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Lisa S. Robison[1], Sabrina Swenson[2], John Hamilton[2] , and Panayotis K. Thanos[2]

[1]Department of Psychology, Stony Brook University, Stony Brook, NY; [2]Behavioral Neuropharmacology and Neuroimaging Laboratory on Addictions (BNNLA), Research Institute on Addictions, University at Buffalo, Buffalo, NY

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Exercise Reduces Dopamine D1R and Increases D2R in Rats: Implications for Addiction

Lisa S. Robison[1], Sabrina Swenson[2], John Hamilton[2] and Panayotis K. Thanos[2]

[1]Department of Psychology, Stony Brook University, Stony Brook, NY, [2]Behavioral Neuropharmacology and Neuroimaging Laboratory on Addictions (BNNLA), Research Institute on Addictions, University at Buffalo, Buffalo, NY

Correspondence should be addressed to:

Panayotis K. Thanos

Address: 1021 Main Street, Buffalo, NY 14203-1016

Email: pthanos@ria.buffalo.edu

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Abstract

Introduction: Exercise has been shown to be effective for preventing and treating substance abuse in both clinical and preclinical studies. Less is known, however, regarding the underlying neurobiological mechanisms driving these changes in drug-seeking behavior. One possibility is that exercise may alter the mesolimbic dopamine pathway in such a way that makes drugs of abuse less salient and/or rewarding. *Methods:* To examine possible exercise-induced changes in dopamine signaling, male and female Lewis rats were split into exercise and sedentary groups at 8 weeks of age. Exercise rats were run on a treadmill at 10m/min, five days per week, for six weeks, while sedentary rats remained in their home cage. Rats were euthanized following the 6 weeks of treatment, and their brains were used for *in vitro* autoradiography using [^{3}H]SCH 23,390, [^{3}H]Spiperone, and [^{3}H]WIN55,428 ligands to quantify D1R-like, D2R-like, and dopamine transporter (DAT) binding, respectively. *Results:* Exercised rats had 18% and 21% lower D1R-like binding levels compared to sedentary rats within the olfactory tubercle (OT) and nucleus accumbens shell (AcbS), respectively. In addition, male and female exercise rats showed greater D2R-like binding levels within the dorsomedial (DM CPu; 30%), ventrolateral (VL CPu; 24%), and ventromedial (VM CPu; 27%) caudate putamen, as well as the OT (19%). Greater D2R-like binding in the nucleus accumbens core (AcbC; 24%) and shell (AcbS; 25%) of exercised rats compared to sedentary rats approached significance. No effects were found for DAT binding. *Conclusions:* These findings support the hypothesis that aerobic exercise results in changes in the mesolimbic pathway that could mediate exercise-induced attenuation of drug-seeking behavior.

Key Words: running, treadmill, autoradiography, reward deficiency syndrome, obesity, substance abuse

Introduction

Aerobic exercise can mitigate the risk of several diseases and negative health outcomes including cancer and obesity-related disorders (e.g. type II diabetes mellitus, stroke, osteoarthritis) (1). Cognitive benefits have also been noted, including enhanced executive function, learning and memory, and protection against cognitive decline during aging and Alzheimer's disease (1, 2). Psychologically, exercise is linked to several mental health benefits, including enhanced mood, and reduced stress, anxiety, and depression (3). Exercise may also serve as a natural and cost-effective means of preventing and treating substance abuse, which in the United States alone affects 21.5 million people (8.1%) and costs over $700 billion a year (4). Epidemiological and experimental clinical studies have shown promise for the efficacy of exercise to combat substance abuse, and exercise has been shown to be effective in preventing the initiation, escalation, and relapse of substance use of several drug classes (alcohol, nicotine, stimulants, and opiates) in animal models. Details concerning the neurobiological mechanisms driving these exercise-induced changes in drug-seeking behavior have yet to be elucidated.

Survey studies in adolescents have discovered a relationship between a lack of physical activity and high-risk behaviors, such as smoking cigarettes, taking drugs, and consuming alcohol (5, 6). It is unclear, however, whether this relationship in humans is due to the shared influence of third variables (e.g. socioeconomic status, social support or standing, biological/genetic factors, personality traits) that drive both of these behaviors, or whether exercise has physiological effects that directly contribute to attenuated drug-taking behavior. Clinical studies suggest that exercise intervention is capable of producing improved outcomes for individuals with stimulant use disorders (7, 8), and a recent meta-analysis of 22 randomized control trials found that exercise increased abstinence rates across follow-up periods, drug class, and exercise type and intensity, while mitigating withdrawal symptoms, anxiety, and depression in adult substance abusers (9).

Animal studies have been performed in an attempt to further explore this association. Generally, exercise is effective in the initiation/acquisition, maintenance, escalation, extinction, and relapse phases of drug-seeking and preference across several drug classes (alcohol, nicotine, stimulants, and opiates) (10-12). Although it is generally accepted that women and female rats are more susceptible to drugs during all phases of the addiction process (13), it is interesting to note that studies have also demonstrated sex differences in the efficacy of exercise to prevent drug-seeking behaviors (14). For example, wheel running decreases cocaine self-administration in female, but not male, Sprague Dawley rats (15). Conversely, we previously found that six weeks of treadmill running significantly attenuated conditioned place preference (CPP) in female rats (though preference was still significant), and inhibited CPP for cocaine in males altogether (12). It remains to be determined exactly how exercise exerts these effects on drug-seeking and taking behavior, as well as how sex differences modulate this relationship.

It has been suggested that exercise can serve as a non-drug reinforcer (16) and as an alternative drug reinforcer under an alcohol 2-bottle choice paradigm (17). Feelings of euphoria associated with drugs of abuse are believed to be a consequence of their ability to increase extracellular dopamine in the nucleus accumbens (18). Similarly, voluntary exercise increases dopamine release in the striatum and DOPAC (a major dopamine metabolite) in the midbrain (19). Exercise may also enhance extracellular levels of dopamine via reduced reuptake, as treadmill exercise has been shown to decrease dopamine transporter immunolabeling (20). Disturbances in brain dopaminergic activity (including low striatal D2 receptor levels) are associated with compulsive behaviors seen in substance abuse, and compulsive eating and obesity, in both humans and rodents (21-24). Studies suggest that increasing DA transmission, particularly via D2R, is capable of mitigating the consumption of drugs and alcohol in rodents

(25, 26) , and a recent review suggests exercise may be a promising adjunct treatment to behavioral therapy for the treatment of substance abuse due to its effects on the DA system (27). Indeed, eight weeks of exercise training significantly increased striatal D2/D3 receptor availability in methamphetamine users in a residential treatment program (28). Endurance training (both treadmill exercise and wheel running) has also been shown to result in increased striatal D2 binding in animals (29, 30). Similarly, it has been found that 6 weeks of voluntary exercise increases DA synthesis, reduces D2 autoreceptor-mediated inhibition of DA neurons in the substantia nigra pars compacta, and increases postsynaptic D2 mRNA in the caudate putamen (30). In MPTP-lesioned rats and mice, chronic treadmill exercise has been shown to increase DA transmission, D2R mRNA, D2R protein levels, and D2R availability (as measured by [18F] fallypride binding potential), as well as lead to behavioral recovery (31, 32).

Although these results suggest that aerobic exercise's enhancement of dopaminergic signaling is one way by which it may be capable of mitigating drug-seeking behaviors, a few major questions remain to be addressed. Many of the prior studies examined the effects of exercise on DA signaling and receptors in the dorsal striatum, whereas the ventral striatum is also of interest when considering the modulation of rewarding effects. Additionally, many studies utilized unlimited voluntary wheel running as exercise intervention. Studies show that unlimited wheel access results in much larger volumes of exercise than what is used in forced treadmill running studies (20, 33, 34). Moreover, most all previous studies examining the effects of exercise on the dopamine system have utilized only one sex (usually males). Considering that sex differences have been seen in the efficacy of exercise to attenuate drug-seeking behavior (12), this begs the question as to whether there are also sex differences in the efficacy of chronic exercise to improve the functioning of the dopamine system. Therefore, in the current study, we

utilized a relatively small "dose" of exercise (six weeks of treadmill running at 10m/min, five days per week) in male and female rats that was previously shown to attenuate cocaine CPP in a sex-dependent manner (12). We found that exercise rats had lower levels of dopamine D1R-like and higher levels of D2R-like binding in key brain regions across both sexes, which may contribute to previously observed reductions in drug-seeking behavior.

Materials and Methods

Animals

Male (n=16) and female (n=16) Lewis rats (Taconic, Hudson, NY) at 8 weeks of age were individually housed under standard laboratory conditions at 22.0^{o}C± 2^{o}C with a 12h reverse light/dark cycle (lights off: 08:00-20:00 hours). Estrous cycles were not monitored and were allowed to randomly vary to allow for generalizability of results across the estrous cycle, as has been done in previous related studies using female rodents (12, 37). Food and water were available *ad-libitum* for the duration of the study. All subjects were handled daily. The experiment was conducted in accordance with the National Academy of Sciences Guide for the Care and Use of Laboratory Animals (1996) and University at Buffalo Institutional Animal Care and Use Committee.

Treadmill

A custom-made motorized treadmill divided into eight lanes by Plexiglas walls and by a sheet of metal at its end to keep the rats enclosed on the treadmill was used, as done previously (38). The dimensions of the running lanes were 56cm long x 9cm wide x 31cm high.

Exercise Regimen

Following one week of habituation, male and female rats were randomly assigned to be in either the sedentary or exercise group (n=8 per group). Rats in the exercise group were placed on the treadmill (10 meters/minute) five days a week for six weeks. Exercise sessions were performed during the animals' dark cycle, between 10:00h and 14:00h. The first day of exercise lasted 10 minutes and was increased by 10 minutes daily until the final duration of 60 minutes was reached. A ten-minute break followed the first half hour of exercise. In the course of the 6 weeks, rats ran a total of approximately 16.5 km. During this period, the sedentary group was restricted to their cages and received no exercise other than normal cage ambulation.

In vitro receptor autoradiography:

Tissue preparation

Twenty-four hours following the last day of exercise, rats were euthanized under deep isoflurane anesthesia (~3.0%). Brains were harvested, flash frozen in 2-methylbutane, and stored at -80°C until cryosectioned at 14 μm (sagittal plane), and mounted on glass microscope slides. Tissue sections were stored at -80°C until in-vitro receptor autoradiography was performed.

[3H] SCH 23390 Binding

Binding was performed as previously described for dopamine type 1-like receptor (D1R) (39). Briefly, slides were pre-incubated for 60 minutes at room temperature in 50mM Tris HCl buffer (120 mM NaCl, 5 mM KCl, 2 mM CaCl2,1 mM MgCl2, pH = 7.4). Slides were then incubated for 60 minutes at room temperature in pre-incubation buffer in the presence of 2.5 nM [3H] SCH 23390 (SA=85 Ci/mmol) and 40 nM ketanserin. Non-specific binding was determined in the presence of 1 μM flupenthixol. Slides were washed 2 x 5 minutes at 4°C in preincubation buffer followed by a dip at 4°C in dH2O.

[3H] Spiperone Binding

Dopamine type 2-like receptor (D2R) binding was performed as previously described (39). Briefly, slides were pre-incubated for 60 minutes at room temperature in 50mM Tris 7 HCl buffer (120 mM NaCl, 5 mM KCl, 2 mM CaCl2,1 mM MgCl2 , pH = 7.4). Slides were incubated for 60 minutes at room temperature in pre-incubation buffer in the presence of 0.5 nM [3H] Spiperone (specific activity = 16.2 Ci/mmol) and 40 nM ketanserin. Non-specific binding was determined in the presence of 10 µM sulpride. Slides were washed 2 x 5 minutes at 4°C in pre-incubation buffer followed by a dip at 4°C in dH2O.

[3H] WIN 35 428 Binding

Binding was performed as previously described for dopamine transporter (DAT) (39). Briefly, slides were pre-incubated for 10 minutes at 4°C in 30mM sodium phosphate buffer (pH = 7.4). Slides were incubated for 90 minutes at 4°C in 30mM sodium phosphate buffer solution with 0.32 M sucrose (pH = 7.4) and in the presence of 6.5 nM [3H] WIN 35, 428 (specific activity = 76 Ci/mmol). Non-specific binding was determined in the presence of 60 µM cocaine. Slides were dipped then washed 2 x 1 minutes in pre-incubation buffer and dipped in dH2O, all at 4°C.

Region of Interest Analysis

Bound slides and tritium standards on glass slides (ART0123, American Radiolabeled Chemicals, Inc., Saint Louis, MO) were apposed to Kodak MR Film (D1: 4 weeks, D2: 10 weeks, DAT: 12 weeks). Film was scanned at 1200 dpi, and images were quantified using Image J software (NIH). **Figure 1** shows the regions of interest (ROI's) analyzed, including the dorsomedial caudate putamen (DM CPu), ventromedial caudate putamen (VM CPu), dorsolateral caudate putamen (DL CPu), ventrolateral caudate putamen (VL CPu), nucleus accumbens core

(AcbC), nucleus accumbens shell (AcbS), and olfactory tubercle (OT). The medial/lateral portions of the caudate putamen were split at approximately 2.8mm from the midline, and dorsal/ventral portions of the caudate putamen were split to make equal divisions on each section analyzed.

Statistical Analysis

For body weight change and binding values of each radioligand and region of interest, a two-way ANOVA was conducted with the factors of Sex [Male, Female] and Treatment [Exercise, Sedentary]. Significance level was set at α=0.05 and all statistical analyses were performed with Sigmaplot 11.0 (Systat Software, Inc., Chicago, Illinois).

Results

The main effect of exercise and the exercise x sex interaction were not significant for percent weight gain during the course of the exercise treatment period. Only the main effect of sex was significant, such that females gained less weight than males during the exercise treatment period [$F(1,24) = 45.732$; $p<0.001$]. Mean $\pm$ SEM for percent weight gain by group: Male Sedentary 154.81 $\pm$ 8.50, Male Exercise 161.10 $\pm$ 9.08, Female Sedentary 94.11 $\pm$ 9.07, Female Exercise 103.81 $\pm$ 8.21.

Significant effects of exercise were found across groups for brain *[3H]SCH 23390* (D1R-like) binding levels. Specifically, chronic exercise rats showed significantly lower D1R-like binding in the nucleus accumbens shell (AcbS) [$F(1,20) = 5.395$; $p<0.05$; **Figure 2**], and the olfactory tubercle (OT) [$F(1,20) = 10.341$; $p<0.01$]. There was no significant difference observed between males and females on D1R-like binding levels ($p>0.05$). In addition, there was no significant interaction between exercise and sex on D1R-like levels across all brain regions

examined ($p>0.05$). These results suggest that exercise reduces D1R-like binding in the ventral striatum in a sex-independent manner.

Significant effects of exercise were also found across groups for brain *[3H]Spiperone* (D2R-like) binding levels. Specifically, chronic exercise treated rats showed greater D2R-like binding levels in the olfactory tubercle (OT) [$F(1,24) = 4.678$; $p<0.05$; **Figure 3**], dorsomedial caudate putamen (DM CPu) [$F(1,24) = 4.571$; $p<0.05$], ventrolateral caudate putamen (VL CPu) [$F(1,24) = 4.724$; $p<0.05$], and ventromedial caudate putamen (VM CPu) [$F(1,24) = 4.270$; $p<0.05$]. The effects of exercise in the nucleus accumbens core (AcbC) and shell (AcbS) approached significance ($p=0.06$ for both). In addition, there was no significant interaction between exercise and sex on D2R-like binding levels across all brain regions examined ($p>0.05$). These results suggest that exercise increases D2R-like binding in several subregions of the dorsal and ventral striatum in a sex-independent manner.

Finally we did not observe any significant effect across groups in *[3H]WIN 35*,428 (DAT) binding levels [**Figure 4**]. Specifically, there was no significant main effects or interaction between exercise and sex on DAT levels across all brain regions examined ($p>0.05$).

Discussion

The current study examined how daily aerobic exercise impacts dopamine (DA) signaling in the brain. Male and female exercise rats showed 18% and 21% lower D1R-like binding in the olfactory tubercle (OT) and nucleus accumbens shell (AcbS), respectively. In addition, exercised rats showed higher D2R-like binding levels within the dorsomedial (DM CPu; 30%), ventrolateral (VL CPu; 24%), and ventromedial (VM CPu; 27%) caudate putamen, as well as the OT (19%). The effect of exercise on D2R-like levels approached significance in the nucleus

accumbens core (AcbC: 24%) and shell (AcbS; 25%). Lastly, there were no effects of exercise observed on dopamine transporter (DAT) binding. These exercise-induced alterations in dopamine receptor binding may represent one neurobiological mechanism by which exercise attenuates drug-seeking behavior.

Whereas we found no changes in DAT binding, a previous study found that exercised animals showed lower DAT immunolabeling in young adult male C57BL/6J mice, which may lead to higher basal levels of DA (32). Although the exercise regimen used in this prior study was similar (60min treadmill running, 5 days/week for a total of 30 days), the maximum velocity was more than double (~22m/min versus 10m/min in the current study) (32). This may account for discrepant results, as striatal DA metabolite levels have been shown to be correlated with speed of running (19).

Additionally, we found that both male and female chronic treadmill exercised rats had lower D1R-like binding in the ventral striatum and higher D2R-like binding in several subregions of the dorsal and ventral striatum. This is in agreement with previous studies that have found that treadmill exercise attenuates D1R and enhances D2R in the basal ganglia (30-32). It has also been shown that chronic high levels, but not low levels, of voluntary wheel running produced lower levels of D2R availability as well as higher levels of delta FosB and TH mRNA in the AcbC resembling a state similar to chronic exposure of drugs of abuse (41). These findings suggest that the effects of exercise on the DA system may be dependent on intensity, and that while moderate exercise may protect against drug use, high levels of exercise may sensitize the reward pathway. This may in part explain increases in addictive behavior (i.e. compulsive exercise/exercise addiction) seen in some individuals.

These findings that exercised animals had lower D1R and higher D2R binding in striatal regions has key implications for addiction. DA receptors are highly expressed in the CPu, which is primarily composed of medium spiny neurons (MSNs) that express either of these DA receptor subtypes. D1-expressing neurons directly project to midbrain regions ("direct pathway") promoting movement, while D2-projecting neurons project to midbrain regions indirectly via the pallidum and subthalamic nuclei ("indirect pathway"), serving to suppress movement. These two receptor subtypes have opposing effects on adenylyl cyclase and consequent intracellular signaling, with D1 receptors being excitatory and D2 receptors being inhibitory (42).

The differential roles of the DA D1 and D2 receptors in motor function, motivation, and consummatory and addictive behaviors have become of interest. D1R antagonism in the AcbS with SCH-23390 dose-dependently attenuates drug-primed reinstatement of cocaine-seeking behavior, while injection of the antagonist into the AcbC had no effect (43). More recently, D1- and D2- expressing MSNs in the dorsal CPu appear to mediate opposing reward-related behaviors, as stimulation of D1 MSNs is reinforcing and enhances locomotor activity, while stimulation of D2 MSNs is aversive/punishing and promotes freezing behavior (44). Additional studies have also shown that the stimulation of D1-expressing MSNs, or the inhibition of D2-expressing MSNs, enhances an individual's sensitivity to psychostimulants (45). In the Acb, the opposing roles of D1 and D2 receptors in reward-related behavior have also been demonstrated. The activation of D1-expressing MSNs has been shown to enhance cocaine conditioned place preference (46, 47). Conditioned place preference for psychostimulants is reduced by the activation of D2-MSNs (46, 47) and increased by their inactivation (48). Moreover, D2-MSN inhibition increases motivation for cocaine (PR breakpoint), while activation of D2-MSNs reduces cocaine self-administration (49). Results of the aforementioned studies then lead to the

conclusion that an increased number of D1R and/or a decreased number of D2R would be associated with enhanced drug-seeking/compulsive behavior, which indeed has been demonstrated in several studies (22, 23, 50, 51); though contradictory findings with D1 and D2 agonism and antagonism have been seen (52-54).

Therefore, interventions for substance abuse that reduce D1R and/or increase D2R, should be effective at mitigating drug-seeking behavior. Indeed, it has been shown that D2R DNA transfer into the nucleus accumbens attenuates cocaine self- administration in rats and reduced ethanol consumption in mice (25, 26). Exercise-associated lower levels of D1R and higher levels of D2R binding reported in the current study therefore provide one possible neurobiological mechanism by which exercise may be protective against addictive behavior, as has been shown in several previous studies in both humans and animals (5, 6, 9-12, 14). Specifically, we previously found that this same exercise regimen attenuated cocaine CPP in females (though significant preference was still present), and inhibited cocaine CPP altogether in male rats (12). It is possible that females need a greater volume of exercise to see equal reductions in drug-seeking behavior compared to males, as it has been shown that females voluntarily run more than males when given free access to a running wheel (55). We chose to utilize the same exercise regimen in males and females to determine whether the previously-observed sex differences in the efficacy of exercise to attenuate cocaine preference could be attributable to sex differences in the effects of exercise on dopamine receptor levels. Although a sex difference was seen in our previous behavioral findings (12), the current study did not find any significant sex differences in the effects of exercise on DA receptor or DAT binding in striatal regions.

Lastly, we chose to use forced exercise (treadmill running) rather than voluntary wheel running in the current study for a number of reasons. As stated, we have previously shown that this same exercise regimen is capable of attenuating drug-seeking behavior (12), and wanted to explore possible mechanisms of these findings. Second, we argue that although forced exercise is a stressor (36, 40), it may better model an average person compared to voluntary wheel running, which yields much greater volumes of exercise. While our exercise regimen resulted in running volumes of 600 m/day, adult rats given daily free access to a running wheel have been shown to run up to 10,000 m/day (33, 34). It has also been demonstrated that rodents given unlimited access to a running wheel will use the wheel for approximately 5 hours per day (56). As has been argued by others (36), most humans do not have such large amounts of leisure time to devote to physical activity, even if motivated to do so.

In conclusion, the present findings indicate that chronic treadmill exercise results in lower levels of D1R-like binding in the ventral striatum and higher levels of D2R-like binding in several subregions of the dorsal and ventral striatum, without any indication of an effect on DAT binding. These differences in DA receptor binding may reflect a potential neuromechanism by which exercise is capable of reducing drug-seeking behavior. Importantly, these effects were seen even with relatively small doses of moderate intensity exercise that did not alter body weight gain throughout the course of the intervention period. Despite previous findings that this same exercise regimen was more effective at attenuating cocaine CPP in male rats compared to females, we did not observe sex differences in the efficacy of exercise to alter dopamine receptor binding. Further investigation is necessary to elucidate additional mechanisms driving exercise-induced changes in drug-seeking behavior (e.g. endogenous opioid signaling), particularly those that may be responsible for sex differences observed previously. It should be noted that we did

not monitor or account for the estrous cycle of females when brains were collected in the current study, similar to our previous behavioral study using similar treatment groups (12). As circulating estrogen levels can influence DA-related measures and DA receptor levels vary throughout different phases of the estrous cycle (57-59), it is possible that this introduced variability in the assessment of females. Future studies should also explore whether exercise can normalize altered DA signaling and receptor levels resulting from chronic drug use, as this may provide insight into neurobiological mechanisms by which exercise could serve as a treatment for substance abuse.

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FIGURE CAPTIONS

Figure 1: Regions of interest (ROI's) analyzed, including the olfactory tubercle (OT; #1), nucleus accumbens shell (AcbS; #2), nucleus accumbens core (AcbC; #3), ventromedial caudate putamen (VM CPu; #4), dorsomedial caudate putamen (DM CPu; #5), dorsolateral caudate putamen (DL CPu; #6), and ventrolateral caudate putamen (VL CPu; #7). The medial/lateral portions of the caudate putamen were split at approximately 2.8mm from the midline, and dorsal/ventral portions of the caudate putamen were split to make equal divisions on each section analyzed.

Figure 2: Mean (+SEM) [3H]SCH 23390 binding for dopamine D1-like receptors in male and female sedentary versus exercised rats. Exercise decreased [3H]SCH 23390 binding in the nucleus accumbens shell (AcbS; $p<0.05$) and olfactory tubercle (OT; $p<0.01$). These results suggest that exercise reduces D1R-like binding in the ventral striatum in a sex-independent manner.

Figure 3: Mean (+SEM) [3H]Spiperone binding for dopamine D2-like receptors in male and female sedentary versus exercised rats. Exercise was found to increase [3H]Spiperone binding in the dorsomedial caudate putamen (DM CPu; $p<0.05$), ventrolateral CPu (VL CPu; $p<0.05$), ventromedial CPu (VM CPu; $p<0.05$), and olfactory tubercle (OT; $p<0.05$). Increases in [3H]Spiperone binding in the nucleus accumbens core (AcbC) and shell (AcbS) of exercised rats approached significance ($p=0.06$ for both). These results suggest that exercise increases D2R-like binding in several subregions of the dorsal and ventral striatum in a sex-independent manner.

Figure 4: Mean (+SEM) [3H]WIN 35,428 binding for the dopamine transporter (DAT) in male and female sedentary versus exercised rats. No differences in [3H]WIN 35,428 binding were observed in any region examined across treatment or sex (p>0.05).

Figure 1

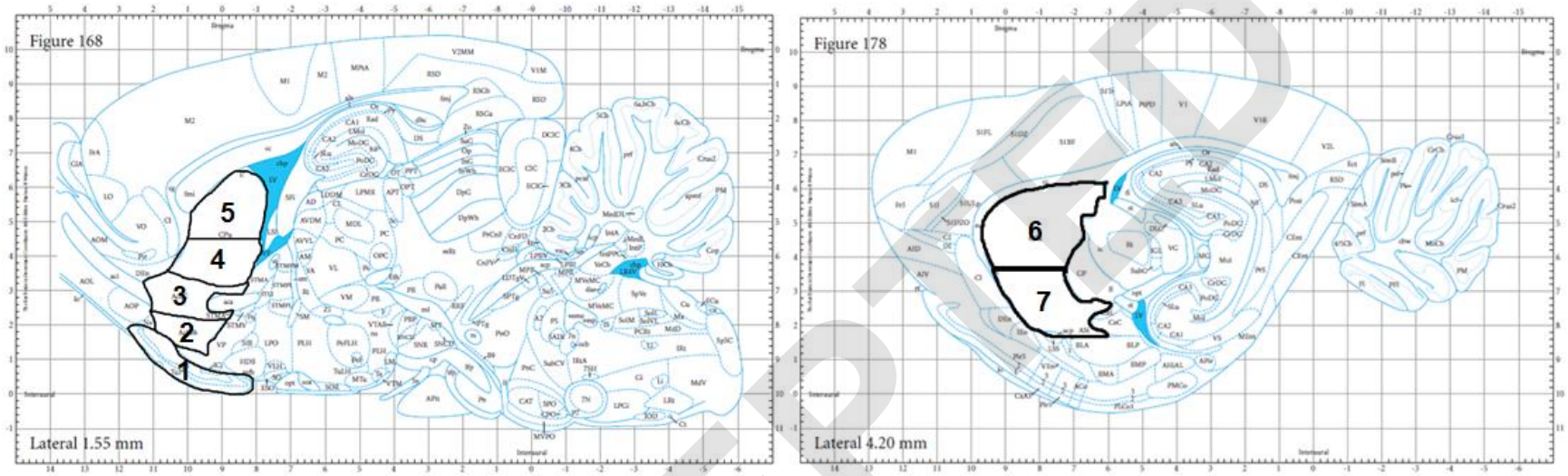

Figure 2

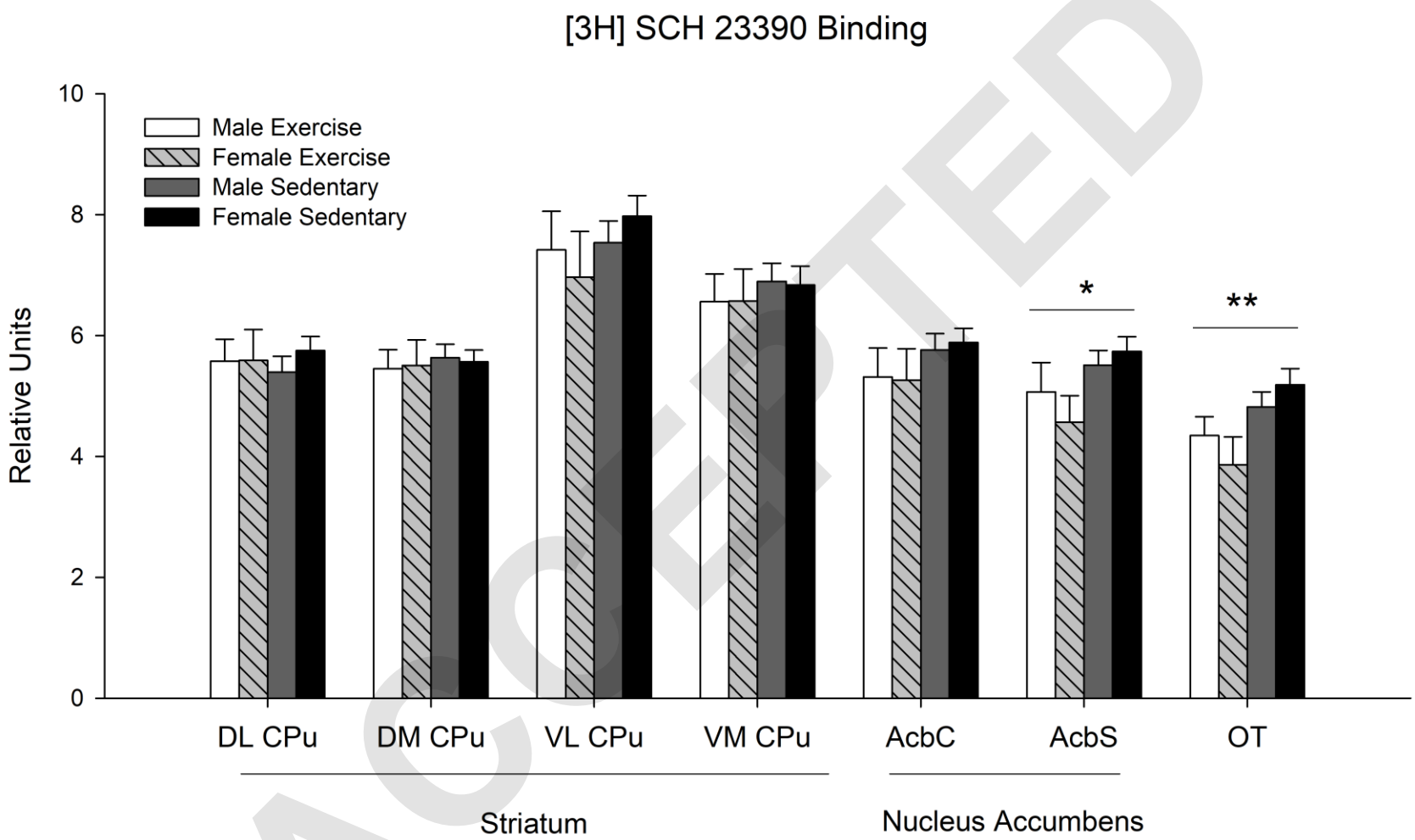

Figure 3

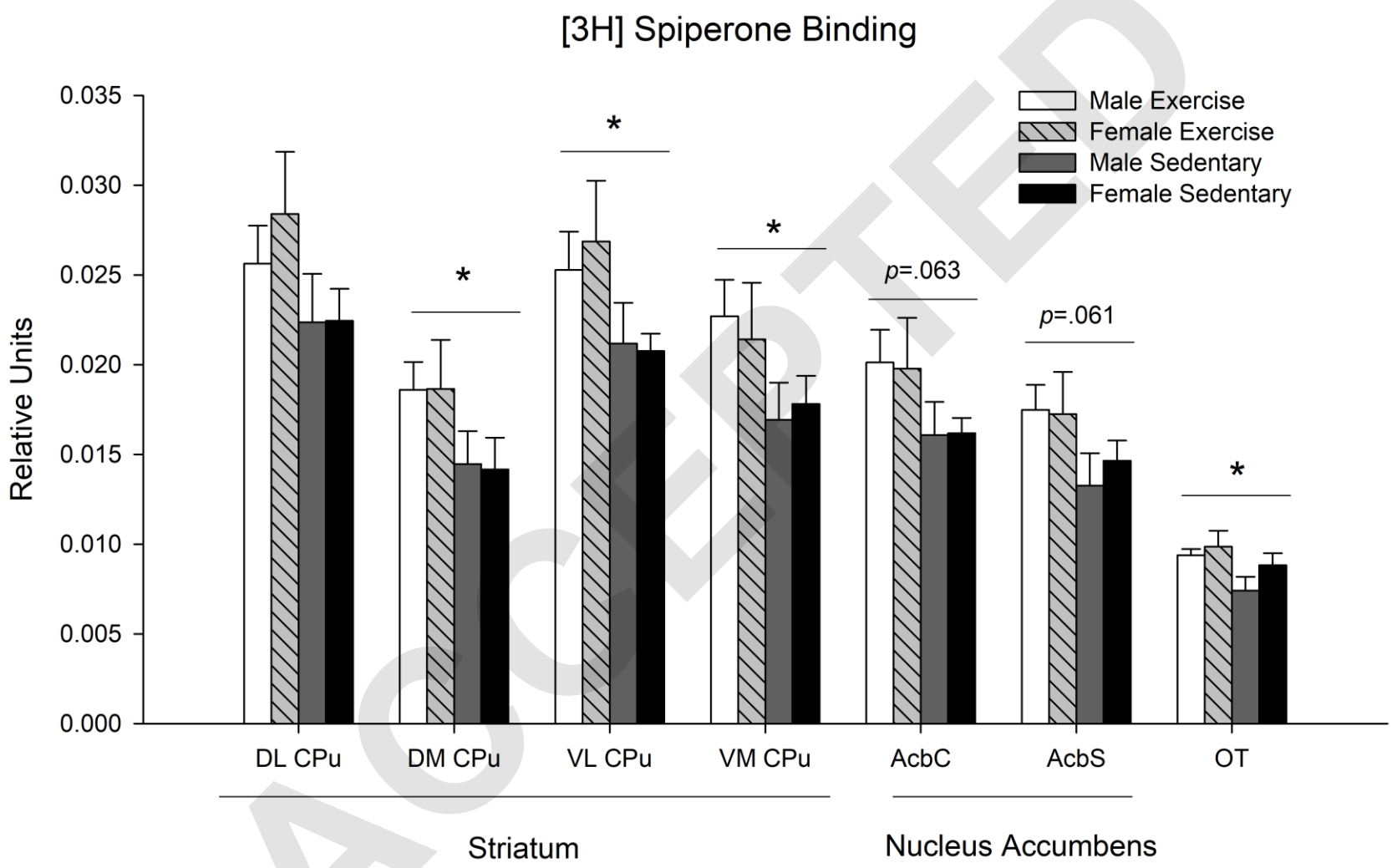

Figure 4