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Ashley Rosenberg April 13, 2021

# Influence of species-typical group size on social preferences and behavioral phenotype

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
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# Abstract

## Influence of species-typical group size on social preferences and behavioral phenotype

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**Background:** Both individual and species differences affect how an animal engages in a social interaction. To inform neurological studies, it is essential to characterize species-typical behaviors, especially for newer model organisms, before looking at how certain neural mechanisms drive these behaviors. A characteristic of behavior that may be particularly informative for social behavioral phenotype is species-typical group size. For group living to evolve, animals may have evolved mechanisms that promote positive social interactions with conspecifics in various contexts - whether related to mating or not. Additionally, there may be a relationship between a drive to affiliate with larger groups and an affinity for novelty. After all, in large [non eusocial] groups, an animal is more likely to encounter novel conspecifics. Therefore, social neophilia may be an important trait for group-living species.

**Purpose:** The purpose of this project is to begin to identify species-typical social behavioral phenotypes and discern if species-typical group sizes in the wild will influence social preferences, such as a preference for affiliating with novel or familiar conspecifics.

**Methods:** To address how species-specific social phenotype influences social interactions, I compared two related species: the moderately social, small family-group living Mongolian gerbil and the highly social, large-group living African spiny mouse. I examined female spiny mouse and gerbil performance in a variety of social behavior tests and analyzed relationships between behaviors.

**Results:** A social interaction test revealed that while female spiny mice are more prosocial than aggressive, female gerbils are not significantly more prosocial than aggressive. However, female gerbils were significantly more aggressive than spiny mice. Spiny mice also spent significantly more time investigating and affiliating with a large group compared to a small group during a group size preference test, and exhibited more social boldness. Gerbils appeared more neophobic, spending significantly more time not interacting with stimulus animals during social preference tests.

**Conclusion:** These findings highlight that spiny mice may be particularly well-suited for studies of prosociality and gregariousness. Furthermore, principal components analysis revealed that spiny mice may have a highly flexible phenotype whereas for gerbils, social boldness, and not a propensity toward social novelty, may predict gregariousness.

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**Introduction:**

Every animal possesses unique strategies for navigating through the social world. Both individual and species differences affect how an animal responds to a social interaction. Internal cues and prior experience are integrated in the brain and then translated into adaptive behavioral responses in the appropriate context. To inform neurological studies, it is essential to characterize species-specific behaviors and compare behaviors between species, especially for newer model organisms, before looking at how certain neural mechanisms drive these behaviors. Differences in life strategies of various species may reflect adaptation to differing environments and potentially, the development of particular central mechanisms involved with the regulation of social behavior. Understanding why and how an animal exhibits certain social behavior is interesting not only for the sake of science, but also for gaining translational insight into human social behavior. For example, identifying the molecular underpinnings of social deficit may yield important clues for the advancement of treatments for diseases associated with altered or deficient social behavior such as depression, social phobia, autism and schizophrenia (Donaldson and Young, 2008). This thesis seeks to explore how species-typical group size influences social preferences and behavioral tendencies when affiliating with conspecifics in a non-reproductive context.

There may be a relationship between a drive to affiliate with larger groups and an affinity for novelty. After all, in large [non eusocial] groups, an animal is more likely to encounter novel conspecifics. Therefore, highly gregarious (a preference to affiliate with large groups) species may have a propensity toward social novelty. On the other hand, animals that display a more aggressive, territorial phenotype may prefer affiliating with familiar individuals, but importantly may still thoroughly investigate novel individuals in order to identify potential threats. To address how species-specific social phenotype influences social interactions, I compared two related species: the moderately social, small family-group living Mongolian gerbil and the highly social, large-group living African spiny mouse. Notably, recent phenotyping data has demonstrated that spiny mice, despite being called a mouse, are a type of gerbil (Chevret et al., 1993). Does species-typical group size influence general social preferences? More specifically, will an animal that lives in large groups in the wild engage with the social world differently than an animal that is territorial and lives in small groups of related individuals?

In territorial species, same-sex conspecifics are typically avoided or attacked (Goodwin, 1983; Gobrogge et al., 2007; Goodson and Kingsburg, 2011), yet, selective bonding often induces prosocial behavior, suggesting that even territorial species can be prosocial in the context of mating (Young and Wang, 2004). Conversely, highly gregarious species commonly live in large groups (Zann, 1996) and display high levels of affiliative behavior towards conspecifics outside of their chosen mate and offspring (Bester-Meredith et al., 1999; Xu et al., 2010). Group living has proven to be evolutionarily adaptive when its benefits outweigh the costs (ie. shared defense against predators and accelerated acquisition of resources (Ebensperger, 2001)). For group living to evolve, animals may have evolved mechanisms that promote positive social interactions with conspecifics in various contexts - whether related to mating or not. Perhaps highly social species lack anxiety or possess higher levels of boldness, encouraging positive interactions with novel individuals. Alternatively, more gregarious species may enjoy interacting with novel individuals and even find it rewarding to do so, whereas territorial and less social species do not find interactions with novel conspecifics to be rewarding.

African spiny mice engage in positive social interactions with both related individuals and strangers (Cizkova et al., 2011; Haughton et al., 2016; Tuckova et al., 2016). Spiny mice are communal breeders such that every group member cares for young pups, regardless of genetic relation. Young pups have been observed suckling from multiple lactating females (Young, 1976), and males also display parental care (Haughton et al., 2016). Spiny mice are widely dispersed in communities across semi dry and arid regions from Africa, the Middle East and Asia (Nowak, 1999). Because they do not dig burrows, researchers predict that they rely on rock crevices and already established burrows for shelter (Deacon, 2009). Although spiny mice are not immune to aggressive behaviors, especially when placed in a novel environment, they exhibit low levels of aggression (Haughton et al., 2016). Much of the social behavioral data about spiny mice has been obtained through studies of captive-bred colonies because the social dynamics of spiny mice in the wild remain largely unknown (Haughton et al., 2016). On the other hand, previous studies have shown that Mongolian gerbils reside in non-overlapping territories and breeding pairs will aggressively reject and attack intruders (Agren et al., 1989a; Agren et al., 1989b). Unlike the African Spiny mouse, the Mongolian gerbils exhibit stranger-directed aggression regardless of sex as a means of territorial defense (Elwood and Broom, 1978; Gerling and Yahr, 1979; Clark and Galef Jr., 1999). Gerbils will behave more aggressively in a familiar site compared to an unfamiliar one, suggesting territorial defense is highly characteristic of the species (Wechkin and Cramer, 1971). Especially during the reproductive season, gerbil territories require thorough protection of complex protective burrow systems needed for shelter, food storage and copulation (Bannikov, 1954; Agren et al., 1976). Furthermore, periods of aggression between founder females and other females have been associated with greater success in raising young and an increased weaning rate, offering an adaptive reason females may behave aggressively: to optimize reproductive success (Scheibler et al., 2004). Aggression can also occur within pairs and towards offspring (Scheibler et al., 2004).

Studies in the Kelly Lab have shown that, when given the choice to affiliate with a large or small group, spiny mice significantly prefer to affiliate with the larger group (Fricker and Kelly, unpub obs.). This strong affiliation for larger groups is unsurprising given that isolated spiny mice are relatively inactive and lethargic, yet activity levels return to normal when placed back into group housing (Haughton et al., 2016). Additionally, this reflects their natural history of living in large groups of up to 50 in the wild (Pers. comm. Dr. Ashley Seifert, University of Kentucky). Alternatively, gerbils live in smaller groups consisting of 2-17 individuals composed of extended family (Agren et al., 1989a), with an average group size of 8 (Wang et al., 2011). In socially monogamous animals, periods of prolonged social contact before copulation facilitates the formation of pair bonds (Razzoli et al., 2003). Research has indicated that gerbils are socially monogamous both in the field and under laboratory conditions (Agren et al., 1989a; Stockman and Novack, 1981). However, this thesis specifically looks at different social behaviors displayed in a non-reproductive context, with an intention of avoiding confounding effects of the motivation to mate. Therefore, all stimulus animals were same-sex conspecifics. This distinguishes this study from previous research that uses Mongolian gerbils to study social aggression and affiliation displayed by females towards males (Razzoli et al., 2003). A group size preference test also conducted in the Kelly Lab with male gerbils showed that male gerbils spend significantly less time investigating either the large or small group compared to male spiny mice (Fricker and Kelly, unpub obs.). This further distinguishes the moderately social gerbils from the highly social spiny mice. Lastly, spiny mice coexist in large groups and welcome newcomers (Cizkova et al., 2011), whereas gerbils do not (Agren, 1989a; Wang et al., 2011; Pan et al., 2020). This suggests that there is something unique about the drive of spiny mice to affiliate with large groups comprised of both related and unrelated individuals. In order to determine if the way an animal engages with social novelty relates to a more gregarious phenotype, in the present study I examine female spiny mouse and gerbil performance in a variety of social behavior tests, and examine statistical relationships between behaviors to begin to identify species-typical social behavioral phenotypes.

## Methods:

### *Animals*

15 adult female African spiny mice (*Acomys cahirinus*) and 16 female Mongolian gerbils (*Meriones unguiculatus*) were used for the purposes of this study. Spiny mice were obtained from the Kelly Lab breeding colony, and breeders were obtained from the breeding colony of Dr. Ashley Seifert (University of Kentucky). Gerbils were ordered from Charles River. Gerbils were group housed (2-4) in standard rat polycarbonate cages (40.64cm X 20.32cm X 20.32cm) and spiny mice were group housed (3-5) in 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. All animals were provided with chow 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 (Protocol PROTO201900126).

### ***Design***

To characterize species-specific patterns of social behavior and begin to denote species-typical social behavioral phenotypes, we conducted four commonly used behavioral paradigms with each subject: social approach, social preference, group size preference, and social interaction. All subjects and stimulus animals were females. This project is part of a larger study composed of both male and female gerbils and spiny mice. To avoid carry-over effects from a particular test or experience, subjects underwent a 1-hour break in between social approach and preference tests, and a 2-hour break after running a social interaction test. The order of tests for all subjects was randomized and a maximum of 3 tests was run per day for each subject. All behavioral tests were video recorded with Sony Handycam HDR-CDX405 1080p Camcorders (Sony, USA) and later quantified with Behavioral Observation Research Interactive Software (BORIS; Friard & Gamba, 2016). Social approach and social preference tests were scored in BORIS for 5 minutes, while social interaction tests and group size preference tests were scored for 8 minutes. Unlike the social approach and social preference tests, social interaction and group size preference tests were scored for a longer interval of time because subjects remain active after 5 minutes during social interaction and group size preference tests because they can freely interact or have more stimuli to investigate, respectively.

### ***Cleaning protocols***

After each test was complete, subjects and stimulus animals were returned to their home cages. The walls and base of the testing chambers were cleaned with Virkon-S followed by water and dried with a microfiber towel before getting used for another test to eliminate any confounding effects of olfactory stimuli (urine and feces) left behind by previous animals.

### ***Behavior***

#### *Social Approach*

To assess general attraction/aversion to social novelty, subjects underwent a social approach test with a novel, same-sex conspecific. Novel stimuli were individuals with whom the subjects had never interacted before. Subjects were placed in a large Plexiglas testing chamber (81.28 cm x 40.64 cm x 38.1 cm) and acclimated in the testing chamber for 3 minutes before stimulus animals were added. After acclimation, subjects were placed on one side of the chamber under a plastic beaker (with a weight on top) and the novel stimulus was added to the opposite end of the chamber under a black pencil holder (8.99 cm x 9.70 cm x 8.99 cm) with a weight on top. After proper placement, subjects were released and latency to approach the stimulus animal (gerbils n=14; spiny mice n=14) and the duration of time spent investigating the stimulus animal (defined as nose inserted into or rearing up on the stimulus' container; gerbils n=16; spiny mice n=15) were recorded during a 6-minute test.

#### *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. Does NOT count when subject is sitting on top of container. |

### *Social Interaction*

To measure behavior during social interactions without any barriers between animals, subjects underwent a social interaction test with a freely moving novel, same sex conspecific. Novel stimuli were individuals with whom the subjects had never interacted before. 20-30 minutes prior to testing, the backs of stimulus animals were colored in order to easily identify the subject vs. the stimulus during the interaction. Subjects acclimated in a testing chamber (40.64 cm x 20.32 cm x 20.32 cm) for 10 minutes. After acclimation, the stimulus animal was placed onto the opposite side of the chamber as the subject, and interactions were recorded during a 10-minute test. Behaviors scored included the subject's prosocial behavior (positive investigation, allogrooming, huddling, and positive side-by-side contact; gerbils n=16; spiny mice n=15) towards the stimulus, aggressive behavior (biting, chasing, pinning, lunging/attacking, rearing, and aggressive side-by-side contact; gerbils n=16; spiny mice n=12) towards the stimulus, and nonsocial behaviors (all other behaviors not considered overtly prosocial or aggressive, including autogrooming, jumping, sitting alone, and nonsocial exploration; gerbils n=16; spiny mice n=15). Autogrooming was also analyzed as its own variable (gerbils n=15; spiny mice n=14).

*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 crisscrossed 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. |
| **Nonsocial** | All behavior instances not included in Prosocial or Aggressive. | Subject is not making contact with or exhibiting behavior direct toward stimulus animal. |

*Social Preference*

To identify any social preferences for novelty or familiarity, subjects underwent a social preference test with a novel same-sex conspecific on one side, and a familiar same-sex conspecific on the other side. Novel stimuli were individuals with whom the subjects had never interacted before. Familiar stimuli consisted of each subject's cage mate/sibling. Subjects acclimated in the testing chamber (81.28 cm x 40.64 cm x 38.1 cm) for 3 minutes, and then were trapped under a beaker in the center of the chamber (with a weight on top to prevent escape). The novel same-sex conspecific and familiar same-sex cage mate/sibling were placed under pencil holders (with a weight on top) at opposite ends of the chamber. Upon subject's release, the duration of time spent in the familiar (gerbils n=16; spiny mice n=15) or novel (gerbils n=16; spiny mice n=15) zone (within one body length of the stimuli) and the duration of time spent investigating the novel (gerbils n=16; spiny mice n=12) or familiar (gerbils n=15; spiny mice n=14) stimuli (defined as nose inserted into or rearing up on the stimulus' container) were recorded during a 6-minute test. Duration of time spent in the neutral zone (gerbils n=16; spiny mice n=15) was later calculated (represents when the subject was not within one body length of either stimulus animal).

*Ethogram*

| **Behavior** | **Description** |
|---|---|
| Social Investigation | Time subject spends poking nose through or rearing up on the stimulus animal's container. Does NOT count when subject is sitting on top of container. |
| In Zone | Time subject spends within one body length (approximately 1/3 of the chamber) from the stimulus container. |

### *Group Size Preference*

To quantify gregariousness and discover any social preferences based on group size, subjects underwent a group size preference test in a large testing chamber (81.28 cm x 40.64 cm x 38.1 cm) and were given the choice to affiliate with a small (2) or large (8) group of novel same-sex conspecifics. Members of both groups were individuals with whom the subjects had never interacted before. The 8 novel same-sex conspecifics (large group) were individually placed under pencil holders (with weights on top) and grouped on one end of the chamber, and the 2 novel same-sex conspecifics (small group) were individually placed under pencil holders (with weights on top) and grouped on the opposite end of the chamber to acclimate for 5 minutes before the subject was added. After acclimation, the subject was placed under a beaker into the center of the chamber between the 2 groups. Upon subject's release, the duration of time spent in the large group (gerbils n=15; spiny mice n=15) or small group (gerbils n=16; spiny mice n=15) zone (within one body length of the stimuli) and the duration of time spent investigating the large (gerbils n=16; spiny mice n=15) group or small (gerbils n=16; spiny mice n=15) group (defined as nose inserted into or rearing up on the stimuli's containers) were recorded during an 8-minute test. Duration of time spent in the neutral zone (gerbils n=15; spiny mice n=15) was later calculated (represents when the subject was not within one body length of either group).

*Ethogram*

| Behavior | Description |
|---|---|
| Social Investigation | Time subject spends poking nose through or rearing up on the stimulus animal/group's container. Does NOT count when subject is sitting on top of container. |
| In Zone | Time subject spends on the stimulus animal/group’s side (approximately 1/3 of the chamber). |

***Statistics***

Behavioral measures for each behavior test type were analyzed using SPSS 26 (IBM Analytics, USA). Due to non-normal distributions for all behavioral measurements, non-parametric tests were used to determine any significant differences in mean values. Normality was assessed using histograms and visually assessing for skewness and kurtosis. Outliers for individual behavioral tests were considered to be 3 standard deviations outside the mean and were removed from the data sets before conducting statistical analyses. For non-parametric comparison of within-species differences, a Wilcoxon signed-rank test was conducted. For non-parametric comparison of between-species differences, a Mann-Whitney U test was performed.

## Behavioral Results

### *Social Approach*

In order to engage in any form of social interaction, an animal must approach another. Therefore, the social approach test is an essential part of understanding how animals engage in social interactions. In this behavioral test, we measured the time it took for the subject to approach the novel stimulus animal and the time spent investigating the stimulus animal. A Mann-Whitney U Test indicated no significant differences in social approach latency ( $\mathrm{Z} = -.276, \mathrm{p} = .808$) or time spent investigating the novel stimulus ($\mathrm{Z} = -1.502, \mathrm{p} = .191$) during the social approach test between female gerbils and spiny mice.

### *Social Interaction*

Compared with a social approach test, the social interaction paradigm may be more indicative of how an animal will interact with same-sex conspecifics in the wild because subjects can freely interact with stimulus animals in the testing chambers without any barriers. Subjects were placed in the testing chamber with one other novel same-sex conspecific. In order to have sufficient statistical power to analyze types of behavior, individual behaviors for each subject were categorized as prosocial, aggressive or nonsocial. Prosocial behaviors included positive investigation, allogrooming, huddling, and positive side-by-side contact. Aggressive behaviors consisted of biting, chasing, pinning, lunging/attacking, rearing and aggressive side-by-side contact. Non-Social behaviors included autogrooming, jumping and cage exploration. We quantified any differences found within female gerbils and female spiny mice interacting prosocially, aggressively and non-socially respectively, as well as compared the values between species for time spent engaging in these types of interactions. A Wilcoxon signed-rank test indicated no significant difference between time spent interacting aggressively or prosocially ($Z = -.672, p = .501$, **Fig. 1A**) in female gerbils. However, female spiny mice spent significantly more time engaging in prosocial behaviors compared to aggressive behaviors ($Z = -3.351, p < .001$, **Fig. 1A**). Both female gerbils and female spiny mice spent the most time engaging in non-social behaviors compared to aggressive behaviors (gerbils, $Z = -2.844, p = .004$; spiny mice, $Z = -3.408, p < .001$, **Fig. 1A**) and prosocial behaviors (gerbils, $Z = -3.464, p < .001$; spiny mice, $Z = -3.408, p < .001$, **Fig. 1A**). For between species comparisons, a Mann-Whitney U test was performed. We found that female gerbils spent significantly more time than female spiny mice interacting aggressively ($Z = -3.324, p < .001$, **Fig. 1A**). Furthermore, female spiny mice spend significantly more time than female gerbils interacting prosocially ($Z = -3.162, p = .002$, **Fig. 1A**). There was no significant difference in time spent interacting non-socially between female gerbils and female spiny mice ($Z = -.237, p = .812$, **Fig. 1A**). Although autogrooming was considered to be a nonsocial behavior, it was also analyzed separately as its own variable. A significant species difference was found in time spent autogrooming between female gerbils and female spiny mice, with spiny mice autogrooming more than gerbils ($Z = -2.076, p = .038$, **Fig. 1B**).

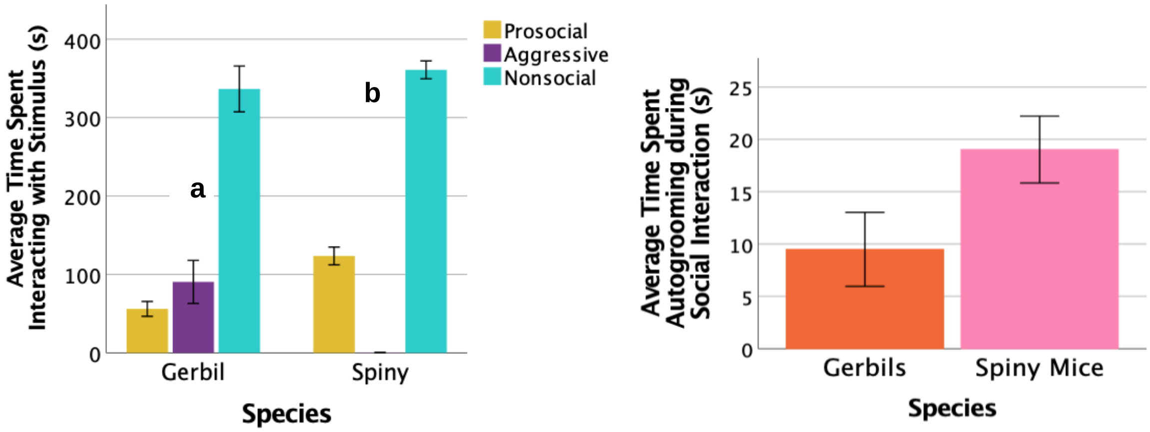

**Figure 1:** Mean (± SEM) duration of time (s) spent engaging in nonsocial (blue), aggressive (purple), and prosocial (yellow) behavior for gerbils and spiny mice during the social interaction test (**A**). Mean (± SEM) duration of time (s) spent autogrooming during the social interaction test for gerbils (orange) and spiny mice (pink) (**B**). Differential letters in comparisons indicates a $p \leq 0.05$.

*Social Preference*

Presumably, the social preference test can help us better understand if a species exhibits a preference to affiliate with novel or familiar same-sex conspecifics. Since both female gerbils and female spiny mice live in groups, both are bound to interact socially with familiar individuals. Additionally, both animals are likely to be approached by novel individuals, some of whom may pose as threats while others may be welcomed into the social group. For these reasons, it is useful to analyze within-species and between-species differences through this behavioral paradigm that presents a novel and a familiar conspecific on either side of the test cage. In the social preference test, we quantified social interactions as time spent in close proximity to the novel or familiar conspecifics, respectively, time spent investigating each of the stimulus animals and time spent in the neutral zone engaging with neither the familiar stimulus animal nor the novel stimulus animal. A Wilcoxon signed-rank test indicated that female gerbils, unlike female spiny mice, spend significantly more time investigating the novel stimulus (gerbils, $Z = -2.017, p = .044$; spiny mice, $Z = -1.477, p = .140$, **Fig. 2A**). However, after conducting a Mann-Whitney U test, there were no species-specific differences found between female gerbils and female spiny mice in time spent investigating the novel ($Z = -.696, p = .486$, **Fig. 2A**) or familiar stimulus ($Z = -1.047, p = .295$, **Fig. 2A**). Interestingly, while female gerbils spent significantly more time investigating the novel stimulus compared to the familiar stimulus, this preference was not apparent for time spent in the novel or familiar zones ($Z = -1.551, p = .121$, **Fig. 2B**). They did, however, spend significantly more time in the novel zone compared to the neutral zone ($Z = -3.516, p < .001$, **Fig. 2B**) and in the familiar zone compared to the neutral zone ($Z = -3.516, p < .001$, **Fig. 2B**) interacting with neither stimulus. This further supports the classification of gerbils as a moderately social species who would rather be near an individual, whether familiar or novel, compared to being alone. Similarly, female spiny mice showed this same pattern of behavior with no significant difference between time spent in the novel or familiar zones ($Z = -.170, p = .865$, **Fig. 2B**), but significantly more time spent in the novel zone compared to the neutral zone ($Z = -3.408, p < .001$, **Fig. 2B**) and in the familiar zone compared to the neutral zone ($Z = -3.408, p < .001$, **Fig. 2B**) interacting with neither stimulus. Using a Mann-Whitney U test, we found significant species-specific differences in time spent in the familiar zone and time spent in the neutral zone, but no difference in time spent in the novel zone ($Z = -.435, p = .664$, **Fig. 2B**). Female gerbils spent significantly more time in the neutral zone avoiding interactions with either stimulus animal than the female spiny mice ($Z = -4.309, p < .001$, **Fig. 2B**). Curiously, spiny mice spent significantly more time in the familiar zone than the female gerbils ($Z = -2.767, p = .006$, **Fig. 2B**). This difference could have been perpetuated by female gerbils spending a fair amount of time in the neutral zone.

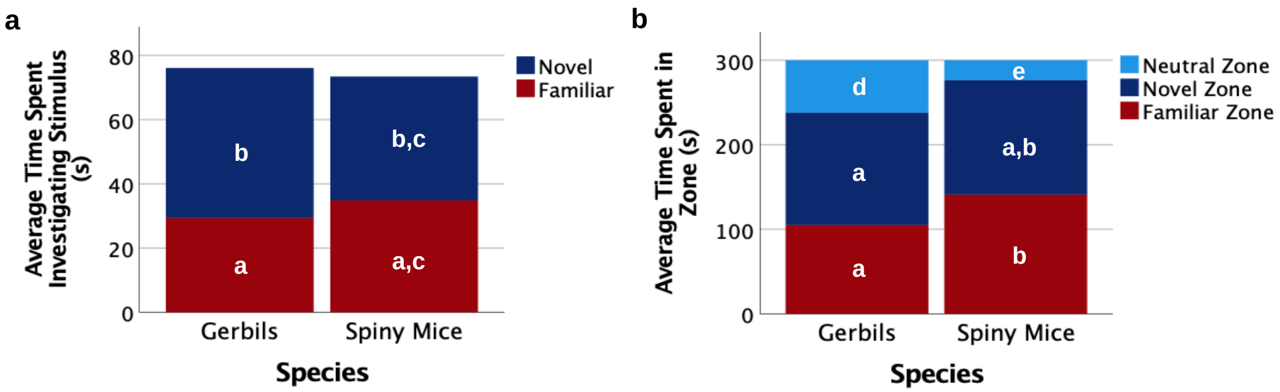

**Figure 2:** Duration of time (s) spent investigating a familiar (red) or novel (dark blue) conspecific during a social preference test (**A**). Duration of time (s) spent in the familiar (red), novel (dark blue) or neutral (light blue) zones during a social preference test (**B**). Time spent in the middle/neutral zone represents when the subject was not near either stimulus animal. Differential letters in comparisons indicates a $p \leq 0.05$.

*Group Size Preference*

The group size preference test serves as a useful tool for determining the gregariousness of a species, or the preference to affiliate with a larger group. The subject is placed in the center of the testing chamber with a large group of 8 novel same sex individuals on one side of the chamber and a small group of 2 novel same-sex individuals on the opposite side of the chamber. We measured time spent engaging in social investigation of either the small group or large group, time spent in the neutral zone interacting with neither group and time spent in close proximity to the small group or large group. A Wilcoxon signed-rank test was performed to determine within-species differences for the female gerbils and female spiny mice, respectively. We determined that both female gerbils and female spiny mice were more likely to investigate a larger group (gerbils, $Z = -3.516, p < .001$; spiny mice, $Z = -3.408, p < .001$,

**Fig. 3A**). Importantly, female spiny mice investigate the large group for significantly more time than female gerbils ($Z = -4.743, p < .001$, **Fig. 3A**). There is no significant difference between the two species of time investigating the small group ($Z = -.791, p = .429$, **Fig. 3A**). Female gerbils spend less than half the time investigating the groups than female spiny mice (**Fig. 3A**). This may be caused by female gerbils' general anxiety to interact with groups of any size, while female spiny mice are willing to spend over ⅔ of the total test time with a group, and preferentially the larger group ($Z = -3.408, p < .001$, **Fig. 3B**). However, female gerbils still do show the pattern of spending significantly more time investigating the large group. This may be due to a larger group posing as more of a threat and needing to be further investigated. The same within-species patterns discovered by another Wilcoxon signed-rank test carried over to the time spent in close proximity to the two groups. Both female gerbils and female spiny mice spent significantly more time near the larger group than the smaller group (gerbils, $Z = -3.408, p < .001$, spiny mice, $Z = -3.408, p < .001$, **Fig. 3B**). However, while the female spiny mice spent significantly more time near the large group compared to the neutral zone ( $Z = -3.408, p < .001$, **Fig. 3B**) and near the small group compared to the neutral zone ( $Z = -3.408, p < .001$, **Fig. 3B**), the female gerbils spent the most time in the neutral zone, significantly more than both the small group ($Z = -3.408, p < .001$, **Fig. 3B**) and large group zones ($Z = -3.408, p < .001$, **Fig. 3B**). A Mann-Whitney U test highlighted a significant species-specific difference between time spent in the neutral zones and time spent in the large group zones, but no significant difference in time spent in the small group zone ($Z = -.079, p = .937$, **Fig. 3B**). The female gerbils spent significantly more time than the female spiny mice in the neutral zone ($Z = -4.309, p < .001$, **Fig. 3B**). Unsurprisingly, the female spiny mice spent significantly more time in close proximity to the large group than female gerbils ( $Z = -4.666, p < .001$, **Fig. 3B**).

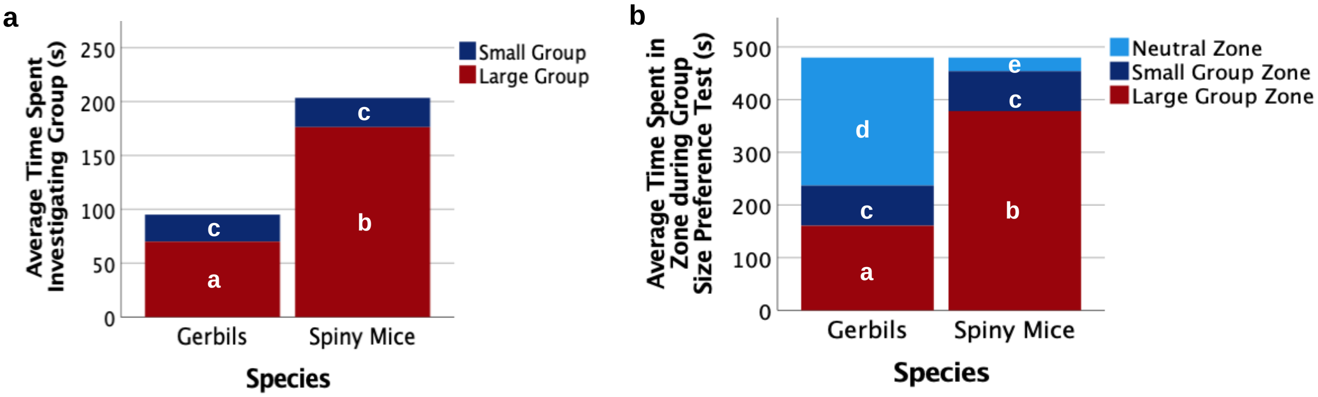

**Figure 3:** Duration of time (s) spent investigating the large group (red) or small group (dark blue) during a group size preference test (**A**). Duration of time (s) spent in the large group (red), small group (dark blue) or neutral zones (light blue) during a group size preference test (**B**). Time spent in the middle/neutral zone represents when the subject was not near either stimulus animal. Differential letters in comparisons indicates a $p \leq 0.05$.

## Behavioral Relationships

### *Principal Component Analysis Results for Female Gerbils and Female Spiny Mice*

A principal component analysis (PCA) was run to explore how different behaviors from the various behavioral analyses may relate to one another in female gerbils and female spiny mice, respectively. This statistical procedure reduces large datasets with multiple variables into smaller components of statistically related variables (although not necessarily linearly related) that account for most of the variance observed in the full dataset. Typically, loadings on the components that are considered are ≤ -0.4 and ≥ 0.4 (Liu et al., 2003), however we adopted a conservative approach and only considered loadings that were ≤ -0.5 and ≥ 0.5. For both female gerbils and female spiny mice, only the first three components were looked at because they had the highest eigenvalues (gerbils:2.311-4.848; spiny mice: 2.233-5.062) or most explanatory power by accounting for the majority of variance in the data. We would expect that the variables within a test would relate to one another. Thus, the third component for female gerbils does not reveal any useful relationship between variables since time spent interacting non-socially, aggressively and prosocially are all determined from the social interaction test (Table 1). However, PC1 for female gerbils identified a relationship between a preference for familiarity and a lack of preference for social novelty (Table 1). PC2 reveals a relationship between gregariousness and social boldness (Table 1). On the other hand, the PCA conducted for female spiny mice is not helpful in uncovering any behavioral patterns (Table 2). The majority of relationships are from the same test types, and therefore, do not reveal any new relationships between variables.

Table 1: Principal Components Matrix of Female Gerbil Behavior

| | **PC1 “Neophobia”** | **PC2 “Gregariousness and Social Boldness”** | **PC3 “Activity”** |
|---|---|---|---|
| **BEHAVIORAL MEASURES** | | | |
| **Social Preference Test** | | | |
| Time Spent (s) in Familiar Zone | **.900** | -.175 | .187 |
| Time Spent (s) Investigating Familiar Stimulus | **.873** | .164 | .118 |
| Time Spent (s) in Novel Zone | **-.771** | .399 | -.261 |
| Time Spent (s) Investigating Novel Stimulus | -.418 | .244 | -.123 |
| **Group Size Preference Test** | | | |
| Time Spent (s) in Large Group Zone | -.060 | **.964** | .087 |
| Time Spent (s) Investigating Large Group | -.193 | **.871** | -.122 |
| Time Spent (s) in Small Group Zone | .253 | -.012 | .018 |
| Time Spent (s) Investigating Small Group | -.147 | .143 | .244 |
| **Social Approach Test** | | | |
| Social Approach Latency (s) | .045 | **-.775** | -.041 |
| Time Spent (s) Investigating Stimulus | .163 | .475 | -.084 |
| **Social Interaction Test** | | | |
| Time Spent (s) Interacting Non-Socially | -.176 | .020 | **-.950** |
| Time Spent (s) Interacting Aggressively | .393 | -.019 | **.843** |
| Time Spent (s) Interacting Prosocially | **-.589** | .060 | **.618** |
| Time Spent (s) Autogrooming | .267 | -.162 | -.155 |

Table 2: Principal Components Matrix of Female Spiny Mouse Behavior

| | **PC1** | **PC2** | **PC3** |
|---|---|---|---|
| **BEHAVIORAL MEASURES** | | | |
| **Social Preference Test** | | | |
| Time Spent (s) in Familiar Zone | .132 | **-.928** | .025 |
| Time Spent (s) Investigating Familiar Stimulus | .060 | -.069 | .101 |
| Time Spent (s) in Novel Zone | -.298 | **.874** | -.096 |
| Time Spent (s) Investigating Novel Stimulus | -.147 | **.792** | -.007 |
| **Group Size Preference Test** | | | |
| Time Spent (s) in Large Group Zone | **.963** | -.167 | -.080 |
| Time Spent (s) Investigating Large Group | **.709** | -.275 | -.219 |
| Time Spent (s) in Small Group Zone | **-.971** | .060 | .028 |
| Time Spent (s) Investigating Small Group | **-.845** | .188 | -.169 |
| **Social Approach Test** | | | |
| Social Approach Latency (s) | **.523** | .132 | .376 |
| Time Spent (s) Investigating Stimulus | .068 | .188 | .321 |
| **Social Interaction Test** | | | |
| Time Spent (s) Interacting Non-Socially | **.715** | -.126 | -.327 |
| Time Spent (s) Interacting Aggressively | .218 | -.074 | **-.886** |
| Time Spent (s) Interacting Prosocially | .021 | -.149 | **.724** |
| Time Spent (s) Autogrooming | .065 | **.604** | .327 |

*Correlation: Neophobia in female gerbils*

Gregariousness score was calculated by subtracting the time spent in close proximity to the small group from the time spent in close proximity to the large group. Higher values indicated a greater level of gregariousness, or propensity to affiliate in a large group. There is a negative correlation between gregariousness score and time spent in the neutral zone during the social preference test ($\mathrm{r} = -.568, \mathrm{p} = .027$, **Fig. 4**). Overall, female gerbils with a higher gregariousness score tend to spend less time in the neutral zone during the social preference test. This suggests that gregariousness may not necessarily be a product of neophilia, or the desire to readily engage with a novel stimulus, but instead, gregariousness relates more to level of sociality or desire to engage in social interactions at all with novel or familiar conspecifics. A less gregarious species may prefer not to interact socially as often. These findings are supported by the negative correlations between time spent in the neutral zone during the social preference test and time spent investigating the large group ($\mathrm{r} = -.574, \mathrm{p} = .020$, **Fig. 5A**) and near the large group ($\mathrm{r} = -.634, \mathrm{p} = .011$, **Fig. 5B**) during the group size preference test. In summary, the more time a species spends in the neutral zone during the social preference test, the less time they will spend in the large group zone and investigating the large group during the group size preference test. This may indicate that gerbils who are generally more asocial are less likely to interact with a larger group or have any interest in the larger group at all. Therefore, the drive to associate with a larger group may stem from a general drive to be affiliative. It is worth noting that we did not run correlations against time spent in the neutral zones in the group size preference test and social preference test in spiny mice because they spent so little time overall in these zones, it is likely the correlations would not be informative.

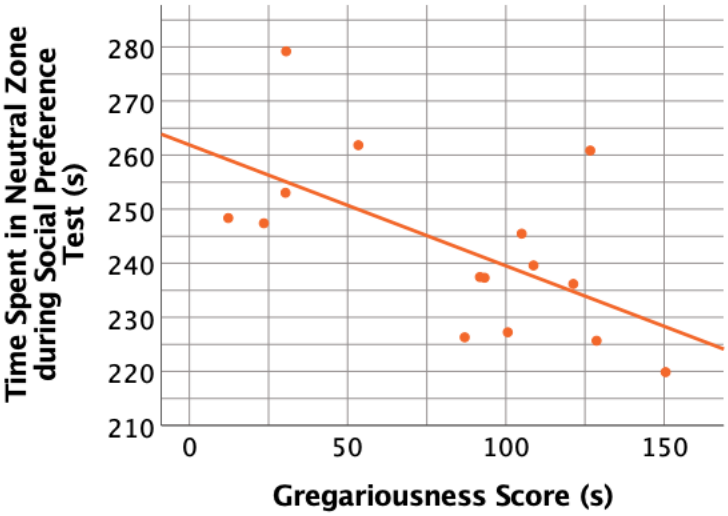

**Figure 4 (above):** Pearson's correlation revealed a negative relationship between female gerbil's time spent in the neutral zone during the social preference test and a gregariousness score from the group size preference test (r=-.568 and p=.027). A higher gregariousness score reflects a more gregarious phenotype.

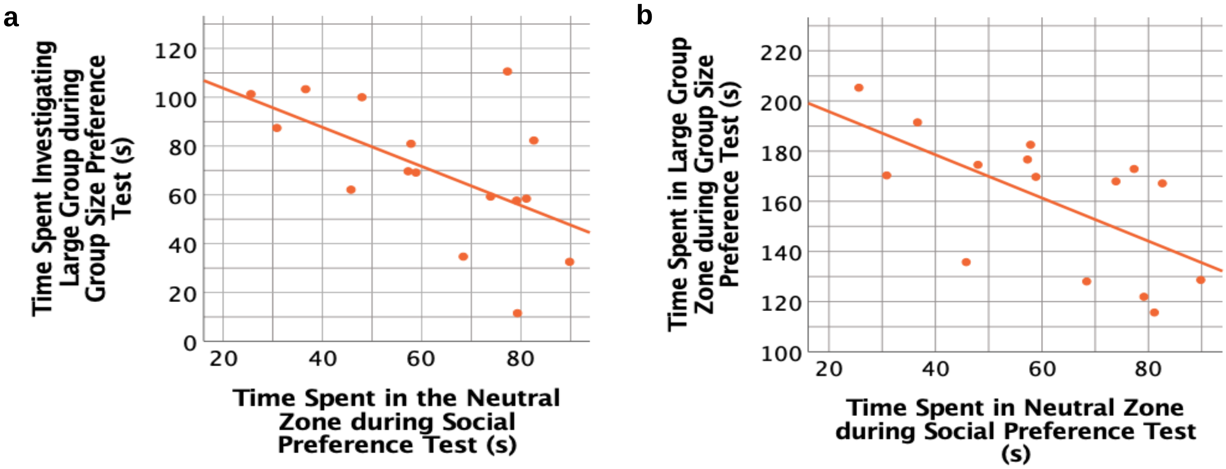

**Figure 5 (above):** Pearson's correlation revealed a negative relationship between female gerbil's time spent in the neutral zone during the social preference test and time spent investigating the large group during the group size preference test (**A**) (r=-.574, p=.020), and a negative relationship between female gerbil's time spent in the neutral zone during the social preference test and time spent in the large group zone during the group size preference test (**B**) (r=-.634, p=.011).

*Correlation: Phenotypic consistency in female gerbils*

There is a positive correlation between time spent in the neutral zone in the group size preference test and time spent in the neutral zone during the social preference test ( $r = .621, p = .014$, **Fig. 6**). Essentially, female gerbils who spend more time in the neutral zone during the group size preference test also spend more time in the neutral zone during the social preference test. This data supports phenotypic consistency. Although open to interpretation, this may be representative of a shy phenotype, or a less socially active phenotype in female gerbils. This data shows that female gerbils who don't want to interact with a large or small group also don't want to interact with a novel or familiar stimulus. During the group size preference test, the smaller group consisted of two individuals. It is possible that female gerbils may have been less inclined to interact with two individuals than they would have with one individual since they readily engage in pairbond partnerships.

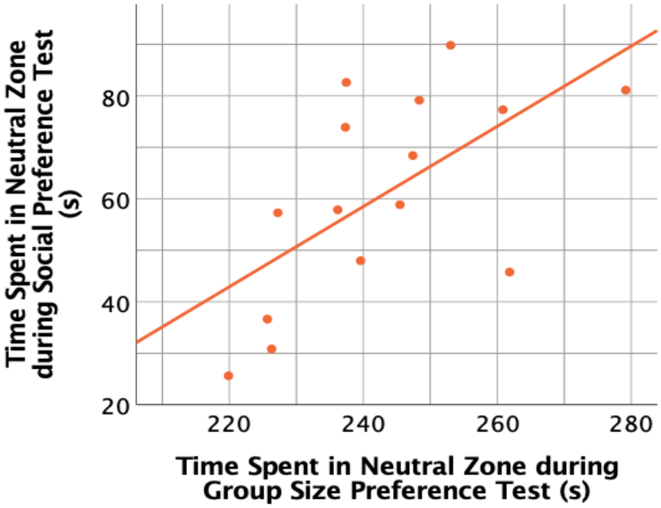

**Figure 6:** Pearson's correlation revealed a positive relationship between female gerbil's time spent in the neutral zone during the group size preference test and time spent in the neutral zone during the social preference test (r=.621, p=.014).

### *Correlation: Neophobia aligns with anxious phenotype in female gerbils and female spiny mice*

For both female gerbils and female spiny mice, autogrooming during the social interaction test may be a display of anxiety but for different reasons. For female gerbils, there is a positive correlation between time spent autogrooming during the social interaction test and time spent investigating ($r = .559, p = .030$, **Fig. 7A**) and near the familiar stimulus during the social preference test ($r = .515, p = .049$, **Fig. 7B**). This indicates that female gerbils who spent more time autogrooming in the social interaction test also spent more time in close proximity to the familiar stimulus and investigating the familiar stimulus. In one study, female white-faced capuchins groomed themselves significantly more while in close proximity to same-sex conspecifics than while alone (Manson and Perry, 2003). Similar to how the capuchins may have felt more comfortable in a nonsocial context, gerbil's may have felt less anxious in the familiar zone because they are used to being with their cage mate. This may shed light on the role of autogrooming in female gerbils as representative of anxiety in the animal as opposed to a method for releasing pheromones to seem more desirable since a more neophobic female gerbil will also spend more time autogrooming. If autogrooming is an indicator of anxiety, a more neophobic animal may have a more anxious phenotype, and thus be less socially bold. Future studies can be conducted, such as the open field test, to measure levels of anxiety in both rodents in nonsocial settings. It would be interesting to see if an animal that is anxious in a nonsocial environment would also be anxious in a social environment. It is also possible that social interactions may be more anxiety inducing for female gerbils or female spiny mice. This could offer more evidence for why spiny mice live in larger groups of familiar and unfamiliar individuals in the wild. Female spiny mice show a negative correlation between time spent autogrooming during the social interaction test and time spent near the familiar stimulus during the social preference test ($r = -.523, p = .046$, **Fig. 7C**). Essentially, the more time female spiny mice spend autogrooming, the less time they spend in the familiar zone during the social preference test. Initially, it may appear confusing why a more anxious spiny mouse would spend less time near a familiar stimulus because one would assume that the animal is more comfortable with a familiar stimulus. However, due to the fact that spiny mice live in larger groups in the wild, dominance hierarchies play a more significant role in social interactions (Cizkova et al., 2011). It is possible that they would be more anxious with a familiar individual if it has something to do with signaling rank in hierarchy. Since a novel individual would not yet have a social rank, it may be less intimidating to be near this individual.

**a** **b** **c**

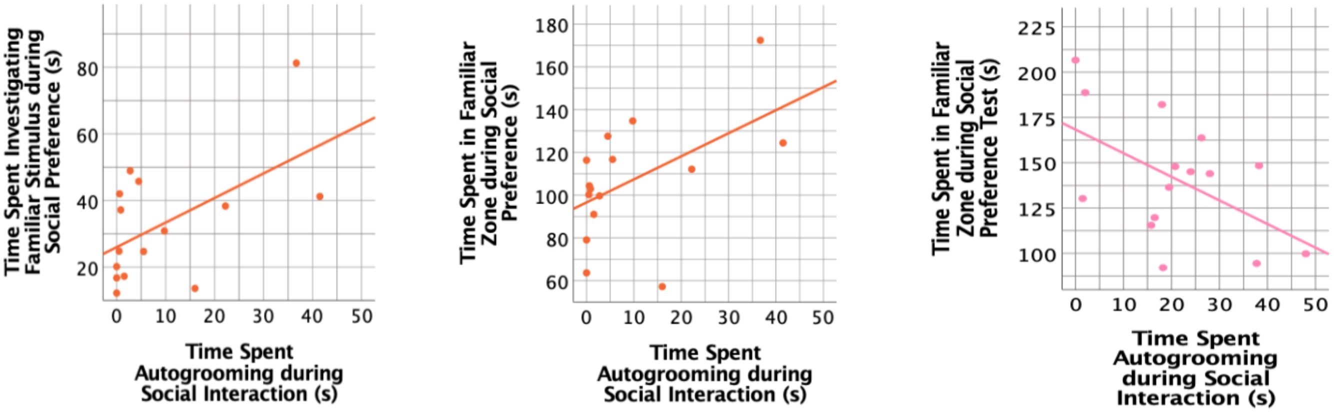

**Figure 7:** Pearson's correlation revealed a positive relationship between time spent autogrooming during the social interaction test and time spent investigating the familiar conspecific during the social preference test in female gerbils (**A**) (r=.559, p=.030), a positive relationship between time spent autogrooming during the social interaction test and time spent in the familiar zone during the social preference test in female gerbils (**B**) (r=-.515, p=.049), and a negative relationship between time spent autogrooming during the social interaction and time spent in the familiar zone during social preference test in female spiny mice (**C**) (r=-.523 and p=.046).

*Correlation: Social approach latency differentially relates to investigation of groups based on size in spiny mice*

There were significant correlations uncovered between social approach latency in the social approach test and time spent investigating the small group and time spent investigating the large group during the group size preference test in female spiny mice. There was a negative correlation between social approach latency and time spent investigating the small group ( $\mathrm{r} = -.515, \mathrm{p} - .049$, **Fig. 8**) during the group size preference test, but a positive correlation between social approach latency and time spent investigating the large group ( $\mathrm{r} = .567, \mathrm{p} = .028$) in the group size preference test in female spiny mice. It is worth noting that overall, the female spiny mice still spend much more time investigating the large group than the small group.

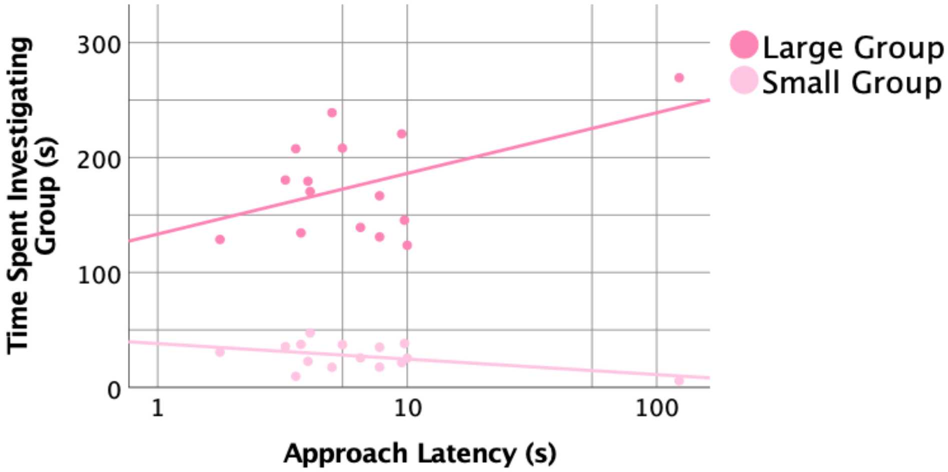

**Figure 8:** Pearson's correlation revealed a negative relationship between approach latency during social approach test and time spent investigating the small group (light pink) (r=-.515, p=.049), and a positive relationship between approach latency during social approach test and time spent investigating the large group (dark pink) (r=.567, p=.028) during group size preference test in female spiny mice.

### *Correlation: Longer approach consistent with aggression in female gerbils*

There was also a significant positive correlation between approach latency in the social approach test and time spent interacting aggressively in the social interaction test in female gerbils ($r = .588, p = .027$, **Fig. 9**). Gerbils that took more time to approach a novel conspecific were also more aggressive with novel conspecifics. Hesitancy to approach a novel stimulus may be indicative of neophobia and/or a preference to affiliate with familiar conspecifics in gerbils.

*Figure*

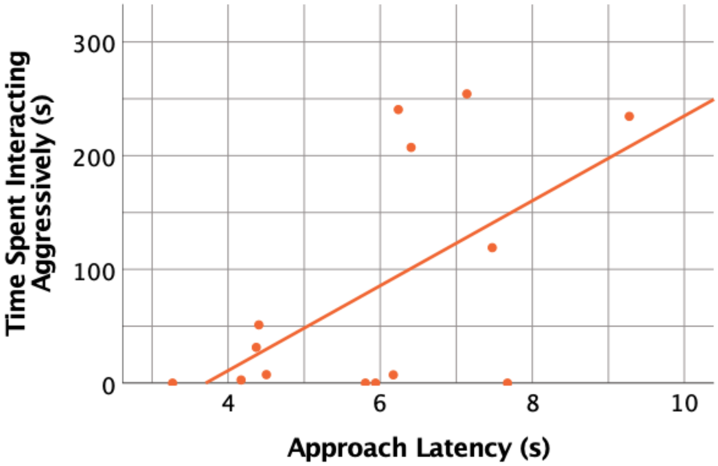

**Figure 9:** Pearson's correlation revealed a positive relationship between approach latency during social approach test and time spent interacting aggressively during social interaction test in female gerbils (r=.588, p=.027).

## Discussion

### ***Species-typical behavior***

#### *Social Approach*

The longer a female spiny mouse took to approach the novel stimulus animal, the less time they spent investigating the small group and the more time they would spend investigating the large group. This may indicate that social approach is not necessarily related to gregariousness in female spiny mice. Alternatively, female spiny mice that prefer to affiliate in large groups may simply have less interest approaching a singular animal or are more cautious because this is a more unfamiliar setting for them to see a lone individual. The female spiny mice appear to clearly prefer investigating the large group more than the small group. This could provide evidence that female spiny mice are so comfortable in large groups that they feel more hesitant to approach a singular animal because they may not have the same protection as when they are with their large group. On the other hand, a female spiny mouse may simply have less interest interacting with a singular individual and therefore, take longer to approach the animal. The longer a female gerbil took to approach the novel stimulus animal, the more time they spent interacting aggressively during the social interaction test. For female gerbils, approach latency can potentially be used to inform us how aggressive the animal will be during a social interaction. Female gerbils may take longer to approach an unfamiliar conspecific because they want to ensure that the novel conspecific is not a threat. Gerbils who are generally more cautious may also be more aggressive during their social interactions because their instinct is to be defensive. This would be consistent with the territoriality that gerbils exhibit toward non-family group members (Wang et al., 2011; Pan et al., 2020). Additionally, previous studies have reported that female gerbils defend their home cages against same-sex and opposite-sex intruders comparably to males (Thiessen and Dawber, 1972). Other studies have confirmed that female gerbils, among themselves, are as aggressive as males (Rieder and Reynierse, 1971; Spencer et al., 1973) and thus, more closely resemble the aggressive patterns of hamsters but differ from rats and mice (Payne and Swanson, 1970).

### *Social Interaction*

The inclination to be prosocial in female spiny mice is consistent with prior evidence of prosocial interactions among strangers, and social curiosity of precocial spiny mice pups between 1 and 5 days old (Ratnayake et al., 2014). It is possible that spiny mice physiology supports a less aggressive behavioral phenotype. It has been hypothesized that microglial stimulated neurogenesis may play a role in modulating prosocial behavior (Zhelezov, 2016). Interestingly, spiny mice complete the majority of neurogenesis before birth (Haughton, 2016). It may be worth exploring a potential relationship between substantial brain development of mice in utero (Brunjes, 1990) and an immediate drive of young pups to be curious and social. The lack of aggression observed in female spiny mice may also relate to a reliably stable and abundant source of breeding opportunities. Female aggression towards a female intruder has been associated with competition for breeding opportunities (Cizkova et al., 2011). If there is no scarcity of breeding opportunities, female spiny mice may exhibit less aggression towards a same-sex novel conspecific. Interestingly, female spiny mice exhibit more aggression and dominance over male companions while in the females’ home cages, but males are not significantly more aggressive towards females while the males’ home cages (Porter, 1976). Additionally, female spiny mice are more likely to treat sexually inexperienced males as intruders compared to males with prior breeding experience (Andres and Deni, 1982). This may indicate that female spiny mice are more selective with mates and consequently, have more influence over breeding opportunities. Unlike the spiny mice, female gerbils were more aggressive during social interactions and they were not significantly more prosocial than aggressive. In fact, female gerbils were significantly more nonsocial, preferring to not engage with a novel, same-sex conspecific. These findings are consistent with the territorial nature of Mongolian gerbils in the wild (Elwood, 1975; Swanson and Lockley, 1978; Clark and Galef Jr., 2000). One study found fighting to be common amongst gerbils housed together without habituation (Buckmaster, 2006).

### *Social Preference*

Given the territorial nature of female gerbils and the tendency to view a novel conspecific as a safety threat, it is unsurprising that they would spend more time investigating a novel stimulus. This also provides evidence that a tendency to investigate novel individuals may be more representative of a mechanism for defense in more territorial species. Female spiny mice may show no preference for a novel or familiar stimulus because they are equally likely to encounter either a novel individual or familiar individual in their large groups consisting of novel and familiar individuals. Therefore, neither a novel nor familiar individual would be particularly exciting to interact with or pose more of a threat to a female spiny mouse.

### *Group Size Preference*

The finding that female gerbils affiliated with and investigated the large group more than the small group was surprising given that Mongolian gerbils live in small family groups and are territorial (Wang et al., 2011; Pan et al., 2020). Although female gerbils may be somewhat gregarious in a group size preference test in the lab, the finding that female spiny mice are significantly more gregarious than female gerbils mirrors spiny mouse behavioral ecology in the wild (i.e., spiny mice live in large groups whereas gerbils do not). It is worth noting that the individual gerbil stimuli in the groups were unable to move freely as they were each placed under a container. The focal female gerbil may have been more inclined to investigate the larger group for this reason, since group members that are restrained may be less intimidating to the lone gerbil. This could also be the case with the female spiny mice, however, considering they are used to interacting with large groups, it is unlikely they would be intimidated by a large group size. To further untangle the gerbil gregarious findings here, future studies could examine behavior of freely interacting small and large groups of novel conspecifics. I expect that under such circumstances, spiny mice would still exhibit high degrees of gregariousness, but that gerbils would exhibit less gregariousness and engage in substantial amounts of nonsocial behavior and aggression.

### ***Behavioral flexibility as an evolutionarily adaptive strategy for spiny mice***

The PCA data confirmed a relatively predictable, stable phenotype exhibited by gerbils and a more scattered, unreliable phenotype for spiny mice. The lack of a stable phenotype in spiny mice may indicate that flexibility has proven to be the most evolutionarily adaptive behavioral characteristic while living in less stable environments. It is possible that spiny mice have developed incredibly flexible social behavior in order to accommodate the shifting dynamics of group living. Behavioral flexibility has served as an important adaptive response to changing environments in other animal species (Dukas, 1998, Shettleworth 1998, Reader and Laland, 2003). Behavioral plasticity is well suited for rapid responses to external environmental changes. Spiny mice display increased aggressive behavior towards a male intruder in the presence of pregnant or lactating females (Cizkova et al., 2011). Thus, aggressive behavior may be associated with the reproductive status of females which is constantly changing. This provides another example of the potential for behavioral flexibility in spiny mice. It will be interesting to see if any new patterns of behavior are revealed in spiny mice or if the flexible behavioral phenotype remains and if initial behavioral phenotypes are confirmed in gerbils once the male data is combined with the female data and statistical power is increased.

### ***Personality/Behavioral Phenotype and Translational Implications***

Research over the past 10 years has established that personality traits exist and can be measured in nonhuman animals (Gosling, 2008). Consistent behavioral phenotypes and characterized individual differences can help researchers better understand the ecological and evolutionary implications of certain traits (Bell and Stamps, 2004; Carere and Eens, 2005; Dingemanse and Reale, 2005; McElreath and Strimling, 2006). If certain constraints limit the flexibility of an individual’s behavior, it is possible that these specific traits serve a particular adaptive function. Certain environments may give rise to evolutionarily adaptive species-typical traits. For example, in an environment with scarce resources, it may have been advantageous for individuals to be highly aggressive in order to defend their territories. In an area with abundant resources, this same highly aggressive phenotype may hinder an animal’s ability to find potential mates (Dingemanse et al., 2004; Groothius and Carere, 2005). Therefore, by characterizing species-typical behaviors as was done in this study, we may be able to better understand how environmental pressures can promote the development of certain animal personalities while simultaneously hindering the development of less advantageous behavioral phenotypes. Although human research remains necessary, animal studies on personality can offer a unique opportunity to address longstanding questions such as the interplay of biological and environmental factors on personality development or the impact of personality on health that have proven difficult to answer with human studies alone (Gosling, 2008). Advantages afforded by animal studies include more flexibility with experimental manipulation over subject's environments, genetics and pharmaceutical treatments (Ferris and Delville, 1994; De Jonge et al., 1996; Chiavegatto et al., 2001). Additionally, researchers have more opportunities to explore underlying neural mechanisms for the expression of certain behavioral traits. From the PCA conducted on female gerbils in this study, PC1 helped to establish a correlation between large group affiliation and preference for novelty. PC1 suggested that the more time an animal spends with a familiar stimulus, the less prosocial it is with novel conspecifics. This fits with our original hypothesis that a highly social, gregarious animal would have to have a preference for novelty at least to some extent; without the preference for novelty, the animal would likely not seek out large groups that contain novel individuals. Similarly, one may expect that animals that are generally less prosocial may also have a tendency to prefer familiarity; familiarity is a known entity. However, PC2 suggested that social boldness may be a more important indicator of gregariousness than a preference for novelty. PC2 suggested that the quicker an animal approaches another novel animal, the more gregarious they are. More specifically, a gerbil that more readily approaches a novel animal also prefers to affiliate with a larger group. If an animal is readily associating with a large group, it is likely they are constantly bombarded with social interactions and would be more inclined to approach a novel conspecific. Due to the formation of long-term, socially monogamous pair bonds and the conservation between mechanisms underlying social behaviors in prairie voles and social cognition in humans (Gingrich et al., 2000; Young et al., 2001; Liu and Wang, 2003), prairie voles have served as a common model organism for understanding social cognitive deficits including autism spectrum disorders (McGraw and Young, 2010). However, both gerbils and spiny mice can serve as model organisms and allow for the study of non-reproductive sociality. In particular, studies of non-reproductive sociality may offer more translational insights into humans on the autism spectrum who can more readily form attachments with family members, but have more difficulty forming attachments with strangers (Chevallier et al., 2012).

### ***Future Studies***

There is a rich literature on the involvement of tyrosine hydroxylase and social behavior. Data across a variety of vertebrates has demonstrated differential activation of neurons containing tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine synthesis dependent upon the type of social stimulus (Charlier et al., 2005; Bharati and Goodson, 2006; Goodson et al., 2009; Northcutt and Lonstein, 2009). Because TH is presumably dopaminergic (Daubner, Le, and Wang 2011), examining TH neural activity can provide insight into how reward circuitry is activated during social interactions. Catecholamines have been implicated in affiliative behavior outside of immediate sexual and parental contexts including partner courtship and clumping behavior (Alger, Juang, and Riters 2011). When male prairie voles cohabitate with females, there has been evidence of elevated Fos expression in TH-positive neurons (Alger, Juang, and Riters 2011). This phenomenon has been identified in other vertebrates such as male zebra finches who court showing an increased number of TH-positive cell bodies compared to males who do not (Alger, Juang, and Riters 2011). More gregarious species may find interactions with novel conspecifics to be more rewarding. Future studies can compare TH-Fos colocalization in dopaminergic rich regions like the ventral tegmental area (VTA) and nucleus accumbens (Nacc) after conducting an immediate early gene (IEG) test where a subject is exposed to a novel conspecific or a novel object to see if social novelty differentially activates the reward neural circuitry. A comparative approach between the highly social African spiny mouse and the moderately social Mongolian gerbil can examine TH-Fos colocalization during exposure to a novel conspecific to see if animals that live in larger groups in the wild find interactions with novel conspecifics to be more rewarding.

### ***Conclusion***

These findings highlight that spiny mice may be particularly well-suited for studies of prosociality and gregariousness. Although there are numerous species for studying social behavior, they are primarily appropriate for studying sociality in a reproductive context. The data from the social interaction and group size preference test in particular emphasize that spiny mice would be ideal for studying prosocial behavior in non-reproductive contexts. Spiny mice may be able to bridge the gap in studies of sociality and serve as a model organism not only for studying reproductive sociality, but also non-reproductive sociality. Although several avian species exhibit non-reproductive sociality (Goodson et al., 2009; Kelly et al., 2011), this phenotypical trait in mammals is rare. Therefore, spiny mice may be able to provide more translational insight into human non-familial and/or non-romantic relationships. Furthermore, principal components analysis revealed that spiny mice may have a highly flexible phenotype whereas for gerbils, social boldness, and not a propensity toward social novelty, may predict gregariousness. The lack of predictable phenotype in spiny mice may reflect an evolutionarily adaptive flexibility needed for large-group living and the ability to exhibit context-appropriate behavior in a changing environment. Whereas for small group living species like gerbils, exhibiting a stable phenotype may be the most adaptive strategy.

## Contributions

I obtained experience conducting behavioral tests like those in this thesis in the Kelly Lab prior to the onset of the coronavirus pandemic. I was trained in conducting social behavior tests, how to do perfusions, and how to section brains on a cryostat in prairie voles. I was unable to physically conduct the behavioral tests in this thesis due to a policy of no undergrad researchers being allowed in the lab for safety reasons surrounding covid. The behavioral tests in this thesis were conducted primarily by Jose Gonzalez, as well as Brandon Fricker. Brandon Fricker scored the spiny mouse behavior videos, while I scored all of the gerbil behavior videos. I conducted all statistical tests for data analysis and wrote the thesis under the guidance of Dr. Kelly.

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