Effects of Dopamine Receptor Activation and Antagonism on Social Motivation in Mice

by

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Abstract

Dopamine (DA), a neurotransmitter, plays a role in motivation, learning, mating, and aggression in humans. The present study experimentally investigates the effects of mesolimbic DA receptor activation and antagonism on social motivation in male and female C57BL/6J mice. Subjects (N=60), aged 8-10 weeks, were randomly assigned to receive Levodopa, DA antagonist, or saline intraperitoneal (i.p.) injections before completing a social motivation task. A stopwatch was used to record the time it took for a mouse to cross barriers of increasing height and the time spent in nose-nose orientation with the stimulus mouse. Two mixed-design, three-way ANOVAs were used to explore the dependent variables of time spent sniffing and time spent crossing barriers. The results indicated a number of significant interactions. Levodopa did not significantly alter time to cross barriers or sniff time as compared to saline, while the DA antagonist significantly increased the time spent crossing barriers and significantly decreased sniff time. Between-subjects comparisons indicated sex effects for both time spent sniffing and time spent crossing barriers.

Table of Contents

List of Tables and Figures

CHAPTER I

Introduction

Dopamine (DA), a neurotransmitter commonly found in the central nervous system (CNS) of humans and many animals, is classically involved in reward-motivated behavior. Neurons located in the ventral midbrain and the cerebral cortex transmit DA in two modes: tonic and phasic mode (Grace et al., 2007). The tonic mode is crucial for enabling the normal functions of neural activity by maintaining a constant, baseline level of DA in neural structures. In the phasic mode, DA neurons dramatically increase or decrease their firing rates, which causes significant changes in DA concentration lasting several seconds (Schultz, 2007). In Schultz's "Predictive Reward Signal of Dopamine Neurons" (1998), it was found that the phasic DA responses are initiated by multiple types of rewards and reward-related sensory signals. The phasic mode also allows for DA to meet its role in motivational control and as an incentive cue that promotes instant reward-seeking (Berridge and Robinson, 1998). Natural rewards, such as food and water, often produce phasic bursts of DA activity. Phasic dopamine signaling is also classically associated with the rewarding properties of addictive substances (Schultz, 1998).

New Findings on the Role of Dopamine in Motivated Behavior

Recent research has suggested that other types of rewarding stimuli are also associated with DA. For example, positron emission tomography (PET) studies demonstrate that monetary rewards won during gambling games are associated with phasic DA release in the striatum of participants (Zald et al., 2004). Likewise, rewarding

activities such as playing video games and listening to pleasurable music have been associated with DA release in the striatum (Koepp et al., 1998; Salimpoor et al., 2011).

DA has newly been associated with social behaviors. Several studies highlight the role of striatal DA for socially motivated behaviors such as mating and maternal care. Data from functional imaging research in humans demonstrate striatal excitation for a range of rewarding social stimuli such as "beautiful faces, positive emotional expressions, own social reputation, and maternal and romantic love" (Krach et al., 2010). Nevertheless, the reward processing of social stimuli goes beyond simply releasing DA in the striatum. Three dopamine pathways—mesolimbic, mesocortical, and nigrostriatal—are regarded as the reward pathways. The rewards these pathways provide are due to the activation of all three circuits in the experiencing and the anticipation of social reward. By doing so, the association between a stimulus, or behavior, and the reward is reinforced. With the reoccurring stimuli resulting in reward, the associations become stronger through a process called long-term potentiation (Meck, 2006).

However, in addition to prosocial interaction, dopamine is also associated with aggression. In a study done by Schwartzer and colleagues (2013), it was found that the observed increases in aggression following repeated winnings in fights may indicate a learned behavior due to DA activity, which acts to reinforce the behavior. In many animal studies, hyperactivity in the DA systems was linked to an increase in impulsive aggression (Harrison et al., 1997). And in rodents, increased DA levels were observed before, during, and after aggressive fights (Seo et al., 2008).

DA has recently been associated with social motivation, also. In an investigation about "Postpartum changes in affect-related behavior and VTA dopamine neuron activity

in rats," the data suggest that parturition results in reduced DA activity and low social motivation in the postpartum period (Rincón-Cortés & Grace, 2020). Because dopaminergic neurotransmission plays a vital role in incentive motivational procedures, recent research seeks to investigate the role of dopamine in social reward—like social play. In a study that explores rats in isolation carrying out an operant conditioning task, it was found that rats that were isolated for a longer duration and treated with the dopamine reuptake inhibitor met the criterion necessary for social play (Achterberg et al., 2016).

The Role of Mice in Research

Mice are the primary species of preference when it comes to pre-clinical trials and are the ideal animal model to study human diseases due to their physiological, anatomical, and genetic resemblance to humans (Bryde, 2013). A number of mouse models, such as inbred strains, knockout (KO), and transgenic mice are used in various research (Tam & Cheung, 2020). For example, mouse strains like BALB/c and C3H while useful in immunological studies, they also carry a high sensitivity to mutagenesis (Beck et al., 2000). Mus musculus, C57BL/6 (B6), is a standard inbred strain of laboratory mice known for its genetic stability. It is widely used in research due to its easy breeding and the availability of congenic strains (Frohlich, 2020). This strain is also utilized in behavioral studies due to its high physical activity and the ability to quickly learn different tasks (Bryant, 2011). Additionally, the strain is isogenic with abundant phenotypic, genetic, and genomic data, including a complete reference genome (Sarsani, 2019).

Theoretically, the principle of an inbred strain is that individuals share the same homozygous allele for every DNA sequence in the genome, making them genetically indistinguishable. However, not all B6 substrains are created equally. The C57BL/6N (B6/N) was derived from the original C57BL/6J (B6/J) strain and has several phenotypic and genetic differences (Bryant, 2011). These phenotypic differences allow for substantial flexibility when it comes to studying different behaviors. Replicable differences such as anxiety and fear learning are greater in B6/N mice than B6/J, while rotarod performance and pain sensitivity are greater in B6/J mice (Bryant et al., 2008; Matsuo et al., 2010). Nonetheless, choosing among the B6 substrains, as opposed to other inbred strains, allows for reverse genetic studies, like transgenic and knockouts, to be possible (Bryant, 2011).

Mice continue to be the species of interest when it comes to attempting to understand the effects of drugs of abuse. The B6/J strain has been especially popular in alcohol studies due to its ability to drink more alcohol in both schedule-controlled and voluntary conditions (McClearn & Rodgers, 1959). However, this strain is less sensitive to other measures of alcohol rewards including sensitization, motor stimulation, and alcohol discriminative stimulus cues when compared to the DBA2/J (DBA) strains (Fish et al., 2009). Due to the DBA strain's increased olfactory and taste sensitivity, it does not drink substantial amounts of alcohol. Nevertheless, when compared to the B6/J strain, the DBA strain has increased cell firing in the VTA, pointing to larger dopaminergic activation by alcohol and dopamine release in the nucleus accumbens (Kapasova & Szumlinski, 2008; Fish et al., 2009).

Genetically manipulated mouse models are also important tools for understanding the role of dopamine in social motivation. Recent studies have observed DA receptor activation in KO mice exhibit increased social interactions in their familiar cage, hyperactivity in novel environments, and decreased depression-associated behaviors (Tanda et al., 2009). Other studies about KO mice demonstrated increased rates of aggression and reactivity following benign social interactions (Rodriguiz et al., 2004).

The Current Study and Rationale

The current study aimed to examine the role of dopamine receptor activation in motivated social behavior. The study accomplished this by measuring the amount of effort C57Bl/6J mice would expend to socialize following the pharmacological manipulation of dopamine receptor activation. The C57Bl/6J inbred mice were chosen, as they display average levels of social behavior, sufficient motor dexterity for the behavioral task, and genetic stability. Both male and female mice were used to determine any sex effects. The measurement of social motivation was performed using the barrier task, which requires animals to cross barriers of increasing heights to access a social stimulus (another mouse). This study measured not only the animal's desire to be social but the amount of effort it is willing to exert to access a social interaction. The pharmacological manipulation will be an injection of a Levodopa to activate dopamine receptors or a dopamine antagonist to deactivate dopamine receptors. The Levodopa injection allowed the researcher to determine the role of dopamine in social motivation in these mice. Additionally, we aimed to validate the measurement of social motivation by the barrier task, which is a behavioral task not previously used in mice.

Hypotheses:

1. Dopamine receptor activation would increase the effort exerted during a behavioral task to reach a social stimulus.
2. Dopamine antagonist would decrease the amount of exerted effort to reach a social stimulus.
3. Females would display higher amounts of social motivation and sniffing in the saline groups, but males would be more affected by pharmacological manipulation.
4. The barrier task would be an appropriate and reliable measurement of social motivation in mice.

CHAPTER II

Method

Subjects

Pretreatment of Animals. Male and female, adult, C57BL/6J mice were bred and housed in the MTSU vivarium for a minimum of ten weeks prior to initiating behavioral experiments. Mice had food and water ad libitum and have been placed on a reverse light/dark cycle. Separate groups of male (30) and female (30) mice received either saline (control), Levodopa (1mg/kg; DA receptor activation) and Benserazide hydrochloride (intended to block the peripheral metabolism of Levodopa; 1mg/kg), or SCH-23390 hydrochloride (0.1mg/kg) and S (-) Raclopride (+)-tartrate salt (0.05 mg/kg; DA-1 and DA-2 antagonist, respectively). The mice were then assigned to undergo a social motivation behavior test under one of the three-drug treatments. The experimental groups are shown in Table 1.

Table 1: Experimental Groups

The number of animals required per group.

Group	Drug	Sex	N
1	Saline	Male	10
2	Saline	Female	10
3	Levodopa	Male	10
4	Levodopa	Female	10
5	DA antagonist	Male	10
6	DA antagonist	Female	10

Design

A mixed-measures design was used in this experiment. Two three-way, mixed measures ANOVAs were used for the three independent variables: sex (between subjects variable), drugs injected (between subjects variable), and barrier difficulty (within subjects variable). Sex was operationally defined as the distinction between male and female as determined at the time of weening. This variable had two levels, male and female. The second independent variable was the drugs injected as operationally defined as the mixed solution injected intraperitoneally. This variable had three levels: 1) saline, 2) Levodopa, 3) DA antagonist. The third independent variable was barrier difficulty, which was operationally defined as the number of perforated panels stacked on top of each other. This third variable had three levels: one stacked barrier, two stacked barriers, and three stacked barriers. Each of the ANOVA analyses determined the effect of these independent variables on one of the two dependent variables measured throughout the study: the time it took to cross the barrier and total nose-nose interactions. The time it took to cross the barrier was measured as soon as the experimental mouse was placed in the arena until all four paws touched the ground on the side of the stimulus mouse. Nose-nose interactions were measured when the nose of the experimental mouse was within 10 mm of the stimulus mouse.

Materials

Behavioral Arena. An 85x35x50 cm plexiglass container that contained an 11.1x11.1x12.7 cm inverted wire pencil cup was the apparatus used to conduct the

experiment. The arena was divided into two sections with the use of sliding, perforated partitions (see Appendix B).

Drugs. The saline solution used contained 0.9 percent sodium chloride. The 3, 4-Dihydroxy-L-phenylalanine (L-DOPA) was used as the DA receptor activation in conjunction with Benserazide hydrochloride, used to prevent the L-DOPA metabolism by the peripheral nervous system. R (+)-SCH-23390 hydrochloride was the DA antagonist used to block D_1 and D_5 DA receptors in conjunction with S (-) Raclopride (+)-tartrate salt, a selective D_2, D_3, and D_4 DA receptor antagonist. All drugs were purchased from Sigma-Aldrich, a biotechnology company that manufactures and distributes chmeicals.

Procedures

Ten mice, five males and five females, were trained to be the "pencil cup mouse," or the stimulus mouse. Their training was done over a period of two weeks by placing a mouse under an upside-down, wired pencil cup for 10-minute intervals for a total of 50 minutes each. It was crucial that the training took place inside the behavioral arena and under the fluorescent lights used when recording. The conditioning was to habituate the mice to the environment of the experiment. It was also done to decrease potential stress pheromones secreted by the stimulus mouse that may have influenced the behavior of the experimental mouse.

Twenty mice, 10 males and 10 females, were selected for the control group condition. They were injected intraperitoneally with saline in a 100:1 ratio to their weight. For example, a 20 g mouse received 0.2 mL of saline. The mice were then habituated to the room for one hour before being placed in the behavioral arena. The mice

had food and water ad libitum for the entire one-hour wait time. Before placing either a stimulus mouse or an experimental mouse, the arena was thoroughly wiped down with 70% ethanol to eliminate any odors that could potentially influence the experimental mouse. A stimulus mouse of the same sex as the experimental mouse was placed under the pencil cup and moved so that the cup was touching the back wall of the arena. The experimental mouse was then placed on the opposite side of the arena with one perforated panel in place. A total of three minutes was given to the experimental mouse to explore the arena and approach the stimulus mouse. If the experimental mouse crossed the barrier, another two minutes were allowed for exploration and approach while a stopwatch was used to record the total nose-nose interactions. After the two minutes were completed, a second perforated panel was stacked on the first, and the experimental mouse was transported back to its side of the arena if it was not already there. Again, a total of three minutes were allocated for the experimental mouse to cross the barrier, and if it crossed, a two-minute window for exploration and approach was allowed and total nose-nose interactions were timed. This process was repeated for the third, and final, barrier. If at any barrier the experimental mouse did not cross within the three minutes, it was removed from the behavioral arena and no other barriers were stacked. This was coded as 180 seconds for the barrier time and zero seconds for total nose-nose in the data analysis.

The same process was again used for Levodopa and DA antagonist conditions. The 3, 4-Dihydroxy-L-phenylalanine (L-DOPA) and the Benserazide hydrochloride were each mixed in a 1:1 ratio with saline and given at a 100:1 ratio to weight. The mice were placed back in their cages for a one-hour wait before they were placed in the behavioral

arena. For the DA antagonist, R (+)-SCH-23390 hydrochloride was mixed at a 200:1 ratio with saline, and S (-) Raclopride (+)-tartrate salt was mixed at a 20:1 ratio with saline. 1:0.1 of each drug was given to the experimental mouse 30 minutes before entering the behavioral arena.

CHAPTER III

Results

Analytical Plan

Two 2 (sex) x 3 (drugs) x 3 (number of barriers) mixed-measures ANOVAs were conducted to measure the effects of the independent variables on time spent crossing the barriers and time spent sniffing.

Assumption Testing

The assumption of normality and constant variance in a linear model was met. The dependent variable was continuous and measured in seconds. For barrier time, detection of outliers through studentized residuals resulted in one outlier that was not excluded due to the outlier's occurrence at one only barrier difficulty level. The sphericity for the repeated measures ANOVA was violated, and the Greenhouse-Geisser correction was used ($\eta_p^2 = 0.433$). For sniff time, the sphericity for the repeated measures ANOVA was violated, and the Greenhouse-Geisser correction was used ($\eta_p^2 = 0.116$).

Descriptive Statistics

The 2 (male/female) x 3 (saline/levodopa/DA antagonist) x 3 (barrier difficulty) design yielded 18 groups. Each group consisted of 10 mice randomly assigned to that group. The group means and standard deviations for the dependent variable of time to cross the barrier are shown in Table 2. The group means and standard deviations for the dependent variable of time spent socially sniffing are shown in Table 3.

Table 2: Barrier Time

Time measured to cross each barrier by drug for males and females.

	Barriers					
	1		2		3	
	M	F	M	F	M	F
Saline	34.14±17.256	57.54±16.299	47.800± 28.631	37.86± 18.129	118.86±59.226	130.3± 58.606
Levodopa	121.7±54.989	43.34±22.513	94.92± 67.528	56.52±37.277	142.70± 55.415	123.14±53.210
Antagonist	180±.000	139.76±66.805	180±.000	157.98± 46.484	180±.000	177.48±7.969

N=10; M ± SD

Table 3: Sniff Time

Time measured in nose-nose interaction for each barrier by drug for males and females.

	Sniff					
	1		2		3	
	M	F	M	F	M	F
Saline	18.11± 4.931	29±8.817	15.98± 8.088	21.36± 10.489	11.76±10.085	14.35± 16.491
Levodopa	14.979±12.234	26.2±5.383	16.69±13.371	28.44±9.716	10.52± 14.263	20.05±20.929
Antagonist	.000±.000	8.6±14.143	.000±.000	3.4± 8.329	.000±.000	2.40±7.589

N=10; M ± SD

Inferential Statistics

*Effects on time spent crossing barriers***.** The mixed-measures ANOAV indicated significant within-subjects effects for barrier time ($F(1.606, 54) = 41.159$, $p < 0.001$). Post hoc tests indicated that across all mice, time spent crossing barrier three ($M = 145.41$, $SD = 50.888$) was significantly greater than time spent crossing barriers one and two (barrier 1: $M = 96.08$, $SD = 65.638$; barrier 2: $M = 95.85$, $SD = 67.011$; $p < 0.05$). The ANOVA analysis also indicated a barrier by drug interaction ($F(3.212, 54) = 6.469$, $p < 0.001$) was observed as demonstrated in Figure 3 and Figure 5.

Significant between-subjects effects for barrier time by drug, sex, and drug by sex interactions were also observed ($F(2, 54) = 56.511$, $\eta_p^2 = 0.677$, $p < 0.001$; $F(1, 54) = 6.285$, $\eta_p^2 = 0.104$, $p < 0.001$; $F(2, 54) = 3.963$, $\eta_p^2 = 0.128$, $p < 0.001$, respectively). Males ($N = 30$, $M = 122.237$, $SD = 63.289$) took longer to cross the barriers than females

(N = 30, M = 102.658, SD = 57.892). Post hoc tests using the Bonferroni correction revealed that there was a significant reduction in social motivation from Levodopa to the antagonist (MD = -72.150, SD = 9.564) and from saline to the antagonist (MD = -198.120, SD = 9.564), but there was not a significant increase from Levodopa to the saline (MD = 25.970, SD = 9.564) at an alpha level of 0.05. Additionally, a significant decrease in barrier crossing was observed between the first and third barrier (MD = -49.333, SD = 7.284) but not between the first and second barrier (MD = 0.233, SD = 4.530). The drug by sex interaction resulted due to Levodopa increasing time spent crossing barriers only in males and the DA antagonist increasing barrier crossing times more in males than in females (see Table 2 and Figure 1).

*Effects on time social sniffing***.** The second mixed-methods ANOVA indicated significant within-subjects effects of barrier difficulty (F (1.606, 54) = 7.108, $\eta_p^2 = 0.116$, $p < 0.001$) with barrier three (M = 9.85, SD = 14.534) being associated with reduced sniff time as compared to barrier 1(M = 16.15, SD = 13.160, $p < 0.05$) (see Figure 2).

The ANOVA indicated significant sex between-subject effects for sex with males (N = 30, M = 8.995, SD = 8.207) spending less time in nose-nose interactions than females (N = 30, M = 19.059, SD = 11.326). Between-subjects effects of drug administered were also significant (F (2, 54) = 32.773, $\eta_p^2 = 0.548$, $p < 0.05$). Post hoc tests using the Bonferroni correction revealed that there was a significant reduction in sniff time from Levodopa to the antagonist (MD = 17.080, SD = 2.366) and from saline to the antagonist (MD = 16.026, SD = 2.366), but again, no significant difference

between the saline to Levodopa (MD = -1.054, SD = 2.366) at alpha level of 0.05 (see Figure 4 and Figure 6).

Figure 1

Comparison of the barrier time and barrier by drug interactions across sex.

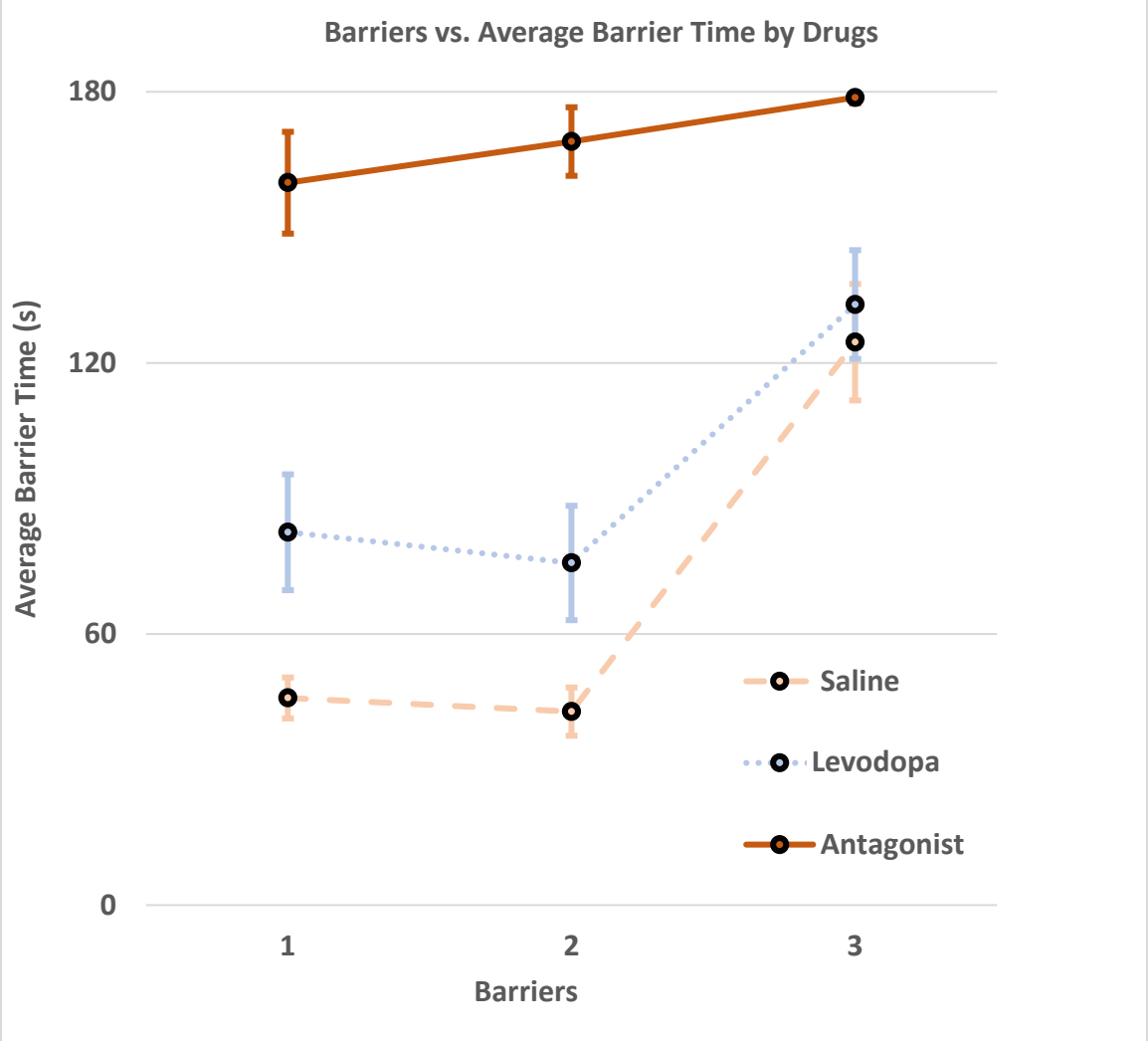


Figure 2

Comparison of the sniff time and barriers by drug interactions across sex.

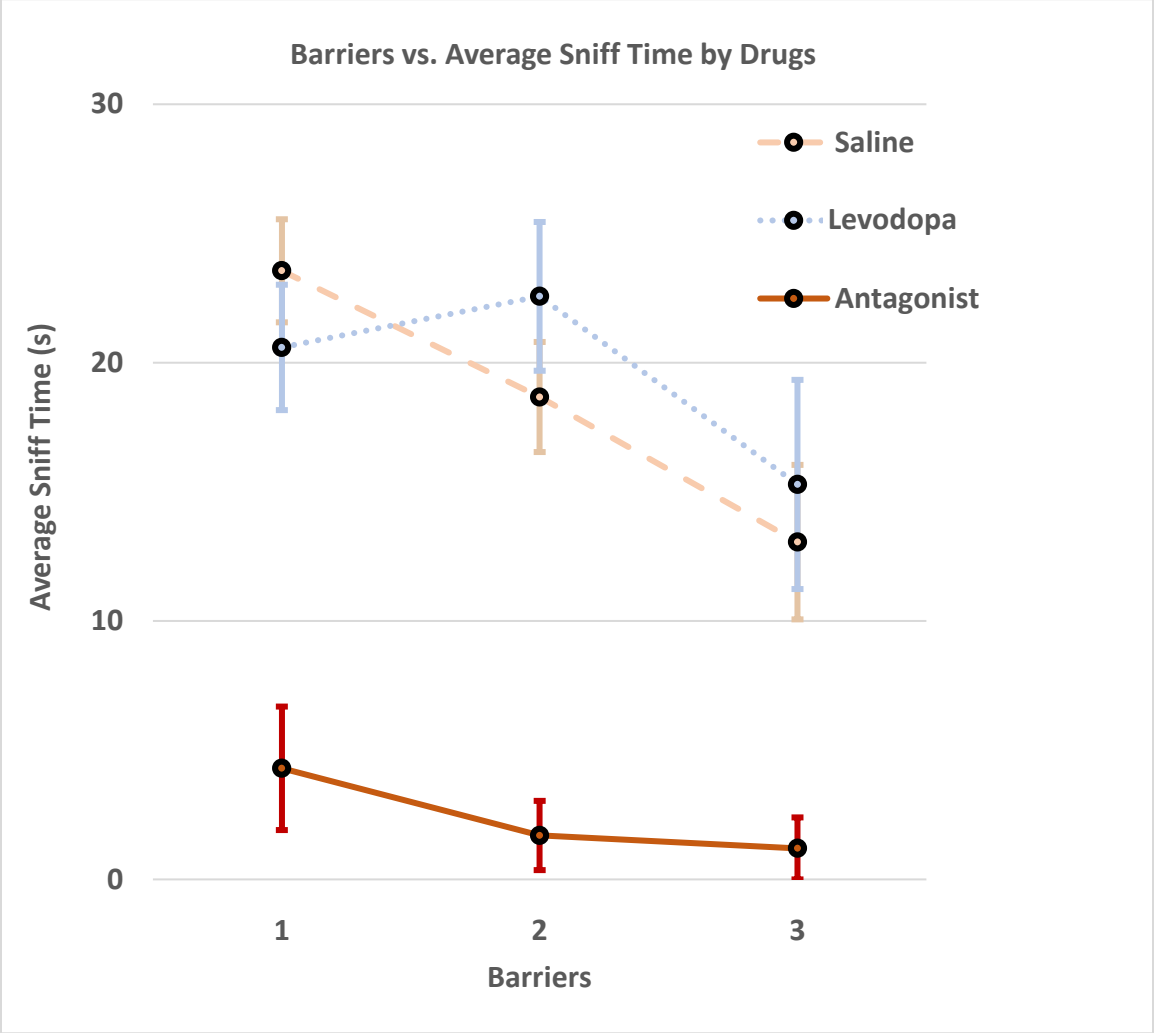


Figure 3

Comparison of the mean barrier time across all drugs by sex.

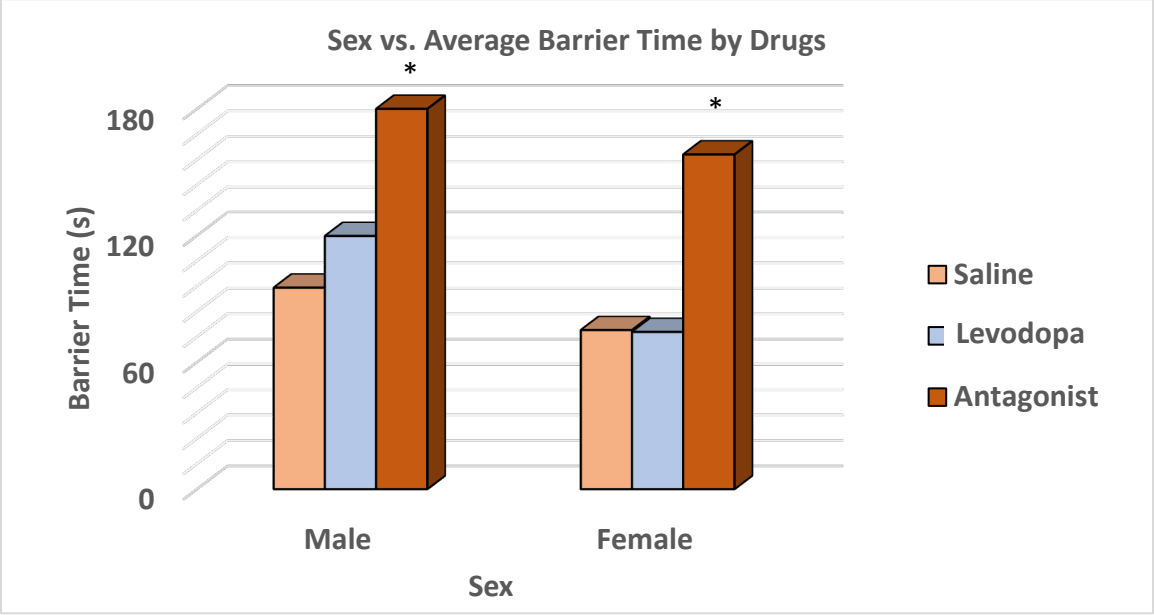


Figure 4

Comparison of the mean sniff time across all drugs by sex.

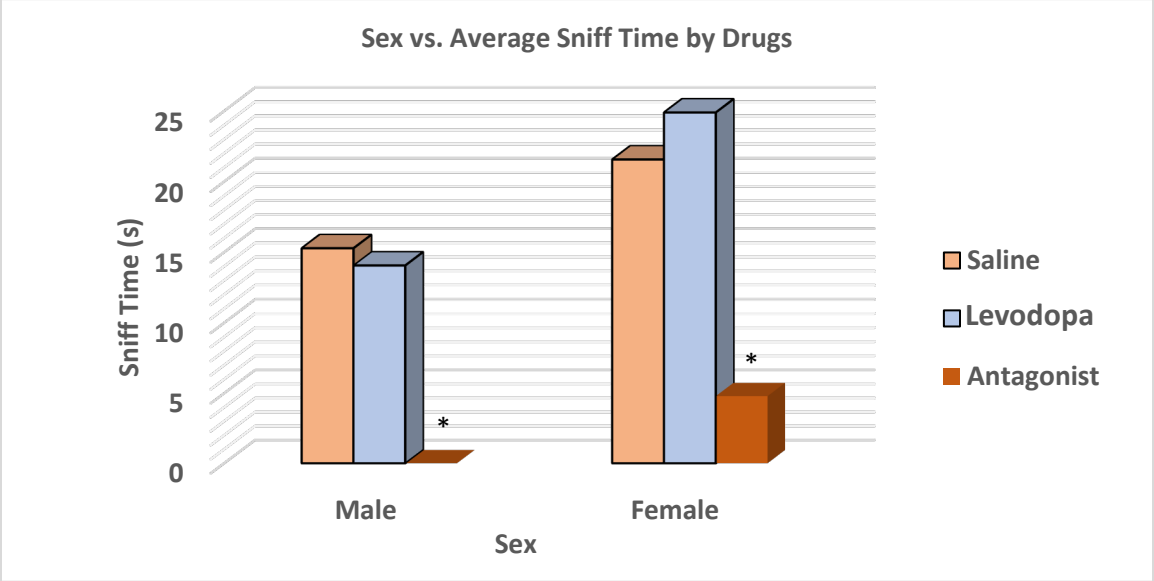


Figure 5

Comparison of the drug interactions across sex and barriers.

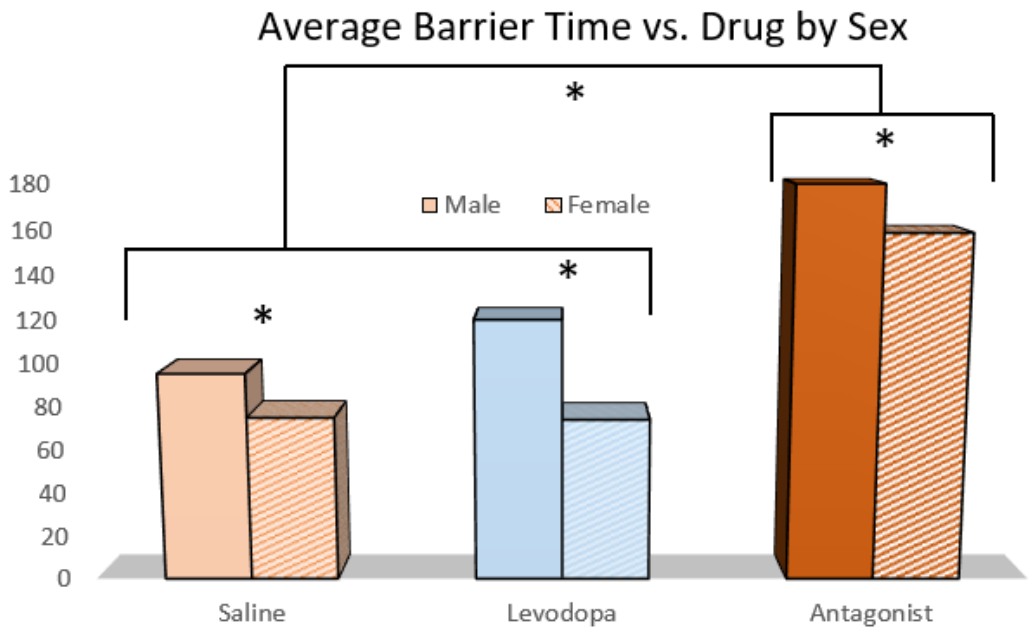
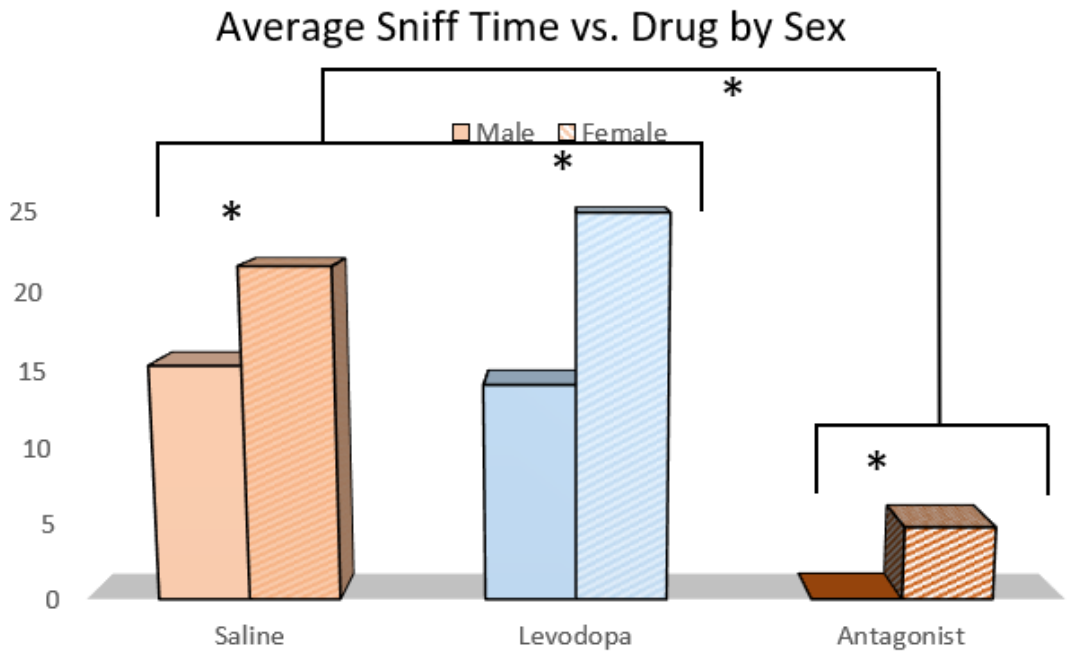


Figure 6

Comparison of the drug interactions across sex and sniff time.



CHAPTER IV

Discussion

The purpose of this study was to gain a better understanding of dopamine's role in social motivation as well as the use of this behavioral task as a measure of social motivation. There were several key findings. Sex differences were found in the analyses of both of the dependent variables. Generally, females expressed higher motivation to reach a social stimulus and higher social sniffing times in the saline conditions. However, males were more sensitive to pharmacological manipulation. The drug applied also made a large impact on each dependent variable. While it was hypothesized that a DA receptor activation would increase effort to reach a stimulus or in other words, would decrease the time to cross the barriers. Our findings indicate that Levodopa increased time to cross barriers when compared to the control group. This pattern of results is consistent with previous literature, which demonstrated our observation of increased exploration could be attributed to DA's role of hyperactivity in novel environments (Tanda et al., 2009). The mice may have experienced a competing motivation to explore the environment that may have delayed crossing the barrier to reach the social stimulus. Increases in barrier crossing time following Levodopa administration were observed more in male mice, while female mice were relatively unaffected by the dopamine receptor stimulation. These results are consistent with previous, unpublished oxytocin research that found that females displayed higher baseline levels of social behavior than males but were more resistant to pharmacological manipulation.

Our findings emphasize the significant increase in barrier time and decrease in sniff time because of DA antagonists. While this pattern is consistent with the hypothesis,

there are potential challenges to the interpretation of the data. Researchers observed that the general locomotion of the mice decreased significantly following dopamine receptor antagonism, which potentially suggests a sedative effect that could have influenced their ability to cross the barriers. These effects may have manifested differently across sex, as female mice expressed more interest in the stimulus mouse by propping themselves over the wall or sniffing near it despite frequently failing to cross. Additionally, general observations demonstrated the males' decreased interest in the behavioral arena as they moved farther from the wall, often staying in the corner.

Despite these limitations, the present study has enhanced our knowledge of the relationship between DA and social behavior. In terms of future research, it would be useful to extend the current findings by examining other routes of drug administration. Potentially, the use of brain region-specific injections through cannula infusions can limit the effects of the drugs on the peripheral nervous system so social motivation can be measured without changes to locomotion or activity levels. In addition, the barrier task might be improved by changing the stimulus mouse as each barrier is added, to maintain a high level of social motivation. Finally, additional tasks to measure social motivation should be developed to determine convergent validity among the tests to better evaluate the usefulness of each test for measuring the construct of interest.

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Appendices

Appendix A: Institutional Review Board Approval Letter

IACUC

INSTITUTIONAL ANIMAL CARE and USE COMMITEE
Office of Research Compliance,
010A Sam Ingram Building,
2269 Middle Tennessee Blvd
Murfreesboro, TN 37129

IACUCN006: FCR PROTOCOL APPROVAL NOTICE

Thursday, May 14, 2020

Senior Investigator	**Tiffany Rogers** (ROLE: Principal Investigator)
Co-Investigators	NONE
Investigator Email(s)	*tiffany.rogers@mtsu.edu*
Department	Psychology
Protocol Title	***Neurochemical and Behavioral Analysis of Social Interaction in Mice***
Protocol ID	**20-3003**

Dear Investigator(s),

The MTSU Institutional Animal Care and Use Committee has reviewed the REVISED animal use proposal identified above under the ***Full Committee Review (FCR) mechanism***. The

IACUC met on 5/12/2020 to determine if your proposal meets the requirements for approval. The Committee determined through a majority of vote that this REVISED protocol meets the guidelines for approval in accordance with PHS policy. In view of the current COVID-19 crisis, the IACUC also introduced a few restrictions. A summary of the IACUC action(s) and other particulars of this this protocol are tabulated below:

IACUC Action	**APPROVED for one year**

Date of Expiration	**5/31/2021**
Number of Animals	192 (ONE HUNDRED and NINETY TWO)
Approved Species	**C57BL/6J Mice (Jackson Laboratories)**
Category Subclassifications	☐ **Teaching** ☐ Classroom ☐ Laboratory ☒ **Research** ☒ Laboratory ☐ Field Research ☐ Field Study ☒ Handling/Manipulation ☐ Observation
	Comment: NONE
Approved Site(s)	MTSU Vivarium: Rooms SCI1170L (housing) and SCI 1170K (procedures)
Restrictions	**1. Must comply with all FCR requirements;** **2. Mandatory compliance with CDC guidelines during COVID-19; Social distancing guidelines are made by the Dean of CBAS.** **3. The PI must make alternative plans to ensure proper animal care, including euthanasia if needed, in the event the research team is quarantined due to COVID19**
Comments	NONE

This approval is effective for three (3) years from the date of this notice **till 5/31/2023** The investigator(s) MUST file a Progress Report annually updating the status of this study. Refer to the schedule for Continuing Review shown below; NO REMINDERS WILL BE SENT. A continuation request (progress report) must be **approved** by the IACUC prior to **5/31/2021** for this protocol to be active for its full term. Once a protocol has expired, it cannot be continued and the investigators must request a fresh protocol.

Continuing Review Schedule:

Reporting Period	Requisition Deadline	IACUC Comments
First year report	4/30/2021	NONE
Second year report	4/30/2022	NONE
Final report	4/30/2023	NONE

Post-approval Amendments:

Date	*Amendment*	*IACUC Notes*
NONE	NONE	NONE

Post-approval Actions:

Date	*Amendment*	*IACUC Notes*

05/14/2020	Mekenzie Meadows, Psychology graduate student) is added as a co-investigator. CITI training and health screening are confirmed	Initial FCR approval

MTSU Policy defines an investigator as someone who has contact with live or dead animals for research or teaching purposes. Anyone meeting this definition must be listed on your protocol and must complete appropriate training through the CITI program. Addition of investigators requires submission of an Addendum request to the Office of Research Compliance.

The IACUC must be notified of any proposed protocol changes prior to their implementation. Unanticipated harms to subjects or adverse events must be reported within 48 hours to the Office of Compliance at (615) 494-8918 and by email – compliance@mtsu.edu.

All records pertaining to the animal care be retained by the MTSU faculty in charge for at least three (3) years AFTER the study is completed. In addition, refer to MTSU Policy 129: Records retention & Disposal (https://www.mtsu.edu/policies/general/129.php) for Tennessee State requirements for data retention. Please be advised that all IACUC approved protocols are subject to audit at any time and all animal facilities are subject to inspections at least biannually. Furthermore, IACUC reserves the right to change, revoke or modify this approval without prior notice.

Sincerely,

Compliance Office

(On behalf of IACUC)

Middle Tennessee State University

Tel: 615 494 8918

Email: iacuc_information@mtsu.edu (for questions) and

Iacuc_submissions@mtsu.edu (for sending documents)

Appendix B: Behavioral Arena