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Characterization of Social Behavior in the Spiny Mouse (*Acomys cahirinus*)

By

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Master of Arts

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B.S. Behavioral Neuroscience, Lehigh University 2019

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An abstract of
A thesis submitted to the Faculty of the
James T. Lanery School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
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2021

Abstract

Characterization of Social Behavior in the Spiny Mouse (*Acomys cahirinus*)

By Brandon Fricker

While there are many species that are commonly used for the study of mammalian social behavior, there remains a need for lab-suitable organisms that are appropriate for examining sociality specifically in non-reproductive contexts (i.e., social behavior not in the context of mating or parenting). The spiny mouse, Acomys cahirinus, is a large-group living, cooperatively breeding rodent that holds great potential for studying a wide range of social behaviors in reproductive and non-reproductive contexts. Here we characterize basic social behaviors in male and female spiny mice to obtain a foundation for future study. We tested adult spiny mice in social approach, social preference, social interaction, social recognition, and group size preference paradigms. Regardless of sex, novelty, or familiarity, we found that both males and females rapidly approach conspecifics demonstrating high social boldness. Additionally, both sexes are significantly more prosocial than aggressive when freely interacting with conspecifics. However, we observed effects of sex on social preferences, such that males exhibit a preference to affiliate with same-sex conspecifics, whereas females exhibit a preference for affiliating with opposite-sex conspecifics. We discuss how this preference may relate to the cooperative breeding system of spiny mice. Lastly, both sexes show a robust preference for affiliating with large over small groups, indicating they may be an ideal species for the study of mammalian gregariousness. These data lay a basic foundation for future studies that seek to assess complex group dynamics and the mechanisms underlying reproductive and non-reproductive social behaviors in a highly social mammal.

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Characterization of Social Behavior in the Spiny Mouse (*Acomys cahirinus*)

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While there are many species that are commonly used for the study of mammalian social behavior, there remains a need for lab-suitable organisms that are appropriate for examining sociality specifically in non-reproductive contexts (i.e., social behavior not in the context of mating or parenting). The spiny mouse, *Acomys cahirinus*, is a large-group living, cooperatively breeding rodent that holds great potential for studying a wide range of social behaviors in reproductive *and* non-reproductive contexts. Here we characterize basic social behaviors in male and female spiny mice to obtain a foundation for future study. We tested adult spiny mice in social approach, social preference, social interaction, social recognition, and group size preference paradigms. Regardless of sex, novelty, or familiarity, we found that both males and females rapidly approach conspecifics demonstrating high social boldness. Additionally, both sexes are significantly more prosocial than aggressive when freely interacting with conspecifics. However, we observed effects of sex on social preferences, such that males exhibit a preference to affiliate with same-sex conspecifics, whereas females exhibit a preference for affiliating with opposite-sex conspecifics. We discuss how this preference may relate to the cooperative breeding system of spiny mice. Lastly, both sexes show a robust preference for affiliating with large over small groups, indicating they may be an ideal species for the study of mammalian gregariousness. These data lay a basic foundation for future studies that seek to assess complex group dynamics and the mechanisms underlying reproductive and non-reproductive social behaviors in a highly social mammal.

Introduction

Examining the evolution of social behavior using a comparative approach across species provides valuable insight into important selection pressures as well as mechanistic commonalities and differences underlying behavior across organisms. However, to utilize comparative strategies, it is necessary to have several species available that exhibit a particular behavioral phenotype of interest. Obtaining a variety of species can be a challenge when searching for species amenable to experimental manipulation in a laboratory setting, especially when the behavioral phenotype of interest is mammalian grouping behavior. Importantly, social behavior is a broad term that encompasses many distinct types of behavior that may not have evolved in a linked manner (Goodson, 2013). For example, a species that is excellent for studying the behavior between bonded mates may not be the ideal species for studying grouping behavior. In order to understand factors that influence or mechanisms underlying grouping behavior, it is crucial to use an organism for which their natural history reflects large, social group-living. Studies have identified several species of birds, fish, and insect that are highly social and suitable for studying grouping behavior (Bazazi et al., 2008; Brandl & Bellwood, 2015; Breed et al., 1990; Kasper et al., 2017; Keller, 1998; Remeš et al., 2015; van Dijk et al., 2014; Voelkl et al., 2015). However, many mammals that live in large groups, such as primates, herding ungulates, and mongooses (i.e., meerkats) simply are not suitable for maintaining large colonies in laboratory settings. Therefore, there is a surprising lack of mammalian species suitable for laboratory studies of grouping behavior. Arguably, mammals most tractable for laboratory experiments are in the Order Rodentia. A few highly social rodents have been examined, including African striped mice (*Rhabdomys pumilio*) and degus (*Octogon degus*), but the majority of social behavior studies for these species occur in field settings (Insel et al., 2020;

Quirici et al., 2011; Schoepf & Schradin, 2012; Schradin & Pillay, 2004). In the present study, we sought to expand the number of highly social rodents available for laboratory and field study by characterizing the basic social behaviors of spiny mice (*Acomys cahirinus*).

Spiny mice are native to Africa, the Middle East, and southern Asia where they preferentially live in rocky outcroppings (Deacon, 2009; Frynta et al., 2011; Nowak, 1999). They are a cooperative breeding, large group living (~30 individuals per group) rodent that holds great potential for lab-based social behavior studies (Haughton et al., 2016). Spiny mice were historically used for studying obesity due to their propensity to overeat and develop Type II diabetes (Gonet et al., 1966; Shafrir, 2000). However, more recently they have emerged as a powerful model for studying complex tissue regeneration (Gawriluk et al., 2016; Seifert et al., 2012), and as a model for human-like reproductive biology given that at least one species of spiny mouse exhibits a menstrual cycle in captivity (Bellofiore et al., 2017, 2018). Their large groups, as well as cooperative breeding strategy, position spiny mice as an excellent candidate for studying sociality and grouping behavior. Indeed, early studies during the 1980’s began exploring some of the social components of their cooperative breeding tactics and kin recognition abilities. Porter and colleagues identified that spiny mouse mothers would nurse unfamiliar neonates as frequently as their own pups, suggesting a high degree of sociality regardless of genetic relation (Porter et al., 1980). This work also proposed the hypothesis that a primary strategy for kin recognition in spiny mice may be phenotype matching based on odors identified during nursing (Porter, 1988; Porter et al., 1983). Recently, there has been a resurgence in spiny mouse behavioral research, and studies have explored both their cooperative breeding strategy (Tučková et al., 2016) and their behavior in standard non-social laboratory paradigms such as the elevated plus maze and open field test (Ratnayake et al., 2014). In order to build upon this growing literature, and to lay a basic foundation for studying highly social behaviors, here we examine spiny mouse behavior in a variety of ethologically-relevant social behavioral paradigms.

To characterize adult social behaviors and preferences, we ran male and female spiny mice through tests in both reproductive (i.e., potential for the motivation to mate) and non-reproductive contexts. Spiny mice underwent variations of social approach, social interaction, social recognition, social preference, and group-size preference tests. Both sexes rapidly approached all stimulus conspecifics and displayed high levels of prosocial behavior, as well as robust gregariousness (i.e., a preference to affiliate with large groups). However, we observed effects of sex on behavioral preferences when spiny mice were given a choice between investigating and affiliating with either a same-sex or opposite-sex conspecific. The findings presented here are suitable for behavioral comparison with other rodents typically used for social behavior studies, and highlight the gregarious nature of the spiny mouse, suggesting they may be uniquely suited to studies examining mammalian grouping behavior.

Materials and Methods

Animals

15 adult female spiny mice (PND 60-200) were used for all behavioral tests in this experiment. 16 adult male spiny mice (PND 60-200) were used for the social approach, social preference, and social recognition tests. Due to Covid-19 pandemic-related lab shutdowns, we were unable to complete behavioral testing in the males. Thus, an additional 15 adult males (PND 60-200) were

used for the group size preferences and social interaction tests, resulting in 31 total males used for this study. All spiny mice were obtained from our breeding colony, and breeders were obtained from the breeding colony of Dr. Ashley Seifert (University of Kentucky). All animals were group housed (2-5) in either standard rat polycarbonate cages (40.64cm X 20.32cm X 20.32cm) or larger two-level polycarbonate cages (32cm X 38cm x 40cm) lined with Sani-Chips bedding, and were provided with nesting material, rodent igloos, and shepherd shacks. Animals were able to obtain food and water ad libitum and were kept on a 14-hour light: 10-hour dark cycle. Ambient temperature was maintained at 24 ±2 °C. All procedures were approved by the Institutional Animal Care and Use Committee of Emory University (PROTO201900126).

Design

To obtain a profile of basic spiny mouse social behavior, we ran male and female spiny mice (PND 60-200) through variations of five common social behavior tests: social approach, social interaction, social preference, social recognition, and group size preference. All behavioral tests were video recorded using Sony Handycam HDR-CX405 1080p Camcorders (Sony, USA) for subsequent scoring using Behavioral Observation Research Interactive Software (BORIS; Friard & Gamba, 2016). Most tests lasted no more than 10 min because, similar to many rodents in lab studies, spiny mice exhibit a burst of behavior at the beginning of the test; in the absence of the ability to freely interact with conspecifics, behavior tapers off after about 10 min and animals choose to sleep or rest. The order of tests for all subjects was randomized. The results were analyzed to identify both species-wide patterns in social behavior as well as potential sex effects.

Behavior

Social Approach

To assess general social attraction/aversion, subjects underwent three social approach tests with three different types of stimuli: familiar same-sex, novel same-sex, and novel opposite-sex individuals. Subjects were placed in a large Plexiglas testing chamber (81.28 cm X 40.64 cm X 38.1 cm) and allowed to acclimate for 3 min. After acclimation, subjects were contained under a plastic beaker on one end of the chamber while a stimulus animal was placed under a wire mesh container on the opposite side of the chamber. The subjects were then released and the latency to approach the stimulus animal, time spent within one body length of the stimulus animal, and time spent investigating the stimulus animal (defined as nose inserted into or rearing up on the stimulus' tube) were recorded during a 5-min test (Table 1).

Social Interaction

In order to quantify behavior during freely behaving social interactions, subjects were run in two tests where they were allowed to freely interact with a novel opposite-sex in one test and a novel same-sex stimulus animal in the other test. In a testing chamber (40.64cm X 20.32cm X 20.32cm), subjects were allowed to acclimate for 3 min. After acclimation, the stimulus animal was released into the chamber, and the interactions were recorded for 8 min. Behaviors scored (Table 2) included the subject's overt prosocial behavior (positive investigation, allogrooming, huddling, and positive side-by-side contact), overt aggressive behavior (biting, chasing, pinning, lunging/attacking, rearing, and aggressive side-by-side contact), and non-overt social behaviors (all other behaviors not considered overtly prosocial or aggressive, including grooming, jumping, sitting alone, and nonsocial exploration).

Social preference

To examine social preferences, subjects were run in two social choice tests: novel versus familiar and same-sex versus opposite-sex. In a large testing chamber (81.28 cm X 40.64 cm X 38.1 cm) subjects acclimated for 3 min. After acclimation, subjects were contained in the middle of the chamber while the 2 stimuli (a novel, same-sex conspecific and a familiar, same-sex cagemate or a novel, same-sex conspecific and a novel, opposite-sex conspecific) were placed under wire mesh containers at opposite ends of the chamber. Upon release, the percentage of total time in a zone spent within one body length of the stimuli, time spent investigating the stimuli (defined as nose inserted into or rearing up on the stimulus' tube), and jumping were recorded during a 5 min test (Table 3).

Social Recognition

In order to examine the ability to discriminate between different conspecifics, subjects were run in a standard habituation-dishabituation paradigm. After a 3 min acclimation period in a large testing chamber (81.28 cm X 40.64 cm X 38.1 cm), subjects were contained under a plastic beaker to one end of the chamber while the first novel, same-sex stimulus animal was placed under a wire mesh container on the opposite side. The subject was then released and the latency to approach the stimulus, time spent within one body length of the stimulus, and time spent investigating the stimulus (defined as nose inserted into or rearing up on the stimulus' tube) were recorded for 5 min (Table 4). This 5-min trial was repeated four times at 15-min intervals with the same stimulus animal. On the fifth and final trial, a new novel, same-sex stimulus was presented with the same measures for 5 min. The first, fourth, and fifth trial were compared. Discrimination between the “old” and “new” stimulus animals is determined by the observation of a steady decline in social investigation from Trial 1 to Trial 4, and a reinvigoration of investigative behavior in Trial 5 when the new animal is presented (Choleris et al., 2003).

Group Size Preference Test

Subjects underwent a group size preference test where they were given the choice to affiliate with either a small (2) or large (8) group of novel, same-sex conspecifics. In a large testing chamber (60.96cm X 45.72cm X 38.1cm), stimulus groups were placed on opposite sides of the chamber under individual wire mesh containers and were allowed to acclimate for 3 min. After acclimation, subjects were placed under a plastic beaker in the middle of the chamber between the large and small groups and were then released. Upon release, the time spent within one body length of the stimulus groups and time spent investigating the stimulus groups (defined as nose inserted into or rearing up on one of the stimulus’ tube) were recorded during an 8-min test. This test is used to determine the degree of gregariousness (a preference to affiliate with large groups) that a species or individual exhibits (Kelly & Goodson, 2013, 2014).

Statistics

Behavioral measurements for each test were analyzed using SPSS 26 (IBM Analytics, USA). Use of parametric or non-parametric tests was based on distribution of the data. In behavioral tests in which no sex effects were identified in any of the variables quantified, the data were combined to include males and females in the analysis in order to increase statistical power. Outliers for individual behavioral tests that were three standard deviations outside the mean were removed from analyses. Effect sizes for normally distributed data were calculated and reported as Cohen's d, whereas effect sizes for nonparametric analyses were reported as r where r = $\frac{z}{\sqrt{n}}$.

Results

Here we aimed to characterize basic social behaviors of spiny mice to lay a foundation for future social behavior and social neuroscience studies. For this characterization, we ran male and female spiny mice through five common social paradigms: a social approach test, a social interaction test, a social preference test, a social recognition test, and a group-size preference test. Behavioral analyses were used to identify both species-wide and sex-specific trends in social behavior. For behavioral tests examining differences in subjects' behavior with stimulus animals that were same-sex or opposite-sex, we did not combine male and female data for analyses because the sex of the stimulus animal is confounded with the sex of the focal animal. However, for all other behavioral tests where no sex effects were identified, male and female data were combined for analyses.

Social Approach

In order for an animal to engage in a social interaction, they must first exhibit approach behavior. Aversion or attraction to conspecifics can be influenced by factors such as novelty, familiarity, and/or the sex of the conspecific. To determine whether spiny mice exhibit differences in the readiness to approach and investigate different types of individuals, subjects were exposed to novel opposite-sex, novel same-sex, and familiar same-sex conspecifics during social approach tests. Friedman's analysis suggested neither males ($X^2(2) = 2$, p=0.37) nor females ($X^2(2) = 1.08$, p=0.58) displayed a difference in approach latency based on stimulus type, and rather subjects rapidly approached all conspecifics (see Supplementary Materials **Fig. S1**). Similarly, there was no difference in time spent investigating novel opposite-sex, novel same-sex, or familiar same-sex conspecifics in male ($X^2(2) = 3.35$, $p=0.15$) or female ($X^2(2) = 1.73$, $p=0.42$) spiny mice (**Fig. 1**). We did identify a sex effect, however, such that female spiny mice spent significantly more time than males investigating a novel, opposite-sex conspecific (Mann Whitney U (n1 = 16, n2 = 15) = 66, z=-2.14, $p = 0.03$; r= 0.38; **Fig. 1**). Thus, whereas males and females readily approached and investigated all types of stimulus animals tested here, female spiny mice spend more time investigating opposite-sex individuals.

Social Interaction

Approach behavior may not necessarily be indicative of the valence of how an animal will interact with a conspecific. Thus, we also conducted social interaction tests where subjects freely interacted with novel, same-sex and novel, opposite-sex conspecifics in order to determine the composition of time spiny mice spend engaging in overtly prosocial, overtly aggressive, and non-overt behaviors. We scored the interactions for overt prosocial (positive investigation, allogrooming, huddling, and positive side-by-side contact), overt aggressive (biting, chasing, pinning, lunging/attacking, rearing, and aggressive side-by-side contact), and non-overt behaviors (self-grooming, solitary sitting, jumping, cage exploration) and compared the total time spent engaging in each of these types of behavior. Overall, both males and females spent significantly more time engaged in non-overt behaviors than prosocial (all $p <0.01$) and aggressive (all $p < 0.01$) behaviors when interacting with either a novel, same-sex or novel, opposite-sex animal (Table 6; **Fig.2a**). However, between the overt behaviors, spiny mice were more prosocial than aggressive (all $p < 0.01$) (Table 6; **Fig.2a**). Notably, very little aggressive behavior was observed for all subjects in either stimulus condition. These results suggest that spiny mice are, generally, highly prosocial.

Next, within each sex we examined whether spiny mice exhibit differences in behavior when interacting with a novel, same-sex conspecific and a novel, opposite-sex individual. A general linear model analysis indicated a significant difference in the time male and female spiny mice spent engaged in overt prosocial and non-overt behaviors with same versus opposite-sex conspecifics (all $p < 0.01$; Table 7), but not for overt aggression ($F = 0.89$, $p = 0.36$; Table 7). *Wilcoxon* signed ranks for males revealed no difference in prosocial, aggressive, or non-overt behavior between interactions with a same- and opposite-sex conspecific (all $p > 0.36$; Table 7). Similarly, we observed no difference in aggressive behavior with same- versus opposite-sex stimuli for females (all $p = 0.06$; Table 7). However, we did find that female spiny mice spent more time exhibiting prosocial behavior towards opposite-sex stimulus animals than they did toward same-sex stimuli ($Z = -2.67$, $p = 0.01$; $r = 0.69$; **Fig. 2b**), as well as more time engaged in non-overt behaviors when with a same-sex individual than an opposite-sex stimulus ($Z=2.67$, $p=0.01$, $r=-0.69$). Together, these findings suggest that although both males and females are highly prosocial, female spiny mice exhibit different degrees of prosociality and non-overt behavior, which may be primarily dependent on the sex of the conspecific.

Social Preference

To determine if spiny mice exhibit a preference to affiliate with specific types of conspecifics, we performed two social preference choice tests: novel versus familiar and same-sex versus opposite-sex. For the novel versus familiar choice test, combined analysis for males and females yielded no significant difference in the time spent investigating each stimulus (Z=-0.26, *p*= 0.80) or the percentage of total time spent in a stimulus zone (Z=-0.32, *p*= 0.75), suggesting that spiny mice do not exhibit a preference for investigating or affiliating with novel or familiar conspecifics (**Fig. 3a**).

For the novel, same-sex versus novel, opposite-sex choice test, a paired t-test revealed that males spent significantly more time investigating a same-sex conspecific than an opposite-sex individual (t(14) = 2.3, *p*= 0.04; d= 0.92; **Fig. 3b**). Similarly, a Wilcoxon signed ranks test showed that males also spent a larger percentage of total contact time in the stimulus zone of the same-sex conspecific (Z= -2.10, *p*= 0.04; r=0.38; **Fig. 3b**). Female spiny mice, on the other hand, displayed the opposite preference. Females spent more time investigating (Z= -2.22, *p*= 0.03; r= 0.40; **Fig. 3c**) the opposite-sex stimulus animal. However, females did not exhibit a significant difference in the percentage of total contact time spent in the zones of either stimuli (Z= -1.59, *p* > 0.05; **Fig. 3c**). Despite the lack of preference for female time spent affiliating with same- or opposite-sex individuals, the female investigation preference observed in this choice test mirrors the results presented above, such that females spend more time investigating novel opposite-sex individuals in a social approach test, and females spend more time engaging in prosocial behavior with opposite-sex conspecifics than with same-sex ones in a social interaction test. Together, these results suggest that, while spiny mice do not display a preference for social novelty or familiarity, male spiny mice prefer investigating and affiliating with same-sex individuals, whereas female spiny mice exhibit a preference for investigating opposite-sex conspecifics.

Social Recognition

Most animals are presumably capable of discriminating between different individuals. However, spiny mice do indiscriminately provide parental care (including female nursing) to pups regardless of genetic relation (Tučková et al., 2016). Spiny mice may not need to identify specific individuals due to their large groups and generalized parental behavior. In order to validate the efficacy of a standard social recognition paradigm and confirm social discrimination abilities in spiny mice, subjects were run in a habituation-dishabituation test. Briefly, this test consisted of five trials of same-sex social exposure, with the same stimulus animal used in Trials 1-4 and a new stimulus used in Trial 5. We compared the difference in time spent investigating the stimulus animal across Trials 1, 2, and 5. We observed no statistically-significant differences between males and females, and so proceeded with combining male and female data. Friedman's analysis revealed a difference in investigation time across the 3 trials ($X^2(2) = 22.50$, $p < 0.001$). Posthoc analyses indicated that spiny mice spent more time investigating the stimulus animal in Trial 1 than in Trial 4 ($Z=-2.17$, $p= 0.03$; $r= 0.28$), suggesting a decrease in interest due to repeated exposure to the same stimulus animal. However, subjects spent more time investigating the stimulus animal in Trial 5 compared to Trial 4 ($Z=-4.21$, $p < 0.001$; $r=0.54$; **Fig. 4**), suggesting a renewed interest in social investigation due to the exposure of a new stimulus animal. These findings reflect the expected results of a habituation-dishabituation test, demonstrating that an animal exhibits social discrimination. Interestingly, though, subjects also spent more time investigating the stimulus animal in Trial 5 compared to Trial 1 ($Z=-3.38$, $p= 0.001$; $r= 0.58$; **Fig. 4**). Typically, results for this test in other species do not report a difference in investigation time between the first trial exposure of the initial stimulus animal and the first (and only) trial exposure of the new stimulus animal. The increase in investigation of the second stimulus animal in the spiny mice may be a byproduct of their highly prosocial nature in both reproductive and non-reproductive contexts, however, this is discussed further below (see Discussion). Regardless, spiny mice do indeed recognize different conspecifics and largely exhibit the expected behavior of an animal tested in a habituation-dishabituation paradigm.

Group-size Preference

Lastly, because spiny mice live in large groups in the wild, they may exhibit higher degrees of gregariousness (a preference to affiliate with large groups). To both confirm this possibility as well as identify whether rodent group size preferences can be accurately captured in the lab (see (Kelly et al., 2011) for group size choice tests in birds), spiny mice were tested in a group size choice test to determine whether they prefer affiliating with smaller (2) or larger (8) groups. We found no statistically-significant differences in males and females, and thus data are combined. Wilcoxon signed ranks tests revealed that spiny mice spent more time investigating the large group than the small group ($Z=-4.78$, $p< 0.001$; $r= 0.62$; **Fig. 5a**) and also spent a significantly larger percentage of total contact time in the stimulus zone of the large group ($Z=-4.78$, $p< 0.001$; $r= 0.62$; **Fig. 5b**). These results reflect the gregariousness observed in field studies and laboratory colonies of spiny mice and demonstrate that we can accurately measure group size preferences in spiny mice in a laboratory setting. Together, these findings demonstrate that male and female spiny mice are not only highly prosocial, but are also highly gregarious.

Discussion

In the present study, we characterized basic social behaviors of male and female spiny mice using five common social paradigms. Generally, we found that male and female spiny mice were highly prosocial in all paradigms. Here we discuss how spiny mouse behavior (specifically *A. cahirinus*) compares to other rodents commonly used in studies of social behavior and consider potential functions of behavioral sex effects.

Spiny Mice Social Behavior Characterization

Social Approach

All social interactions begin with an initial approach. Examination of social approach can be indicative of social characteristics of a species, including levels of boldness, sociability, and neophobia. Social approach tests are frequently used to determine levels of sociability in mouse models of autism (Yang et al., 2011). In the social approach tests in the present study, spiny mice of both sexes rapidly approached and investigated all stimulus animals at similar rates. Female spiny mice, however, spent significantly more time investigating novel, opposite-sex conspecifics, whereas males showed no difference in investigation based on stimulus type. This sex effect underscores the importance of distinguishing affiliation time and investigation time as only investigation time differed between males and females. Together, these variables may be more indicative of a preference for one stimulus type over another, however, investigation may largely reflect information-seeking to determine whether the conspecific is a threat, potential mate, or familiar kin. Our findings here are consistent with previous studies in spiny mice that showed that females are more aggressive and dominant toward opposite-sex intruders than males (Porter, 1976), and that if given a choice between sexually experienced or inexperienced males, females display more aggression toward the sexually inexperienced male (Andres & Deni, 1982).

These previous findings and the effects of sex on social investigation in the present study suggest that female spiny mice may be significantly more choosey than males.

In addition, the rapid approach times for all stimulus animals demonstrates that spiny mice have a high degree of social boldness and/or interest in conspecifics. Comparing these approach times to other rodents highlights this high level of sociability. While it can take some species, such as standardly-reared prairie voles (*Microtus ochrogaster*) an average of 82 sec (A. M. Kelly et al., 2020) and Mongolian gerbils (*Meriones unguiculates*) an average of 29 sec (Kelly et al. unpub obs.) to approach a novel, same-sex conspecific, spiny mice approached novel, same-sex conspecifics, on average, in 7 sec (note that the gerbils and spiny mice had the same distance to travel in these studies, whereas the voles had a shorter distance to travel to reach the stimulus animal). The swift social approach and high sociability of spiny mice may be particularly appealing for studies interested in using approach latency as an important metric of social boldness, social attraction, or social avoidance.

Social Interaction

While social approach tests may capture initial social attraction or avoidance, a social interaction test in which animals are allowed to freely interact can provide a fine-grained evaluation of social responses to conspecifics. Such tests can also be used to identify the degree to which a species is prosocial and/or aggressive. In the social interaction tests used in the present study, we grouped behaviors into three major categories: prosocial, aggressive, and non-overt (i.e., presumably nonsocial behaviors). When comparing these three types of behavior, we found that male and female spiny mice spent significantly more time engaged in non-overt behaviors regardless of the type of stimulus animal (i.e., novel, opposite-sex or novel, same-sex) with which they interacted. Notably, this abundance of non-overt behavior may, at times, communicate important social information that we were unable to detect and/or interpret. For example, self-grooming, which was categorized under non-overt behavior here, may be indicative of stress or anxiety, parasite loads, social signals, or body temperature regulation in rodents (Ferkin & Leonard, 2005; Kalueff & Tuohimaa, 2005). Because non-overtly prosocial and aggressive behaviors are difficult to interpret, we cannot explain why spiny mice spend more time engaged in non-overt behaviors. This finding, however, has also been shown in prairie voles when they interact with a novel, same-sex conspecific (A. M. Kelly et al., 2018), suggesting that it may be common for rodents to choose to spend time alone even when in a social context. In order to understand potential social signals embedded within these seemingly non-overt behaviors, future studies could examine non-behavioral social signals including scent marking and ultrasonic vocalizations.

Considering the overt behaviors displayed in the social interaction tests, we can place spiny mice as a predominantly prosocial species given that males and females spent significantly more time engaged in prosocial than aggressive behavior. Generally, very low levels of aggression were observed in all subjects, and we did not find that stimulus type (novel, same-sex or novel, opposite-sex) influenced aggressive behavior for either males or females. Males exhibited similar behavior profiles when interacting with male and female conspecifics. However, we found that females displayed different levels of prosocial behavior depending on the sex of the conspecific, such that females spent significantly more time engaged in prosocial behaviors with opposite-sex stimulus animals. This difference in prosociality is consistent with the results from the social approach test and suggests that not only do females more thoroughly investigate an opposite-sex conspecific, but they also exhibit more prosocial behavior towards males than other females when freely interacting. Notably, previous studies demonstrated that female spiny mice are more dominant and aggressive than males, and that more aggression is observed in all-female cages than in all-male cages (Porter, 1976). Together, these findings suggest that female spiny mice may prefer affiliating with opposite-sex over same-sex conspecifics, while males are less socially discriminate. This may be a unique social feature about male spiny mice given that studies in other species show patterns similar to the female spiny mice in our study – typically an animal modifies its behavior based on the sex of the conspecific. For example, both male and female Mongolian gerbils, prairie voles, and Sprague-Dawley rats exhibit differential prosocial and aggressive behavior dependent on the sex of the conspecific they are interacting with (Argue & McCarthy, 2015; DeVries et al., 1997; Swanson, 1974). Why male spiny mice behave similarly with same- and opposite-sex conspecifics warrants further study but may suggest males of this species are more socially tolerant. Interestingly, this stands in contrast to Norway rats, which are also a large group living species, at least in the wild, such that male Norway rats are less socially tolerant and more territorial than females (Brain, 1976; Calhoun, 1979).

The high proportion of overtly prosocial behavior exhibited by spiny mice in both novel, same-sex and novel, opposite-sex interactions sets this species apart from others as being highly social in multiple social contexts. This sociability stands in contrast to mice (e.g. Falkner et al., 2016; Golden et al., 2017, 2019; van Heukelum et al., 2019), hamsters (e.g. Grieb et al., 2021; McDonald et al., 2012; Olvera-Ramos et al., 2020) , and Sprague-Dawley rats, which will engage in lethal aggression towards novel conspecifics (Blanchard et al., 1975). Meanwhile, prairie voles are highly prosocial in reproductive contexts with pairbond partners and family members, but can be very aggressive toward novel, same-sex conspecifics (Kelly & Ophir, 2015; Lee et al., 2019). Meadow voles, on the other hand, are more prosocial in non-reproductive contexts than reproductive contexts, which is demonstrated by characteristic high levels of intrasexual female sociality and high levels of intersexual aggression (Kelly & Ophir, 2015; Lee et al., 2019). Thus, spiny mice may be a unique, lab-tractable mammalian organism for the study of *both* reproductive and non-reproductive prosociality.

Social Preference

Although social interaction tests evaluate social responses to conspecifics, they do not indicate which type of conspecifics may be preferred over others. Social preference tests allow for a direct comparison between conspecifics to identify any such preferences. In the present study, we exposed spiny mice to either novel and familiar same-sex conspecifics or to same-sex and opposite-sex novel individuals and evaluated subject preference based on how they divided affiliation and investigation time with the two social choices. When choosing between interacting with novel or familiar conspecifics, both male and female spiny mice showed no preference for either individual. While lack of preference for novel or familiar conspecifics is not uncommon in rodents, it may be a function of this species' sociality. More colonial species, including spiny mice, degus (Insel et al., 2020) and adult mice (*Mus musculus*) (Beery et al., 2018), tend to show no preference for novel or familiar conspecifics. However, juvenile inbred mice strains do prefer novel conspecifics (Moy et al., 2004). Territorial species, on the other hand, typically prefer familiar individuals. In a similar social preference paradigm to the one in this study, Mongolian gerbils show a preference for familiar same-sex individuals (Kelly et al.

pers. obs.), as do prairie voles (Beery et al., 2018) and meadow voles *(Microtus pennsylvanicus)* (Anacker et al., 2016). Non-territorial, large group living may allow for more freedom in social interactions, while territorial, small group living species may benefit from more stringent interactions with familiar over novel conspecifics. Alternatively, the high levels of prosociality we described in the social interaction tests may mask any preference that spiny mice possess. From our findings, we can conclude that spiny mice exhibit no immediate preference, however, future studies may utilize significantly longer tests in order to determine if novel versus familiar social preferences emerge.

In the same-sex versus opposite-sex preference test, we found that spiny mouse preferences differed based on sex. Males preferred investigating and affiliating with same-sex conspecifics, whereas females preferred investigating opposite-sex individuals. This sex effect is in line with our social approach test results indicating a difference in affiliation and investigation time between the sexes, as well as previous studies showing that females are more aggressive towards opposite-sex individuals than males (Porter, 1976). Together, our findings suggest not only that female spiny mice may be more selective in mate choice than males, but they may also be the primary driver of these reproductive behaviors. Furthermore, this effect of sex on social preference stands in contrast to other rodents often studied for their sociality. For example, while both prairie and meadow vole females successfully form same-sex preferences, male voles of either species engage in high levels of aggression towards novel same-sex individuals (Lee et al., 2019). The significant preference to affiliate and investigate same-sex individuals in male spiny mice reinforces their potential as a unique, lab-tractable mammalian organism for studying non-reproductive prosociality. Indeed, several non-reproductive social behaviors, such as coordinated cooperation (Avital et al., 2016), juvenile play (Berg et al., 1999; Panksepp, 1981), and sentinel behavior (Dr. Ashley Seifert, pers. comm.), along with their neural underpinnings, could be elucidated through studies utilizing male spiny mice.

Social Recognition

Social recognition is critical for the structure and stability of groups and informs decision-making processes that promote context-appropriate behaviors in relation to established social hierarchies (Kaidanovich-Beilin et al., 2011; Lee et al., 2018). Even though we observed no preference for novel over familiar conspecifics in the spiny mice, trajectories of social interactions likely differ based on whether or not an individual recognizes a conspecific. A social recognition test is commonly used in rodent behavioral testing (Kercmar et al., 2011; Thor et al., 1982) and evaluates whether animals can distinguish between a repeatedly introduced conspecific and subsequently introduced novel conspecific. Typically, the subject habituates to the increasingly familiar first stimulus animal as it becomes familiar, and exhibits a decrease in investigation time; upon introduction of the novel stimulus animal, investigation time increases, confirming that the subject is capable of social discrimination (Hölter et al., 2015). Here we found that spiny mice were successful at discriminating between two separate novel, same-sex conspecifics. This is consistent with previous studies suggesting spiny mouse weanlings are able to differentiate between kin and non-kin based on olfactory cues (Porter et al., 1983; Porter et al., 1986). Interestingly, male and female spiny mice spent more time investigating the second novel conspecific presented in Trial 5 than they did the first novel conspecific in Trial 1. While it is not uncommon to find slight behavioral variations in a social habituation-dishabituation paradigm (Zheng et al., 2013), our findings may indicate increasing interest as more conspecifics are presented. Such increased interest could act as a mechanism that promotes affiliation with, and potentially the formation and/or maintenance of, large group sizes.

Social recognition capabilities may be especially important in large group living species where there are more individuals to differentiate between and greater complexity in social hierarchies (Pusey & Packer, 1997). To our knowledge, extremely little is known about spiny mouse social hierarchies, and thus the extent of social memory in spiny mice remains unknown. However, in sciurid rodents (i.e., squirrels, marmots, prairie dogs), variation between individual animal alarm vocalizations increases as group size increases (Pollard & Blumstein, 2011); this may act as a way to better discriminate between individuals for species that frequently encounter novel conspecifics and/or live in large groups. Because spiny mice emit ultrasonic vocalizations (pers. obs.), future studies could seek to elucidate whether spiny mice identify individuals through vocalization in addition to olfactory cues.

Group-Size Preference

While gregariousness (a preference to affiliate with large groups) is well characterized in avian species (Goodson et al., 2009;Kelly et al., 2011), it is surprisingly understudied in mammals – particularly the mechanisms of gregariousness. This is especially true in a laboratory setting. Because spiny mice live in especially large groups and are lab-tractable, they offer the unique opportunity for the study of mammalian gregariousness. Thus, we aimed to develop a group-size preference test that would allow us to capture gregarious behavior in spiny mice in a lab setting. This test was modeled after a group size preference test previously used in several species of Estrildid finches (Kelly & Goodson, 2013; Kelly et al., 2011).When presented with 2 or 8 novel, same-sex conspecifics, spiny mice preferred to affiliate with and investigate the larger group. This preference reflects the highly gregarious behavior observed in spiny mouse in the field (Haughton et al., 2016). To our knowledge, group size preference has not been directly tested in other highly social rodents, however, it is worth noting that other rodents do exhibit gregarious behaviors including rats (Latané, 1969), CD-1 mice (Lee et al., 2018), and African striped mice (Schradin et al., 2010). Performance in a group size preference test and the degree of gregariousness in the lab presumably reflects the behavioral ecology and species-typical group size of a species. For example, the Mongolian gerbil, a relative of the spiny mouse (Alhajeri et al., 2015; Fabre et al., 2012; Steppan & Schenk, 2017), may exhibit a preference for the smaller group over the larger group due to their small group sizes and high territory defense in the wild (Deng et al., 2017). Additional studies could compare these closely related species to identify any potential differences in gregariousness and their underlying mechanisms to better understand the evolution of grouping behaviors.

It remains unclear what aspect of spiny mouse behavioral ecology may drive gregariousness. Breeding strategy and mating systems likely relate to gregariousness, but whether one precedes the other is unknown and difficult to disentangle. Importantly, breeding strategy does not always predict gregariousness given that Estrildid finches are socially monogamous, yet flock in large groups and are highly gregarious (Goodson et al., 2012). However, for spiny mice, the group care for offspring involved in their communal breeding strategy may indeed encourage gregarious behavior. Increased alloparental care for offspring can decrease the amount of time individuals spend parenting, thus allowing more time for resource acquisition for mates, food, or territories, which is particularly beneficial in unpredictable environments (Guindre-Parker & Rubenstein, 2018). Indeed, some mammalian species form groups specifically when their young are most vulnerable in order to take advantage of communal-rearing benefits (White et al., 2010). However, our group size preference test used novel, same-sex stimulus animals, so any motivation to interact with the larger group is unlikely to stem exclusively from their breeding strategy and motivations to mate. This test design does not negate the possibility that gregariousness could still be a byproduct of communal breeding. Given the high levels of prosociality we observed in the social interaction tests, it is also feasible that spiny mice generally find social interactions rewarding, which may facilitate a preference to affiliate with larger group. Future studies could examine reward circuitry of spiny mice interacting with small versus large groups in order to address proximate questions about spiny mouse gregariousness. Together, these findings demonstrate that spiny mice are an ideal species for examining gregariousness, grouping behavior, and inter-group dynamics due to their high levels of sociality and communal breeding (Porter et al., 1980; Tučková et al., 2016).

Considerations for Social Behavior Tests in A. cahirinus

When testing spiny mice, we discovered that modifications of existing rodent behavioral tests were needed to properly test social behavior in the spiny mice as compared to other species, including prairie voles or Mongolian gerbils (Kelly et al. *pers obs*). For most species, social approach/investigation/recognition tests using stimulus chambers with 1cm diameter holes in one wall is sufficient to maintain the subject's interest in the stimulus animals (Castro et al., 2020; Kelly et al., 2020). In spiny mice, however, we required access holes surrounding the entire stimulus container that were at least 2cm in diameter in order for the spiny mice to engage in social investigation for more than a few seconds. It is clear that, while spiny mice may learn olfactory cues quickly (Janus, 1993) and form preferences based off them (Janus, 1988; Porter et al., 1983, 1986 1986), olfactory cues alone are not sufficient motivation for social interactions. This preference for physical contact during an interaction may explain some differences across studies where spiny mouse behavior is examined. For example, Ratnayake et al. (2014) designed their social interaction paradigm with two chambers that allowed the subject to remain separated from the stimulus animal and also restrained the stimulus animal in a cylinder. Our study, on the other hand, allowed for free interactions in a single chamber. This methodological difference may account for the larger interest in investigation that we report here compared to the findings presented by Ratnayake et al. (2014).

We also noted that extensive acclimation times appeared to decrease interest in the behavioral tests. While it is common to allow other rodent species acclimation periods as long as 30 minutes for behavioral tests in novel apparatuses (Kelly et al., 2020), 3 minute acclimations were more than sufficient for the spiny mice in all of the current paradigms. In our experience, prairie voles are an easily stressed rodent where longer periods to acclimate to new surroundings are necessary, whereas the spiny mice are quite bold and readily explore new environments. Additionally, we found that spiny mice frequently succeed at jumping out of 2 foot. tall testing chambers (by, rather impressively, doing back flips), so chambers with high walls and lids are recommended for testing.

A Consideration of Spiny Mouse Social Phenotype from the Perspective of Cooperative Breeding

The sex effects identified in our study – in the social approach, social interaction, and social preference tests – may be related to the cooperative breeding strategy of spiny mice. While it is currently unknown how spiny mice parse breeding dominance among female spiny mice, mammalian cooperative breeders often have a single dominant female engaging in the majority of breeding relations (Lukas & Clutton-Brock, 2012). The greater interest in opposite-sex individuals exhibited by female spiny mice may serve as a mechanism to maintain breeding dominance through consistently searching for mating opportunities. However, within our colony, females in the same breeding cage appear to all reproduce (pers obs.). Rather, there may be no dominant female and all females compete for access to the available males. Consistent competition seems unlikely, though, as high reproductive competition tends to favor solitary living, not cooperative communities, in other rodents (Schradin et al., 2010). Therefore, it may be more likely that there are dominant females that drive a potentially matrilineal system in spiny mice. If female spiny mice drive a matrilineal reproductive system, then several factors would be expected within their groups. Three of these factors include large groups, small litter sizes, and male-biased dispersal rates (Holekamp & Sawdy, 2019). While spiny mice do live in large groups and produce small litters in the field (Haughton et al., 2016), it is yet unknown whether they have a male-biased dispersal rate. Given that subordinate females sometimes receive higher inclusive fitness benefits than males in cooperatively breeding species (Richardson et al., 2002), it seems plausible that spiny mice could display this biased dispersal. Future field and lab studies in spiny mice are required to further characterize their cooperative breeding system and hierarchy.

Conclusion

In this study, we characterized the basic social behavior of spiny mice through social approach, social interaction, social preference, social recognition, and group size preference tests. We identified a high propensity for prosocial behaviors in all of our behavioral paradigms. We also noted several sex effects that indicate that male spiny mice prefer engaging in non-reproductive prosocial behaviors with same-sex conspecifics, whereas females prefer interacting with opposite-sex conspecifics. These preferences highlight the uniqueness of spiny mice as being suitable for studies of *both* reproductive and non-reproductive prosociality. Further, the preference for males and females to affiliate with large groups demonstrates that spiny mice are an ideal, lab-tractable organism for studying mammalian gregariousness. Together, this study lays a basic foundation for future exploration of mechanisms underlying social behaviors in a group-living mammal and for the study of complex group dynamics.

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Figure Captions:

Fig. 1. Spiny mice exhibit an effect of sex on investigation of conspecifics during social approaches. Mean (± SEM) time in seconds both male (magenta) and female (orange) spiny mice spent investigating novel opposite-sex, novel same-sex, and familiar same-sex conspecifics. Male and female spiny mice investigated all conspecific equally, but female spiny mice investigated novel opposite-sex conspecifics longer than males. Dots represent individual data. Different letters indicate $P \leq 0.05$.

Fig. 2. Spiny mice engage in high levels of non-overt and overt prosocial behavior in social interactions. Breakdown of time male (**A**) and female (**B**) spiny mice spent engaging in non-overt (light purple; yellow), overt prosocial (dark purple; orange), and overt aggressive (gray) behavior during a 5-minute social interaction test with same- and opposite-sex, novel conspecifics. Both male and female spiny mice spent significantly more time exhibiting non-overt behavior than overt prosocial and overt aggressive behaviors and more time engaged in overt prosocial behavior than aggressive behavior. Female spiny mice spent more time interacting prosocially with novel opposite-sex conspecifics than same-sex conspecifics.

Fig. 3. Spiny mice exhibit social preferences based on sex. (**A**) Combined male and female spiny mice mean (± SEM) time in seconds for investigation (left) and percentage of total time spent in a stimulus zone (right) during the novel (dark green) vs familiar (light green) conspecific social preference test. Spiny mice did not display a significant preference for novel or familiar conspecifics. (**B**) Male spiny mean (± SEM) time in seconds for investigation (left) and percentage of total time spent in a stimulus zone (right) during the same- (dark purple) versus opposite-sex (light purple) conspecific social preference test. Males spent significantly more in

the zone of and investigating same-sex than opposite-sex conspecifics. (**C**) Female spiny mean (± SEM) time in seconds for investigation (left) and percentage of total time spent in a stimulus zone (right) during the same- (orange) versus opposite-sex (yellow) conspecific social preference test. Females spent more time investigating, but not more time in the zone of, opposite-sex than same-sex conspecifics. Dots represent individual data. Different letters indicate $P \leq 0.05$.

Fig. 4. Spiny mice display social discrimination in social recognition test. Combined male and female spiny mice mean (± SEM) time in seconds for investigation of novel, same-sex conspecifics during the social recognition test. Spiny mice show significantly decreased interest after repeated exposure to the same novel conspecific (Trial 1 vs Trial 4), but renewed interest upon exposure to a new, novel conspecific (Trial 4 vs Trial 5). Dots represent individual data. Different letters indicate $P \leq 0.05$.

Fig. 5. Spiny mice prefer larger groups over smaller groups. Combined male and female spiny mice mean (± SEM) time in seconds for investigation (**A**) and percentage of total time spent in a stimulus zone (**B**) during a group size preference test. Spiny mice spent significantly more in the zone of and investigating large groups (8; light blue) over small groups (2; dark blue). Dots represent individual data. Different letters indicate $P \leq 0.05$.

Table 1. Social approach ethogram.

Behavior	Description
Approach Latency	Latency for subject to approach the stimulus animal. Active from moment the subject is released from the beaker to the point when the subject reaches the stimulus animal.
Social Investigation	Time subject spends poking nose through or rearing up on the stimulus animal's container. Time when the subject is on top of stimulus container is not included.
Jumping	Time subject spends jumping.

Table 2. Social interaction ethogram.

	Behavior	Description
Prosocial	Positive Investigation	Subject sniffing or positively investigating the stimulus animal.
	Allogrooming	Subject grooms the stimulus animal.
	Huddling	Subject and stimulus are either touching flanks or criss-crossed on top of each other.
	Positive Side-by-Side Contact	Subject showing positive, prosocial contact side-by-side with the stimulus that is not specifically huddling.
Aggressive	Biting	Subject biting at the stimulus animal, mouth making contact with the stimulus animal's body.
	Chasing	Subject aggressively chasing the stimulus animal. Initiator is chaser for entire event.
	Pinning	Pinning the stimulus down
	Rearing	Subject rearing up on hind paws for either offense or defense.
	Aggressive Side-by-Side Contact	Subject and stimulus are touching flanks but in an aggressive manner. May be between aggression bouts.
Non-overt	All behavior instances not included in Prosocial or Aggressive.	Subject is not making contact with or exhibiting behavior direct toward stimulus animal.

Table 3. Social preference ethogram.

Behavior	Description
Social Investigation	Time subject spends poking nose through or rearing up on the stimulus animal's container. Time when the subject is on top of stimulus container is not included.
In Zone	Time subject spends within one body length (approximately 1/3 of the chamber) from the stimulus container.
Jumping	Time subject spends jumping.

Table 4. Social recognition ethogram.

Behavior	Description
Social Approach (Trials 1-5)	Latency for subject to approach the stimulus animal. Includes moment the subject is released from the beaker to the point when the subject reaches the stimulus animal. Trial 5 is new stimulus animal.
Social Investigation (Trials 1-5)	Time subject spends poking nose through or rearing up on the stimulus animal's container. Time when the subject is on top of stimulus container is not included. Trial 5 is new stimulus animal.
Jumping (Trials 1-5)	Time subject spends jumping.

Table 5. Group size preference ethogram.

Behavior	Description
Social Investigation	Time subject spends poking nose through or rearing up on the stimulus animal/group's container. Time when the subject is on top of stimulus container is not included.
In Zone	Time subject spends on the stimulus animal/group’s side (approximately 1/3 of the chamber).
Jumping	Time subject spends jumping.

Table 6. Breakdown of behavior during the social interaction tests.

Sex	Conspecific	Comparison	Test	χ2/Z	P	r
Male	Same-Sex	Overall	Friedman’s test	25.20	<0.01*	
		Overt Prosocial vs Overt Aggression	Wilcoxon	-3.41	<0.01*	0.62
		Overt Prosocial vs Non-Overt*	Wilcoxon	-2.61	<0.01*	0.67
		Overt Aggression vs Non-Overt*	Wilcoxon	-3.308	<0.01*	0.88

	Opposite-Sex	Overall	Friedman's test	25.20	<0.01*	
		Overt Prosocial* vs Overt Aggression	Wilcoxon	-3.41	<0.01*	0.62
		Overt Prosocial vs Non-Overt*	Wilcoxon	-3.00	<0.01*	0.76
		Overt Aggression vs Non-Overt*	Wilcoxon	-3.41	<0.01*	0.88
Female	Same-Sex	Overall	Friedman's test	28.13	<0.01*	
		Overt Prosocial* vs Overt Aggression	Wilcoxon	-3.25	<0.01*	0.59
		Overt Prosocial vs Non-Overt*	Wilcoxon	-3.41	<0.01*	0.88
		Overt Aggression vs Non-Overt*	Wilcoxon	-3.41	<0.01*	0.88
	Opposite-Sex	overall	Friedman's test	28.13	<0.01*	
		Overt Prosocial* vs Overt Aggression	Wilcoxon	-3.41	<0.01*	0.88
		Overt Prosocial vs Non-Overt*	Wilcoxon	-3.35	<0.01*	0.87
		Overt Aggression vs Non-Overt*	Wilcoxon	-3.41	<0.01*	0.88

Note: under comparison * means greater time.

Table 7. Differences in time engaged in behavior based on stimulus sex during the social interaction tests.

Behavior	Comparison	Test	F/Z	P	r
Overt Prosocial	Overall	GLM	4.63	0.04*	
	Male Same vs Opposite-Sex	Wilcoxon	-1.02	0.31	
	Female Same vs Opposite-Sex*	Wilcoxon	-2.70	<0.01*	0.69
Overt Aggression	Overall	GLM	0.89	0.36	
Non-Overt	Overall	GLM	4.48	0.04*	
	Male Same vs Opposite-Sex	Wilcoxon	-0.97	0.33	
	Female Same* vs Opposite-Sex	Wilcoxon	-2.67	<0.01*	0.69

Note: * in under comparison means greater time.

Figure 1

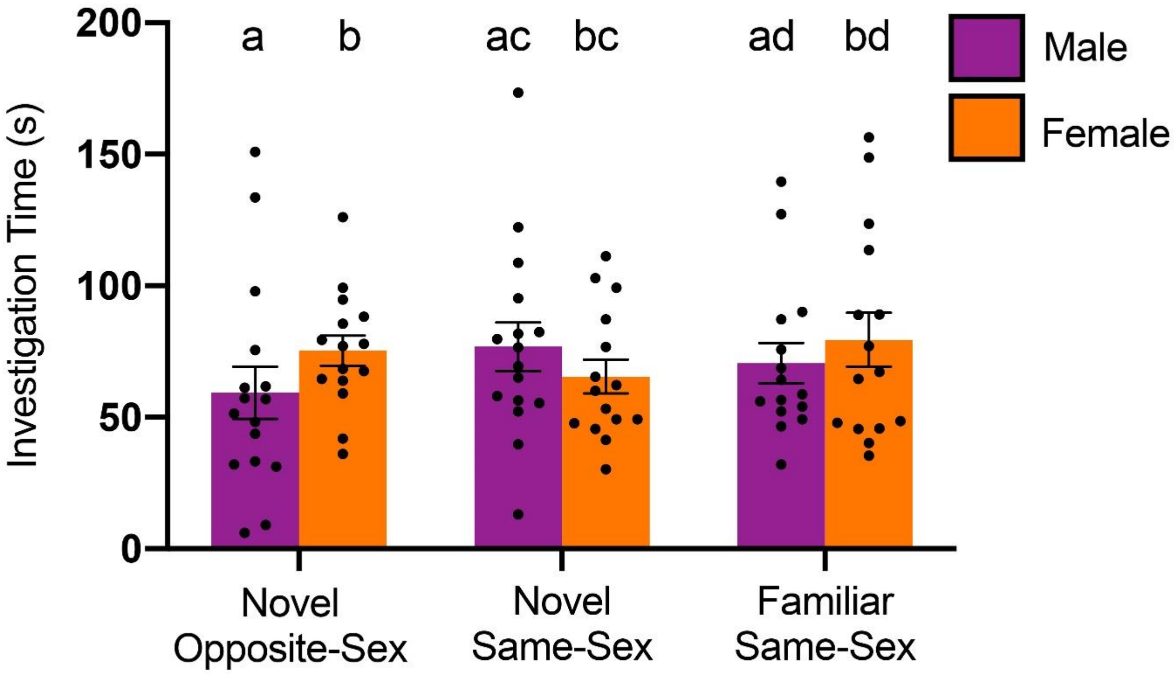


Figure 2

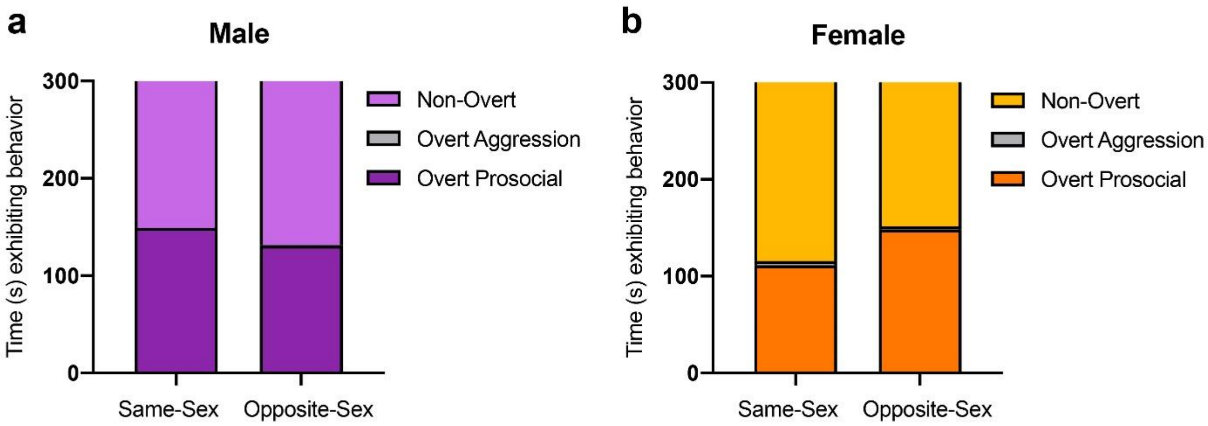


Figure 3

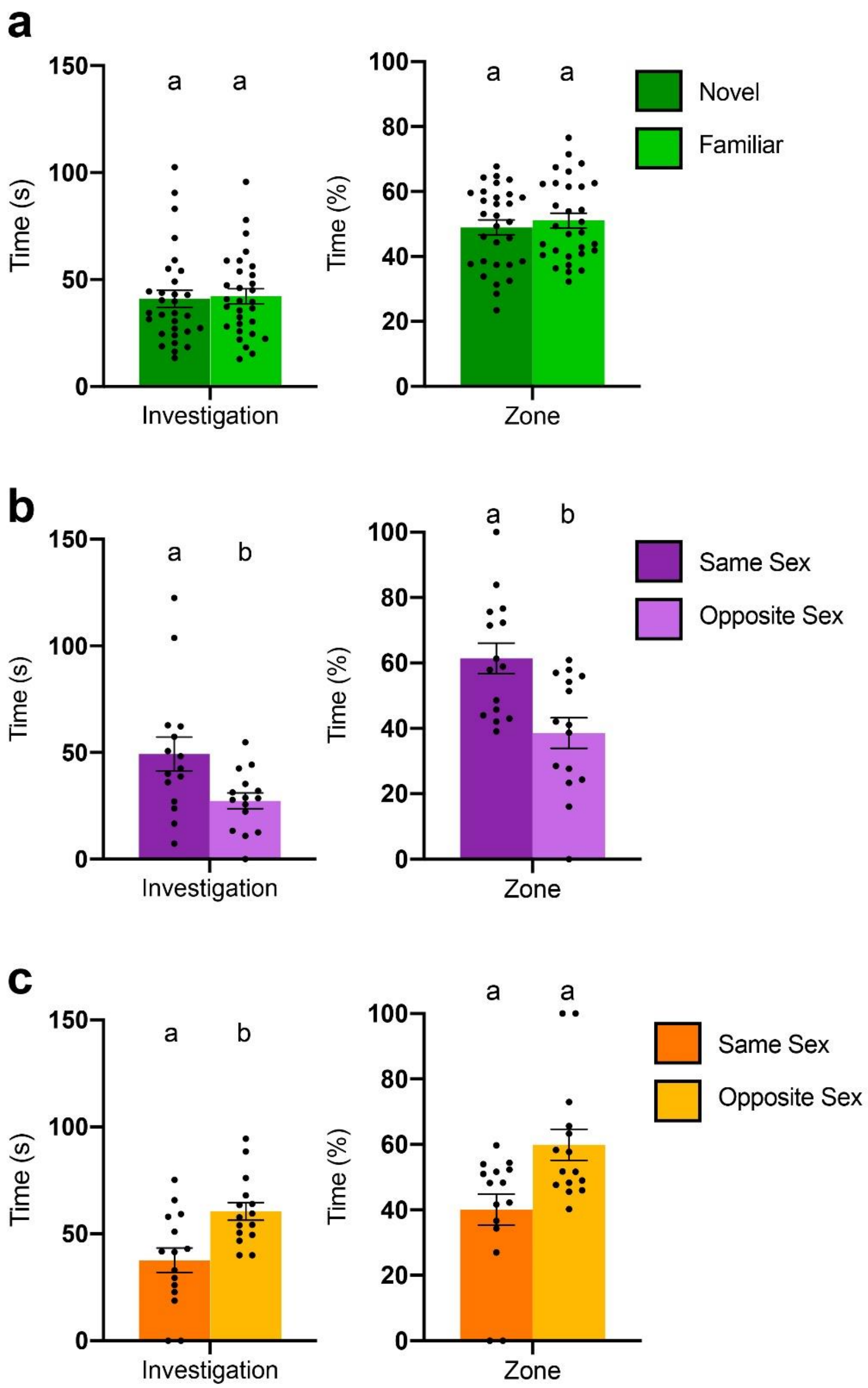
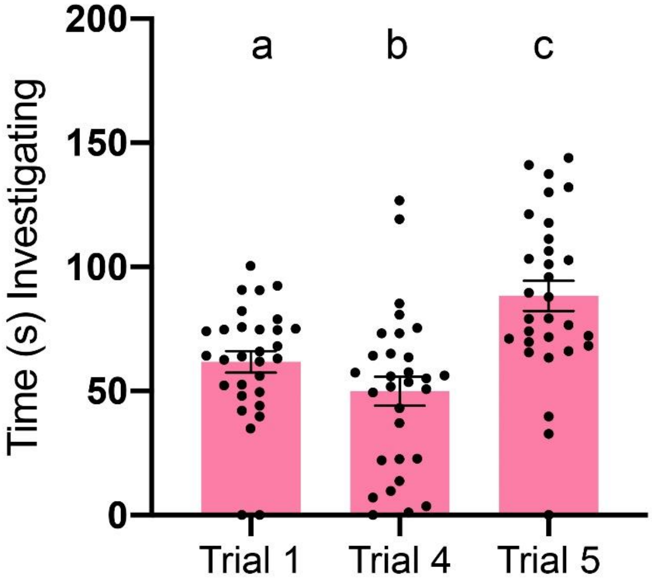

a
Time (s)
150
100
50
0
a
a
Investigation
Time (%)
100
80
60
40
20
0
a
a
Zone
Novel
Familiar
b
Time (s)
150
100
50
0
a
b
Investigation
Time (%)
100
80
60
40
20
0
a
b
Zone
Same Sex
Opposite Sex
c
Time (s)
150
100
50
0
a
b
Investigation
Time (%)
100
80
60
40
20
0
a
a
Zone
Same Sex
Opposite Sex

Figure 4



200
150
100
50
0
Time (s) Investigating
a
b
c
Trial 1
Trial 4
Trial 5

Figure 5

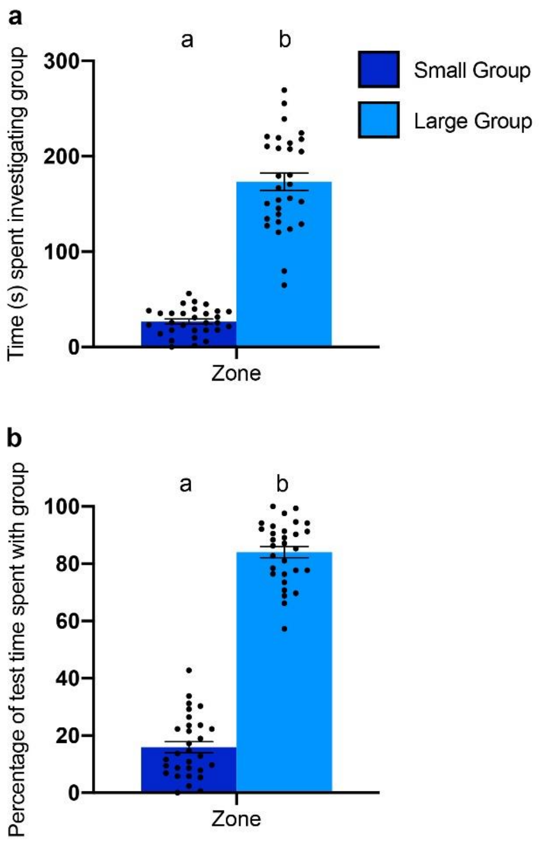


Figure S1