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Individual and Combined Effects of Physical Exercise and Methylphenidate on Orienting Behavior and Social Interaction in Spontaneously Hypertensive Rats

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Abstract

This study determined the duration of exercise and amount of methylphenidate that is needed to affect attentional function and social behavior in Spontaneously Hypertensive Rats (SHR), a commonly-used animal model of Attention-Deficit/Hyperactivity Disorder (ADHD). Attention was assessed by measuring the orienting response to repeated presentations of a non-reinforced visual cue. Social behavior was examined by allowing rats to freely explore a large arena containing an unfamiliar conspecific rat. Consistent with their hyper-responsive phenotype, non-exercising SHRs exhibited a high level of orienting behavior and little habituation, as well as hyper social behavior compared to normo-active rats. Exercise or methylphenidate decreased orienting behavior and social behavior in a dose-dependent fashion. In addition, we found an additive effect of combining doses of exercise and methylphenidate that alone were ineffective in altering behavior. These data indicate that physical exercise and methylphenidate can reduce hyper-responsiveness to irrelevant stimuli and reduce hyper-social behavior in SHR. Moreover, sub-threshold doses of methylphenidate can be used in combination with moderate amounts of exercise to reduce distractibility, supporting the notion that exercise may be useful as an adjunctive or replacement therapy in ADHD.

Keywords

Attention; Attention-Deficit/Hyperactivity Disorder; rat; methylphenidate

Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) is a common neurodevelopmental condition (5% of school-age children, American Psychological Association, 2013) that is characterized by developmentally inappropriate symptoms of inattention, hyperactivity, and impulsivity. ADHD typically emerges in childhood and persists into adolescence and adulthood, resulting in poor social, academic, and occupational outcomes (Barkley, 2002; Mannuzza & Klein, 2000). Numerous studies have shown that psychostimulants can have positive effects on the behavioral symptoms of ADHD (Chronis, Jones, & Raggi, 2006), presumably by enhancing catecholamine transmission through inhibition of the dopamine

(DAT) and norepinephrine transporters (NET). Indeed, drugs such as methylphenidate (MPH, Ritalin™) are used to treat approximately 85% of children diagnosed with the disorder (Chronis et al., 2006). Nonetheless, there are several significant limitations to relying on psychostimulants as the primary intervention for ADHD. For example, up to 30% of children do not exhibit any behavioral improvements with stimulant use or are unable to tolerate the side effects (Biederman, Spencer, & Wilens, 2004). Moreover, the benefits of psychostimulants wane once treatment is discontinued (Chronis et al., 2006; Jensen et al., 2007; Steinberg-Epstein, Book, & Wigal, 2011) and there is also concern about abuse potential and long-term health effects. Together, these limitations have stimulated research on possible adjunctive or alternative therapies for ADHD (Halperin & Healey, 2011; Scherer et al., 2010; Swanson et al., 2007; Volkow & Insel, 2003).

Physical exercise has recently emerged as a potential treatment for individuals with ADHD because of its beneficial effects on cognitive functioning and mental health in both children and adults (Carek, Laibstain, & Carek, 2011; Sibley & Etnier, 2003; Strong et al., 2011) and because exercise, like MPH, has been shown to increase dopamine and norepinephrine levels (Hattori, Naoi, & Nishino, 1994; Pagliari & Peyrin, 1995; Winter et al., 2007). Indeed, several studies have now reported beneficial effects of an exercise intervention in children with ADHD (Chang, Liu, Yu, & Lee, 2012; Pontifex, Saliba, Raine, Picchietti, & Hillman, 2013). As a result, there is current research interest in using animal models of ADHD to determine the parameters and mechanisms that mediate the effects of exercise (Hopkins, Sharma, Evans, & Bucci, 2009; Naylor, Persson, Ericksson, Jonsdottir, & Thorlin, 2005; Robinson, Hopkins, & Bucci, 2011; Robinson, Eggleston, & Bucci, 2012). One such model is the spontaneously hypertensive rat strain (SHR; Davids, Zhang, Tarazi, & Baldessarini, 2003; Sagvolden, 2000; Sagvolden, Russell, Aase, Johansen, & Farshbaf, 2005). SHRs exhibit the behavioral and cognitive impairments typically associated with the disorder, including hyperactivity, impulsivity, and inattention (Hopkins et al. 2009; Kantak et al., 2008; Robinson et al., 2011; 2012; Russell, 2007; Sagvolden et al., 2005). SHRs also exhibit altered dopamine and norepinephrine neurotransmission (Heal, Smith, Kulkarni, & Rowley, 2008; Russell, 2000; 2002; Solanto & Conners, 1982). Interestingly, female SHRs display more attentional impairments and hyper-social behavior than male SHRs, consistent with a growing evidence that females diagnosed with ADHD may be more cognitively impaired than males (Gershon, 2002; Nigg, Blaskey, Huang-Pollock, & Rappley, 2002; Spencer, Biederman, & Mick, 2007).

We have recently shown that 21 days of access to running wheel is as effective as a 0.125 mg/kg dose of MPH in improving attention and reducing hyper-social behavior in female SHRs (Hopkins et al., 2009; Robinson et al., 2012). The present study sought to extend this finding by determining the minimum amounts of MPH (Experiment 1) or exercise (Experiment 2) that are necessary to affect attention and social behavior. A final study (Experiment 3) tested whether the doses of exercise and MPH that alone were insufficient to affect behavior could be combined to produce a significant effect on attention and social behavior. The latter experiment was particularly useful for testing whether exercise might be used as an adjunctive therapy to reduce the amount of MPH needed to affect behavior.

In all three experiments, attentional function was assessed by observing orienting responses to repeated presentations of a non-reinforced visual stimulus. Orienting is defined as rearing up on the hind legs towards the stimulus (Holland, 1977; 1984) and is an often-used measure of attentional processing (Gallagher, Graham, & Holland, 1990; Kaye & Pearce, 1984; Lang, Simons, & Balaban, 1997). In normal rats, rearing behavior rapidly decreases (habituates) when the cue is not followed by reinforcement, reflecting an adaptive decrease in attention to a behaviorally-irrelevant stimulus (Gallagher et al., 1990; Holland, 1997; Kaye & Pearce, 1984). We have shown previously that SHR rats exhibit hyper-orienting behavior with little or no habituation compared to normo-active controls strains (Hopkins et al., 2009; Robinson et al., 2011, 2012) indicating that they are more prone to respond to distracting, irrelevant stimuli. Social interaction was assessed in the current study using a procedure adapted from File and colleagues (File, 1980; File & Seth, 2003) and used previously to demonstrate that SHR rats exhibit hyper-social behavior. Indeed, compared to normo-active control rats, SHR rats initiate more interactions with an unfamiliar rat (Hopkins et al. 2009; Robinson et al., 2011, 2012).

MATERIALS AND METHODS

Subjects

Experiment 1—Fifty-five female SHR rats were obtained from Harlan Laboratories (Indianapolis, IN) or Charles River Laboratories (Wilmington, MA) and 8 female Wistar rats (7–8 weeks old) were obtained from Harlan Laboratories. Rats were group housed (3–4 per cage) and maintained on a 14/10 h light-dark cycle throughout the experiment. Group housing was chosen because housing juvenile rats in isolation has been shown to be anxiogenic (Muchimapura, Fulford, Mason, & Marsden, 2002; Wiberg & Grice, 1963), which could confound interpreting the effects of exercise on cognitive function (Leasure & Decker, 2009; Stranahan, Khalil, & Gould, 2006). For 7 days, rats were allowed to acclimate to the vivarium before behavioral testing began. SHR rats were divided into five treatment groups that received an injection of saline or MPH (0.015625, 0.03125, 0.0625, or 0.125mg/kg) prior to the orienting procedure. Wistar rats were treated with saline. The same doses were used for the social interaction test, except that the 0.015625mg/kg dose was excluded since the 0.03125 and 0.0625mg/kg doses were without effect. Sample sizes are noted in Table 1.

Experiment 2—Sixty-eight female SHR rats were obtained from Harlan or Charles River Laboratories and maintained as in Experiment 1. Rats were divided into five groups. One group was a non-exercise control group that remained in the home cage without access to a running wheel. The remaining 4 groups of rats had access to a running wheel attached to the home cage for 2, 5, 10, or 21 days. Sample sizes are noted in Table 1.

Experiment 3—Twenty-four female SHR rats (Charles River Laboratories) were maintained as described in Experiment 1 and were divided into two groups (n=12 each): a saline-treated non-exercise control group (SAL-No EX) and a MPH-treated exercise group (MPH-EX). This experiment was specifically designed to determine if doses of exercise or MPH that alone were *insufficient* to affect orienting or social behavior would have significant effect

when they were combined. Thus, the highest doses that were without effect in Experiments 1 and 2 were used in Experiment 3. For orienting behavior, those doses were 0.015625 mg/kg of MPH and 2 days of exercise. For social behavior, they were 0.0625 mg/kg of MPH and 10 days of exercise. All procedures were conducted in accordance with Association for Assessment and Accreditation of Laboratory Animal Care guidelines and the Dartmouth College Institutional Animal Care and Use Committee.

Apparatus

Exercise—Rats in the exercise groups in Experiments 2 and 3 had access to a stainless steel running wheel (35.6cm diameter, 4.8mm rods placed 1.6 cm apart; Med Associates Inc., St. Albans, VT) via an opening in the side of the cage. An automatic counter mounted on the side of the apparatus monitored wheel rotations.

Orienting behavior—Unconditioned orienting behavior was assessed in a standard conditioning chamber (24cm × 30.5cm × 29cm; Med Associates, Inc.) connected to a computer and enclosed in a sound-attenuating chamber (62cm × 56cm × 56cm) equipped with an exhaust fan to provide airflow and background noise (~68dB). The chambers consisted of aluminum front and back walls with clear acrylic side walls and ceiling, and a grid floor. A dimly illuminated food cup was recessed in the center of one wall at a height of 5cm. The stimulus light was a 2.8-W bulb located on the center of the chamber wall opposite the food cup, 1 cm from the ceiling. A red house light (2.8W) was located on the ceiling of the sound-attenuating chambers to provide background lighting. Three pairs of photobeam sensors were mounted in the chamber and used to detect rearing behavior. The sensors were placed 15 cm above the grid floor and were evenly spaced along the wall so that a rearing response produced anywhere in the chamber would be detected by one of the sensors. A photobeam was also located across front of the food cup.

Social interaction—The social interaction procedure was conducted in a white plastic tub measuring 119.4 cm × 59.7 cm × 59.7 cm. In the center of the tub was a clear plexiglass cylinder (27.9 cm long × 7.6 cm diameter) containing an unfamiliar rat of the same strain and gender ('target rat'). There were five holes on each side of the cylinder (1.9 cm diameter), two holes on top (1.9 cm diameter), and one hole on either end (3.2cm and 1.9cm). A camera was mounted directly above the center of the tub and was used to videotape the session.

Drug treatment

In Experiments 1 and 3, MPH (Sigma-Aldrich, St. Louis, MO) was dissolved in saline and injected into the intraperitoneal cavity in a volume of 1.0 ml/kg. MPH was injected 10 min before the start of a behavioral test session. Rats in the control group were injected with saline 10 min before behavioral testing began.

Behavioral procedure

Wheel running—SHRs in Experiment 2 were provided with 24 hr access to a running wheel attached to their home cage for 2, 5, 10, or 21 days before behavioral testing. In Experiment 3, rats in the MPH-treated exercise group had access to the wheel for 2 days

before the orienting procedure because 2 days was the highest dose exercise that did not affect orienting. These rats were then placed back on the wheels for an additional 8 days before they were tested in the social interaction task because 10 days of exercise was the highest dose that did not affect social behavior. In both experiments, the door to the wheel was blocked off 2 hours prior to behavioral testing in order to minimize the potential for exercise-related fatigue. The number of wheel rotations was recorded daily.

Orienting behavior—During a single 32-min session, rats received 12 non-reinforced presentations of the stimulus light (10-sec duration). During the 10sec presentation period, the red house light was extinguished and the stimulus light flashed on/off at a frequency of 1Hz. The average inter-stimulus interval was 2.75 min.

Social interaction—The day following completion of the orienting procedure, rats were exposed to the social interaction apparatus (except in Experiment 3 where social interaction took place 8 days after the orienting procedure). At the beginning of the session each rat was placed in the corner of the tub and was allowed to explore the tub for 10 min. After the session all surfaces were cleaned with disinfectant to remove any odors.

Behavioral measures and data analysis

Exercise—Since a group of rats shared each wheel it was not possible to know the exact distance run by each individual rat. However, previous studies have shown that group-housed rodents spend approximately equal amounts of time on the wheel when it is freely accessible (Fox, Hammack, & Falls, 2008). Thus, we divided the number of wheel rotations recorded each day by the number of rats in the cage. The average daily distance run by an individual rat was then calculated by multiplying the number of rotations by the circumference of the wheel (1.12 m) to convert to meters.

Orienting behavior—During the unconditioned orienting session, breaks in the photobeams mounted on the walls of the chamber were monitored by a computer and used to measure orienting behavior during non-reinforced presentations of the light. Orienting was defined as rearing on the hind legs with both forepaws off the ground (Holland, 1977). The number of breaks in the three photobeams used to detect rearing behavior was summed for each trial, because previous studies indicate that it is unlikely that a rearing response will simultaneously break more than one photobeam (Keene & Bucci, 2007). The number of beam breaks was then summed across blocks of 4 trials in order to assess habituation of the unconditioned orienting response. A repeated measures analysis of variance (ANOVA) was used to assess differences in rearing using Block as the within-subjects variable and Group as the between-subjects variable. P-values were corrected with the Huynh-Feldt procedure and significant differences were subsequently analyzed using Fisher's PLSD test.

Social interaction—The primary measure of social interaction was the number of times the experimental rat approached and sniffed inside one of the holes in the cylinder that contained the target rat. In addition, walking around the cylinder with continuous sniffing was counted as an interaction. Exploration of other parts of the cylinder (i.e., areas without holes) was not counted as an interaction. Interactions initiated by the target rat when they

poked their noses out of the front hole were not scored unless the experimental rat reciprocated the interaction. The number of social interactions was analyzed using a one-way ANOVA with Group as the between-subject variable. Significant differences were subsequently analyzed using Fisher's PLSD test.

Locomotor activity—The recording of the social interaction session was also scored to assess the levels of general locomotor activity in each group of rats. Locomotor activity was measured to assess whether any differences that emerged in orienting behavior or social interaction might be due to drug effects on general activity levels. To measure locomotion, two lines were drawn perpendicular to the long side of the tub on the video screen, thus dividing the tub into three equal areas. A line crossing was counted when all four paws crossed over onto the other side of the line. The number of line crossings was analyzed using a one-way ANOVA with Group as the between-subject variable and significant differences were subsequently analyzed using Fisher's PLSD test. All statistical analyses were conducted using an alpha level of 0.05.

RESULTS

EXPERIMENT 1

Orienting behavior—The amount of orienting behavior displayed by SHR in the MPH groups is illustrated in Figure 1. A repeated measures ANOVA revealed a significant main effect of Block [$F(3, 112) = 16.7, p < 0.001$]. There was also a main effect of Group [$F(5, 56) = 3.6, p < 0.008$] and a significant Block $\times$ Group interaction [$F(10, 112) = 1.9, p < 0.05$]. Post hoc analysis of the main effect of Group revealed that the saline treated SHR exhibited more rearing behavior than all other groups ($ps < 0.03$), with the exception of the 0.015625 mg/kg MPH group ($p > 0.11$).

The Block $\times$ Group interaction was subsequently decomposed using repeated measures ANOVAs that compared rearing behavior across blocks in each group. Rearing behavior did not habituate in the saline treated SHR or SHR treated with a 0.015625 mg/kg dose of MPH ($p > 0.3$); in contrast, all other groups exhibited a significant decrease in rearing over blocks of trials ($ps < 0.03$). In addition, one-way ANOVAs were carried out to compare differences between the groups during each block. During Block 1 there were no differences in rearing behavior between groups [$F(5, 61) = 1.0, p > 0.4$], indicating that the amount of rearing behavior was initially comparable in all groups. However, there were significant group differences in the amount of rearing during Block 2 [$F(5, 61) = 4.7, p < 0.001$], with post-hoc tests revealing that saline-treated SHR reared more than all groups ($ps < 0.04$), except the 0.125 mg/kg MPH treated group, which was marginally significant ($p > .05$). During Block 3, there was also a significant group difference in the amount of orienting behavior [$F(5, 61) = 2.8, p < 0.03$], with post hoc tests revealing a significant difference between saline treated SHR and all other groups ($ps < 0.03$), except the 0.015625 mg/kg MPH treated group ($p > .2$).

Social interaction—Figure 2A illustrates the number of social contacts made during the social interaction task. A one-way ANOVA revealed a significant difference between groups in the number of interactions made with the target rat, [$F(4, 57) = 4.4, p < 0.004$]. Post hoc

tests revealed a significant difference between the saline treated SHR_s and the 0.125 mg/kg MPH group and saline treated Wistar_s ($p < .002$). No other MPH groups were significantly different from the saline treated SHR_s group ($p > 0.1$).

Locomotor activity—Locomotor activity during the social interaction task is shown in Figure 2B. A one-way ANOVA revealed no significant differences between groups in the number of line crossings made during the 10-min social interaction session, [$F(4, 57) = 1.4, p > 0.2$].

EXPERIMENT 2

Exercise—The average daily distance run by each rat in the exercise groups was 3.5 km for the 2 day exercise group, 4.4 km for the 5 day exercise group, 4.3 km for the 10 day exercise group, and 4.8 km for the 21 day exercise group.

Orienting behavior—The amount of rearing behavior exhibited by rats in each exercise group during non-reinforced presentations of the stimulus light is illustrated in Figure 3. A repeated measures ANOVA revealed a significant main effect of Block [$F(2, 126) = 16.1, p < 0.001$] and a main effect of Group [$F(4, 63) = 4.3, p < 0.005$] but no significant Block $\times$ Group interaction [$F(8, 126) = 0.6, p > 0.7$]. Post hoc analysis of the main effect of Group revealed that the non-exercise control group exhibited more rearing behavior than all other groups ($p < 0.02$), with the exception of the lowest dose of 2 days EX ($p > 0.09$). The first trial was compared across groups in order to rule out any confounding effects of exercise on rearing. A one-way ANOVA did not reveal any group differences in rearing during the first trial, [$F(4, 67) = 0.8, p > 0.5$], indicating that rats in all groups displayed similar amounts of orienting behavior to the initial presentation of the light.

Social interaction—Figure 4A illustrates the number of social interactions made during the 10 minute free-exploration session. A one-way ANOVA revealed a significant difference between groups in the number of interactions made with the target rat [$F(3, 49) = 3.3, p < 0.03$]. Post hoc tests revealed a significant difference between the non-exercise control group and the 21 day exercise group ($p < 0.01$). However, there were no differences between non-exercising SHR_s and the 5- and 10-day exercise groups ($p > 0.09$).

Locomotor activity—Locomotor activity during the social interaction task is shown in Figure 4B. A one-way ANOVA revealed no significant difference between groups in the number of line crossings made during the 10-min social interaction session [$F(3, 49) = 2.2, p > 0.09$].

EXPERIMENT 3

Exercise—The average daily distance run by each rat in the exercise group was 3.3 km for the first 2 days of exercise and 3.2 km for the entire 10 days of exercise.

Orienting behavior—Rearing behavior during the unconditioned orienting session is shown in Figure 5. A repeated measures ANOVA revealed a significant main effect of Block [$F(2, 44) = 12.9, p < 0.001$]. There was also a main effect of Group [$F(1, 22) = 13.0,$

$p < 0.003$] and a significant Block $\times$ Group interaction [$F(2, 44) = 4.1, p < 0.03$]. The Block $\times$ Group interaction was subsequently decomposed using repeated measures ANOVAs that compared rearing behavior across blocks in each group. As expected, rearing behavior did not habituate in the non-exercise control group ($p > 0.3$), while the MPH-treated exercise group significantly decreased rearing across blocks ($p < 0.001$). In addition, one-way ANOVAs comparing the groups on each block revealed no significant differences during Block 1, indicating that the amount of orienting behavior was initially comparable across both groups [$F(1,23) = 0.7, p > 0.4$]. However, there were significant group differences in the amount of rearing behavior during Block 2 [$F(1,23) = 9.3, p < 0.007$] and Block 3 [$F(1,23) = 20.7, p < 0.001$].

Social interaction—Figure 6A illustrates the number of social interactions made during the social interaction task. A one-way ANOVA revealed no significant difference between groups in the number of interactions made with the target rat, [$F(1,23) = 0.23, p > 0.8$].

Locomotor activity—Figure 6B shows the number of line crossings made during the social interaction task. A one-way ANOVA indicated no significant difference between groups in locomotor activity during the 10-min session [$F(1,23) = 3.04, p > 0.09$].

DISCUSSION

The present study tested the effects of exercise, MPH, or a combination of exercise and MPH on ADHD-like behaviors in the SHR strain, a commonly used animal model of ADHD. One goal of this study was to determine the amount of MPH or exercise that is necessary to affect orienting behavior and social interaction. A second goal was to determine if exercise and MPH could be combined to produce additive effects on these behaviors.

Orienting behavior

The results of Experiments 1 and 2 demonstrate that non-exercising or saline-treated SHRs exhibit high levels of rearing behavior to a non-reinforced visual cue. One interpretation of this finding is that SHRs have difficulty ignoring distracting, irrelevant stimuli. Indeed, orienting behavior towards a visual stimulus is an often used measure of attentional processing of the stimulus (Gallagher et al., 1990; Kaye & Pearce, 1984; Lang et al., 1997) and rearing behavior should rapidly decrease (habituate) when the cue is not followed by reinforcement. The habituation of the orienting response is thought to reflect an adaptive decrease in attention to a behaviorally irrelevant stimulus (Gallagher et al., 1990; Holland, 1997; Kaye & Pearce, 1984). The high levels of rearing behavior displayed by SHRs are consistent with their hyper-responsive phenotype (Sagvolden et al., 2005) and suggest they are easily distracted by irrelevant stimuli.

MPH treatment significantly reduced excessive orienting behavior in SHRs, suggesting that treated rats were better able to ignore the irrelevant visual stimulus. Rats that were treated with either 0.03125, 0.0625, or 0.125 mg/kg dose of MPH exhibited a robust decrease in orienting behavior across trials and their rearing behavior was indistinguishable from normo-active Wistar rats, while the lowest dose of 0.015625 mg/kg did not reduce rearing behavior in SHRs. Similar to these findings, other studies have found that MPH can

facilitate other aspects of attentional function, including sustained attention and the ability to maintain attention to behaviorally relevant cues (Thanos et al., 2010; Berridge et al., 2006). The present data add to these studies by focusing on distractibility, and aspect of attentional function that has received comparatively less research attention. MPH increases the levels of dopamine and norepinephrine in brain tissue by blocking the transporters responsible for their reuptake. For instance, MPH has been shown to increase the extracellular levels of dopamine and norepinephrine in the prefrontal cortex (PFC) and to increase levels of dopamine in the striatum and nucleus accumbens (Berridge et al., 2006; Bymaster et al., 2002). Although it was not directly tested here, the doses of MPH used in the present experiment likely affected transmitter levels in PFC rather than in the striatum since prior studies have found that low doses of MPH improve PFC-dependent cognitive functions and substantially increase norepinephrine and dopamine efflux within the PFC (Berridge et al., 2006). In contrast, in areas outside of the PFC these doses have minimal impact on norepinephrine and dopamine efflux (Berridge et al., 2006).

Exercise also reduced excessive orienting behavior. SHRs that had access to a running wheel for 5, 10, or 21 days exhibited a robust decrease in orienting behavior across trials. In comparison, 2 days of access to the running wheel was insufficient to affect rearing behavior compared to non-exercising SHRs, indicating more than 2 days of exercise is necessary to improve this aspect of attentional function. Because the effects of exercise on orienting behavior could reflect increased fatigue or other non-specific effects of exercise, rearing behavior during the first presentation of the visual stimulus was analyzed. There were no group differences in the amount of rearing behavior exhibited during the first presentation of the visual stimulus indicating that exercise did not have a general effect on the ability to rear due to fatigue. Moreover, the wheels were blocked 2 hours before rats were tested, further minimizing the chance that fatigue could affect the orienting response.

A comparison of effect sizes (Cohen's d) indicated that 5 days of exercise ($d = 1.04$) was nearly as efficacious as the 0.03125 mg/kg dose of MPH ($d = 1.5$) in reducing orienting behavior. The finding that exercise is as effective as MPH in reducing orienting behavior suggests that physical exercise might be a useful adjunctive or replacement therapy for attentional dysfunction in persons with ADHD. Indeed, other studies that have compared the effects of exercise to MPH in SHRs and found that exercise was as effective as MPH in reducing hyperactivity and improving spatial learning and short-term memory (Kim et al., 2011). Interestingly, when doses of exercise (2 days) and MPH (0.015625 mg/kg) that alone were unable to reduce orienting behavior were combined, there was an additive effect of the two treatments. Notably, exercise and MPH have similar neurobiological effects in that both increase the expression of tyrosine hydroxylase and BDNF expression in the striatum and hippocampus, respectively, which are decreased in SHRs compared to control rats (Kim et al., 2011). Together these findings indicate that exercise and MPH may act through similar neurobiological mechanisms to improve ADHD-like behaviors in SHRs.

In most of our prior studies (Robinson et al., 2011, 2012) we have consistently found that untreated female SHRs exhibit little or no habituation of the orienting response. This was also the case in Experiments 1 and 3 in the present study. However, on occasion, we have observed some habituation in female SHRs as illustrated in Experiment 2. In the current

study, this appears to be attributable to the behavior of a few rats that exhibited substantially lower levels of orienting compared to the rest of the rats in the control group. Since the behavior of these rats did not reach a threshold for exclusion, their data was included in the analysis and thus contributed to the lower levels of orienting observed particularly during the second and third blocks. Nonetheless, the SHR control group in Experiment 2 still exhibited significantly more orienting behavior than the rats in the experimental group. Moreover, the amount of orienting behavior was comparable between the groups on the very first trial, indicating that the effect was not merely due to exercise-induced fatigue in the experimental group.

Social interaction

To date, there have been very few studies that examined the effects of exercise or MPH on social behavior in SHRs. Social deficits are a common symptom in ADHD (Pelham, Fabiano, & Massetti, 2005; Whalen & Henker, 1992), but a relatively understudied aspect of the disorder (Swanson et al., 2001). The results from Experiments 1 and 2 indicate that MPH or exercise can decrease hyper-social behavior in SHRs. Indeed, 21 days of exercise or a 0.125 mg/kg dose of MPH reduced the number of interactions initiated by SHRs during the 10-minute social interaction session. The reduction in social interactions was not accompanied by a decrease in locomotor activity indicating the results were not due to fatigue effects or disruptions in general activity levels. Thus, physical exercise may have potential use as a replacement therapy for psychostimulants in treating impairments in social behavior. However, additional research is needed on the nature of social dysfunction in ADHD, which is likely, a complex phenomenon with characteristics that are not fully realized in the rat model used here.

Interestingly, a longer duration of exercise was needed to impact social behavior compared to orienting behavior. Only 5 days of exercise was needed to affect orienting behavior, whereas 21 days of exercise was needed to impact social behavior. One explanation of these results is that more exercise is needed to produce sufficient neurobiological changes to affect social behavior. A higher dose of MPH was also needed to impact social behavior compared to orienting behavior, supporting the idea that a sufficient alteration to the noradrenergic and/or dopaminergic systems is needed to improve social behavior. Indeed, higher doses of MPH have been shown to elicit more dopamine and norepinephrine release in the PFC, compared to lower doses (Berridge et al., 2006). In addition, we found that exercise and MPH could be combined to affect orienting behavior but not social behavior. The different effects of exercise on attention and social behavior suggest that exercise may have dissociable effects on these behaviors.

Alternatively, it is possible that hyper-orienting and hyper-social behavior observed in SHRs both reflect a general deficit in habituation rather than deficits in attention and social behavior per se. Thus, the differences in sensitivity of orienting behavior and social behavior to exercise or MPH could merely be due to different habituation processes to a novel light compared to an unfamiliar rat. Although future studies are needed to test this directly, it is notable that other studies have found that exercise and MPH can impact social behavior in rats using the same procedures used here (Hopkins et al., 2009) as well as different

procedures (Ko et al., 2013; Vanderschuren et al., 2008). Exercise was found to decrease aggressive social behaviors in SHR (Ko et al., 2013) and MPH treatment decreased social play behavior in adolescent Wistar rats (Vanderschuren et al., 2008). The effect of MPH on social behavior was blocked by pretreatment with the α -2 adrenergic receptor antagonist RX 821002 but was not altered by the dopamine receptor antagonist cis-(Z)-flupenthixol, the α -1 adrenergic receptor antagonist prazosin, or the β -adrenergic receptor antagonist propranolol, suggesting MPH impacts social behavior specifically through an α -2 receptor-mediated mechanism (Vanderschuren et al., 2008). Because exercise decreased hyper-social behavior in the SHR, much like MPH, it may suggest that exercise impacts social interaction through a similar noradrenergic mechanism.

Children with ADHD also experience improvements in social behavior after exercise interventions (Chronis et al., 2006; Kang et al., 2011). For example, children with ADHD that participated in a 90-min athletic activity twice a week exhibited enhanced social skills and cooperativeness (Kang, Welcher, Shelton, & Schuman, 2011). In addition, less anxiety and depression and fewer social problems were observed in ADHD children after a 10-week physical activity-training program (Verret, Guay, Berthiaume, Gardiner, & Béliveau, 2012). These studies suggest that exercise may be a useful therapeutic tool in treating social dysfunction in children with ADHD. However, additional research into the types of social behaviors that can be affected by exercise and the mechanism through which exercise may be impacting social behavior is needed.

Conclusions

In summary, the present study systematically compared the effects of physical exercise and MPH on ADHD-like behaviors. Physical exercise and MPH both reduced excessive orienting behavior and hyper-social behavior in SHRs. These findings replicate the results of our prior study (Robinson et al., 2012) that used only a single dose of exercise (21 days) or MPH (0.125 mg/kg) and extend that work identifying the minimum doses of MPH and exercise that are needed to improve attention and social behavior. Moreover, combining 21 days of exercise with 0.125 mg/kg of MPH in our prior study did not produce an additive effect, likely due to a floor effect. That was confirmed here by demonstrating that doses of exercise (2 days) and MPH (0.015625 mg/kg) that had no effect alone could be combined to significantly impact orienting behavior. However, the exact mechanism through which exercise improves attentional function remains unclear and awaits future experiments. Given the intense interest in developing new therapies for ADHD, these findings have important implications for using physical exercise as an adjunctive or replacement therapy for psychostimulants. In addition, these findings address current gaps in the literature regarding the paucity of studies on ADHD in adults, and the lack of research on female subjects.

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Figure 1.

Unconditioned orienting (rearing behavior) observed during repeated presentations of a non-reinforced visual stimulus. The number of breaks in the photobeams used to detect rearing behavior is shown on the y-axis. Blocks of trials (first 4, middle 4, and last 4 trials) are shown on the x-axis. Rearing behavior did not habituate in the saline-treated SHRs. MPH treated SHRs did show habituation of the orienting response across blocks of trials, with the exception of the 0.015 mg/kg dose. Data are means $\pm$ the *SE*.

A. **B.** **Figure 2.**

The average number of social interactions made by each group (panel A). The number of social interactions reflects the number of contacts made by an experimental rat with holes in the cylinder containing the target rat during a 10-min session. A 0.125 mg/kg dose of MPH reduced the number of social interactions initiated, as compared to saline-treated SHRs. No other MPH doses significantly reduced social interaction behavior. There were no differences in locomotor activity during the session (panel B). Locomotor activity data

reflect the number of times rats in each group crossed either of two lines that separated the arena into thirds. Data are means $\pm SE$.

Figure 3.

Unconditioned orienting (rearing behavior) observed during repeated presentations of a non-reinforced visual stimulus. The number of breaks in the photobeams used to detect rearing behavior is shown on the y-axis. Blocks of trials (first 4, middle 4, and last 4 trials) are shown on the x-axis. There was a significant difference between non-exercising SHR and all other groups, with the exception of the 2 day exercise group. Data are means $\pm$ the *SE*.

A. **B.** **Figure 4.**

The average number of social interactions made by each group in (panel A). The number of social interactions reflects the number of contacts made by an experimental rat with holes in the cylinder containing the target rat during a 10-min session. 21 days of exercise reduced the number of social interactions initiated, as compared to non-exercising SHRs. 5 and 10 days of exercise did not alter social interaction behavior. Locomotor activity data reflect the number of times rats in each group crossed a line that separated the arena into thirds (panel

B). There were no differences in locomotor activity between controls and exercising SHR. Data are means $\pm$ SE.

Figure 5.

Unconditioned orienting (rearing behavior) observed during repeated presentations of a non-reinforced visual stimulus. The number of breaks in the photobeams used to detect rearing behavior is shown on the y-axis. Blocks of trials (first 4, middle 4, and last 4 trials) are shown on the x-axis. Rearing behavior did not habituate in the non-exercising, saline-treated SHRs. SHRs that received 2 days of exercise and a 0.015625 mg/kg MPH did show habituation of the orienting response across blocks of trials. Data are means $\pm$ the *SE*.

Figure 6.

The average number of social interactions made by each group in (panel A). The number of social interactions reflects the number of contacts made by an experimental rat with holes in the cylinder containing the target rat during a 10-min session. A 0.0625 mg/kg dose of MPH combined with 10 days of exercise did not reduce the number of social interactions initiated, as compared to saline-treated non-exercising SHR. There were no differences in locomotor activity during the session (panel B). Locomotor activity data reflect the number of times

rats in each group crossed either of two lines that separated the arena into thirds. Data are means $\pm SE$.

Table 1

Samples sizes for the groups in Experiments 1 and 2

Experiment 1	Orienting behavior	Social interaction
SHR saline	13	13
0.015625 mg/kg MPH	10	n/a
0.03125 mg/kg MPH	10	10
0.0625 mg/kg MPH	12	14
0.125 mg/kg MPH	9	13
Wistar saline	8	8
Experiment 2		
NX	16	13
2 days EX	12	n/a
5 days EX	12	15
10 days EX	16	11
21 days EX	12	11