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The Effects of Resistance Exercise on Cocaine Self-Administration, Muscle Hypertrophy, and BDNF Expression in the Nucleus Accumbens

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Abstract

Background—Exercise is associated with positive outcomes in drug abusing populations and reduces drug self-administration in laboratory animals. To date, most research has focused on aerobic exercise, and other types of exercise have not been examined. This study examined the effects of resistance exercise (strength training) on cocaine self-administration and BDNF expression, a marker of neuronal activation regulated by aerobic exercise.

Methods—Female rats were assigned to either exercising or sedentary conditions. Exercising rats climbed a ladder wearing a weighted vest and trained six days/week. Training consisted of a three-set “pyramid” in which the number of repetitions and resistance varied across three sets: eight climbs carrying 70% body weight (BW), six climbs carrying 85% BW, and four climbs carrying 100% BW. Rats were implanted with intravenous catheters and cocaine self-administration was examined. Behavioral economic measures of demand intensity and demand elasticity were derived from the behavioral data. BDNF mRNA expression was measured via qRT-PCR in the nucleus accumbens following behavioral testing.

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Contributors

M.A. Smith, R.T. Lacy, and J.C. Strickland developed the study concept and data collection measures. Data were collected by J.C. Strickland, M.A. Witte, and R.T. Lacy. J.C. Strickland, M.A. Smith, J.S. Beckmann, J.M. Abel, and W.J. Lynch assisted in data analysis and interpretation. M.A. Smith drafted the manuscript, and J.C. Strickland and W.J. Lynch provided critical reviews. All authors approved the manuscript for submission.

Conflict of Interest

No conflict declared.

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Results—Exercising rats self-administered significantly less cocaine than sedentary rats. A behavioral economic analysis revealed that exercise increased demand elasticity for cocaine, reducing consumption at higher unit prices. Exercising rats had lower BDNF expression in the nucleus accumbens core than sedentary rats.

Conclusions—These data indicate that resistance exercise decreases cocaine self-administration and reduces BDNF expression in the nucleus accumbens after a history of cocaine exposure. Collectively, these findings suggest that strength training reduces the positive reinforcing effects of cocaine and may decrease cocaine use in human populations.

Keywords

demand curve; drug; physical activity; rat; strength training

1. INTRODUCTION

Exercise, defined as engagement in physical activity to improve health and fitness, is a promising intervention that may reduce drug use and the probability of relapse. Epidemiological studies report that engagement in exercise-related activities is associated with lower rates of drug use (Korhonen et al., 2009; Strohle et al., 2007). Moreover, individuals who participate in exercise-related activities over the course of treatment maintain higher abstinence rates than those who do not (Brown et al., 2010; Weinstock et al., 2008). Preclinical studies have generally supported these findings, reporting that physical activity decreases cocaine, methamphetamine, nicotine, heroin, and morphine self-administration (Hosseini et al., 2009; Lacy et al., 2014; Miller et al., 2012; Sanchez et al., 2015; Smith et al., 2008; Smith and Pitts, 2012).

To date, most epidemiological studies examining the link between physical activity and substance use have only studied aerobic exercise, and preclinical studies have used aerobic manipulations exclusively (e.g., wheel running). This exclusive focus on aerobic exercise represents a limitation of existing studies given the growing popularity of strength training and the difficulty some populations have engaging in aerobic exercise (e.g., those with compromised respiratory functioning). There is a small body of literature reporting that resistance exercise produces several positive effects on mental health that may confer protection against substance abuse. For instance, resistance exercise increases self-esteem (Ossip-Klein et al., 1989) and cognition (Cassilhas et al., 2007), and decreases depression (Timonen et al., 2002) and anxiety (Bibeau et al., 2010; Strickland and Smith, 2014). Improvements in mental health have also been shown in substance-abusing populations, with one study reporting decreases in depressive symptoms after a 4-week bodybuilding program (Palmer et al., 1995); however, measures of drug intake were not collected.

The primary objective of this study was to examine the effects of resistance exercise on cocaine self-administration in female rats. This was accomplished by using a model of resistance exercise that minimizes pain and stress to the organism. In most existing models of resistance exercise, dynamic movements of the hindlimbs are motivated by electric shock, such that the subject must repeatedly jump to escape or avoid the shock (Fluckey et al., 1995; Hernandez et al., 2000; Nilsson et al., 2010; Tamaki et al., 1992). In other models,

subjects are placed in a weighted vest and submerged in water, requiring the animal to jump repeatedly to the surface in order to breathe (Cunha et al., 2005; De Souza et al., 2011; Haraguchi et al., 2011). The use of these methods limits the appeal of these models because repeated exposure to pain (e.g., electric shock) and stress (e.g., water submersion) can confound behavioral and neurobiological measures relevant to substance use.

Very little is known about the behavioral mechanisms by which physical activity reduces drug self-administration. Knowledge of these behavioral mechanisms is necessary to inform clinical practice and predict which populations will benefit most from exercise-based interventions. Consequently, a secondary objective of this study was to perform a behavioral economic analysis on the cocaine self-administration data. An advantage of econometric analyses is that they provide an efficient and multidimensional view of demand and motivation for a drug (Hursh and Roma, 2013). Factor analytic studies have revealed two broad domains of demand captured by economic equations, which are typically identified as 1) demand intensity and 2) demand elasticity (e.g., Bidwell et al., 2012; MacKillop et al., 2009). In the latter case, interventions that increase the demand elasticity for a drug decrease consumption of that drug at higher prices, which is particularly relevant for populations that are sensitive to monetary costs (e.g., adolescents).

Whereas the neurobiological effects of aerobic exercise have been studied extensively (see reviews by Lynch et al., 2013; Morgan et al., 2015), research examining the neurobiological effects of resistance exercise is still in its infancy. Consequently, a final objective of this project was to examine the effects of resistance exercise on the expression of brain derived neurotrophic factor (BDNF) mRNA in the nucleus accumbens. BDNF is from the neurotrophin family of growth factors that mediates activity-dependent dendritic growth and arborization (Horch, 2004). Cocaine self-administration increases BDNF expression in the nucleus accumbens, ventral tegmental area, and amygdala in a time-dependent manner (Grimm et al., 2003). Wheel running normalizes BDNF expression in brain areas relevant to drug self-administration, and this may serve as a mechanism by which aerobic exercise reduces drug self-administration and other measures of drug-seeking behavior (Li and Wolf, 2015; Lynch et al., 2013; Peterson et al., 2014).

2. MATERIALS AND METHODS

2.1 Subjects

Female Long-Evans rats (Charles River Laboratories, Raleigh, NC) were obtained on PND 42 and assigned to exercising or sedentary conditions. Estrous cycle was not monitored and allowed to cycle freely. Females were selected for two reasons. First, females are under-represented in preclinical research and there have been recent calls to increase their use in research relevant to human health (Clayton and Collins, 2014; Klein et al., 2015). Second, a slower growth rate in females was seen as advantageous because it limited the range and variability of the resistance loads (determined as a percentage of body weight in grams) that would be used over the course of the multi-week study.

All rats were housed individually in polycarbonate cages for the duration of the study. Subjects were kept on a 12-h light-dark cycle (lights on: 0500) in a temperature- and

humidity-controlled environment. Each rat had free access to food and drinking water, with the exception of light food restriction during lever-press training. All animals were kept in accordance with the guidelines of the Institutional Animal Care and Use Committee of Davidson College.

2.2 Apparatus

Self-administration sessions took place in operant conditioning chambers from Med Associates, Inc. (St. Albans, VT). Chambers contained two response levers, two stimulus lights located above the levers, and one house-light located on the ceiling above the rear wall. Food pellets were delivered into a food hopper located between the levers. Drug infusions were delivered via a pump mounted outside the chamber through a Tygon tube attached to a swivel on top of the chamber. During all sessions, one lever was designated as the active lever; responses on the opposite lever were recorded but had no programmed consequences.

2.3 Resistance Training

Exercising rats were trained to climb a ladder (46 cm long, 2 cm separating each rung, 90° incline) with a weighted harness (Figure 1). The load apparatus consisted of weights attached to a vest harness. The bottom of the ladder was placed on a countertop, approximately 80 cm above the floor. The top of the ladder contained a three-sided enclosure painted black. The enclosure provided a “safe area” that allowed rats to escape the light from a 60-watt, incandescent desk lamp that was placed on the floor and directed toward the ladder. A stack of towels was positioned below the apparatus to cushion the impact of falls. The length of the ladder required rats to make six to eight dynamic movements per climb.

Resistance training was conducted six days per week. During these sessions, exercising rats carried increasing loads relative to their body weight (BW) using a 3-set “pyramid” regimen: eight climbs carrying 70% BW, six climbs carrying 85% BW, and four climbs carrying 100% BW. Rats were allowed to rest 120 s between sets. Loads incrementally increased each session during the first two weeks of training to acclimate subjects to the procedure and prevent injury, with all rats reaching terminal loads (i.e., the 70/85/100% BW pyramid) approximately one week before catheter implantation. Resistance training occurred each evening (1700), approximately 14 h prior to self-administration testing the following morning (0900). Sedentary rats wearing a vest harness without weights were placed repeatedly on the ladder oriented on its side and according to the same schedule to control for possible enrichment-related effects that might be attributed to experimenter handling and exposure to novelty. Sedentary subjects were placed in their home cage between sets to remove them from the light stimulus. Resistance training continued in this manner for the full duration of the study and terminated on the day of tissue collection.

2.4 Lever-press Training

Two weeks prior to catheter implantation and one week after arrival, subjects were restricted to 90% of their free-feeding BW and trained to press a response lever using food reinforcement. In these sessions, lever pressing was reinforced with a single food pellet on a

fixed-ratio (FR1) schedule of reinforcement. Training was terminated when subjects obtained 40 reinforcers per session over four training sessions. All subjects met this criterion within seven days.

2.5 Catheter Implantation

Three weeks after arrival, each rat was implanted with an intravenous catheter. Each subject was anesthetized with a combination of ketamine (100 mg/kg, ip) and xylazine HCl (15 mg/kg, ip). An intravenous catheter was inserted into the right jugular vein and exited on the dorsal surface of the scapulae. Ticarcillin (20 mg/kg, iv) and heparinized saline was infused through the catheter daily to prevent infection and maintain patency. After one week, ticarcillin administration ended and catheter patency was maintained by heparinized saline. Resistance training was suspended for two days following surgery.

2.6 Cocaine Self-Administration Training

Self-administration training began three days after surgery. Each training session began with an intravenous infusion of the training dose of cocaine, illumination of the stimulus light above the active response lever, and illumination of the house light. During training, cocaine (0.5 mg/kg/infusion; National Institute of Drug Abuse, Research Triangle Institute, Research Triangle Park, NC) was available on a FR1 schedule of reinforcement. Coincident with each infusion, a 5 s tone sounded and the stimulus light above the lever turned off for 20 s to signal a timeout during which cocaine was not available. Training sessions terminated automatically after 2 h elapsed. Training continued in this manner for three consecutive days. The maximum number of infusions was limited to 21 on the first day of training to prevent overdose.

2.7 Cocaine Self-Administration Testing

During testing, responding was reinforced on a FR1 schedule of reinforcement during five sessions conducted over five consecutive days. Each session lasted 2 h, and a different dose of cocaine was tested during each session. No limit was placed on the maximum number of infusions. Doses were tested in an irregular order with the stipulation that no more than two ascending or descending doses could be tested in a row. Each dose was tested in a single session conducted on separate days. All rats were tested with 0.0 (saline), 0.03, 0.1, 0.3, and 1.0 mg/kg/infusion cocaine. All other conditions were identical to those present during training.

2.8 Hindlimb Grip Strength and Muscle Mass

Hindlimb grip strength was measured 24 h following self-administration testing using a grip strength meter (Columbus Instruments, Columbus, OH). The hindlimb of each rat was placed on a metal grid attached to a force meter and the subject pulled back by the base of the tail until the grid was released. Two measures were taken in succession and the body-mass corrected average was used for data analysis.

Subjects were sacrificed via rapid decapitation after grip strength testing. Brains were removed and flash frozen on dry ice and then placed in a -80°C freezer for storage. The right extensor digitorum longus (EDL), soleus (SOL), gastrocnemius (GAS), tibialis anterior

(TA), and vastus lateralis (VL) were removed and immediately weighed to the nearest 0.1 mg.

2.9 Nucleus Accumbens BDNF

Bdnf gene expression was measured from punches obtained from the nucleus accumbens core and shell using quantitative real-time PCR (qRT-PCR). An RNeasy®Lipid Tissue Mini Kit (Qiagen, Valencia, CA) was used to isolate total RNA according to the manufacturer's protocol. The quantity and quality of the RNA were determined using a NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Scientific). cDNA templates were prepared from RNA samples using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems; ABI) according to the manufacturer's protocol. The ABI StepOnePlus real-time PCR system was used to perform qRT-PCR. TaqMan®Probe Detection (ABI) was used to detect total *Bdnf* gene expression via TaqMan®Gene Expression Assay, Rn02531967_s1 normalized to the endogenous control assay, beta-2 microglobulin (B2m Rn00560865_m1). For each cDNA sample examined, target and endogenous control genes were measured in triplicate and *Bdnf* gene expression was analyzed by the comparative CT (CT) quantitation method using StepOne™ software. To determine relative gene expression, values were analyzed by scaling to a reference sample that was equal to 1 and defined as the lowest value observed in each region. The remaining samples in the Core and Shell were calculated as *Bdnf* gene expression levels scaled relative to their respective reference sample.

2.10 Data Analysis

Two separate cohorts of rats containing an equal number of sedentary and exercising rats were obtained 3 months apart. Self-administration data were initially analyzed via three-way, mixed-factor ANOVA, with cohort and group (sedentary vs. exercise) serving as between-subjects factors and dose serving as the within-subjects factor. There was no main effect or interaction that involved a significant effect of cohort, so this factor was removed from the analysis and all data were analyzed via two-way, mixed-factor ANOVA. Post hoc comparisons were made at each dose using Fisher's Least Significant Difference Test. As a secondary analysis, area under the curve (AUC) estimates were determined for each group using the trapezoid rule; these group effects were then compared using an independent samples *t*-test. Effects sizes were calculated as partial eta-squared (η_p^2) for the dose-response data and as Cohen's *d* for all other between-group comparisons.

Demand intensity and demand elasticity for cocaine were determined using the exponential demand equation (Hursh and Silberberg, 2008):

$$\text{Log}_{10}Q = \text{log}_{10}(Q_0) + k(e^{-\alpha * Q_0 * C} - 1)$$

Where *Q* = consumption at each unit price; *Q*₀ = derived demand intensity (consumption at unconstrained price); *k* = a constant that denotes the range of consumption values in log₁₀ units (set to *k* = 3); *C* = the unit price of the commodity (responses/mg cocaine); and α = derived essential value (a measure of demand elasticity). Greater values of *Q*₀ indicate greater consumption at unconstrained price (i.e., a hypothetical zero price). Greater values of α indicate greater elasticity (i.e., a greater sensitivity to change in unit price). Models were

fit using nonlinear mixed effects modeling (NLME) in the NLME package in R statistical software (Pinheiro et al., 2007), with exercise defined as a fixed, between-subjects factor and subject defined as a random factor.

Grip strength data were first corrected for body mass by dividing grams force by kilograms body mass. Muscle mass data were corrected for body mass by dividing grams muscle by kilograms body mass. Adjustment for body mass to correct for potential individual differences in body mass is consistent with previous studies examining the effects of resistance training on muscle mass and strength (e.g., Tamaki et al. 1992). Grip, muscle, and *Bdnf* data were analyzed using independent samples *t*-tests. Values three standard deviations from the mean were considered outliers and removed for statistical analyses (one data point from the nucleus accumbens core and two from the shell). Bivariate correlations were also conducted correlating these data with AUC, economic demand parameters, and *Bdnf* expression data in the exercising group.

3. RESULTS

3.1 Cocaine Self-Administration

All subjects responded on the first day of self-administration training and stable patterns of responding characterized by regular post-infusion pauses were apparent within three days. The number of infusions varied across sessions (main effect of day: $F_{2,48} = 33.603$, $p < .001$), which could be attributed to fewer infusions on the first day when the maximum number of infusions was limited to 21 (Figure 2). No main effect of group or group x day interaction was observed.

In the dose-response analysis (Figure 3), responding maintained by cocaine generally decreased as a function of dose (main effect of dose: $F_{3,72} = 61.368$, $p < .001$, $\eta_p^2 = .719$). Exercising rats responded less than sedentary rats (main effects of group: $F_{1,24} = 5.813$, $p = .024$, $\eta_p^2 = .195$) and this effect was moderated by dose (dose x group interaction: $F_{3,72} = 3.140$, $p = .030$, $\eta_p^2 = .116$). Post hoc tests revealed that exercising rats responded significantly less than sedentary rats at the lowest (0.03 mg/kg) and highest (1.0 mg/kg) doses tested (p values $< .035$). An AUC analysis revealed a similar effect, with exercising rats responding significantly less than sedentary rats ($t_{24} = 2.264$, $p = .033$). The effect size was large regardless of the method of analysis (dose-response analysis: $\eta_p^2 = .195$; AUC analysis: $d = 0.922$).

No differences in responding were observed during the saline substitution test, and no differences in inactive lever responding were observed under any dose condition (data not shown).

3.2 Behavioral Economic Analysis of Demand

Using the dose-response data, a demand analysis isolated the effects of demand intensity and demand elasticity for cocaine. In this analysis, consumption was plotted as a function of unit price and an exponential demand curve was fit to the data (Figure 4). NLME modeling indicated that demand intensity (Q_0) did not differ between groups ($F_{1,75} = 0.410$, $p = .524$). In contrast, demand elasticity (α) was greater in exercising rats than sedentary rats ($F_{1,75} =$

4.537, $p = .036$), indicating that exercising rats were more sensitive to the price manipulation and responded significantly less at higher unit prices than sedentary rats.

3.3 Hindlimb Grip Strength and Muscle Mass

Following self-administration testing, body weight, grip strength, and muscle mass were measured in all rats (Figure 5). No significant differences in body mass were observed between groups (data not shown); however, exercising rats exhibited greater hindlimb grip strength ($t_{24} = 2.948$, $p = .007$) and greater gastrocnemius muscle mass than sedentary rats ($t_{24} = 2.951$, $p = .007$). Grip strength was 34% greater in exercising than sedentary rats, corresponding to a large effect size ($d = 1.138$). Gastrocnemius muscle mass was 11% greater in exercising than sedentary rats, with a similarly large effect size ($d = 1.133$). Measures of other muscles did not differ significantly between groups. Neither grip strength nor gastrocnemius muscle mass was correlated with AUC values or economic measures of demand.

3.4 Nucleus Accumbens BDNF

Total *Bdnf* gene expression (Figure 6) was significantly lower in exercising rats than sedentary rats in the nucleus accumbens core ($t_{10} = 4.567$, $p = .001$) and numerically lower in the nucleus accumbens shell ($t_9 = 1.900$, $p = .090$). The observed effect sizes were large for both the core ($d = 2.45$) and shell ($d = 1.09$). These values were not significantly correlated with AUC scores, economic measures of demand, hindlimb grip strength, or gastrocnemius muscle mass.

4. DISCUSSION

The primary finding of this study is that resistance exercise decreased the positive reinforcing effects of cocaine in female rats. The effects of resistance exercise were robust with large effect sizes, as determined in both a dose-response analysis and an AUC analysis (which functionally collapses across dose). Moreover, the direction and magnitude of these effects were replicated in two cohorts of rats obtained at different time points and tested approximately three months apart. These effects are similar to those reported for aerobic exercise (Lacy et al., 2014; Smith et al., 2008), suggesting that strength training may be an effective intervention in drug abuse prevention and treatment programs.

In the present study, we observed a significant decrease in the lowest and highest doses of cocaine tested, 0.03 and 1.0 mg/kg/infusion, respectively. Although an ascending limb was not readily apparent in either group, responding in the exercising group numerically peaked at 0.1 mg/kg/infusion, suggesting that lower doses would not have engendered greater levels of responding. Given the absence of a pronounced horizontal shift in the dose-effect curve between the two groups, these data suggest that the curve was shifted downward in the exercising group, reflecting a decrease in the reinforcing effectiveness of cocaine.

Recent investigators have argued for the expanded use of quantitative analyses of behavior to isolate potential mechanisms that may be responsible for drug effects (Pitts, 2014). Using a demand analysis, consumption was plotted as a function of unit price, and an exponential curve was fit to the data to obtain two measures of demand: (1) demand intensity and (2)

demand elasticity. Demand intensity (also known as “unconstrained demand” and represented by Q_0) corresponds to the Y intercept and reflects the consumption of a commodity at a unit price of zero (i.e., when the commodity is free). Demand elasticity (represented by α) is derived from the slope of the curve and reflects how sensitive consumption is to changes in unit price (i.e., how rapidly does consumption decrease when the price of the commodity increases). Each of these factors independently influence the shape and position of the curve, and each factor is a behavioral determinant of drug self-administration. By assessing the entire shape of the demand function as opposed to a single ratio metric (e.g., breakpoints on progressive ratio schedules), behavioral economic analyses are less sensitive to parametric manipulations (e.g., step size) that can complicate comparisons across experimental procedures and obscure underlying functional relationships (Hursh and Roma, 2013; Hursh and Silberberg, 2008).

In the present study, demand intensity (Q_0) did not differ between sedentary and exercising rats, indicating that demand for cocaine at a hypothetical zero price point was not influenced by resistance exercise. In contrast, demand elasticity was greater in exercising rats than sedentary rats, meaning that greater reductions in consumption were observed in exercising rats at higher unit prices (i.e., lower doses). This latter finding is important because it reveals that resistance exercise may be particularly effective in populations that are sensitive to cost manipulations. In regard to drug use, these populations include adolescents and individuals of low economic status, both of which are more likely to curtail drug use if the behavioral or monetary costs of obtaining the drug are high (Carpenter and Cook, 2008; Choi, 2014; Lee, 2008; Powell et al., 2005).

Whereas the neurobiological effects of aerobic exercise have been well described (Lynch et al., 2013), the effects of resistance exercise have received less attention. We observed a robust attenuation of total *Bdnf* gene expression in the nucleus accumbens core and a smaller nonsignificant decrease in the shell following resistance training. Previous studies indicate that BDNF regulates a diverse range of cocaine-related behaviors, including drug self-administration, and that these effects are region-dependent (Li and Wolf, 2015). For example, short-access cocaine self-administration (i.e., 2–4 h/day) increases BDNF in the nucleus accumbens (Fumagalli et al., 2013; Graham et al., 2007), with the core affecting early withdrawal behavior and the shell influencing later stages of withdrawal (Li et al., 2013). Furthermore, intra-accumbens infusion of BDNF in the shell increases cocaine intake, enhances reinstatement, and increases extinction responding, whereas infusion of a BDNF antibody produces the opposite effects (Graham et al., 2007). Clinically, serum BDNF concentrations after three weeks of abstinence are positively correlated with relapse in patients with cocaine use disorder (D’Sa et al., 2011). Taken together, these findings suggest that changes in cocaine-induced BDNF expression may be a neurobiological mechanism by which resistance exercise reduces the positive reinforcing effects of cocaine and decreases cocaine intake.

One aim of this study was to adapt a model of resistance exercise that would be suitable for drug abuse research but that minimized exposure to painful or stressful stimuli. Consequently, we avoided the use of electric shock and water submersion, both of which are often employed in animal models of resistance exercise (e.g., Aguiar et al., 2010; Fluckey et

al., 2002; Hornberger and Farrar, 2004; Soufi et al., 2011; Tamaki et al., 1992). Similar to previous studies, we used ladder climbing to induce muscle hypertrophy; however, we avoided the use of electric shock and other painful stimuli in a manner similar to that recently described by Nokia and colleagues (2016). Specifically, in the present study, a ladder was placed on a tabletop and a desk lamp was placed directly beneath the ladder; rats could escape the light from the lamp by reaching the top of the ladder and entering a small enclosure. Although stress behaviors were observed early in training (e.g., defecation, vocalization), these behaviors were observed equally in both groups and extinguished within the first few days of training. That stress behaviors were also observed in sedentary subjects upon initial exposure to the apparatus suggests that the behaviors were due to the novel environment and experimenter handling, and not due to the experimental procedures *per se*. Furthermore, stress reliably increases drug self-administration (Koob and Kreek, 2007; Sarnyai, 1998), an effect that is directly opposite of that observed in the present study. If stress were involved, our results likely represent a conservative estimate of the effects of resistance exercise on drug self-administration.

In order to validate our model of resistance exercise, we took functional and anatomical measures of muscle tissue in both groups. The hindlimb grip apparatus measures flexor muscle strength, and previous studies indicate ladder climbing evokes increased flexor movement (e.g., Bolton et al., 2006; Ross et al., 1997). Exercising rats had greater hindlimb grip strength and GAS muscle mass relative to sedentary control rats. These findings are similar to those observed in other models of resistance exercise in which muscle hypertrophy was a primary outcome measure (Tamaki et al., 1992; Wong and Booth, 1988). The GAS is a muscle of the lower hind limb and is responsible for plantar flexing the hind paw at the ankle. The GAS increases in contractile response after resistance exercise (Fitts, 2003; Norenberg and Fitts, 2004), and manipulations that increase GAS neuromuscular junction formation increase the contractile force of the hindlimb (Guerron et al., 2010; Huang et al., 2013). The concordance between the present data and those of previous studies examining the functional and anatomical effects of resistance exercise supports the validity of the present model.

No differences were observed between sedentary and exercising rats in a saline substitution test. Similarly, no differences were observed between groups in the number of responses on an inactive lever across any dose condition. These data indicate that exercise did not produce generalized decreases in operant responding that might be due to impairments in motor functioning. This latter point is particularly relevant in studies of exercise because it rules out muscle fatigue as a contributing factor.

The experimental parameters of the drug self-administration procedure may be manipulated to model different transitional phases of substance use (e.g., acquisition, maintenance, escalation, relapse), with previous research demonstrating the protective effects of aerobic exercise across all these phases (Smith and Lynch, 2012). In the current study, resistance exercise decreased the reinforcing effects of cocaine during a stable period of drug intake (i.e., maintenance). It is unknown if similar outcomes following resistance exercise would be observed at other points in the abuse continuum. However, the effects of resistance exercise on other health outcomes suggest several mechanisms by which resistance training may

prove beneficial. For example, resistance exercise decreases measures of anxiety and depression that are known to serve as risk factors for the initiation of drug use (e.g., Strickland and Smith, 2012; Swadi 1999; Timonen et al., 2002). Similarly, resistance exercise modulates activity along hormonal and stress pathways (e.g., hypothalamic pituitary adrenal axis; Crewther et al. 2011) known to regulate drug-taking behavior and contribute to relapse (e.g., Armario 2010; Koob and Kreek, 2007). Future studies will be needed to test predictions about the generality of these effects from resistance exercise across the abuse continuum.

The present study was designed as an initial characterization and validation study on the effects of resistance exercise on cocaine self-administration, and several issues should be addressed in follow-up studies. First, the present study only examined females, and we do not know whether similar findings would be obtained in males. A previous study reported that concurrent access to a running wheel decreased cocaine self-administration in females but not males (Cosgrove et al., 2002); however, we failed to find sex differences in the effects of aerobic exercise when the running wheel was available only in the home cage (Smith et al., 2011; 2012). Second, rats were obtained during late adolescence (PND 42), and we do not know whether resistance exercise would produce similar effects at other ages or during other developmental periods. Third, we also do not know whether the effects of resistance exercise extend to the positive reinforcing effects of nondrug reinforcers. Aerobic exercise typically increases the reinforcing effects of food (Belke, 2006; Smith and Witte, 2012), suggesting that it has different effects on drug versus nondrug reinforcers. Finally, we do not have a baseline of BDNF expression in control animals that did not self-administer cocaine. Consequently, we do not know whether the decrease in BDNF expression in the present study is specific to subjects with a history of cocaine self-administration or whether it is a general effect that would also be apparent in naïve subjects.

Clinical patients who engage in aerobic exercise over the course of treatment maintain better treatment outcomes relative to those who don't engage in exercise (Brown et al., 2010; Weinstock et al., 2008). Research on resistance exercise has lagged behind research on aerobic exercise, partly because of a lack of validated preclinical models. In the present study, we used a model of resistance exercise that produces both functional and anatomical changes in muscle tissue. Using this model, we showed that resistance exercise decreases drug self-administration and alters BDNF expression in the nucleus accumbens in a manner similar to that reported previously for aerobic exercise, suggesting that strength training may represent a novel and effective intervention for cocaine abuse.

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References

- Aguiar AF, Aguiar DH, Felisberto AD, Carani FR, Milanezi RC, Padovani CR, Dal-Pai-Silva M. Effects of creatine supplementation during resistance training on myosin heavy chain (MHC) expression in rat skeletal muscle fibers. *J Strength Cond Res.* 2010; 24:88–96. [PubMed: 19816211]
- Armario A. Activation of the hypothalamic-pituitary-adrenal axis by addictive drugs: different pathways, common outcome. *Trends Pharmacol Sci.* 2010; 31:318–325. [PubMed: 20537734]
- Belke TW. Responding for sucrose and wheel-running reinforcement: effect of pre-running. *Behav Processes.* 2006; 71:1–7. [PubMed: 16165314]
- Bibeau WS, Moore JB, Mitchell NG, Vargas-Tonsing T, Bartholomew JB. Effects of acute resistance training of different intensities and rest periods on anxiety and affect. *J Strength Cond Res.* 2010; 24:2184–2191. [PubMed: 19834350]
- Bidwell LC, MacKillop J, Murphy JG, Tidey JW, Colby SM. Latent factor structure of a behavioral economic cigarette demand curve in adolescent smokers. *Addict Behav.* 2012; 37:1257–1263. [PubMed: 22727784]
- Bolton DA, Tse AD, Ballermann M, Misiaszek JE, Fouad K. Task specific adaptations in rat locomotion: runway versus horizontal ladder. *Behav Brain Res.* 2006; 168:272–279. [PubMed: 16406145]
- Brown RA, Abrantes AM, Read JP, Marcus BH, Jakicic J, Strong DR, Oakley JR, Ramsey SE, Kahler CW, Stuart GG, Dubreuil ME, Gordon AA. A pilot study of aerobic exercise as an adjunctive treatment for drug dependence. *Ment Health Phys Act.* 2010; 3:27–34. [PubMed: 20582151]
- Carpenter C, Cook PJ. Cigarette taxes and youth smoking: new evidence from national, state, and local Youth Risk Behavior Surveys. *J Health Econ.* 2008; 27:287–299. [PubMed: 18242745]
- Cassilhas RC, Viana VA, Grassmann V, Santos RT, Santos RF, Tufik S, Mello MT. The impact of resistance exercise on the cognitive function of the elderly. *Med Sci Sports Exerc.* 2007; 39:1401–1407. [PubMed: 17762374]
- Choi SE. Are lower income smokers more price sensitive? the evidence from Korean cigarette tax increases. *Tob Control Epub.* 2014
- Clayton JA, Collins FS. Policy: NIH to balance sex in cell and animal studies. *Nature.* 2014; 509:282–283. [PubMed: 24834516]
- Cosgrove KP, Hunter RG, Carroll ME. Wheel-running attenuates intravenous cocaine self-administration in rats: sex differences. *Pharmacol Biochem Behav.* 2002; 73:663–671. [PubMed: 12151042]
- Crewther BT, Cook C, Cardinale M, Weatherby RP, Lowe T. Two emerging concepts for elite athletes: the short-term effects of testosterone and cortisol on the neuromuscular system and the dose-response training role of these endogenous hormones. *Spots Med.* 2011; 41:103–123.
- Cunha TS, Tanno AP, Costa Sampaio Moura MJ, Marcondes FK. Influence of high-intensity exercise training and anabolic androgenic steroid treatment on rat tissue glycogen content. *Life Sci.* 2005; 77:1030–1043. [PubMed: 15904936]
- De Souza RW, Aguiar AF, Carani FR, Campos GE, Padovani CR, Silva MD. High-intensity resistance training with insufficient recovery time between bouts induce atrophy and alterations in myosin heavy chain content in rat skeletal muscle. *Anat Rec.* 2011; 294:1393–1400.
- D'Sa C, Fox HC, Hong AK, Dileone RJ, Sinha R. Increased serum brain-derived neurotrophic factor is predictive of cocaine relapse outcomes: a prospective study. *Biol Psychiatry.* 2011; 70:706–711. [PubMed: 21741029]
- Fitts RH. Effects of regular exercise training on skeletal muscle contractile function. *Am J Phys Med Rehab.* 2003; 82:320–331.
- Fluckey JD, Dupont-Versteegden EE, Montague DC, Knox M, Tesch P, Peterson CA, Gaddy-Kurten D. A rat resistance exercise regimen attenuates losses of musculoskeletal mass during hindlimb suspension. *Acta Physiol Scand.* 2002; 176:293–300. [PubMed: 12444935]
- Fluckey JD, Kraemer WJ, Farrell PA. Pancreatic islet insulin secretion is increased after resistance exercise in rats. *J Appl Physiol.* 1995; 79:1100–1105. [PubMed: 8567549]
- Fumagalli F, Moro F, Caffino L, Orru A, Cassina C, Giannotti G, Di Clemente A, Racagni G, Riva MA, Cervo L. Region-specific effects on BDNF expression after contingent or non-contingent

- cocaine i.v self-administration in rats. *Int J Neuropsychopharmacol.* 2013; 16:913–918. [PubMed: 23164369]
- Graham DL, Edwards S, Bachtell RK, DiLeone RJ, Rios M, Self DW. Dynamic BDNF activity in nucleus accumbens with cocaine use increases self-administration and relapse. *Nat Neurosci.* 2007; 10:1029–1037. [PubMed: 17618281]
- Grimm JW, Lu L, Hayashi T, Hope BT, Su TP, Shaham Y. Time-dependent increases in brain-derived neurotrophic factor protein levels within the mesolimbic dopamine system after withdrawal from cocaine: implications for incubation of cocaine craving. *J Neurosci.* 2003; 23:742–747. [PubMed: 12574402]
- Guerron AD, Rawat R, Sali A, Spurney CF, Pistilli E, Cha HJ, Pandey GS, Gernapudi R, Francia D, Farajian V, Escobar DM, Bossi L, Becker M, Zerr P, de la Porte S, Gordish-Dressman H, Partridge T, Hoffman EP, Nagaraju K. Functional and molecular effects of arginine butyrate and prednisone on muscle and heart in the mdx mouse model of Duchenne Muscular Dystrophy. *PloS One.* 2010; 5:e11220. [PubMed: 20574530]
- Haraguchi FK, Silva ME, Neves LX, dos Santos RC, Pedrosa ML. Whey protein precludes lipid and protein oxidation and improves body weight gain in resistance-exercised rats. *Eur J Nutr.* 2011; 50:331–339. [PubMed: 21046124]
- Hernandez JM, Fedele MJ, Farrell PA. Time course evaluation of protein synthesis and glucose uptake after acute resistance exercise in rats. *J Appl Physiol.* 2000; 88:1142–1149. [PubMed: 10710414]
- Horch HW. Local effects of BDNF on dendritic growth. *Rev Neurosci.* 2004; 15:117–129. [PubMed: 15202684]
- Hornberger TA, Farrar RP. Physiological hypertrophy of the FHL muscle following 8 weeks of progressive resistance training in the rat. *Can J Appl Physiol.* 2004; 29:16–31. [PubMed: 15001801]
- Hosseini M, Alaei HA, Naderi A, Sharifi MR, Zahed R. Treadmill exercise reduces self-administration of morphine in male rats. *Pathophysiology.* 2009; 16:3–7. [PubMed: 19131225]
- Huang G, Lee X, Bian Y, Shao Z, Sheng G, Pepinsky RB, Mi S. Death receptor 6 (DR6) antagonist antibody is neuroprotective in the mouse SOD1G93A model of amyotrophic lateral sclerosis. *Cell Death Dis.* 2013; 4:e841. [PubMed: 24113175]
- Hursh SR, Roma PG. Behavioral economics and empirical public policy. *J Exp Anal Behav.* 2013; 99:98–124. [PubMed: 23344991]
- Hursh SR, Silberberg A. Economic demand and essential value. *Psychol Rev.* 2008; 115:186–198. [PubMed: 18211190]
- Klein SL, Schiebinger L, Stefanick ML, Cahill L, Danska J, de Vries GJ, Kibbe MR, McCarthy MM, Mogil JS, Woodruff TK, Zucker I. Opinion: sex inclusion in basic research drives discovery. *Proc Natl Acad Sci.* 2015; 112:5257–5258. [PubMed: 25902532]
- Koob G, Kreek MJ. Stress, dysregulation of drug reward pathways, and the transition to drug dependence. *Am J Psychiatry.* 2007; 164:1149–1159. [PubMed: 17671276]
- Korhonen T, Kujala UM, Rose RJ, Kaprio J. Physical activity in adolescence as a predictor of alcohol and illicit drug use in early adulthood: a longitudinal population-based twin study. *Twin Res Hum Genet.* 2009; 12:261–268. [PubMed: 19456218]
- Lacy RT, Strickland JC, Brophy MK, Witte MA, Smith MA. Exercise decreases speedball self-administration. *Life Sci.* 2014; 114:86–92. [PubMed: 25132360]
- Lee JM. Effect of a large increase in cigarette tax on cigarette consumption: an empirical analysis of cross-sectional survey data. *Public Health.* 2008; 122:1061–1067. [PubMed: 18602655]
- Li X, DeJoseph MR, Urban JH, Bahi A, Dreyer JL, Meredith GE, Ford KA, Ferrario CR, Loweth JA, Wolf ME. Different roles of BDNF in nucleus accumbens core versus shell during the incubation of cue-induced cocaine craving and its long-term maintenance. *J Neurosci.* 2013; 33:1130–1142. [PubMed: 23325250]
- Li X, Wolf ME. Multiple faces of BDNF in cocaine addiction. *Behav Brain Res.* 2015; 279:240–254. [PubMed: 25449839]
- Lynch WJ, Peterson AB, Sanchez V, Abel J, Smith MA. Exercise as a novel treatment for drug addiction: a neurobiological and stage-dependent hypothesis. *Neurosci Behav R.* 2013; 37:1622–1644.

- MacKillop J, Murphy JG, Tidey JW, Kahler CW, Ray LA, Bickel WK. Latent structure of facets of alcohol reinforcement from a behavioral economic demand curve. *Psychopharmacol.* 2009; 203:33–40.
- Miller ML, Vaillancourt BD, Wright MJ Jr, Aarde SM, Vandewater SA, Creehan KM, Taffe MA. Reciprocal inhibitory effects of intravenous d-methamphetamine self-administration and wheel activity in rats. *Drug Alcohol Depend.* 2012; 121:90–96. [PubMed: 21899959]
- Morgan JA, Corrigan F, Baune BT. Effects of physical exercise on central nervous system functions: a review of brain region specific adaptations. *J Mol Psychiatry.* 2015; 3:e3.
- Nilsson MI, Greene NP, Dobson JP, Wiggs MP, Gasier HG, Macias BR, Shimkus KL, Fluckey JD. Insulin resistance syndrome blunts the mitochondrial anabolic response following resistance exercise. *Am J Physiol-Endoc.* 2010; 299:E466–474.
- Nokia MS, Lensu S, Ahtiaainen JP, Johansson PP, Koch LG, Britton SL, Kainulainen H. Physical exercise increases adult hippocampal neurogenesis in male rats provided it is aerobic and sustained. *J Physiol.* 2016; 594:1855–1873. [PubMed: 26844666]
- Norenberg KM, Fitts RH. Contractile responses of the rat gastrocnemius and soleus muscles to isotonic resistance exercise. *J Appl Physiol.* 2004; 97:2322–2332. [PubMed: 15322071]
- Ossip-Klein DJ, Doynne EJ, Bowman ED, Osborn KM, McDougall-Wilson IB, Neimeyer RA. Effects of running or weight lifting on self-concept in clinically depressed women. *J Consult Clin Psych.* 1989; 57:158–161.
- Palmer JA, Palmer LK, Michiels K, Thigpen B. Effects of type of exercise on depression in recovering substance abusers. *Percep Motor Skill.* 1995; 80:523–530.
- Peterson AB, Abel JM, Lynch WJ. Dose-dependent effects of wheel running on cocaine-seeking and prefrontal cortex Bdnf exon IV expression in rats. *Psychopharmacol.* 2014; 231:1305–1314.
- Pinheiro J, Bates D, DebRoy S, Sarkar D. Linear and nonlinear mixed effects models. R package version 3. 2007:1–89.
- Pitts RC. Reconsidering the concept of behavioral mechanisms of drug action. *J Exp Anal Behav.* 2014; 101:422–441. [PubMed: 24585427]
- Powell LM, Tauras JA, Ross H. The importance of peer effects, cigarette prices and tobacco control policies for youth smoking behavior. *J Health Econ.* 2005; 24:950–968. [PubMed: 15990184]
- Ross JF, Handley DE, Fix AS, Lawhorn GT, Carr GJ. Quantification of the hindlimb extensor thrust response in rats. *Neurotoxicol Teratol.* 1997; 19:405–411. [PubMed: 9380008]
- Sanchez V, Lycas MD, Lynch WJ, Brunzell DH. Wheel running exercise attenuates vulnerability to self-administer nicotine in rats. *Drug Alcohol Depend.* 2015; 156:193–198. [PubMed: 26433561]
- Sarnyai Z. Neurobiology of stress and cocaine addiction. Studies on corticotropin-releasing factor in rats, monkeys, and humans. *Ann NY Acad Sci.* 1998; 851:371–387. [PubMed: 9668628]
- Smith MA, Lynch WJ. Exercise as a potential treatment for drug abuse: evidence from preclinical studies. *Front Psychiatry.* 2012; 2:e82.
- Smith MA, Pennock MM, Walker KL, Lang KC. Access to a running wheel decreases cocaine-primed and cue-induced reinstatement in male and female rats. *Drug Alcohol Depend.* 2012; 121:54–61. [PubMed: 21885215]
- Smith MA, Pitts EG. Wheel running decreases the positive reinforcing effects of heroin. *Pharmacol Rep.* 2012; 64:960–964. [PubMed: 23087148]
- Smith MA, Schmidt KT, Iordanou JC, Mustroph ML. Aerobic exercise decreases the positive-reinforcing effects of cocaine. *Drug Alcohol Depend.* 2008; 98:129–135. [PubMed: 18585870]
- Smith MA, Walker KL, Cole KT, Lang KC. The effects of aerobic exercise on cocaine self-administration in male and female rats. *Psychopharmacol.* 2011; 218:357–369.
- Smith MA, Witte MA. The effects of exercise on cocaine self-administration, food-maintained responding, and locomotor activity in female rats: importance of the temporal relationship between physical activity and initial drug exposure. *Exp Clin Psychopharmacol.* 2012; 20:437–446. [PubMed: 22924703]
- Soufi FG, Saber MM, Ghiassie R, Alipour M. Role of 12-week resistance training in preserving the heart against ischemia-reperfusion-induced injury. *Cardiol J.* 2011; 18:140–145. [PubMed: 21432819]

- Strickland JC, Smith MA. The anxiolytic effects of resistance exercise. *Front Psychol.* 2014; 5:e753.
- Strohle A, Hofler M, Pfister H, Muller AG, Hoyer J, Wittchen HU, Lieb R. Physical activity and prevalence and incidence of mental disorders in adolescents and young adults. *Psychol Med.* 2007; 37:1657–1666. [PubMed: 17579930]
- Swadi H. Individual risk factors for adolescent substance use. *Drug Alcohol Depend.* 1999; 55:209–224. [PubMed: 10428362]
- Tamaki T, Uchiyama S, Nakano S. A weight-lifting exercise model for inducing hypertrophy in the hindlimb muscles of rats. *Med Sci Sport Exer.* 1992; 24:881–886.
- Timonen L, Rantanen T, Timonen TE, Sulkava R. Effects of a group-based exercise program on the mood state of frail older women after discharge from hospital. *Int J Geriatr Psychol.* 2002; 17:1106–1111.
- Weinstock J, Barry D, Petry NM. Exercise-related activities are associated with positive outcome in contingency management treatment for substance use disorders. *Addict Behav.* 2008; 33:1072–1075. [PubMed: 18486352]
- Wong TS, Booth FW. Skeletal muscle enlargement with weight-lifting exercise by rats. *J Appl Physiol.* 1988; 65:950–954. [PubMed: 2459101]

Highlights

- The effects of resistance exercise on cocaine self-administration were examined
- Resistance exercise reduced cocaine intake by increasing the elasticity of demand
- Resistance exercise decreased *Bdnf* gene expression in the nucleus accumbens
- Muscle mass and hindlimb grip strength increased following resistance exercise
- Resistance exercise may be effective for treating cocaine use disorder

Figure 1. Rodent model of resistance exercise

In our model of resistance exercise, a rat is placed on a vertical ladder (left panel) wearing a weighted vest (not shown). The rat climbs to the top (center 2 panels), and remains in a darkened enclosure until the next trial (right panel). A bright light projects onto the ladder, which is raised approximately 80 cm above the floor. A stack of towels is placed below the ladder to cushion falls; however, falls are infrequent, occurring on approximately 2% of climbs. We can manipulate number of climbs (reps), number of sets, number of sessions, and amount of weight (present study schedule: bottom panel). Sedentary control rats are placed repeatedly on the apparatus when oriented on its side to equate handling and exposure to the apparatus. All sessions are conducted at the beginning of the dark cycle in a dimly lit room.

Figure 2. Cocaine self-administration training did not differ between groups

Cocaine self-administration during training in sedentary ($n = 11$; black circles) and resistance exercise ($n = 15$; white triangles) rats. Vertical axis depicts number of infusions during a 2-h session. Horizontal axis depicts daily training session. Vertical lines surrounding data points represent the SEM; where not indicated, the SEM fell within the data point. The maximum number of infusions was limited to 21 on the first day of cocaine exposure.

Figure 3. Resistance exercise decreases cocaine self-administration

Left Panel: Dose-response data of cocaine self-administration in sedentary ($n = 11$; black circles) and resistance exercise ($n = 15$; white triangles) rats. Vertical axis depicts number of infusions during a 2-h session. Horizontal axis depicts doses of cocaine in mg/kg/infusion. Points above "0" depict the effects of saline. Right Panel: Area under the curve (AUC) estimates of the dose-response data. Vertical axis depicts AUC estimates obtained from four doses of cocaine in sedentary (black bar) and resistance exercise (white bar) rats. Vertical lines surrounding data points represent the SEM; where not indicated, the SEM fell within the data point. Asterisk (*) indicates significant difference.

Figure 4. Resistance exercise increases demand elasticity for cocaine

Demand curves computed from the dose-response data of sedentary (black circles) and resistance exercise (white triangles) rats depicted in Figure 3. Vertical axis depicts consumption (measured as intake in mg/kg in log units). Horizontal axis depicts unit price (depicted as responses/mg/kg in log units). An exponential demand equation was fit to the data and plotted as mean values. Vertical lines surrounding data points represent the SEM; where not indicated, the SEM fell within the data point.

Figure 5. Resistance exercise increases functional and anatomical measures of muscle mass
Hindlimb grip strength (left panel) and gastrocnemius muscle mass (right panel) of sedentary (black bars) and resistance exercise (white bars) rats. Vertical axes depict hindlimb grip strength ($\text{g/kg} \times 100$) and gastrocnemius muscle mass (g/kg). Vertical lines represent the SEM. Asterisks (*) indicates significant differences.

Figure 6. Resistance exercise reduces *Bdnf* gene expression in the nucleus accumbens
 Relative *Bdnf* mRNA expression in the nucleus accumbens core (left) and shell (right) for sedentary (black bars) and resistance exercise (white bars) rats. For each sample, *Bdnf* gene expression was first normalized to its endogenous control, beta-2 microglobulin. The vertical axes depict *Bdnf* mRNA expression relative to a reference sample. The reference sample was equal to 1 and defined as the lowest value observed in each region analyzed. The remaining samples in the core and shell were calculated as *Bdnf* gene expression levels scaled relative to their respective reference sample. Vertical lines represent the SEM. Asterisk (*) indicates significant difference.