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Chronic wheel running reduces maladaptive patterns of methamphetamine intake: regulation by attenuation of methamphetamine-induced neuronal nitric oxide synthase

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Abstract

We investigated whether prior exposure to chronic wheel running (WR) alters maladaptive patterns of excessive and escalating methamphetamine intake under extended access conditions, and intravenous methamphetamine self-administration-induced neurotoxicity. Adult rats were given access to WR or no wheel (sedentary) in their home cage for 6 weeks. A set of WR rats were injected with 5-bromo-2'-deoxyuridine (BrdU) to determine WR-induced changes in proliferation (2-h old) and survival (28-day old) of hippocampal progenitors. Another set of WR rats were withdrawn (WRw) or continued (WRc) to have access to running wheels in their home

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cages during self-administration days. Following self-administration [6 h/day], rats were tested on the progressive ratio (PR) schedule. Following PR, BrdU was injected to determine levels of proliferating progenitors (2-h old). WRc rats self-administered significantly less methamphetamine than sedentary rats during acquisition and escalation sessions, and demonstrated reduced motivation for methamphetamine seeking. Methamphetamine reduced daily running activity of WRc rats compared with that of pre-methamphetamine days. WRw rats self-administered significantly more methamphetamine than sedentary rats during acquisition, an effect that was not observed during escalation and PR sessions. WR-induced beneficial effects on methamphetamine self-administration were not attributable to neuroplasticity effects in the hippocampus and medial prefrontal cortex, but were attributable to WR-induced inhibition of methamphetamine-induced increases in the number of neuronal nitric oxide synthase expressing neurons and apoptosis in the nucleus accumbens shell. Our results demonstrate that WR prevents methamphetamine-induced damage to forebrain neurons to provide a beneficial effect on drug-taking behavior. Importantly, WR-induced neuroprotective effects are transient and continued WR activity is necessary to prevent compulsive methamphetamine intake.

Keywords

Self-administration; Subgranular zone; Hippocampus; Medial prefrontal cortex; BrdU; Ki-67; nNOS; AC-3

Introduction

Successfully reducing risk behaviors such as substance abuse can potentially result in large public health gains (Ali et al. 2006; USPSTF 2008). The need for effective interventions for psychostimulant abuse and dependence in primary care settings are increasing, and clinical research supports the use of nondrug alternative reinforcers (money, vouchers) to reduce drug consumption (Higgins et al. 1994; Silverman et al. 1999; Hart et al. 2000; Petry et al. 2005; Gross et al. 2006). Preclinical research in animal models of drug self-administration and dependence has demonstrated that enriched environment with alternative nondrug reinforcers is a promising approach to reduce the initiation, maintenance, motivation and reinstatement of drug seeking. For example, enriching the animal's home cage with novel objects, social partners, and providing access to nondrug reinforcers (sweeteners, sugar) has reduced acquisition and maintenance of cocaine and amphetamines (Comer et al. 1994, 1996; Bardo et al. 2001; Klebaur et al. 2001).

Furthermore, use of physical activity as a nondrug reinforcer has been beneficial in clinical settings and preclinical studies. In humans, randomized controlled trials examining physical exercise to improve outcomes in smoking and alcohol cessation has provided evidence that physical activity can reduce reinforcing effects of nicotine and alcohol and lower relapse (Sinyor et al. 1982; Palmer et al. 1988; Marcus et al. 1991, 1999; Martin et al. 1997; Ussher et al. 2001; Sundblad et al. 2008). These findings also provide some of the most convincing support for investigating the use of exercise (and most frequently, vigorous intensity exercise) to improve outcomes in stimulant abuse (Collingwood et al. 1991, 1994). Preclinical findings demonstrate that voluntary wheel running (WR) in rodents performed prior to or concurrent with drug self-administration decreases self-administration of amphetamines and cocaine (Kanarek et al. 1995; Cosgrove et al. 2002; Miller et al. 2011; Smith and Pitts 2011), reduces positive reinforcing effects of cocaine (Smith et al. 2008), and prevents maladaptive patterns of excessive cocaine intake (Smith et al. 2011) compared with sedentary animals. Similarly, running experience before any cocaine experience or during forced abstinence also reduces cocaine seeking triggered by cocaine priming and cocaine cues (Lynch et al. 2010; Zlebnik et al. 2010; Smith et al. 2012) compared with

sedentary animals. These findings suggest that WR may attenuate some of the underlying behavioral manifestations that are responsible for maladaptive patterns of drug intake. In addition, WR may produce neuroadaptations in components of the extended reward system (e.g., striatum, medial prefrontal cortex, hippocampus) to provide beneficial effects on drug taking and seeking (Macias et al. 2007; Ahmad et al. 2009; Khabour et al. 2010).

The primary pharmacological activity of methamphetamine and amphetamine-like substances is the release of monoamine neurotransmitters (dopamine, serotonin and norepinephrine) from axon terminals in the brain reward regions by inhibiting/reversing monoamine reuptake transporters (Sulzer et al. 2005). Supporting this, accumulating evidence demonstrates that dopamine transporters play a major role in cellular mechanisms of methamphetamine-induced neurotoxicity. For example, recent causal and correlative studies support the role for dopamine transporters in binge methamphetamine-induced striatal neurotoxicity (Fumagalli et al. 1998; Numachi et al. 2007). Most notable is that disruption of striatal dopamine homeostasis by binge methamphetamine administration leads to striatal neurotoxicity expressed as increases in oxidative stress, apoptosis and neuroinflammatory responses (Thomas et al. 2004; Quinton and Yamamoto 2006). In support of these hypotheses, recent self-administration paradigms of methamphetamine intake (clinically relevant models of drug-taking behaviors) have reported that extended access to methamphetamine self-administration (>4 h of access per day) produces significant neurotoxic effects in the striatum [persistent decreases in levels of dopamine and tyrosine hydroxylase; decreases in levels and activity of dopamine transporter and vesicular monoamine transporter-2, and increases in astrogliosis (Schwendt et al. 2009; Krasnova et al. 2010; McFadden et al. 2011, 2012)]. These studies suggest that progressive increases in the intake of methamphetamine over extended access schedules of reinforcement lead to persistent neuroadaptive changes in the extended reward system.

Particularly interesting are accumulating reports on WR-induced inhibition of neuroadaptations produced by neurotoxic regimens of cocaine and methamphetamine (Lynch et al. 2010; O'Dell et al. 2012). For example, the experience of WR before and after a neurotoxic binge regimen of methamphetamine ameliorated methamphetamine-induced reduction in dopamine and serotonin transporters (O'Dell et al. 2012). Because the study did not assess distinct effects of prior WR and WR post-methamphetamine experience on methamphetamine neurotoxicity, we can hypothesize that prior WR experience before methamphetamine exposure produced a neuroprotective effect, and WR experienced after methamphetamine exposure enhanced compensatory changes to ameliorate methamphetamine-induced brain injury. In the context of the above hypothesis, the present study evaluated whether chronic WR before extended access to methamphetamine (that produces escalation and maladaptive patterns of methamphetamine self-administration) produces neuroprotective effects in the components of the brain reward system. We particularly focused on neuronal nitric oxide synthase expressing neurons, as reactive nitrogen species (nitric oxide and peroxynitrites) and nitric oxide synthesis in the striatum contribute to binge methamphetamine-induced striatal neurotoxicity (Itzhak 1997; Itzhak et al. 1998, 1999, 2000, 2004; Yamamoto and Bankson 2005; Eyerman and Yamamoto 2007; Wang et al. 2008; Zhu et al. 2009; Wang and Angulo 2011b). In addition, the present study also determined whether the behavioral and neuroprotective effects of WR are long lasting and whether continued WR is required to reduce maladaptive patterns of methamphetamine self-administration and motivation to seek methamphetamine.

Materials and methods

Animals

Surgical and experimental procedures were carried out in strict adherence to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication number 85–23, revised 1996) and approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute. Fifty-eight adult, male Wistar rats (Charles River), weighing 200–250 g at the start of the experiment, were housed two per cage in a temperature-controlled vivarium under a reverse light/dark cycle (lights off 10.00 am to 10.00 pm) for at least 1 week. After the first week, all rats were single housed for the remaining period until euthanasia. Food and water were available *ad libitum*. Thirty-three rats were separated and single housed in cages equipped with running wheels (Nalgene activity wheels 34.5-cm diameter $\times$ 9.7-cm wide with magnetic switches connected to a PC). Daily running activity was monitored and wheel revolutions were collected in 10 min bins with VitalView Software (Minimitter Inc.). Six rats received one intraperitoneal injection of 5-bromo-2'-deoxyuridine (BrdU, dissolved in 0.9 % saline and 0.007 N NaOH at 20 mg/ml; 150 mg/kg, *i.p.*) 2 weeks into their 6-week running activity to label neural progenitors in the S phase of the cell cycle and were killed 4 weeks later. Twelve additional rats experienced 6 weeks of running activity; 6 rats were killed 2 h after BrdU injection, and 6 rats were withdrawn from WR for 8 days and were killed 2 h after BrdU injection (*i.p.*) (Fig. 1a). Two groups of sedentary rats were injected with BrdU (*i.p.*) and were killed 2 h later or 4 weeks later. These rats were 14- to 15-week old at the end of the experiment.

Fifteen rats experienced WR for 6 weeks and underwent surgery for catheter implantation for intravenous methamphetamine self-administration (Mandyam et al. 2007). Thirteen rats housed under sedentary conditions (no wheel, sedentary) also underwent surgery at the same time. For surgery, rats were anesthetized with 2–3 % of isoflurane mixed in oxygen. They were implanted with a silastic catheter (0.3 $\times$ 0.64 mm OD; Dow Corning Co.) into the right external jugular vein under aseptic conditions. The distal end of the catheter was s.c. threaded over the shoulder of the rat where it exited the rat via a metal guide cannule (22G, Plastics One Inc.) that was anchored onto the back of the rat. After surgery, rats were given an analgesic (Flunixin, 2.5 mg/kg, *s.c.*). Antibiotic (Timentins, 20 mg, *i.v.*; SmithKline Beecham) was administered daily to the rats for at least 5 days. To extend catheter patency, the catheters were flushed once daily with 0.1 ml of an antibiotic solution of cefazolin (10.0 mg/mL; SavMart Pharmaceuticals) dissolved in heparinized saline (70 U/mL; Baxter Health Care Corp) before each self-administration session and with 0.1 ml of heparinized saline (70 U/mL) after each session. The patency of catheters in the rats was tested using the ultra short-acting barbiturate Brevital (methohexital sodium, 10 mg/ml, 2 mg/rat) whenever a catheter failure was suspected during the study.

WR rats after surgery were allowed to recover in cages without running wheels for 4 days. Following recovery, six WR rats continued to have access to running wheels in their home cage (WRc). Nine WR rats were withdrawn from running activity (WRw). Eight days after surgery, rats ($n = 6$, WRc; $n = 9$, WRw; $n = 9$, sedentary) were trained to press a lever according to an FR 1 schedule of methamphetamine reinforcement (0.05 mg/kg/injection of methamphetamine hydrochloride, generously provided by the national institute on drug abuse) in operant boxes (Med Associates) under baseline (acquisition) conditions (1 h access per day for 10 days). All animals were housed on a reverse cycle (lights off at 10 am) and were transferred from their home cages (with or without access to running wheels) to their operant chambers (no access to running wheels) between 9 and 10 am (Fig. 3c). WRc animals were not presented with a running wheel and methamphetamine concurrently, and access to methamphetamine occurred during a period when running activity was minimal

(Figs. 1b, 3c). The sessions for the 1-h schedule occurred between 9 am and 11 am (Fig. 3c; 1 h) and the sessions for the 6-h schedule occurred between 9 am and 4 pm (Fig. 3c; 6 h).

Training on the first and second day was initiated with two–three priming (noncontingent) infusions of methamphetamine during the first 10 min. Rats were allowed to respond for the remaining 50 min. Acquiring methamphetamine self-administration was defined as maintenance of similar number of infusions over three consecutive days during baseline training sessions. After baseline (acquisition) training, the rats were subjected to long access schedule of methamphetamine reinforcement (LgA, 6 h per day under an FR1 schedule). Methamphetamine self-administration was performed 5 days per week and all rats experienced 22 LgA sessions. After LgA sessions, responding was reinforced on a progressive ratio (PR) schedule of reinforcement (Richardson and Roberts 1996). On this schedule, the number of responses required for reinforcement incremented progressively, and each session continued until a breakpoint (defined as the number of infusions obtained before 1 h elapsed with no infusions) was reached. Breakpoints were determined for saline, and 0.025, 0.05, 0.75 and 0.1 mg/kg/infusion methamphetamine. Sixteen hours after the last PR session, rats from WRc, WRw, sedentary and drug naïve ($n = 4$) groups received one injection of a saturating dose of 20 mg/kg BrdU (i.v.), and survived for 2 h. These rats were 23- to 24-week old at the end of the experiment.

After the last experimental session, rats with catheters were fully anaesthetized using chloral hydrate (14 mg/kg, i.v.), while rats without catheters were anesthetized using chloral hydrate (240 mg/kg, i.p.). Rats were then transcardially perfused with phosphate-buffered saline (over 2 min at 15 ml/min and 4 % paraformaldehyde (over 20 min at 15 ml/min). The brains were dissected out and postfixed in 4 % paraformaldehyde at 4 °C for 16–20 h and sectioned in the coronal plane at a thickness of 40 μ m on a freezing microtome. The sections through the brain were collected in nine vials [containing 0.1 % NaN₃ in 1X phosphate-buffered saline (PBS)] and stored at 4 °C. One-ninth of the brain region was used for immunohistochemical analysis.

Antibodies, immunohistochemistry, microscopic analysis, and quantification

The following primary antibodies were used for immunohistochemistry (IHC): Ki-67 [1:1000, catalog # RM-9106-S, Thermo Scientific (Mandyam et al. 2008)], BrdU [1:400, catalog # MCA2060, Serotec (Mandyam et al. 2008)], activated caspase-3 [AC-3; 1:500, catalog # 9664, Cell Signaling (Mandyam et al. 2008)], c-Fos [1:1000, catalog # sc-52, Santa Cruz Biotechnology (Recinto et al. 2012)] and neuronal nitric oxide synthase [nNOS; 1:2000, catalog # sc-55521, Santa Cruz Biotechnology (Wang and Angulo 2011b)]. The left and right hemispheres of every ninth (BrdU, Ki-67, AC-3, Fos) section through the rat medial prefrontal cortex or hippocampus were slide-mounted, coded, and dried overnight prior to IHC. The sections were pretreated (Mandyam et al. 2004), blocked, and incubated with the primary antibodies followed by biotin-tagged (BrdU, Ki-67, Fos, nNOS and AC-3) secondary antibodies. Immunoreactive cells in the subgranular zone (SGZ; i.e., cells that touched and were within three cell widths inside and outside the hippocampal granule cell-hilus border for 2 h BrdU) or granule cell layer (GCL; 28 day BrdU, AC-3) were visually quantified with a Zeiss primostar photomicroscope (600 $\times$ magnification) and absolute cell counting rather than unbiased stereological estimates in sections through the dentate gyrus [–1.4 to –6.7 mm from bregma; (Paxinos and Watson 1997)] were quantified (Noori and Fornal 2011). Cells in the SGZ and GCL were summed and multiplied by 9 to give the total number of cells (Eisch et al. 2000).

Ki-67 labeled cells in the medial prefrontal cortex (mPFC; 3.7–2.2 mm from bregma), Fos-labeled cells in the granule cell layer of the dentate gyrus (–1.4 to –6.7 mm from bregma), AC-3 labeled cells in the nucleus accumbens shell (2.7 mm from bregma) and nNOS labeled

cells in the orbital cortex (2.7 mm from bregma), medial prefrontal cortex (2.7 mm from bregma), nucleus accumbens shell (2.7 mm from bregma), nucleus accumbens core (2.2 mm from bregma), medial septal nucleus (−0.26 mm from bregma), bed nucleus of the stria terminalis-dorsal, bed nucleus of the stria terminalis-ventral (−0.26 mm from bregma), caudate putamen (striatum) (−0.26 mm from bregma), amygdala (−2.3 mm from bregma), dorsal dentate gyrus (−2.3 mm from bregma), periaqueductal gray (−6.04 mm from bregma), ventral dentate gyrus (−6.04 mm from bregma), ventral tegmental area [−6.04 mm from bregma Paxinos and Watson 1997]) were examined with a Zeiss Axiophot Microscope equipped with Micro-BrightField Stereo Investigator software, a three-axis Mac 5000 motorized stage, a Zeiss digital charge-coupled device ZVS video camera, PCI color frame grabber, and computer workstation. Live video images were used to draw contours delineating the brain regions analyzed. The fields of the brain regions for quantification were traced separately at 50× magnification. A 150 × 150 μm frame was randomly placed over the regions of interest using the Stereo Investigator stereology platform. The frame was moved randomly over the tissue to cover the entire contoured area and labeled cells in each region falling entirely within the borders of the contour were marked and analyzed. Immunoreactive cells were quantified bilaterally and were summed up for each brain region.

Data analysis

WR data are expressed as revolutions per day or per hour and miles per week [calculated by multiplying the number of revolutions by the radius of the wheel in kilometers and by 0.62 for conversion to miles]. The effect of weeks or hours during the day was examined over the 6–15 weeks period or 24 h period using a two-way repeated measures analysis of variance (ANOVA; duration × day) followed by the Student–Newman–Keuls post hoc test. The methamphetamine self-administration data are expressed as the mean mg/kg per session of methamphetamine self-administration for each group of rats. The effect of session duration on methamphetamine self-administration per session and during the first hour of a session was examined over the 22 escalation sessions using a two-way repeated measures ANOVA (session duration × daily session) followed by the Student–Newman–Keuls post hoc test. The pattern of responding for methamphetamine is expressed as the mean mg/kg per hour over 6-h sessions in LgA rats and were compared between the first and >10th escalation sessions. Differences in the rate of responding between the first and other escalation sessions were evaluated using the paired *t* test. For the effect of WR on methamphetamine self-administration and PR responding, the three groups (sedentary LgA, WRc-LgA and WRw-LgA) were used as between-subjects factors, and differences were assessed by ANOVA. For the BrdU, Ki-67, AC-3, Fos and nNOS analyses, one-way ANOVA or unpaired Students *t* test was used. The data are expressed as mean ± SEM in all graphs. Pearson's correlations were used to examine the relationship between wheel running and methamphetamine self-administration (methamphetamine consumed during acquisition and escalation).

Results

Effects of continued access to WR and withdrawal from WR on proliferation of neural progenitors and neuronal activation of granule cell neurons in the dentate gyrus of the hippocampus

Rats were housed in home cages without running wheels (sedentary) or with running wheels (WR) for 6 weeks and were killed immediately after running activity (WRc) or after 8 days of cessation of running activity (WRw) (Fig. 1a). BrdU was injected at two time points in two different cohorts of WRc group to estimate the levels of cell proliferation (2-h-old BrdU cells) and cell survival (28-day-old BrdU cells) altered by running activity (Fig. 1a). BrdU was injected after 8 days of withdrawal from running activity and rats were killed 2 h later. Running activity escalated during initial weeks of activity, and was maintained thereafter

(effect of days, $F_{5,161} = 30.47$, $p < 0.001$). Post hoc analyses indicate a significant increase in running output between weeks 1 and 2 ($p < 0.001$), and weeks 2 and 3 ($p < 0.01$). No significant differences were seen between weeks 3 and 6 (Fig. 1c). Microanalysis of running activity demonstrates a circadian effect on running output with almost all activities occurring during the dark cycle (Fig. 1b). Mathematical analysis of wheel-running data demonstrated that during the dark cycle, Wistar rats perform 97.6 % of their running activity. During the light cycle, Wistar rats perform 2.4 % of the running activity; most of the activity is seen immediately following the dark phase (Fig. 1b). Running output increased during the dark phase (effect of time of day, $F_{23,503} = 10.1$, $p < 0.001$), with maximal activity occurring 5–10 h into the dark phase ($p < 0.01$ vs. light phase).

Running activity increased the number of 2-h- and 28-day-old BrdU cells in the dentate gyrus compared with sedentary conditions (Fig. 1d; p 's < 0.05). Eight days of withdrawal from running activity normalized the levels of 2-h-old BrdU cells (Fig. 1e; $p < 0.05$ vs. WR animals). Running activity produced significant activation of granule cell neurons in the dentate gyrus (Fig. 1f; $F_{2,24} = 11.6$; $p < 0.001$). Post hoc analysis indicates a significant increase in neuronal activation in runners compared with sedentary rats and wheel-withdrawn rats ($p < 0.01$), and a reduction in neuronal activation in wheel-withdrawn rats compared with runners and sedentary controls ($p < 0.05$).

Effects of prior and continued access to running wheels and cessation of chronic running on responding for methamphetamine infusions

Following chronic WR or sedentary conditions, all rats underwent surgery for intravenous catheters and were tested for operant responding for intravenous methamphetamine self-administration (Fig. 2a). Sedentary, WRc and WRw rats responded on the first day of self-administration, and stable response rates were observed by the third session. Repeated measures ANOVA detected a significant increase in the number of active lever presses over acquisition days ($F_{9,89} = 3.09$, $p = 0.002$) in sedentary rats but not WRc and WRw rats. Repeated measures two-way ANOVA detected a significant increase in the number of active lever presses compared with inactive lever presses over acquisition days in sedentary rats ($F_{9,89} = 12.63$, $p = 0.002$), WRc rats ($F_{9,50} = 8.01$, $p = 0.01$) and WRw rats ($F_{9,89} = 11.03$, $p = 0.004$) (Fig. 2b). Repeated measures two-way ANOVA did not detect an interaction ($F_{18,180} = 0.8$, $p = 0.69$), but revealed a significant main effect of wheel running ($F_{2,180} = 4.7$, $p = 0.02$; Fig. 2c). Post hoc analysis indicates a higher intake of methamphetamine in WRw rats compared with sedentary and WRc rats ($p < 0.05$), and higher intake in sedentary rats compared with WRc rats ($p < 0.05$).

Effects of continued access to running wheels on escalation of methamphetamine self-administration under extended access conditions

Repeated measures two-way ANOVA for daily 6 h self-administration revealed a significant interaction ($F_{42,357} = 1.4$, $p = 0.05$), a significant effect of session ($F_{21,357} = 14.8$, $p = 0.001$) and a strong trend for the effect of running activity on daily methamphetamine intake ($F_{2,357} = 2.99$, $p = 0.07$). Repeated measures two-way ANOVA for the first-hour self-administration revealed no interaction ($F_{42,357} = 1.1$, $p = 0.26$), a significant effect of session ($F_{21,357} = 6.7$, $p = 0.001$) and no effect of running activity on the first-hour methamphetamine intake ($F_{2,357} = 1.9$, $p = 0.17$).

Daily 6 h methamphetamine intake in sedentary LgA rats significantly increased over self-administration days, and escalation in daily methamphetamine intake was evident after ten sessions of self-administration (Fig. 2d; effect of days of self-administration: $F_{21,197} = 5.9$, $p < 0.001$). Post hoc analysis revealed an escalation in methamphetamine intake in sedentary LgA rats during sessions 10–22 compared with earlier sessions (all $p < 0.05$).

Methamphetamine intake during the first hour of the 6-h session in sedentary LgA rats significantly increased over self-administration days, and escalation in methamphetamine intake was evident after nine sessions of self-administration (Fig. 2e; $F_{21,197} = 6.8$, $p < 0.001$).

Daily 6 h methamphetamine intake in WRw rats significantly increased over self-administration days, and escalation in daily methamphetamine intake was evident after 16 sessions of self-administration (Fig. 2d; $F_{21,197} = 11.07$, $p < 0.001$). Post hoc analysis revealed an escalation in methamphetamine intake in WRw LgA rats during sessions 16–22 compared with the earlier sessions ($p < 0.05$). Methamphetamine intake during the first hour of the 6-h session in WRw LgA rats significantly increased over self-administration days, and escalation in methamphetamine intake was evident after nine sessions of self-administration (Fig. 2e; $F_{21,197} = 2.9$, $p < 0.01$).

Daily 6 h methamphetamine intake in WRc rats significantly increased over self-administration days (Fig. 2d; $F_{21,131} = 1.7$, $p = 0.03$). However, post hoc analysis did not detect differences in daily methamphetamine intake in WRc LgA rats over 22 sessions. Methamphetamine intake during the first hour of the 6-h session in WRc LgA rats did not increase over self-administration days (Fig. 2e; $F_{21,131} = 1.09$, $p = 0.36$).

Effects of prior and continued access to running wheels on the motivational effects of methamphetamine

Following 22 sessions of extended access methamphetamine self-administration, sedentary, WRc and WRw rats were tested on the PR schedule. Repeated measures two-way ANOVA did not detect an interaction ($F_{6,57} = 0.63$, $p = 0.69$), did not show dose-dependent changes in break-points ($F_{3,57} = 1.2$, $p = 0.31$), but revealed a significant effect of running ($F_{2,57} = 6.3$, $p = 0.007$; Fig. 2f). Post hoc analysis indicates a higher intake of methamphetamine in WRw rats compared with sedentary and WRc rats ($p < 0.05$), and higher intake in sedentary rats compared with WRc rats ($p < 0.05$).

Relationship between running output and amount of methamphetamine consumed

Pearson's correlations revealed a significant positive relationship between wheel running (measured as revolutions per day after surgery) and methamphetamine self-administration during acquisition (measured as amount of methamphetamine consumed during the 10-day period; Fig. 3a, $R^2 = 0.80$, $p = 0.014$) only in WRc rats. Similarly, wheel running was also predictive of enhanced methamphetamine intake (escalation) during the first-hour access of the 6-h sessions (Fig. 3a, $R^2 = 0.94$, $p = 0.001$). Notably, wheel running was not predictive of total methamphetamine intake during the entire 6-h session (extended access; $R^2 = 0.5$, $p = 0.1$; data not shown). Wheel running in WRw rats did not correlate with methamphetamine intake during acquisition or escalation sessions.

Effects of methamphetamine self-administration on running output

Rats in the WRc group increased their running activity during the 6 weeks and an escalation in running output was evident at a rate similar to all other running groups (effect of days, $F_{13,83} = 9.1$, $p < 0.001$). Running output decreased sharply after the first and following each methamphetamine baseline session, and this effect was maintained until the end of the study, such that running activity did not return to pre-surgery levels (Fig. 3b; $p < 0.05$ vs. pre-methamphetamine weeks). Microanalysis of running activity demonstrates an overall reduction in running activity during the dark cycle without altering the circadian effect on running output (Fig. 3c).

Effects of prior and continued access to wheel running and methamphetamine on levels in progenitors in the dentate gyrus and the mPFC

To understand the underlying mechanism for the effect of wheel running on reduced methamphetamine self-administration, markers of cell proliferation (BrdU, Ki-67) were examined in the SGZ of the dentate gyrus and the mPFC (Fig. 4a–c). Consistent with previous findings, extended access methamphetamine self-administration in sedentary rats reduced the levels of newly born progenitors in the SGZ (Fig. 4d; $F_{3,29} = 18.5$, $p < 0.001$) and mPFC (Fig. 4f; $F_{3,27} = 3.9$, $p = 0.02$) compared with drug-naïve controls (Teuchert-Noodt and Dawirs 1991; Hildebrandt et al. 1999; Teuchert-Noodt et al. 2000; Mandyam et al. 2007, 2008; Yuan et al. 2011; Recinto et al. 2012). Surprisingly, extended access methamphetamine self-administration in WRc and WRw rats reduced the levels of newly born progenitors in the SGZ to a greater extent compared with sedentary methamphetamine rats ($p < 0.01$). The greater reduction in progenitors in the SGZ in WRc and WRw rats is perhaps attributable to enhanced apoptosis as both these groups demonstrated a strong trend toward enhanced levels of AC-3 labeled cells in the granule cell layer of the dentate gyrus (Fig. 4e; $F_{3,32} = 2.7$, $p = 0.060$). The enhanced reduction in newly born progenitors in WRc and WRw rats was not evident in the mPFC compared with sedentary methamphetamine rats (Fig. 4f), suggesting that the effect was specific to the progenitors in the SGZ.

Effects of prior and continued access to wheel running and methamphetamine on the levels of nNOS expressing neurons and activated caspase-3 cells in the nucleus accumbens shell

The number of nNOS expressing neurons and AC-3 cells was quantified in various regions of the brain including the striatal regions (Fig. 5a–d; Table 1) to determine an underlying neuroprotective mechanism for WR-induced reduction in methamphetamine self-administration. Sedentary methamphetamine rats demonstrated greatest number of nNOS expressing neurons in the nucleus accumbens shell region (Fig. 5e; $F_{5,38} = 9.7$, $p < 0.0001$). Post hoc analysis revealed a significantly higher number of nNOS expressing neurons in sedentary methamphetamine rats compared with drug-naïve controls, methamphetamine naïve WRc and WRw rats, and methamphetamine WRc and WRw rats ($p < 0.05$). Methamphetamine did not alter the number of nNOS expressing neurons in the nucleus accumbens core region (Fig. 5d; $F_{5,38} = 1.5$, $p = 0.18$), and several other regions in the brain (Table 1). WRc and WRw rats demonstrated significantly lower levels of nNOS expressing neurons in the ventral tegmental area compared with sedentary methamphetamine rats (Table 1; $p < 0.05$). Sedentary methamphetamine rats also showed significantly higher levels of AC-3 cells compared to WRc rats in the nucleus accumbens shell region ($p = 0.04$, unpaired t test), and this effect was not seen with WRw rats (Fig. 6).

Discussion

The present study demonstrates that chronic continued running activity decreases acquisition of methamphetamine self-administration, reduces maladaptive patterns of escalation in methamphetamine intake under extended access conditions and diminishes the positive reinforcing effects of methamphetamine compared with sedentary controls. Notably, our findings demonstrate that the effects of chronic running activity under the conditions of the present study are limited to active running. For example, cessation of chronic running activity before methamphetamine access resulted in enhanced rate of acquisition of methamphetamine self-administration. Furthermore, cessation of chronic running activity before methamphetamine access failed to produce protective effects on escalating patterns of methamphetamine intake, and did not reduce the positive reinforcing effects of methamphetamine. Most importantly, the present findings provide concurrent evidence that prior and continued running activity exerts protection against methamphetamine addiction

by ameliorating methamphetamine-induced increases in the levels of neuronal nitric oxide synthase and apoptosis with significant effects in the nucleus accumbens shell. Our findings provide novel evidence that prior and continued access to wheel activity protects against striatal methamphetamine toxicity. These results add to a growing body of preclinical literature supporting a promising role for physical activity in reducing drug self-administration, drug reward and drug-induced neuroadaptations in the extended reward regions (Cosgrove et al. 2002; Smith et al. 2008, 2011, 2012; El Rawas et al. 2009; Hosseini et al. 2009; Zlebnik et al. 2010; Miller et al. 2011; Mustroph et al. 2011; Smith and Pitts 2011; O'Dell et al. 2012).

Accumulating recent evidence from clinically relevant models of drug self-administration and drug dependence undisputedly demonstrate that wheel running can be used as an alternative reinforcer to reduce drug self-administration and reinstatement of drug seeking (Cosgrove et al. 2002; Smith et al. 2008, 2011, 2012; Lynch et al. 2010; Zlebnik et al. 2010; Miller et al. 2011; Smith and Pitts 2011). The present results are consistent with a previous study that examined wheel access and extended access cocaine self-administration (Smith et al. 2011), but significantly extend the findings with methamphetamine, a mechanistically distinct, more potent and toxic-stimulant drug of abuse. Importantly, the generality of the effect of prior and continued wheel activity during self-administration was confirmed on the acquisition of self-administration, escalating compulsive-like patterns of self-administration and motivation for methamphetamine (Smith et al. 2008, 2011; Smith and Pitts 2011). These results indicate that running activity in rats is an operant response, can act as a nondrug reinforcing agent, and competed with the reinforcing efficacy of methamphetamine, thereby decreasing the positive reinforcing effects of methamphetamine (Kanarek et al. 1995; Miller et al. 2011). However, it is important to note that the access to a running wheel is a form of environmental enrichment, and the reduced methamphetamine intake may be due to an enrichment-related effect. Environmental enrichment has been shown to influence the positive reinforcing effects of cocaine (Smith et al. 2009), and its protective effects on measures of amphetamine- and cocaine-seeking behavior have been well documented (Bardo et al. 2001; Stairs et al. 2006; Thiel et al. 2010, 2011). Although traditional models of environmental enrichment include multiple enriching stimuli (e.g., access to running wheels or climbing devices, novel objects or toys, and same-sex cagemates), the present data suggest that access to an unlocked running wheel in the absence of other enriching stimuli is sufficient for decreasing methamphetamine-seeking behavior in acquisition and extended access procedures. Alternately, other effects of chronic wheel running such as alterations in the metabolism of methamphetamine, dynorphin levels (Werme et al. 2000), dopamine neuron activity (Ahmad et al. 2009), delta FosB levels (Werme et al. 2002) and brain-derived neurotrophic factor levels (Macias et al. 2007; Khabour et al. 2010) in reward-associated brain regions could have contributed to reduced methamphetamine-seeking behavior in acquisition and extended access procedures.

Recent studies also show that there is an inverse relation between nondrug reinforcers and drug use. For example, in preclinical models, deprivation of nondrug reinforcers (sweetened solution) increases the likelihood of cocaine use (Carroll and Boe, 1982). Furthermore, in clinical studies, cessation or restriction from physical activity produces maladaptive traits, such as enhanced drug use, mood disturbances and psychological distress (Chan and Grossman 1988; Modin et al. 1996; Pate et al. 2000; Lejoyeux et al. 2008). Thus, it appears that continued exercise programs or maintenance of rigorous physical activity may protect individuals from drug use and prevent psychological effects associated with exercise cessation. The present preclinical results demonstrate that cessation or withdrawal from chronic wheel activity enhances methamphetamine self-administration, and that the enhanced methamphetamine consumption during exercise withdrawal is not relative to the amount of exercise output achieved before the initiation of methamphetamine experience.

The increase in acquisition in methamphetamine self-administration was evident in WRw rats from the first day of methamphetamine access and was maintained during the limited-access sessions. The increase in methamphetamine intake after cessation of chronic wheel activity could be attributed to exercise deprivation effect as seen with deprivation of other substances such as food and sweetened solutions (Katz et al. 1978; Carroll et al. 1981; Carroll and Boe 1982). In addition, increased methamphetamine self-administration in WRw rats could be attributed to the negative affect symptoms associated with the withdrawal from chronic running activity (Kanarek et al. 2009) and the associated negative neuroadaptations as shown in the current study. However, the increased self-administration in WRw rats was not evident during the extended access sessions, suggesting that the deprivation effects were transient. Therefore, it appears that concurrent access or continued access to wheel activity is more important than prior access to wheel activity to reduce maladaptive patterns of methamphetamine intake.

Linear regression analysis indicated that exercise output predicts the enhanced methamphetamine consumption during initial methamphetamine experience and during the first-hour access of the extended access schedule in the WRc group. This suggests that the physical activity-induced synthesis and release of dopamine in the basal ganglia (Meeusen et al. 1997) and the resulting alterations in the dopaminergic mesocorticolimbic system may lead to alterations in the reinforcing value of methamphetamine. Furthermore, differences in plasma concentrations of methamphetamine in WRc animals compared to sedentary controls could have contributed to the effects of methamphetamine on exercise output (Han et al. 1996). Importantly, and consistent with previous reports (Kanarek et al. 1995; Serwatkiwicz et al. 2000; Miller et al. 2011), methamphetamine intake significantly reduced running activity compared with pre-methamphetamine levels (decreased running output by 60 %), and these effects were evident after the initial days of acquisition with limited-access self-administration and remained consistent until the end of behavioral testing. This is important since a gradual escalation in the amount of running activity was seen in all the rats prior to any methamphetamine exposure; that escalation in running output may be due to a shift in the reinforcing value of wheel activity over extended access paradigms (Lattanzio and Eikelboom 2003; Ferreira et al. 2006; Kanarek et al. 2009). It is unlikely that the reductions in running activity during methamphetamine acquisition were entirely due to stress associated with surgery per se, as surgery did not significantly reduce exercise output compared with prior surgery levels. It is also unlikely that the reductions in running activity during acquisition can be attributed to the fact that rats were prevented from running during self-administration sessions as microanalysis studies revealed that although over 97 % of wheel running occurred during the dark phase of the light/dark cycle, less than 1 % occurred during the first 3 h of the dark phase of the cycle (Eikelboom and Mills 1988; Klante et al. 1999; Ferguson and Cada 2003; Johnson and Mitchell 2003), the period of time in which the self-administration sessions for acquisition were conducted. Therefore, a reduction in running output post methamphetamine acquisition could be due to reduced reinforcing value of the wheel relative to its reinforcing value prior methamphetamine experience (Serwatkiwicz et al. 2000). However, reduction in running output post methamphetamine escalation could be due to forced abstinence from wheel during the hours of escalation sessions and the impact of having access to one reinforcer (methamphetamine) during the period when the second reinforcer (running wheel) was normally available.

Studies over the last four decades have revealed that the adult brain possesses the capacity for spontaneous neurogenesis; and that newly born neurons in the hippocampus assist with maintaining activity, function and behavior dependent on the hippocampus (Aimone et al. 2006; Lacefield et al. 2012). Most notably, running activity and methamphetamine self-administration have opposing effects on the levels of newly born neurons in the hippocampal dentate gyrus and newly born glia in the mPFC (van Praag et al. 1999;

Mandyam et al. 2007, 2008); brain regions implicated in drug reward and relapse to drug seeking (Mandyam and Koob 2012). Therefore, we hypothesized that the reduced methamphetamine taking and reduced maladaptive patterns of methamphetamine seeking after prior and continued chronic running activity could be caused by an inhibition or prevention of methamphetamine-induced neuroplastic events in the dentate gyrus and mPFC. Our findings failed to detect any protective effects of running activity on newly born dentate gyrus and mPFC progenitors in WRw and WRc methamphetamine rats, suggesting that running activity prevented other measures of toxicity produced by methamphetamine. However, it was surprising to see that the progenitors in the dentate gyrus in WRw and WRc methamphetamine rats were reduced to a greater extent compared with sedentary methamphetamine rats. This effect could be attributed to increased neuroinflammatory responses in the brain and the periphery (e.g., interleukin-6 levels) after chronic wheel running experience (Colbert et al. 2001; Funk et al. 2011), which could negatively affect the levels of progenitors in the hippocampus (Vallieres et al. 2002; Monje et al. 2003; Hoehn et al. 2005).

Methamphetamine's neurotoxicity is mainly attributed to its long-term neuroadaptations in the striatum (Eisch et al. 1992; Fumagalli et al. 1998; Xu et al. 2005; Numachi et al. 2007; Wang and Angulo 2011a, b). Converging lines of evidence from preclinical and clinical studies have revealed that high doses of binge methamphetamine exposure increases extracellular concentrations of dopamine (Sulzer and Rayport 1990), and causes selective degeneration of dopaminergic terminals in the striatum (Hotchkiss and Gibb 1980; Ricaurte et al. 1982). Binge methamphetamine administration increases extracellular dopamine levels and produces striatal neurotoxicity by overproduction of toxic metabolites of dopamine oxidation (Wrona et al. 1997), which result in oxidative stress and damage (Yamamoto and Zhu 1998). The protracted surplus of dopamine induced by binge methamphetamine also promotes several other cascades of events that contribute to neurotoxicity: e.g., excess glutamate release (Nash and Yamamoto 1992), nitric oxide production (Taraska and Finnegan 1997; Zhu et al. 2009) and microglial activation (LaVoie et al. 2004; Thomas et al. 2004). Particularly interesting is the role of reactive nitrogen species in methamphetamine toxicity (Itzhak 1997; Itzhak et al. 1998; Anderson and Itzhak 2006; Wang et al. 2008), and involvement of striatal neurokinin-1 receptor-expressing interneurons in binge methamphetamine-induced nitric oxide activation and apoptosis. These studies suggest an important role of striatal nitric oxide synthesis in methamphetamine-induced apoptosis (Zhu et al. 2009). Furthermore, neuropeptides such as substance P are associated with neurokinin-1 receptor-induced striatal nitric oxide synthesis and apoptosis, perhaps suggesting functional role of striatal neuropeptides in binge methamphetamine-induced striatal neurotoxicity (Wang and Angulo 2011a, b). Altogether, the neurotoxic cascade activated by nitric oxide has been implicated in methamphetamine-induced neurotoxicity and neurodegeneration in the striatum, supporting a mechanistic role of neuronal nitric oxide synthesis in binge methamphetamine toxicity (Itzhak 1997; Itzhak et al. 1998; Anderson and Itzhak, 2006; Wang et al. 2008). These studies suggest that methamphetamine-induced neuroadaptations in the striatum contribute to maladaptive patterns of compulsive methamphetamine seeking demonstrated in extended access self-administration models, and these neurotoxic effects in the striatum by methamphetamine may perpetuate dependence-like behavior (Schwendt et al. 2009; Krasnova et al. 2010; McFadden et al. 2011, 2012).

In the context of the above hypothesis, the dorsal striatum and nucleus accumbens have been implicated in the long-term maintenance of compulsive drug taking (Everitt et al. 2008; Wise 2009). Therefore, interventions such as physical activity may prevent or dysregulate methamphetamine-induced neurotoxicity in the striatum to inhibit maladaptive patterns of methamphetamine seeking observed in extended access models of methamphetamine self-administration. Given the recent evidence that prior running activity ameliorates binge

methamphetamine-induced depletion of monoamine transporters in the striatal regions (O'Dell et al. 2012), we hypothesized that prior running activity exerted neuroprotection by reducing other forms of methamphetamine self-administration-induced neurotoxicity in the striatum, specifically by inhibiting neuronal nitric oxide synthesis. Our findings clearly indicate that prior and continued running activity prevented maladaptive patterns of methamphetamine self-administration-induced enhancement of neuronal nitric oxide synthase expressing neurons and apoptosis, and these effects were most significant in the nucleus accumbens shell region of the striatum and were not observed in various other regions analyzed. Because rewarding properties of amphetamines can be prevented by inhibiting the neuronal adaptations occurring in the nucleus accumbens shell region (Robbins et al. 1983; Velazquez-Sanchez et al. 2011), these results also suggest that prior and continued running activity could have wider therapeutic applications in stimulant-spectrum disorders than those previously recognized.

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Fig. 1.

Wheel running increases proliferation and survival of newly born progenitors in the dentate gyrus of the hippocampus, and increases neuronal activation of hippocampal granule cell neurons in the dentate gyrus. **a** Schematic of experimental procedure and BrdU injections during a 6-week chronic running activity paradigm; BrdU injections are indicated by a syringe. **b** Total number of revolutions per hour during week 6 during a 24-h period. **c** Total number of revolutions per week (left y-axis); total miles run per week (right y-axis) during the 6 week running period. **d** Quantitative analysis of the number of BrdU labeled cells in the subgranular zone (2-h BrdU, left y-axis) or granule cell layer (28-day BrdU, right y-axis) of the hippocampal dentate gyrus in sedentary and wheel running (WRc) rats. **e** Quantitative analysis of 2-h-old BrdU cells in the subgranular zone from sedentary, WRc and WRw rats. **f** Stereological estimates of the number of Fos cells in the granule cell layer of the dentate gyrus of the hippocampus. Data are expressed as mean $\pm$ SEM; $n = 18$ in **b** and **c**; $n = 6$ in each group in **d** and **e**; $n = 6-12$ in each group in **f**. * $p < 0.05$ vs. sedentary rats; # $p < 0.05$ vs. WRc rats

Fig. 2.

Wheel running decreases methamphetamine self-administration. **a** Schematic of methamphetamine self-administration behavior (0.05 mg/kg/inj, FR1 schedule) and BrdU injections (indicated by syringes). **b–f** Active and inactive lever responses for methamphetamine during acquisition sessions (**b**); methamphetamine intake (mg/kg) during acquisition sessions (**c**); during escalation sessions (**d, e**), and during PR sessions (**f**). Data are expressed as mean $\pm$ SEM. $n = 9$ sedentary group; $n = 9$ WRw; $n = 6$ WRc. * $p < 0.05$ vs. sedentary methamphetamine rats in (**b**); vs. session 1 in (**c–d**); § $p < 0.05$ vs. WRc in (**b, e**); # $p < 0.05$ vs. WRw in (**e**)

Fig. 3.

Running output before methamphetamine experience positively correlates with the amount of methamphetamine self-administered (**a**). Exercise output in revolutions per day (*x*-axis) plotted against amount of methamphetamine consumed; *left* y-axis represents acquisition in mg/kg and *right* y-axis represents escalation in mg/kg. **b–c** Weekly running output from WRC rats before surgery (SUR), after SUR and after initiation of methamphetamine self-administration (Meth) (**b**) and daily running output before and after methamphetamine self-administration (**c**). Data are expressed as mean $\pm$ SEM. $n = 9$ sedentary group; $n = 9$ WRw; $n = 6$ WRC. ¹ $p < 0.05$ vs. week 1; ² $p < 0.05$ vs. week 2; ⁶ $p < 0.05$ vs. week 6 in (**b**)

Fig. 4.

Methamphetamine reduced proliferating cells in the dentate gyrus and mPFC. **a–c** Photomicrographs of DAB stained 2-h BrdU cells (**a**), AC-3 cells (**b**) and Ki-67 cells (**c**). Scale bar in **c** applies **a–c** = 20 μ m. **d–f** Quantitative analysis of immunoreactive cells stained for BrdU (**d**), AC-3 (**e**) and Ki-67 (**f**). Data are expressed as mean $\pm$ SEM; n = 9 sedentary group; n = 9 WRw; n = 6 WRc. * p < 0.05 vs. drug naïve rats; # p < 0.05 vs. sedentary methamphetamine rats

Fig. 5. Methamphetamine self-administration increases the number of nNOS expressing neurons; wheel running prior to methamphetamine exposure prevents methamphetamine-induced increase in nNOS neurons. **a** Schematic of a coronal brain section 2.70 from bregma, a region analyzed for nNOS quantification. **b–d** Low magnification image of nNOS neurons in the nucleus accumbens shell region [indicated as a *square box* in **a**] showing nNOS stained neurons from drug naïve (**b**) and sedentary methamphetamine (**c**) rat. **d** High magnification of nNOS neurons in the nucleus accumbens shell region. Scale bar in **d** is 200 μ m in **b**, **c** and 20 μ m in **d**. **e** Quantitative analysis of nNOS expressing neurons in the nucleus accumbens shell and core regions. Data are expressed as mean $\pm$ SEM. $n = 9$ sedentary group; $n = 9$ WRw; $n = 6$ WRc. * $p < 0.05$ vs. drug naïve; # $p < 0.05$ vs. sedentary methamphetamine; \$ $p < 0.05$ vs. WRw methamphetamine rats

Fig. 6.

Methamphetamine self-administration increases the number of AC-3 expressing cells; wheel running prior to methamphetamine exposure prevents methamphetamine-induced increase in the number of AC-3 cells. Quantitative analysis of AC-3 expressing cells in the nucleus accumbens shell region. Data are expressed as mean $\pm$ SEM. $n = 9$ sedentary group; $n = 9$ WRw; $n = 6$ WRc. # $p < 0.05$ vs. sedentary methamphetamine by unpaired t test

Table 1

Quantitative analysis of the number of nNOS expressing neurons in each brain region

Brain region	Drug naive	Sedentary meth	WRc meth	WRw meth
Orbital cortex	22 ± 2	17 ± 2	17 ± 2	19 ± 3
Medial prefrontal cortex	46 ± 5	45 ± 4	43 ± 7	45 ± 3
Medial septal nucleus	30 ± 10	30 ± 6	40 ± 5	30 ± 9
Bed nucleus of the stria terminalis-dorsal	10 ± 3	15 ± 2	13 ± 3	10 ± 1
Bed nucleus of the stria terminalis-ventral	5 ± 2	7 ± 2	6 ± 2	6 ± 1
Caudate putamen (striatum)	98 ± 9	85 ± 6	95 ± 9	87 ± 6
Amygdala	24 ± 3	32 ± 3	33 ± 4	28 ± 2
Hippocampal dentate gyrus	24 ± 8	29 ± 4	27 ± 6	26 ± 4
Periaqueductal gray	53 ± 20	67 ± 10	42 ± 10	60 ± 13
Ventral tegmental area	6 ± 3	9 ± 3	4 ± 2 [#]	3 ± 1 [#]

Data are expressed as mean ± SEM. *n* = 9 sedentary group; *n* = 9 WRw; *n* = 6 WRc.[#]
p < 0.05 vs. sedentary methamphetamine rats