ATTACHMENT #1

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File name: FRCAC Fall 2020 Rogers.pdf
Number of pages: 14

TITLE: Determining the role of mesolimbic dopamine activity in social motivation in mice

PRINCIPAL INVESTIGATOR: Tiffany Rogers, Ph.D.

NONTECHNICAL ABSTRACT:

Dopamine, a neurotransmitter in the central nervous system of humans and animals, is well known for its role in motivation for natural rewards and in motivation for addictive substances. However, research has recently begun to demonstrate its role in social behavior. Dopamine release in the mesolimbic pathway is correlated with engagement in social interaction in humans and in animals. However, dopamine's specific role in social behavior is poorly understood. I hypothesize that dopamine moderates social behaviors by promoting motivation for social interaction. In order to test this hypothesis, I will design and create testing arenas for mice to measure social motivation. Mice have a natural proclivity for social interaction. However, the testing arenas will measure their motivation to interact by requiring the experimental mice to overcome a specific obstacle prior to being allowed to engage socially with a stimulus mouse. The amount of effort the mouse is willing to exert to access a social stimulus will be a measurement of social motivation. Dopamine will be manipulated by intracerebral infusion of dopaminergic drugs into target nuclei in the brains of the experimental mice during behavioral tasks. Three dopaminergic agents will be infused in separate groups of mice. Two dopaminergic drugs, SCH23390 and raclopride, are dopamine receptor antagonists which will block the dopamine activity in two separate populations of dopamine receptors. Dopamine hydrochloride will also be infused into the target nuclei in order to increase dopamine activity. Phosphate-buffered saline will be infused as a control. Therefore, across the groups of mice, effects on social motivation following both increases and decreases in dopamine activity can be compared to controls. Mouse behaviors during the tasks will be video recorded and analyzed using a combination of software packages and hand coding. Performance on the behavioral tasks will be compared across groups to determine if blocking or promoting dopamine activity at mesolimbic receptors sites altered the motivation of mice to engage in social interactions.

PROJECT DESCRIPTION:

Introduction

Dopamine is a catecholaminergic neurotransmitter in the central nervous system. Multiple dopamine pathways extend from the midbrain to various targets in the cerebrum. The mesolimbic pathway extending from the ventral tegmental area in the midbrain to the nucleus accumbens in the ventral striatum is particularly important for motivation to natural and learned rewards as well as addictive substances (Nestler, 2005). While dopamine is thoroughly described as a mediator of reward for many types of stimuli, it has only recently been associated with the rewarding properties of social interaction.

Dopamine has been correlated with multiple social behaviors in humans. Dopamine release in the nucleus accumbens is associated with prosocial behaviors such as viewing positive emotional expressions on the faces of others, maternal care behaviors, and feelings of romantic love (Krach et al., 2010). Positive social interactions such as social play are also associated with dopamine release in rodents (Manduca et al., 2016). One potential explanation of dopamine's role, then, would be that it simply mediates the rewarding properties of social interaction. However, recent studies have shown that dopamine is associated with negative social behaviors such as aggression. Dopamine measurements before, during, and after acts of aggression indicate increased mesolimbic dopamine in nonhuman primates (Miczek, DeBold, & Van Erp, 1994) and in mice (Hadfield, 1983; Tidey & Miczek, 1996), and reductions in dopamine activity in clinical patients results in lowered aggression (Nelson & Trainor, 2007). Therefore, dopamine's role in social behavior may be that it increases motivation for social interaction regardless of whether the interaction is positive (affiliative) or negative (aggressive).

As the field of research addressing the role of dopamine in social motivation remains sparse, the current study will address whether pharmacological manipulation of dopamine will result in changes in social motivation. Some recent research has addressed motivation for dams to access their pups or for males to access a sexual partner. However, very little research has been done to examine social motivation for a same-sex conspecific. The current study will therefore use a same-sex conspecific as a social stimulus reward for expended effort in behavioral tasks.

Background and Specific Aims

Aim 1: Does mesolimbic dopamine alter social behavior by modifying levels of social motivation?

The first aim will address whether dopamine drives social motivation. Mice will be exposed to one of two tests for social motivation requiring increasing amounts of effort in successive rounds to access a social stimulus (a same-sex conspecific). During the behavioral tasks, dopamine activity in the nucleus accumbens will be increased or inhibited by the intracerebral infusion of pharmacological agents. I hypothesize that increasing dopamine activity in the nucleus accumbens will result in increased social motivation and decreasing dopamine activity through either D1-like receptors or D2-like receptors will decrease social motivation as compared to controls.

Aim 2: Does the alteration of dopaminergic activity in mesolimbic nuclei produce differential social responses across sexes?

Both male and female mice will be included in all behavioral tests to determine sex-specific effects of dopamine on social behaviors. I hypothesize that males and females will both exhibit the social motivation changes outlined in Aim 1 but that males will exhibit these changes to a larger degree.

Aim 3: Do the novel behavioral tasks demonstrate validity for future use in the field of social neuroscience?

Convergent validity will be evaluated across the two behavioral tests for social motivation. Additional tests analyzing aggression and anxiety will be utilized to control for alternative social behavior effects in mice and to allow for testing of divergent validity between the social motivation tests and other tests. I hypothesize that the two social motivation tests will exhibit convergent validity within behavioral scores and that divergent validity will be demonstrated between scores on social motivation tests and tests for anxiety and aggression.

Rationale for Model. Mice make excellent reduced models to explore social behavior as they have a natural proclivity to be in groups (Shemesh et al., 2013), develop elaborate social hierarchies (Williamson et al., 2016), and communicate via ultrasonic vocalizations (Ferhat et al., 2016). The genes and environment of mice can be tightly controlled to reduce extraneous variables in ways that cannot be accomplished in humans. Additionally, both male and female mice will be used in the current study as recent research has demonstrated sexually distinct patterns of social behavior, and granting agencies have initiated requirements for investigated sex differences in federal funded work.

Rationale for Methodology. Cannula infusions of drugs into central nervous system nuclei allows for restricted temporal effects and avoids peripheral effects. The drugs selected will both increase dopamine activity (dopamine hydrochloride) and inhibit dopamine activity across each of the two major categories of dopamine receptors (D1-like receptors inhibited by SCH23390 and D2 receptors inhibited by raclopride). By inhibiting each receptor type individually, the unique effects of each on social motivation can be determined. Novel testing arenas provide the field with new tools that will allow the measurement of social motivation in mice. Currently, measurements of general sociability and social recognition tests are commonly utilized. However, very few tests for social motivation exist. Most attempt to retrofit existing operant chambers to a social paradigm. These have been utilized with mixed success depending on the type of alterations performed on the chamber. However, the behavior arenas proposed in the current study will provide the field with specific measurements of social motivation by determining the amount of effort exerted by mice to access a social stimulus.

Methodology

Animals. Male and female adult C57BL/6J mice will be ordered from Jackson Laboratories and housed in the animal vivarium at MTSU. Mice will have food and water ad libitum and will be placed on a reverse light/dark cycle.

Stereotaxic Implantation of Cannulas and Drug Delivery. Dopaminergic manipulations during behavior will consist of bilateral cannula infusions into the nucleus accumbens. For the chronic implantation of cannulas, mice will be anesthetized via isoflurane inhalation and placed within a stereotaxic surgery frame and temperature-regulated heating pad. An incision of the skin on the top of the head will be made and two holes, one above each hemisphere, will be drilled into the skull at the appropriate coordinates for each brain area (Franklin & Paxinos, 2019. A sterile cannula guide (stainless steel with plastic pedestal; P1 Technologies) will be inserted into brain tissue 1mm above the target site. Dental cement will be used to fix the cannula guide to the skull.

Group	Sex	Drug	N
1	Male	SCH23390	12
2	Male	Raclopride	12
3	Male	Dopamine Hydrochloride	12
4	Male	Phosphate-Buffered Saline (Control)	12
5	Female	SCH23390	12
6	Female	Raclopride	12
7	Female	Dopamine Hydrochloride	12
8	Female	Phosphate-Buffered Saline (Control)	12
		EXPERIMENTAL ANIMAL N:	96
		ADDITIONAL STIMULUS MICE N:	4
		TOTAL N:	**100**

Table 1. Experimental groups and the number of animals required

A sterile dummy cannula will be placed in the cannula guide to keep the guide from becoming blocked or contaminated. The animal will be removed from the stereotaxic frame and isoflurane and will be directly observed until normally ambulating. To alleviate postsurgery pain, mice will be injected immediately after surgery with ketoprofen given subcutaneously. Daily health checks will follow all surgeries. During each behavioral task, the infusion cannula will be placed into the cannula guide and attached to a head block, tether, swivel with lever arm, and infusion pump to administer infusions beginning 10 minutes prior to behaviors. Separate groups of male and female mice will receive either dopamine hydrochloride, SCH23390, raclopride, or phosphate-buffered saline in an intracerebral infusion targeted at the nucleus accumbens during the behavioral tasks. The experimental groups are shown in Table 1.

Elevated Plus Maze. To test anxiety in experimental mice, each mouse will first undergo the elevated plus maze. The plus-shaped maze consists of two open arms (without walled barriers) and two closed arms (with walled barriers). The test pits the need of mice to explore versus the need to stay in enclosed areas. The more the mice explore the open arms the less anxious they are judged to be. The mice will be exposed to the test for 5 minutes and time spent in the open arms will be scored by Noldus Ethovision software.

Social Motivation Barrier Test. The second behavior test will address social motivation. The test arena consists of an 85x35x50 cm plexiglass container. A stimulus mouse (same-sex conspecific) will be located in a chamber on one end and the experimental mouse will be placed on the other end. Between the mice, plexiglass partitions, which range from 2 cm to 10 cm, will be placed in successive trials. Each trial will last 1 minute to determine if the experimental mouse will expend the required effort to cross the partition. Hand coding will be used to record the following: latency to cross each barrier and the number of barriers the mouse is willing to cross.

Social Motivation Door Test. The third behavior test will also measure social motivation. This apparatus is made of a clear acrylic material that is divided into one large and two smaller chambers which are separated by a one-way, upright swinging door. A stimulus mouse (same-sex conspecific) is placed in one of the two chambers. Four weights are added to the door in successive trials each lasting one minute. Weights are added to the door to increase the degree of effort the subject must expend to reach the social reward. Hand coding will be used to record the following: time spent in pushing the dividing door and the amount of weight pushed for social interaction.

T-maze Test. Lastly, the T-maze will be utilized to test aggression changes in the mice. It is a T-shaped maze with mice initially placed on the main walkway and then given the option to

explore the left or right arm of the "T". A cagemate will be placed in one arm and an unfamiliar mouse will be placed in the other arm. Mice displaying increased aggression will approach the unfamiliar mouse more often (a social challenge) and will attack and/or bite the stimulus mice more often. The mice will be exposed to the test for 5 minutes and hand coding will be used to record the following: time spent in a nose to nose orientation, time spent in the social approach of the experimental mouse to the stimulus mouse, number of times the experimental mouse bites.

Data Analysis. To evaluate the behavioral data, the dependent variables described above will be quantified, and multiple factorial ANOVAs will be utilized to determine the impact of sex and drug administration on the dependent variable outcomes.

Milestones and Timeline

Table 2 outlines the proposed timeline and milestones. Should any additional assistance be needed, undergraduate volunteers will be recruited to assist in the lab. If the currently proposed behavioral tasks do not demonstrate group differences, they may be substituted with other social behavior tasks.

Dates	Milestones
January 1-14, 2021	Order mice from Jackson Laboratories
January 15-31, 2021	Allow mice to habituate
February 2021	Initiate surgical implantations
March 2021	Continue surgeries; begin behavior tests
April – August 2021	Continue surgeries; continue behavior tests
September – October 2021	Code and analyze data
November – December 2021	Write the results and submit for publication

Table 2. Proposed Timeline and Milestones

Necessary Resources

Item	Quantity	Supplier	Cost
C56Bl/6J Mice (Male and Female)	100	Jackson Laboratories	2739.65
Mice Shipping Charge	1	Jackson Laboratories	424.00
Bilateral Cannula Guides	96	P1 Technologies	1645.44
Dummy Cannulas	40	P1 Technologies	359.60
Internal Cannulas	40	P1 Technologies	347.60
PE50 Tubing	1 package	P1 Technologies	11.63
Cannula Construction Set Up Charge	1	P1 Technologies	150.00
D1R Antagonist SCH23390	1 (25 mg)	Millipore Sigma	384.00
D2R Antagonist Raclopride	1 (25 mg)	Millipore Sigma	156.00
Dopamine Hydrochloride	1 (25 mg)	Millipore Sigma	88.20
Student Worker	3 hours per week for 52 weeks at $15.00/hr		2340
		TOTAL:	**8646.12**

Table 3. Proposal Budget

Budget Justification. The items listed within the budget proposal (Table 3) are consumable resources used during the proposed projects. The study design includes 8 groups which will include 12 mice each. Four additional mice have been placed on the budget to be used as social stimuli during the behavioral experiments. The justification for the number of subjects and power analysis is included in the appendix. Mice utilized within the study will be euthanized and the cannula

implantation site will be confirmed in the fixed brain tissue via histology. The necessary components for cannula implantations are included within the budget. The dummy cannulas and internal cannulas can be reused 2-3 times and therefore only 40 are proposed to be ordered; cannula guides cannot be reused. Tubing necessary to mediate drug administration is also included. P1 also charges a mandatory set up fee of $150.00 for the construction of bilateral cannulas which require specific widths and lengths in their construction. The drugs to be administered are listed above and are all commercially available at the Millipore Sigma website. Feeding, watering, and health checks must be done daily, and cage changes must be performed weekly. In order to ensure that the PI has sufficient time to conduct and oversee the proposed experiments, a student worker is requested to provide these daily and weekly care tasks.

The Rogers Lab either has or will plan to purchase any additional supplies needed. The lab has allotted space (two rooms) to carry out all planned procedures within the animal facility. All homecages, watering bottles, scales for weighing mice and drugs, and anesthesia machines are provided by the MTSU animal facility. The Rogers Lab currently has all behavioral chambers or the supplies to construct all behavioral chambers, stereotaxic rig, phosphate-buffered saline, surgical tools, and pipettes and tips. The Rogers Lab will supply all food and bedding necessary for the mice, dental cement, medications for post-surgery care, and gloves and all necessary PPE from startup funds allocated by ORSP.

Future external funding

I, in collaboration with Dr. Deranda Lester of The University of Memphis, have performed experiments attempting to understand the effects of subchronic systemic oxytocin administration on endogenous dopamine levels in mice (data not published). Our results led us to submit an NIH R15/AREA grant in February of this year to attempt to leverage our findings into exploring the interaction of dopamine and oxytocin in social behaviors. The application was not funded, but feedback through the summary statement was overall positive. The feedback did indicate areas of improvement that could be addressed through the funding of the proposal. First, the reviewers indicated that I, being a junior faculty member, have not yet had a large number of undergraduate trainees. Second, the reviewers stressed the importance of having published using the techniques included in the proposal (the same techniques proposed in the current proposal) and the importance of having published as an independent investigator. Lastly, the reviewers cited the limited available research supporting dopamine's causal relationship with social motivation. The completion and publication of the projects outlined in the current proposal would address each of these areas of weakness in our last R15/AREA proposal and would place us in an excellent position to resubmit. Additionally, the construction and validation of novel behavior arenas will be added to the "Innovation" portion of our R15/AREA resubmission.

Dissemination

The proposed project is innovative as it explores a new application for the well-known neurotransmitter dopamine and it seeks to produce new behavioral tasks to the field of social neuroscience. Should the tasks be used by others in the field, the resulting publication would be cited in their work bringing attention to the Rogers Lab at MTSU. The results from the proposed study would serve as important preliminary data for an R15/AREA resubmission and would fuel future NIH and NSF proposals as well.

References

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Manduca, A., Servadio, M., Damsteegt, R., Campolongo, P., Vanderschuren, L., & Trezza, V. (2016). Dopaminergic neurotransmission in the nucleus accumbens modulates social play behavior in rats. *Neuropsychophamracology, 41*, 2215-2223.

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Shemesh, Y., Sztainberg, Y., Forkosh, O., Shlapobersky, T., Chen, A., & Schneidman, E. (2013). High-order social interaction in groups of mice. *eLife, 2*, e00759.

Tidey JW & Miczek KA. (1996). Social defeat stress selectively alters mesocorticolimbic dopamine release: an in vivo microdialysis study. *Brain Research,721,*140–149.

Williamson, C.M., Franks, B., & Curley, J.P. (2016). Mouse social network dynamics and community structure are associated with plasticity-related brain gene expression. *Frontiers in Behavioral Neuroscience, 10(152)*, 1-16.

BIOGRAPHICAL SKETCH

NAME: Tiffany Rogers

POSITION TITLE: Assistant Professor of Psychology, Middle Tennessee State University

EDUCATION/TRAINING:

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Lipscomb University	B.S.	05/2006	Psychology
University of Memphis	M.A.	08/2009	Psychology
University of Memphis	Ph.D.	08/2012	Behavioral Neuroscience
Vanderbilt University	Postdoctoral Fellow	2012-2014	Molecular and Cellular Neuroscience
Vanderbilt University	Postdoctoral Fellow	2015	Molecular and Cellular Neuroscience

A. Personal Statement

As an early career investigator, I am continuing to hone my career goals as a scientist. I am now able to leverage my experiences across multiple techniques and mouse models to pursue an understanding of the neurochemical underpinnings of social behaviors in mice. I am currently interested in understanding the role of dopamine and oxytocin and their interactions to produce complex social behaviors.

B. Positions and Honors

Positions and Employment

2009 – 2012 Lecturer, Psychology Department, The University of Memphis, Memphis, TN
2012 – 2014 Postdoctoral Fellow, Psychiatry Department, Vanderbilt University, Nashville, TN
2015 Postdoctoral Fellow, Pharmacology Department, Vanderbilt University, Nashville, TN
2015 Scientific Director of Conte Behavioral Core, Vanderbilt University, Nashville, TN
2014 – 2018 Lecturer, Psychology Department, Lipscomb University, Nashville, TN
2015 – 2019 Full-time Lecturer, Psychology Department, Middle Tennessee State University, Murfreesboro, TN
2016 – 2018 Course Developer, Professional Studies Department, Lipscomb University, Nashville, TN
2017 – 2018 Lecturer, Biology Department, Lipscomb University, Nashville, TN
2019 – Assistant Professor, Psychology Department, Middle Tennessee State University, Murfreesboro, TN

Other Experience and Memberships

2013 – Member of Society for Neuroscience
2018 – Co-Sponsor for the Collegiate Neuroscience Society of Middle Tennessee State University

C. Contributions to Science

1. In my early work, I investigated a novel pathway originating in the cerebellum and terminating in the prefrontal cortex and its potential alterations in mouse models of autism using fixed potential amperometry. This work has provided evidence that cerebellar abnormalities observed in autism may be associated with some behavioral symptoms of autism via downstream cerebellar-prefrontal cortex circuitry alterations. The work also may prove useful in providing therapeutic targets for future treatments for patients with autism spectrum disorders.

a. **Rogers, T.D.**, Dickson, P.E., Heck, D.H., Goldowitz, D., Mittleman, G., & Blaha, C.D. (2011). Connecting the dots of the cerebro-cerebellar role in cognitive function: neuronal pathways for cerebellar modulation of dopamine release in the prefrontal cortex. *Synapse, 65(11)*,1204-12. PMID: 21638338
b. **Rogers, T.D.**, Dickson, P.E., McKimm, E., Heck, D.H., Goldowitz, D., & Blaha, C.D., & Mittleman, G. (2013). Reorganization of circuits underlying cerebellar modulation of prefrontal cortical dopamine in mouse models of autism spectrum disorder. *Cerebellum, 12(4)*, 547-56. PMID: 23436049
c. **Rogers, T.D.**, Lester, D.B., Dickson, P.E., Miller, M.M., Heck, D.H., Goldowitz, D., Mittleman, G., Blaha, C.D. (2010). Connecting the dots of the autism disconnection hypothesis: Neural pathways for cerebellar modulation of dopamine release in the prefrontal cortex. *Society for Neuroscience Abstracts*, 562.21.

d. **Rogers, T.D.**, Spight, V., Heck, D.H., Goldowitz, D., Mittleman, G., Blaha, C.D. (2011). Cerebellar Purkinje cell loss results in a shift in modulatory control of cortical dopamine release by two distinct cerebellar-prefrontal cortex pathways: Relevance to the Autism disconnection hypothesis. *Society for Neuroscience Abstracts*, 56.08.

2. I have worked with multiple mouse models of autism to attempt to better understand the neurobiological underpinnings of social behavior and social behavior deficits such as those seen in clinical populations diagnosed with autism. My research in this area has focused on three mouse models of autism specifically: the fragile X mental retardation 1 (*Fmr1*) null mouse, the Gly56Ala (*G56A*) transgenic mouse, and the K-Cl cotransporter 2 (*KCC2b*) mutant mouse. In the *Fmr1* mouse, I characterized the patterns of neuronal activation following the presentation of a social stimulus. I performed RNA sequencing in the prefrontal cortex and amygdala and compared *Fmr1* null mice to genetic controls and to mice that had been exposed to a novel non-social stimulus as a control for social exposure. This allowed me to identify genetic expression changes that were specific to social interaction in the prefrontal cortex and amygdala and to determine aberrant patterns of gene expression changes in the *Fmr1* null mice in the same social exposure. I also used RNA sequencing to explore the prefrontal cortex and amygdala gene expression changes in the *G56A* transgenic mouse, a mouse model of hyperserotonemia observed in autism due to the rare variant in the serotonin transporter gene *SLC6A4.* Additionally, I performed behavioral tasks to characterize two proposed autism mouse models, the KCC2b and the *Lurcher* mouse. In the *KCC2b* mouse I measured the social and anxiety behaviors and found that the *KCC2b* mutant mouse has disturbed social dominance behaviors. In the *Lurcher* mouse, I measured behavioral flexibility via a serial reversal learning task and found reduced behavioral flexibility in the *Lurcher* mouse as compared to controls. Autism is particularly noted for being associated with a broad spectrum of symptoms, genetic mutations, and differences in macro and micro anatomy of the nervous system. The behavioral characterization of mouse models of autism allows for the investigation of a diverse range of neurobiological alterations and behavioral symptoms identified in this neurodevelopmental disorder.

a. **Rogers, T.D.**, Anacker, A.M.J., Kerr, T.M., Forsberg, C.G., Wang, J., Zhang, B., & Veenstra-VanderWeele, J. (2017). Effects of a social stimulus on gene expression in a mouse model of fragile X syndrome. *Mol Autism, Jun 23;8:30*. PMID: 28649315
b. Anacker, A.M.J., Moran, J.T., Santarelli, S., Forsberg, C.G., **Rogers, T.D.**, Stanwood, G.D., Hall, B.J., Delpire, E., Veenstra-VanderWeele, J., & Saxe, M.D. (2019). Enhanced Social Dominance and Altered Neuronal Excitability in the Prefrontal Cortex of Male KCC2b Mutant Mice. *Autism Res, 12(5)*,732-743. PMID: 30977597
c. **Rogers, T.D.**, Forsberg, C.G., Veenstra-VanderWeele, J. (2014). Differences in neuronal activation and gene expression in the fragile X mouse. *International Meeting for Autism Research Abstracts*, 18132.
d. Dickson, P.E., **Rogers, T.D.**, Del Mar, N., Martin, L.A., Heck, D., Blaha, C.D., Goldowitz, D., & Mittleman, G. (2010). Behavioral flexibility in a mouse model of developmental cerebellar Purkinje cell loss. *Neurobiol Learn Mem, 94(2)*, 220-228. PMID 20566377

3. In more recent work, I have begun to explore the role of oxytocin in social behaviors in mice. I explored the effects of social isolation in juvenile C57BL/6J mice on social behaviors such as sociability and preference for social novelty as tested by the Three-Chamber Sociability Task. Subchronic pretreatment of i.p. oxytocin elicited sex-specific patterns of social discrimination between a familiar and novel social stimulus. Similarly, social isolation caused sex-specific patterns in social discrimination providing a three-way pretreatment by housing condition by sex interaction. I also measured stimulation-evoked mesolimbic dopamine release in mice having undergone the same social isolation and oxytocin pretreatment. In these results, we found that while oxytocin changed the levels of dopamine release, social isolations altered dopamine release levels in a sex-specific manner. These studies were completed in collaboration with Dr. Deranda Lester, the Co-PI of The University of Memphis. These results are currently in preparation to be submitted for publication.

a. Berry, K., Estes, M.K., Paige, N.B., Meadows, M., Lester, D.B., & **Rogers, T.D.** (2019). Effects of subchronic oxytocin treatment on social behavior following social isolation in juvenile mice. *Society for Neuroscience Abstracts*, 647.11.
b. Estes, M.K., Paige, N.B., Mills, M.N., **Rogers, T.D.**, & Lester, D.B. (2019). Systemic oxytocin treatment reverses the effect of social isolation on mesolimbic dopamine release. *Society for Neuroscience Abstracts*, 647.12.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/tiffany.rogers.1/bibliography/public/.

IRB/IACUC STATUS
FRCAC APPLICATION

☐ No protocol submitted to IRB/IACUC

☑ Protocol submitted to ☐ IRB ☑ IACUC
on: 12/19/2019

☑ Compliance Office Protocol # 20-3003

☐ IRB ☑ IACUC Approval Received on: 05/14/2020

FRCAC comments (for committee use only):

Justification of Animal Number

Tiffany Rogers
FRCAC Proposal Fall 2020
"Determining the role of mesolimbic dopamine activity in social motivation in mice"

The current protocol requests a total of 96 mice to be used as experimental subjects in the proposed study. Additionally, 4 mice to be used as social stimuli are requested. This number results from a power calculation typically used by behavioral, rodent researchers to ensure detection of any group differences while accounting for inherent behavioral variability and attrition.

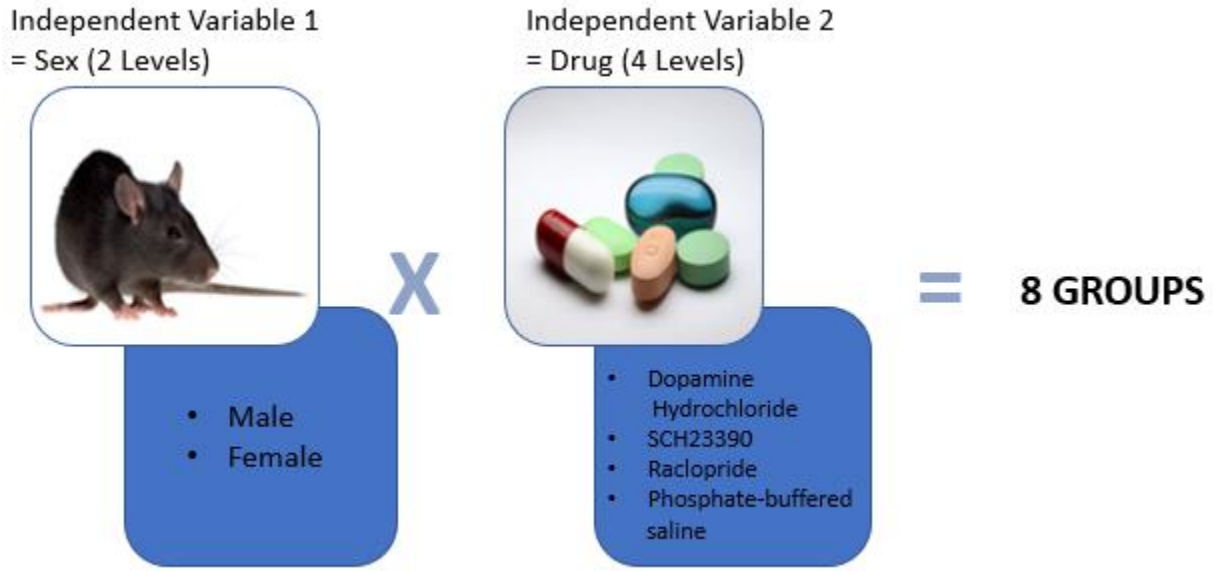

Figure 1. – Proposed Experimental Procedures and Groups

The experimental procedures are shown in Figure 1. Both male and female mice will be assessed for behavior changes following intracerebral infusions into the nucleus accumbens. During these infusions a battery of behavioral tasks will be performed on each mouse. The total number of groups included in the proposed experimental procedure below is 8 (2 levels of sex x 4 levels of drug infusion). According to the power analysis conducted by excel calculator provided by The University of Boston (attached, description below) and according the experience of the researcher and published data, the required number of mice per groups is 10-12. Due to the nature of the study, the researcher will use 12 mice per group. The cannula infusions can be misplaced, and the researcher would remain unaware until histology is performed at the conclusion of that trial. Even a half of a millimeter of misplacement of the cannula can result in a lack of behavioral manipulation. To protect against inherent variability in behavior and the likely attrition of subjects due to cannula misplacement, I am electing to use the higher end of the recommended range of subjects per group (12 as opposed to 10). ***With 12 mice assigned to each group and a total of 16 groups, the total number of requested mice is 96.***

In the attached excel spreadsheet, the power calculator provided by The University of Boston (https://www.bu.edu/researchsupport/compliance/animal-care/working-with-animals/research/sample-size-calculations-iacuc/) uses example group means and standard deviations (cells B4-5 and C4-5) to determine required sample size. I have input data from mouse behavior results currently being prepared for publication that examined sniffing time in mice in the three-chamber sociability task. The two groups demonstrated a 15% mean difference (Group A – M = 80 sec, SD = 12 sec; Group B – M = 92 sec, SD = 8 sec). With a desired alpha level of 0.05 and a desired power of 80% the recommended number of subjects per group is 11 which is very much in line with published literature of 10-12 animals per group discussed above.

I - Sample Size Calculations for Means												Table for (Z1-alpha/2+Z1-beta)squared				
	Anticipated Values													beta		
	Mean	Stan. Dev										alpha	0.05	0.1	0.2	0.5
Group 1	80	12		Difference in means		15	%					0.1	10.8	8.6	6.2	2.7
Group 2	92	8										0.05	13	10.5	7.8	3.8
												0.02	15.8	13	10	5.4
The cells in the table below show the estimated number of subjects needed in each												0.01	17.8	14.9	11.7	6.6
group in order to demonstrate a statistically significant difference at "p" values																
ranging from 0.10 - 0.01 and at varying levels of "power".																
Power is the probability of finding a statistically significant difference at																
a given "P" value with the specified number of subjects in each group.																
		Sample Size Needed in Each Group														
alpha level			Power													
("p" value)		95%	90%	80%	50%											
0.10		16	12	9	4											
0.05		19	15	11	5											
0.02		23	19	14	8											
0.01		26	22	17	10											

Purchase Order

Purchase Order Date	PO/Reference No.	Revision No.
Jun 12, 2020	**P0080543**	**0**

Buyer Information

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Murfreesboro, TN 37132
United States

ShipTo Address Code	MAIN

Delivery Information

Requested Delivery Date	
Expedite	No
Ship Via	Best Carrier-Best Way

Shipping Instructions

Owner Department	Psychology
Note to Supplier	Tiffany Rogers Please process asap.
Attachments for supplier	
	Q105329-JAX QUOTE...
Supplier Terms and Conditions	PO_Terms_and_Conditions.pdf (45k)

Line No.	Product Description	Catalog No.	Size / Packaging	Unit Price	Quantity	Ext. Price
1 of 1	ITEM 1= 000664 (C57BL/6J) 50-F->29.49 ITEM 2=000664 (C57BL/6J)50-M->29.08 ITEM 3=SMF0001 (PROD TRANSPORT CONTAINER)->13.00 S/H=424.00 TOTAL 3310.08	*see below*	EA	3,163.65 USD	1 EA	3,163.65 USD

Taxable	No
Capital Asset	No
PO Clauses	Refer below

Shipping, Handling and Tax charges are calculated and charged by each supplier. Total **3,163.65 USD**

Billing Information

Charge to PO Listed Above	
Payment Terms	
F.O.B.	Destination
Contract	*no value*

Billing Address

Middle Tennessee State University
MTSU
Attn: Accounts Payable
Cope Administration Building - Room 106

Pricing Code	
Account Code	
Quote number	
PO Clauses	Refer below

CART
Invoice Email: invoice@mtsu.edu
Murfreesboro, TN 37132
United States

PO Terms

Header	5	Portal Invoice Suppliers	Portal Invoice Suppliers - Please attach/upload a copy of the invoice document when submitting invoices. THIS IS REQUIRED ON ALL INVOICE SUBMISSIONS.

Purchase Order History

Line No.	Date/Time	User	Action	Field Name	From	To
1	6/15/2020 10:46 AM	Alexis Guy	PO modified	Unit Price	3,310.08	3,163.65

Section 1: Manufacture Information

P1 Technologies Inc.
6591 Merriman Road.
Roanoke Va. 24018
1.540.772.7950

Linda Pham
Sales Engineer
1.540.772.7960 ext. 100
lindapham@p1tec.com

Section 2: Customer Information

VISA/MASTERCARD SALES
MIDDLE TENNESSEE STATE UNIV
1301 E. MAIN ST.
MURFREESBORO TN 37132

tiffany.rogers@mtsu.edu

Section 3: Quote Information

Quote #	Revision	Customer Account #	Payment terms	Start Date	Expiration Date	Customer RFQ #
32919	1	E00100	CREDIT CARD	03/06/2020	11/30/2020	

Section 4.1: Part Pricing Module

Item #	Part Number	Description	96 EA
1	8IC315GMNG11 C/C 3.2MM	C315GMN(2)-G11/SP GUIDE DBL 26GA MINI G11	$14.34 *{$1,376.64}*
2	8IC315FDMN02	C315FDMN/SPC DUMMY "FIT" .008"/.2MM MINI (INT CAP)	**40** EA $8.99 *{$359.60}*
3	8IC315IMNSPC	C315IMN/SPC INTERNAL 33GA 42595 MINI	**40** EA $8.69 *{$347.60}*
4	8F023X050P01 8F023X050P01	C313CT/ PKG TUBING 023 X 050 PE50 10'	**1** EA $11.73 *{$11.73}*
5	SU2 Must be purchased with lines 1	SET UP CHARGE	**1** EA $150.00 *{$150.00}*

Section 5: Notes

1) Should the design concept change from that which was used for quotation due to product needs or customer preferences, P1 Technologies Inc. Reserves the right to reassess cost.
2) Quote is subject to P1 Technologies Inc. standard terms and conditions.
3) Leadtime provided is best estimate based on current production backlog and material availability. Delivery dates to be determined after receipt of order.
4) Blanket orders are acceptable for higher quantities.
5) Prices are in US Dollars and FOB shipping point, Roanoke, VA (USA).
6) Forms of payment accepted are: Visa, Master Card, Discover, AMEX, wire transfer and checks. Our bank information will be sent upon request should you choose wire transfer method of payment.

TERMS AND CONDITIONS OF SALE

All quotations and sales are subject to the following terms and conditions:

1. **Offer and Acceptance.** Buyer has offered to purchase from P1 Technologies, Inc. ("Seller") the products ("Goods") described in the attached quote and/or sales order. These terms and conditions and any attached quote and/or sales order are hereafter referred to collectively as the "Acknowledgement." Seller's acceptance of this offer is expressly conditioned upon Buyer's assent to the exclusive terms and conditions set forth herein. If the Acknowledgement is submitted in response to a purchase order or other written or oral offer of the Buyer to purchase the Goods, to the extent that the Buyer's offer contains material conflicts, differences or additions (collectively, the "Conflicting Terms"), the Acknowledgement shall be considered a counteroffer to sell the Goods to Buyer under the terms and conditions herein contained, and any Conflicting Terms shall be void and of no force or effect and shall be deemed rejected and objected to by Seller without further notice. Buyer's acceptance of any such counteroffer is exclusively limited to the terms and conditions set forth herein. Furthermore, acceptance of Goods constitutes acceptance of these Terms and Conditions of Sale, and these Terms and Conditions of Sale supersede any and all other agreements, whether written or verbal, that may or may not have been made previously between the parties.

2. **Material.** Buyer agrees that any material or components provided to Seller by Buyer for use in manufacture and completion of the Goods specified in the Acknowledgement shall be deemed to meet any requirements and specifications required by the Buyer unless Seller is notified in writing within 30 days of receipt of such material or components.

3. **Payment.** Unless otherwise provided in writing, Buyer shall pay the invoice amount within thirty (30) days of the invoice date. If the credit of Buyer is impaired at any time, in the sole discretion of Seller, Seller may require payment in advance before further shipment.

4. **Shipment; Delays.** All prices are EX-WORKS, Roanoke VA USA and do not include any shipping charges. Risk of loss and title to all Goods furnished by Seller shall pass directly to Buyer at the FOB point of shipment. Shipping dates are estimated and under no circumstances does Seller guarantee date of shipment. Seller is not liable to the Buyer for any production or delivery delay or for any damages suffered by Buyer due to such delay, if such delay is, directly or indirectly, caused by war, acts of God, fires, floods, accidents, labor disputes, civil disturbances, terrorism, action of government, shortages or failure of supply of labor, fuel, materials or equipment, transportation delays, or other causes beyond Seller's control. In the event any of the above contingencies occurs, Seller may cancel this Acknowledgement or any part thereof without any resulting liability.

5. **Warranty; Exclusion of Warranties.** There are no express warranties hereunder, except that the goods will, at the time of shipment, be free from defects in material and workmanship.

   SELLER DISCLAIMS ALL IMPLIED WARRANTIES AND SIMILAR OBLIGATIONS (OTHER THAN GOOD TITLE) INCLUDING BUT NOT LIMITED TO THOSE OF FITNESS FOR A PARTICULAR PURPOSE, AND MERCHANTABILITY, WHETHER OTHERWISE ARISING BY LAW, CUSTOM, USAGE, TRADE PRACTICE, COURSE OF DEALING, OR COURSE OF PERFORMANCE.

   There are no warranties which extend beyond those express warranties contained in the Agreement. Buyer affirms that it has not relied upon Seller's skill nor judgment to select or furnish the Goods for any particular purpose beyond the specific express warranties in the Agreement. Any design provided by Seller is based on information provided by Buyer. Any modifications of drawings, prototypes and other work of Seller after approval by Buyer will be at Buyer's expense at Seller's normal rates for services and materials. Seller does not warrant the Goods will comply with the requirements of any safety or environmental code or regulation of any federal, state, municipality or other jurisdiction.

6. **Buyer's Remedy.** The parties agree that the buyer's exclusive remedy shall be repair, replacement or credit, at the sole option of seller. In no event shall seller be liable or responsible for any costs, damages, lost profits, liquidated damages or penalties or for other direct, indirect, special, incidental or consequential damages, nor for any claim against buyer by any third party, nor for any amount in excess of the purchase price of any defective goods.

7. **Cancellation.** Buyer cannot cancel an order accepted by Seller and cannot return non-defective Goods without Seller's written consent and upon terms indemnifying Seller against loss. There shall be no cancellation of orders for Goods built to Buyer's specifications after preparation for manufacture/assembly begins. There shall be no return of non-defective Goods manufactured to Buyer's specifications. Cancelled charges may include, but are not limited to, costs and expenses incurred in the production of the "Goods", lost profit, and the cost of items and special materials purchased. Cancelled charges may be the total of the "Finished Goods".

8. **Taxes, Duties and Licenses.** Buyer agrees to pay any and all applicable federal, state and local taxes (domestic and foreign) to which the Goods may be subject, including, without limitation, excise taxes, sales taxes, value-added taxes and use taxes, duties and license fees.

9. **Claims.** ALL SALES ARE FINAL. In no case are Goods to be returned without first obtaining Seller's return material authorization ("RMA") number. Failure to make written claims for defects, damage or shortages within ten (10) days after delivery shall constitute Buyer's irrevocable acceptance of the Goods and admission that the Goods fully comply with the terms, conditions and specifications of the Acknowledgement.

10. **Limitations on Actions.** Any action brought against Seller must be commenced within one (1) year of the date of accrual of the cause of action, or it shall be barred.

11. **Seller's Remedies.** In the event of any breach or default by the Buyer, Seller may pursue any of the following remedies, none of which are exclusive: (a) terminate or cancel the Acknowledgement; (b) retain any down payment made by the Buyer and apply it first in reduction of damages to the extent of and as an offset to such damages, and second, to the reduction of any other indebtedness of the Buyer to Seller, and (c) pursue any other remedies available at law or in equity. If Seller incurs expenses, including, without limitation, court costs, expenses and reasonable attorneys' fees, in attempting to collect any amount owed or to enforce any term or condition of the Acknowledgement, then Buyer agrees to pay to Seller, in addition to any other sums owed or relief sought, all such expenses to the fullest extent permitted by law.

12. **Indemnity.** Buyer shall indemnify, save and hold harmless Seller from any and all loss, cost, expenses and damages, including reasonable attorneys' fees, on account of any and all manner of claims, demands, actions and proceedings, concerning any Goods sold, that may be instituted against Seller: (a) alleging infringement for Goods made to Buyer's specification; (b) arising out of the change to, or alteration of the Goods by Buyer or any third party; (c) involving the use by Buyer or any third party of the Goods in a manner or application not normally intended by Seller; or (d) involving any negligence whatsoever on the part of the Buyer or any third party.

13. **Non-Waiver.** No waiver by Seller of any breach of the Acknowledgement shall operate as a waiver of such breach, or of any subsequent breach thereof.

14. **Severability.** If any portion of the Acknowledgement shall be held invalid, those parts of the Acknowledgementthat are not held invalid shall continue in full force and effect.

15. **Choice of Law.** The Acknowledgement, any offer by the Buyer and any matter related thereto shall be governed by the laws of the Commonwealth of Virginia, without regard to principles of conflict of laws. Any action, suit, or other legal proceeding which is commenced to resolve any matter arising under or relating to the Acknowledgement or this transaction shall be commenced and prosecuted only in a state court located in the County of Roanoke, Virginia.

16. **Credit.** The extension of credit or the acceptance of a check, note, trade acceptance or guarantee of payment shall not affect any of Seller's rights hereunder and Buyer agrees Seller may change credit terms at any time.

17. **Tools and Equipment.** All tools, dies, jigs, gauges, fixtures, patterns, molds or other equipment used by Seller in producing Goods shall remain the property of Seller, unless provided otherwise in writing.

18. **Entire Agreement, Assignment and Modification.** The Acknowledgement, which exclusively sets forth the rights and obligations of the parties, (a) constitutes the final and entire agreement between the parties, superseding all prior written or oral communications between the parties, and (b) may not be modified or assigned except in a writing signed by both parties.