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The effects of post-extinction exercise on cocaine-primed and stress-induced reinstatement of cocaine seeking in rats

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Abstract

Rationale—Voluntary aerobic exercise has shown promise as a treatment for substance abuse, reducing relapse in cocaine-dependent people. Wheel running also attenuates drug-primed and cue-induced reinstatement of cocaine seeking in rats, an animal model of relapse. However, in most of these studies, wheel access was provided throughout cocaine self-administration and/or extinction and had effects on several parameters of drug seeking. Moreover, the effects of exercise on footshock stress-induced reinstatement have not been investigated.

Objectives—The purposes of this study were to isolate and specifically examine the protective effect of exercise on relapse-like behavior elicited by a drug prime or stress.

Methods—Rats were trained to self-administer cocaine at a stable level, followed by extinction training. Once extinction criteria were met, rats were split into exercise (24 h, continuous access to running wheel) and sedentary groups for three weeks, after which drug-seeking behavior was assessed following a cocaine prime or footshock. We also measured galanin mRNA in the locus coeruleus and A2 noradrenergic nucleus.

Results—Exercising rats ran ~4-6 km/d, comparable to levels previously reported for rats without a history of cocaine self-administration. Post-extinction exercise significantly attenuated cocaine-primed, but not footshock stress-induced, reinstatement of cocaine seeking, and increased galanin mRNA expression in the LC but not A2.

Conclusion—These results indicate that chronic wheel running can attenuate some forms of reinstatement, even when initiated after the cessation of cocaine self-administration, supporting the idea that voluntary exercise programs may help maintain abstinence in clinical populations.

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Conflict of interest

DW is co-inventor on a patent concerning the use of selective dopamine β-hydroxylase inhibitors for the treatment of cocaine dependence (US-2010-0105748-A1; “Methods and Compositions for Treatment of Drug Addiction”). The other authors declare no conflicts of interest.

Keywords

cocaine; rat; reinstatement; exercise; stress; locus coeruleus

Introduction

The efficacy of exercise in maintaining physical and mental health is well established, and voluntary aerobic exercise is an effective intervention for a variety of stress-related neuropsychiatric and neurological disorders, including depression, anxiety, and epilepsy (Blumenthal et al. 2012; Cooney et al. 2013; Veale et al. 1992). Exercise has also shown promise as a treatment for drug addiction. The results from several small clinical studies indicate that individuals with a history of substance abuse who voluntarily participate in physical activity exhibit lower rates of relapse (Brown et al. 2010; Sinyor et al. 1982; Weinstock et al. 2008).

Likewise, several studies have demonstrated the potential therapeutic efficacy of voluntary, chronic aerobic exercise in animal models of psychiatric illnesses. We found that exercise confers resilience against seizures, stress, and depression-like behavior (Epps et al. 2013; Sciolino et al. 2012), and several groups have reported reductions in various relapse paradigms. Rats trained to self-administer cocaine exhibit lower rates of drug-seeking behavior during cocaine-primed or cue-induced reinstatement if they had a history of voluntary chronic aerobic exercise (Lynch et al. 2010; Peterson et al. 2014; Smith et al. 2012; Zlebnik et al. 2010). However, in most of these studies, rats had access to running wheels before and during the acquisition and maintenance of cocaine self-administration (Smith et al. 2012), prior to extinction (Lynch et al. 2010; Peterson et al. 2014), or throughout extinction (Zlebnik et al. 2010). Because exercise also attenuated the acquisition, escalation, breakpoint, and extinction of cocaine self-administration under some conditions (Lynch et al. 2010; Smith and Pitts 2011; Smith et al. 2008; Smith et al. 2011; Zlebnik et al. 2012; Zlebnik et al. 2010), it was not clear whether exercise was reducing reinstatement per se or whether its effects on relapse-like behavior were secondary to changes in self-administration or extinction. This is a key issue from a therapeutic standpoint because interventions to curb addiction are most easily instituted to help prevent relapse after individuals have achieved abstinence. Moreover, the anxiolytic properties of exercise suggest that it would effectively inhibit stress-induced reinstatement, but this has yet to be rigorously tested; there is only a single report that exercise can attenuate reinstatement elicited by the pharmacological stressor yohimbine (Zlebnik et al. 2014), and none that examined a physiological stressor like footshock. Determining the ability of exercise to protect against different relapse trigger modalities may also help identify individuals who are most likely to benefit from this type of therapy.

The molecular mechanisms behind the therapeutic effects of exercise on relapse behavior are not well understood, but they may involve the neuropeptide galanin. We have previously shown that aerobic exercise increases galanin mRNA specifically in the noradrenergic locus coeruleus (LC) (Holmes et al. 2006; Murray et al. 2010; O'Neal et al. 2001; Reiss et al. 2009). Galanin inhibits opiate- and psychostimulant-induced locomotor activity and place

preference (Hawes et al. 2008; Narasimhaiah et al. 2009; Picciotto 2008; Zachariou et al. 2003) and suppresses NE transmission (Holmes and Picciotto 2006; Sciolino and Holmes 2012), which is required for multiple forms of relapse-like behavior (Leri et al. 2002; Schroeder et al. 2010; Schroeder et al. 2013; Smith and Aston-Jones 2011; Weinshenker and Schroeder 2007; Zhang and Kosten 2005). Furthermore, we have recently shown that the galanin receptor agonist, galnon, attenuates cocaine-primed reinstatement (Ogbonmwan et al. 2014). Although these data indicate that galanin may contribute to the effects of exercise on drug seeking, the ability of chronic exercise to increase galanin expression in the LC has not been assessed in animals with a history of cocaine exposure.

This study had three main objectives. First, we determined whether chronic voluntary aerobic exercise, when provided only *after* cocaine self-administration and extinction, would attenuate cocaine-primed reinstatement. Second, we assessed the effects of exercise on footshock stress-induced reinstatement of cocaine seeking. Finally, we evaluated the ability of exercise to increase galanin in the LC in cocaine-experienced animals. Because NE derived from the A2 noradrenergic nucleus is specifically required for stress-induced reinstatement (Erb et al. 2000; Leri et al. 2002; Shaham et al. 2000), we also measured galanin mRNA in this brain region.

Methods

Subjects

Male Sprague-Dawley rats (151-175 g, ~7-8 weeks of age upon arrival) (n=28) were used for cocaine-primed reinstatement experiments. Male Long-Evans rats (151-175 g) (n=14) were used for the stress-induced reinstatement experiments in accordance with the literature (Brown et al. 2009; Erb et al. 2000; Kupferschmidt et al. 2011; Leri et al. 2002). All rats were obtained from Charles River (Wilmington, MA). Rats were individually housed in clear polycarbonate cages (50 × 30 × 30 cm) and given ad libitum access to food and water unless otherwise specified. Rats were housed in a temperature- and humidity-controlled animal facility and maintained on a 12-h reverse light/dark cycle. Testing occurred during the dark phase with background noise emitted by a white noise generator. Animals were allowed to acclimate to the vivarium for one week prior to surgery. All experiments were conducted at Emory University in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and approved by the Emory IACUC.

Food training

To facilitate the acquisition of drug self-administration, animals were first trained to lever press for food (45 mg pellets) in an operant conditioning chamber (Med Associates, St Albans, VT) prior to surgery. Each chamber was equipped with a house light and two retractable levers, with a stimulus light above each lever. Animals were trained on a fixed ratio 1 (FR1) schedule with a 20-s time out. Each active lever response resulted in the delivery of 1 food pellet. Presses on the inactive lever had no programmed consequences. Food training sessions lasted 8 h, or until the animal met criteria, defined as at least 70% active lever selection and at least 100 food pellets obtained. Most rats met criteria on the

first day of food training, but a few required 2–3 days. There were no significant differences between groups.

Jugular catheter surgery

All instruments and implants were sterilized prior to surgery. Rats were surgically implanted with a catheter into the right jugular vein after food training, as described (Schroeder et al. 2010; Schroeder et al. 2013). Rats were anesthetized with isoflurane administered by vaporizer with oxygen delivered through a nose cone, and the surgical site was shaved and cleaned with Betadine. Catheter tubing was threaded subcutaneously from the back and guided over the shoulder into the right jugular vein, and tubing was sutured down. Rats received meloxicam (1 mg/kg, s.c.) immediately following surgery and were allowed to recover for 1 week prior to cocaine self-administration. Catheters were flushed daily with 0.05 ml gentamicin (3 mg/ml) and 0.1 ml heparinized saline (30 ml in sterile saline) to help maintain patency. Catheter patency was verified prior to cocaine self-administration by administering 0.08-0.12 ml of the short-acting barbiturate methohexital sodium (10 mg/ml, IV; Eli Lilly, Indianapolis, IN, USA), which rapidly produces moderate sedation.

Cocaine self-administration for cocaine-primed reinstatement experiment

Daily cocaine self-administration sessions were run for 2 h on a FR1 schedule. At the start of each session, both active and inactive levers were extended, and each press of the active lever resulted in a cocaine infusion (0.5 mg/kg, 167 μl/kg, i.v., dissolved in 0.9% physiological saline) (NIDA Chemical Synthesis & Drug Supply Program, Bethesda, MD) accompanied by illumination of the stimulus light located above the lever. Following a 20-s timeout period (during which time active lever presses were counted but did not result in drug infusion), the stimulus light was extinguished and responses were again reinforced. Responses on the inactive lever had no programmed consequences. The session was terminated early to prevent overdose if the number of cocaine infusions exceeded 40. Maintenance criteria were considered met when the number of active lever presses varied by < 20% of the mean, preference for the active lever exceeded 75% and at least 20 cocaine infusions were obtained for 3 consecutive days, with a minimum of 5 total days of cocaine self-administration.

Extinction for cocaine-primed reinstatement experiment

The day after meeting maintenance criteria was reached, lever pressing was extinguished. Rats were placed in the self-administration chambers for daily 2-h sessions, during which responses on the previously active lever no longer resulted in cocaine delivery or presentation of cocaine-paired cues. Extinction criteria were met when active lever presses over 3 consecutive days were <30% of the average number of active lever presses during the last 3 days of maintenance.

Voluntary exercise for cocaine-primed reinstatement experiment

The day that rats reached extinction criteria, they were randomly assigned to either the sedentary group (n=13) or one of two exercise groups (n=7-8; see below). Exercise rats had

continuous access to a running wheel placed in their home cage for 21 d, while running wheels were not introduced into the home cages of sedentary rats.

Cocaine-primed reinstatement

Reinstatement of cocaine-seeking behavior was tested after 21 days of wheel running in a cocaine-primed reinstatement session. Because we were interested in the chronic rather than the acute effects of exercise, running wheels were removed from the cages of a subset of exercise rats (n=8) on day 21, and cocaine-primed reinstatement was tested on day 22. We controlled for the potential stress of running wheel removal by leaving the wheels in the cages of the rest of the exercise rats (n=7). Notably, rats exercise almost exclusively during the dark cycle, so running was virtually nonexistent during the 12-h light cycle just prior to reinstatement testing, even when wheels were not removed. Rats were given a non-contingent priming injection of cocaine (10 mg/kg, i.p.) and placed in operant conditioning chambers under extinction conditions (i.e., presses on the formerly active lever had no programmed consequences) for 2 h. Immediately following the reinstatement session, rats were euthanized by CO2 asphyxiation, and brains were removed, blocked (a single mid-sagittal cut), frozen on dry ice, and stored at −80 C

Cocaine self-administration, extinction, and stress-induced reinstatement

Stress-induced reinstatement was conducted as previously described (Kupferschmidt et al. 2011; Schroeder et al. 2013). Briefly, subjects received a single 2-h long habituation session in the operant conditioning chambers. Rats were then trained in 3-h cocaine self-administration sessions on a FR1 schedule. Each self-administration session began with extension of the inactive lever for 5 min. The house light was then illuminated, and the active lever was extended. Responses on the active lever resulted in a cocaine infusion (0.5 mg/kg) accompanied by illumination of a discrete light cue located above the lever. Following a 20-s timeout period, the stimulus light was extinguished, and responses were again reinforced. Responses on the inactive lever had no programmed consequences. To prevent overdose, the session was terminated early upon the attainment of 60 cocaine infusions. Following 10 d of cocaine self-administration, subjects were given 3-4 daily massed 1-h extinction trials, during which active lever responses resulted in the illumination of the stimulus light and activation of the infusion pump, but no delivery of cocaine. Subjects were extinguished until active lever presses over 3 consecutive extinction sessions were <25% of the average number of active lever presses during the last 3 days of maintenance.

The day that rats reached extinction criteria, they were randomly assigned to either an exercise or sedentary group. Exercise rats had ad libitum access to a stainless steel rodent activity wheel (Mini Mitter, Bend, OR) in their home cage for 21 days. Each wheel was connected to an electromagnetic counter that measured the number of wheel revolutions, which were recorded daily. The daily distance run was calculated for each rat by multiplying the number of wheel rotations by the wheel circumference (107.75 cm) and converting the result to km. Running wheels were not introduced into the home cages of sedentary rats. On day 22, subjects were placed in the operant conditioning chambers with both levers retracted. Following a 10-min habituation period, they were exposed to 15 min of

intermittent footshock (0.6 mA; 0.5 s/shock, range of 4-80 s between shocks). The reinstatement session began with extension of the inactive lever for 5 min, followed by illumination of the house light and extension of the active lever. Responses on the active lever resulted in illumination of the cue light above the lever and activation of the infusion pump, which did not contain a syringe, so no cocaine was delivered. Following a 20-s timeout period, the stimulus light was extinguished, and responses were again accompanied by the cue light and infusion pump activation. Responses on the inactive lever had no programmed consequences.

In situ hybridization and densitometry

A subset of the brains collected from the rats in the cocaine-primed reinstatement experiment (6 sedentary, 4 exercise) were used for in situ hybridization for galanin mRNA. Only a subset was used because tissue from half of the animals was used for other analyses that are not included in the present manuscript, and some of the remaining samples were unusable due to experimenter error. There were no significant differences in reinstatement magnitude between the rats that were used for the in situ analysis and those that were not. Tissue from one hemisphere was cut in 12-μm sagittal sections on a cryostat and subsequently thaw-mounted onto gelatin-coated glass microscope slides (2 sections/slide), which were stored at −80%C until further processing. Anatomical location was also verified in adjacent sections using 0.1% thionin stain and a rat brain atlas (Paxinos and Watson 1998).

Tissue was processed as previously described (Murray et al. 2010; Sciolino et al. 2012). For pretreatment, tissue was fixed in 4% formaldehyde in 0.12 M phosphate-buffered saline (PBS), rinsed in PBS, soaked in 0.25% acetic anhydride in 0.1 M triethanolamine HCl and 0.9% NaCl, dehydrated in a series of EtOH washes, delipidated in chloroform, and washed in EtOH. A oligonucleotide probe complementary to preprogalanin mRNA (5′-G AAG GTA GCC AGC GCT GTT CAG GGT CCA GCC TCT CTT CTC CTT T-3′; Oligos etc, Wilsonville, OR) was labeled at the 3’ end with ^{35}S-dATP (1 mCi; Perkin Elmer, Boston, MA), tailing buffer, CoCl2, and terminal deoxynucleotransferase (Roche, Indianapolis, IN). Unbound radionucelotide was removed using column separation (Micro Bio-Spin P30 in Tris, Bio-Rad, Hercules, CA), and bound radionucelotide was stabilized using 1 M dithiothreitol. Sections were covered with radiolabeled probe in hybridization buffer (25% formamide, 72 mM NaCl, 3.2 mM Tris HCl, 0.0032 mM EDTA, 0.001% sodium pyrophosphate, 0.004% sodium dodecyl sulfate, 0.002 mg/ml heparin sulfate, and 2% dextran sulfate) and incubated for 24 h at 37%C. Sections underwent a series of washes in 1% SSC and 2% SSC-formamide (50:50) at 40%C and room temperature, as well as in distilled H2O and EtOH. Sections were allowed to dry and subsequently opposed to 35S-sensitive film (Kodak BioMax MR, Rochester, NY) for 14 d. Films were developed in Kodak GBX fixer and developer and air dried.

Film images were captured under optimized conditions using a light table (Northern Light D95, Imaging Research Inc., Piscataway, NJ) and digital camera equipped with a macro lens (Nikon D5000, Micro-NIKKOR 55mmf/2.8 lens, Melville, NY). Images were processed on a Macintosh computer (Apple, Inc., Cupertino, CA) using NIH Image (Bethesda, MD; http://

rsb.info.nih.gov/nih-image/). Images of the LC and A2 were selected and measured using a uniform area. Mean grayscale brightness values were obtained from 2–4 sections per subject. Densitometry was performed on original images that were in no way digitally manipulated. Example photomicrographs were uniformly transformed across groups to a color scale using NIH Image.

Data analysis

Self-administration data were analyzed by t-test when comparing 2 groups and one-way ANOVA followed by Tukey's multiple comparisons post hoc tests and two-way ANOVA followed by Sidak's multiple comparisons post hoc tests when comparing more than 2 groups, wheel-running data were analyzed with linear regression, and in situ hybridization data were analyzed by *t*-test. All analyses were performed with Prism 6.0 for Macintosh.

RESULTS

Voluntary wheel running in rats with a history of cocaine self-administration

Twenty-one days of voluntary wheel running were recorded in rats following cocaine self-administration and extinction and prior to cocaine-primed and stress-induced reinstatement. Rats in both groups initially ran ~1-2 km/d, which increased to ~4-6 km/d by the end of the exercise phase (linear regression for cocaine-primed reinstatement group: $F_{1,313}$=35.30, p<0.0001; linear regression for stress-induced reinstatement group: $F_{1,145}$=30.98, p<0.0001) (Fig. 1). The line slopes for the cocaine-primed and stress-induced reinstatement groups were not significantly different from each other. These distances are comparable to those we have reported for drug-naïve rats (Epps et al. 2013; Reiss et al. 2009; Sciolino et al. 2012).

Post-extinction exercise attenuates cocaine-primed reinstatement

Rats went through cocaine self-administration and extinction, then were divided into a sedentary group ("SED"), an exercise group that had continuous (22 days) running wheel access ("WHEEL"), and an exercise group that had the wheels removed after 21days ("WHEEL-RM"). All rats were tested for cocaine-primed reinstatement 22 days after completing extinction training. No differences between the groups were observed for the number of days to reach maintenance criteria (SED 9±1.48, WHEEL 8.14±1.16, WHEEL RM 8.25±0.73), number of cocaine infusions obtained during the maintenance phase (SED 35.15±1.40, WHEEL 35.29±2.22, WHEEL RM 31.5±3.15) or active lever presses during the maintenance phase (SED 66.46±17.77, WHEEL 56.14±11.36, WHEEL RM 43±4.60), as assessed by one-way ANOVA. By contrast, we found that active lever responding during cocaine-primed reinstatement was attenuated in both exercise groups (Fig. 2). A two-way ANOVA (repeated measures by phase) showed a main effect of experimental phase (i.e, extinction vs. reinstatement; $F_{1, 25}$=15.48, P<0.001) and treatment ($F_{2, 25}$=3.68, p<0.05), and a borderline significant interaction ($F_{2, 25}$=3.32, P=0.05). Post hoc tests revealed that sedentary rats significantly reinstated compared to extinction (t=5.12, p<0.0001), while the exercise rats did not. Furthermore, reinstatement responding was significantly lower in both exercise groups compared to the sedentary group (WHEEL, t=3.01, p<0.05; WHEEL-RM, t=3.12, p<0.05). Inactive lever responding was low during all phases and no significant differences were observed between groups (Maintenance: SED 1.08±0.42, WHEEL

2.38±0.80, WHEEL RM 0.57±0.30; Extinction: SED 5.54±3.02, WHEEL 3.71±1.29, WHEEL RM 6.50±1.62; Reinstatement: SED 10.38±5.22, WHEEL 2.29±0.42, WHEEL RM 6.13±2.23).

Exercise has no effect on footshock-induced reinstatement

Following cocaine self-administration, extinction, and 3 weeks under exercise or sedentary conditions, drug-seeking behavior was assessed after 15 min of intermittent footshock (0.6 mA, 0.5 s/shock, 4-80 s between shocks). No differences between the groups were observed for the number of cocaine infusions obtained during the maintenance phase (sedentary 45.81±3.95, exercise 47.86±6.16) or active lever presses during the maintenance phase (sedentary 50.04±3.23, exercise 59.86±7.10), as assessed by t-test. In contrast to the cocaine-primed reinstatement results, we found that exercise did not attenuate footshock-induced reinstatement of active lever pressing (Fig. 3). A two-way ANOVA (repeated measures by phase) showed a main effect only of experimental phase ($F_{1, 12}$=28.87, P<0.001). Post hoc tests revealed that both sedentary (t=4.42, p<0.05) and exercise (t=3.18, p<0.05) rats significantly reinstated compared to extinction, and there was no difference between sedentary and exercise groups. Inactive lever responding was low during all phases and no significant differences were observed between groups (Maintenance: sedentary 3.29±1.00, exercise 4.05±1.27; Extinction: sedentary 3.43±1.39, exercise 6.14±1.59).

Exercise increases galanin mRNA in the LC but not A2

Galanin mRNA abundance in the LC was significantly higher in exercising rats compared to sedentary rats (t=2.50, p<0.05) (Fig. 4A, 4B). The magnitude of the increase was comparable to what we have reported for drug-naïve rats (Reiss et al. 2009; Sciolino et al. 2012; Van Hoomissen et al. 2004). Galanin mRNA levels in A2 were not above background in either the exercise or sedentary groups (Fig. 4A, 4B and data not shown).

DISCUSSION

Post-extinction exercise attenuates cocaine-primed reinstatement

Our results are consistent with several other reports that chronic voluntary aerobic exercise can attenuate cocaine-primed reinstatement (Smith et al. 2012; Zlebnik et al. 2010). However, in contrast to previously published paradigms, we provided access to the running wheel only after extinction had occurred. This allowed us to specifically test the ability of exercise to attenuate reinstatement and rule out potential secondary effects stemming from alterations in the reinforcing efficacy of cocaine during self-administration or facilitation of extinction learning. We found that even after drug self-administration ceased, chronic voluntary exercise significantly reduced drug-seeking behavior. Attenuation of cocaine-primed reinstatement was similar in rats that had access to running wheels until just prior to the reinstatement test and in rats that lost access to running wheels 24 hours before the reinstatement test, suggesting that the acute effects (i.e. < 24 hours) of the exercise were not required for its efficacy. Because we did not test a control group that has access to a locked wheel, we cannot distinguish between physical activity and environmental enrichment with certainty; indeed, these stimuli will always be confounded to varying degrees because rats will often spend a significant amount of time climbing on locked wheels. Thus, we used a

sedentary group with no wheel access as our control group and consider voluntary wheel running an extreme form of environmental enrichment. Regardless, these results suggest that exercise therapy initiated after abstinence has been achieved will help prevent relapse in treatment-seeking individuals.

Exercise has no effect on footshock-induced reinstatement

While many studies have shown the ability of exercise to reduce drug-primed and cue-induced reinstatement of cocaine seeking, only one (Zlebnik et al. 2014) has assessed stress-induced reinstatement. In that set of experiments, rats self-administered cocaine for 10 days, were given access to running wheels during extinction training for 14 days, and then tested for reinstatement following administration of the anxiogenic drug yohimbine, with or without concurrent presentation of cocaine-associated cues. Exercise significantly attenuated yohimbine-induced reinstatement under both conditions. While yohimbine is considered a pharmacological stressor, its mechanism of action in the reinstatement of cocaine seeking is somewhat obscure and does not appear to require the canonical stress machinery in the brain, such as norepinephrine (NE) and corticotropin-releasing factor (CRF) (Brown et al. 2009). By contrast, the neurochemical and neuroanatomical substrates underlying footshock stress-induced reinstatement are well defined and involve an A2-central nucleus of the amygdala-bed nucleus of the stria terminalis circuit driven by NE and CRF (Erb et al. 2000; Leri et al. 2002; Shaham et al. 2000). Unlike its ability to attenuate reinstatement elicited by other modalities, we found that exercise failed to block footshock-induced reinstatement. However, this result should be interpreted with caution, since overall responding was somewhat lower in exercise rats compared to sedentary rats. We do not think that the differences in the two procedures (e.g. Sprague-Dawley vs. Long Evans rats, single daily extinction trials without cues vs. massed extinction trials with cues, etc) can explain the ability of exercise to attenuate cocaine-primed, but not footshock-induced, reinstatement because there are many examples of interventions that work for both. For example, we have shown that the dopamine β-hydroxylase inhibitor, nepicastat, similarly attenuates both cocaine-primed and footshock-induced reinstatement of cocaine seeking using the distinct paradigms (Schroeder et al. 2010; Schroeder et al. 2013). Nevertheless, our results combined with reports from the literature suggest that exercise might be more effective at preventing relapse caused by drug re-exposure or drug-associated cues than stress.

Possible mechanisms underlying reduction of relapse-like behavior by exercise

We have shown that chronic exercise increases galanin mRNA in the LC of drug-naïve rats (Holmes et al. 2006; Murray et al. 2010; O'Neal et al. 2001; Reiss et al. 2009), and our new finding that wheel running also increases galanin mRNA in the LC of rats with a history of cocaine self-administration suggests a potential mechanism to explain the ability of exercise to attenuate cocaine-primed reinstatement. We know that NE, most likely released from LC projections to the prefrontal cortex and/or ventral tegmental area, is required for the rewarding effects of cocaine and cocaine-primed reinstatement (Gaval-Cruz and Weinshenker 2009; Mitrano et al. 2012; Schroeder et al. 2010; Schroeder et al. 2013; Ventura et al. 2007; Weinshenker and Schroeder 2007; Zhang and Kosten 2005). Because somatodendritically released galanin suppresses LC firing (Vila-Porcile et al. 2009; Xu et al. 2005), an increase in LC galanin could dampen the activation of this noradrenergic circuit

and prevent drug-seeking behavior triggered by cocaine re-exposure. LC-derived galanin could also directly suppress the mesocortical dopamine transmission that is necessary for cocaine-primed reinstatement. Galaninergic fibers of the LC project to and modulate the activity of ventral tegmental area (VTA) neurons (Jones and Moore 1977; Mejias-Aponte et al. 2009; Simon et al. 1979; Weiss et al. 2005), and we have shown that the galanin agonist, galnon, blocks cocaine-induced DA overflow in the frontal cortex and drug-primed cocaine seeking (Ogbonmwan et al. 2014). Other proposed molecules and mechanisms that may also contribute include phosphorylated extracellular signal-related kinase (pERK), brain-derived neurotrophic factor (BDNF), and epigenetic modifications (Lynch et al. 2013; Lynch et al. 2010; Peterson et al. 2014). Our failure to observe an upregulation of galanin mRNA in A2 could explain why exercise did not impact footshock stress-induced reinstatement in the present study. Although NE is also required for footshock-induced reinstatement, its origin is A2, not the LC, and involves the ventral noradrenergic bundle-central nucleus of the amygdala-bed nucleus of the stria terminalis circuit (Erb et al. 2000; Leri et al. 2002; Shaham et al. 2000). One limitation of this interpretation is that the galanin mRNA data came only from rats in the cocaine-primed reinstatement experiment.

However, the increase of galanin mRNA in the LC but not A2 is a pattern that we have reported many times in naïve rats (Holmes et al. 2006; Murray et al. 2010; O'Neal et al. 2001; Reiss et al. 2009). Because the rats in the cocaine-primed reinstatement experiment and the footshock-induced reinstatement experiment had very similar levels of cocaine exposure and identical amounts of exercise, it is very unlikely that the pattern of galanin expression would be different.

Conclusion

This study adds to the growing body of evidence that exercise may be beneficial in the treatment of substance abuse. Chronic voluntary aerobic exercise administered after cocaine self-administration and extinction was sufficient to attenuate cocaine-primed but not stress-induced reinstatement. Future studies will help identify the neurobiological mechanisms behind this, as well as test the contribution made by LC-derived galanin.

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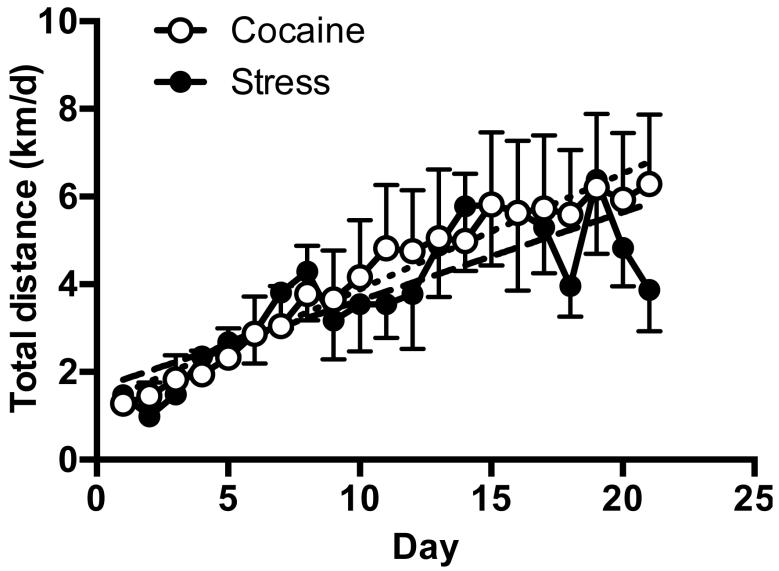

Fig. 1.

Voluntary wheel running after cocaine self-administration and extinction. Rats destined for cocaine-primed ("Cocaine") and stress-induced ("Stress") reinstatement were given continuous access to running wheels in their home cages for 3 weeks following cocaine self-administration and extinction. Mean±SEM distance run per day in kilometers is shown. Line of best fit is also shown (dotted line for Cocaine rats, dashed line for Stresss rats).

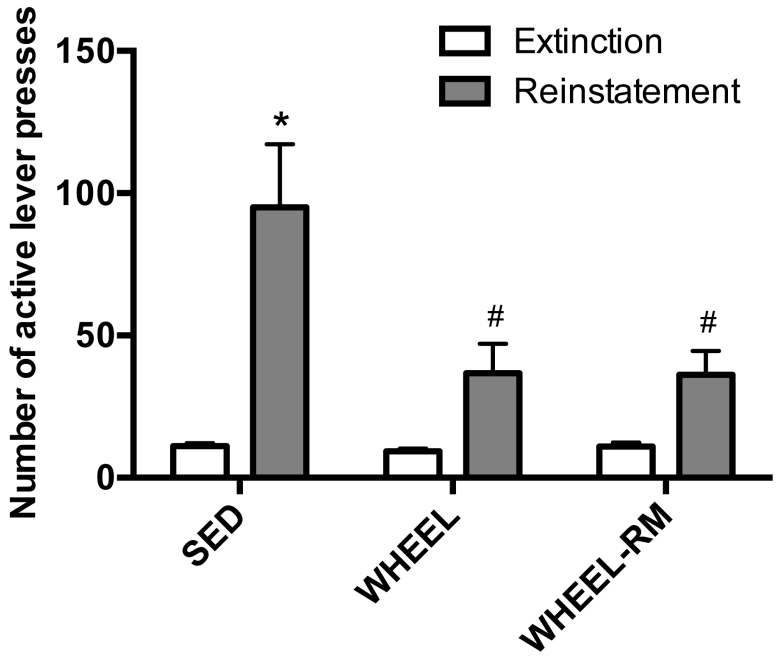

Fig. 2.

Post-extinction exercise attenuates cocaine-primed reinstatement. Following cocaine self-administration and extinction, rats were divided into 2 exercise groups ("WHEEL", running wheel in home cage; "WHEEL-RM", running wheel removed from home cage the day before the reinstatement test) or sedentary ("SED", home cage with no running wheel) groups for 3 weeks, and then given a cocaine-primed reinstatement test. Shown is the mean ±SEM number of active lever presses during the last 3 days of extinction and the single reinstatement test. *$p<0.0001$ compared to Ext, $^{\#}p<0.05$ compared to SED.

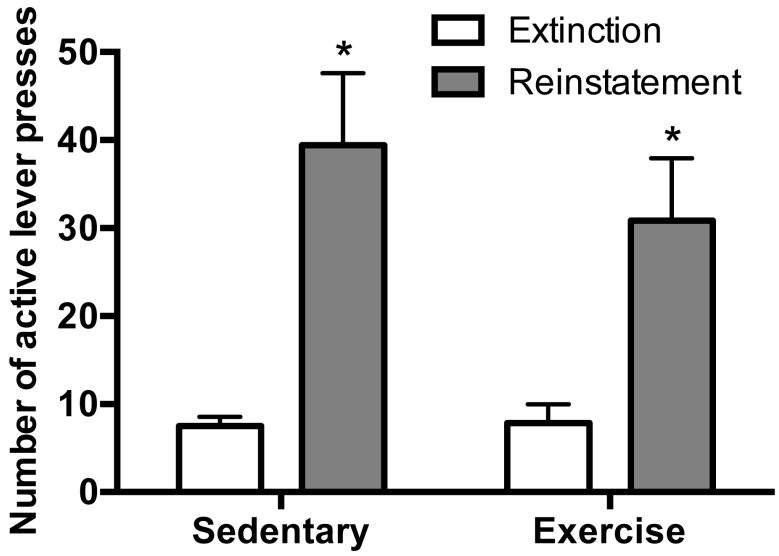

Fig. 3.

Exercise has no effect on footshock-induced reinstatement. Following cocaine self-administration and extinction, rats were divided into exercise (running wheel in home cage) and sedentary (home cage with no running wheel) groups for 3 weeks, and then given a footshock-induced (15 min, 0.6 mA, 0.5 s/shock, 4–80 s between shocks) reinstatement test. Shown is the mean±SEM number of active lever presses during the last 3 extinction sessions and during the single reinstatement test. *$p<0.0001$ compared to Ext, $^{\#}p<0.05$ compared to SED. *$p<0.05$ compared to extinction.

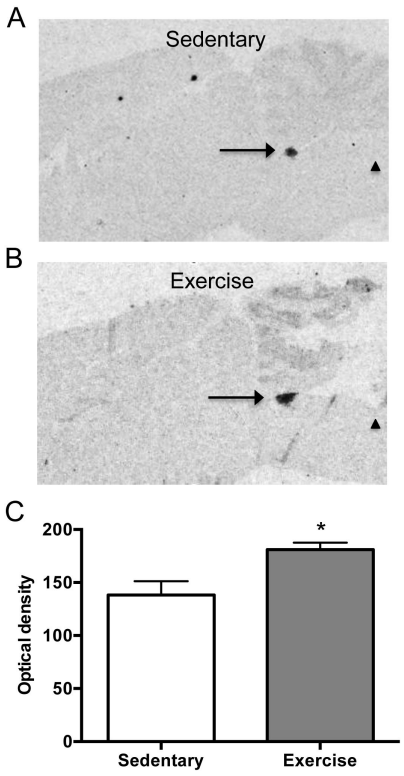

Fig. 4.

Exercise increases galanin mRNA in the LC but not A2. Representative in situ hybridization micrographs from an exercise rat (A) and a sedentary rat (B) showing galanin mRNA expression in the brain. Arrows indicate the galanin mRNA signal in the LC, arrowheads indicate the lack of galanin mRNA signal in the approximate location of A2. Shown in (C) is the mean±SEM optical density in the LC. *$p<0.05$ compared to Sedentary.