Effects of a Selective D^4 Antagonist on Repetitive Behaviors in a Genetic Model of ADHD in Rats

By

LINA PARKS

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Approved by:

Dr. Bethany Plakke Anderson

Abstract

This study aimed to investigate the efficacy of the selective D4 antagonist, A-412997, on repetitive behaviors of a rodent model of ADHD. We hypothesized that the drug would have no significant effect on repetitive behavior compared to the control groups. This would indicate that the D4 antagonist is comparable to and more effective than other treatments of ADHD, such as methylphenidate. Our study found significant strain effects for all three behaviors (digging, inactivity, and marble interaction) between the Long Evens control group and the spontaneously hypertensive rat (SHR) experiment group. We found that there were no significant impacts of drug exposure on marble interaction time and bouts, time spent inactive, bouts inactive, or digging time. Overall, this indicated that A-412997 could be an effective treatment for ADHD when looking at the possible side effects. Future directions should investigate other possible repetitive behaviors, in order to be sure that there is no confounding behavior that is impacted by this drug.

Attention-Deficit/Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder that displays persistent patterns of inattentiveness and/or hyperactivity (DSM-V, 2017). ADHD most often affects children, having an impact on their functioning in school and social settings, but symptoms may continue past childhood and into adulthood. Studies show that ADHD occurs in about 3%-6% of school-aged children, with boys being diagnosed more than girls in a ratio of 3:1 (Tannock, 1998). Those with ADHD have been known to struggle with motivation, attention, executive functioning, and emotional control.

The dopaminergic system and dysregulation of the dopamine receptors (D1-D5) are hypothesized to be one mechanism behind the disorder. Dopamine (DA) has been known to help regulate neural motor control, cognition, emotion, and motivational and reward processes (Wu et al., 2012). Dysfunction of the dopaminergic system can account for many of the symptoms of ADHD, such as executive impairments, hyperactivity, impulsivity, inattentiveness, and emotional dysregulation. Treatments for ADHD have focused on the D4 receptor for its role in the cognitive symptoms. Previous research has investigated this question at length and studies have found that the D4 receptor has a possible link to ADHD as a candidate receptor to target in treatment (Brownman et al., 2005).

ADHD Treatment

One of the most common treatments for ADHD is Dopamine (DA) reuptake inhibitors, such as methylphenidate (MPH). Ritalin and Concerta are examples of MPH-based treatments for ADHD that inhibit the reuptake in the DA transporter, DAT, increasing extracellular DA (Volkow et al., 1998). Another common treatment of ADHD is DA promoters, such as Adderall, which stimulates the release of DA at the synapse, therefore increasing

extracellular DA levels (Woolley et al., 2008). These medications also change levels of other catecholamines which may result in other side effects (Wilens, 2008).

Due to the nonspecific DA actions of these drugs, which result in unwanted side effects, this project investigated the impacts of a specific DA receptor agonist. The drug used in this experiment, A-412997, is a highly selective DA-D4 receptor agonist that has been shown to have a procognitive effect on a variety of cognitive tasks (Woolley et al., 2008). Along with that, this drug has been shown to have less of an effect on the rest of the catecholaminergic system, which can reduce the unwanted side effects that are triggered by these interactions (Wooley et al., 2008). By testing this more selective form of treatment, we may be able to decrease the number of side effects associated with other ADHD treatments, such as potential drug abuse linked to methylphenidate, because the D4 drug does not result in reward-seeking behaviors (Woolley et al., 2008).

Genetic Model of ADHD in Rats

To investigate the effectiveness of the DA antagonist on repetitive behaviors, we used spontaneously hypertensive rats (SHR) as a genetic model of ADHD. Previous studies have found that SHRs are a useful model of ADHD in rats (Brownman et al., 2005; Coa et al., 2012; Sagvolden et al., 2000). When investigated as a genetic model of ADHD, SHRs exhibit all the major characteristics of ADHD, such as difficulties with sustained attention, hyperactivity, motor impulsiveness, and cognitive impulsiveness (Sagvolden et al., 2000)

When SHRs were studied in an attentional set-shifting task (ASST), SHRs exhibited poorer performance on the task compared to control strains. SHR showed the fastest latency in ASST, showing impulsivity when it comes to decision-making. The set-shifting task with SHR showed that they are impaired in discrimination learning, reversal learning, and

attentional set-shifting (Coa et al., 2012), which is consistent with the symptoms of ADHD in humans. Since SHR performed similarly to humans with ADHD, it suggests that SHR can serve as a genetic model that exhibits face and construct validity for ADHD, compared to control strains (Sagvolden et al., 2000).

Current Experiment

Although there are still medications available for treating ADHD, such as Methylphenidate and Amphetamines, it is still important to seek more effective and efficient options for those diagnosed with ADHD. Our current experiment aimed to use the information from previous studies to investigate the efficacy of the highly specific dopamine receptor antagonist on repetitive behaviors in an ADHD model of rats. Our lab project assessed the effectiveness of the drug in treating cognitive attention symptoms in SHRs. Other projects within the lab are aimed at testing the overall efficacy of the drug, through tasks such as an attentional set-shifting task (ASST). This project, though, was designed to examine the side effects, therefore, no significant differences between experimental groups' repetitive behavior were expected. This would indicate that the treatment could be classified as an effective treatment for ADHD, competing with more common treatments, such as Methylphenidate.

Methods

Subjects. A total of 41 rats were used in this experiment. SHR was utilized as a validated genetic model of ADHD in rodents and Long-Evans (LE) rats were used as a control strain in our experiment. The rats used arrived at Kansas State University at a target weight of 225-2500 grams, which would be Postnatal Day (PND) 56-63 for both strains. The rats were housed as pairs with their same strain in a room with a 12-hours light: dark schedule (lights in at 7:00 a.m.)

with free access to water. All procedures proposed were in accordance with the Institutional Animal Care and Use Committee at Kansas State University.

Procedure

Marble-Burying Test. For the Marble-Burying (MB) test, rats were placed in a double-wide cage filled with pine bedding. Rats were then given 5-minutes to habituate to the box and then transferred to a holding cage. While in the holding cage, twenty marbles were arranged in the double-wide cage in a 4x5 grid pattern in the center of the arena. The rat was placed back into the arena for 10-minutes and taken out once the time expired. A marble was classified as buried if 3/4ths were covered by bedding. Trials were also recorded and coded later using LimeLight video software (ActiMetrics; USA). All behaviors were coded by the same person to assure that there was little to no variability in what the researcher classified as that specific behavior. Behaviors coded using LimeLight consisted of marble interaction, inactivity, and digging. The study by Ku et al., (2016) provided the definitions of marble interaction, inactivity, and digging. Behaviors had to occur for at least 1 s to be coded. A before and after picture of the marble arrangement was taken and scored by another observer to ensure the correct marble counts were made.

Drug. A-412997 is a highly selective D4 agonist, such that it shows almost no effect on other DA receptors and shows a higher DA level compared to other commonly used D4 drugs (Moreland et al., 2005). This drug has been shown to be present in the blood-brain barrier up to over 60 minutes after administration (Moreland et al., 2005). The drug was mixed in sterile saline water in Bluemont Hall and was stored properly at -5 Celsius after mixing. The drug was administered orally to the rats for 14 days to mimic treatment for ADHD in humans and maintained throughout training and testing. Chronic oral administration was chosen because it is

the most similar to how humans receive their treatment, compared to one or a few acute treatment doses.

Data Analysis

A two-way ANOVA was used to investigate group differences, in which strain and condition are two-level categorical predictors. An analysis was run for each of the three behaviors: digging, inactivity, and marble interaction. An analysis was run for both the percent time of each behavior, as well as the bouts of the behavior for each rat. Significant interactions were followed by tukey post hoc analysis.

Results

Digging Behavior

This first behavior that was analyzed was digging. For percent time spent digging, there was a significant main effect of strain ($F(1, 41) = 12.28$, $p = 0.0011$) and an interaction ($F(1, 41) = 4.128$, $p = 0.0487$), See Figure 1.

Tukey post hocs that were significantly different were the comparison between LE drug and SHR control groups ($p = 0.0068$, 95% CI= -4.712, -0.5968) as well as the comparison between strains in control groups ($p = 0.0017$, 95% CI= -5.223, -0.9972). The important comparison between the drug on LE digging time was not significant nor was the effect of the drug on the SHR group. Analyzing the bouts of digging showed that there was a significant effect of strain ($F(1, 41) = 7.837$, $p = 0.0078$) and interaction ($F(1, 41) = 2.867$, $p = 0.0980$), See Figure 2. Tukey

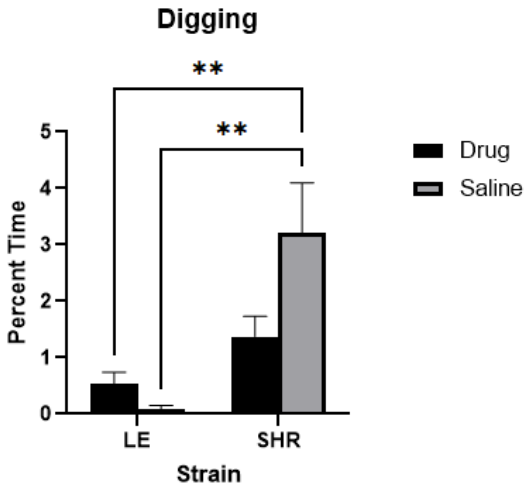

Figure 1: Percent time of digging. SHR rats dug for a greater percent time compared to LE rat

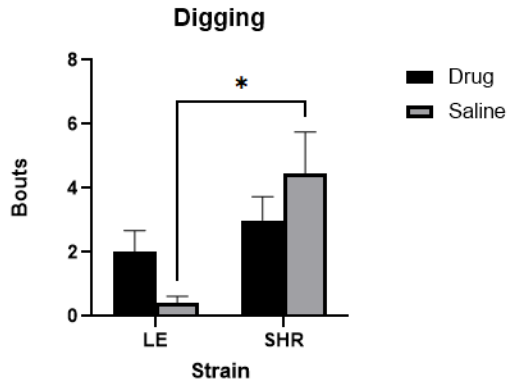

Figure 2: Bouts Digging. SHR had more bouts of digging compared to LE rats

post hoc that was significantly different was the comparison between the control groups (p= 0.0136, 95% CI= -7.461, -0.6616). There was no significant impact of the drug on the bouts of digging between the groups. While there was a significant effect of strain in both percent time and bouts, the amount of digging for SHRs is uncharacteristically low, which is surprising. It would be beneficial to look at other behaviors not examined here to understand if time was spent on other repetitive behaviors such as grooming.

Inactive time

There was a significant main effect of strain when looking at the percent time inactive ($F(1, 41) = 19.22$, $p = <0.0001$), Figure 3. The main effect illustrates that SHR rats overall (regardless of drug administration) were more inactive than controls. This is a surprising result as SHRs are usually more impulsive and hyperactive than LE rats. The analysis of bouts of inactivity shows a significant strain effect ($F (1, 41) = 5.816$, $p=0.0204$) and no effects of the drug, Figure 4, meaning that SHR is more inactive compared to LE rats. The fact that there was no effect of the drug was the predicted outcome and demonstrates it is not

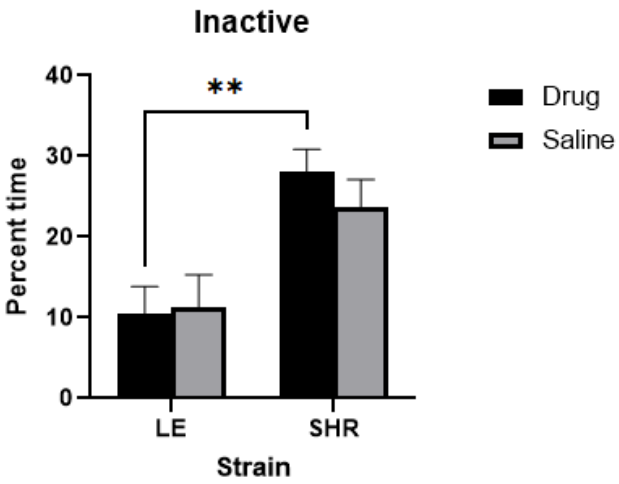

Figure 3: Percent Time inactive. SHR spent a greater time inactive compared to LE rats.

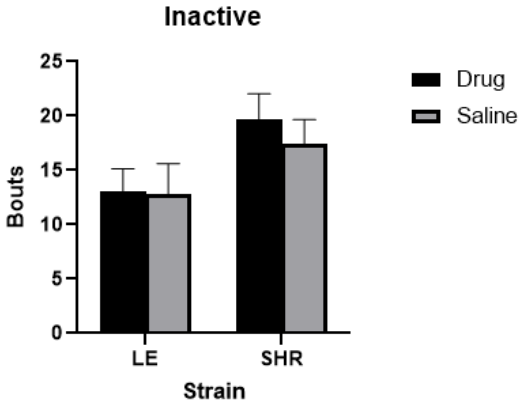

Figure 4: Bouts of inactivity. SHR had more bouts of inactivity compared to LE rats.

driving different behavior profiles in LEs or SHRs.

Marble Interaction

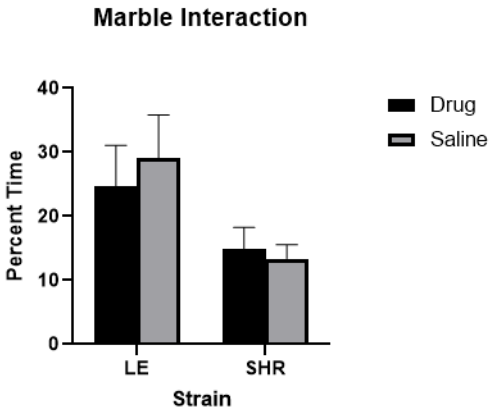

Figure 5: Percent time spent interacting with marbles. LE rats spent more time interacting with the marbles compared to SHR.

For the overall percent time spent interacting with the marbles, there were significant strain effects (F (1, 41) = 7.040, p=0.0113), Figure 5, which is inconsistent with the background of the strains. We expect SHR to be interacting more with marbles, but our results show that they are consistently interacting with the marbles less than LEs. It could be beneficial to investigate other repetitive behaviors to possibly explain why less time was spent interacting with the marbles. There were no significant interactions to report for the percent time of marble interaction. Similarly, when looking at bouts of marble interaction, there were no significant interactions to report, as well as no drug or strain differences, Figure 6. It is interesting to note that, while the percent time spent interacting with the marbles was low, the bouts of interaction were fairly similar between the two strains. I hypothesize that this may be due to impulsivity and hyperactivity. SHR seems to not be spending much time interacting with the marbles with each bout, while LE rats will spend more time interacting with the marble for each bout.

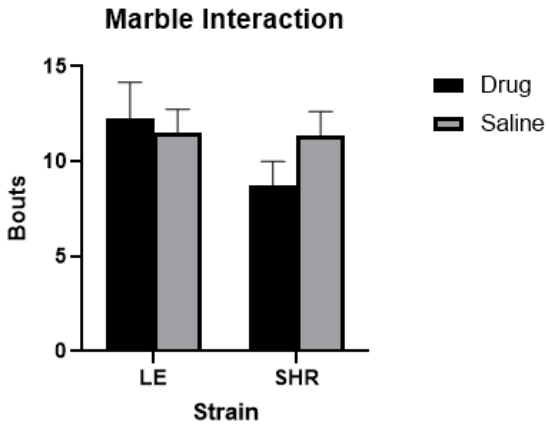

Figure 6: Bouts of Marble Interaction. There were no significant strain differences in terms of bouts of marble interacction.

After analyzing these behaviors, we also ran an ANOVA and multiple comparisons on the number of marbles buried for each rat. What we found was that there was so significant interaction or difference between the marble buried, regardless of strain or treatment, see Figure 7.

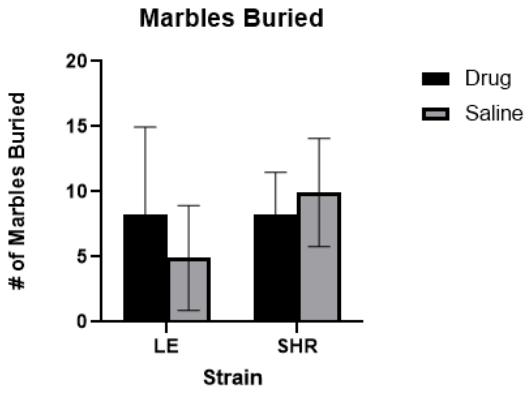

Figure 7: Number of Marbles Buried. There was so significant difference between strain or treatment for the number of marbles buried.

Discussion

Summary

This experiment aimed to investigate the impacts of a highly selective dopamine receptor antagonist on repetitive behaviors of a genetic model of ADHD in rats, compared to a control strain. We hope that this research will assist in limiting side effects that are commonly associated with current ADHD treatments, such as methylphenidate. We expected to find no differences between drug and no drug groups when SHRs were treated with the DA antagonist. The marble task was run to assess excessive repetitive behavior. SHRs had more bouts of digging and spent slightly more time digging than LEs. While SHRs dug more than LEs, most rats did not dig much. SHRs were also more inactive than LEs which is an unexpected result. The strain background of SHRs indicates that they should be locomoting more than controls when it comes to activity. SHR should be doing more to show repetitive behaviors, as indicated by previous research. A way to truly investigate this phenomenon would be to track and analyze the total distance traveled. It could be a case that they are running more but taking more breaks in between the hyperactivity. We found that there were no significant impacts of drug exposure on marble interaction time and bouts, time spent inactive, bouts inactive, or digging time. This indicates that the selective

dopamine antagonist, A-412997, may be an effective treatment for ADHD since it shows the little modulating effect on repetitive behaviors in the experimental group, compared to controls.

Limitations

This project coded a few behaviors but other behaviors that were not coded were exploration and grooming. This means that there could have been substantial time spent on these other behaviors that indicate ADHD-like behaviors since rats did not spend much time on behaviors like digging. Another limitation is that there is evidence that Long-Evans may not be the best control strain to use to compare ADHD symptoms. When conducting future research, a Wistar strain should be considered to properly evaluate the effects of ADHD on SHRs (Sagvolden et al., 2009).

Future Directions

The results of this experiment have a great impact on the discussion regarding the treatment of ADHD. Moving forward, it would be beneficial to examine the other behaviors, such as grooming and exploration, since there is an indication that there is a significant amount of time that the rats are participating in other behaviors than the ones examined here, along with the question of why the SHR rats did not engage in more of the repetitive behaviors that we analyzed here. It could be the case that they spent more time grooming or exploring. Future studies should also look at the total distance traveled to account for hyperactivity compared to inactivity. Another aspect that could be examined in the future is the effects of gender and age on the effects of this drug, in order to see if this treatment is generalizable to all populations.

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