, $F_{2,45}=5.39$, $P=.0080$) or baclofen (1-way ANOVA, $F_{2,35}=6.04$, $P=.0056$) and again was not present when intracellular calcium was chelated with 10 mM BAPTA (1-way ANOVAs, $F_{2,26}=0.637$, $P=.54$ and $F_{2,23}=0.186$, $P=.83$ for dopamine and baclofen, respectively). This suggests that GIRK channel-mediated currents in dopamine neurons are decreased in response to METH self-administration and that this effect is not observable when intracellular calcium is chelated. Furthermore, since the effect was observed in neurons from both short and long access mice, the threshold drug exposure for this adaptation is fairly low.

Finally, we sought to determine if the decrease in GIRK channel-mediated currents was dependent on contingency of drug administration. The control group received daily noncontingent injections of saline vehicle for 14 consecutive days. The treated mice received either 14 days of 2 mg/kg METH or 11 days of saline vehicle followed by 3 days of 2 mg/kg METH. Mice were sacrificed the day after their last injection, and we examined currents induced by iontophoresis of dopamine in the VTA. While a 1-way ANOVA fell just short of significance for a main effect of treatment ($F_{2,36}=3.05$, $P=.0597$), Dunnett's test did reveal a significant difference between the saline group and the METH

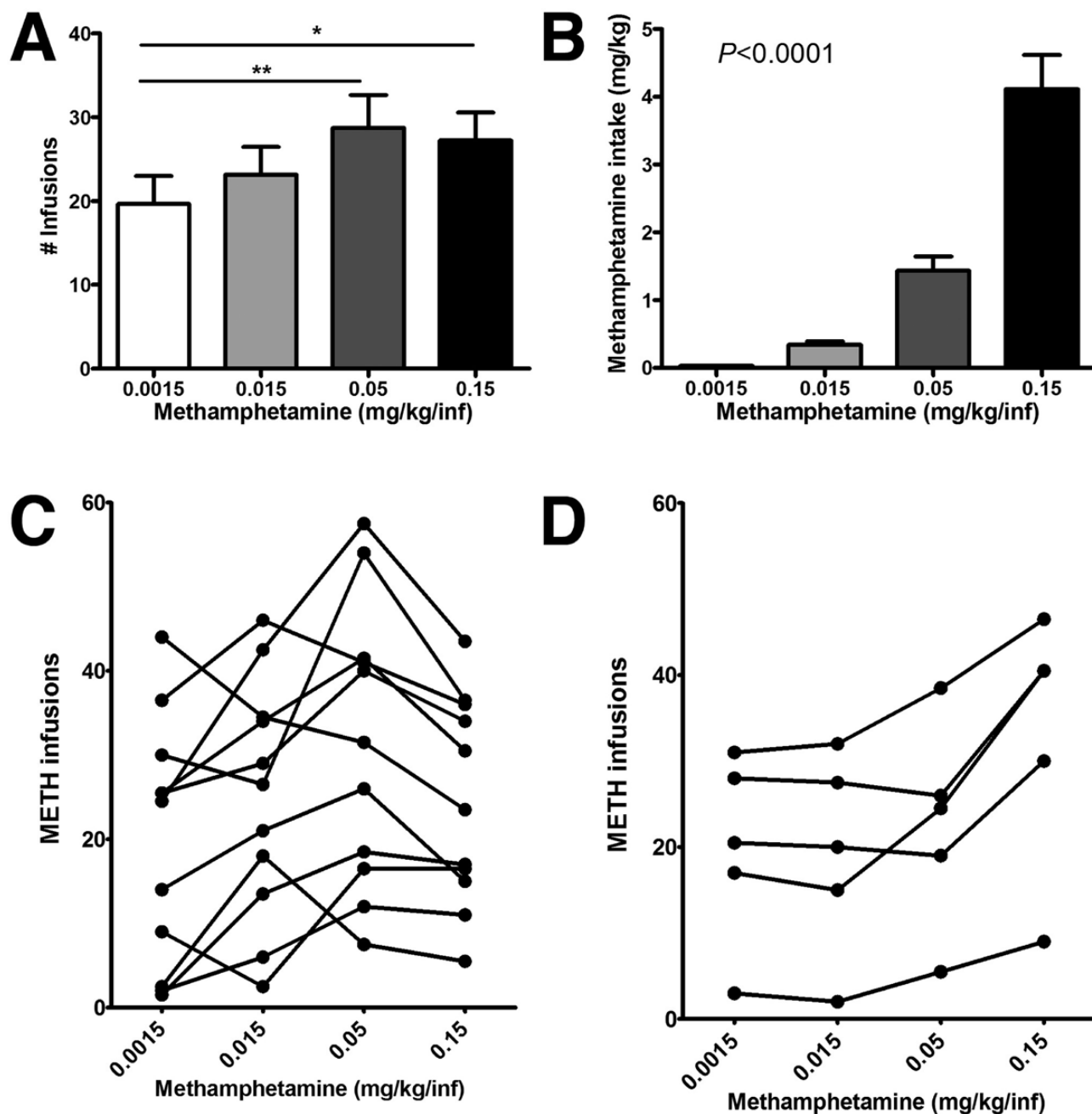


Figure 1. Methamphetamine (METH) intake increases with the amount of METH administered per infusion. When expressed as number of infusions earned per session (A), intake increased significantly when the dose was increased from 0.0015 to 0.05 and 0.15 mg/kg/infusion. Individual mouse data show that the intake of 10 of the 16 mice exhibited an inverse U-shaped curve (C) with only 5 mice showing greatest number of infusions at the highest dose tested (D). Intake expressed as mg/kg METH taken per session (B) showed significant differences in intake as analyzed by Tukey's Multiple Comparison post-hoc between every pair of concentrations tested except between 0.0015 and 0.015 mg/kg/infusion. * $P < .05$, ** $P < .01$.

x14 group ($P < .05$; Figure 5). Consistent with published findings, this suggests that METH is capable of decreasing GIRK channel-mediated currents in the VTA independent of self-administration, although these effects may not be as pronounced.

Discussion

Mouse Model of METH Self-Administration

Here we show that ad libitum-fed DBA/2J mice will reliably self-administer METH at doses that are behaviorally relevant. Rodent

models of drug self-administration often employ food restriction as a strategy to increase acquisition or expression of drug self-administration (Carroll et al., 1979; Carroll and Meisch, 1984; de la Garza and Johanson, 1987; Macenski and Meisch, 1999). Food restriction also enhances behavior measured in response to diverse classes of reinforcers, including opiate-induced stereotypies and amphetamine-induced locomotion (Carroll et al., 1979; Stuber et al., 2002). Work from our laboratory recently identified a plausible cellular mechanism for some of these effects by showing that food restriction increases dopamine neuron excitability through adaptations in glutamate and dopamine

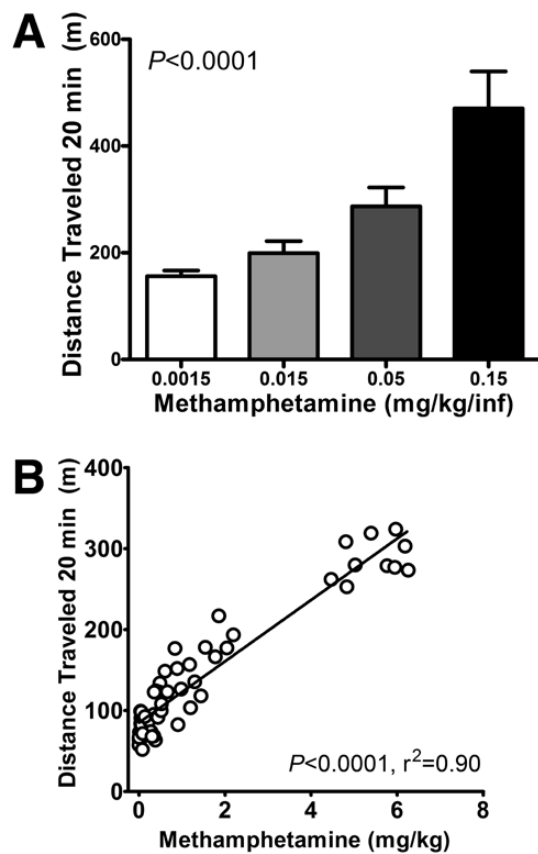


Figure 2. Mice increase horizontal distance traveled in response to increasing concentrations of methamphetamine (METH) available for self-administration. A repeated-measures analysis of variance (ANOVA) revealed a significant effect of METH concentration on the distance traveled in the 20 minutes after the self-administration session (A), with mice ($n = 7$) exhibiting more locomotor stimulation as the available concentration of METH increased. There was a significant correlation between the amount of METH self-administered and the amount of subsequent locomotion (B; $P < .0001$). Inf, infusion.

receptor signaling (Branch et al., 2013). These effects persist for at least 10 days following the return of mice to ad libitum feeding (Branch et al., 2013). Thus, to eliminate the potentially confounding effects of food restriction on dopamine neuron excitability, we developed a self-administration procedure that used ad libitum-fed mice. Approximately two-thirds of the mice tested established stable self-administration behavior on an FR3 operant schedule, and responses in the active hole were $>85\%$ of total pokes in both holes. Furthermore, our results suggest that in 1-hour daily sessions, these mice will self-administer METH in amounts that increase forward locomotion linearly with intake.

Our METH self-administration procedure also avoided the use of natural reinforcers (i.e., food) while training the mice on the operant task. Mice previously trained to make an operant response for food and a cue (such as a light) may continue responding for the cue alone in a manner that is very similar to that observed when a drug of abuse is substituted for food (Thomsen and Caine, 2011). This brings into question if the mouse is actually responding for the drug of abuse (as it is usually interpreted) or for the cues that were conditioned with the food reinforcement. Ultimately, this makes it difficult to interpret the motivation for responding. Rodent models of drug self-administration have greater face validity than non-contingent administration to human drug use and may yield

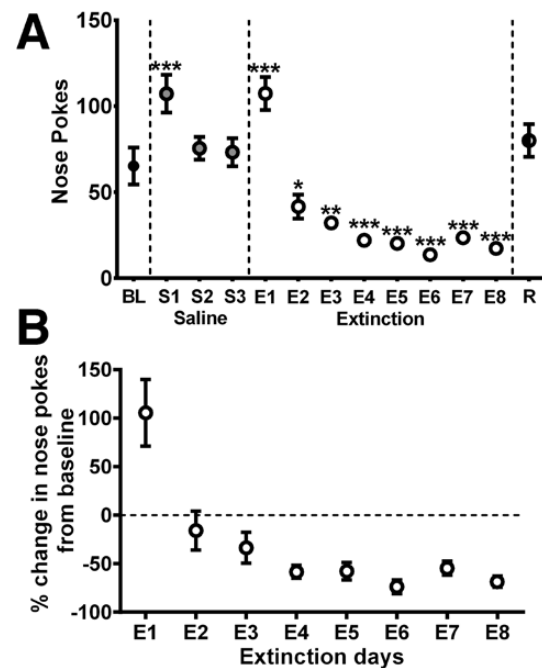


Figure 3. Mice with a history of methamphetamine (METH) self-administration exhibit extinction and cue-induced reinstatement. Mice ($n = 10$) trained to self-administer METH in daily operant sessions showed a significant increase in nose pokes on the first day of saline substitution but not on days 2 or 3 (A). When extinction conditions (no stimulus light or METH infusion) were instituted, responding increased on day 1 compared with baseline (last day before saline substitution; 0.15 mg/kg/infusion METH). However, days 2 to 8 of extinction conditions resulted in a significant decrease from baseline responding (A-B). When the stimulus light previously paired with METH infusion was reinstated on a fixed ratio 3 (FR3) schedule of nose poking (with no delivery of METH), mice increased responding from the eighth day of extinction conditions (A). Dunnett's multiple comparisons post hoc; * $P < 0.05$ vs baseline, ** $P < .01$ vs baseline, *** $P < .001$ vs baseline.

greater insight to neuroadaptations occurring in human drug users. Contingency was a central component to this study, as the majority of published animal studies investigating the effect of in vivo psychostimulants on ionic currents in single neurons have used noncontingent, experimenter delivery of the drugs. Previous reports have identified differences between experimenter-delivered and self-administered METH (Stefanski et al., 1999, 2002) as well as cocaine effects on the dopamine system (Hemby et al., 1997; Paladini et al., 2004). Thus, we felt that the most valid model for studying the effects of METH would incorporate self-administration without use of prior operant food training or food restriction.

Although the summary graph describing the number of infusions self-administered across increasing doses of METH did exhibit an ascending limb, it did not show a clear descending limb consistent with the inverted-U shape typically observed with psychostimulant self-administration (Moffett and Goeders, 2005). If we had been able to increase the dose to 0.5 mg/kg/infusion, we would have expected a decreased number of infusions. However, after 3 mice died in the operant chambers on day 3 of self-administering 0.15 mg/kg/infusion, we made the ethical decision to cease the dose manipulation. When individual mice were analyzed for number of infusions, we did indeed observe inverted U-shape curves in most cases, although there was individual variability of the peak dose of METH. The shape of the summary graph may also have been affected by running the dose-response in ascending order to minimize exposure-dependent

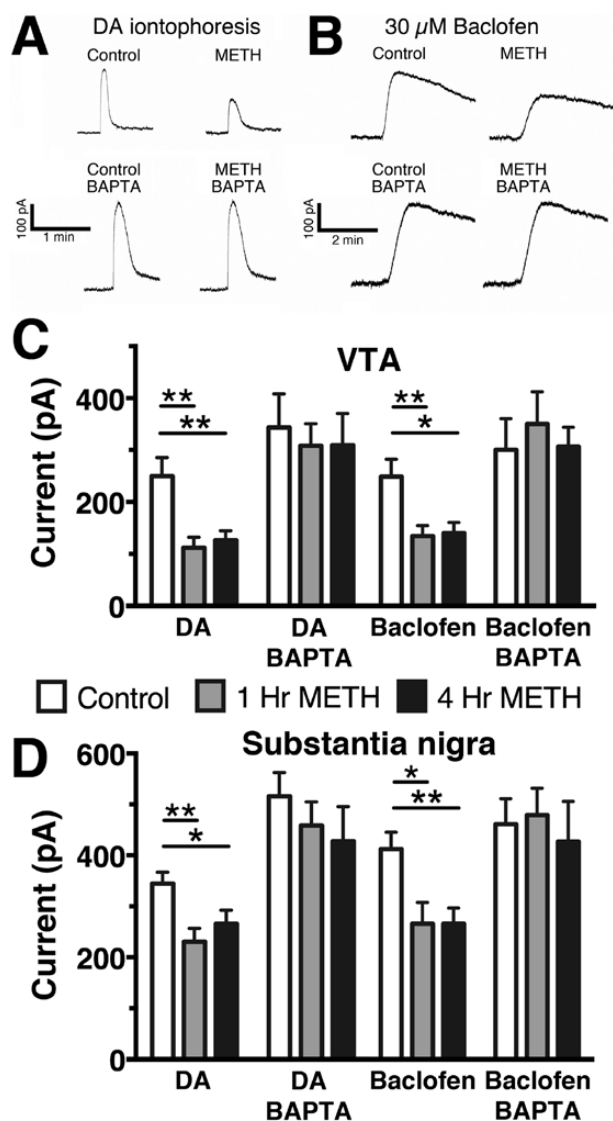


Figure 4. Methamphetamine (METH) self-administration decreases G-protein coupled inwardly rectifying potassium (GIRK) channel-mediated currents in dopamine neurons. We obtained whole-cell voltage clamp (-55 mV) recordings of ventral tegmental area (VTA) and substantia nigra dopamine neurons in brain slices. To determine maximal GIRK channel-mediated currents, we applied dopamine (DA) by iontophoresis followed in most cells by bath perfusion of baclofen. In the VTA, DA neurons in slices from mice that had self-administered METH in 1- or 4-hour daily sessions exhibited smaller currents in response to both dopamine (A,C) and baclofen (B-C), but this effect was absent when intracellular calcium was chelated with 10mM BAPTA in the recording pipette ($n = 8-12$ cells from 4-7 mice/group). Sample recording traces in panels A and B were obtained from the VTA of mice in the control and short access groups. In the substantia nigra, while currents were generally larger, dopamine neurons from self-administering mice exhibited smaller currents in response to both DA and baclofen (D). This effect was not observed when calcium was chelated by the addition of 10mM BAPTA to the recording pipette ($n = 8-19$ cells from 5-10 mice/group). Dunnett's posthoc test; * $P < .05$, ** $P < .01$.

effects such as desensitization and tolerance. Thus, we cannot rule out the possibility that cumulative experience with METH produced one or more confounds at the last, highest concentration tested. As expected from previous reports with METH and other drugs of abuse, removing METH along with the delivery cues decreased responding to the point of extinction criteria in nearly every mouse in <8 days. Furthermore, when cues (but

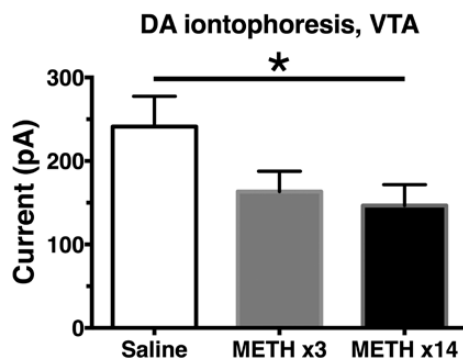


Figure 5. Noncontingent administration of methamphetamine (METH) decreases dopamine-mediated currents in the ventral tegmental area (VTA). We obtained whole-cell patch clamp recordings of VTA dopamine (DA) neurons in slices from mice that had received 14 daily injections of saline, 11 daily injections of saline followed by 3 daily injections of 2mg/kg METH (METH x3) or 14 daily injections of 2mg/kg METH (METH x14). Currents induced by a maximal iontophoresis of dopamine were smaller in amplitude in neurons from mice that had received METH, although this effect did not appear to be as pronounced as the decrease observed in the self-administration experiment ($n = 12-15$ cells from 3 mice/group). Dunnett's test; * $P < .05$.

not METH) were resumed, operant responding was reinstated to levels similar to that observed prior to the extinction protocol (cue-induced reinstatement; [Kufahl and Olive, 2011](#); [Thomsen and Caine, 2011](#); [Yan et al., 2006](#)).

Effects of METH on GIRK Channel Signaling

The results show that GIRK channel-mediated currents in dopamine neurons are decreased by METH self-administration. This effect exhibited the following features: (1) Decreased GIRK currents were observed regardless of whether the dopamine neuron was located in the substantia nigra or the VTA; (2) The decrease was observed in slices from mice that had been self-administering METH in either 1- or 4-hour sessions; (3) The decrease was not receptor specific and was observed subsequent to activation of either D2 dopamine or GABA_B receptors; and (4) No decrease was observed when intracellular calcium was chelated with 10mM BAPTA in the recording pipette. GIRK channel currents hyperpolarize dopamine neurons and provide a strong inhibition of firing subsequent to multiple types of inhibitory synaptic input ([Lacey et al., 1988](#); [Beckstead et al., 2004](#)). The decrease in GIRK currents produced by METH would thus be expected to increase dopamine neuron excitability through disinhibition and possibly increase motivated behaviors associated with dopamine release in forebrain terminal regions. Acute administration of psychostimulants increases extracellular dopamine in the somatodendritic compartment as well as in the ventral striatum ([Di Chiara and Imperato, 1988](#); [Kalivas and Duffy, 1988](#)). Extracellular dopamine can subsequently activate GIRK channels through dopamine D2 autoreceptors (eg, [Branch and Beckstead, 2012](#)). Our findings thus suggest that METH self-administration may activate a feed-forward neuroadaptive process, decreasing the ability of dopamine autoreceptors to act as a brake on cell excitability and increasing the likelihood and/or the quantity of future METH intake. Consistent with this, we did observe an increase in intake in mice that self-administered METH over several weeks (in this study intake for the last 5 days before sacrifice was 177% of intake over the first 5 days of baseline intake for the short access group).

Two recently published studies suggest that a single non-contingent injection of psychostimulants can induce a transient

decrease in GABA_B receptor-mediated GIRK currents in VTA dopamine neurons. Arora et al. (2011) observed a VTA-specific decrease in GIRK currents and surface expression of GIRK2-containing subunits after a single injection of cocaine. Padgett et al. (2012) described a decrease in GABA_B currents in dopamine neurons of the VTA after a single injection of METH (but not cocaine) in P15-35 C57Bl/6 mice. Our findings also show decreases in GABA_B receptor-mediated currents but additionally demonstrate that self-administration of METH can decrease GIRK currents in both the substantia nigra and VTA when the channels are activated by either GABA_B or dopamine D2 receptors. We observed decreased GIRK channel currents in adult DBA/2J mice that had been subjected to both short and long access to METH, supporting the notion put forth by these 2 studies that a decrease in GIRK signaling is an adaptation occurring early in drug exposure that could promote self-administration. This assertion was further supported by the decrease in dopamine-mediated currents we observed subsequent to noncontingent administration of METH, although the effect required more than 3 daily injections of 2 mg/kg before it reached statistical significance. It will be instructive to determine the full relationship between GIRK channel signaling in dopamine neurons and self-administration of psychostimulants.

Interestingly, the effect of METH experience on GIRK currents was not observed when intracellular calcium was chelated with 10 mM BAPTA. Although it is not clear that calcium chelation produces a true reversal of the effect of METH experience, it is consistent with previous work from our group and others suggesting an inverse relationship between intracellular calcium and D2 autoreceptor-mediated GIRK signaling. Low frequency electrical stimulation produces a calcium-dependent long-term depression of dopamine-mediated inhibitory postsynaptic currents (Beckstead and Williams, 2007). Maximal GIRK current amplitudes produced by activation of D2 or GABA_B receptors are larger when intracellular calcium is chelated (Beckstead and Williams, 2007), an effect recapitulated in the present study. Furthermore, depletion of intracellular calcium stores increases D2 autoreceptor-mediated GIRK currents and decreases autoreceptor desensitization in VTA dopamine neurons (Perra et al., 2011). Both of the studies describing decreased GABA_B currents following single injections of psychostimulants used an intermediate concentration of calcium chelator (1.1 mM EGTA) in the recording pipette that, based on published work, might be expected to partially enhance GIRK signaling (Beckstead and Williams, 2007; Arora et al., 2011; Padgett et al., 2012). Although the specific targets for calcium that are responsible for decreased GIRK currents following contingent METH have not been determined, the lack of receptor specificity suggests an effect at the level of the G-protein coupling or the GIRK channel. One strong possibility for this effect is via decreased surface expression of GIRK channels (Arora et al., 2011; Padgett et al., 2012). Another recently published report suggests that amphetamine self-administration can enhance regulator of G-protein signaling 2 to decouple inhibitory dopamine receptors from its G-protein effector (specifically, G α i2; Calipari et al., 2014). Regardless of the mechanism(s) responsible for our findings, it is encouraging that the neuroadaptation that was produced by weeks of METH self-administration disappeared when calcium was chelated, lending hope to the prospect of one day developing a therapeutic strategy to reverse drug related-changes in neurotransmission that are responsible for increasing drug use. Chronic METH users often struggle to overcome the enhanced reinforcing properties that are consistent with chronic use. A therapeutic agent could thus be developed to increase D2 autoreceptor-mediated

GIRK currents, returning METH sensitivity to levels associated with drug-naïve individuals.

In summary, ad libitum-fed mice that self-administer METH for several weeks exhibit a decrease in GIRK channel-mediated currents in dopamine neurons of the substantia nigra and VTA. This effect occurs independent of the neurotransmitter receptor and is not observed when intracellular calcium is chelated. Work is underway to elucidate the full relationship between GIRK channel signaling in dopamine neurons and self-administration of psychostimulants.

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Statement of Interest

None

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