Assessing the Effects of Pairing on Social Preferences in Rats

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DEDICATIONS

I want to dedicate my master's thesis to my fiancé, family, and my dog. I extend my sincere gratitude to my faithful fiancé, George, who has been an unwavering source of support throughout this journey. Thanks for sharing the financial burden that my degree has entailed and for always being a source of encouragement. I cherish the early-graduation gift you gave me as a tangible reminder of your belief in me. Your presence has helped me navigate the unpredictable waves of life during this challenging time.

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ABSTRACT

Pairing in ABA therapy for children with ASD involves therapists engaging with children through imitation, preferred stimuli, non-contingent reinforcement, and minimizing demands to enhance compliance and session engagement. While various studies have explored pairing techniques, the mechanisms behind its effectiveness remain unclear. Proposed theories include conditioned reinforcement and aversive deconditioning, but limited research confirms which mechanisms are most influential. This translational experiment aimed to examine pairing in a lab setting with rats. In the first procedure, primary rats participated in preference assessments for food and toys, with peanut halves, Cheerios, banana chips, crawl tubes, and hiding trunks being the most preferred items. The second procedure involved a social olfactory choice task where rats selected between two other rats' scents. The less preferred rat became the pairing partner, while the more preferred rat remained a stranger. The final procedure introduced pairing sessions with the primary rat, their partner rat, and preferred items. Preliminary results show significant individual differences; some rats preferred their partner, one favored the stranger rat, and the others were indifferent. For instance, R1 showed consistent responding for their partner rat on multiple days; R2 initially showed consistent responding for their partner rat on multiple days but then dropped off toward the end; R3 showed consistent responding for their stranger rat on multiple days; and R4, R5, and R6 showed indifference. These findings suggest that traditional pairing methodologies may not sufficiently condition a partner rat as a reinforcing stimulus. This highlights the need for further research to understand the dynamics of rat pairing and the factors influencing preferences, indicating that mere exposure to a partner may not create a strong bond.

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CHAPTER 1: INTRODUCTION

Social Behavior

Social behavior encompasses the multitude of methods by which an individual communicates or interacts with another being (Jabarin et al., 2022). Social behavior emerges from the interplay of various factors, including biological components such as genetics, temperament, neurological function, and cognitive abilities (Curley & Champagne, 2023; Malik & Marwaha, 2022). Additionally, environmental influences play a critical role, encompassing aspects such as cultural context, parent-child dynamics, and interactions within broader social settings, including schools and neighborhoods. Relationships with family members and peers also significantly contribute to the development of social behavior (Malik & Marwaha, 2022). Moreover, psychological factors, including the ability to imitate social skills, emotional responses, and cognitive processes, further shape these interactions (Curley & Champagne, 2023; Malik & Marwaha, 2022). In humans, social interaction involves using interpersonal, emotional, cognitive, and behavioral skills to adapt to and navigate society (Mylett et al., 2024). There are numerous benefits that can arise from adaptive social behavior among humans, including reduced mortality rates, promotion of positive health behaviors, enhancement of subjective well-being, emotional support, decreased depression rates, and resilience to various adverse health outcomes (Huxhold et al., 2014; Umberson & Montez, 2010).

On the other hand, maladaptive social behaviors, such as persistent rumination on previous aversive social events, a continual search for validation and comfort from others, and heightened susceptibility to stressful social interactions, can have concerning implications (Kupferberg & Hasler, 2023). Individuals exhibiting these behaviors often respond to various social situations with inappropriate behaviors that do not align with the context, leading to self-

isolation from social engagements. Furthermore, there may be an increased expression of negative emotions such as frustration and hostility directed towards others, a decreased perception of self-worth, and a heightened risk of developing or exacerbating depressive symptoms. Maladaptive social behaviors can also lead to negative reactions from others, resulting in rejection, diminished relationships, and inadequate support (Kupferberg & Hasler, 2023). Further scientific investigation into the dimensions of social behavior is essential for identifying strategies to enhance and refine social interactions. Such efforts will ensure that diverse populations, including individuals diagnosed with disorders associated with social challenges, benefit from the advantages associated with adaptive social behavior while minimizing the adverse effects that can arise from maladaptive behaviors.

Disorders associated with social challenges encompass a diverse array of conditions that significantly impact an individual's ability to interact effectively in various social settings. Among these, attention-deficit/hyperactivity disorder (ADHD) is a prominent example. This neurodevelopmental disorder is characterized by symptoms of inattention, impulsivity, and hyperactivity, which can lead to difficulties in forming and maintaining peer relationships (National Collaborating Centre for Mental Health et al., 2011). Research indicates that children and adolescents with ADHD often experience social rejection, which can exacerbate feelings of isolation and negatively affect self-esteem (Carpenter et al., 2009).

Another notable condition is generalized anxiety disorder (GAD), which affects individuals in multiple contexts, including familial, professional, and community settings. The pervasive anxiety associated with GAD can hinder effective communication and social interactions, thereby limiting one's ability to engage with others and diminishing their quality of

life. Individuals with GAD often struggle to navigate social scenarios, which can lead to avoidance behaviors and further social withdrawal (National Collaborating Centre for Mental Health et al., 2011). Obsessive-compulsive disorder (OCD) is also associated with significant social impairments. The intrusive thoughts and compulsive behaviors characteristic of OCD can interfere with personal relationships and professional commitments. Individuals may find themselves increasingly isolated as their symptoms create barriers to social engagement, often resulting in strained relationships with partners, family members, and colleagues (National Collaborating Centre for Mental Health et al., 2011).

Social difficulties are also particularly prevalent among individuals with depression. Research indicates that about 3.7 million adolescents aged between 12 and 17 in the United States experienced at least one major depressive episode with intense impairment in 2020. Additionally, about 14.5 million U.S. adults, which accounts for over 5% of the U.S. adult population, within the year of 2020 had at least one major depressive episode with intense impairment across that year (National Institute of Mental Health, 2023). Depression is a condition that is characterized by a persistent low mood, fatigue, and feelings of hopelessness, all of which can significantly impair interpersonal relationships. Individuals with depression often experience decreased productivity at work or school, which can exacerbate feelings of isolation and contribute to neurotic emotions that strain relationships with family, friends, and peers (National Collaborating Centre for Mental Health et al., 2011). Youth struggling with depression frequently face a myriad of challenges in daily living, compounded by social difficulties. Insufficient conversational skills, limited social knowledge, and diminished motivation for social engagement can heighten vulnerability to depression during childhood (Mylett et al., 2024).

These social deficits can lead to further complications, creating a cyclical pattern where social isolation exacerbates depressive symptoms, making recovery more challenging.

Similarly, some individuals with autism spectrum disorder (ASD) exhibit social skill challenges that can hinder their development compared to their peers. Research indicates these individuals may experience difficulties in social interactions, reduced social-emotional awareness, and a lack of cooperative engagement with peers, making it harder for them to establish and maintain meaningful relationships (Carpenter et al., 2009). According to the U.S. Centers for Disease Control and Prevention, about 1 in 36 children have been diagnosed with ASD, which indicates how common the disorder is (U.S. Centers for Disease Control and Prevention, 2025). The significance of the social challenges experienced by individuals with ASD and similar conditions underscores the importance of targeted interventions to enhance social skills and emotional well-being, ultimately improving the quality of life for affected individuals.

Translational Research

Given the need for interventions that promote social behavior in humans, applied research focused on identifying and understanding these interventions is essential. However, the complexity of the human experience creates a substantial challenge for research designed to isolate the relevant variables and implement the controls necessary for a rigorous study of social behavior. Translational research is a practical approach that uses highly controlled methods of basic research to address applied concerns, such as social behavior (Critchfield, 2011; Pilgrim, 2011). Translational research plays a pivotal role in bridging the gap between experimental research and applied settings. Vollmer (2011) identified several variations of translational

research, including (1) the application of basic principles and (2) the use of laboratory research to solve applied problems. In the first variation, the results of basic experimental studies conducted under controlled conditions, such as in laboratories, may be generalizable to practical applications in real-world settings. For example, the use of positive reinforcement in applied behavior analysis therapy for children with ASD was first documented by Skinner in the basic lab with rats and pigeons (Critchfield, 2011; Vollmer, 2011). Applied environments are generally less controlled and more dynamic, and various factors may influence the results of translational work (Kyonka & Subramaniam, 2018). While the principles and results derived from controlled studies can still be relevant, the actual implementation of those findings requires careful consideration and adaptation (Nieto et al., 2021). The shift from controlled environments to practical applications highlights the importance of additional testing and refinement before widespread implementation to produce socially significant changes among humans. As researchers strive to ensure the efficacy and safety of their findings, a critical step in this process involves the exploration of new concepts in experimental settings.

A significant portion of translational research is dedicated to integrating findings from experimental behavior analysis into practical, real-world applications. However, translational research also plays a crucial role in identifying challenges encountered in applied settings and evaluating alternative methods of exploring those struggles within experimental settings. In the second variation described by Vollmer (2011), translational research can be conducted by identifying applied problems and using laboratory research to solve them. For example, when new medications are being considered for use in clinical populations, they may first be tested with animal subjects in the basic laboratory (Critchfield, 2011; Vollmer, 2011). This bi-

directionality of translational research – moving from the lab to the field or from the field to the lab – makes it a unique and practical approach for understanding complex problems, including the exploration of interventions that affect social behavior. Either way, before applying new or refined concepts or interventions directly to human participants, it is crucial to explore and evaluate these ideas in experimental settings, often using animal models. This approach is not only ethical but also practical, as it allows researchers to conduct preliminary studies without the complications and risks associated with human participants (Bryda, 2013). Particularly within the realm of behavioral research, laboratory rats and mice have emerged as favored models due to significant similarities in their social behaviors, anatomical structures, and physiological processes compared to humans (Bryda, 2013; Nieto et al., 2021). Given the inherent complexity of human social behavior, having robust animal models helps researchers investigate social behaviors and related phenomena in a controlled environment.

Rat Models

Animal models offer remarkable value in research as they allow for systematic manipulation of genetic, environmental, and experiential factors (Nieto et al., 2021). This flexibility enables researchers to recreate conditions that closely mirror those experienced by target populations in applied settings. However, it is critical to acknowledge that while these animal models can replicate many aspects of human behavior and conditions, they do not encompass every facet of human experience. Their utility lies in their ability to simulate similar reactions to diverse stimuli and conditions, thereby facilitating the translation of research findings into appropriate applications in human contexts (Nieto et al., 2021).

Rodent models, particularly rats, have become crucial for studying social behavior due to several advantages. These animals are not only socially interactive but also have a well-documented genetic makeup, allowing for extensive research into their behavioral patterns (Bryda, 2013; Guo & Sun, 2023). While mice are commonly employed in behavioral neuroscience due to their size and ease of care, rats are often preferred for social behavior studies. Their larger size fosters more complex social interactions, and their behavioral patterns exhibit similarities to human social behaviors, making them particularly valuable for such research (Bryda, 2013). Similar social behavioral patterns between rats and humans can be exemplified through social dominance, motivation, and recognition (Guo & Sun, 2023). Social dominance among rats is analogous to human behaviors concerning social classes and the reactions of individuals from different "classes." Rats exhibit their dominance through variations in age, size, and strength, which can lead to physical and reproductive implications for the lower-ranked rodents, such as decreased access to resources and reproductive partners when in competition with higher-ranked rodents (Choi et al., 2024). In humans, depression can manifest as an excessive focus on dominance and social status, which may contribute to feelings of inferiority (Guo & Sun, 2023).

Translational Relevance of Rodent Research to Human Behavior

Social motivation in rats can relate to human behaviors through the effects of social isolation and behaviors relative to effort exertions to gain access to social interaction. The three-chamber apparatus test is a method among rat subjects to assess the social motivation of the rats to determine how valuable social interaction is to them based on how hard they will work to gain access to another rat (Guo & Sun, 2023). A study by Schulingkamp and colleagues (2023) used a

three-chamber social interaction test, which showed that the fourth female rat in the study engaged in 80 lever presses to gain brief access to another female rat. A study by Lehman and Adams (1977) suggested that a prolonged period of social isolation (5 weeks) between male DA agouti, Fischer albino, and WAG-Rij albino rats contributed to aggressive behaviors such as biting and kicking when reintroduced to social interaction with another male rat. Another modern example of the effects of social isolation on human interactions is the effects of the COVID-19 pandemic, and how the prolonged periods of isolation led to symptoms of anxiety, depression, anger, and agitation among some individuals (Hwang et al., 2020).

Social recognition involves various cues used by individuals to express their internal sensations, indicating whether a social interaction is welcoming or confrontational. Interestingly, the way rats exhibit social recognition mirrors human behavior through their sensitivity to social signals (Guo & Sun, 2023). These signals can encompass both vocal communication, such as spoken language, and non-vocal expressions, including body movements, facial expressions, and vocalizations that convey emotions like pleasure or discomfort. For instance, a furrowed brow may signal disagreement or confusion, while laughter and smiling can indicate joy or contentment. In humans, additional non-vocal communicative methods involve sounds like grunts, groans, and squeals, which can be likened to rats' vocalizations, such as hissing or squealing. During play or aggressive interactions, rats produce different vocalizations to communicate whether a social encounter is intended as an invitation or a warning (Venkatraman et al., 2024). Furthermore, rats also utilize body language, like raised fur, to signal pain, discomfort, defensive behavior, or grooming challenges (Ebbesen & Froemke, 2021).

In addition to these experimental advantages and relevant comparisons to human behaviors, rats present several logistical benefits when compared to human participants. Their shorter lifespans result in quicker developmental cycles, allowing researchers to observe changes across generations within reasonable time frames (Bryda, 2013). Furthermore, they require significantly fewer resources to care for, which makes extended studies more feasible. Using rats in laboratory settings enables researchers to maintain strict experimental controls over critical variables, such as social learning histories, housing conditions, genetics, and access to social reinforcers (Bryda, 2013). These controls would be either unethical or impractical to replicate in human participants, thereby emphasizing the importance of using rat models in behavioral research (Guo & Sun, 2023).

A prominent example of the applicability of rat experiments to human social behavior was a series of studies conducted starting in 1978 known as the "Rat Park" (Alexander et al., 1978; Gage & Sumnall, 2019). These studies sought to explore the effects of housing conditions and social isolation on morphine self-administration in rats. The findings revealed that female rats were significantly more affected by periods of isolation, as evidenced by their higher morphine consumption following isolation compared to male rats under similar conditions or female rats not subjected to isolation. Remarkably, the studies demonstrated a decrease in morphine self-administration among all rats when they were housed in a social environment, as opposed to those in isolation, highlighting a correlation between social context and substance use (Alexander et al., 1978). These results offer a valuable analogy for human behavior, suggesting that individuals who are socially integrated may exhibit lower levels of substance use than those who experience social isolation. Subsequent research conducted by Alexander and colleagues

further explored these findings, including a 1981 study that examined the impact of environmental enrichment in contrast to more austere housing conditions. The results indicated that rats residing in enriched environments consumed less morphine than those housed in environments lacking engaging stimuli (Gage & Sumnall, 2019). This research spotlights the significant role of environmental factors in shaping behavior, highlighting the potential benefits of enriched environments for humans. People in enriching environments with social networks are likely to show lower levels of substance use compared to those in less stimulating settings (Galaj et al., 2020).

Considering the results of the prior studies mentioned, the contributions of rats to behavioral research are not only ongoing but essential. The insights gained from studies involving these animals continue to inform our understanding of human behavior, paving the way for new interventions and applications that can benefit society as a whole (Bryda, 2013). The interplay between animal research and human application ultimately reflects a comprehensive approach to understanding complex behaviors, leading to innovative solutions backed by empirical evidence. Based on this understanding, a translational approach is likely to yield practical benefits when exploring various interventions for promoting social behavior.

Assessing Social Behavior in Rats

When investigating social behavior from a translational perspective, rat models are a valuable tool because they are a highly social species. Social behavior in rats encompasses a range of activities that reflect their interactions with others, including parenting, play, aggression, nesting, grooming, licking, climbing, and social following (Acikgoz et al., 2022; Anderson & Layton, 2012; Galizio & Odum, 2022). These behaviors are crucial for understanding the social

dynamics within rat populations and have been instrumental in studying various psychological disorders such as ASD, depression, and bipolar disorder (Acikgoz et al., 2022). Rats are often utilized as model organisms in behavioral research due to their social nature and the ease with which their interactions can be observed and quantified. There exist broadly at least three distinct methods commonly employed by researchers to evaluate social behavior in rodents: the social interaction test, the social preference test, and the social choice procedure. These methodologies are supported by a substantial body of literature (Acikgoz et al., 2022).

Social Interaction Open Field Test

One of the simplest methods to evaluate social interactions is the social interaction open field test. In a study conducted by Galizio and Odum (2022), this test was employed to investigate social interaction differences between typical rats and rats exposed to valproate in utero (thought to be a model for ASD). The social interaction test typically entails isolating a rodent from its conspecifics for 24 hours. Following this isolation period, the rodent is reintroduced to a peer, and researchers subsequently analyze the social behaviors displayed by the previously isolated rodent. During these sessions, behaviors such as licking, climbing, chasing, sniffing, and playing with the stimulus rats were documented. These actions reflect the underlying social dynamics and can provide insights into whether deficits are present in experimental rats. One of the limitations of the open field test within the study pertains to the behavioral definitions of the social behaviors exhibited by the rats. It remains ambiguous whether certain behaviors, such as orienting toward another rat or maintaining proximity to another rat, accurately reflect social interaction. Furthermore, an additional limitation of the assessment involves the lack of evaluation regarding the inherent value of the social stimuli

presented. Lastly, there was no isolation of the aspects of social interaction deemed more critical, such as determining whether the behaviors associated with olfactory interaction with another rat are of greater significance than engaging in play with that rat, among other factors.

Social Preference Test

The social preference test, commonly referred to as the social avoidance or social approach test, serves as a prominent methodology for evaluating social behaviors and preferences for social interaction in rodent models, particularly in rats (Acikgoz et al., 2022). This experimental paradigm quantifies the duration of time a rat spends engaging with a social partner, compared to its interactions with non-social stimuli, such as food items, during the observation period. For instance, Reppucci et al. (2020) employed a three-chamber setup. Rats are placed in the center chamber and allowed to access either side chamber at any time. When one chamber contains another rat and the other chamber contains nothing, rats generally choose to spend more time with the other rat than in isolation. A critical addition to this experimental framework was the incorporation of alternative stimuli, such as food, which further enriched the environmental context for the rats. The findings from these experiments markedly illustrated that adolescent rats exhibited a pronounced preference for social interaction when they were not subjected to food deprivation. Conversely, under conditions of food deprivation, these rats exhibited a distinct preference for food over social engagement, indicating that preference for social interaction can be disrupted by competing reinforcers (Reppucci et al., 2020). Similar to the open field assessment, this procedure is limited in that it does not isolate the relevant aspects of the other rat that drive preference, and it does not quantify how valuable social interaction is

to rats. There are also many other variations of the social preference test, including other approach and avoidance paradigms, each with similar limitations (Acikgoz et al., 2022).

Social Choice Procedures

Social choice procedures assess whether rats will work to gain access to other rats (e.g., Hackenberg et al., 2021). This test typically involves observing rodents in situations where they can choose to respond for social or nonsocial stimuli. Hackenberg et al. (2021) implemented this test by using an operant chamber with three separate sections where the target rats were placed in the center of the chamber where, instead of having unrestricted access as in the social preference paradigm, they were able to press levers to enter the left or right side of the chamber. One of these lateral sections contained either a restrained cage mate or a novel rat. The other contained either a different rat or an empty chamber. This methodology permitted an assessment of whether rats would exert effort to gain access to another rat rather than to an empty room or one that only contained a harness without another rat present. The results showed that rats reliably responded to access another rat. However, one rat also responded for non-social stimuli (e.g., an empty harness). A strength of this procedure is that it has the capability of assessing the relative value of social stimuli compared to each other or to nonsocial stimuli by recording the number of responses rats are willing to exert. However, because rats are responding to access a restrained rat in a harness, it is still unclear which aspects of the chamber rats are responding to obtain. It is possible that they are working for the other rat, but it is also possible, as was the case with at least one rat, that they were responding to access nonsocial stimuli in the chamber.

Another type of social choice procedure has been proposed more recently and addresses these limitations. It is known as the free operant social olfactory choice procedure (Hinnenkamp

et al., 2025). The free operant social olfactory choice procedure was designed to evaluate female Long Evans rats' preferences for different types of olfactory stimuli. The initial experiment detailed in the paper featured the presentation of used bedding from other rats to represent social scents, along with cotton pads infused with essential oil droplets, such as vanilla and strawberry, to represent non-social scents. Clean bedding was utilized as the control scent. In the operant chamber, two nose ports were available to the rats, allowing them to choose between air scented with one of the social odors or air infused with the non-social scents and the control scent through the opposing port. The findings revealed that the rats exhibited a preference for both social and non-social scents in comparison to the control scent. Furthermore, the researchers investigated the rats' preferences for social scents versus non-social scents when both were presented simultaneously, without the control scent. In this setup, one nose port presented one of four social scents derived from the used bedding of four different rats, while the opposing port offered one of four non-social scents from the essential oil-infused cotton pads. The results indicated individual differences among the six participating rats. One rat showed indifference, three rats favored the social scents over the non-social ones, and two rats appeared to exhibit a bias, as they continued to respond more frequently to one port even after a reversal was implemented (Hinnenkamp et al., 2025).

The free operant social olfactory choice procedure offers several advantages. One of its main benefits is that the operant chamber is automated, which helps reduce the risks of human error, observer bias, or unnecessary human interaction. This automation also streamlines data collection, eliminating the need for a person to monitor the experiment throughout the entire hour, making it more time-efficient. Additionally, the scents can be carefully controlled to limit

the introduction of extra variables, such as exposure to other odors. For instance, this method can uncover individual differences in preferences for social scents, as shown by the varied results among participants in the second experiment (Hinnenkamp et al., 2025). Beyond just preferences for location and social compared to nonsocial scents, there were also significant differences in how much each rat responded, with some being more active than others (Hinnenkamp et al., 2025). Another strength of this method is its ability to directly compare two or more different scents within the same session. Since the procedure gathers data over multiple days, it is particularly effective for assessing how behavioral or environmental changes can influence preferences. After establishing a baseline for social preferences, researchers can systematically test the effects of various interventions. However, while there are clear benefits to this procedure, some limitations must also be considered. For example, a side bias may skew the results, and it can be challenging to manage the scents in the experimental room since nearby boxes could introduce additional odors through the airports. One additional limitation of this procedure is that it solely assesses the preferences of the rats for social olfactory stimuli, without evaluating other sensory interactions, such as those involving visual or tactile systems. While the free operant social olfactory choice procedure does have certain limitations, it offers advantages that surpass those of competing methodologies.

Enhancing the Value of Social Stimuli

One common strategy thought to enhance social behavior in ABA therapy for children with ASD is pairing. Pairing within applied settings includes practices such as imitating the behaviors of the child, presenting the child with preferred stimuli, amounts of non-contingent

reinforcement[1], engaging with the child and individuals that have previous rapport with the child, and minimizing demands (Lugo et al., 2017; Ensor et al., 2024). Pairing is beneficial in increasing compliance, decreasing instances of challenging behaviors, reducing the aversiveness of therapeutic sessions, and reducing off-task behaviors (Gormley et al., 2020).

The implementation of pairing in clinical settings varies across different studies. Shillingsburg et al. (2018) used a multiple baseline design to demonstrate the effectiveness of pairing in reducing problem behaviors among four young children with ASD diagnoses. The methods of the study consisted of four stages that began with the presentation of various items non-contingently, then the requirements progressed to the therapist coming into closer proximity to the participant, then increasing the response requirement by making access to the items contingent on the child remaining in close proximity to the therapist, and the final stage consisted of the presentation of demands (Shillingsburg et al., 2018). In another study, Fanning Tacoaman et al. (2024) defined pairing as a combination of distinct therapist behaviors, including maintaining close proximity to the child, imitating vocalizations and movements exhibited by the participating children, and modeling various play actions with the toys available, among others, a short period of time before the instructional session to engage with the child and the items the child preferred. During this time, the instructor provided various statements related to the activity and waited for natural breaks in the activities before wrapping them up. This approach allowed

[1] Non-contingent reinforcement (NCR) is a problematic term in the field of behavior analysis since the name of the concept suggests that all contingencies are removed, which is inaccurate. Contingencies remain during NCR such as proximity, time, and natural contingencies that may remain. In the context of this paper, the term non-contingent reinforcement will refer to the antecedent manipulation strategy where demands are minimized, and potential reinforcers are presented at a higher frequency than in the absence of that procedure.

for a smooth transition into the instructional tasks, such as concluding a song that the child had selected (Fanning Tacoaman et al., 2024). A study conducted by Ensor et al. (2024) explored pairing to establish rapport between therapists and clients. The pairing procedure included having therapists maintain a close proximity to the child, imitate the child's vocalizations and play actions, offer additional potential reinforcers during the pairing session, provide specific praise related to behavior, and engage in novel play activities with items that captured the child's interest (Ensor et al., 2024). Despite the research that has been conducted on specific pairing procedures, the mechanisms by which these procedures are effective are unclear.

One noteworthy intervention that utilizes similar procedures to pairing is Parent-Child Interaction Therapy (PCIT), a specialized program designed to train parents to adaptively interact with their children in play-based settings through the guidance of trained therapists (Hembree-Kigin & McNeil, 1995). PCIT focuses on helping parents develop skills to engage in positive child-led play interactions while managing challenging behaviors. Although pairing is focused primarily on these kinds of positive social play interactions, PCIT is particularly beneficial for addressing issues such as non-compliance and challenging behaviors that may cause harm to the child or others (Hembree-Kigin & McNeil, 1995). In PCIT, parents are taught how to encourage positive play while minimizing reinforcement of challenging behaviors (Hembree-Kigin & McNeil, 1995). PCIT sessions are often structured with specific time allocations, typically lasting about 15 minutes, with approximately 10 minutes dedicated to direct parent-child engagement (Hembree-Kigin & McNeil, 1995). In addition to procedural similarities, PCIT also draws strong connections to pairing procedures through its treatment effects. Outcomes stemming from this approach often include increased compliance, reduced

maladaptive behaviors, and a tailored fit to the needs of the participants involved (Cooley et al., 2014; Gormley et al., 2020), similar to pairing. The combined results foster more positive interactions between the treatment administrator, whether a parent, teacher, or therapist, and the child (Cooley et al., 2014; Gormley et al., 2020). Both procedures are also highly versatile in accommodating various ages, behavioral challenges, diagnoses, and family dynamics. Another element of PCIT that also aligns with pairing is its methods for facilitating transitions between activities, ultimately helping shift control from the child back to the parent or therapist (Shillingsburg et al., 2018; Cooley et al., 2014).

Despite the clear benefits of pairing and similar procedures (e.g., PCIT), the underlying mechanisms of these results are unclear. Numerous theories have suggested several possible components of the pairing procedure that may lead to its ability to enhance clinical outcomes. One notable concept is the theory of conditioned reinforcement. This theory suggests that the therapist becomes a generalized reinforcer through consistent and repeated interactions between the therapist, the child, and the child's existing reinforcers (McLaughlin & Carr, 2005). According to Kelly et al. (2015), conditioned reinforcement involves the association of the instructor with potent reinforcers that have previously been established, thereby rendering the instructor a conditioned reinforcer (Kelly et al., 2015). In his book, *Science and Human Behavior*, Skinner (1953) elaborates on how effective generalized reinforcers are, particularly concerning the concepts of satiation and deprivation of the primary reinforcers linked to these generalized reinforcers. Unlike primary reinforcers like food, one does not directly experience deprivation or satiation with a generalized reinforcer such as money. Instead, money acts as a powerful motivator because of its connections to various primary reinforcers, including essential

items like food and water. The drive to acquire more money stems from the deprivation of those goods that can be used to purchase. This analogy can also be applied to therapists' work, who utilize the principles of deprivation and satiation of both primary and conditioned reinforcers to effectively influence their clients' motivations (Skinner, 1953). Many of the pairing procedures used in clinical settings seem to rely on this theory of conditioned reinforcement (e.g., making sure the client and therapist are in close proximity, pairing oneself with multiple previously established reinforcers, etc.), which emphasizes the necessity of gaining a deeper understanding of the mechanics of this theory.

Current pairing procedures predominantly rely on methods that optimize the likelihood of the therapist becoming a generalized conditioned reinforcer. However, alternative theories regarding the efficacy of the pairing intervention have been proposed. Kelly et al. (2015) outlined various rationales concerning the behavioral effects observed in participants during sessions that follow the introduction of presession pairing. One theory involves non-contingent reinforcement, which provides an individual with access to reinforcers regardless of specific behavioral responses. This approach can diminish the motivation to engage in challenging behaviors to obtain those reinforcers, serving as an abolishing operation. Another theory is that the pairing sessions may have deconditioned the association between the instructor and aversive stimuli. In many cases, the instructor and the learning environment are associated with worsening conditions signified by a lower rate of reinforcement and a higher rate of demands in the presence of those conditions. By conducting pairing sessions with a high rate of reinforcement before the instructional phase, the instructor is associated less with the potentially aversive learning environment and more associated with appetitive stimuli. Another theory

proposed that the mechanism of pairing centers around the theory of behavioral momentum. Behavioral momentum theory presents the idea that a richer schedule of reinforcement is more resistant to extinction when environmental conditions alter. This theory suggests that tolerating the transition to less preferred instructional tasks is facilitated by a history of the high reinforcer rates inherent to presession pairing (Kelly et al., 2015).

At least four theories, including conditioned reinforcement, behavioral momentum, aversive deconditioning, and non-contingent reinforcement, have been proposed to explain the effective processes involved in the pairing procedures. Unfortunately, very little research has been done to test and understand these potential theories, so it is unclear which theory best explains the effectiveness of presession pairing. As illustrated in the specific pairing procedural details described previously, many common steps seem to be informed by various theories. For example, most of the steps involved in the pairing procedures described by Fanning Tacoaman et al. (2024) and Ensor et al. (2024) are primarily informed by the conditioned reinforcement theory (e.g., maintaining close proximity to the child's reinforcers). However, PCIT places emphasis on reconditioning parent-child interactions so that the child no longer finds these interactions aversive, consistent with aversive deconditioning. If we fully understand the underlying mechanisms of pairing, we can optimize pairing procedures to include only the most critical components using component or parametric analyses. Therefore, more research is needed to fully understand the effectiveness of pairing, the optimal procedures involved in pairing, and the theories that support them. Translational research is ideal for testing these theories and informing clinical practice by bridging the gap between laboratory discoveries and practical applications in patient care and ensuring that scientific advancements are effectively translated into real-world

solutions. This translational research approach has the potential to not only validate theoretical models through rigorous experimentation but also provide opportunities to refine these models based on clinical feedback.

Purpose

Although pairing is a frequently used intervention in applied settings, the mechanism of how pairing may enhance clinical outcomes remains unknown. Utilizing translational research could be advantageous in this context, allowing for the refinement of models and mechanisms associated with pairing, informed by clinical feedback, for further analysis in experimental settings. The purpose of the present translational experiment is to examine the pairing intervention in a basic lab setting with rats. By studying the procedure in a highly controlled experimental setting, we hope to shed light on the proposed explanations, particularly the conditioned reinforcement theory. According to this theory, the therapist acquires reinforcing value simply by being paired with the client's preferred items and activities. To test this idea, we propose to model pairing sessions in rats by placing two rats in an arena with access to preferred treats and toys (e.g., cereal, dried fruit, nuts, chew toys, crawl tubes, hideaway toys).

We addressed this question by first assessing the reinforcing value of social scents in rats using the free operant social olfactory choice procedure. It was hypothesized that, consistent with previous research, rats would work to access social scents associated with another rat and that there would be individual differences in this preference (Hinnenkamp et al., 2025). After evaluating the initial reinforcing value of social scents, the goal was to investigate the effect of pairing sessions on rats' preference for social olfactory stimuli. If the mechanism underlying the pairing intervention was conditioned reinforcement, we hypothesized that rats exposed to pairing

sessions with a partner rat would prefer the partner rat's social scent over that of other rats. The results of this study could have implications for the effectiveness of and mechanisms underlying pairing in clinical settings.

CHAPTER 2: METHOD

2.1 Subjects

Approval was obtained from the MTSU Institutional Animal Care and Use Committee before the study began, which involved fourteen rats (see Appendix A). Fourteen female brown rats (*Rattus norvegicus*), approximately five weeks old, were used. Six of the rats (R1-R6) served as primary subjects, and eight (SA-SH) served as stimulus rats acting as scent donors and potential pairing partners. Upon arrival to the colony, all rats were individually housed until preference assessment sessions had been completed (see below). After that, the primary rats were pair-housed to encourage normal social interaction during development, while stimulus rats remained individually housed to isolate their scents. All rats were kept in clear plastic cages with stainless steel lids, provided with standard rat chow and water, and lined with dried corncob bedding. All were maintained on a free-feeding schedule, ensuring they were not deprived of food or water.

All rat home cages were located in the colony room, which is temperature- and humidity-controlled and operates on a reverse 12-hour light/dark cycle (lights on from 6 PM to 6 AM). All testing sessions were conducted during the dark cycle, and a red light was present in the colony room from 11 AM to 6 PM so the researchers could work while minimizing disruption to the rats' resting cycle (Hinnenkamp et al., 2025). Experimental sessions were conducted up to six days per week.

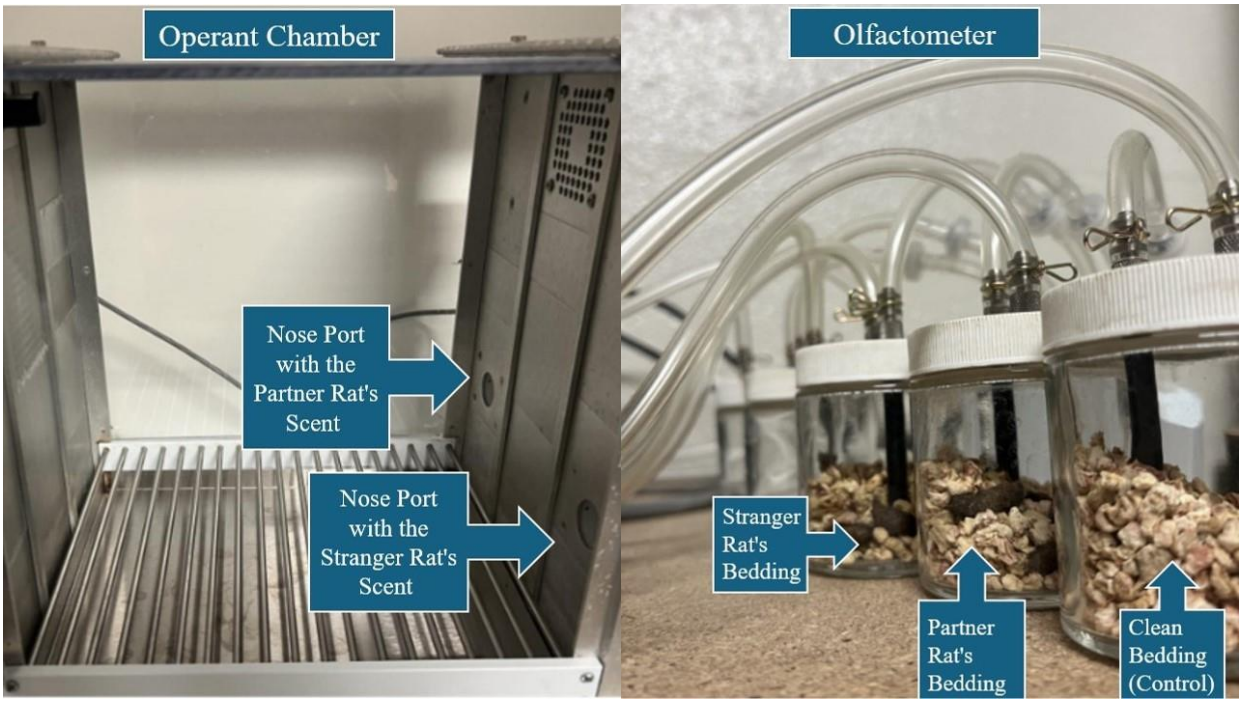
2.2 Apparatus

In the experimental setting, two Med Associates operant chambers with nose poke ports and olfactometers were utilized to perform the free operant social olfactory choice procedure

(see Figure 1). Each chamber (ENV-007) was enclosed in a sound-attenuating cubicle (ENV-018MD) and equipped with an overhead LED house light, two nose-poke ports (ENV-275-NPP) on the front wall of the chamber, arranged horizontally adjacent to one another, and bars lining the bottom of the chamber with intervals between each bar. A tray was located at the bottom of the chamber to collect the rats' excrement. Each nose-poke opening contained an internal light that briefly illuminated when a body part or object is inserted, prompting the recording of a response with a photo beam. Externally, the chamber was connected to an olfactometer (ENV-275-5), which housed five pressurized jars containing the social scents, specifically used bedding that included both excrement and corn cob bedding sourced from the home cages of the individual stimulus rats. Jars were filled to approximately one-third capacity with the social scents. The contents of the jars were replaced daily, and new samples of the social scents were collected weekly on Mondays. Clean bedding in one of the five jars served as a control scent to clear residual social odor, with clean air cycled through the operant chamber after each social scent presentation. The lids of the jars were fitted with airtight tubing that extended from the jars to the operant chamber, allowing pressurized air to be introduced into the chamber upon insertion of a body part or object into the nose-poke openings. The olfactometer was controlled by a computer running Med-PC software, which systematically recorded data pertaining to the duration and frequency of nose pokes.

Figure 1.

Operant Chamber and Olfactometer



Pairing sessions occurred in the colony room where the rats were housed. During each session, the pairing room was illuminated with red light. The pairing apparatus included a plastic home cage with stainless steel open-air lids. The cage was lined with dried corncob bedding, a small dish containing preferred edibles (e.g., nuts, dried fruit, or cereal), and a play item (e.g., chew toys, hideout trunk, or crawl tube).

2.3 Procedure

2.3.1 Preference Assessment for Food and Toys

Eight free operant preference assessment sessions were conducted with each primary rat to assess which food items and play items they preferred to consume or engage with. Each day,

up to one food preference assessment session and one toy preference assessment session were conducted per rat until four sessions of each have been completed. The information gathered from all eight preference assessments for each primary rat provided insight into which foods and toys to present during their pairing sessions (see Data Analysis for details about ranking).

During the food preference assessments, four different food items in separate containers were placed in the four corners of each primary rat's home cage. During setup, the number of food items in each container was counted to get the initial amount for later scoring. Four food preference assessment sessions were conducted, with different food container placements to counterbalance and reduce location bias. For each food preference assessment, food containers were placed in the rat's home cage for 15 minutes. Then, the rat was removed, and the number of food items present in each food container was counted and recorded. Food items that had been moved outside their allocated food containers were also counted, but these quantities were recorded separately. Even though these items had not been consumed, their removal from the container indicated that the rat had engaged with the food items in some way. The ranking of food items was established based on the quantity change of each food item remaining following the assessment. Food items that have been consumed or removed from the food containers in increased quantities were assigned a higher ranking.

Figure 2.

Food Preference Assessment Items

Note: The images show the front and top view of the food items used during the free operant choice food preference assessments. The top left of the images shows Froot Loops, the top right shows Cheerios, the bottom left shows unsalted peanut halves, and the bottom right shows freeze dried banana chips.

The four toy preference assessment sessions were based on the four play items the rat could engage with. The items were placed into each of the four corners of a large arena. Primary rats were placed individually in the arena for 10 minutes, and each session was video recorded. Each preference assessment involved placing the items in different locations to counterbalance and reduce location bias. Data were collected from the videos by recording the frequency and duration of engagement with each toy item, which was defined as physical contact between any part of the rat (excluding the tail) and any part of the toy (e.g., chewing, lying on, dragging, sitting on, climbing on, etc.). The rank was determined by which items they engaged with for the longest duration.

Figure 3.

Toy Preference Assessment Items

Note: The images show the front and top view of the toys used during the free operant choice toy preference assessments. The top left of the images shows the crawl tube, the top right shows the hideaway trunk, the bottom left shows the round chew, and the bottom right shows the stick chew toy.

The results of the preference assessments are shown in Table 1. For food items, all rats preferred Peanuts most, closely followed by either Cheerios (R1, R5, and R6) or Banana Chips (R2, R3, and R4). Food rankings were primarily determined based on the amount of food consumed (i.e., number of items missing); however, the ranking for R3 required consideration of consumption and general interaction (i.e., number of items displaced from the container). R3 only consumed one peanut half across all four sessions, so her second preferred item was determined by which items were most commonly displaced from the container, banana chips, indicating that she had interacted with them in some way, even though she did not consume them. For toys, all rats except for R1 preferred the Crawl Tube most, while R1 preferred the Hiding Trunk most. The second-most preferred toy was either the Crawl Tube (R1), the Hiding

Trunk (R2, R4, R5, and R6), or the Stick Chew (R3). The two most preferred food items and the two most preferred toys for each subject were subsequently used during their pairing sessions.

Table 1.

Preference Assessment Results

Food Preference Assessment

Subject	Rank 1	Rank 2	Rank 3	Rank 4
R1	Peanuts	Cheerios	-	Froot Loops / Banana Chips
R2	Peanuts	Banana Chips	Froot Loops	Cheerios
R3	Peanuts	Banana Chips	-	Froot Loops / Cheerios
R4	Peanuts	Banana Chips	Cheerios	Froot Loops
R5	Peanuts / Cheerios	-	Froot Loops	Banana Chips
R6	Peanuts	Cheerios	Banana Chips	Froot Loops

Toy Preference Assessment

Subject	Rank 1	Rank 2	Rank 3	Rank 4
R1	Hiding Trunk	Crawl Tube	Round Chew	Stick Chew
R2	Crawl Tube	Hiding Trunk	Stick Chew	Round Chew
R3	Crawl Tube	Stick Chew	Hiding Trunk	Round Chew
R4	Crawl Tube	Hiding Trunk	Stick Chew	Round Chew
R5	Crawl Tube	Hiding Trunk	Stick Chew	Round Chew
R6	Crawl Tube	Hiding Trunk	Round Chew	Stick Chew

Note: Rank 1 indicates the most preferred item. When there was a tie, both items were ranked together. The two most preferred items from each category were used during subsequent pairing sessions.

2.3.2 Baseline Social Preference: Free Operant Social Olfactory Choice Procedure

The initial phase of the experiment involved measuring the primary rats' baseline preference for different stimulus rats using the free operant social olfactory choice procedure. At least 6 sessions of the free operant social olfactory choice procedure were conducted with each rat. Sessions were conducted three days per week (Mondays, Wednesdays, and Fridays) at approximately the same time each day. At the start of the session, the primary rat was placed in the operant chamber. The house light turned on, both nose-poke ports were illuminated, and the olfactometer delivered control air (air blown through a jar containing unused rat bedding) into both nose-poke ports. When the rat placed their nose in one of the ports, a 0.3-s burst of air was blown through a jar containing the used bedding of a stimulus rat into the port. One port was connected to a jar containing the used bedding of one stimulus rat (e.g., Rat A), and the other port was connected to a jar containing the used bedding of a different stimulus rat (e.g., Rat B). After the scent was delivered, the control air resumed. A 0.5-s interresponse interval was enforced, so that the rat must remove their nose from the port for at least 0.5 s before new responses could be registered. The session continued for one hour, and the rat had the opportunity to respond to either port as many times as they could.

Following the completion of at least six baseline sessions, preferences for stability were analyzed to identify pairing partners for the primary rats. Stability during baseline was determined through several criteria. First, the range of the frequency of nose pokes to each nose-poke port across the last three sessions had to fall within a maximum range of 10 responses, indicating that overall responding was similar across days. Second, the proportion frequency of nose pokes to each nose-poke port across had to indicate a proportion responding greater than 0.5

for one stimulus rat over the other in two of the last three sessions (or one of the last three sessions, if the other two sessions were 0.5), with no steady trend of proportion in the direction of the less-preferred rat. After these criteria were met, the less-preferred stimulus rat was selected as the partner rat for the primary rat (due to experimenter error, the more-preferred stimulus rat was erroneously selected as the partner rat for R4). A maximum of nine baseline sessions was conducted; if stability criteria had not been met at that point, the partner was randomly selected. The subjects that met the maximum of nine sessions and required a random assignment of their partner rat included R2 and R6. Table 2 provides an overview of the rat assignments. To reduce the number of stimulus rats required for the current experiment, the first four primary rats completed baseline sessions first. The rats not assigned as partners for these rats were then used as stimulus rats for the remaining two primary rats.

Table 2.

Rat Assignments During and Following Baseline

Primary Rat	Stimulus Rat – Left Port	Stimulus Rat – Right Port	Assigned Partner Rat
R1	Stimulus Rat A	Stimulus Rat C	Stimulus Rat C
R2	Stimulus Rat E	Stimulus Rat G	Stimulus Rat E
R3	Stimulus Rat B	Stimulus Rat D	Stimulus Rat D
R4	Stimulus Rat F	Stimulus Rat H	Stimulus Rat H*
R5	Stimulus Rat A	Stimulus Rat B	Stimulus Rat B
R6	Stimulus Rat F	Stimulus Rat G	Stimulus Rat G

Note: Whichever stimulus rat was less preferred during baseline was assigned as the partner rat for the primary rat during pairing sessions. An asterisk (*) indicates an error during rat assignments, leading to the higher preferred rat being assigned as the partner rat due to an error in data analysis.

2.3.3 Pairing Intervention

After each rat met the stability criteria for baseline, pairing sessions were initiated. Pairing sessions were conducted three days per week (Tuesdays, Thursdays, and Saturdays), and the rats continued to complete sessions of the free operant olfactory choice procedure on the alternate days (Mondays, Wednesdays, and Fridays). The free operant olfactory choice procedure was identical to baseline, using the same scents, one of which came from the rat that was now assigned as their partner rat for the primary rat.

During each pairing session, the primary rat and their assigned partner stimulus rat were placed in a plastic home cage equipped with food and toys corresponding to the outcomes of the preference assessments conducted with the primary rats. Specifically, the cage contained one of the primary rat's top two most preferred food items and one of the primary rat's two most preferred toys, randomly selected each day. Each session began with a 10-minute habituation period during which the primary rat was placed in the empty home cage, without preferred food or toys, and without the partner rat. After 10 minutes, the partner rat, toy, and food were added to the cage simultaneously, and the pairing session began. These pairing sessions lasted for 15 minutes (Hembree-Kigin & McNeil, 1995). Each session was video recorded and observed by an experimenter in real time. If one of the rats engaged in severe aggression and caused injury to the other rat, the experimenter was prepared to separate the rats to ensure their safety; however, this never occurred. Pairing sessions were conducted for up to 4 weeks. During this time, the proportion of responses to the partner rat's scent in the operant chamber was analyzed and compared to baseline to determine whether preference shifted towards the partner rat.

2.4 Data Analysis

The data collected from the preference assessments were systematically evaluated as described in the Procedure. A ranking of food items was established based on the quantity of food remaining in the presented containers. Items that were consumed or displaced in larger quantities from the food containers were assigned higher rankings. The data from each food preference assessment were compiled and collectively ranked to identify the two most preferred food items from the initial selection of four, which were subsequently utilized during the pairing sessions. A ranking of toys was established based on the amount of time the rat engaged with each toy item. Items that the rat spent the most time engaging with received higher rankings. The data from each toy preference assessment were compiled and collectively ranked to identify the two most preferred play items from the initial selection of four, which were subsequently used during the pairing sessions. Rankings for food and toy preference assessments are shown in Table 1. Interobserver agreement (IOA) for the duration data was evaluated for 33.33% of the total toy preference assessment sessions, resulting in an agreement rate of 87.5%.

The dependent variables in the free operant olfactory choice procedure portion of the experiment included frequency, duration, and proportion data for both. Frequency was the number of responses in each nose-poke port, and duration was the total amount of time in seconds spent in each port. Absolute frequency and duration data were used to calculate the proportion of responses (responses or time to partner rat / total responses or time) in the nose-poke port for the partner rat. A proportion exceeding 0.5 signified a preference for the partner rat over the stranger rat. In contrast, a proportion below 0.5 indicated a preference for the stranger

rat over the partner rat. A proportion of value of 0.5 suggested indifference or the absence of a distinct preference between the partner and the stranger rat.

Data was analyzed visually and through statistical testing. Given the small sample size and possible issues with normality, we performed a non-parametric Wilcoxon matched-pairs signed-rank test on the median proportions obtained from the last six baseline sessions and the last six pairing sessions (or all available sessions if the rats hadn't completed six pairing sessions). This test allowed us to statistically assess whether there was a significant difference between the two dependent samples, being baseline and during the pairing sessions.

Before conducting the study, an a priori power analysis was conducted utilizing G* Power version 3.1.9.7 (Faul et al., 2007) to determine the minimum sample size required for the evaluation of the study's hypothesis. Because the initial experimental design involved three phases, the original power analysis was conducted for a repeated-measures ANOVA. Due to time constraints, the third phase was omitted, making the Wilcoxon matched-pairs signed-rank test a more appropriate analysis, as described previously. However, the original power analysis indicated that a sample size of N = 6 per group would be adequate to detect a large effect (dz = 1.3), assuming a significance level of $\alpha = .05$ and power of 0.8, for a repeated-measures ANOVA. Because no previous studies have used these procedures, we estimated our effect size to be large, because a large effect is more likely to have potential clinical significance. Although more subjects would have been ideal and would have permitted us to detect smaller effect sizes, current resources were limited. Therefore, the final sample size of N = 6 per group was regarded as sufficient for the testing of the hypothesis.

CHAPTER 3: RESULTS

The primary objective of this study is to demonstrate whether exposure to pairing sessions would enhance rats' preference for olfactory stimuli associated with a partner rat. To achieve this goal, we compared the proportion of responses to the scent of the partner rat in the free operant olfactory choice procedure, both prior to (baseline) and after the initiation of pairing sessions. Table 3 presents the number of free operant olfactory choice sessions required for each rat to meet the stability criteria during the baseline phase, as well as the number of free operant olfactory choice sessions completed during the pairing phase. Additionally, it outlines the number of pairing sessions experienced by each rat. Rats R1, R3, and R4 have completed the pairing phase, which consisted of 4 weeks of pairing sessions, each totaling 13 days of pairing. Data collection is currently ongoing for rats R2, R5, and R6.

Table 3.

Number of Choice and Pairing Sessions Completed Across Phases

Subject	Choice Sessions - Baseline	Choice Sessions - Pairing	Pairing Sessions
R1	6	12	13
R2	9	11	12
R3	8	12	13
R4	6	12	13
R5	6	5	6
R6	9	2	3

Tables 4 and 5 present descriptive statistics for the primary dependent measures analyzed from performance within the free operant olfactory choice procedure across various phases. Specifically, Table 4 displays the absolute frequency, defined as the number of nose pokes directed toward each nose-poke port per session, and the absolute duration, which indicates the total seconds spent at each nose-poke port per session. In contrast, Table 5 outlines the proportion frequency in the port associated with the scent of the partner rat, calculated as the frequency of nose pokes to the partner port divided by the total nose pokes, along with the proportion of duration spent in the port with the scent of the partner rat, computed as the time spent in the partner port divided by the total duration. These tables include the median and interquartile range for each measure during the final six baseline sessions and the last six pairing sessions. For the rats that have not completed six sessions within the pairing phase, the median and interquartile range were determined from all pairing sessions conducted to date.

Table 4.

Descriptive Statistics for Absolute Frequency and Absolute Duration

	Frequency				Duration (s)			
	Baseline		Pairing		Baseline		Pairing	
Subject	Partner	Stranger	Partner	Stranger	Partner	Stranger	Partner	Stranger
R1	5.50 (2.50)	7.00 (2.00)	4.00 (1.50)	2.00 (0.75)	4.43 (1.12)	5.70 (2.04)	2.24 (1.47)	1.95 (0.67)
R2	8.50 (1.75)	10.00 (4.50)	3.50 (1.75)	3.50 (1.00)	8.01 (8.61)	8.60 (7.42)	1.72 (0.63)	2.81 (1.53)
R3	12.00 (8.50)	14.50 (1.75)	6.50 (4.00)	9.00 (3.00)	7.21 (9.66)	8.96 (5.03)	2.69 (2.94)	10.20 (5.57)
R4	9.50 (1.00)	8.00 (1.50)	5.00 (1.50)	4.50 (1.75)	10.87 (4.80)	9.59 (3.73)	7.53 (9.14)	7.44 (5.91)
R5	11.50 (9.50)	15.00 (1.50)	13.00 (1.00)	12.00 (3.00)	21.95 (25.63)	51.88 (9.50)	14.82 (10.24)	23.35 (23.48)
R6	6.50 (2.50)	8.50 (3.25)	8.00 (1.00)	8.00 (2.00)	7.01 (2.46)	11.72 (2.50)	7.40 (0.95)	9.83 (3.06)

Note: This table displays the median and interquartile range from the last 6 sessions of each phase (or all sessions if fewer than 6 sessions have been completed in the phase so far).

Table 5.

Descriptive Statistics for Proportion Frequency and Proportion Duration

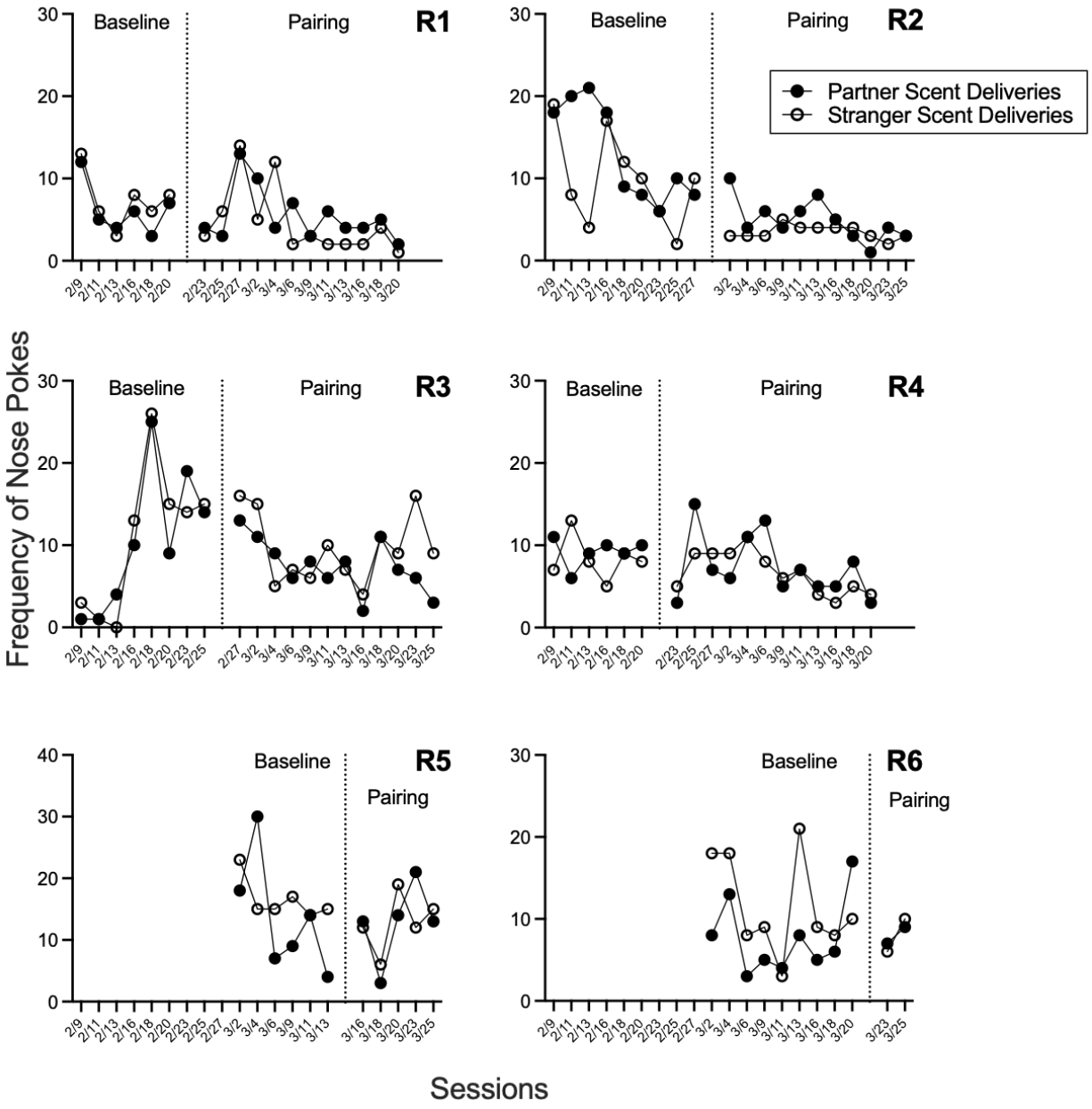
	Proportion Frequency		Proportion Duration	
Subject	Baseline	Pairing	Baseline	Pairing
R1	0.46 (0.04)	0.67 (0.08)	0.43 (0.12)	0.54 (0.07)
R2	0.47 (0.07)	0.53 (0.19)	0.49 (0.25)	0.42 (0.37)
R3	0.49 (0.11)	0.39 (0.20)	0.53 (0.14)	0.34 (0.12)
R4	0.54 (0.09)	0.53 (0.13)	0.60 (0.23)	0.52 (0.12)
R5	0.39 (0.16)	0.47 (0.10)	0.29 (0.31)	0.31 (0.27)
R6	0.48 (0.19)	0.50 (0.03)	0.34 (0.32)	0.44 (0.11)

Note: This table displays the median and interquartile range from the last 6 sessions of each phase (or all sessions if fewer than 6 sessions have been completed in the phase so far).

Figure 4 illustrates the frequency of nose pokes allocated to each port within the context of the free operant olfactory choice procedure throughout the experiment. As presented in Table 4 and Figure 4, notable individual differences were observed in the number of nose pokes executed by the rats at each port, with certain individuals exhibiting a significantly higher frequency of responses per session compared to others. For instance, the highest number of responses recorded across both ports during a single session was 51 for R3, whereas R4 achieved a maximum of only 24. Within individual rats, the frequency of responses displayed considerable variability over the course of the experiment. For example, R3 recorded a mere two responses in one session while achieving up to 51 responses in another session. Overall, the trend indicated that the frequency of responding decreased as the experiment progressed for the majority of the rats, a phenomenon consistent with findings reported previously (Hinnenkamp et al., 2025).

Figure 4.

Absolute Frequency of Nose Pokes Allocated to Each Port

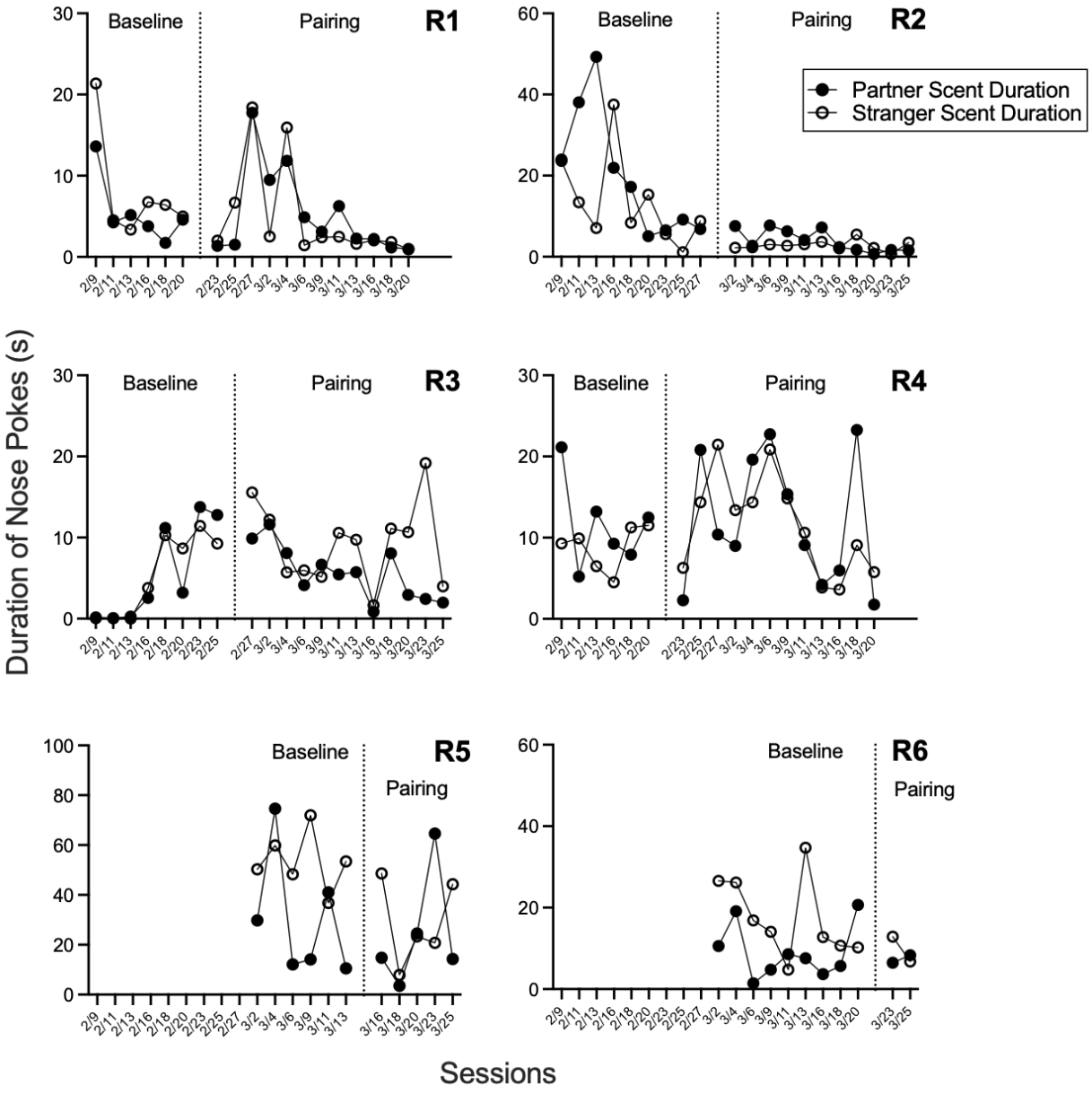


Note: The y-axis range differs for R5. Filled and unfilled circles represent responding to the partner and stranger scents, respectively.

The data presented in Figure 5 illustrate the duration of time allocated by each rat to the nose-poke ports during the free operant olfactory choice procedure throughout the course of the experiment. As highlighted in Table 4 and Figure 5, there were significant individual variations in the time that rats dedicated to each port, with certain rats exhibiting a markedly greater amount of time spent per session compared to others. For instance, the highest recorded time spent in both ports during a single session was 134.55 seconds for subject R5, in contrast to merely 25.43 seconds for subject R3. Additionally, for certain rats, specifically R1 and R2, the duration of responding demonstrated a trend analogous to response frequency, whereby the duration decreased as the experiment progressed. However, it is noteworthy that durations varied considerably throughout the study for the remaining rats.

Figure 5.

Duration of Time Spent in Each Port (seconds)



Note: The y-axis range differs for R2, R5, and R6. Filled and unfilled circles represent responding to the partner and stranger scents, respectively.

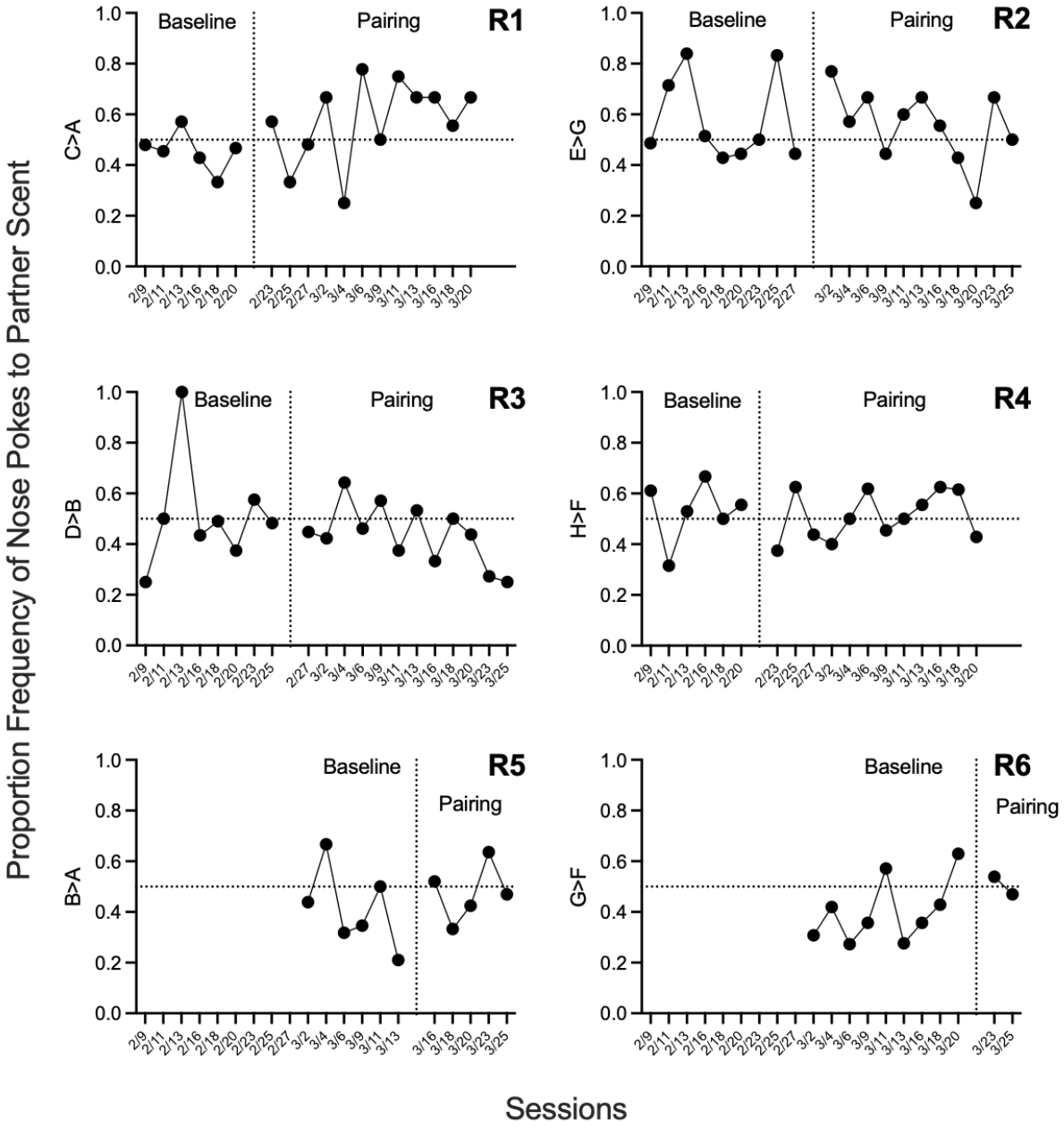
Figure 6 displays the proportion of nose pokes allocated to the partner rat in comparison to the stranger rat. In this study, the rat that showed the least preference during the baseline phase was labeled as the partner rat. Consequently, all rats exhibited indifference or a slight preference for the stranger rat prior to pairing, except for R4, who was mistakenly paired with a rat linked to the slightly more preferred scent due to an error by the experimenter. Once the pairing sessions began, the hypothesis suggested that preference would shift toward the partner rat. R1, R3, and R4 have completed their pairing sessions, allowing for a full interpretation of their data.

For R1, after the phase change, there was a slight upward trend in responding, with most data points clustering above the 0.5 indifference line. There was a period of increased responding toward their partner rat, as evidenced by the final 7 sessions, which had values at or above 0.5. For R2, sustained responding towards their partner rat was not consistently observed, as data points alternated around 0.5 with only brief runs (2–3 consecutive sessions) above the line. The data for R2 is still ongoing. During the pairing phase, R3 exhibited a gradual downward trend, with its levels shifting from around 0.5 to mostly below 0.5. The variability was moderate, showing noticeable fluctuations from session to session. There was increased responding toward the stranger rat, which was evident as R3 consistently responded to the stranger rat for five consecutive days during the final days of the pairing phase. During pairing, R4 displayed a consistent level of responding that resembled their behavior during the baseline phase, with the level centering around 0.5. R4 showed a tendency towards indifference, as evidenced by brief periods (three to four consecutive sessions) where they would respond consistently towards the partner rat, as well as similar brief periods of responding towards the stranger rat. R5 showed a level with values fluctuating around 0.5. Sustained responding towards the stranger or partner rat

was not observed. However, there were only five data points collected so far during this phase, making it difficult to draw definitive conclusions. For R6, there were only two data points available so far during the implementation of the pairing phase, which also makes it challenging to infer meaningful insights. Nevertheless, the data indicate that R6 showed a response toward the partner rat on the first day following the pairing sessions and shifted to responding toward the stranger rat on the second observed day after the second pairing session.

Figure 6.

Proportion Frequency of Nose Pokes Allocated to the Partner Rat Relative to the Stranger Rat

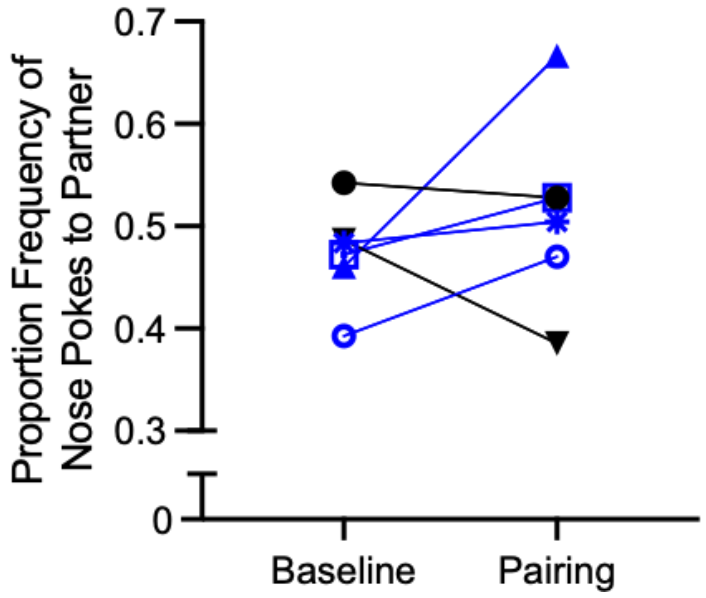


Note: The horizontal line at 0.5 indicates indifference. All data points above the line indicate a preference for the partner rat.

Given these inconsistent findings, comparisons between the proportion frequency in baseline and pairing were not statistically significantly different. A Wilcoxon matched-pairs signed rank test indicated that proportion preference during pairing (*Mdn* = 0.52, *IQR* = 0.09) was not significantly greater than baseline (*Mdn* = 0.48, *IQR* = 0.06), $W = 9.000$, $p = 0.4375$. However, examining individual subject data does reveal potentially interesting findings. Figure 7 shows that four of the six primary subjects displayed at least a small increase in proportion frequency of nose pokes to the partner rat from baseline to pairing.

Figure 7.

Median Proportion Frequency of Nose Pokes to Partner During Baseline and Pairing

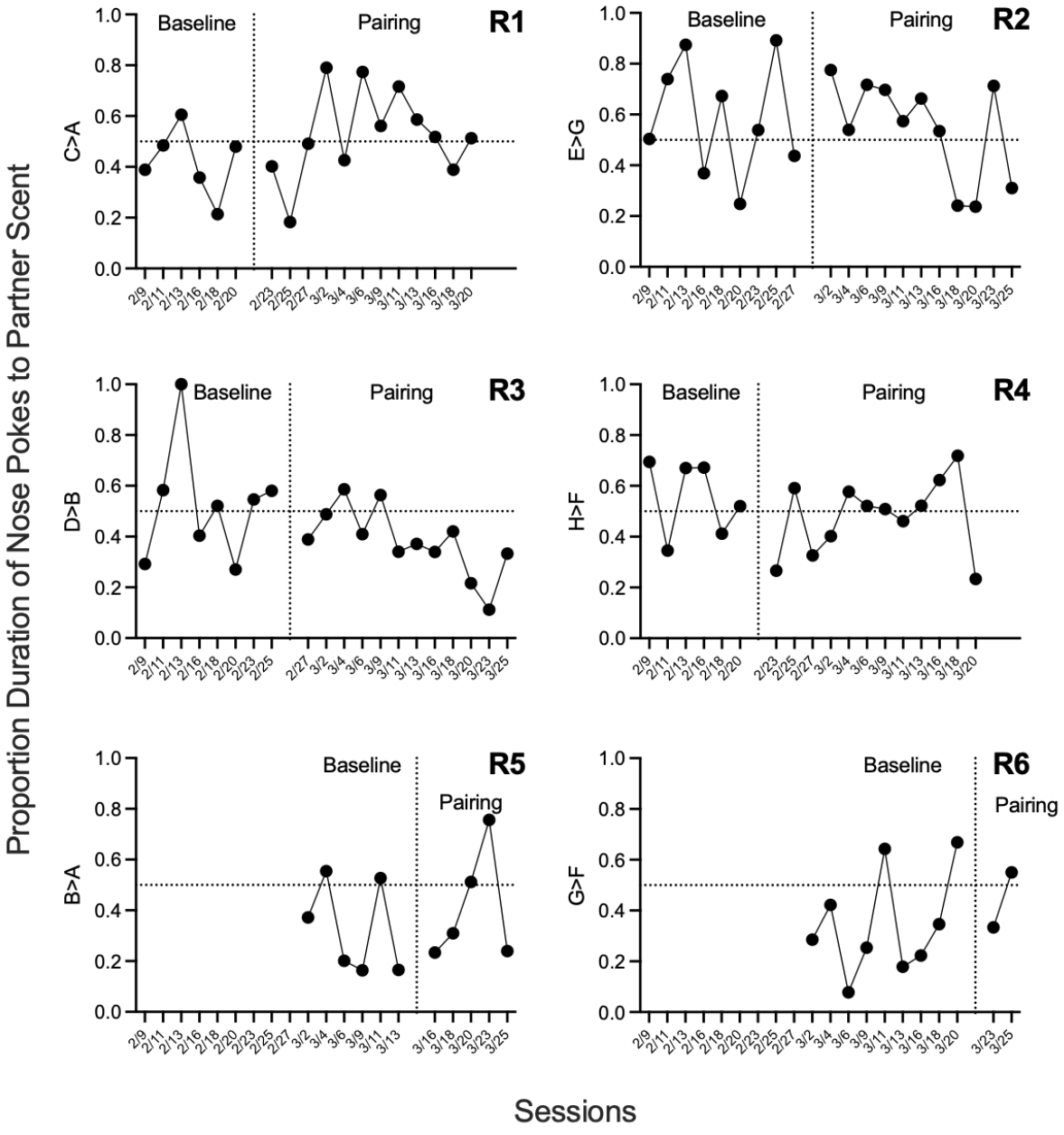


Note: Different symbols represent individual subjects (triangles for R1, unfilled squares for R2, reverse triangles for R3, filled circles for R4, unfilled circles for R5, asterisks for R6). Blue and black data points indicate an increase and decrease in the proportion frequency from baseline to pairing, respectively.

Figure 8 shows the proportion duration of responding to the partner port relative to the stranger port. Notably, there is more individual variation in proportion duration, indicating that the duration of time spent across ports was less consistent across days. R1 did not show sustained time spent in either the partner rat port or the stranger rat port, as indicated by the proportion duration data. The data points fluctuated around 0.5, with only brief runs (3-4 consecutive sessions) above and below the 0.5 indifference line. In contrast, R2 demonstrated a trend of increased time spent towards their partner rat, particularly during the first seven consecutive days. However, it should be noted that this effect seemed to dissipate later in the phase and data collection for R2 is ongoing. R3 exhibited increased time spent towards the stranger rat, particularly in the final seven consecutive days. R4 displayed more evidence of indifference, with brief runs (2-3 consecutive sessions) both above and below the 0.5 indifference line. Initially, R5 showed more time spent towards the stranger rat in the first two days following the pairing sessions; however, this changed to more responding towards the partner rat in the following two days, and then back to the stranger rat on the final day. The data for R5 is still ongoing, but current results indicate indifference with high variability. Similarly, data for R6 is also ongoing, with only two data points available for analysis. The first day showed more responding towards their stranger rat, while the subsequent data point indicated more responding towards the partner rat.

Figure 8.

Proportion Duration of Nose Pokes Allocated to the Partner Rat Relative to the Stranger Rat



Note: The horizontal line at 0.5 indicates indifference. All data points above the line indicate a preference for the partner rat.

CHAPTER 4: DISCUSSION

The main goal of this study was to determine whether pairing sessions would increase rats' preference for olfactory stimuli linked to a partner rat. Grounded in the principles of conditioned reinforcement, we hypothesized that repeated exposure to a partner rat alongside preferred stimuli would increase the reinforcing value of that rat's scent. The findings from our investigation lend partial support to this hypothesis but also highlight significant variability among the subjects, suggesting that multiple behavioral mechanisms might be at play. Comparing the data from all of the subjects, we found no statistically significant increase in preference for the partner's scent following the pairing sessions. The Wilcoxon matched-pairs test, which contrasted baseline preferences with those recorded during the pairing phases, revealed no significant differences in preference, as measured by the proportion of responses to the partner rat, suggesting that the pairing sessions did not consistently sway odor preferences across all subjects. Nonetheless, a closer look at individual responses reveals important differences.

Significant variations among the rats were observed. For example, R1 responded more to their pairing partner, as shown in the proportion frequency preference data presented in Figure 6. These data indicate a period of increased responding toward their partner rat, particularly during the final 7 sessions, which had values at or above 0.5. However, the duration proportion preference data in Figure 8 reveal that consecutive days of responding toward the partner rat were not consistently observed for the amount of time spent in each port. The data fluctuated around 0.5, with only brief runs (3-4 consecutive sessions) above and below the indifference line. In the case of R2, although there was no sustained preference in responding towards the

partner rat as indicated in Figure 6, the data in Figure 8 showed increased time spent in the nose port that had the scent of their partner during the first 7 consecutive sessions, however this duration of responding towards their partner rat diminished after those initial sessions with data collection for R2 still ongoing. R3 demonstrated consistent responding towards their stranger rat, as indicated in both Figures 6 and 8. In Figure 8, R3 responded to the stranger rat for the last 7 days and in Figure 6, she maintained consistent responses for five consecutive days in the final part of the pairing phase. R4 exhibited more evidence of indifference across both Figures 6 and 8, with brief runs (2-4 consecutive sessions) of responding both above and below the 0.5 indifference line. Data collection for R5 and R6 is still ongoing, so their assessments are preliminary. Currently, both R5 and R6 exhibit indifference, as shown in Figures 6 and 8. This variability in preference among the rats suggests that individual social dynamics could play a significant role in their interactions and underscores the need for further investigation into the factors that influence these preferences. Moreover, both the frequency and duration of responses generally declined over time, a pattern consistent with previous research (Hinnenkamp et al., 2025). This declining engagement may have contributed to the study's inability to detect clear changes in preference.

These results indicate that the formation of social or olfactory preferences is more complex than initially thought and may hinge on additional factors, such as individual differences and procedural elements. Although partner preferences can emerge in certain cases, they do not constitute a consistent or robust phenomenon, highlighting the need for further research to uncover the conditions under which such preferences might reliably develop. Consistent with previous research on social motivation in rodents (e.g., Guo & Sun, 2023;

Hinnenkamp et al., 2025), rats reliably responded to access social olfactory stimuli throughout the experiment, indicating that such cues can effectively serve as reinforcers. Additionally, we observed substantial individual differences in both the frequency and duration of responses, mirroring variability noted in previous operant social paradigms (e.g., Schulingkamp et al., 2023). These results stress the importance of considering individual differences in our assessments of social reinforcement processes.

After implementing the pairing sessions, the data revealed mixed outcomes. Three subjects showed varying responses towards either their partner or their stranger rat. R3 was the only subject that maintained sustained responses towards the stranger rat across both proportion frequency and proportion duration measurements. R1 consistently responded to the partner rat in the proportion frequency data but demonstrated indifference in the proportion duration data. R2 initially exhibited consecutive responses towards the partner rat in the proportion duration data. However, these responses did not persist in the final days of the pairing phase, and she showed indifference in the proportion frequency data. The remaining subjects displayed indifference across both the proportion frequency and proportion duration data. The data from R1 aligns with predictions from conditioned reinforcement theory (e.g., McLaughlin & Carr, 2005; Skinner, 1953), suggesting that the scent associated with the partner acquired reinforcing properties through repeated associations with preferred items and activities. However, this response was not consistent across all subjects; others showed the opposite or did not demonstrate stable shifts in preference between the baseline and pairing phases. These findings indicate that pairing alone may not be enough to reliably establish conditioned reinforcement under the evaluated conditions.

Research suggests that simple associations are often insufficient for establishing conditioned reinforcers. McLaughlin and Carr (2005) emphasized that repeated and consistent interactions are essential for establishing something as a generalized reinforcer. It is possible that the 13 pairing sessions allotted to each participant were insufficient to solidify their partner's role as a generalized reinforcer. Kelly (2015) highlighted that the potency of the reinforcer is a significant factor. The stimuli presented may not have been sufficiently compelling for each participant, particularly for Participant R3, who did not exhibit a reliable preference for any of the foods across the four preference assessments. In addition, Fanning Tacoaman et al. (2024) demonstrated the importance of allowing participants to select toys during the session, highlighting the need to reassess preferences frequently. Not having more frequent preference assessments throughout the study with a larger array of items may have limited the value of the items presented during the pairing sessions. Within the framework of generalized reinforcers, Skinner (1953) pointed out the importance of motivating operations, particularly in relation to the deprivation of certain items. Although the animals had limited opportunities to interact with the toy items during the pairing sessions and access to food items was restricted to those same sessions, their unrestricted access to general rat chow may have diminished feelings of food deprivation. The lack of food deprivation could have impeded the partner rats' connections to primary reinforcers, as these connections were not reinforced under conditions of deprivation.

The overall decline in response frequency across sessions may indicate underlying processes such as habituation or satiation to the olfactory stimuli. Given the minimal effort required to access these stimuli, coupled with subjects' exposure to the scents 6 days per week, either in the operant chamber or during pairing sessions, they were afforded repeated exposure at

minimal cost. This extensive exposure may have diminished the reinforcing value of the scents over time. As a result, decreased motivation to engage in operant responding may have emerged, contributing to the observed decline in response rates across sessions. Together, these results suggest that the effects of pairing are likely complex, and multiple theoretical approaches may be needed to fully account for the observed patterns of behavior.

A significant contribution of this study is the clear display of individual variability in response to the pairing procedures. While some subjects showed meaningful changes in their preferences, others exhibited minimal to no effect. This variability resonates with findings in both animal and human research. For instance, increased responding as a result of social isolation has been documented in rodents (e.g., Lehman & Adams, 1977). These connections highlight the translational relevance of our findings, suggesting that sensitivity to social reinforcement and pairing procedures can vary widely from one individual to another. The implications of our results are noteworthy for applied behavior analytic interventions, especially the pairing procedures commonly used in clinical settings (e.g., Shillingsburg et al., 2018; Ensor et al., 2024). While pairing is frequently applied to establish therapists as conditioned reinforcers, our findings suggest that its effectiveness may not be universal. Instead, the success of pairing procedures may hinge on factors that could not be controlled within the experiment such as being client-led or interacting with the toys in a manner preferred by the child/client. These insights advocate for detailed component and parametric analyses aimed at pinpointing the key elements of pairing procedures to enhance their effectiveness in applied contexts.

From a translational perspective, this study illustrates the value of modeling clinically relevant interventions within controlled laboratory settings. In a manner similar to the insights

derived from the Rat Park studies (e.g., Alexander et al., 1978; Gage & Sumnall, 2019), which underscored the significance of environmental context in influencing behavior, the present findings highlight the intricate nature of social reinforcement processes and the critical role of environmental and procedural variables. By bridging basic and applied research, translational models such as the one implemented in this study can enhance theoretical frameworks and improve the efficacy of clinical interventions.

The current study has several limitations that must be considered when interpreting the results. These limitations arise from the experimental conditions, available resources, and uncontrolled elements of the study. With respect to the apparatus utilized for the preference assessments, contextual differences may have posed challenges. The varying assessment environments, such as employing the home cage for food assessments versus a novel arena for toy evaluations, may have influenced engagement levels and the generalizability of the identified preferences across different settings. In the context of the free operant preference assessment procedure, particularly regarding toy preference assessments, behaviors exhibited by certain rats, such as jumping off items or attempting to escape using larger toys, suggested potential aversion or disinterest in the assessed items. Although some toys were coded as having a higher preference, this classification could be misleading if the rats were merely engaging with these items as a means of exiting the arena.

In the food preference assessments, overall food consumption was low, which necessitated the use of both consumption and displacement of items as factors considered in determining rankings. The measurement system introduces ambiguity in interpretation. Displacement alone does not necessarily signify preference; rather, it may reflect exploratory

behavior or non-consumptive actions. This limitation was particularly evident with rat R3, where reliance on indirect indicators of engagement was necessary due to low levels of consumption. Furthermore, the resources utilized for preference assessments may not have been appealing to all rats. For instance, R3 demonstrated no items of highly preferred food based on consumption, thus highlighting the discrepancy between displacement and actual preference. It is also possible that the rats were less likely to sample food items because they had unlimited access to food throughout the study, thus no establishing operation was in place to make additional food items valuable as reinforcers. Multiple preference assessment sessions were conducted, but it is possible that these sessions were insufficient for the rats to habituate to the novelty of the items.

While the free operant preference assessments aimed to identify highly preferred food and toy items, they did not directly assess the reinforcing efficacy of these stimuli. The study did not aim to determine whether the stimuli functioned as reinforcers in a formal sense. Although the study did not incorporate a programmed response requirement to access social interactions, toys, or food items, it was designed for the stimuli to serve as valuable resources, potentially functioning as unconditioned or conditioned reinforcers during pairing, thereby increasing the likelihood of the social partner acquiring positive value. However, because these stimuli were employed in a pairing procedure without programmed contingencies, their capacity to function as reinforcers was not directly evaluated, leading to the possibility that items deemed more preferred may not have been effective in establishing the social partner as a conditioned reinforcer.

Another limitation associated with the free operant social olfactory choice procedure is the use of olfactory stimuli rather than direct social interactions as reinforcers. Although this

method permits controlled and repeated measurement, it may not entirely capture the complexity of social preference. Variability in the salience of olfactory stimuli, such as differences in bedding freshness or odor strength, could have introduced uncontrolled variability across sessions. Additionally, the extended duration of the sessions (one hour) may have resulted in within-session changes in responding due to habituation or fatigue. Consequently, the response patterns observed may not consistently reflect preferences throughout the entirety of the session. Furthermore, rats may have initially responded to the social olfactory stimuli due to their reinforcing value. However, after having experience with directly contacting a partner rat during pairing sessions, responding to the scents in the operant chambers may have inadvertently been placed on extinction, because the scent of their partner never led to direct access to the partner in the procedure. Furthermore, using the free operant olfactory choice procedure to assess preference for social stimuli assumes that the reinforcing aspects of the olfactory stimuli are truly social in nature. Although the bedding contains the scent of the other rat, there are also other nonsocial aspects of the stimulus (e.g., scent of the bedding itself, scent of the other rat's waste which could contain the residual smell of food, etc.).

Concerning the pairing intervention, there exists the potential for preferences to change over time. The preference assessments were conducted solely prior to the pairing intervention, with no subsequent reassessment to account for possible shifts in stimulus value following the initial sessions. It is important to acknowledge the uncontrollable interaction between the rats, wherein the pairing partner may have engaged with the toys or with the primary rat in a manner that would be more preferred by the primary rat, which may have influenced preferences. The type of social interaction occurring between the two rats may also have been an important factor.

Rats have been known to develop social hierarchies, in which one rat exhibits dominant behaviors towards the other rat (e.g., aggression, grooming, etc.). If the primary rat was the more submissive in a pairing, it is possible that the social interactions may have been less preferred due to the formation of the social hierarchy, irrespective of the presence of preferred stimuli. One limitation of the pairing procedures was that some sessions took place under red light in the colony room, while others were conducted under full white light, depending on the time of day. Additionally, since these pairing sessions were scheduled after other projects and lab shifts, various uncontrollable factors came into play, including delays caused by previous shifts on other projects or by the experimenter. Finally, the valence of the interactions was uncontrolled. Anecdotally, some of the rats interacted primarily through grooming, whereas others interacted primarily through aggressive fighting, and others may have had only limited interactions at all. If certain interactions were more preferable, these experiences may have impacted the rats' responding in the free operant olfactory choice task. Overall, these limitations must be considered in the context of the study's findings and their implications for understanding preference behaviors in rats.

Future studies can delve deeper into this topic by examining the effects of altering the experimental setup. Future research could compare how rats interact with their pair-housed rat versus a stranger rat with whom they have not previously interacted. Additionally, implementing pair housing for the stimulus rats may provide them with more opportunities for social interaction, which could influence their behavior and the value of social interaction during the pairing sessions. Moreover, increasing the sample size of primary rats in these studies could enhance the internal validity of the findings, such as in the case of the statistical analyses being

able to detect more subtle differences across the subjects. Regular reassessment of preferences for toys and treats would also be beneficial, as it could reveal changes over time or in response to different conditions. Furthermore, incorporating a broader range of toys and treats into the experiments could yield more comprehensive insights into the factors that drive rats' preferences and interactions.

In conclusion, the current study offers preliminary evidence that pairing procedures can augment the reinforcing value of social stimuli under specific conditions for one subject, providing partial support for conditioned reinforcement theory. However, the observed variability and the absence of consistent effects across all subjects indicate that pairing cannot be regarded as a singular or universally effective mechanism. Rather, the findings advocate for a more subtle, multi-theoretical perspective on pairing and emphasize the importance of translational research in advancing both theoretical understanding and practical applications within the field of behavior analysis.

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APPENDIX A: IACUC Approval

4/7/26, 12:25 PM

PI :	Galizio, Ann Irene Bruce
Protocol #	2025-0075
Status :	Approved (w/o Stipulation)
Approved :	07/16/2025
Expires :	07/16/2028
Title :	Effects of Pairing on Preference for Social Olfactory Stimuli in Rats

Table of Contents

Protocol Introduction

Tips on Navigation:

As you complete each page, you will receive a green checkmark on the left side of the screen in the Table of Contents. When all pages have green checkmarks, PIs will be able to submit the protocol for review. To navigate between pages, you can use the navigation arrows at the bottom of each page when the page is complete. You can also click on the page title in the Table of Contents. Some pages have attachment grids at the bottom of the page. Please feel free to attach any relevant documentation to those pages.

Sections on this page will populate the protocol form. Please feel free to return to this page to update selections as needed.

Federal/Foundation funded?

☐

Internally funded?

✓

Private/Commercially/Other funded?

☐

Will you be using tissues from animals not on this protocol?

Yes ○ No *

Will antibodies be specifically generated for this study?

Yes ○ No *

Will you be collaborating with an outside institution?

Yes ○ No *

Will any live animal research be conducted off campus?

Yes ○ No *

Will animals be housed outside central housing facilities for more than 12 hours?

Yes ○ No *

Will animals be moved through public access areas?

Yes ○ No *

Will human clinical areas be used

Yes ○ No *

Will field studies or research be conducted?

Yes ○ No *

Protocol Species Grid

To add a species, click the "ADD" button on the bottom of the grid, select the species from the picklist then place checkmarks
in each column as applicable. Save the species selection by clicking the Save button on the bottom of the grid, then save the
page by clicking the Save button below the grid.

Species	Breeding?	Non-Surgical Procedures?	Restraint?	Surgery?	Vet Drugs?	Test Agents?	Euthanize?	Biohazard (IBC)?
Rat	No	Yes	No	No	No	No	No	No

Protocol Overview

Note that you may click and drag the bottom right corner of any text area to resize it.

Enter title for this Protocol

Effects of Pairing on Preference for Social Olfactory Stimuli in Rats

How would you explain to a non-scientist the long term or overall scientific goals and objectives of the proposed work?

The goal of this experiment is to investigate the mechanisms underlying a common clinical behavioral intervention, pairing, using a procedure with strong experimental control already established in our lab. Pairing is a procedure commonly used in clinical settings (e.g., applied behavior analysis therapy for individuals with autism spectrum disorder), in which the therapist interacts with the client while engaging with the client's preferred items and activities. The theory is that by pairing themselves with these preferred items, the therapist will acquire reinforcing value and the client will be more motivated to work with them. However, although evidence shows that pairing effectively reduces challenging behavior during therapy, very little research has been conducted to understand the mechanism of this practice, which means we have no way of knowing WHY it is effective. Therefore, the primary goal of the proposed study is investigate possible explanations for the efficacy of the pairing intervention. Given the challenges involved in addressing this question with humans in clinical settings, we propose to test the effects of the pairing in rats using an established procedure to assess social preference.

Previous work in our lab has shown that rats will respond to access social scents (Hinnenkamp et al., under review), but we have not yet thoroughly investigated the factors that influence rats' preference for social olfactory stimuli. The aim of the present study is to test whether rats will respond more to social scents after experiencing a modified version of the pairing intervention typically used in clinical settings. We will simulate pairing in rats by allowing two rats to interact in an enriched environment with treats and toys to which they usually do not have access. This would be similar to a therapist and client's interacting with the client's preferred treats and toys. If humans are more motivated to respond for the therapist after this pairing process, we hypothesize that rats will also respond more for the scent of the rat with which they experienced the pairing process. This finding would support the use of pairing in clinical intervention. However, if we do not see an increased preference for the rat associated with pairing, it will call into question the mechanism by which pairing is effective in humans, prompting the need to investigate this question further.

How would you explain to a non-scientist the way the proposed animal use might benefit human or animal health, the advancement of knowledge, or the good of society?

The results of this study are directly applicable to clinical practice. By simulating pairing in a lab setting with rats, we can address questions about its mechanisms while controlling for extraneous variables that pose major challenges in human research. If we observe the results we anticipate (that rats

respond more for a rat associated with pairing), it would support the use of pairing in clinical intervention. However, if we do not see an increased preference for the rat associated with pairing, it will call into question the mechanism by which pairing is effective in humans, prompting the need to investigate this question further. Either outcome could lead to improved clinical practices in the future.

Please provide a brief summary of the proposed experiments. The summary should allow the committee to understand what combination
of experimental interventions/procedures will be used for all animals, or groups of animals requested on the protocol. Please do not include
specific experimental details here. These will be provided in the methodology section. The goal of this section is to capture the rationale and
scope of the work to be conducted.

Experimental Summary

The proposed study involves testing the effects of pairing sessions on the value of social stimuli in rats. Value will be determined by allowing rats to make a nose-poke response to produce social scents, with more nose-pokes indicating more valuable stimuli. Throughout the study, we will be collecting data through daily behavioral sessions.

Baseline Assessment: The study will begin with a baseline procedure. During baseline conditions, each subject will respond to one nose-poke port to produce the scent of one Stimulus Rat (e.g., Rat A) and to the other port to produce the scent of another Stimulus Rat (e.g., Rat B). During baseline, the subject will never have interacted with either stimulus rat. This procedure will be repeated until a preference is established and responding stabilizes.

Pairing Sessions: After establishing initial baseline preference for Rat A or Rat B or indifference, we will randomly select one of those Stimulus Rats to serve as the Partner Rat for the subject. The other Stimulus Rat will remain a stranger. Each day, the Subject Rat will be placed in an enriched environment with their Partner Rat for 30 minutes. During this time, they will be in a larger arena and will have access to extra nesting material, treats (e.g., cereal, fruit, nuts, etc.), and toys (e.g., running wheel, crawl tube, cave, etc.). Then preference will be re-assessed using the same procedure as baseline. This process will be repeated several times. If pairing increases the reinforcing value of the Partner Rat, we expect that the Subject will subsequently respond more to the Partner Rat than the other Stimulus Rat in the assessment.

Protocol Internal Funding

Add each Internal source that will supply funding for this work.

Organization	Department	Fund Title	Sponsored Projects #
Presidents Office	Psychology	Costs will be paid by the Psychology Department or myself (Dr. Galizio).	

Organization/Department

Presidents Office Psychology

Fund Title

Costs will be paid by the Psychology Department or myself (Dr. Galizio).

Sponsored Projects Fund Number

Org Name

Presidents Office

Type of Animal Use

Identify all types of animal use for this protocol.
Your choices here and on subsequent pages will determine the correct USDA pain category.

Which of the following describe the type of animal use proposed in this application? (check all that apply)
Research Type (Basic, Applied, Experimental)

Basic

Instructional or Training

☐

Field Studies

☐

Field Research

☐

Rat

Species Information

Complete required fields

Age Range of study animals

21 days to 3 years

Target weight range

50-500 g

Will animals be Specific Pathogen Free?

Yes ● No ○

Animal Identification Method

Rats' tails will be marked upon arrival using colored sharpies so that they are easily identifiable. For example, one rat may be labeled "Purple 3" and she will have three purple lines drawn on her tail. If the marks begin to fade, they will be re-drawn, so that research assistants can easily identify the rats. This is the strategy we have used to identify rats in our lab for several years.

You did not select "Euthanize" on the species grid. What will happen to the animals at the end of the study?

After completing this study, animals will be used in future behavioral experiments. If at any point rats are not taking part in an experiment, they will be kept in the colony room with free access to food and water and regular care. Our rats will be used in behavioral studies until they die or exhibit any signs of pain or distress, at which point they will be euthanized only if necessary. If rats need to euthanized for health concerns, carbon dioxide asphyxiation will be used. Euthanasia and verification of death will always be performed by the faculty advisor and will follow the guidelines outlined by the National Institutes of Health (https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/b5_euthanasia_of_rodents_using_carbon_dioxide.pdf).

Justification for Choice of Species

Justify the choice of species by stating why a species lower on the phylogenic scale is not appropriate.

Justification for Choice of Species/Animal Model

This study will utilize female rats. Our lab has used this sex and species in similar experiments in the past. Rats are a preferred species to study social behavior, as they tend to be social creatures and their behavior is relatively easy to train. Female rats will be used because previous research has shown that they exhibit higher social responsiveness than male rats (Vaderhooft et al., 2019). Our lab has experience working with and housing female rats (Hinnenkamp et al., under review).

We will be procuring rats from a reputable local breeder. For more details, see the attached document.

Use Locations

Indicate all of the locations where surgeries, procedures and/or euthanasia will be performed.

Location/Building	Room	Type
Science Building	1170H	Housing
Science Building	1170I	Procedur
Science Building	1170N	Procedur

Location/Room

Science Building 1170H

Location Type

Housing

Location/Room

Science Building 1170I

Location Type

Procedur

Location/Room

Science Building 1170N

Location Type

Procedur

Strain Information

Choose a strain from the pick list. If the strain you want is not on the pick list, enter it in the other field.

Strain	Age	Phenotype	Weight	Edited
Dumbo	21 days - 3 years		50-500 g	Edited

Press the space bar within the Strain text box to bring up available strains. If you do not see your selection, please contact Research Compliance Officer at RCO@mtsu.edu to have it added to the dictionary.

Species Strain

Dumbo

Age

21 days - 3 years

Phenotype

Weight

50-500 g

Non-Surgical Procedures

Procedure Name
Open Field
Operant Chamber

Procedure Name (If you don't see your Procedure, select Holding Procedure and select No below. Contact Research Compliance Officer at RCO@mtsu.edu to have the new procedure added to dictionary. Continue to fill out your protocol. You will be notified when it has been loaded into the dictionary and you can add the procedure to your protocol. Note: You cannot submit a protocol with any Holding Procedure selected.)

Open Field

Describe the procedure, giving enough detail so that another individual could carry it out (e.g. volume, dose, duration, frequency, ...)

Pairing Sessions will be completed in an open-field arena.

Pairing Sessions: After establishing initial baseline preference for Rat A or Rat B or indifference, we will randomly select one of those Stimulus Rats to serve as the Partner Rat for the subject. The other Stimulus Rat will remain a stranger. Each day, the Subject Rat will be placed in an enriched environment with their Partner Rat for 30 minutes. During this time, they will be in a large arena and will have access to extra nesting material, treats

(e.g., cereal, fruit, nuts, etc.), and toys (e.g., running wheel, crawl tube, cave, etc.). Then preference will be re-assessed using the same procedure as baseline (Operant Chamber). This process will be repeated several times.

Please describe any special precautions you have taken or will take to assure that the procedure can be conducted safely for animals and humans.

All student researchers will be extensively trained how to handle rats and how to use the software that controls and collects data for the experiment. During all phases/conditions of the experiment, rats will have unlimited access to food and water while in their home cages. Rats will be weighed and visually inspected daily to ensure their health.

Procedure Name (If you don't see your Procedure, select Holding Procedure and select No below. Contact Research Compliance Officer at RCO@mtsu.edu to have the new procedure added to dictionary. Continue to fill out your protocol. You will be notified when it has been loaded into the dictionary and you can add the procedure to your protocol. Note: You cannot submit a protocol with any Holding Procedure selected.)

Operant Chamber

Describe the procedure, giving enough detail so that another individual could carry it out (e.g. volume, dose, duration, frequency, ...)

Rats will will run daily behavioral sessions in an operant chamber. Operant chambers will contain two lighted nose-poke ports that are connected to an olfactometer (5 jars and an apparatus to blow air through the jars and into the chamber).

Baseline: The study will begin with a baseline procedure. During baseline conditions, the olfactometer will be set up so that one jar contains used bedding from one stimulus rat from the colony room (e.g., Rat A) and another jar contains used rat bedding from a different stimulus rat from the colony room (e.g., Rat B). Rats will be placed in the operant chamber, and both nose-poke ports will be illuminated. If the rat places her nose in the left nose-poke port (or right, sides are counterbalanced), a photo beam will detect the nose-poke response and the olfactometer will produce a 0.4-s burst of air through the jar containing used bedding of Rat A into the nose-poke port. If the rat nose-pokes in the other port, the olfactometer will produce a 0.4-s burst of air through the jar containing used bedding of Rat B. After responding, the rat must remove their nose and 0.5 s must pass before another response may be made. The rat can respond to either port as many times as they want during the 60-min session. Rats will complete baseline sessions every day for at least 10 days. The proportion of responses made to each nose-poke port will be analyzed and baseline sessions will continue until preference is stable for at least the last 6 days.

Please describe any special precautions you have taken or will take to assure that the procedure can be conducted safely for animals and humans.

All student researchers will be extensively trained how to handle rats and how to use the software that controls and collects data for the experiment. During all phases/conditions of the experiment, rats will have unlimited access to food and water while in their home cages. Rats will be weighed and visually inspected daily to ensure their health.

Humane Use Animal Categories

*The USDA categories marked as required (with a red *) below were determined to be appropriate based on the procedures you reported.*

Please enter the number of animals requested for each of the required categories. The total of the required fields may not exceed your total requested number.

For reference the USDA Categories are defined
B = Breeding or Holding Colony Protocols
C = No more than momentary or slight pain or distress and no use of pain-relieving drugs, or no pain or distress. For example: euthanatized for tissues; just observed under normal conditions; positive reward projects; routine procedures; injections; and blood sampling.
D = Pain or distress appropriately relieved with anesthetics, analgesics and/or tranquilizer drugs or other methods for relieving pain or distress.
E = Pain or distress or potential pain or distress that is not relieved with anesthetics, analgesics and/or tranquilizer drugs or other methods for relieving pain or distress.

At the bottom of the page, please attach your Statistical Jusificaiton for Animal Numbers.

USDA Category	# of Animals
B	0
C	14
D	0
E	0

Justify the numbers of animals to be used.

- **Describe the statistical method (or other method) used to justify the number of animals per group.**
  Federal guidance states that statistical methods must be used in order to justify the number of animals requested.
- **Describe mortality or exclusion rates if applicable.**
- **Be sure to include breeding colony numbers (production, maintenance, undesired genotypes).**
- **The numbers provided in this section must also match the total amount in the # of animals column above.**

This study will use a total of 14 rats. Of these, 6 rats will be the primary subjects and the other 8 rats will be Stimulus Rats, randomly determined.

Our lab was one of the first to demonstrate that rats will nose-poke to access social stimuli using an olfactometer (Hinnenkamp et al., under review), and little research exists on this topic. We will use a single-subject experimental design to examine the extent to which each rat will respond to access social stimuli over time. Using a single-subject design allows us to use smaller sample sizes than would be necessary in a group design. Each rat will be tested daily throughout the experiment, and their data will each be analyzed separately to determine the reinforcing value of social stimuli. This approach is consistent with other similar behavioral research in the field. We will use 6 subjects, because 6 subjects were used in our previous work (Hinnenkamp et al., under review).

We need 8 Stimulus Rats to serve as scent donors and potential Partner Rats for our 6 subjects. The reason we need 8 Stimulus Rats is because, during Baseline, if a subject shows a preference for one of the Stimulus Rats, we will select the less-preferred Stimulus Rat as the Partner Rat (we hypothesize that preference will increase for the Partner Rat, so it is most experimentally sound to ensure that the Partner Rat is initially less preferred so that an increase can be observed; i.e., to avoid a ceiling effect). Each Partner Rat may only be paired with one subject (otherwise, their experiences with one subject may impact their interactions with another subject). We diagrammed the possible combinations and determined that a minimum of 8 Stimulus Rats are needed (see below).

Subject A - Stimulus Rat (SR) 1 vs. SR 2
Subject B - SR 3 vs. SR 4
Subject C - SR 5 vs. SR 6
Subject D - SR 7 vs. SR 8
Subject E - SR 1 or 2 (whichever is not identified as A's Partner Rat) vs. SR 3 or 4 (whichever is not identified as B's Partner Rat)
Subject F - SR 5 or 6 (whichever is not identified as C's Partner Rat) vs. SR 7 or 8 (whichever is not identified as D's Partner Rat)

Two of the 8 Stimulus Rats will not be selected as Partner Rats and so will not experience any experimental procedures. However, they will still be kept in the colony to serve as scent donors (used bedding). They will be eligible to participate in other experiments in the future.

Methodology

*For each species, **describe in narrative form all experimental or instructional procedures to be performed on the animals** (e.g. blood collection, surgery, behavioral training, administration of substances or test compounds, breeding, tumor induction, etc.). Include the time frames and intervals and describe the procedures in the order in which they will be performed. Include a description of procedures performed on anesthetized animals. All procedures checked on the procedures page should be described below.*

Include the rationale for use of tissues in vitro but do not describe in vitro procedures performed on tissues taken from animals or procedures performed on animals after they are euthanized.

*Please list the interventions/procedures in chronological order, indicating the time interval between each procedure, and the final disposition of the animals at the end of the experiment. **All procedures listed in the table on the procedures page must be included.***

Flowcharts or other graphical representation of the methodology can be very helpful. This text box allows for tables, pictures and flowcharts to be created in Cayuse or they can be pasted into the text box. It is preferred that all tables, charts, and pictures be inserted in the text box but you may attach the files below if needed. Please contact a member of the IACUC or veterinary staff should you require guidance regarding the information and level of detail that should be provided here.

Procedure Description

All student researchers will be extensively trained how to handle rats and how to use the software that controls and collects data for the experiment. During all phases/conditions of the experiment, rats will have unlimited access to food and water while in their home cages. Rats will be weighed and visually inspected daily to ensure their health.

Baseline: The study will begin with a baseline procedure. Rats will will run daily behavioral sessions in an operant chamber. Operant chambers will contain two lighted nose-poke ports that are connected to an olfactometer (5 jars and an apparatus to blow air through the jars and into the chamber). During baseline conditions, the olfactometer will be set up so that one jar contains used bedding from one stimulus rat from the colony room

olfactometer will be set up so that one jar contains used bedding from one stimulus rat from the colony room (e.g., Rat A) and another jar contains used rat bedding from a different stimulus rat from the colony room (e.g., Rat B). Rats will be placed in the operant chamber, and both nose-poke ports will be illuminated. If the rat places her nose in the left nose-poke port (or right, sides are counterbalanced), a photo beam will detect the nose-poke response and the olfactometer will produce a 0.4-s burst of air through the jar containing used bedding of Rat A into the nose-poke port. If the rat nose-pokes in the other port, the olfactometer will produce a 0.4-s burst of air through the jar containing used bedding of Rat B. After responding, the rat must remove their nose and 0.5 s must pass before another response may be made. The rat can respond to either port as many times as they want during the 60-min session. Rats will complete baseline sessions every day for at least 10 days. The proportion of responses made to each nose-poke port will be analyzed and baseline sessions will continue until preference is stable for at least the last 6 days.

Pairing Sessions: After establishing initial baseline preference for Rat A or Rat B or indifference, we will randomly select one of those Stimulus Rats to serve as the Partner Rat for the subject. The other Stimulus Rat will remain a stranger. Each day, the Subject Rat will be placed in an enriched environment with their Partner Rat for 30 minutes. During this time, they will be in a large arena and will have access to extra nesting material, treats (e.g., cereal, fruit, nuts, etc.), and toys (e.g., running wheel, crawl tube, cave, etc.). Then preference will be re-assessed using the same procedure as baseline (Operant Chamber). This process will be repeated several times.

Info

Will animals be subjected to procedures involving unrelieved pain or distress (category E)?

Yes ○ No *

Indicate how pain and/or distress will be monitored during all phases of all procedures and experiments (do not include post-op monitoring here). Include each procedure, the frequency, duration, and rationale of observations, and the management plan for any adverse reactions.

Each day, rats will be weighed, fed/watered, and checked for visual signs of illness, injury, pain, or distress. A data sheet containing each rats' daily weight and comments will be stored in the colony room and will be available for inspection at any time.

To ensure daily care, a care schedule will be created each semester. This care schedule will outline which individual (co-investigators or student research assistants) will be in charge of daily care for the rats and facilities. If the assigned individual cannot complete their daily care, caretakers will be trained to contact a backup. This backup will be any of the co-investigators or other student research assistants.

Animal caretakers will be trained to recognize visual signs of illness, injury, pain, or distress. If there is any sign of abnormal behavior (e.g., weight loss, lack of grooming, disoriented gait), caretakers will be instructed to contact the primary investigator (Dr. Galizio) or co-investigator (Dr. Hinennkamp). When the observation is verified and determined to be of concern, the attending veterinarian will be contacted.

Reduce, Refine, Replace

REDUCTION of animal use (i.e., modifying the experimental paradigm or performing statistical analysis to allow the use of fewer animals to obtain the needed information).

Please state how the number of animals you have requested is the minimum needed in order to obtain valid scientific conclusions.

This study will use a total of 14 rats. Of these, 6 rats will be the primary subjects and the other 8 rats will be Stimulus Rats, randomly determined.

Our lab was one of the first to demonstrate that rats will nose-poke to access social stimuli using an olfactometer (Hinnenkamp et al., under review), and little research exists on this topic. We will use a single-subject experimental design to examine the extent to which each rat will respond to access social stimuli over time. Using a single-subject design allows us to use smaller sample sizes than would be necessary in a group design. Each rat will be tested daily throughout the experiment, and their data will each be analyzed separately to determine the reinforcing value of social stimuli. This approach is consistent with other similar behavioral research in the field. We will use 6 subjects, because 6 subjects were used in our previous work (Hinnenkamp et al., under review).

We need 8 Stimulus Rats to serve as scent donors and potential Partner Rats for our 6 subjects. The reason we need 8 Stimulus Rats is because, during Baseline, if a subject shows a preference for one of the Stimulus Rats, we will select the less-preferred Stimulus Rat as the Partner Rat (we hypothesize that preference will increase for the Partner Rat, so it is most experimentally sound to ensure that the Partner Rat is initially less preferred so that an increase can be observed; i.e., to avoid a ceiling effect). Each Partner Rat may only be paired with one subject (otherwise, their experiences with one subject may impact their interactions with another subject). We diagrammed the possible combinations and determined that a minimum of 8 Stimulus Rats are needed (see below).

Subject A - Stimulus Rat (SR) 1 vs. SR 2
Subject B - SR 3 vs. SR 4
Subject C - SR 5 vs. SR 6
Subject D - SR 7 vs. SR 8

Subject E - SR 1 or 2 (whichever is not identified as A's Partner Rat) vs. SR 3 or 4 (whichever is not identified as B's Partner Rat)
Subject F - SR 5 or 6 (whichever is not identified as C's Partner Rat) vs. SR 7 or 8 (whichever is not identified as D's Partner Rat)

Two of the 8 Stimulus Rats will not be selected as Partner Rats and so will not experience any experimental procedures. However, they will still be kept in the colony to serve as scent donors (used bedding). They will be eligible to participate in other experiments in the future.

REFINEMENT of animal use such as modifying manipulations or measurement techniques to reduce the pain and/or distress experienced by the animals as compared to prior techniques, or using less sentient species (e.g., frog instead of mouse, mouse instead of dog).

Please describe why less invasive procedures or procedures which may cause less pain or distress cannot be used.

The rats used in this behavioral experiment should encounter no or minimal pain or distress. No invasive procedures are being used.

REPLACEMENT of animals with non-animal techniques (e.g., using tissue culture, computer simulations, etc.).

Please describe why non animal models or invertebrate models will not suffice for your study.

This research cannot be examined in vitro, through computer simulation (no such simulations are available), through statistical analysis (insufficient data currently exist on this topic), or with invertebrate animals (no accepted practices for studying social olfactory stimuli in invertebrates and no standard equipment available for studying operant behavior invertebrates).

Exemptions

Are you requesting any exceptions to the Guide or to IACUC policies?

Yes ○ No *

Will Animals be subject to Food and/or Water Restriction?

Yes ○ No *

Will there be any special husbandry requirements?

Yes ○ No *

Personnel List

Name all MTSU Faculty and Staff working on this project. To add new faculty/staff to this protocol, click *+Add Perrsonnel*.
To add undergraduate and graduate students, fill out Add Student Request Form (https://www.mtsu.edu/research/forms.php) and submit.

For personnel already listed, you will need to select them and click *Edit Personnel* and answer all required information, indicated by the red asterisk.

Name	Business Role	Phone	Email	Organization	Department	Additional Access	Primary?	Requester?	Edited
Galizio, Ann Irene Bruce	Principal Investigator	1-615-898-2319	ann.galizio@mtsu.edu	Presidents Office	Psychology	No	Yes	No	Edited
Hinnenkamp, Jay Evan	Principal Investigator	1-615-494-8873	jay.hinnenkamp@mtsu.edu	Presidents Office	Psychology	No	No	No	Edited
Feild, Jordan Patrice	Researcher Staff Members		jpf3q@mtmail.mtsu.edu	Presidents Office	Psychology	No	No	No	Edited

Business Role

Principal Investigator

Name

Galizio, Ann Irene Bruce

Organization Department

Presidents Office Psychology

Email

ann.galizio@mtsu.edu

Office Phone

1-615-898-2319

Cell Phone

Alternate Phone

Will need additional access to the vivarium.

☐

Home Phone

Primary Contact?

✓

Copy on all Emails

✓

Will person be handling animal species?

Yes • No ○

Species Name	Type	Procedure Description
Rat	Procedures	Open Field
Rat	Procedures	Operant Chamber

Degrees

Experience and Qualifications

Training Event	Description	Type	Date Certified	Training ID	Certification Flag	Date Completed
Health Screen	Form for Health Screen	Health Screen	/ /	Health Screen	No	/ /

Business Role

Principal Investigator

Name

Hinnenkamp, Jay Evan

Organization Department

Presidents Office Psychology

Email

jay.hinnenkamp@mtsu.edu

Office Phone

1-615-494-8873

Cell Phone

Alternate Phone

Will need additional access to the vivarium.

☐

Home Phone

Primary Contact?

☐

Copy on all Emails

☐

Will person be handling animal species?

Yes • No ○

Species Name	Type	Procedure Description
Rat	Procedures	Open Field
Rat	Procedures	Operant Chamber

Degrees

Experience and Qualifications

Training Event	Description	Type	Date Certified	Training ID	Certification Flag	Date Completed
Health Screen	Form for Health Screen	Health Screen	/ /	Health Screen	No	/ /

Business Role

Researcher Staff Members

Name

Feild, Jordan Patrice

Organization Department

Presidents Office Psychology

Email

jpf3q@mtmail.mtsu.edu

Office Phone

Cell Phone

Alternate Phone

Will need additional access to the vivarium.

☐

Home Phone

Primary Contact?

☐

Copy on all Emails

☐

Will person be handling animal species?

Yes • No ○

Species Name	Type	Procedure Description
Rat	Procedures	Open Field
Rat	Procedures	Operant Chamber

Degrees

Experience and Qualifications

Training Event	Description	Type	Date Certified	Training ID	Certification Flag	Date Completed
Health Screen	Form for Health Screen	Health Screen	/ /	Health Screen	No	/ /

Databases Searched

USDA Policies state that alternative searches are required for all animal use Protocols causing pain or distress to animal subjects.

Details of the search must include keywords used, years covered, and databases searched.

Did you record any animals in pain level D or E on the USDA Categories page?

Yes ○ No •

Endpoints & Assurances

List clinical parameters that would lead to early euthanasia in any of the procedures described in this protocol

After completing this study, animals will be used in future behavioral experiments. If at any point rats are not taking part in an experiment, they will be kept in the colony room with free access to food and water and regular care. Our rats will be used in behavioral studies until they die or exhibit any signs of pain or distress, at which point they will be euthanized only if necessary. If rats need to euthanized for health concerns, carbon dioxide asphyxiation will be used. Euthanasia and verification of death will always be performed by the faculty advisor and will follow the guidelines outlined by the National Institutes of Health (https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/b5_euthanasia_of_rodents_using_carbon_dioxide.pdf).

The information contained in this protocol is true and accurate, and that, to the best of my knowledge, it conforms to Institutional Animal Care and Use Committee (IACUC), and USDA, policies on the use of animals in research.

✓

Protocol Attachments

The following is a list of all attachments listed on this Protocol

Page	File Name	Description	Original File Name
Protocol	2025-0075_1_0001_Rat Supplier MOU 2025 signed copy.pdf	Rat	Rat Supplier MOU
Attachments	(https://mtsur4.app.cayuse.com/attachment/2025-0075_1_0001_Rat Supplier MOU 2025 signed copy.pdf)	Supplier MOU	2025 signed copy.pdf