

An assessment of social olfactory stimuli as reinforcers in female rats

by

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ABSTRACT

Deficits in social behavior are a hallmark of autism spectrum disorder (ASD). A tool which may help us isolate and understand how genes, prenatal, perinatal, and postnatal factors influence social behavior is the rodent model. The current study investigated if six female Long-Evans rats would work to obtain olfactory stimuli and their preference for social and nonsocial olfactory stimuli. In Experiment 1, rats were given a choice between a social or nonsocial scent and a control scent. In Experiment 2, the rats were given a choice between social scents and nonsocial scents within the same session. The results of Experiment 1 showed that rats preferred social and nonsocial scents over control scents. Experiment 2 results suggested that rats preferred social and nonsocial stimuli similarly. By studying the value of social stimuli in rats we eventually hope to understand the processes that establish and influence the value of social stimuli in humans.

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Introduction

Deficits in social behavior are a defining feature of autism spectrum disorder (ASD; Frye, 2018). Individuals diagnosed with ASD often have difficulties with social communication, facial expressions, social cognition, social cues, and responding to others in distress (Dawson et. al., 1998; Celani, Battachi, & Arcidiacono, 1999; Bacon et al., 1998; Weiss & Harris, 2001; & Laugeson et al., 2012). These deficits are concerning given that one in 44 children in the United States are estimated to be diagnosed with ASD (Maenner et al., 2021). Given the high prevalence of ASD diagnoses and the potential level of impairment in functioning, it is critical to study social behaviors. By investigating the factors that contribute to social behavior, researchers may discover new and superior methods to teach these behaviors.

Social behavior is varied and multifaceted, and no single unified definition has yet arisen, perhaps due to its complexity. This lack of consensus is likely a result of researchers investigating the many different aspects of social interaction (Simpson, 1987). Recent definitions of social behavior include the ways individuals act and react in relation to one another (Cerulo, 2009), reciprocal behavior that is influenced by partner's sensory signals (Feldman, 2021), and observable or discreet responses to environmental stimuli that allow individuals to adapt and cope (Matson & Wilkins, 2007), among other ways. Within the field of applied behavior analysis (ABA) social behavior is viewed as a type of operant behavior (Weiss & Harris, 2001). According to this perspective, social behavior can be understood and taught by studying the contexts in which it occurs and its consequences (Skinner, 1938). Flynn and Healy (2012) conducted a literature review looking at effective treatments for social behavior and self-help skills for individuals with

ASD. They identified several different interventions that have shown strong results in helping those with ASD develop social skills. All seven interventions utilized operant conditioning. If social behavior is viewed as operant behavior that is maintained by its consequences, it is important to understand the types of consequences that maintain social behavior and how they acquire their value.

The etiology underlying ASD and its associated social behavior deficits are poorly understood (Haratizadeh et al., 2020). One class of factors likely to influence social behavior are genes (Lazaro & Golshani, 2015 & Haratizadeh et al., 2020). Over 1,000 genes have been associated with ASD (Lazaro & Golshani, 2015 & Haratizadeh et al., 2020). A second factor likely to influence social behavior are environmental hazards. These factors include maternal and paternal age, caesarian delivery, and birth weight (Wang et al., 2017). Finally, the effectiveness of early behavioral interventions suggests that social behavior is also likely influenced by early learning experiences (Haratizadeh et al., 2020, Weiss & Harris, 2001). To study and understand the etiology of social behavior deficits it will be necessary to identify research procedures which can disentangle these complex and interacting factors.

A framework which may help us to better isolate and understand how genes, prenatal, perinatal, and postnatal factors may influence social behavior are rodent models (Haratizadeh et al., 2020). Rodent models are useful because rodents and humans have remarkably similar genes, biological processes, and brain circuitries because of our close evolutionary relationship (Lazaro & Golshani, 2015). Rodent models also allow researchers to manipulate genes, early learning history, and exposure to harmful

environmental stimuli more ethically and with more scientific control. Identifying tracking, and manipulating these same factors in humans would be nearly impossible both ethically and practically. For example, Lazaro and Golshani (2015) suggested that rodent models can help researchers to track and understand microcircuits in the brain that may contribute to ASD and to identify genes that rats share with humans that can help determine outcomes and responses to treatment.

To study social behavior in rodents, a wide variety of behavioral tasks have been developed. These procedures include social release (Bartal et al., 2011; Bartal et al. 2014; Bartal et al., 2016; Silberberg et al., 2014), social choice (Schwartz et al., 2017; Hackenberg et al., 2021), social conditioned place preference (Fitchett et al., 2006; Kummer et al., 2011; Fritz et al., 2011), and hybrid procedures which combine components of the above three paradigms (Hiura et al., 2018; Sato et al., 2015; Hachiga et al., 2018). In social release procedures rats are given the opportunity to emit a response to release a rat that is being restrained in a confined or wet space chamber (Bartal et al., 2011; Bartal et al., 2014; Bartal et al., 2016; Silberberg et al., 2014). The response a rat emits to release the constrained rat is typically a lever press, although touch sensor doors (Silberberg et al., 2014) and weighted doors (Bartal et al., 2011; Bartal et al. 2014; Bartal et al., 2016) have also been used. If the constrained/distressed rat is released into the chamber with the free rat, the free rat is typically allowed to interact with the constrained rat for between 10 seconds (Silberberg et al., 2014) and 60 minutes (Bartal et al., 2014). Preference for releasing the constrained/distressed rat over leaving the rat constrained is typically tested across 12 (Bartal et al., 2011; Bartal et al., 2014; Bartal et al., 2016) to 15

(Silberberg et al., 2014) trials and the dependent measures of interest are the total number and rate of release responses and latency to release.

Using social release paradigms researchers have investigated if rats are more likely to free a constrained/distressed rat when it is a cagemate (Bartal et al., 2011; Bartal et al., 2014; Bartal et al., 2016), non-cagemate (Bartal et al., 2014), different strain (Bartal et al., 2014), same strain (Bartal et al., 2011; Bartal et al., 2014), housed in the same chamber (Bartal et al., 2011; Bartal et al., 2014; Bartal et al., 2016; Silberberg et al., 2014), or housed in a different chamber (Bartal et al., 2014; Silberberg et al., 2014). Research using the social release paradigm has shown that rats will work to free another rat (Bartal et al., 2011, Bartal et al., 2014; Bartal et al., 2016; Silberberg et al., 2014), that non-cagemates are freed more often and quicker than cagemates (Bartal et al., 2014), and that familiar strains of rat (i.e., Long-Evans or Sprague Dawley) are more likely to be freed than non-familiar strains (Bartal et al., 2011; Bartal et al., 2014).

A second procedure commonly used to study social behavior is the social choice procedure. In this procedure a rat chooses between one-of-two options. The first option is access to a social stimulus like a restrained or unrestrained familiar rat (Schwartz et al., 2017; Hackenberg et al., 2021), or the scent and fur of a mother rat (Alberts & May, 1984). The second option varies but has included an empty chamber (Schwartz et al., 2017; Hackenberg et al., 2021), a familiar or non-familiar rat (Hackenberg et al., 2021), the scent and fur of a nonlactating virgin rat mother (i.e., a female rat that has not given birth to pups but that has adopted another rats' pups; Alberts & May, 1984), and a scented and heated fur tube (Alberts & May, 1984). Choice procedures differ from

release procedures because there is more than one possible programmed consequence. To make a choice, rats have been required to press a lever (Hackenberg et al., 2021), physically contact a stimulus (Alberts & May, 1984), or run left/right in an E maze (Schwartz et al., 2017).

Within the social choice procedure rats are commonly given between one (Alberts & May, 1984) and 60 choices (Schwartz et al., 2017) to demonstrate preference. Once a choice is made, rats are given between 45 seconds (Hackenberg et al., 2021) and 4 hours (Alberts & May, 1984) to interact with their choice option. Within social choice procedures the dependent measure is the percentage of social choices made by rats and latency to choose. Using social choice procedures researchers have found that rats prefer a constrained cagemate rat over an empty chamber (Hackenberg et al., 2021), a non-cagemate over a cagemate (Hackenberg et al., 2021), a distressed rat over a non-distressed rat (Schwartz et al., 2017), a non-distressed rat over an empty chamber (Schwartz et al., 2017), and a scent that has been paired with thermotactile stimuli, a stimulus that the rat can touch and is heated, over novel scents (Alberts & May, 1984).

The social conditioned place preference procedure (SCPPP) is a third way that social behavior has been studied. In the SCPPP a rat is first exposed to two different chambers across subsequent training sessions. These chambers are typically the two arms of an E maze. During training only one of the two chambers is available to rats on any given session. When available, each chamber is associated with a unique visual, tactile, or olfactory stimulus to distinguish it from the other chamber and a social stimulus like another rat is only present in one of the two chambers (Bardo & Bevins, 2000).

Following training sessions, rats are given a test session where they are allowed to enter either of the chambers, but the social stimulus typically associated with one of the chambers is no longer present. Two features of the SCPPP make it different from the social choice procedure. First, during the SCPPP test trial, the social stimulus is absent. Second, during the SCPPP test trial rats can run between and access both choice options/chambers.

Within the SCPPP rats are typically given eight training sessions (Fitchett et al., 2006; Kummer et al., 2011; Fritz et al., 2011) and one test session to demonstrate their preference. This test session lasts between 10 (Fitchett et al., 2006) and 15 minutes (Kummer et al., 2011; Fritz et al., 2011). The dependent measure in the SCPPP procedure is the amount of time spent within each chamber. Using the SCPPP researchers have investigated if rats and mice prefer their own bedding over another mouse's bedding (Fitchett et al., 2006) and if rats prefer free access to a rat in a small space over restricted access (i.e., access through bars or a plastic wall) in a large space (Kummer et al., 2011). Research has also investigated if rats prefer a chamber associated with social interaction over a chamber associated with cocaine (Fritz et al., 2011). In general, rodents demonstrate a preference for the chamber that contains a social stimulus over a chamber associated with cocaine or their own bedding (Fitchett et al., 2006; Fritz et al., 2011). Rats also prefer chambers that give free access to another rat over chambers that restrict access, even when free access occurs in a confined space (Kummer et al., 2011).

Social behavior has also been studied using combinations of the above three designs. These hybrid designs are typically a combination of the social release procedure

and choice procedure (Hiura et al., 2018; Sato et al., 2015; Hachiga et al., 2018). For example, Hachiga et al. (2018) gave rats a choice between entering a chamber with a restrained rat or a chamber with a free rat. Trials within these hybrid procedures last between 3 (Hachiga et al., 2018) and 20 minutes (Sato et al., 2015) and rats have been given between 3 (Hiura et al., 2018) and 48 choices (Hachiga et al., 2018) to demonstrate preference. The results of hybrid studies typically replicate the results of nonhybrid studies which show rats choose to free distressed cagemates over non-distressed cagemates (Schwartz et al., 2017; Bartal et al. 2014). They also show that rats prefer access to free rats over restrained rats (Hachiga et al., 2018).

Although the above experimental procedures have contributed to researcher's understanding of the factors that influence and determine social behavior in rodents, they also have limitations. For example, in all the proceeding procedures it is difficult to isolate what stimulus changes impact rats' behavior. Early social release research by Bartal and colleagues (Bartal et al., 2011, Bartal et al., 2014; Bartal et al., 2016) argued that rats release constrained/distressed rats because they feel empathy for those rats. Research by Hachiga et al., (2018) and Schwartz et al., (2017) have suggested rats release constrained rats because released rats allow for social interaction. Hackenberg et al. (2021), Sato et al., (2015), and Schwartz et al. (2017) have provided a third explanation: rats choices in these procedures are controlled by access to touch or taste stimuli. Collectively, these studies raise questions about why rats make choices in these social paradigms. Do rats emit choices because of empathy, social stimulation, tangible items such as water or a harness, or some combination of these and other factors? In the

procedures currently used to study social preference, it is difficult to separate out and identify the critical factors.

A second limitation of the social release and social choice procedures is that it is difficult to evaluate the strength of animals' preference. When a rat is given a choice between a social stimulus and a second nonsocial stimulus, it is possible to determine which stimulus the animal prefers, but not how strong that preference is. Being able to assess the strength of rats' preference for social stimuli, and if there are large individual differences in rats' preference for social stimuli may help researchers parametrically manipulate and understand the factors contributing to social behavior.

An additional limitation of the SCPPP is that it removes the social stimuli present during training trials during the testing trial. Bardo and Bevins (2000) suggest that the removal of social stimuli during CPPP test trials may function similarly to the introduction of a novel stimulus. This is a problem because rats have been shown to prefer novel stimuli and contexts over familiar ones (Bardo et al., 1993; Bardo and Bevins, 2000). Thus, when an animal spends more time in the chamber previously associated with a social stimulus, this might be because of a preference for the previously present social stimulus or because that environment is now novel and interesting to explore. This problem may further be exacerbated by the fact that CPPP test sessions typically last for a single session, which may not be sufficient time for the novelty of the new context to wear off. Thus, while rodent models of social behavior show great promise for helping researchers to understand the factors that control social behavior,

new procedures or controls will be necessary to ensure rodents' preference for social stimuli and interactions is actually being assessed.

The goal of the following two studies was threefold. The first goal was to evaluate a novel choice procedure for assessing the value of social stimuli in rats. This procedure was explicitly designed to better control for the limitations in other social preference assessment procedures. The second goal was to determine if social olfactory stimuli (i.e., air blown through another rats used bedding) will function as a reinforcer for rats' nose poking behavior. There is currently no published research which has attempted to establish and maintain behavior using only social scents when animals have not been deprived of food or water. The third goal was to assess the relative value of social and nonsocial olfactory stimuli. While rats may work to access social olfactory stimuli when they are presented with a control stimulus, it will also be important to assess the relative value of social and nonsocial olfactory stimuli.

In Experiment 1 rats were given a choice between either a social scent and a control scent or a nonsocial scent and a control scent. By presenting just scents instead of another rat, water, a novel item, or a new space, we hoped to reduce the number of stimuli that influence rats' choices. Because rats are a social species that heavily utilizes scents (Slotnick, 2001), we believe rats will spend more time smelling and make more responses to obtain social and nonsocial scents than a control scent. A control scent was included in the procedure to control for the possibility that rats may chose a scent option just to feel or lick the air stream and not to smell the scent (Hendry & Rasche, 1961). If

rats were emitting a choice response just for the air stream, we expected them to be indifferent between the scent and control options.

An additional advantage of the novel choice procedure being used in Experiment 1 is that it allowed us to better assess how valuable social olfactory stimuli are to rats and to explore individual differences in value. Unlike the social release, hybrid, and the SCPPP, which give rats a predetermined number of choice trials, the choice procedure that was used in this research is a free-operant procedure. This means that during experimental sessions rats' choices are not limited, and they can emit a response to produce an olfactory stimulus at any point in the session. To reduce the possibility that rats' behavior is being influenced by novelty, and not social scents, rats ran daily choice sessions until their choice proportions were stable. This procedural modification differs from the SCPPP and the social release procedures which typically run a predetermined and fixed number of testing/choice sessions.

The results of Experiment 1 allowed us to determine if rats can detect and if they prefer social or nonsocial scents over a control scent. They did not allow us to directly compare the relative value of social and nonsocial scents. Experiment 2 gave rats a choice between social and nonsocial scents within the same session. By giving rats the option to choose between social and nonsocial scents, we attempted to assess if rats prefer social or nonsocial scents and the strength of that preference.

To summarize, a novel choice procedure was used to assess rats' preference for social and nonsocial olfactory stimuli across two experiments. This novel choice procedure was developed to allow researchers to (1) isolate and better identify the

stimulus changes influencing rats' choices, (2) assess the strength of social stimuli reinforcers, and (3) control for the possibility that rats behavior is being influenced by novelty. In Experiment 1 rats chose between control stimulus and either a social olfactory stimulus or a nonsocial olfactory stimulus. This allowed us to evaluate if rats can detect and if rats will repeatedly work to obtain olfactory stimuli. In Experiment 2, rats chose between social stimuli and nonsocial olfactory stimuli. This allowed us to evaluate the relative value of social and nonsocial olfactory stimuli in rats.

Experiment 1 Methods

2.1 Subjects

Six female rats were used in the experiment. Four of the rats were experimentally naïve and two had previous olfactory research experience. Each rat was individually housed in a clear plastic cage on a reverse 12-hr light/dark cycle (6 AM – 6 PM). Rats were individually housed in this experiment because previous research investigating the effects of individual and joint housing on rats' social behavior has produced mixed results. Douglas et al (2004) reported that social isolation strengthened the rewarding properties of social interaction, but studies by Tanaka et al (2019) and Galal (2021) reported that socially isolated rats showed impairment in social preference. Future research might further explore the effects of individual and pair housing on rats' preference for social and nonsocial scents. Rats had free access to food and water throughout the experiment. Female rats were used because research has indicated they more quickly and consistently respond for social stimuli than male rats (Bartal et al., 2011).

2.2 Apparatus

Three Med Associates Operant Chambers (ENV-007) housed inside sound attenuating cubicles (ENV-018MD) was used in Experiment 1. The front and rear walls of each chamber are made of aluminum (10”L and 13”H) and the side walls (13”W and 13”H) and ceiling (10”L and 13”W) are made of plexiglass. The floor of the operant chamber is made up of (10”D and 1/4”W) aluminum bars spaced .5” apart. Below the floor an aluminum drop pan collected rat waste. Two nose poke ports (ENV-254-CB) were mounted on the front wall. Each nose poke port was mounted 3” from the floor and can be illuminated with a white light. An olfactometer (ENV-275-5) capable of presenting and removing up to five scents was attached to both nose poke ports. The rear wall contains an LED (ENV-215M-LED) house light mounted 11” above the floor that can be used to illuminate the operant chamber. Experimental events were controlled by a windows computer running version 5.1 of Med-PC.

2.3 Procedure

In Experiment 1 rats were given a choice to nose poke in a port that delivers air mixed with four scents (either four social or four nonsocial) and a control port that delivers air blown through clean bedding (control). Four scents were used because pilot research in our lab indicated that most rats will emit responses for a single scent, but that a single scent may maintain limited responding in some rats. By using multiple social or nonsocial scents we hoped to increase the number of nose pokes emitted by rats.

Social scents were created by blowing air through glass containers containing used rat bedding. The bedding used for social scents were obtained from eight different rat cages once per week. The eight rats were from our rat colony and included the six rats

running in the experiment. Nonsocial scents were created by blowing air through glass containers containing bedding and a small cotton swab containing essential oil. To create the nonsocial scents 100g of clean bedding was stored with a large cotton swab containing .2mL of essential oil and 10 small cotton swabs, each containing .03mL of essential oil. Nonsocial scents were created once a week and stored in an airtight bag. A list of the eight essential oils that were used to create the nonsocial scents is displayed in Table 1. These eight scents were used because the rats had never been exposed to them before, had no prior learning history associated with the scents, and because rats were able to detect and discriminate between these scents in previous research (Galizio, Mason & Bruce, 2020). The type of scent (social or nonsocial) and the four (out of eight) scents delivered each day were pseudo randomly determined. This means that the type of scent and the four specific scents used each day were randomly determined, with the constraint that all scent types and individual scents need to be presented before any are repeated. With the constraint that all scents need to be presented before any are repeated. To control for position place preference four of the six rats were randomly assigned to receive the scents in the left port and two received it in the right port.

Table 1

Nonsocial scents and the companies that made them.

Coconut – Great American Spice	Honey– Great American Spice
Strawberry – Eternal Essence	Apple– Great American Spice
Almond– Great American Spice	Clove– Great American Spice
Vanilla – Sun Essential Oils	Blueberry– Great American Spice

Note. This table lists the essential oils used as the nonsocial scents for all Experiments as well as the vendors they came from.

At the start of each daily session rats were placed in the operant chamber, the house light was turned on, and control air began to flow through both nose pokes. When a rat breaks an infra-red beam in the nose poke associated with the social or nonsocial scent, the control air stopped flowing, the nose poke was illuminated, and one-of-four social or nonsocial scents was delivered for .3 s. After the delivery of the scent, the control scent was turned back on, and rats can once again emit a nose poke at either of the ports. When a rat breaks the infra-red beam in the nose poke associated with the control scent, the control air stopped flowing, the nose poke was illuminated, and the control was turned back on. The control scent was turned on and off repeatedly to make sure that the only difference in the scented and control port choices is the presence of the social or nonsocial scent. Once a scent has been delivered the rats will need to remove their nose and break the infrared beam again to obtain another scent. This contingency was programmed to ensure rats could not keep their nose in the port and continuously receive a scent. To reduce the likelihood that scents remain in a chamber, each nose poke contains a small hole that is connected to an air pump which continuously pulls air from

the chamber. To further keep rats from continually receiving the same scent from one delivery the scent was evacuated from the chamber after .3 s.

Daily session lasted one hour, and the number of nose pokes and the time rats spend in each nose poke was recorded. Following each daily session, the chamber was sprayed and wiped with a bleach solution to clean and reduce residual scents from previous rats. Daily sessions continued until rats' nose-poking behavior is stable. To be considered stable the proportion of response that rats make to the social and nonsocial scent will need to meet the following criteria: (a) the last three two-day means do not trend up or down and (b) none of the last six proportions are the highest or lowest proportions in the experiment. After stability criteria was met, the location of the scent and control were reversed to rule out the possibility of a side bias and to ensure rats prefer scents. Rats ran in the reversal condition until the stability criteria was once again met.

In Experiment 1 we hypothesized that rats would show a preference for the social and nonsocial scents over control scents. To evaluate this hypothesis rats' responses to social, nonsocial, and control ports during stability sessions were analyzed using a repeated measures ANOVA. The seconds spent in the social, nonsocial, and control ports were also analyzed with a repeated measure ANOVA. An alpha level of .05 was used for all statistical tests. To control for type 1 error Bonferroni-adjusted post hoc tests were used.

Experiment 1 Results

Figure 1 displays the proportion of responses that each rat made to social and nonsocial scents during Conditions 1 and 2. The dotted line in the figure at .5 indicates indifference. If rats made identical numbers of responses to social (blue data path) or nonsocial (purple data path) scents and control scents, data would fall on this line. If the data path is above this line, it indicates that rats made more response to the social (nonsocial) scent than to the control scent. The number of days it took for rats' choice proportions to stabilize in Condition 1 ranged from 42 (Orange) to 80 (Blue) days. Rats' choice proportions for social scents over a control scent ranged from .38 (Black) to 1 (i.e., exclusive choice) (Black & Brown) in Condition 1, while rats' choice proportions for nonsocial stimuli ranged from .33 (Black) to 1 (Brown & Orange). At the start of Condition 1 rats quickly learned which port delivered the social and nonsocial scents and the choice proportions for all six rats predominately favored that port throughout the entire condition.

In Condition 2 of Experiment 1 the ports that delivered the social/nonsocial and control scents were reversed. The number of days it took rats to reach stability following the reversal ranged from 30 (Brown) to 90 (Turquoise). As in Condition 1, rats quickly located which port delivered social and nonsocial scents at the beginning of Condition 2 and their choices proportions predominately favored the port which delivered social or nonsocial stimuli over the control port. In Condition 2 Rats' social choice proportions ranged from 0 (Black) to 1 (Black), while rats' choice proportions for nonsocial stimuli ranged from .30 (Brown) to 1 (Black). Black 1's choice proportions in Condition 2 were

quite variable because of the low number of responses it emitted. This is discussed in further detail below.

Figure 1

Proportion of Responses Spent With the Scent Verses the Control

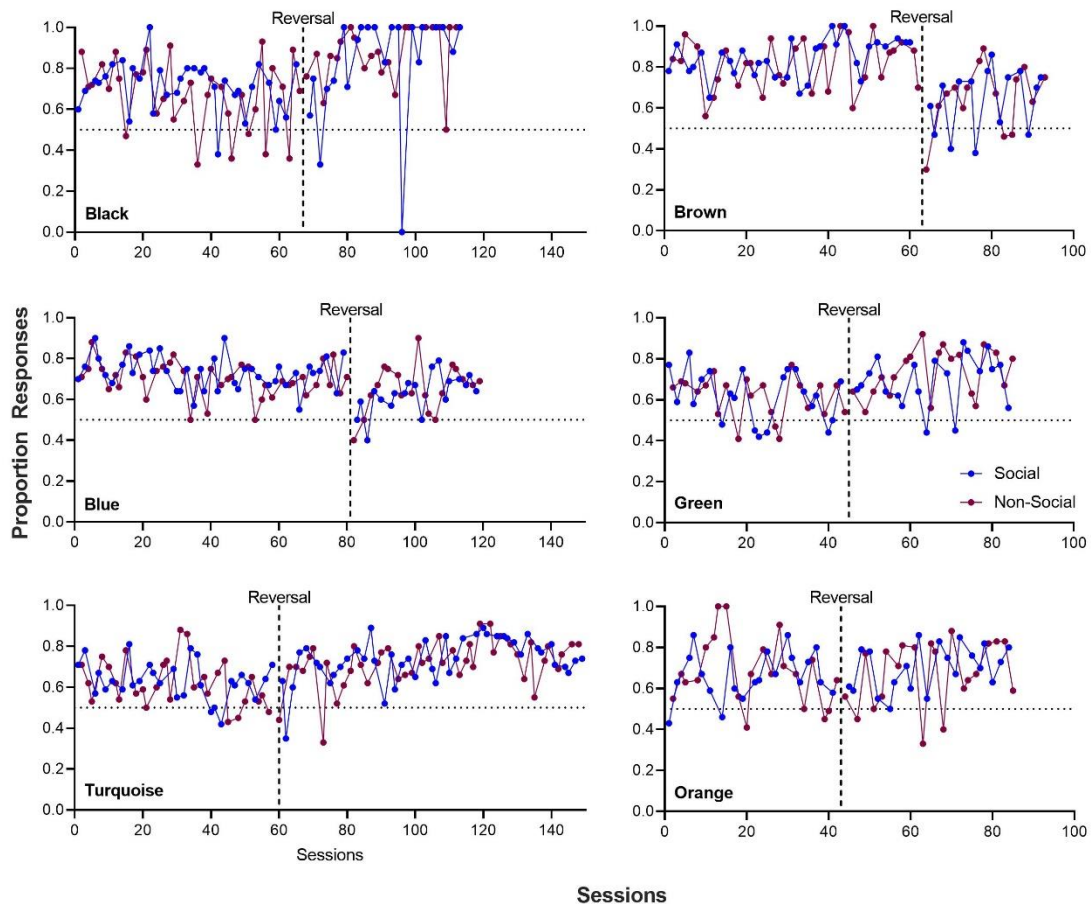


Figure 2 displays the total number of responses made to the social, non-social, and control ports in each daily session of Conditions 1 and 2. In Condition 1, the number of responses rats made to social stimuli ranged from 3 (Black, Orange & Blue) to 67 (Brown), while the number of responses made to nonsocial stimuli ranged from 1 (Orange) to 70 (Brown). Responses made to the control scent ranged from 0 (Black, Brown & Orange) to 33 (Black). In Condition 1 and Condition 2 several rats showed an

initial spike in responding that quickly decreased across several days. This spike was not observed in Figure 1 because the increase in responding occurred similarly to social, nonsocial and control scents.

At the start of Condition 2 a spike in total responses once again occurred for five of six rats. Blue was the only rat that did not show an immediate spike in responding following the reversal. In Condition 2 the number of responses made to social stimuli ranged from 0 (Black) to 54 (Turquoise), while the number of responses made to nonsocial stimuli ranged from 0 (Black) to 45 (Green). The number of responses made to control stimuli ranged from 0 (Black) to 31 (Turquoise). Because Black emitted very few responses (commonly between 1 and 5) to the social, nonsocial, and control ports in Conditions 1 and 2, this resulted in the high degree of variability in her choice proportions. Black had two sessions (sessions 99 and 103) in which she made zero responses.

Figure 2

Total Number of Social, Nonsocial, and Control Responses

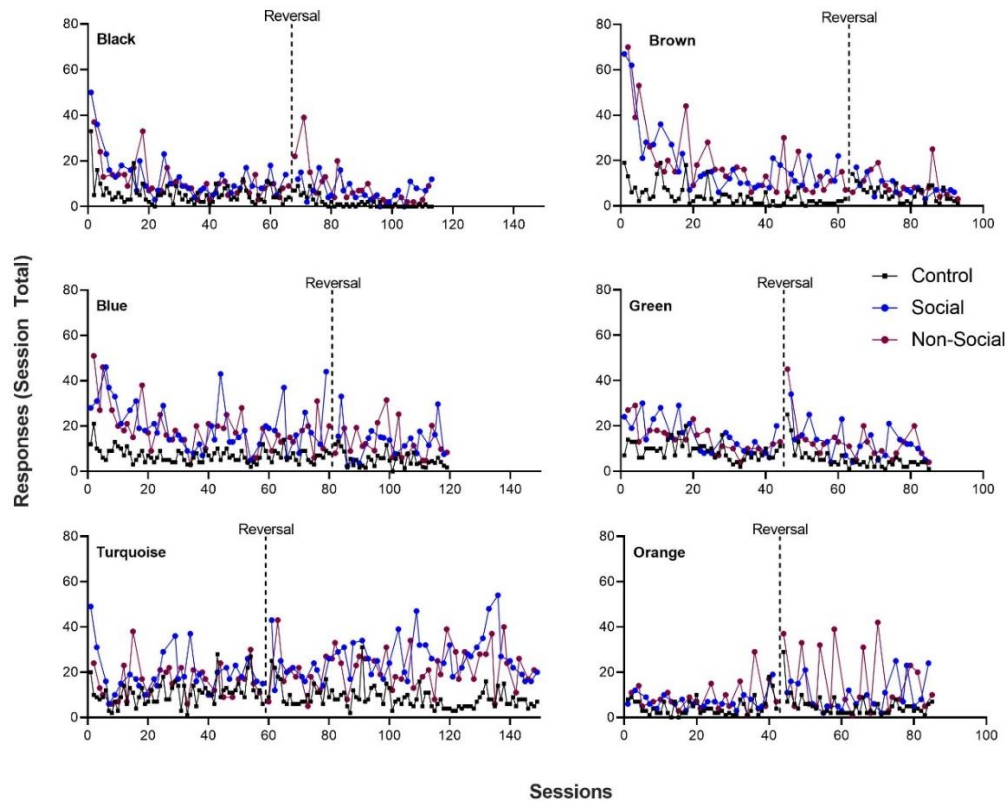


Figure 3 displays the proportion of time (in seconds) rats spent with their nose in the social or nonsocial port as compared to the control port during each session. In Condition 1, the proportion of time rats spent in the social port ranged from .21 (Black) to 1 (Black & Brown) and the proportion of time spent in the nonsocial port ranged from .22 (Black) to 1 (Brown & Orange). In Condition 1, the rats time proportions predominately favored the port which delivered social or nonsocial stimuli over the control port.

In Condition 2, the proportion of time with the social stimuli ranged from 0 (Black) to 1 (Black), the proportion of time spent with the nonsocial stimuli ranged from

.23 (Orange) to 1 (Black). Following the reversal there was a small dip in the proportion time Blue spent with the social and nonsocial stimuli that steadily increased over time. A small dip in proportion time also occurred for Brown on the first session following the reversal. None of the other rats saw a significant spike or drop in either social or nonsocial proportion time. As in Condition 1, the rats time proportions predominately favored the port which delivered social or nonsocial stimuli over the control port.

Figure 3

The Proportion of Time With Social and Nonsocial

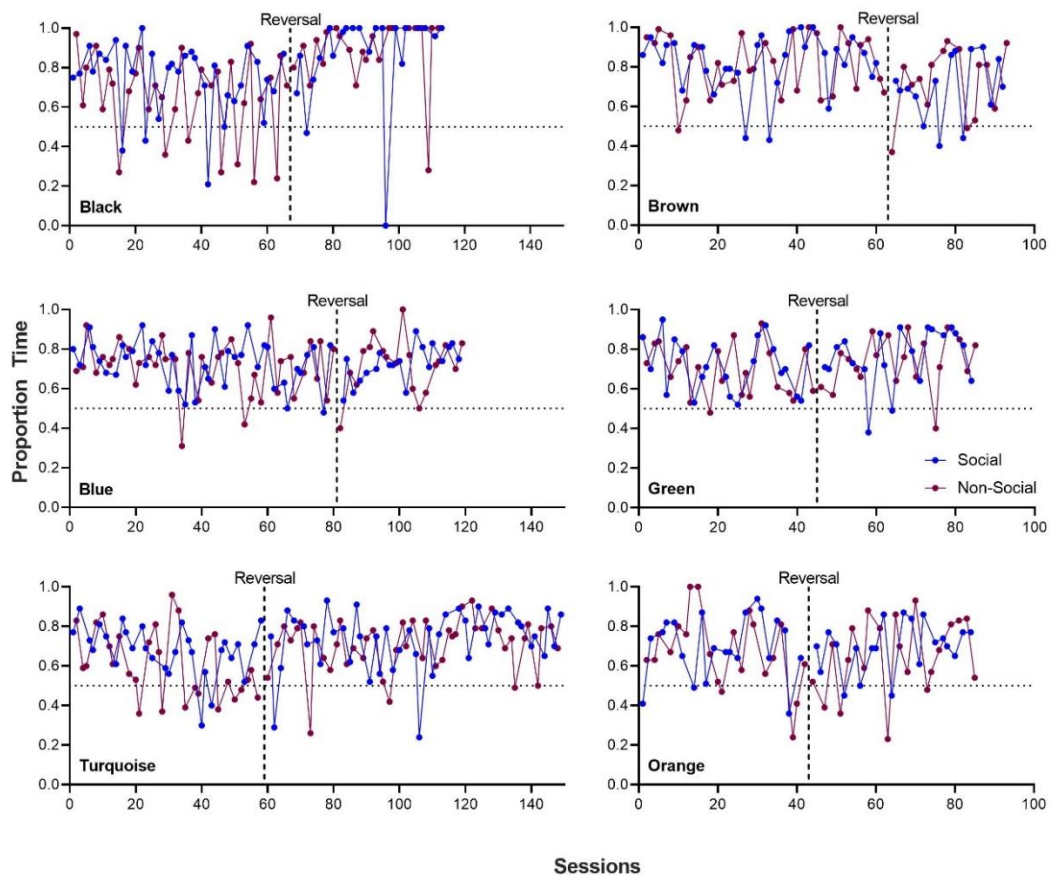
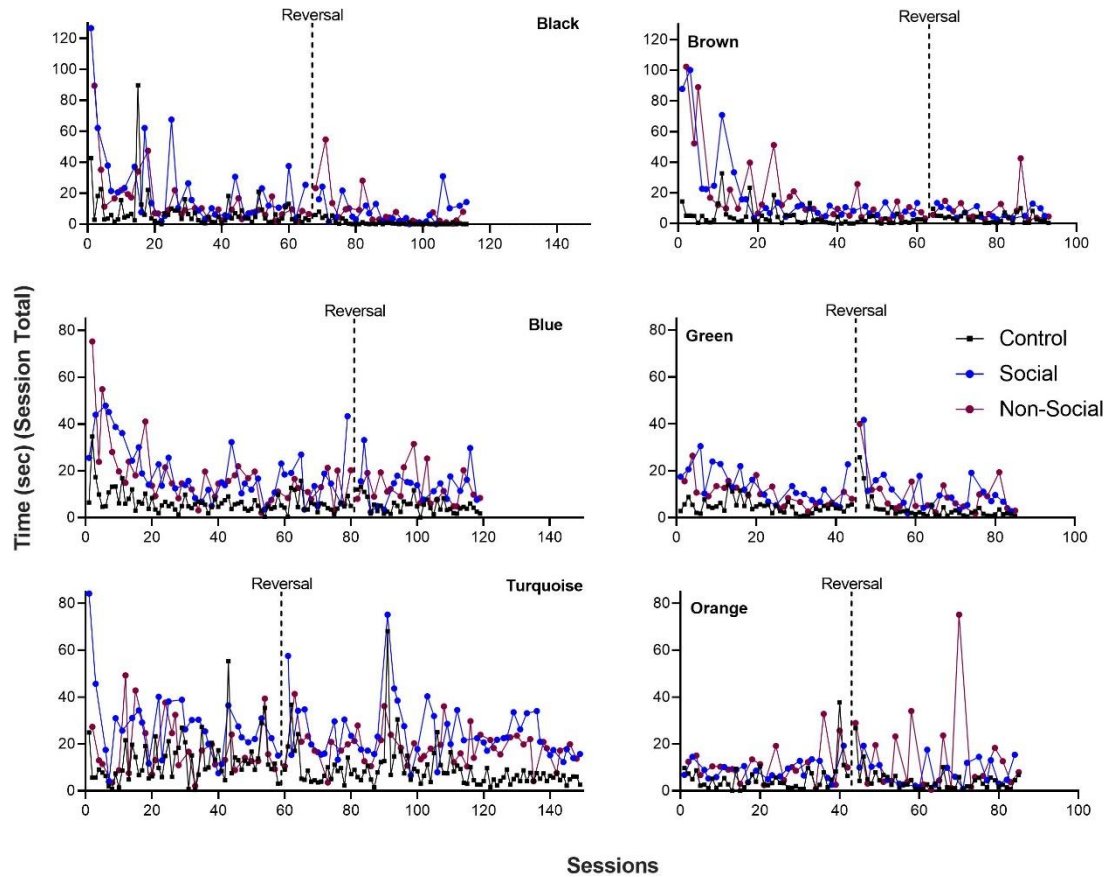


Figure 4 shows the total seconds each rat spent in the control, social, or nonsocial ports during each session of Experiment 1. In Condition 1, four rats (Black, Brown, Blue,

and Turquoise) initially spent large amounts of time (> 75 s) in the social, nonsocial, and control ports. The remaining two rats (Green and Orange) did not demonstrate this pattern of time allocation. In Condition 1, the amount of time spent in the port when it delivered social scents ranged from 1.88 (Black) to 126.61 s (Black), while the amount of time spent in the port when it delivered nonsocial stimuli ranged from .99 (Orange) to 102.32 s (Brown). The time spent in the port associated with the control scent ranged from 0 (Black, Brown & Orange) to 89.75 s (Black).

At the beginning of Condition 2, three rats (Black, Green, and Turquoise) showed a small spike in the amount of time spent in the social, nonsocial, and control scents. The amount of time spent in the port when it delivered social scents ranged from 0 (Black) to 75.19 (Turquoise), while the amount of time spent in the port when it delivered nonsocial stimuli ranged from 0 (Black) to 75.2 (Orange). The amount of time spent in the control port ranged from 0 (Black) to 36.75 (Turquoise). On session 69 orange spent a total of 75.2 s in the nonsocial port, which was the highest amount of time orange spent in any port during the experiment. Following this session this port was checked to make sure it was correctly recording data and that the correct scents were used. No errors were found.

Figure 4*Total Amount of Time Spent in Second*

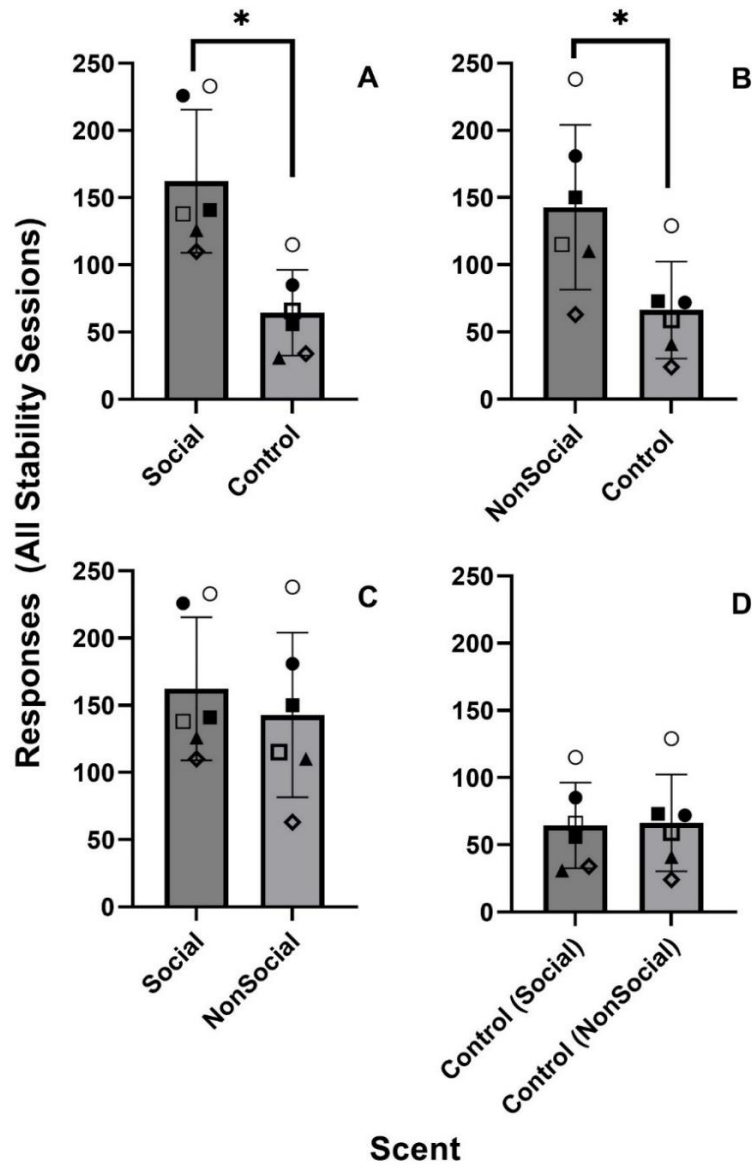
Note. The y-axis on the top two graphs is different from the y-axis on the bottom four graphs.

To further evaluate if rats preferred social and nonsocial scents over control scents the total responses allocated to social, nonsocial, and control scents were each summed across the 12 stability sessions (six stability sessions from Condition 1 and six stability sessions from Condition 2). The resulting four sums of responses (i.e., social, nonsocial, social control, nonsocial control) were checked for normality using Shapiro-Wilk's tests and analyzed using repeated measures ANOVAs. A Greenhouse-Geisser corrected

repeated measures ANOVA determined that there was a significant difference in the number of responses emitted to the social, nonsocial, social control, and nonsocial control ports ($F(1.811, 9.056) = 42.73, P < 0.01$). Bonferroni-adjusted pairwise comparisons indicated the total number of responses made to social scents were statistically greater than the total responses made to the social control scent ($t = 8.95$ (95% CI, 56.17 to 139.5F), $p < .01$) and that the total number of responses made to nonsocial scents were significantly greater than the total number of responses made to the nonsocial control scent ($t = 6.63$ (95% CI, 32.53 to 120.5), $p < 0.01$) (Figure 5 – Panels A & B). The total number of responses made to social scents did not differ from the total number of responses made to nonsocial scents ($t = 2.00$ (95% CI, -17.59 to 56.59), $p = 0.41$), nor did the number of responses made to social control and nonsocial control scents ($t = 0.34$ (95% CI, -22.50 to 18.83), $p > 0.99$) (Figure 5 – Panels C & D)).

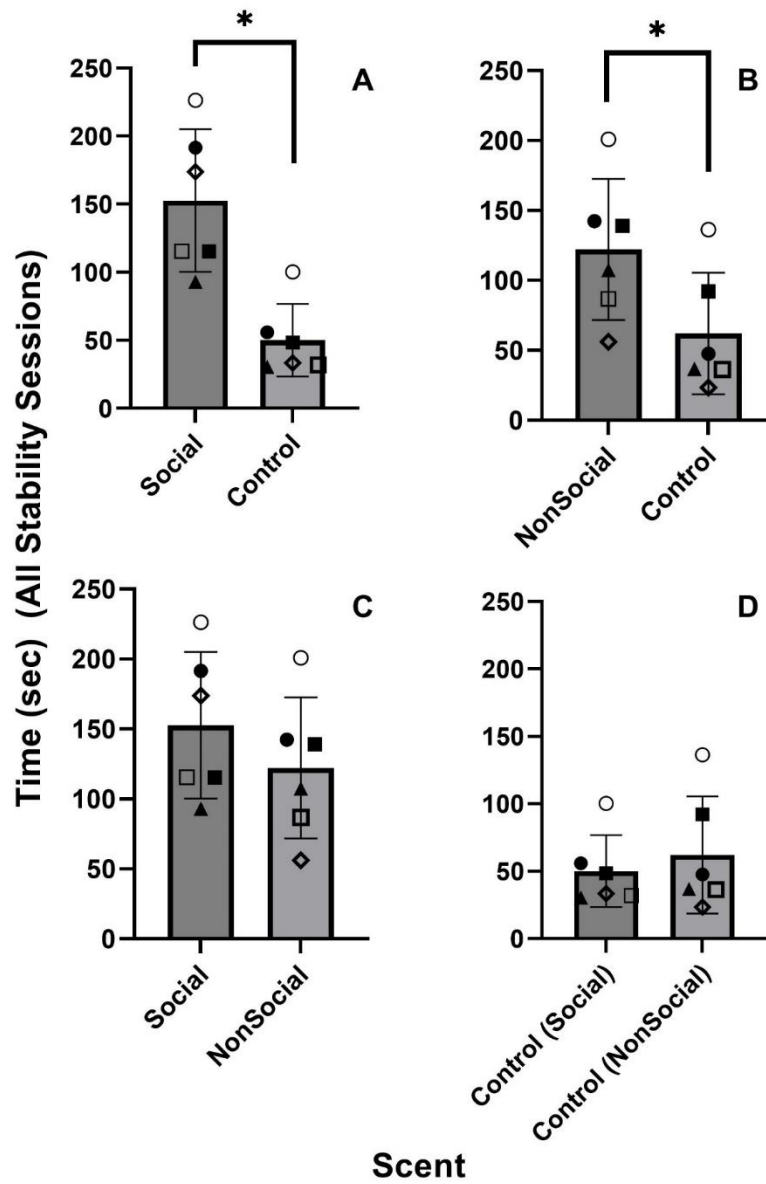
Figure 5

Results From the Total Responses ANOVA



Note: The bar represents the average number of responses that rats made to scents during stability sessions. The shapes represent individual subject data. The shape associated with each rat is held constant across all figures.

To further evaluate if rats spent different amounts of time in the social, nonsocial, and control ports, the time spent in those ports were each summed across the 12 stability sessions (six stability sessions from Condition 1 and six stability sessions from Condition 2). The resulting four sums of time (s) (i.e., social, nonsocial, social control, nonsocial control) were checked for normality using Shapiro-Wilk's tests and analyzed using a repeated measures ANOVA. A Greenhouse-Geisser corrected repeated measure ANOVA determined there was a significant difference in the amount of time spent in the social, nonsocial, social control, and nonsocial control ports across the final 12 stability sessions ($F(1.539, 7.695) = 21.10, P < 0.01$). Bonferroni-adjusted pairwise comparison revealed the time spent in the social port was significantly higher than the time spent in social control port ($t = 7.06$ (95% CI, 47.23 to 157.9), $p < 0.01$) and that time spent in the nonsocial port was statistically higher than the time spent in the nonsocial control port ($t = 6.78$ (95% CI, 26.29 to 93.79), $p < 0.01$) (Figure 6 – Panels A & B). Time spent in the social port did not differ significantly from the time spent in the nonsocial port ($t = 1.47$ (95% CI, -48.61 to 109.6), $p = 0.81$), nor did the time spent in the social control port differ from the time spent in the nonsocial control port ($t = 1.29$ (95% CI, -47.43 to 23.40), $p > 0.99$) (Figure 6 – Panels C & D).

Figure 6*Results From the Total Time ANOVA*

Note. The bar represents the average time each rat spent in the port associated each scent during stability sessions. The shapes represent individual subject data.

Experiment 1 Discussion

The primary goal of Experiment 1 was to evaluate if social and nonsocial scents would function as reinforcers for rats' nose-poking behavior. The results from Experiment 1 suggest that rats will reliably respond for both social and nonsocial scents, even when they have not been deprived of food or water, indicating that both types of scents can function as reinforcers. These findings align with previous research showing that rats prefer access to another rat over an empty chamber (Bartal et al., 2011; Bartal et al. 2014; Bartal et al., 2016; Silberberg et al., 2014; Schwartz et al., 2017 & Hackenberg et al., 2021). Additionally, these findings show that social olfactory stimuli themselves will influence behavior, which calls into question the interpretation of previous research that rats release other rats because of empathy; rats may in fact release other rats simply to access social olfactory stimulation.

A secondary goal of Experiment 1 was to evaluate a novel free-operant choice procedure for studying rats' preferences for social and nonsocial scents. One advantage of this procedure is that it allows rats to make repeated responses for scents across successive sessions. This feature of the procedure reduces the likelihood that novelty is influencing rats' choices, which may be a problem in other social assessments (Bardo & Bevins, 2000). Evidence that novelty may influence the value of olfactory reinforcers is present at the beginning of Experiment 1 and following the reversal. At both these points in the experiment, spikes in responding, which declined over time, were observed in 5 out of 6 rats. These spikes in responding are consistent with previous research showing novelty of stimuli impacts rats' choices. (Bardo et al., 1993).

A second advantage of the free-operant choice procedure used in Experiment 1 is that it allows for the strength of rats' preference for social and nonsocial stimuli to be evaluated. While one rat (Black) made an average of 7.21 responses to social and nonsocial stimuli during stability sessions, another rat (Turquoise) emitted nearly three times that number of responses (19.63). Given that the procedure is sensitive enough to detect these individual differences, it is ideal for the systematic investigation of the factors which lead to individual differences in preference for social and nonsocial stimuli.

A final observation from Experiment 1 is that even though rats overwhelmingly responded to the scent port more than the control port, several rats continued to emit control responses across more than 100 sessions. One possible explanation for this observation is that rats continued to occasionally check the control port to see if new scents would occur in that port. A second possibility is that occasionally smelling the control scent may have increased the reinforcing value of the social and nonsocial scents. In other words, if rats only smelled social or nonsocial scents, rats may have begun to habituate to those scents within a session. By sampling both the scent and control ports, rats may have postponed habituation and maximized the reinforcing value they obtained from social and nonsocial scents.

Experiment 2 Methods

Experiment 1 allowed us to determine if rats can detect a social and nonsocial scent and if they prefer a social or nonsocial scent over a control scent. The results could not tell us the relative value of social and nonsocial scents. For example, rats may nose poke the same number of times in the port during social scent and nonsocial scent days. If

this were to occur, it would be difficult to determine rats' preference for social and nonsocial scents.

To determine if rats prefer social or nonsocial scents, Experiment 2 investigated rats' preference for social and nonsocial scents when rats can choose between both in the same session. In Experiment 2, one nose port presented one of four social scents and the other presented one of four nonsocial scents. This choice condition helped us to determine the relative value of social and nonsocial scents.

5.1 Subjects

The subjects, housing conditions, and feeding conditions used in Experiment 2 were identical to those used in Experiment 1.

5.2 Apparatus

The apparatus described in Experiment 1 was used in Experiment 2 with one exception. A second olfactometer was added to each operant chamber. The addition of a second olfactometer allowed eight (four social and four nonsocial) scents to be delivered within the same session.

5.3 Procedure

The procedure used in Experiment 2 was identical to the procedure used in Experiment 1, but with two exceptions. First, instead of being given a choice between a control scent and either a social or nonsocial scent rats chose between a social scent and a nonsocial scent. Second, the amounts of nonsocial scents made each week were doubled to compensate for running nonsocial and social since every day. This mean that there was now 200g of bedding used, two full cotton swobs, 20 pieces of cotton swob, and a total of

1mL of essential oil. The scents used each day were pseudo randomly assigned with the constraint that all scents needed to be presented once before any scent is repeated.

Each daily session in Experiment 2 lasted one hour and the number of nose pokes and the time rats spent in each nose poke was recorded. Daily Sessions continued until rats' response proportions are stable. The stability criteria used in Experiment 2 was be identical to the stability criteria from Experiment 1.

In Experiment 2 we hypothesized that the rats would show a preference for the social scent over the nonsocial scent. To evaluate this hypothesis rats' total social responses were compared to their total nonsocial responses and their total social time was compared to their total nonsocial time using a repeated measures *t* test.

Experiment 2 Results

At the beginning of Experiment 2 a programming error led to two rats (black and orange) receiving a scent in the port opposite from where they responded for 5 sessions. The data from these first five sessions was not included in graphs, stability criteria, or data analysis.

Figure 7 displays the proportion of responses that each rat made during Conditions 1 and 2 of Experiment 2. The dotted line in the figure at .5 indicates indifference. If rats made identical response to social (blue data path) and nonsocial scents, data would fall on this line. If the data path is above this line, it indicates that rats made more response to the social scents than to the nonsocial scents. The number of days it took for rats' choice proportions to stabilize in Condition 1 ranged from 15 (Orange) to

24 (Brown) days. Rats' choice proportions for social scents over nonsocial scents ranged from 0 (Black) to .86 (Green) in Condition 1. In Condition 1 four rats (Blue, Brown, Green, Turquoise) favored the social scent port, 1 rat (Orange) favored the nonsocial port, and 1 rat (Black) appeared to be indifferent between the two ports.

In the second condition of Experiment 2, the scent locations were reversed. Following this reversal, it took rats from 16 (Black & Blue) to 25 (Brown) days to meet the stability criteria. In Condition 2, Rats' choice proportions ranged from .25 (Orange & Brown) to .78 (Black). Following the reversal, only the choice proportions from Orange consistently favored the port delivering social scents, while Brown's choice proportion favored the nonsocial stimuli. The remaining four rats' choice proportions tended to hover at or near indifference.

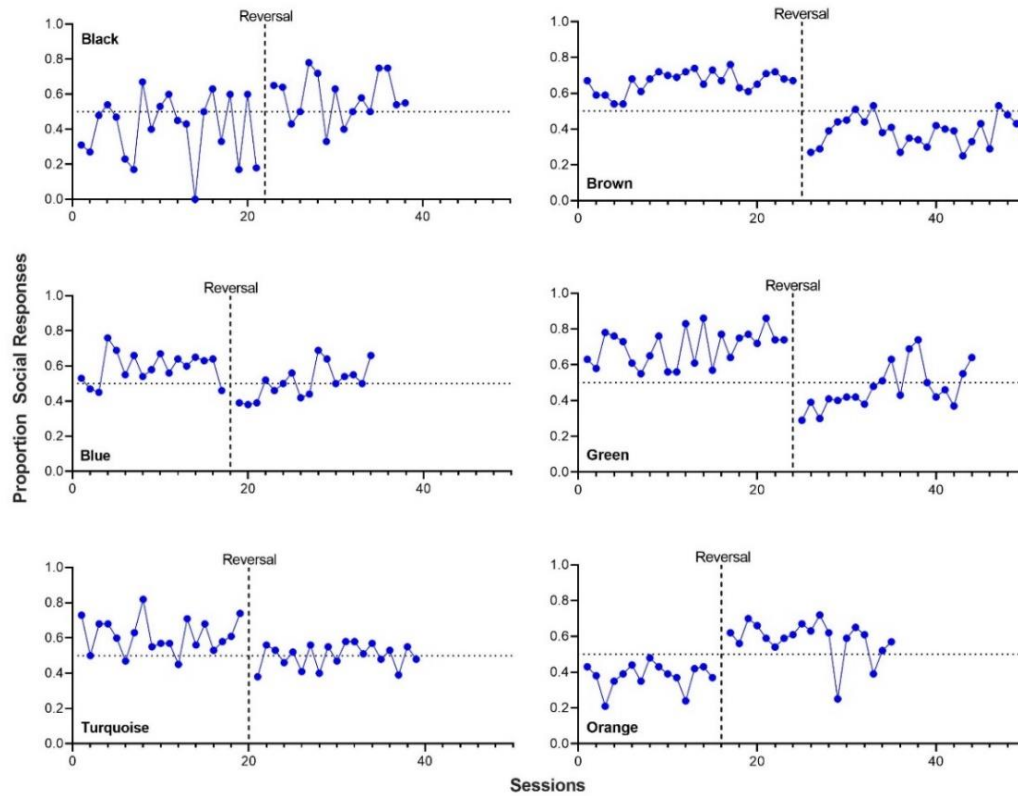
Figure 7*Proportion of Social Responses*

Figure 8 displays the total number of responses made to the social and non-social ports in each daily session of Conditions 1 and 2. In Condition 1, the number of responses rats made to social stimuli ranged from 0 (Black) to 78 (Brown), while the number of responses made to nonsocial stimuli ranged from 2 (Black & Green) to 41 (Orange). Following the reversal, small spikes in responding occurred for some rats (Black, Brown, & Orange), but these spikes in responding tended to be smaller than those observed in Experiment 1. In Condition 2, the number of responses rats made to social stimuli ranged

from 1 (Black) to 54 (Turquoise), while the number of responses made to nonsocial stimuli ranged from 1 (Black) to 57 (Brown).

Figure 8

Total Number of Social and Nonsocial Responses

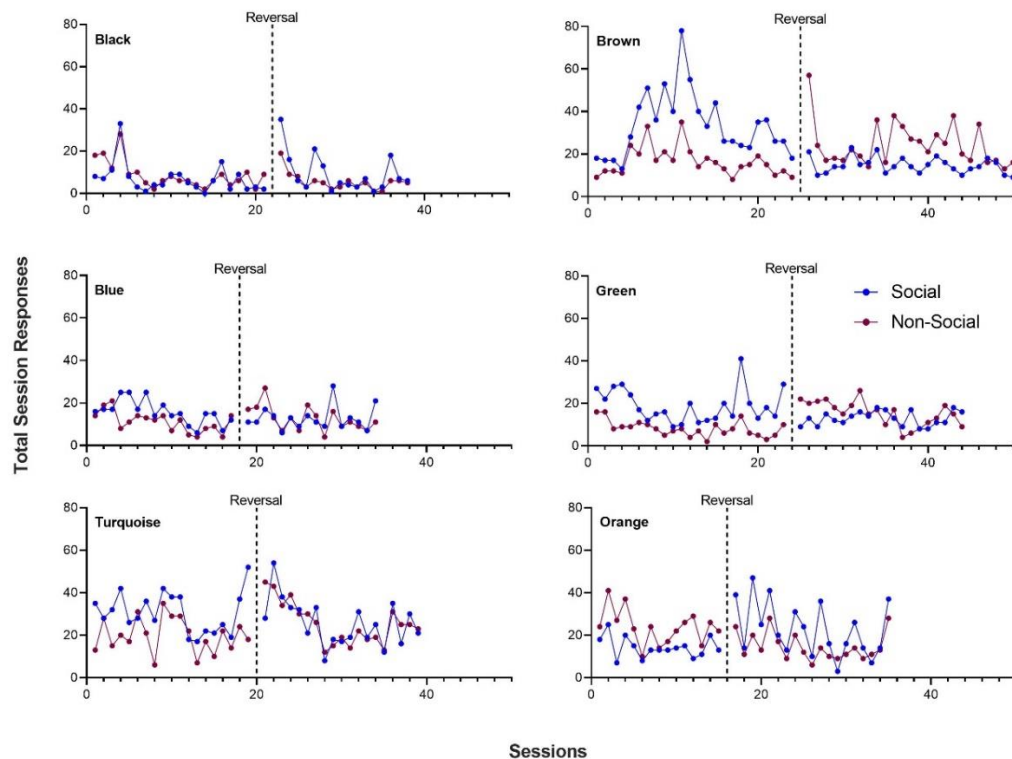


Figure 9 displays the proportion of time (in seconds) rats spent with their nose in the social port as compared to the nonsocial port during each session. In Condition 1, the proportion of time rats spent in the social port ranged from 0 (Black) to .9 (Green). In Condition 1 four (Brown, Blue, Green, and Turquoise) of the six rats spent more time in the social stimulus port, while two rats (Black and Orange) spent more time in the nonsocial stimulus port. In Condition 2, the proportion of time in the social scents port

ranged from .17 (Turquoise) to .77 (Orange). Only the choice proportions of Brown differed consistently from indifference. The five remaining rats time proportions tended to hover near or at indifference.

Figure 9

Proportion of Time with Social Scent

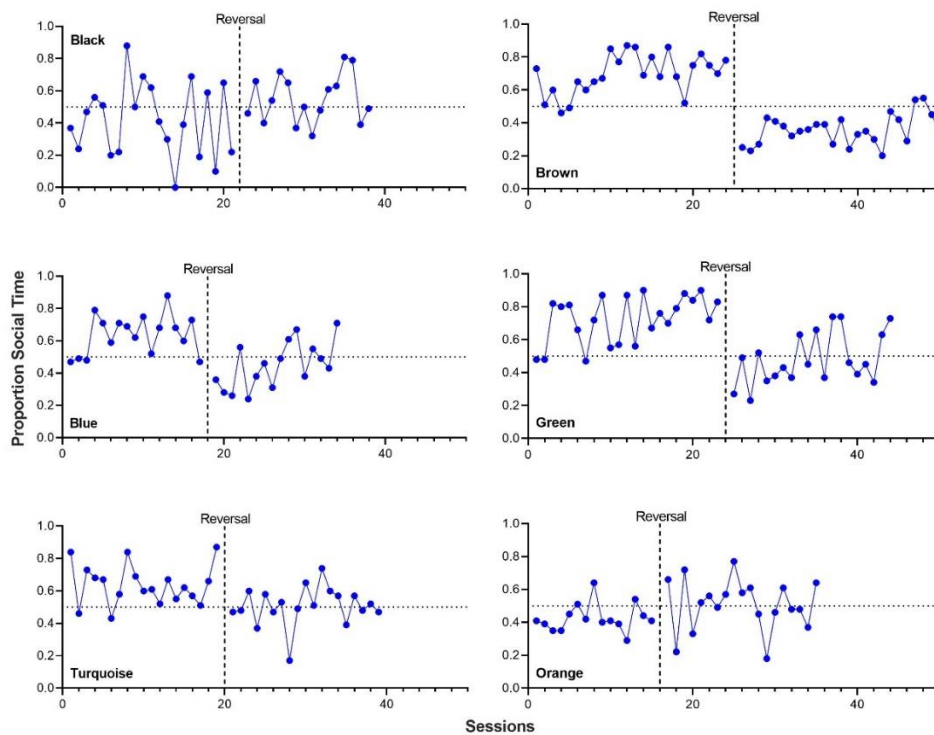
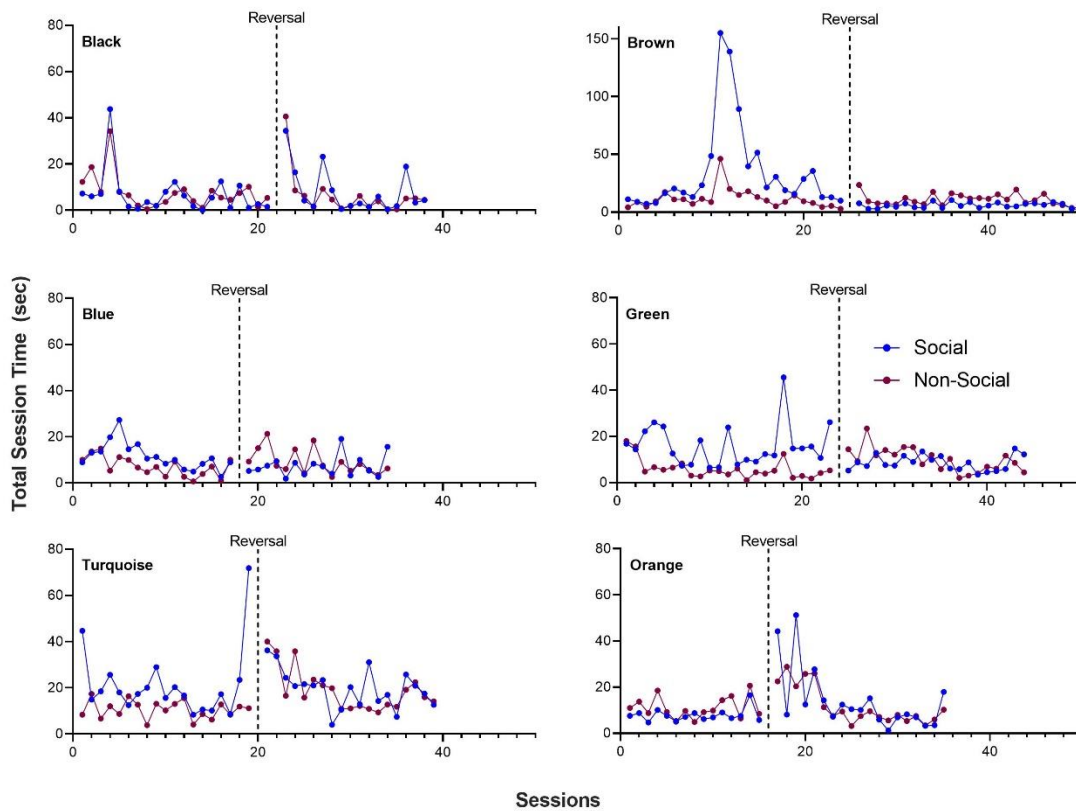


Figure 10 shows the total amount of time (sec) each rat spent in the social or nonsocial ports during each session of Experiment 2. In Condition 1, the amount of time spent in the social port ranged from 0 (Black) to 155.16 s (Brown), while the amount of time spent in the nonsocial port ranged from .5 (Black) to 46.23 s (Brown). Following the reversal, three rats (Black, Orange, and Turquoise) showed a spike in the amount of time spent in the social and nonsocial ports, while the remaining rats showed a small change,

or no change. Turquoise showed a drop-in social time but a spike in nonsocial time. The amount of time spent in the social port ranged from 0.49 (Black) to 51.26 (Orange), while the amount of time spent in the nonsocial port ranged from 0.3 (Black) to 40.57 (Black). On sessions 11 and 12 Orange spent a total of 155.16 and 139.02 s in the social port, which was the highest amount of time orange spent in any port during the experiment. Following these sessions this port was checked to make sure it was correctly recording data and that the correct scents were used. No faculty equipment or errors were identified.

Figure 10

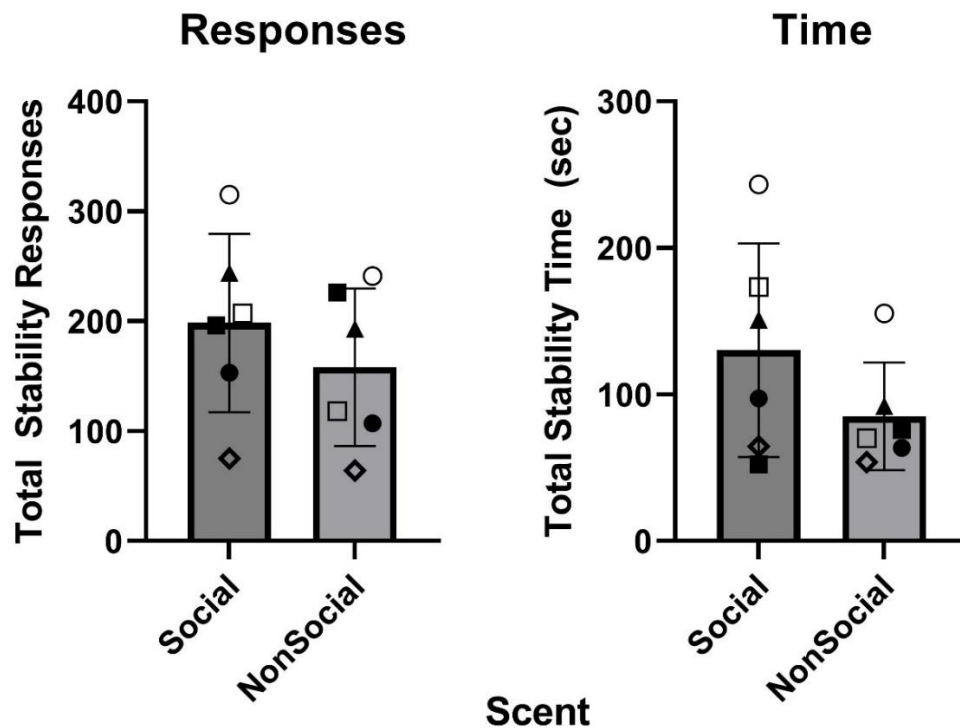
Total Time with Social and Nonsocial Scents



To evaluate if rats preferred social scents over nonsocial scents the total responses allocated to social and nonsocial scents were each summed across the 12 stability sessions (six stability sessions from Condition 1 and six stability sessions from Condition 2). The resulting two response counts and times (i.e., social and nonsocial) were checked for normality using Shapiro-Wilk's tests and analyzed using repeated measures *t* tests. There was no significant difference between the number of responses made to social and nonsocial ports ($M = -40.17$, $SD = 43.49$), $t(5) = 2.26$, $p = 0.07$ (Figure 11 – Panel A). The difference in the amount of time spent in the social and nonsocial ports also did not significantly differ ($M = -45.19$, $SD = 47.81$), $t(5) = 2.32$, $p = 0.07$ (Figure 11 – Panel B).

Figure 11

Repeated Measures T Test of Total Responses and Time



Experiment 2 Discussion

The primary goal of Experiment 2 was to evaluate the relative value of social and nonsocial scents in rats. Although four rats (Blue, Brown, Green, and Turquoise) demonstrated a slight preference for social stimuli over nonsocial stimuli in Condition 1, no preference was observed once the scent locations were reversed. The finding that social stimuli are equally preferred to nonsocial stimuli differs from the findings of Fritz et al. (2011) who found a preference for a chamber paired with another rat over a chamber paired with cocaine using the SCPPP. Our findings also differ from Sato et al. (2015) who found that rats will open a door to release a rat standing in water before opening a door to access food in a hybrid choice/social release procedure.

It is unclear why some research has observed that rats prefer social stimuli over powerful reinforcers like food and drugs but we found no preference for social over nonsocial scents. One possible explanation is that social interactions are commonly composed of multiple social stimuli (i.e., touch, smells, warmth) and that the combination of these stimuli is more valuable than any single stimulus. Future research should investigate if combinations of social stimuli, such as physical interaction, smell, and warmth, are more strongly preferred over a single social stimulus like scent.

At the beginning of Experiment 2, and following the reversal, temporary spikes in rats' responses were once again observed; however, these spikes tended to be smaller than those observed in Experiment 1. One possible explanation for this observation is that after running both conditions in Experiment 1, the social and nonsocial scents may have been even more familiar to rats in Experiment 2. If novelty contributes to the value of

stimuli (Bardo & Bevins, 2000), it follows that smaller spikes in responding would occur in Experiment 2.

As mentioned previously, a possible limitation of Experiment 2 is that a brief programming error occurred for two rats (Orange and Black). The two rats that the error impacted were the only two rats that did not demonstrate a preference for the social scents in Condition 1. It is unclear if the programming error led to this difference or if the difference was the result of other factors. It is worth repeating that none of the four rats who initially demonstrated a preference for social stimuli continued to demonstrate that preference following the reversal. To better understand the impact that the programming error may have had on the results from Experiment 2, and to further explore the relative value of social and nonsocial stimuli, Experiment 2 should be replicated. Future research should also explore using different stability criteria. Compared to Experiment 1, where rats ran for an average of 106.33 sessions, rats in Experiment 2 completed the two conditions in an average of 39 sessions. Using a more stringent stability criteria, or running a greater number of sessions before evaluating stability, might allow small preferences for certain olfactory stimuli to become more apparent.

General Discussion

When designing the two experiments in this paper, we hoped to address the following three questions. The first question was if rats prefer social and nonsocial scents over a control scent. The results from Experiment 1 provided evidence that rats do prefer social and nonsocial olfactory stimuli over control olfactory stimuli, and that this preference persists after a location reversal. The results from Experiment 1 also suggest

that there are individual differences in the strength of rats' preference for social and nonsocial olfactory stimuli. A goal for future research will be to investigate the factors that give rise to these individual differences.

The second question we hoped to address was the relative value of social and nonsocial scents for rats. The results from Experiment 2 do not suggest that rats strongly prefer social or nonsocial scents when the two are simultaneously presented. These findings were also supported by the results from Experiment 1 that showed rats emitted similar numbers of responses to social and nonsocial stimuli when they were presented with a control stimulus. There are several potential explanations for these results. Although similar responding to the two options can be interpreted as indifference, or a similar preference, for social and nonsocial olfactory stimuli, it is also possible that similar responding would be observed if the rats were unable to detect or distinguish between the scents. The results of Experiment 1, which showed that rats would reliably respond to social or nonsocial scents over the control scent and adjust their behavior when the locations were reversed, indicate that the latter explanation is unlikely. Taken together, the data from Experiments 1 and 2 suggest that rats can indeed detect social and nonsocial olfactory stimuli, but that they do not show a preference when both are available concurrently. To increase our confidence in this interpretation, an immediate future direction for our research will involve directly replicating Experiments 1 and 2.

Although a preference for social scents over nonsocial scents was not observed in Experiments 1 and 2, there are several factors that future research could investigate to potentially alter the value of social scents. First, the current study only used female rats to

generate scents and to assess preference. Female rats were used because research has indicated they more quickly and consistently respond for social stimuli than male rats (Bartal et al., 2011). It is unclear if male rats would demonstrate similar preferences for social scents as female rats, or how male or female rats would behave if scents from the opposite sex were presented. Future research should explore how the sex of the individual providing the social scent and the sex of the individual smelling the social scents impact preference.

A second factor future research should explore is the age of rats. In the present experiment two rats were approximately 455 days old at the start of the experiment and the remaining four rats were 90 days old. Future research could investigate if rats prefer social scents that come from younger or older rats, and if scent preference changes with age. A third factor that may impact the reinforcing value of scents is the strain of rat providing the social scents (Bartal et al., 2014). All rats used in Experiments 1 and 2 were Long-Evans rats and research should explore rats' preference for social scents when the scents originate from different strains.

A final factor which may impact the value of social scents is how rats are housed. During Experiments 1 and 2 rats were individually housed but all individually housed rats were kept in the same colony room. This means that rats in Experiments 1 and 2 could potentially see, hear, or smell the same rats that were producing the social stimuli used in the experiment. Being able to smell the other rats in the colony room may have decreased the value of social scents as reinforcers in Experiments 1 and 2. Future studies should evaluate the impact of social isolation prior to the experimental session on

preference for social stimuli as this has been shown to impact social preference (Douglas et al., 2004). Future research might also compare rats' preference for social scents from rats within the same colony to rats from a different colony, such as rats from a different university.

The final question we hoped to address in Experiments 1 and 2 was whether a novel free operant procedure, which gave rats choices for social and nonsocial scents, could help researchers study factors that influence social behavior. This procedure was developed for three reasons. First, it allows researchers to determine the strength of rats' preference for social stimuli by measuring the number of responses made each session. Second, the procedure allows researchers to more easily control alternative explanations (i.e., touch, warmth, avoidance) that may influence rats' preferences for social interactions. Third, the procedure allows researchers to better control the effects of novelty, by extending the number of sessions that social scents are presented and by extending the amount of time that social behavior is assessed. A second advantage of extending the length of time that data are collected is that it also allows researchers to evaluate changes in preference for social stimuli over time. Collectively, the results from Experiments 1 and 2 suggest that the free operant olfactory procedure can produce consistent and replicable data and that its additional controls can help researchers better isolate and study the factors underling and contributing to social behavior and the value of social stimuli.

A potential limitation of Experiments 1 and 2 is that some of the nonsocial scents used might be considered food items (e.g., blueberries, strawberries, and apples).

Although none of the rats used in this experiment were deprived of food, or had ever experienced these scents paired with food, the odors may share similarities to food items typically encountered by rats in the wild. To rule out this possibility that responding to nonsocial stimuli was related to food, future research should explore rats' preferences for scents that have and have not been explicitly associated with food. For example, researchers might explore how many nose pokes rats will emit to air that has been blown through rat chow and compare those response totals to scents never associated with food (e.g., pine or cedar).

The present experiments used rodent models to begin investigating what makes social interactions and stimuli reinforcing for some individuals, but not others. A rodent model was used to investigate this question because it allows for more systematic control of early learning experiences and allows for the manipulating of variables that would be difficult to study ethically with humans. The results of the present two experiments indicate that scents are reinforcers for rats' nose-poking behavior, but that social scents appear to be no more valuable than nonsocial scents. Future research should begin to explore the necessary learning experiences that impact the value of social stimuli as understanding these processes may lead to novel interventions for individuals struggling with social behavior and interactions.

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Appendix A

IACUC Approval

Appendix A shows the original IACUC approval as well as an updated IACUC approval form that cover the entire duration of the study.

IACUC

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



IACUCN006: FCR PROTOCOL APPROVAL NOTICE

Thursday, August 05, 2021

Senior Investigator **Jay Hinnenkamp & Tiffany Rodgers** (ROLE: Principal Investigator)
Co-Investigators NONE
Investigator Email(s) *jay.hinnenkamp@mtsu.edu; tiffany.rodgers@mtsu.edu*
Department Psychology

Protocol Title *An investigation of Olfactory Stimuli as Reinforcers for Rats' nose-poking behavior*
Protocol ID **22-3003**
Funding None

Dear Dr. Walker,

The MTSU Institutional Animal Care and Use Committee has reviewed the REVISED animal use proposal identified above under the **Full Committee Review (FCR) mechanism**. The IACUC initially met on 8/4/2021 to determine if your proposal meets the requirements for approval. The IACUC has determined through an unanimous vote that your animal use protocol meets the guidelines for approval in accordance with PHS policy. In view of the current COVID-19 restrictions, the IACUC also introduced a few policies to protect students and junior staff. A summary of the IACUC action(s) and other particulars of this this protocol are tabulated below:

IACUC Action	APPROVED for one year	
Date of Expiration	8/31/2022	
Number of Animals	FOUR (4)	
Approved Species	<i>Rattus norvegicus (rat)</i>	
Category	☐ Teaching ☒ Research	
Subclassifications	☐ Classroom ☒ Laboratory ☐ Field Research ☐ Field Study	
	☐ Laboratory ☒ Handling/Manipulation ☐ Observation	
	Comment: NONE	
Approved Site(s)	Field Research and MTSU Vivarium: Rooms (SCI 1170H for housing)	
Restrictions	1. Must comply with all FCR requirements; 2. Mandatory compliance with CDC guidelines during COVID-19; Social distancing guidelines are made by the Dean of CBAS. 3. The PI must make alternative plans to ensure proper animal care, including euthanasia if needed, in the event the research team is quarantined due to COVID19	
Comments	NONE	

IACUC**INSTITUTIONAL ANIMAL CARE and USE COMMITTEE**

Office of Research Compliance,
010A Sam Ingram Building,
2269 Middle Tennessee Blvd
Murfreesboro, TN 37129

**IACUCN006: FCR PROTOCOL APPROVAL NOTICE**

Date of original notice: Thursday, August 05, 2021

Date of most recent update: Tuesday, January 4, 2023

Senior Investigator **Jay Hinnenkamp & Tiffany Rogers** (ROLE: Principal Investigator)
 Co-Investigators NONE
 Investigator Email(s) jay.hinnenkamp@mtsu.edu; tiffany.rogers@mtsu.edu
 Department Psychology
 Protocol Title *An investigation of Olfactory Stimuli as Reinforcers for Rats' nose-poking behavior*
 Protocol ID **22-3003**
 Funding None

Dear Dr. Hinnenkamp and Dr. Rogers,

The MTSU Institutional Animal Care and Use Committee has reviewed the REVISED animal use proposal identified above under the **Full Committee Review (FCR) mechanism**. The IACUC initially met on 8/4/2021 to determine if your proposal meets the requirements for approval. The IACUC has determined through unanimous vote that your animal use protocol meets the guidelines for approval in accordance with PHS policy. In view of the current COVID-19 restrictions, the IACUC also introduced a few policies to protect students and junior staff. A summary of the IACUC action(s) and other particulars of this protocol are tabulated below:

IACUC Action	APPROVED for one year	
Date of Expiration	8/31/2023	
Number of Animals	SIX (6)	
Approved Species	<i>Rattus norvegicus (rat)</i>	
Category	☐ Teaching ☒ Research	
Subclassifications	☐ Classroom ☒ Laboratory ☐ Field Research ☐ Field Study ☒ Handling/Manipulation ☐ Observation	
	Comment: NONE	
Approved Site(s)	Field Research and MTSU Vivarium: Rooms (SCI 1170H for housing)	
Restrictions	1. Must comply with all FCR requirements; 2. Mandatory compliance with CDC guidelines during COVID-19; Social distancing guidelines are made by the Dean of CBAS. 3. The PI must make alternative plans to ensure proper animal care, including euthanasia if needed, in the event the research team is quarantined due to COVID19	
Comments	NONE	