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I. Daphne Calma

Rush University

Amanda L. Persons

Rush University

T. Celeste Napier (✉ celeste_napier@rush.edu)

Rush University

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Mitochondrial Function Influences Expression of Methamphetamine-Induced Motor Sensitization

I. Daphne Calma^{1,3}, Amanda L. Persons^{1,2,3}, and T. Celeste Napier PhD^{1,3}

Departments of ¹Psychiatry, ²Physician Assistant Studies, the ³Center for Compulsive Behavior and Addiction, Rush University Medical Center, Chicago, IL 60612

Corresponding author:

T. Celeste Napier, PhD
Professor, Department of Psychiatry and Behavioral Sciences
Director, Center for Compulsive Behavior and Addiction
Rush University Medical Center
Suite 424, Cohn Research Building
1735 W. Harrison Street
Chicago, Illinois 60610
Voice phone: 312-563-2428
Celeste_Napier@rush.edu

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ABSTRACT

Repeated methamphetamine use leads to neuronal maladaptations resulting in addictions. Mechanisms that underpin such adaptations have high energy requirements, implicating mitochondria involvement in addiction-related processes. We pharmacologically explored this possibility in methamphetamine self-administering rats. Motor sensitization a readout that is thought to reflect brain maladaptations akin to those occurring in methamphetamine abusing humans. This was assessed to determine (i) if a short access self-administration protocol results in the expression of motor sensitization, (ii) if and when, mitochondrial function is critical for protracted maintenance and (iii) whether drug contingency influences motor sensitization. Rats self-administered iv methamphetamine for 3 hours per day for 14 days. Those with iv cannula that failed patency were redeployed as non-contingent methamphetamine-matched animals. At three different times during forced abstinence, mitochondrial function was impaired with a low dose of the toxin, rotenone (1mg/kg/day) *via* a osmotic minipump. Motor sensitization was determined during an acute treatment of methamphetamine (1.25mg/kg sc) on abstinence day 62. Methamphetamine self-administration was sufficient to induce motor sensitization. Rotenone administration prevented the expression of sensitization, but the profile depended on abstinence time exposure. Drug contingency also influenced sensitization profiles. Mitochondrial function underpins neuronal plasticity associated with maintenance of motor sensitization induced by methamphetamine self-administration.

INTRODUCTION

Methamphetamine (meth) is a highly abused psychomotor stimulant, and repeated use of meth can lead to the development of addiction. Addiction reflects maladaptive plasticity of neuronal systems that are engaged during drug-associated learning^{5,26,31,33,53} and persist long after drug-taking ceases.^{41,49,56} In laboratory animals, behavioral manifestations of meth-induced plasticity include sensitization^{30,43,66,67} and is used to describe motor responding to bolus administration of moderately high doses of abused drugs wherein the experimenter determines treatment dose and time (i.e., non-contingent administration),^{1,3,6,8,11,34,66} e.g., 5-20 mg/kg of drug given once daily for 5 days.^{15,18,30,43,66} Sensitization is expressed following an acute challenge of the stimulant, which is administered after a period of abstinence.^{30,43,61,66} The hypothetical construct for this model is that the adaptive processes in brain circuits that govern motor function parallel those that govern reward-motivated behavior; therefore, motor readouts from drug-treated rats model the adaptive processes that occur in the brains of humans that abuse drugs.^{51,52} However, it is not clear if the dosing strategy employed by self-administering individuals (i.e., small divided doses that are self-titrated over several hours) results in the hallmark motor profile of classical sensitization treatment protocols. Another unknown is the contribution of contingency to the sensitization process, i.e., the role of performing a learned behavior required to get the drug, as in self-administration paradigms. These unknowns were considered in the current study by non-contingently injecting rats with meth in doses that matched those self-administered by a yoked partner, and testing both treatment groups for motor sensitization.

Meth has a complex pharmacology with direct and indirect consequences that vary depending on the dosing protocols used. Moderate non-contingent bolus doses of meth given *in vivo* (e.g., 2.5-10 mg/kg) increases neuronal cytosolic, synaptic and extra-synaptic levels of dopamine, norepinephrine and serotonin.^{20,60} The increase in transmission can lead to neuroplasticity that involves profound and persistent biochemical and structural changes in neuronal elements, including cytoskeleton reorganization, synthesis and translocation of key proteins, influx of calcium, and activation of kinases.^{9,17,37,46} Such neuroplastic events increase neuronal energy demands. Mitochondria are the primary source of cellular energy (i.e., ATP), and mitochondrial dysfunction leads to long term or permanent damage in the brain.^{13,22,38,39} These dynamic processes continue long after drug exposure has ended,^{27,32,42,64,65} implicating a role for mitochondria during processes associated with abstinence from chronic exposure to meth. For example, non-contingently administered meth (7.5-10mg/kg to rats) reduces mitochondrial respiration and inhibits complex II of the electron transport chain when meth is no longer in the system,^{10,35} but the persistence of the mitochondrial effects is unclear.

The current study was designed to interrogate the long-term involvement of mitochondria in meth-induced motor sensitization using the mitochondrial toxin rotenone. We previously determined that motor sensitization develops during the time of repeated exposure to meth,^{27,43} and that this effect persists for up to 60 days, wherein expression intensity directly correlates with abstinence duration.^{43,44} Thus, we posed that if sensitization is a dynamic, mitochondrial-dependent process, then the effects of rotenone would differ depending on the time of rotenone exposure after terminating meth self-administration. Study outcomes revealed that (i) self-administered meth resulted in modest behavioral sensitization that was expressed during an acute injection of meth at 60 days of abstinence, (ii) meth treatment contingency influenced outcomes, and (iii) during abstinence, the later time periods were more vulnerable to the ability of rotenone to disrupt the maintenance of sensitization.

METHODS

Animals

Male Sprague-Dawley rats (n=104; Envigo, Indianapolis, IN) weighing 225-250g upon arrival were housed in pairs, handled daily, and acclimated to environmentally-controlled conditions (temperature set point 22°C, humidity set 40-45%) for at least one week prior to the start of the experimental protocols. Rats had access food and water *ad libitum*. All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, Washington DC) with protocols approved by the Rush University Institutional Animal Care and Use Committee and with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

Surgical procedures

Jugular vein catheter implantation followed our published protocols.^{24,36,48} Rats were deeply anesthetized with 2-3% isoflurane. Catheters constructed of silastic tubing (0.3mm x 0.64mm; Dow Corning Co., Midland, MI) were inserted into the right jugular vein and secured with sutures. The distal end of the tubing passed subcutaneously over the mid-scapular region and exited through a metal guide cannula (22 gauge; Plastics One Inc., Roanoke, VA). Rats recovered for one week during which the catheters were flushed daily with 0.1-0.2ml sterile saline to maintain patency.

Figure 1. Study Timelines. **(a)** Alzet® minipumps set to deliver vehicle, 1mg/kg/day or 3mg/kg/day of rotenone were implanted subcutaneously. Behavioral measures were conducted on days 1, 3 and 6 of treatment (Red numbers). Pumps were then removed and tissues were harvested at the end of treatment day 6. **(b)** Rats self-administered meth 3hrs/day for 14 days *via* lever pressing or were non-contingently given meth *via* subcutaneous injection. Alzet® minipumps containing vehicle or 1mg/kg/day of rotenone were implanted subcutaneously on meth forced abstinence day 0, 14, or 28. Pumps were then removed and tissues were harvested at the end of treatment day 6. Rats abstained from all treatment until forced abstinence day 61, which is when all rats were subcutaneously administered 1mg/kg meth (meth acute challenge) and behavioral measures were collected. On forced abstinence day 62, rats were sacrificed and tissues were collected.

For implantation of osmotic minipumps, rats were anaesthetized with 2-3% isoflurane. The pumps (Model 2001, Alzet, Cupertino, CA) were inserted subcutaneous (sc) between the shoulder blades for the rotenone dose-response pilot study (Fig. 1a), and above the left hind limb for rats tested during meth abstinence (Fig. 1b). For both studies, the pumps were removed after six days under isoflurane anesthesia.

Drugs and treatment protocols

Rotenone (Sigma-Aldrich, St. Louis, MO) was dissolved in a vehicle solution of 1:1 mixture of dimethyl sulfoxide (DMSO; Sigma-Aldrich) and polyethylene glycol (PEG; Sigma-Aldrich) and was administered *via* sc implanted minipumps. Guided by prior studies,^{7,12,16,57} a pilot dose-response study evaluated motor responses after rotenone administered as 1mg/kg/day (n=4) or 3mg/kg/day (n=4) *versus* vehicle (n=4) for six days. The objectives of the pilot study were to validate a dosing protocol that was sufficient to alter motor function, and to identify a dose that was subthreshold to this effect, i.e., a dose that did not alter motor function. Motor assessments were conducted on the first, third and last day of the rotenone treatment (Fig. 1a). To do so, rats were transported from the housing area to the test area 30min prior to being placed in motor boxes for the 60min test session. As previously published with these boxes,^{28,29,62,63,65} the rats were the most active for the first 15min, and data from this time period were used to define 'threshold' and 'subthreshold' doses of rotenone.

(+) Methamphetamine HCl (Sigma-Aldrich, St. Louis, MO) was dissolved in sterile saline. *Self-administration*: Meth was administered intravenously (iv) as 0.1mg/kg per 0.1mL infusion. Self-administration sessions were 3h per day for a total of 14 days (Fig. 1b). Rats self-administered meth on a fixed ratio-1 (FR-1) schedule of reinforcement wherein one press on the active lever resulted in an infusion of meth and illumination of the cue light for 6s; the house light also illuminated for a total of 20s indicating a time-out period. Presses on the inactive lever had no programmed consequences. Control rats were yoked to a meth counterpart; each infusion of meth resulted in a 0.1mL infusion of saline vehicle. Lever presses by saline-yoked rats had no programmed consequences. Number of active lever presses, inactive lever presses, and the number of infusions were recorded. *Repeated non-contingent administration of meth*: Rats that lost catheter patency within the first 5 days of self-administration were moved to a non-contingent group to serve as controls for the operant task. These rats were "matched" with a meth self-administering rat, and they received a sc bolus injection that equaled the daily intake of their self-administering counterpart. *Acute meth challenge*: On forced abstinence day 61, rats were transported from the housing area to the test area 30min prior the start of motor assessments. Rats were placed in the motor boxes (AccuScan Instruments, Inc., Columbus, OH) for 30min, then returned to their home cage, non-contingently administered 1mg (base)/kg sc injection of meth (referred to as an acute challenge) and immediately placed back into the motor boxes. Behavioral responses to the acute challenge was measured for 90min (described in Table 1). The motor boxes were equipped with two banks of infrared photocells, which allowed for quantification of motor function in three dimensional space. The data were tallied for two test session times i.e., the onset and peak of meth-induced effects. Data for onset were summed for the 20–30min period after the meth injection, illustrating when meth begins to act in the brain. Data for the peak effect were summed for 30–50min post injection, illustrating when meth concentrations are likely the highest in the brain.⁵⁴

Motor Measure	Description
Horizontal Activity	The number of interruptions of the horizontal sensors.
Total Distance	The horizontal distanced traveled measured by the interruptions of the horizontal sensors reported in centimeters.
Vertical Activity	The number of beam interruptions that occurred in the vertical sensor.
Vertical Movement Number	The number of occurrences the rat spends at least 1s between two successive beam interruptions of the vertical sensors.
Vertical Time	The time between two successive beam interruptions of the vertical sensors.
Stereotypy Count	The number of occurrences when the rat interrupts the same sensors repeatedly.
Stereotypy Number	The number of occurrences when there is at least 1s between successive repetitive interruptions of the same sensor.
Stereotypy Time	The time between successive repetitive interruptions of the same sensor.

Table 1. Descriptions of parameters utilized for motoric measures.

At the end of the operant task, rats were randomly assigned to receive a sc minipump containing 1mg/kg/day of rotenone or vehicle for six days during forced abstinence. A 2x2 factorial design was used: saline/vehicle, saline/rotenone, meth/vehicle, and meth/rotenone. The study was conducted in two runs, each run containing equal number of rats from the four treatment groups. To determine if the abstinence time from meth had an effect on the impact of rotenone treatment, the toxin (or its vehicle) were administered at three different forced abstinence time periods: abstinence day 0 thru day 6 (termed R0), forced abstinence day 14 thru day 20 (R14), or abstinence day 28 thru day 34 (R28) (Fig. 1b).

Statistical analyses

Rotenone Dose-Response study: A one-way ANOVA with a *post hoc* Newman-Keuls was conducted to detect difference between animals treated with vehicle, 1mg/kg/day rotenone, and 3mg/kg/day rotenone.

Self-Administration study: Statistical tests between saline/vehicle and meth/vehicle rats were analyzed using a one-tailed Student's *t*-test to determine meth-induced effects. Rotenone assessments were done using a two-way ANOVA with a *post hoc* Newman-Keuls. Saline/rotenone vs saline/rotenone and meth/rotenone vs meth/rotenone comparisons among the three different rotenone administration times (R0, R14, and R28) were compared to determine whether the time of rotenone treatment altered motor activity.

Saline/rotenone vs meth/rotenone comparisons within each treatment time frame were conducted to determine whether rotenone altered meth-induced motor sensitization using a *post hoc* Newman-Keuls. Contingency comparisons were done with a one-tailed Student's *t*-test with a Bonferroni adjusted *alpha* of 0.025 to account for multiple comparisons. All data are presented as mean+SEM. Statistical analyses were performed using Graphpad Prism software v 8.4.2, (La Jolla, CA).

RESULTS

Rotenone Dose Determination

A subthreshold dose of rotenone was identified using motor function as the outcome. Two doses of rotenone (1mg/kg/day and 3mg/kg/day) and vehicle were compared for days 1, 3 and 6 of the six day treatment protocol. Fig. 2 demonstrates that 1mg/kg/day of rotenone did not alter any motoric readouts while 3 mg/kg/day of rotenone significantly reduced locomotion measures on rotenone treatment day 3 (Horizontal Activity: $F_{(2,8)} = 4.19$, $p < 0.05$; Total Distance: $F_{(2,8)} = 6.20$, $p < 0.05$) and rotenone treatment day 6 (Horizontal Activity $F_{(2,8)} = 22.84$, $p < 0.01$; Total Distance $F_{(2,8)} = 21.33$, $p < 0.01$). Rearing measures were also significantly reduced on day 3 (Vertical Activity $F_{(2,8)} = 11.64$, $p < 0.01$; Vertical Movement Number, $F_{(2,8)} = 10.56$, $p < 0.01$; Vertical Time $F_{(2,8)} = 11.08$, $p < 0.01$) and day 6 (Vertical Activity $F_{(2,8)} = 29.97$, $p < 0.01$; Vertical Movement Number $F_{(2,8)} = 20.18$, $p < 0.01$; Vertical Time $F_{(2,8)} = 30.01$, $p < 0.01$) in rats that received 3mg/kg/day of rotenone as compared to vehicle or to 1mg/kg/day rotenone (Fig. 2). Stereotypy measures were not altered by either rotenone treatment at any time point. Based on the lack of a motor effect, six days of 1mg/kg/day rotenone was deemed to be 'subthreshold' and this protocol was used for testing in the subsequent experiments.

Figure 2. Rotenone dose response study. Vehicle, 1mg/kg/day or 3mg/kg/day rotenone was administered for 6 days via a subcutaneous osmotic minipump. Assessments of locomotion, rearing and stereotypic behaviors were conducted. Locomotion and rearing behaviors were significantly decreased in rats treated with 3mg/kg rotenone on rotenone treatment days 3 and 6. One-way ANOVA with a Newman-Keuls *post hoc* test, * $p < 0.05$. Planned contrasts are depicted in the figures.

Acquisition of meth self-administration

All rats readily acquired the operant task (Fig. 3); during the first day of training, pressing of the active lever was significantly higher than pressing on the inactive lever ($F_{(2,1722)} = 323.3, p < 0.001$), indicating that rats were able to differentiate between the reinforced (active) and non-reinforced (inactive) levers. Saline-yoked rats exhibited minimal lever pressing on either lever across all sessions. The average lifetime intake of meth for the self-administering rats ($n=41$) was 22.0 ± 13.9 mg/kg, with an average daily intake of 1.6 ± 1.0 mg/kg (Table 2). These intakes fall within the calculated rodent equivalency range (1.5-4.0 mg/kg) for doses that humans take during recreational use of meth, 0.25-0.67 mg/kg (Goodman and Gilman 1985). However, there was a significant difference in the average lifetime ($F_{(2,39)} = 7.41, p < 0.01$) and average daily intake ($F_{(2,39)} = 4.95, p < 0.05$) among the three groups. *Post hoc* analysis revealed that the R0 treatment group had significantly lower lifetime and daily meth intake compared to R14 and R28 rats (Table 2). Overall, data indicate that rats acquired the operant task immediately.

Figure 3. Methamphetamine (meth) self-administration operant task. Meth self-administering rats readily acquired the operant lever pressing task. There was a significant difference between the active and inactive lever presses throughout the operant task. Repeated measures mixed model ANOVA followed by a Newman-Keuls *post hoc* analysis, # $p < 0.05$, ## $p < 0.01$.

	R0	R14	R28
Cumulative Intake (mg/kg total of 14 days)	12.59±5.22 **	26.07±14.73	30.09±14.09
Daily Average Intake (mg/kg/day)	0.90±0.40 *	1.90±1.10	2.10±1.00

Table 2. Methamphetamine self-administration intake data. One-way ANOVA with a *post hoc* Newman-Keuls, * $p < 0.05$, ** $p < 0.01$.

Expression of sensitization following meth acute challenge

Motor scores to the meth acute challenge were pooled for all rats with a meth/vehicle history and compared to the scores for the pooled saline/vehicle rats at two different time frames highlighted in Figure 4. As shown in Figure 5, during the first 15 minutes after the meth challenge (i.e., onset) meth/vehicle rats exhibited increased rearing behavior (vertical activity $t_{(124)} = 3.09, p < 0.01$; vertical time $t_{(124)} = 2.74, p < 0.01$) and reduced stereotypy count ($t_{(124)} = 1.76, p < 0.05$) compared to scores obtained with saline/vehicle rats during this phase. At 25–45min post-injection (i.e., the peak phase), meth/vehicle rats exhibited increased rearing behavior (vertical activity $t_{(166)} = 1.68, p < 0.05$; vertical time $t_{(166)} = 2.58, p < 0.01$) and stereotypic and horizontal behaviors were lower compared saline/vehicle; therefore, the increase in rearing appeared to be robust enough to compete with stereotypy (time $t_{(166)} = 3.17, p < 0.01$; count $t_{(166)} = 2.81, p < 0.01$; number $t_{(166)} = 2.02, p < 0.05$) as well as horizontal activity ($t_{(166)} = 3.22, p < 0.0001$ (Fig. 6). These findings demonstrated meth self-administration was sufficient to induce behavioral sensitization that could be expressed at 61 days of meth abstinence.

Figure 4. Graphical representation of the response to an acute challenge. There was no difference amongst the rats on how they habituated to the motor boxes prior to administration of the acute challenge (Habituation). The green box represents the time frame analyzed when meth begins to act in the brain (onset). The blue box represents the time frame analyzed when meth is fully in the brain of the rats (peak).

Figure 5. Methamphetamine (meth)-induced motor sensitization during onset phase. Meth/vehicle rats exhibited enhanced vertical measures (vertical activity, movement number, and time) and reduced stereotypy count compared to saline vehicle rats during the onset period. One tailed Student's *t*-test, * $p<0.05$, ** $p<0.01$. Bar graphs contain the samples size analyzed for that treatment group.

Figure 6. Methamphetamine (meth)-induced motor sensitization during peak phase. Vertical activity and time remained were enhanced in meth/vehicle rats during. Horizontal activity and stereotypy measures (stereotypy count, number, time) were reduced in meth/vehicle rats compared to saline/vehicle rats. One tailed Student's *t*-test, * $p < 0.05$, ** $p < 0.01$. Bar graphs contain the samples size analyzed for that treatment group.

Time-dependent effect of rotenone on the expression of sensitization

Responses from saline/rotenone rats were compared among the early (R0), mid (R14), and late (R28) abstinence rotenone treatment groups. Compared to R0 and R14 saline/rotenone scores (Fig. 7 and 8, Table 3 for qualitative comparison with saline/vehicle rats, and Table 4 for *post hoc* comparisons), R28 saline/rotenone rats had lower scores for several behaviors, including horizontal (onset $F_{(2, 129)} = 11.76$, $p < 0.01$; peak $F_{(2, 174)} = 18.80$, $p < 0.01$) and vertical activity (onset $F_{(2, 129)} = 8.90$, $p > 0.01$; peak $F_{(2, 174)} = 35.86$, $p < 0.01$), vertical movement number (onset $F_{(2, 129)} = 16.18$, $p < 0.01$; peak $F_{(2, 174)} = 56.52$, $p < 0.01$), vertical time (onset $F_{(2, 129)} = 6.88$, $p < 0.01$; peak $F_{(2, 174)} = 29.23$, $p < 0.01$) stereotypy count (onset $F_{(2, 129)} = 10.45$, $p < 0.01$; peak $F_{(2, 174)} = 6.25$, $p < 0.01$), and stereotypy time (onset $F_{(2, 129)} = 7.36$, $p < 0.01$). R14 saline/rotenone rats had lower vertical movement number scores than R0 saline rotenone rats (onset $F_{(2, 129)} = 16.18$, $p < 0.01$) during the onset phase (Fig. 7 and Table 4). During the peak phase, R14 saline/rotenone rats exhibited reduced total distance measures ($F_{(2, 174)} = 13.56$, $p < 0.01$) and rearing measures (vertical activity $F_{(2, 174)} = 35.86$, $p < 0.01$; vertical movement number $F_{(2, 174)} = 56.52$, $p < 0.01$; vertical time $F_{(2, 174)} = 29.23$, $p < 0.01$) compare to R0 saline rotenone rats (Fig. 8 and Table 4). The mid and late administration of rotenone altered the effects of the meth acute challenge compared to R0 saline/rotenone rats.

		Onset	Peak
R0	Horizontal Activity	915.60±63.79	903.80±58.31
	Total Distance Traveled	412.50±28.38	384.50±21.10
	Vertical Activity	227.00±31.79	263.00±26.21
	Vertical Time	79.54±23.37	66.89±9.89
	Vertical Movement Number	34.71±4.18	39.22±3.44
	Stereotypy Count	339.60±21.46	310.60±18.79
	Stereotypy Time	60.38±4.35	56.12±4.06
	Stereotypy Number	48.50±1.74	48.09±1.46
R14	Horizontal Activity	904.30±61.76	858.80±45.44
	Total Distance Traveled	488.50±34.58	376.40±21.82
	Vertical Activity	199.40±30.29	216.10±30.28
	Vertical Time	52.07±9.51	56.86±7.99
	Vertical Movement Number	28.73±3.40	32.93±3.11
	Stereotypy Count	347.90±29.70	343.90±18.78
	Stereotypy Time	60.27±4.10	62.02±3.27
	Stereotypy Number	50.10±1.67	49.50±1.30
R28	Horizontal Activity	706.80±25.77	826.60±94.57
	Total Distance Traveled	492.80±54.00	369.30±32.94
	Vertical Activity	164.30±35.40	198.50±41.65
	Vertical Time	54.47±15.77	53.75±13.44
	Vertical Movement Number	28.58±3.01	33.75±4.68
	Stereotypy Count	456.90±50.68	367.60±24.66
	Stereotypy Time	63.43±6.38	64.42±5.72
	Stereotypy Number	48.83±2.04	47.25±1.21

Table 3. Saline/vehicle motoric measure averages and standard error of the mean.

		Onset		Peak	
		saline/rotenone vs saline/rotenone	meth/rotenone vs meth/rotenone	saline/rotenone vs saline/rotenone	meth/rotenone vs meth/rotenone
Horizontal Activity	R0 vs R14	$p \geq 0.05$	** $p < 0.01$	$p \geq 0.05$	** $p < 0.01$
	R0 vs R28	** $p < 0.01$	$p \geq 0.05$	** $p < 0.01$	$p \geq 0.05$
	R14 vs R28	** $p < 0.01$	** $p < 0.01$	** $p < 0.01$	** $p < 0.01$
Total Distance Traveled	R0 vs R14	$p \geq 0.05$	* $p < 0.05$	* $p < 0.05$	** $p < 0.01$
	R0 vs R28	$p \geq 0.05$	$p \geq 0.05$	** $p < 0.01$	$p \geq 0.05$
	R14 vs R28	$p \geq 0.05$	$p \geq 0.05$	** $p < 0.01$	* $p < 0.05$
Vertical Activity	R0 vs R14	$p \geq 0.05$	$p \geq 0.05$	** $p < 0.01$	$p \geq 0.05$
	R0 vs R28	* $p < 0.05$	$p \geq 0.05$	** $p < 0.01$	** $p < 0.01$
	R14 vs R28	* $p < 0.05$	$p \geq 0.05$	** $p < 0.01$	** $p < 0.01$
Vertical Time	R0 vs R14	$p \geq 0.05$	$p \geq 0.05$	** $p < 0.01$	$p \geq 0.05$
	R0 vs R28	* $p < 0.05$	$p \geq 0.05$	** $p < 0.01$	** $p < 0.01$
	R14 vs R28	* $p < 0.05$	$p \geq 0.05$	** $p < 0.01$	** $p < 0.01$
Vertical Movement Number	R0 vs R14	* $p < 0.05$	$p \geq 0.05$	** $p < 0.01$	$p \geq 0.05$
	R0 vs R28	** $p < 0.01$	** $p < 0.01$	** $p < 0.01$	** $p < 0.01$
	R14 vs R28	* $p < 0.05$	* $p < 0.05$	** $p < 0.01$	** $p < 0.01$
Stereotypy Count	R0 vs R14	$p \geq 0.05$	* $p < 0.05$	$p \geq 0.05$	$p \geq 0.05$
	R0 vs R28	** $p < 0.01$	* $p < 0.05$	$p \geq 0.05$	$p \geq 0.05$
	R14 vs R28	* $p < 0.05$	* $p < 0.05$	$p \geq 0.05$	$p \geq 0.05$
Stereotypy Time	R0 vs R14	$p \geq 0.05$	$p \geq 0.05$	$p \geq 0.05$	$p \geq 0.05$
	R0 vs R28	** $p < 0.01$	$p \geq 0.05$	$p \geq 0.05$	$p \geq 0.05$
	R14 vs R28	* $p < 0.05$	$p \geq 0.05$	$p \geq 0.05$	$p \geq 0.05$
Stereotypy Number	R0 vs R14	$p \geq 0.05$	$p \geq 0.05$	$p \geq 0.05$	$p \geq 0.05$
	R0 vs R28	$p \geq 0.05$	$p \geq 0.05$	$p \geq 0.05$	$p \geq 0.05$
	R14 vs R28	$p \geq 0.05$	$p \geq 0.05$	$p \geq 0.05$	$p \geq 0.05$

Table 4. Rotenone administration time *post hoc* Newman-Keuls comparisons.

Meth/rotenone rats were compared to determine if the time of rotenone administration had similar temporal effect in rats with a background of meth self-administration (Fig. 7 and 8). Qualitative assessments of the motor averages of meth/rotenone rats to the motor averages of saline/vehicle (Table 3) show that meth/rotenone rats exhibit similar or lower motor responses for most of the outcome measures. R14 meth/rotenone rats exhibited lower horizontal activity (onset $F_{(2, 129)} = 11.76$, $p < 0.01$; peak $F_{(2, 174)} = 18.80$, $p < 0.01$) and total distance (onset $F_{(2, 129)} = 1.38$, $p > 0.05$; peak $F_{(2, 174)} = 13.56$, $p < 0.01$) scores compared to R0 meth/rotenone rats (Fig. 7 and 8). During the onset phase, R14 and R28 rats exhibited low stereotypy count scores compared to R0 rats and R28 rats exhibited lower scores compared to R14 rats ($F_{(2, 129)} = 10.4$, $p < 0.01$); these differences did not persist to the peak phase. All R28 rotenone-treated rats experienced an adverse reaction to the meth acute challenge. Acute meth exposure resulted in cataleptic-like behaviors soon after the motor test, followed by brief, generalized seizures and death; R28 meth/rotenone rats died within a few hours after the acute challenge, while R28 saline/rotenone rats died several hours later. Similar to meth-naïve rats, the administration of rotenone in rats with a history of meth altered their response to the meth acute challenge and this response varied with the administration time frame.

Figure 7. Rotenone-induced effects on expression of motor sensitization during onset phase. In response to the acute methamphetamine (meth) challenge, R28 administration of rotenone reduced horizontal activity, vertical activity, vertical movement number, vertical time, stereotypy count, and stereotypy time for R28 saline/rotenone rats compared to R0 saline/rotenone rats and horizontal activity, vertical activity, stereotypy count, and stereotypy number when compared to R14 saline/rotenone rats. R14 administration reduced in vertical movement number in R14 saline/rotenone rats compared to R0 saline/rotenone rats. R0 meth/rotenone rats had reduced horizontal activity, total distance traveled, and stereotypy count compare to R0 saline rotenone rats. R14 meth/rotenone rats had increased horizontal activity, total distance traveled, and stereotypy counts compared to R0 meth/rotenone rats. R28 meth/rotenone rats had reduced horizontal activity, vertical movement numbers, and stereotypy counts compared to R14 meth/rotenone rats and lower vertical activity, vertical movement number, and stereotypy counts compared to R0 meth/rotenone rats. Two-way ANOVA with a Newman-Keuls *post hoc* test, *, $p < 0.05$, ** $p < 0.01$. Bar graphs contain the samples size analyzed for that treatment group.

Figure 8. Rotenone-induced effects on expression of motor sensitization during peak phase. In response to the acute methamphetamine (meth) challenge, R28 administration of rotenone reduced horizontal activity, total distance traveled, vertical activity, vertical movement number, and vertical time for R28 saline/rotenone rats compared to R0 and R14 saline/rotenone rats. R14 administration reduced in total distance traveled, vertical activity, vertical movement number, and vertical time in R14 saline/rotenone rats compared to R0 saline/rotenone rats. R0 meth/rotenone rats had reduced horizontal activity, total distance traveled, vertical activity, vertical movement number and vertical time compare to R0 saline rotenone rats. R14 meth/rotenone rats had increased vertical time compared to R14 saline/rotenone rats. R14 meth/rotenone rats had increased horizontal activity and total distance traveled compared to R0 meth/rotenone rats. R28 meth/rotenone rats had reduced horizontal activity, total distance traveled, vertical activity, vertical movement number, and vertical time compared to R0 and R14 meth/rotenone rats. Two-way ANOVA with a Newman-Keuls *post hoc* test, *, $p < 0.05$, ** $p < 0.01$. Bar graphs contain the samples size analyzed for that treatment group.

Rotenone inhibitory effect on the expression of motor sensitization

During the onset phase of responding to the acute challenge, meth/rotenone rats did not exhibit increased rearing compared to their corresponding saline/rotenone rats (Fig. 7). R0 meth/rotenone rats exhibited reduced scores for several behaviors. An interaction between rotenone and meth occurred for horizontal activity ($F_{(2, 129)} = 9.372, p < 0.01$), total distance traveled ($F_{(2, 129)} = 5.243, p < 0.01$), stereotypy count ($F_{(2, 129)} = 5.598, p < 0.01$) and stereotypy time ($F_{(2, 129)} = 4.681, p < 0.05$). *Post hoc* evaluations within each of the three abstinence times, revealed that R0 meth/rotenone rats had decreased horizontal activity ($p < 0.01$), total distance traveled ($p < 0.01$), and stereotypy count ($p < 0.05$) compared to R0 saline/rotenone rats (Table 5).

saline/rotenone vs meth/rotenone		Onset	Peak
Horizontal Activity	R0	** $p < 0.01$	** $p < 0.01$
	R14	$p \geq 0.05$	$p \geq 0.05$
	R28	$p \geq 0.05$	$p \geq 0.05$
Total Distance Traveled	R0	** $p < 0.01$	** $p < 0.01$
	R14	$p \geq 0.05$	$p \geq 0.05$
	R28	$p \geq 0.05$	$p \geq 0.05$
Vertical Activity	R0	$p \geq 0.05$	** $p < 0.01$
	R14	$p \geq 0.05$	$p \geq 0.05$
	R28	$p \geq 0.05$	$p \geq 0.05$
Vertical Time	R0	$p \geq 0.05$	* $p < 0.05$
	R14	$p \geq 0.05$	* $p < 0.05$
	R28	$p \geq 0.05$	$p \geq 0.05$
Vertical Movement Number	R0	$p \geq 0.05$	** $p < 0.01$
	R14	$p \geq 0.05$	$p \geq 0.05$
	R28	$p \geq 0.05$	$p \geq 0.05$
Stereotypy Count	R0	* $p < 0.05$	$p \geq 0.05$
	R14	$p \geq 0.05$	$p \geq 0.05$
	R28	$p \geq 0.05$	$p \geq 0.05$
Stereotypy Time	R0	$p \geq 0.05$	$p \geq 0.05$
	R14	$p \geq 0.05$	$p \geq 0.05$
	R28	$p \geq 0.05$	$p \geq 0.05$
Stereotypy Number	R0	$p \geq 0.05$	$p \geq 0.05$
	R14	$p \geq 0.05$	$p \geq 0.05$
	R28	$p \geq 0.05$	$p \geq 0.05$

Table 5. Saline/rotenone vs meth/rotenone *post hoc* Newman-Keuls comparisons.

During the peak phase, meth/rotenone rats had reduced horizontal and vertical motor measures compared to their saline/rotenone counterparts (Fig. 8). Rotenone administration at any time point appeared to diminish the meth-induced expression of motor sensitization exhibited by meth/vehicle rats. There was a significant rotenone effect for horizontal activity ($F_{(2, 174)} = 18.80, p < 0.01$), total distance traveled ($F_{(2, 174)} = 13.56, p < 0.01$), vertical activity ($F_{(2, 174)} = 35.86, p < 0.01$), vertical movement number ($F_{(2, 174)} = 56.52, p < 0.01$), and vertical time ($F_{(2, 174)} = 29.23, p < 0.01$). There was also a significant meth effect for horizontal activity ($F_{(2, 174)} = 5.579, p < 0.05$) and total distance traveled ($F_{(2, 174)} = 6.423, p < 0.05$). There was a significant interaction for horizontal activity ($F_{(2, 174)} = 10.61, p < 0.01$), total distance traveled ($F_{(2, 174)} = 13.67, p < 0.01$), vertical activity ($F_{(2, 174)} = 5.711, p < 0.01$), vertical movement number ($F_{(2, 174)} = 3.359, p < 0.05$), and vertical time ($F_{(2, 174)} = 6.975, p < 0.01$). *Post hoc* evaluations, seen in Table 5, revealed that R0 meth/rotenone rats had decreased horizontal and vertical behaviors compared to R0 saline/rotenone

rats (horizontal activity ($p<0.01$), total distance traveled ($p<0.01$), vertical activity ($p<0.01$), vertical movement number ($p<0.01$), and vertical time ($p<0.05$)). R14 meth/rotenone rats had increased vertical time ($p<0.05$) compared to R14 saline/rotenone rats. R28 rats exhibited stereotypy but no horizontal or vertical motoric behaviors during this phase. The data suggest that functioning mitochondria during abstinence are required for expression of meth-induced motor sensitization. Unlike meth/vehicle rats, meth/rotenone treated rats, independent of the rotenone treatment time, did not exhibit the enhanced vertical behaviors during the acute challenge.

Effect of contingency on expression of meth-induced sensitization

The effect of treatment contingency was considered by comparing responding to an acute challenge between R14 meth self-administration/rotenone rats to meth non-contingent yoked rats (Table 6). No motoric differences were obtained between the two exposure modalities for the onset phase. However in the peak phase, non-contingent administration reduced total distance travel as compared to distance traveled by the self-administering rats ($p<0.01$). Thus, contingency of drug administration can influence drug-induced motor sensitization.

	Onset	Peak
Horizontal Activity	$p= 0.13, t(46)= 1.55$	$p= 0.10, t(62)= 1.68$
Total Distance Traveled	$p= 0.12, t(46)= 1.60$	** $p= 0.004, t(62)= 3.03$
Vertical Activity	$p= 0.57, t(46)= 0.57$	$p= 0.25, t(62)= 1.17$
Vertical Time	$p= 0.37, t(46)= 0.90$	$p= 0.71, t(62)= 0.37$
Vertical Movement Number	$p= 0.45, t(46)= 0.76$	$p= 0.73, t(62)= 0.35$
Stereotypy Count	$p= 0.28, t(46)= 1.08$	$p= 0.52, t(62)= 0.64$
Stereotypy Time	$p= 0.67, t(46)= 0.43$	$p= 0.84, t(62)= 0.20$
Stereotypy Number	$p= 0.33, t(46)= 0.98$	$p= 0.94, t(62)= 0.07$

Table 6. R14 self-administration methamphetamine (meth)/rotenone vs non-contingent meth/rotenone Students *t* test with Bonferroni α correction of 0.025 for multiple comparisons.

DISCUSSION

Study outcomes revealed three previously undescribed features of meth-induced motor sensitization: (i) the ability of low self-titrated doses of meth to produce motor sensitization, (ii) the effect of contingency, and (iii) the time-related role of mitochondrial function in the persistence of sensitization. The data presented here suggest that rotenone administration disrupts ongoing processes during meth forced abstinence days 0–34 to prevent the expression of meth-induced expression of motor sensitization on abstinence day 60. The data also indicate that the effect of rotenone alone is transient and appears to recover over time. This ability of rotenone to prevent expression of meth-induced motor sensitization highlights the importance of normal mitochondrial function during the maintenance phase. This demonstrated that sensitization was induced during self-administration and that mitochondrial function was necessary to maintain meth-induced maladaptations throughout drug abstinence. Administration of rotenone during mid (days 14-20) and late (days 28-34) abstinence periods reduced the capacity of rats with a history of saline treatments to react to their first exposure of meth, and the later time frame rendered meth toxic. These outcomes reveal the importance of normal mitochondrial function in the processes occurring during meth forced abstinence, and in the ability of small self-administer doses of meth to exert lasting effects on meth-induced motor behavior.

We previously demonstrated that meth-induced motor sensitization is induced using repeated, non-contingent administration of moderately low doses of meth (2.5 mg/kg as the base) and maintained for

at least 60 days^{43,44} This persistent expression suggests that molecular changes were initiated by meth self-administration. The nature of these molecular changes, either long-term or continuously occurring during abstinence, could not be answered by the current experimental design. We previously demonstrated that repeated meth treatments produced molecular changes that lead to the development of incubation and motor sensitization.^{27,43,44,65} Incubation refers to a time-dependent increase in drug-seeking behaviors that is observed during abstinence from self-administration of abused drugs.^{14,25,49} Incubation was not the focus of this research, but like motor sensitization it is a measure of drug-induced neuronal changes, observations from this study may suggest that these changes observed with meth-induced motor sensation may also be reflective of changes in incubation.

Several studies demonstrate behavioral and biochemical differences in drug-induced effects between self-administration and non-contingent treatment protocols.^{21,45,47,50,59} These differences suggest that the two drug administration protocols influence different processes involved in drug-induced plasticity. We aimed to determine whether low doses of meth self-administered over a 3h session was sufficient to for the induction and expression of motor sensitization. Unlike non-contingent protocols, self-administration protocols allow rats to control the amount and timing of drug intake, which engages motivational aspects that maybe important in associative learning. To our knowledge, the only published self-administration protocol used to assess stimulant-induced motor sensitization was extended access (6h session for 16 or 21 days) which result in high self-administered doses of stimulant drug.¹⁹ We revealed that the short-access self-administration protocol used in the current study (3h session/day for 14 days) was sufficient to induce brain adaptations that led to the expression of motor sensitization at 61 days of abstinence. We previously observed that similar low doses of meth administered non-contingently produced the inverted “U” which reflects initial increases in locomotor activity when meth concentrations are low in the brain that are reduced while repetitive behaviors, like stereotypy, are increased as brain concentrations rise and begin to affect more brain regions.^{43,58} The rats in this study did not exhibit the inverted “U” response curve. Differences in sensitization profiles could be due to several factors, including doses self-administered by individual rats, differences in experienced stress during repeated drug administration. By comparing self-administered meth with meth-yoked counterparts, although no meth-treated rats exhibited the inverted “U” response curve, the current study indicated that contingency of administration influences drug-induced effects; however, these other factors need also to be studied before the particular underpinnings can be deciphered.

The expression of motor sensitization after repeated drug exposure is a behavioral index of drug-induced neuronal plasticity. The processes engaged during neuronal plasticity have high energy requirements.^{24,9,23,40,55} The presence of behavioral measures of neuronal plasticity after 60 days of abstinence suggests that processes are actively happening during abstinence to maintain these neuronal changes. This maintenance phase implicates the mitochondria in drug-induced processes. We hypothesized that meth-induced plasticity occurring during abstinence can be modified *via* manipulation of mitochondrial function during the maintenance phase associated with drug abstinence. Rotenone was utilized as a tool to highlight the contribution of mitochondrial function on maintaining sensitization during abstinence. We determined a subthreshold dose and treatment duration of rotenone, and we used this treatment protocol to alter mitochondrial function during early (days 0-6), mid (days 14-20) and late (days 28-34) phases of meth abstinence. Meth self-administering rats treated with rotenone at all three time points tested during abstinence did not exhibit meth-induced enhancement in vertical behaviors seen in the meth/vehicle group while horizontal behaviors were unchanged by meth self-administration. These results suggest that dynamic processes that maintain meth-induced neuronal plasticity are sensitive to reductions in energy. These data support our hypothesis that meth-induced plasticity can be altered by manipulation of mitochondrial function.

Rotenone, administered during mid and late abstinence altered how rats respond to their first exposure to meth. Meth-induces a variety of cellular responses that require energy.^{9,17,37,46} The data presented here suggest that administration of rotenone treatment closer to when the acute challenge is administered does not allow enough time for damaged mitochondria recover. Saline/rotenone rats qualitatively exhibited lower vertical motor responses to the meth challenge compared to saline/vehicle rats when rotenone was administered during mid and late abstinence. The reduction in responsiveness suggests that the requirements, likely energy dependent, for cells to appropriately respond to meth were not met. This reduction in motor response was not evident for the horizontal and stereotypic measure of motor response. The effect of rotenone on vertical aspects of motor activity and the lack of effect by rotenone on stereotypic behaviors suggest that the brain regions that govern these behaviors, basal ganglia vs. mesolimbic, have different vulnerability to a mitochondrial challenge. To our knowledge, there are no studies that compare the energy consumption of limbic brain regions and the basal ganglia. Differences in sensitivity to the effect of rotenone observed through behavior revealed that the mitochondria in these brain regions likely react to the effects of rotenone differently. These differences could suggest that the energy requirements of the cells that reside in the basal ganglia compared to mesolimbic regions may differ.

Administration of rotenone later in the abstinence period either (i) lowered the capacity of mitochondria to produce energy necessary to respond to an acute challenge of meth, or (ii) the capacity was the same, but not enough time to recover so that the meth challenge was rendered toxic. This toxic effect was evident in rats treated with rotenone during late abstinence with or without a history of meth exposure. These rats exhibited adverse reactions to the acute meth challenge and experienced seizures. Seizures can result when the brain is unable to meet increased energy requirements imposed by the imbalance between excitatory and inhibitory transmitters.⁶⁸ Meth is known to cause a massive efflux of these transmitters. If the mitochondria of these rats were damaged, the effects of meth could be toxic and could lead to seizures. It is difficult to deduce what exactly caused the seizures in these animals, but because only rotenone-treated animals experienced them, the results implicate mitochondrial involvement. Further studies are needed to understand the relationship between mitochondrial function and the neuronal process influenced by meth.

Energy is a key requirement for all cellular function, including the dynamic processes needed for maintenance of homeostasis, plasticity. This high energy demand implicates the mitochondria significantly in meth-induced neuronal changes. Mild alterations to mitochondrial function can lead to detrimental results. For example, rats treated with rotenone during the late phase of abstinence appeared to be healthy. The animals in this study did not lose weight, were not sick, and were grooming and moving around normally. Despite their appearance, these animals were not able to appropriately react to the acute challenge of meth, which resulted in seizures and death. Rotenone administration also diminished the expression of motor sensitization exhibited by meth/vehicle rats. This indicates that disrupting energy production during meth abstinence altered the maintenance of brain changes reflected by the expression of motor sensitization induced by meth. Increasing our understanding of exactly how the mitochondria respond to the effects of meth would provide evidence on the role of the mitochondria in meth-induced neurotoxicity and maladaptation. The exact relationship between mitochondrial processes and drug-induced maladaptation remains to be determined, but this study is the first to suggest that the mitochondria are key in the ongoing process that maintain these changes.

Reference List

1. Ago Y, Nakamura S, Kajita N, Uda M, Hashimoto H, Baba A, Matsuda T. Ritanserin reverses repeated methamphetamine-induced behavioral and neurochemical sensitization in mice. *Synapse* 61:757-763 (2007).
2. Alkon DL. Molecular mechanisms of associative memory and their clinical implications. *Behav Brain Res* 66:151-160 (1995).
3. Anagnostaras SG, Robinson TE. Sensitization to the psychomotor stimulant effects of amphetamine: modulation by associative learning. *Behav Neurosci* 110:1397-1414 (1996).
4. Atkins CM, Selcher JC, Petraitis JJ, Trzaskos JM, Sweatt JD. The MAPK cascade is required for mammalian associative learning. *Nat Neurosci* 1:602-609 (1998).
5. Baler RD, Volkow ND. Drug addiction: the neurobiology of disrupted self-control. *Trends Mol Med* 12:559-566 (2006).
6. Ben-Shahar O, Ettenberg A. Repeated stimulation of the ventral tegmental area sensitizes the hyperlocomotor response to amphetamine. *Pharmacol Biochem Behav* 48:1005-1009 (1994).
7. Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci* 3:1301-1306 (2000).
8. Brady AM, Glick SD, O'Donnell P. Selective disruption of nucleus accumbens gating mechanisms in rats behaviorally sensitized to methamphetamine. *J Neurosci* 25:6687-6695 (2005).
9. Brodin L, Bakeeva L, Shupliakov O. Presynaptic mitochondria and the temporal pattern of neurotransmitter release. *Philos Trans R Soc Lond B Biol Sci* 354:365-372 (1999).
10. Brown JM, Quinton MS, Yamamoto BK. Methamphetamine-induced inhibition of mitochondrial complex II: roles of glutamate and peroxynitrite. *J Neurochem* 95:429-436 (2005).
11. Brown TE *et al.* A silent synapse-based mechanism for cocaine-induced locomotor sensitization. *J Neurosci* 31:8163-8174 (2011).
12. Cannon JR, Tapias V, Na HM, Honick AS, Drolet RE, Greenamyre JT. A highly reproducible rotenone model of Parkinson's disease. *Neurobiol Dis* 34:279-290 (2009).
13. Cheng A, Hou Y, Mattson MP. Mitochondria and neuroplasticity. *ASN Neuro* 2:e00045 (2010).

14. Childress AR, Mozley PD, McElgin W, Fitzgerald J, Reivich M, O'Brien CP. Limbic activation during cue-induced cocaine craving. *Am J Psychiatry* 156:11-18 (1999).
15. Davidson C, Lee TH, Ellinwood EH. Acute and chronic continuous methamphetamine have different long-term behavioral and neurochemical consequences. *Neurochem Int* 46:189-203 (2005).
16. Drolet RE, Cannon JR, Montero L, Greenamyre JT. Chronic rotenone exposure reproduces Parkinson's disease gastrointestinal neuropathology. *Neurobiol Dis* 36:96-102 (2009).
17. Du H *et al.* Dopaminergic inputs in the dentate gyrus direct the choice of memory encoding. *Proc Natl Acad Sci U S A* 113:E5501-E5510 (2016).
18. Eradiri OL, Starr MS. Striatal dopamine depletion and behavioural sensitization induced by methamphetamine and 3-nitropropionic acid. *Eur J Pharmacol* 386:217-226 (1999).
19. Ferrario CR, Gorny G, Crombag HS, Li Y, Kolb B, Robinson TE. Neural and behavioral plasticity associated with the transition from controlled to escalated cocaine use. *Biol Psychiatry* 58:751-759 (2005).
20. Fleckenstein AE, Volz TJ, Riddle EL, Gibb JW, Hanson GR. New insights into the mechanism of action of amphetamines. *Annu Rev Pharmacol Toxicol* 47:681-698 (2007).
21. Frankel PS *et al.* Effect of methamphetamine self-administration on neurotensin systems of the basal ganglia. *J Pharmacol Exp Ther* 336:809-815 (2011).
22. Fu ZX *et al.* Dendritic mitoflash as a putative signal for stabilizing long-term synaptic plasticity. *Nat Commun* 8:31 (2017).
23. Geinisman Y *et al.* Remodeling of hippocampal synapses after hippocampus-dependent associative learning. *J Comp Neurol* 417:49-59 (2000).
24. Graves SM, Napier TC. Mirtazapine alters cue-associated methamphetamine seeking in rats. *Biol Psychiatry* 69:275-281 (2011).
25. Grimm JW, Hope BT, Wise RA, Shaham Y. Neuroadaptation. Incubation of cocaine craving after withdrawal. *Nature* 412:141-142 (2001).
26. Harris GC, Wimmer M, Byrne R, Aston-Jones G. Glutamate-associated plasticity in the ventral tegmental area is necessary for conditioning environmental stimuli with morphine. *Neuroscience* 129:841-847 (2004).
27. Herrold AA, Persons AL, Napier TC. Cellular distribution of AMPA receptor subunits and mGlu5 following acute and repeated administration of morphine or methamphetamine. *J Neurochem* (2013).

28. Herrold AA, Voigt RM, Napier TC. Brain region selective cellular redistribution of mGlu5 but not GABA(B) receptors following methamphetamine-induced associative learning. *Synapse* 65:1333-1343 (2011).
29. Herrold AA, Voigt RM, Napier TC. mGluR5 is necessary for maintenance of methamphetamine-induced associative learning. *Eur Neuropsychopharmacol* 23(7):691-6 (2012).
30. Itzhak Y. Modulation of cocaine- and methamphetamine-induced behavioral sensitization by inhibition of brain nitric oxide synthase. *J Pharmacol Exp Ther* 282:521-527 (1997).
31. Kalivas PW, Alesdatter JE. Involvement of N-methyl-D-aspartate receptor stimulation in the ventral tegmental area and amygdala in behavioral sensitization to cocaine. *J Pharmacol Exp Ther* 267:486-495 (1993).
32. Kalivas PW, Hu XT. Exciting inhibition in psychostimulant addiction. *Trends Neurosci* 29:610-616 (2006).
33. Karler R, Calder LD, Chaudhry IA, Turkanis SA. Blockade of "reverse tolerance" to cocaine and amphetamine by MK-801. *Life Sci* 45:599-606 (1989).
34. Kesby JP *et al.* HIV-1 TAT protein enhances sensitization to methamphetamine by affecting dopaminergic function. *Brain Behav Immun* 65:210-221 (2017).
35. Killinger B, Shah M, Moszczynska A. Co-administration of betulinic acid and methamphetamine causes toxicity to dopaminergic and serotonergic nerve terminals in the striatum of late adolescent rats. *J Neurochem* 128:764-775 (2014).
36. Kousik SM, Carvey PM, Napier TC. Methamphetamine self-administration results in persistent dopaminergic pathology: implications for Parkinson's disease risk and reward-seeking. *Eur J Neurosci* 40:2707-2714 (2014).
37. Kwon SK, Hirabayashi Y, Polleux F. Organelle-Specific Sensors for Monitoring Ca(2+) Dynamics in Neurons. *Front Synaptic Neurosci* 8:29 (2016).
38. Li Y, Kauer JA. Repeated exposure to amphetamine disrupts dopaminergic modulation of excitatory synaptic plasticity and neurotransmission in nucleus accumbens. *Synapse* 51:1-10 (2004).
39. Li Y, Rempe DA. During hypoxia, HUMMR joins the mitochondrial dance. *Cell Cycle* 9:50-57 (2010).
40. Lonze BE, Ginty DD. Function and regulation of CREB family transcription factors in the nervous system. *Neuron* 35:605-623 (2002).

41. Lu L, Grimm JW, Dempsey J, Shaham Y. Cocaine seeking over extended withdrawal periods in rats: different time courses of responding induced by cocaine cues versus cocaine priming over the first 6 months. *Psychopharmacology (Berl)* 176:101-108 (2004).
42. Mao LM, Wang JQ. Amphetamine-induced Conditioned Place Preference and Changes in mGlu1/5 Receptor Expression and Signaling in the Rat Medial Prefrontal Cortex. *Neuroscience* 400:110-119 (2019).
43. McDaid J, Graham MP, Napier TC. Methamphetamine-induced sensitization differentially alters pCREB and Δ FosB throughout the limbic circuit of the mammalian brain. *Molecular Pharmacology* 70:2064-2074 (2006).
44. McDaid J *et al.* Nullifying drug-induced sensitization: behavioral and electrophysiological evaluations of dopaminergic and serotonergic ligands in methamphetamine-sensitized rats. *Drug Alcohol Depend* 86:55-66 (2007).
45. Miguens M *et al.* Differential cocaine-induced modulation of glutamate and dopamine transporters after contingent and non-contingent administration. *Neuropharmacology* 55(5):771-9 (2008).
46. Moy SS, Nadler JJ. Advances in behavioral genetics: mouse models of autism. *Mol Psychiatry* 13:4-26 (2008).
47. Palamarchouk V, Smagin G, Goeders NE. Self-administered and passive cocaine infusions produce different effects on corticosterone concentrations in the medial prefrontal cortex (MPC) of rats. *Pharmacol Biochem Behav* 94:163-168 (2009).
48. Persons AL *et al.* Gut and brain profiles that resemble pre-symptomatic Parkinson's disease in methamphetamine self-administering rats. *Drug Alcohol Depend* 225:108746 (2021).
49. Pickens CL, Airavaara M, Theberge F, Fanous S, Hope BT, Shaham Y. Neurobiology of the incubation of drug craving. *Trends Neurosci* 34:411-420 (2011).
50. Reichel CM, Chan CH, Ghee SM, See RE. Sex differences in escalation of methamphetamine self-administration: cognitive and motivational consequences in rats. *Psychopharmacology (Berl)* 223:371-380 (2012).
51. Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Research Reviews* 18:247-291 (1993).
52. Robinson TE, Berridge KC. Addiction. *Annu Rev Psychol* 54:25-53 (2003).
53. Schenk S *et al.* Development and expression of sensitization to cocaine's reinforcing properties: Role of NMDA receptors. *Psychopharmacology (Berl)* 111:332-338 (1993).

54. Segal DS, Kuczenski R. Repeated binge exposures to amphetamine and methamphetamine: behavioral and neurochemical characterization. *J Pharmacol Exp Ther* 282:561-573 (1997).
55. Selcher JC, Weeber EJ, Varga AW, Sweatt JD, Swank M. Protein kinase signal transduction cascades in mammalian associative conditioning. *Neuroscientist* 8:122-131 (2002).
56. Selvas A *et al.* Rat-strain dependent changes of dendritic and spine morphology in the hippocampus after cocaine self-administration. *Addict Biol* 22:78-92 (2017).
57. Sherer TB, Kim JH, Betarbet R, Greenamyre JT. Subcutaneous rotenone exposure causes highly selective dopaminergic degeneration and alpha-synuclein aggregation. *Exp Neurol* 179:9-16 (2003).
58. Sprague RL, Sleator EK. Methylphenidate in hyperkinetic children: differences in dose effects on learning and social behavior. *Science* 198:1274-1276 (1977).
59. Stefanski R, Ladenheim B, Lee SH, Cadet JL, Goldberg SR. Neuroadaptations in the dopaminergic system after active self-administration but not after passive administration of methamphetamine. *Eur J Pharmacol* 371:123-135 (1999).
60. Sulzer D, Sonders MS, Poulsen NW, Galli A. Mechanisms of neurotransmitter release by amphetamines: a review. *Prog Neurobiol* 75:406-433 (2005).
61. Vezina P. Sensitization of midbrain dopamine neuron reactivity and the self-administration of psychomotor stimulant drugs. *Neurosci Biobehav Rev* 27:827-839 (2004).
62. Voigt RM, Herrold AA, Napier TC. Baclofen facilitates the extinction of methamphetamine-induced conditioned place preference in rats. *Behav Neurosci* 125(2):261-7 (2011a).
63. Voigt RM, Herrold AA, Riddle JL, Napier TC. Administration of GABA(B) receptor positive allosteric modulators inhibit the expression of previously established methamphetamine-induced conditioned place preference. *Behav Brain Res* 216:419-423 (2011b).
64. Voigt RM, Mickiewicz AL, Napier TC. Repeated mirtazapine nullifies the maintenance of previously established methamphetamine-induced conditioned place preference in rats. *Behav Brain Res* 225:91-96 (2011c).
65. Voigt RM, Riddle JL, Napier TC. Effect of fendiline on the maintenance and expression of methamphetamine-induced conditioned place preference in Sprague-Dawley rats. *Psychopharmacology (Berl)* 231:2019-2029 (2014).
66. Wearne TA, Mirzaei M, Franklin JL, Goodchild AK, Haynes PA, Cornish JL. Methamphetamine-induced sensitization is associated with alterations to the proteome

of the prefrontal cortex: implications for the maintenance of psychotic disorders. *J Proteome Res* 14:397-410 (2015).

67. Wearne TA, Parker LM, Franklin JL, Goodchild AK, Cornish JL. GABAergic mRNA expression is upregulated in the prefrontal cortex of rats sensitized to methamphetamine. *Behav Brain Res* 297:224-230 (2016).
68. Zsurka G, Kunz WS. Mitochondrial dysfunction and seizures: the neuronal energy crisis. *Lancet Neurol* 14:956-966 (2015).