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GETTING TO KNOW YOU: RELATIONSHIP FORMATION ACROSS DEVELOPMENT IN *OCTODON DEGUS*

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Getting to Know You: Relationship Formation Across the Lifespan in *Octodon degus*

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Social relationships are a necessary part of group formation and cohesion across many animal species and can increase the health and fitness of the individuals involved. These benefits are seen across the lifespan, although the exact advantages and functions differ across developmental stages. Our current understanding of how relationships are formed and maintained is incomplete. Previous findings suggest that a measure of stability may be a useful method for understanding how relationships are formed and how this process may differ across development. The goal of this dissertation is to investigate the variability of social interactions over time and how it is affected by familiarity, sex, and age. We focused on same-sex peer relationships in degus, a highly gregarious rodent species native to Chile. Animals were repeatedly exposed to familiar and unfamiliar same-sex conspecifics in two sets of experiments. The first assessed the effects of sex and familiarity on social interaction variability in adults, while the second examined how this changed over stages of development. We found that total interaction levels depended on familiarity, sex, and age. Variability of interactions differed between strangers and cagemates in adult degus, with female strangers displaying more consistency in their social interactions than female cagemates and adult males. Juvenile and adolescent female degus did not show differences in the variability of social interactions due to familiarity, and variability of the interactions decreased over time. Together these results, while unexpected, suggest that the variability of social interactions cannot be used alone as a general method of describing relationship development. They do indicate that in females, familiar individuals have more variability of interactions while unfamiliar individuals act in a less variable, more predictable manner. These differences warrant further research using variability, in addition to other measures, to describe and understand social relationships and their development.

Keywords: social relationships, Octodon degus, development, relationship formation, familiarization, support vector machine, dyad classification, variability

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Chapter 1: What are social relationships in non-humans and how are they studied?

Many researchers have taken a functional approach to describing social groups and ‘relationships’ in non-human animals and often focused on specific types of relationships such as dominance and affiliative relationships (Kummer, 1978). In more recent years, however, research on relationships has departed from the lens of Darwinian selection and has begun to show that non-human relationships can be more than just a strategy to increase survival. As more research turns its eye towards understanding the nuances of animal relationships, it becomes more apparent that these animals are forming relationships that are highly complex. How relationships are studied and classified, however, is still inconsistent across species. A more generalizable method of understanding relationships is therefore useful, highlighting the need for more research on this topic.

Importance of Social Relationships

Within the animal kingdom, many species form and maintain social groups in order to survive. This survival strategy comes with various costs and benefits, such as an increased risk of disease and decreased risk of predation (Hinde, 1976; Majolo et al., 2008). Relationships that form between individuals within a social group have been shown to influence the cohesion and success of the group, and even the health and overall fitness of the individuals. New groups of animals that do not have an established group dynamic or even relationships with others within the group have been shown to result in an increase in stress hormones. One example of this trend can be seen in a study of rhesus macaques. Hair cortisol concentrations were collected from stable and unstable social groups. The number of aggressive episodes and changes in the group hierarchy was used to distinguish group stability. Hair cortisol concentrations in the unstable group were significantly higher than in the stable group (Vandeleest et al., 2019). These increased stress levels can have negative consequences on the health of the individuals, indicating that stable group dynamics with established relationships are crucial to group cohesion and to the health of each individual in the group (Sapolsky, 1990).

On the individual level, unstable or unestablished relationships also have negative consequences. Many species of fish, for example, have increased stress levels when confronted with an unfamiliar other, but as the relationship becomes more established (i.e., as they fight to determine the dominant and submissive roles) the stress levels decrease and eventually return to baseline levels (Corrêa et al., 2003; Overli et al., 1999). This demonstrates that the formation of established relationships also influences the health of each individual.

When group dynamics and individual relationships are well established, stress levels decrease, and benefits of group-living and social relationships become apparent. Hyenas, for instance, use their close social associations to gain access to food and are more likely to provide aid to a close association in a conflict (Holekamp et al., 2007). Furthermore, the more social relationships an individual has lowers their mortality risk (Ellis et al., 2017), increased survival rates for that animal (Brent et al., 2017; McFarland & Majolo, 2013), and increased survival rates for the offspring of that individual (Cheney et al., 2016; McFarland & Majolo, 2013). Altogether, this indicates that social relationships are beneficial for the mental, physical, and overall well-being of the animals.

As the stability of relationships within a group influence not only the group dynamics but also the health and overall fitness of those involved, it is important to understand how stable relationships are formed. This is essential for species where new individuals frequently immigrate into the group. A constant influx of new individuals requires the original members of the group to quickly form a relationship with the newcomers. The importance of stability of social groups and individual relationships highlights the need for a cross-species marker of relationship development and stability.

How are Social Relationships Measured in Non-humans?

Hinde (1979) defines a relationship as the outcome of multiple interactions between two individuals and measuring these relationships must come from observations of these interactions. The description of the interactions requires the content, quality, and how the interactions are patterned across time and between the individuals. While Hinde's suggested definition and methods have been widely implemented, there are some critiques of this definition that are important to consider. Whitehead and Dufaut (1999), for example, argue the importance of creating a definition of relationships based on the species of interest. The content and quality of interactions can differ greatly between species. Indeed, baboons will use grooming as both an affiliative and agonistic behavior while other species of primates (and other mammals) only use grooming as an affiliative behavior (Barrett & Henzi, 2002). It is, therefore, necessary to make the patterning of the content and quality of interactions species-specific. Furthermore, Barrett and Henzi (2002) point out that the push to operationally define relationships places the focus of the research onto the outcomes of the behaviors rather than the dynamics of sociality. This view may then lead to an incomplete picture of the social processes of that species. The partial view of the species' social processes is further emphasized by Whitehead and Dufaut (1999) who also argue that the summary of interactions would be the most beneficial and informative under a multivariate representation. A multivariate approach would be beneficial to understanding the social processes of the species of interest as there are multiple factors that could be used to define relationships (content, quality, quantity, etc.). Additionally, the factors included in the multivariate approach can also capture the dynamics of the interactions taking the concerns of Barrett and Henzi (2002) into account.

Silk, Cheney, and Seyfarth (2013) expanded on the measurement of relationships provided by Hinde (1979) and worked to add multiple factors or behavioral dimensions for researchers to use when describing a relationship. Frequency of the interactions, diversity of behaviors engaged in, the tension of the interactions, the overall tenor (agonistic, affiliative, etc.), predictability of the behaviors, and the responses and stability of the interactions over time were all proposed to describe and quantify a relationship. The values of each behavioral dimension can then be used to understand the type of relationship a dyad might have. If, for example, a dyad has a high frequency of interaction with an overall affiliative tenor that is predictable and stable over time, it can be determined that this dyad has an established affiliative relationship (Silk et al., 2013). These behavioral dimensions can then also be used to determine the familiarity level of the individuals involved. Not only are the values of certain dimensions found to differ between familiar and unfamiliar dyads, but the stability of these values should also be considered. Stability in the case of animal relationships has been defined as the consistency and continuity of the interactions (Hinde, 1979). Continuity refers to how many interactions two individuals have over a set time and when they interact (time of year, after being separated, etc.) while consistency refers to the behaviors used during the interaction. For example, a relationship between two individuals who frequently interact but their interactions contain different behaviors would be continuous but not consistent. The majority of research in this area uses either the continuity of the relationships of certain behaviors and/or the consistency of the interactions and behaviors to determine the stability of relationships. Either one of these descriptors or both can be used because established and stable relationships can be continuous, but not consistent in certain behavior types or they can be continuous and consistent over time.

Overall, researchers in this field seem to agree that when initially investigating the social relationships of a species, including as many behavioral dimensions as possible is the best method. There is no consensus on a method of research to capture relationships in a more universal (across species) way; however, the use of ‘continuity’ and ‘consistency’ of relationships may provide a promising lead.

Measuring the Formation of Social Relationships

If consistency is an important measure of relationships, this raises questions of how to study the formation of relationships, which might be expected to be inconsistent. Much of the research on the development of new relationships has been focused on specific types of relationships. While this focus on specific relationship types and their formation is valuable to the species of interest, it does not often translate across species.

Some research has used time spent in proximity, grooming, and aggressive interactions to mark the differences between established and unestablished relationships. Male chimpanzees and female baboons, for example, have been found to consistently groom and spend time in proximity to those with whom they have formed a social bond over the course of many years (Massen & Koski, 2014; Massen & Sterck, 2013). Crayfish, lobsters, and even mice demonstrate increased latency to fight and a reduction in the amount or severity of fights when confronted with a familiar conspecific (another individual of the same species) and in subsequent interactions with that same conspecific (Gherardi et al., 2010; Graham & Herberholz, 2009). The initial change in the latency to the first behavior, agonistic or otherwise, indicates the switch from an unfamiliar to a familiar dyad. This latency then stabilizes across interactions, indicating that the individuals have formed an established or stable relationship.

Other indicators of the familiarity of the individuals interacting that have been used in research are vocalizations and variability in individual behaviors. Vocalizations have been shown to increase in frequency when directed at unfamiliar individuals (Sasaki et al., 2020). Individual behavior during social interactions, such as the types of agonistic behaviors used or the boldness (e.g., number of approaches to the conspecific) of certain individuals has also been shown to differ depending on familiarity. When male crickets interact with familiar individuals, they display less variance, as measured by observed changes in aggressive behaviors displayed, in individual aggressive behaviors and these behaviors are more stable with repeated interactions. In other words, the behaviors displayed during the first interaction were consistently displayed in subsequent interactions (Jäger et al., 2019).

The changes in the variability of aggressive behaviors indicate that as the behaviors are decreasing in their variability as the relationship progresses, the established relationship may be a stable one. This idea can also be found in other research done in mice. Lee and colleagues (2019) determined whether the relationship between two male mice was established through the frequency of certain behavior types (e.g., aggressive, or submissive) and whether those frequencies are performed equally by both individuals or not (symmetry). The stability of these frequencies and their symmetry also indicated the time course of the relationship. A dyad was only considered to have an established relationship when the symmetry and frequency of their behaviors remained stable across all subsequent interactions (Lee et al., 2019). This provides further support for the idea that the variability of the relationship may be a useful marker to identify when an established relationship between two individuals is formed.

Another example of the use of stability to indicate the formation of a relationship can be seen in the formation of food-sharing relationships in female vampire bats. Carter and colleagues (2020) tracked the process through which these bats test and grow relationships, using the first instance of food-sharing as their marker of an established relationship. When testing whether a stranger would make a trustworthy partner, all bats slowly increased bouts of grooming to see whether the stranger reciprocated. Exchanges of grooming increased until one of the bats shared their meal, or until the grooming was not reciprocated, in which case, food-sharing did not occur. Much like the male mice, each dyad demonstrated the same process of testing and forming relationships. The process of testing and forming new relationships was variable, in that the amount of grooming that occurred between a dyad would increase across interactions until the first instance of food-sharing occurred. This instance of food sharing marked the point of an established relationship and further evidence for this claim can be seen in the grooming rates reported afterward. The amount of grooming between the two individuals with an established relationship stabilized across subsequent interactions, meaning that the time they spent grooming did not change between interactions.

The common theme that appears across all studies that implement different behavioral dimensions to measure the formation of relationships is stability. While these studies do not explicitly report on stability, it is implied through their findings. In the study on male mice, for example, relationships, once established, were stable. The dominant and submissive mice only displayed behaviors related to their status and this did not change across sessions (Lee et al., 2019). Furthermore, the grooming rates of vampire bats stabilized after their relationship was established (marked by the first instance of food-sharing; Carter et al., 2020). Regardless of the type of behavioral dimension that is used, familiar individuals tend to have some aspect of their social interactions remain stable over time.

“Some aspect” of their social interactions is highlighted here in an attempt to demonstrate that not all of the behaviors or behavioral dimensions used during interactions will remain consistent or continuous as the relationship progresses. In fact, some amount of change is expected to occur in a relationship between two individuals, as outside forces, such as food supply, group dynamics, and the individuals themselves are subject to change as well (Hinde, 1979). Male chimpanzees, for example, have demonstrated consistency in the amount of time they will spend in proximity to one another, but the symmetry of the interactions is not consistent and decreases over time (Koski et al., 2012). This demonstrates the importance of using multiple behaviors and behavioral dimensions to describe relationships. Without the inclusion of proximity scores and symmetry of interactions, we would not have a clear picture of the stability of long-term relationships. It is therefore crucial, especially when investigating a species in which the dynamics of long-term relationships are unknown, to include multiple behaviors and behavioral dimensions initially.

Few studies have investigated the process through which two same-sex individuals form a relationship and those that have focused on the development of a specific type of relationship, such as opposite-sex bonds or dominant-subordinate relationships. In order to compare relationship formation across species and even across the lifespan, however, a more general method of describing relationships that is independent of the type of relationship formed is necessary. When looking at the overall findings of the research presented here, we see that the consistency or stability of behaviors change during the formation of agonistic, affiliative, and dominance relationships. Measures of stability of behaviors, such as the variability of behaviors within and between interactions, may therefore be a more general method that could be used for direct comparisons between species and ages.

Chapter 2: Relationships Across the Lifespan

Introduction

Bekoff (1972) said that in order to obtain a comprehensive understanding of relationships within and between species, it is necessary to investigate these processes across the lifespan. There is much research on social behaviors across the lifespan, but how it is studied (i.e., the measures used, the methods, etc.) varies greatly. There is very little research, however, on how new relationships are formed at different developmental stages. Even in humans, the process of friendship formation, especially in children, is not well known (Bowker & Weingarten, 2022). The characteristics of social interactions and relationships, as well as the function of social interactions does change across the lifespan, suggesting that relationship formation may also differ with age.

Differences in the Function of Relationships Across the Lifespan

The function of social interactions in adults likely includes physical benefits that come from group living and social relationships. For example, the reproductive fitness of a female baboon has been found to increase with larger numbers of social relationships (Cheney et al., 2016). In younger animals, however, the function of social interactions is related to their overall developmental success. The function of social relationships and the interactions can be seen best through studies of social deprivation. Learning appropriate social responses, cognition, memory, and stress responses are also affected by social isolation or deprivation of play (Palagi, 2018; Pellis et al., 2010). For example, adult rats who were deprived of play during their development tend to be hyper defensive and more aggressive when interacting with unfamiliar peers. In addition to the behavioral and cognitive consequences of play deprivation, the necessary pruning of neurons, and the formation of necessary connections within the brain can also be impaired (Bell et al., 2010; Blakemore, 2008). In rats, neurons in the orbitofrontal cortex and medial prefrontal cortex require social experiences such as play during development in order to develop normal responses (Bell et al., 2010). Furthermore, patterns of c-Fos activation in response to social stimuli have been found to differ in strength and effect between adolescent and adult rats (Varlinskaya et al., 2012). The differences between the developmental stages in the neural processes. Cognitive, and behavioral responses that underlie sociality and responses to social stimuli further support that the function of social interaction differ between adults and young animals.

The differing functions of social behaviors for adults and juveniles/adolescents can be seen more directly during play. Juvenile rats play more than adults and are more likely to test boundaries and new strategies when playing, suggesting that they are using the play experience to learn what behaviors warrant certain responses and what may work for them when playing with others in the future (Pellis et al., 2005). This finding also suggests that juvenile rats have more variable play interactions than adults do. In humans, relationships between age-matched infants and preschool-aged children aid in the improvement of social skills such as, perspective-taking, communication, and amicable play behaviors (Bukowski et al., 1998; Gottman et al., 1975). As the function of relationships during the early developmental periods is to learn and test out different behavioral strategies, then the social interactions that occur during these stages are more likely to be variable (less stable). Previous research has shown that the more variability that an individual encounters when learning certain tasks, improves their performance on those tasks during later testing. This finding occurs across multiple disciplines, but whether this is true for social behaviors is still not well known (Raviv et al., 2022).

The differences the previous work has focused on highlights the differences of behaviors between juveniles and adults, and adolescents and adults. There is research that has also identified that the function of social behaviors in juveniles also differs from those seen in adolescents. In freely interacting wild macaques, for example, social play and grooming behaviors have been shown to provide a different function for juveniles than for adolescents. Social play and grooming in juvenile macaques help to strengthen social bonds, with play influencing the strength of the bond more than grooming (Shimada & Sueur, 2018). Social play and grooming in adolescence, however, tend to perform the function of defining their place in the hierarchy and maintaining group cohesion (Kulik et al., 2015).

Relationship Formation Across the Lifespan

As the function of social relationships differs across the lifespan, it then follows that how relationships are formed and maintained would also differ with age. There is evidence that social interactions differ between familiar and unfamiliar individuals in young animals. For example, Yamada-Haga (2002) demonstrated differences in the behavioral sequences of agonistic interactions in unfamiliar male mice between juveniles and adults. Juvenile mice often would use pouncing to transition between different agonistic behaviors, while adults had more diverse transitions such as lateral display following nosing while upright posture transitioned from pawing, nosing, and approaching. However, there is currently no research investigating how these social interactions may change over time as the unfamiliar dyads became familiar with one another.

One example of the effects of familiarity on social interaction comes from work with female rats. Periadolescent female rats displayed higher levels of social investigation and play behavior, and less time in passive contact or allogrooming with unfamiliar compared to familiar dyads. Additionally, females showed higher levels of digging with familiar partners and lower levels of play soliciting with unfamiliar partners (Cirulli et al., 1996; Livia Terranova et al., 1999). These findings suggest that as there is an observable difference in the social interactions of familiar, and unfamiliar individuals.

The common theme of the research on adult relationship formation was that relationships became more stable as they progressed, whether this trend is also present in younger animals, is hard to determine. The process through which relationships are formed in juvenile and adolescents is not yet well researched and no study, to our knowledge, has investigated social relationships over multiple encounters in juvenile or adolescent non-human animals. Much of the previous work has focused instead on the stability of social networks across development. For example, male juvenile ground squirrels show a partner preference, which remained stable over time (Nunes et al., 2015). The results of these studies indicate that the choice of partners may be stable over time, but they do not explore the variability of the dyad's interactions. In other words, these results suggest that the social network of these individuals is stable, but a stable social network does not necessarily mean that the interactions of these individuals are stable.

There are a few studies that suggest juvenile and adolescent non-human animals do form stable social relationships over time which indicates that new relationships may be variable, and therefore unstable. The stabilization of relationships in younger individuals may, however, take longer to occur. In humans, for example, five- and six-year-old children demonstrated relationship stabilization over the course of a year (Wang et al., 2019). In non-human animals we see similar findings. Wild female juvenile and adolescent gelada baboons, for instance, demonstrate stable grooming partners and grooming rates over multiple years (Barale et al., 2015).

Further support for the potential importance of stability of social relationships can be seen in studies that manipulate social stressors by creating unstable social situations. In adulthood, unstable social relationships are linked to increases in stress, as measured through cortisol (Corrêa et al., 2003; Vandeleest et al., 2019). In adolescent and juvenile golden hamsters as well as male and female adolescent rats, social instability increases cortisol levels (Asgari et al., 2021; Hodges et al., 2017; McCormick et al., 2007). Unlike the research done on social instability in adults, these studies define social instability as the changing of housing conditions. It may therefore be that juvenile and adolescent animals prefer stable partners, rather than constantly being exposed to new individuals. Whether social instability in regard to the variability of interactions with other individuals is stressful for young animals is not yet known. It may therefore be true that while young animals prefer stability in their peer group, the interactions of those individuals may be highly variable.

The current body of literature on social relationships during early development is limited but does suggest that much like adults, juvenile and adolescent animals interact differently with unfamiliar individuals and prefer a stable social network. The specifics of the interactions of juvenile and adolescent animals, however, do differ from those seen in adults. Furthermore, the function of social relationships changes across the lifespan, with learning being one of the main functions for juveniles and adolescents. The differing functions and interactions from adult animals suggest that how young animals form new relationships may also be different, but this has yet to be directly investigated.

Chapter 3: Researching Relationship Formation in Octodon degus

The importance of social relationships has been well documented across many taxa but understanding how relationships form and how this process differs at different developmental stages is still not well understood. One reason for this lack of understanding may come from the absence of an agreed-upon measure for social relationship that is generalizable across species, development, and relationship type. Consideration of the *stability* of social relationships (i.e., consistency in the interaction patterns between two individuals) is a common trend found across much of the research on relationships and their formation. This measure, however, is often treated informally and differently across different studies, in this dissertation I apply new, formal measures of "interaction variability" to better gauge the stability of relationships in adult and developing animals. Specifically, I use this to study relationship formation in Chilean degus.

Why degus are valuable for studying relationship formation

In order to research the process through which new relationships are formed, a highly gregarious species that tends to interact and form new relationships with strangers was needed. This is true of *Octodon degus* (the degu) a species of caviomorph rodent native to South America. Degus have many unique behaviors, social structures, and traits that make them a useful model for the study of social behavior. Their group dynamics, stability of their groups, their ability to recognize familiar peers, and differences in interactions over time and between the sexes make this animal a useful model for the investigation of how relationships are formed and change over time. Laboratory-based research on this species has strengthened the claim that the degus are particularly predisposed to be affiliative with unfamiliar others, are highly motivated to be social, and can be beneficial to the area of social behavior research (Colonnello et al., 2011). Social recognition studies done with degus have demonstrated that degus spend more time investigating unfamiliar individuals than familiar ones (Uekita & Okanoya, 2011).

Degu behaviors during interactions with individuals differ in many ways from other rodent species. Rats, for example, play fight much more often than degus (Pellis et al., 2010). Male mice, compared to male degus, can be very aggressive. They fight and establish dominance during an interaction with a same-sex stranger (Kondrakiewicz et al., 2018). This effect is dependent on the strain and life history of the mouse. While male degus do engage in agonistic behavior when presented with an unfamiliar male, the types of agonistic behaviors used differ from mice and they are often less severe, resulting in fewer injuries; (Fischer et al., 1986; Fischer & Meunier, 1985; Lee et al., 2019; Meaney & Stewart, 1981). Male-male social interactions also become more tolerant and less aggressive during the non-breeding seasons (Soto-Gamboa et al., 2005). Female degus, when compared to prairie voles, are much less aggressive and agonistic towards same-sex others. When female prairie voles have pair-bonded they are much more likely to act aggressively towards other females (Lee et al., 2019). Aggression between female degus, on the other hand, is rare (Fischer & Meunier, 1985; Fulk, 1976).

A systematic examination of differences between degus and other rodent species uses the partner preference tests. Rats and mice do not show a peer preference and prefer to remain in neutral areas (Beery et al., 2018; Lee et al., 2019). Prairie voles and meadow voles display a partner preference for same-sex familiar peers (Lee et al., 2019). Degus do not show a partner preference but will split their time huddling with both the stranger and familiar conspecific (Insel et al., 2020). This may indicate that degus have an innate drive to interact with strangers and familiar individuals, unlike other rodent species such as the prairie vole. This is emphasized by the finding that degus are the only rodent species that display huddling behaviors with unfamiliar conspecifics (Beery & Shambaugh, 2021).

Further justification for the use of this species can be seen when comparing their developmental periods to those of other rodent species. Compared to rats and mice, degus have a longer period of adolescence and reach puberty between two and three months of age. The slightly longer adolescent period can be useful for the study of long-term social relationships in younger animals, which is not as easily accomplished in rats or mice (Colonnello et al., 2011).

Social behavioral ecology of the degu

The family of caviomorph rodents to which degus belong are highly variable in their social structures and behaviors. Of those, only a few have been studied in the laboratory setting, e.g., *Cavia porcellus* (guinea pigs) and *Ctenomys sociabilis* (tuco-tucos). Like degus, the tuco-tuco does not display a preference for familiar or unfamiliar peers in partner preference tests; unlike degus, however, they also tend to have highly flexible social strategies and will often remain solitary, suggesting that they are not as motivated to be social and tend towards social tolerance rather than social bonding (Ebensperger & Hayes, 2016). Domestic guinea pigs, on the other hand, live in social groups consisting of dominant males, subordinate males, and harems of females. Female guinea pigs display social hierarchies, although they are weak and tend to change frequently. Females also do not appear to display preferred social associations, other than with their dominant males. These social patterns differ from those of degus, which form consistent same-sex social associations (Ebensperger & Hayes, 2016; King, 1956).

In the wild, degus typically are found in large communities within one area that consists of multiple smaller groups made up of one to two males and two to five females (Ebensperger et al., 2004, 2006). Unlike many species, a non-kin group structure is typical of this species with natal philopatry, non-sex-based dispersal, and high predation rates leading to high turnover which drives group formation (Davis et al., 2016). This species has also been found to communally raise their young (Ebensperger et al., 2004). Within their communities' female degus tend to form distinct social units of around two to four individuals. These females shared nest sites at night, and during the day interacted more with their co-nesting group members than with other females. Another study observed and reported that male and female degus, in their natural habitat, act more agonistically towards intruders and were more affiliative with other residents of their groups (Fulk, 1976). Interestingly, this ability does not seem to be entirely kin-dependent. Unlike most other species that rely on the olfactory system and chemo signaling, degus will differentially investigate a conspecific's urine based on familiarity level of the individuals when reared apart, regardless of their genetic relatedness (Villavicencio et al., 2009). This indicates that degus may change their behaviors and interactions with others based on their level of familiarity and not their relatedness.

Groups of wild degus can experience high rates of turnover. Young animals immigrate into new groups and emigrate from their own groups, and high predation rates lead to the disappearance of many group members. The individuals in the group, especially females, are therefore exposed to new unfamiliar individuals often and must find ways to live and interact with each other quickly (Davis et al., 2016). Interestingly, the impact that the group dynamics has on the degu tend to be sex specific. Stable group dynamics has been found to increase reproductive fitness in female degus, while males receive more reproductive fitness when their group is unstable (Ebensperger et al., 2009, 2017). The fitness benefits that are seen for males and females in stable and unstable groups suggests that females may be more motivated to form stable relationships with new individuals to maintain group stability, while males do not.

Another study by Davis (1975) compared the interactions of two unfamiliar male degus to interactions of the same two males one month later. It was observed that the initial encounter was asymmetrical in nature, with one degu initiating most of the interactions. Interactions tended to begin with olfactory investigation of the head and rear of each individual and fighting also tended to occur early in the interaction. Subsequent interactions were reported to be more symmetrical, with less fighting, increased latency to the first behavior, and more variable in the behaviors that were demonstrated (Davis, 1975). These findings, however, were not analyzed with any statistical methods, nor did they compare repeated interactions with unfamiliar individuals to those of familiar individuals. Fischer and colleagues (1986) investigated potential sex differences in initial encounters with unfamiliar same-sex partners in degus. It was found that females interacted more than males, and males engaged in more agonistic behaviors than females, who were more affiliative than males. This study was unfortunately underpowered and also did not investigate how these interactions changed over time so inferences cannot be confidently drawn from them.

One recent study investigated differences in the frequency of interaction and frequency of behavior types used in an initial interaction of two individuals. They compared the behaviors and sequences of those behaviors used during interactions with two familiar and unfamiliar individuals. A significant difference in the total time spent rear-sniffing and vocalizing was observed with unfamiliar dyads engaging in more rear-sniffing and vocalizing than familiar dyads. Additionally, the total time unfamiliar dyads spent interacting was higher than familiar dyads, but the difference approached significance (Lidhar et al., 2021). These findings suggest that there are behavioral differences in how individuals interact with familiar and unfamiliar individuals, although, how these differences change over time is still not known. There is also no work on how these differences differ between the sexes or change across the lifespan.

The previous research done with degus demonstrates their usefulness for studying relationships and their formation. The behaviors the degu displays both in the wild and in laboratory-based research highlight the differences in relationships between the sexes and make this species ideal for the search of a method to describe relationships generally. Furthermore, the motivation of female degus to interact with new individuals and form stable social bonds with their peers suggests that this species is unique and lends themselves to the investigation of the variability of social interactions as a measure of relationship formation.

Chapter 4: Goals, Hypotheses, and Procedures

The overarching goal of this dissertation is to gain a better understanding of the process by which degus form peer relationships by considering the stability (or conversely, variability) of their interactive behaviors. The main hypothesis of this dissertation is that new relationships (i.e., between initially unfamiliar degus) are more variable in the sets and levels of social interaction than familiar relationships. This predicts that A) measures of variability will be lower in long-term cagemate dyads relative to dyads that are initially strangers, and B) with increasing time and familiarity, initial stranger da will become less variable.

This dissertation additionally asks a second question: whether relationship formation differs development and adult stages lifespan stages. Research suggests that younger animals interact with familiar and unfamiliar individuals differently (Chapter 2). The function and types of social interaction also differ across these stages (Cheney et al., 2016; Palagi, 2018; Pellis & Pellis, 2007). Social learning is important during development, and play behavior is known to take place with both familiar and unfamiliar peers. Here I test the hypothesis that juvenile and adolescent degus display high variability in their social interactions regardless of the familiarity levels of the individuals. This predicts that when presented with a repeated-reunion protocol, juvenile and adolescent degus will not display variability differences between unfamiliar and familiar peers, nor will variability decrease over sessions. A brief overview of the methods and statistics used to test these hypotheses is given below. A more detailed explanation of the methods and statistics are presented in subsequent chapters.

Experimental Procedure

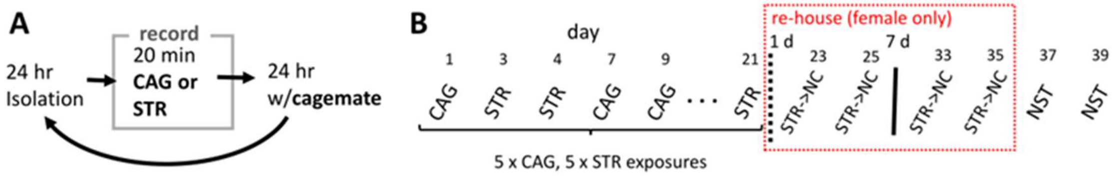
In order to investigate how the variability of social interactions changes over time, we repeatedly reunited familiar and unfamiliar same-sex dyads over the course of 20 to 40 days. Prior to the reunions, animals were acclimated to a testing chamber by placing them alone in the chamber for at least 5 minutes each day for 5 days. Then, over a 20-day period, each degu experienced five, 20-minute “reunion” sessions with a familiar partner (cagemate) and five, 20-minute reunion sessions with an unfamiliar partner (stranger; Figure1A). The order of these sessions was pseudo-randomized and counterbalanced to avoid order effects. The use of five-session is consistent with previous work examining relationship development in mice (Lee et al., 2019). All degus were isolated for 24 hours prior to each reunion to maximize the time that animals interact during the recording session (consistent with observations made by Lidhar et al., 2021).

Preliminary results from the adult female reunion indicated that even after 5 sessions, stranger dyads continue to show higher levels of interaction than cagemate dyads. To allow the strangers more time to form stable relationships, they were co-housed in their home cages and then were reunited for two more sessions. A subset of adult female strangers was further co-housed for a period of 7-days and experienced 2 additional 20-minute reunion sessions (see Figure1B). Adult males were not run through this paradigm due to the potential risk of injury due to fighting in the homecage.

An additional two reunion sessions with a brand-new individual were run following the conclusion of the co-housing period (females) or the 10 reunion sessions (males) to control for behavioral changes over the course of the protocol that are not related to social novelty. This also allows for comparisons in the behavior types and strategies between two unknown conspecifics. This comparison highlights whether the behavioral differences are explained by the level of familiarity or individual differences. If there was no significant difference between the behavioral types and strategies in stranger dyad A and stranger dyad B, it could then be inferred that degus use a general strategy to interact with unknown individuals. Furthermore, this comparison allowed us to control for the potential influence of increased time or increased social exposure as we were able to compare whether the behaviors and stability of the dimensions are solely due to increased experience.

Figure 1

Experimental Design



Note. A) Degus received a repeated sequence of 24 hr isolation, 20-minute reunion recording sessions, and 24 hr co-habitation with cagemates. CAG = cagemate reunion, STR = stranger. B) Sequence of recording sessions over days. Cagemate and stranger reunions were interleaved. Degus were subsequently housed with the initial strangers creating “new cagemates” (STR->NC) tested after one day, and then after one week. In two final reunion sessions, they were presented with a new stranger (NST).

In order to investigate potential differences in relationships and relationship development across the lifespan, additional groups of degus were tested at two developmental stages. First, degus were tested when they were juveniles, and then again as adolescents, soon after they displayed signs of sexual maturity (vaginal opening in females and penile spikes in males). These two age groups were chosen based on previous findings indicating that there are differences in the social behaviors and the function of relationships between juveniles, adolescents, and adults (Kulik et al., 2015; Pellis et al., 2005; Shimada & Sueur, 2018). Degus are considered to be juveniles from PND 28 to 35 and adolescents from PND 56 to 112. Prior to PND 28 young degus are still nursing. To maintain the same protocol used in adults, which involved 24 hours of isolation, we did not test degus before they were weaned. Juvenile and adolescent female degus experienced the same paradigm as adults, with one exception. Due to the relatively short juvenile developmental period, juveniles were not used in the 7-day co-housing tests.

Analysis of behaviors and behavioral dimensions

Video recordings were scored using Behavioral Observation Interactive Research Software (BORIS; Friard & Gamba, 2016). This software can be used to observe animal behavior and log specific events. The logs of events that are produced by the BORIS software were analyzed using MATLAB. The ethogram used consisted of 13 different behaviors which are described in Table 1 and are grouped into 5 behavior categories; agonistic (biting, wrestling, avoiding, marking, rear-push, mounting, and tail shaking), affiliative (huddling, grooming, and in-proximity), and investigative (face-to-face, rear sniffing, body sniffing).

Scoring was primarily done by Amber Thatcher, who also trained other scorers. Training of new scorers was done through the use of a written protocol and video recording of an example video. After the trainee completed a single session, they received detailed feedback about the scoring choices to minimize inter-scorer differences. This process then was repeated until the scoring of the trainee matched the scoring of the trainer. Out of the 1,042 videos scored for this dataset, 449 were completed by Amber

Thatcher. The remaining 593 were scored by undergraduates trained by Amber Thatcher. The interrater reliability (IRR) was calculated on the interaction vectors of a subset of sessions and compared between randomly selected scorers. This statistic is useful for experimental designs with three or more scorers and for data with interval variables (Hallgren, 2012). A subset of observations was chosen at random and scored by a second individual. Previous literature states that a subset of at least 10% of the original data is needed to perform these analyses (O’Connor & Joffe, 2020). The ICC model chosen reports the degree of absolute agreement of measurements that are averages of k independent measurements on randomly selected objects (McGraw & Wong, 1996). The median ICC we obtained indicated that variability between raters was high (*M* = 0.83, *Mdn* = 0.90).

Statistical Analyses

The primary measure used in this research was the proportion of session time animals spent interacting, and the time budgets (i.e., proportion of time spent in each individual behavior) across the five behavior categories. The time budgets of each animal within a dyad together comprised an “interaction vector”, which could then be used to compare similarity across sessions. These measures were used to examine potential differences in how familiar and unfamiliar dyads interact and how these interactions might change over time. To test for significant differences between groups and across days, multiple mixed-effects ANOVAs were used, with corrections for false discovery rates when running separate analyses for each of the five interaction types.

In order to determine the overall variability of a dyad a vector-distance measure was used. This allowed us to compare the overall variability (how similar each interaction vector is within a dyad) for strangers and cagemate groups, and to see whether this variability changed across days. We chose to use the cosine distance metric for this analysis as Euclidean distances can be influenced by the size of the vectors, or in this case, the total amount of interactions, which we expected to be higher in strangers.

Another method to test variability is to examine whether a dyad is consistent enough in its behavior to be distinguished from other dyads. Approaching the question in this way allows for the use of classification methods that can ignore behaviors that are irrelevant for assessing variability. For example, if rear-sniffing is a behavior that depends on other contextual variables, then it may appear that a dyad has a lot of variability even if it has a strong social relationship. We therefore used “dyad classification success” as an additional measure that considers within- versus between-dyad variability. We then trained support vector machine classifiers to distinguish between each pair of dyads within a group (adult female cagemate, adult female stranger, adult male cagemate, etc.). The SVM models were trained to discriminate the five full-session interaction vectors of two dyads within the same group. The models used were trained using a leave-one-out cross-validation method. This training method uses all observations, except one, for training, then the model is used to predict the category of the one observation left out. This process was then repeated for each observation, and the final result was a “success rate” used as our variability measure. Success rates of classification fell between 0 and 1 with a success rate of 0.5 being the probability expected by chance. The success rates of dyad classification were then compared using a 2-way mixed-effects ANOVA.

Chapter 5: Female Degus Show More Stable Social Interactions in New Relationships

Introduction

Much research has shown that social investigation decreases with familiarity (Winslow, 2003), and that certain behavioral responses change with increasing familiarity (Curtis et al., 2003). Whether the variability of social behaviors is affected by familiarity, however, it still not well studied. Some research has indicated that the variability of interactions between individuals may change as the individuals become more familiar with one another, but this has not been investigated directly (Carter et al., 2020; Lee et al., 2019; details of these studies can be found in chapter one). For agonistic relationships it has been shown that relationships become more stable over time and the variability of these interactions decreased (Jäger et al., 2019). These examples focus on agonistic relationships, and still leave us with the question of whether social familiarity is also associated with reduced behavioral variability in other relationship types. Affiliative relationships do tend to show similar trends, with the frequency and symmetry of grooming behaviors becoming less variable as the relationship develops (Carter et al., 2020). These findings imply that behavioral variability may in fact be a useful measure in understanding the process of relationship formation, independent of the type of relationship that is formed.

The goal of the present study was to investigate the effects of familiarity on the behavioral variability of the degu (*Octodon degus*), a highly gregarious rodent native to Chile. The principal hypothesis of this study is that novel relationships between unfamiliar degu dyads have more variable social interactions than those of familiar degu dyads. We predicted that the variability of social interactions differs between familiar cagemate and initial stranger dyads, and furthermore that they decrease over time (become more stable), indicating that a relationship is being formed, regardless of the type of relationship or the sex of the individuals. In order to test this hypothesis, we repeatedly reunited familiar and unfamiliar same-sex peers over the course of 20 to 40 days.

Methods

Subjects

Fifty-two adult female degus aged seven to twenty-nine months (*M* = 11.9 mo, *Mdn* = 11 mo) and 44 male degus aged six to twenty months (*M* = 10.4, *Mdn*= 10) were used. Degus reach developmental maturity by 3.5 months (Hummer et al., 2007; Mahoney et al., 2011) and can live in laboratory settings for up to 8 years (T. M. Lee, 2004). Age effects are not reported due to uneven sampling and potential history threats (e.g., the oldest dyads had been transferred from another colony). All degus had been weaned from parents and separated from opposite-sex siblings between postnatal days 30 and 60 (*M* = 42 days).

All animals were housed in same-sex pairs in 50.8 x 40.6 x 21.6 cm plastic cages in a breeding vivarium at the University of Montana. Degus were fed a 1:1 mixture of chinchilla and guinea pig “Teklad” feeds (Envigo; Indianapolis, IN). Animals were housed on a 12:12 h light/dark cycle, with all tests occurring during the light (active) cycle. Each cage was also equipped with additional enrichment (e.g., hay, cardboard enclosures, plastic “bones”, and wooden blocks) and, prior to the start of the study, provided with occasional dust baths and handling sessions.

Cagemate dyads were created by pairing two same-sex individuals together at the time of weaning, resulting in 24 cagemate dyads that were full siblings (highly familiar and genetically related) and 2 that were not (highly familiar but not genetically related). Animals used for stranger dyads were the same as those used for cagemates, and pairings were selected in an attempt to keep animals matched for age but were otherwise random. Strangers inhabited the same vivarium, so olfactory, auditory, and visual contact was possible prior to testing; however, in no cases had strangers been in physical contact with one another. These conditions for strangers are the same as those used in prior studies from our lab (Insel et al., 2020; Lidhar et al., 2021).

Apparatus

All sessions recorded video and audio using a Logitech HD Pro Webcam C920 USB2 camera and recorded at a sampling rate of 30 frames/s. The recording chamber was a 50 x 50 x 50 cm wooden box (Figure 1B). Following each social exposure, the enclosure was cleaned using 70% ethanol and then dried to prevent residual olfactory cues from affecting the behavior of subsequent animal pairs.

Testing

During the week prior to testing, each animal was pre-exposed to the testing chamber for at least 5 minutes each day over five days. Pre-exposures to the recording chamber were performed without other animals present. Twenty-four hours prior to the first testing session, all individuals were separated into individual cages within the same vivarium room. Testing began after degus were transported to the testing room and the backside of one degu was marked for identification (Pet Paint; Camarillo, CA). The painted degu was then placed in the chamber with an unpainted conspecific, either its cagemate or a stranger. The pair were allowed to interact freely in the recording chamber for 20 minutes. Once animals completed their 20-minute session they were returned to their home cage with their cagemate and were left undisturbed for 24 hours. Animals were placed back with their cagemate following each reunion session to allow the dyad to "recalibrate" their relationships, preserving their dyadic interactions over the weeks and minimizing the impact of the stranger exposures. At this point, this cycle of co-housing, isolation, and testing would repeat, with exposures to strangers and cagemates interleaved in a consistent, pseudo-randomized order for a total of 5 cagemate and 5 stranger sessions (counterbalancing stranger-first and cagemate-first across dyads).

Following 10 reunion sessions, adult female stranger dyads were co-housed together for a period of 24-hours there were again isolated for 24-hours and finally tested for an additional reunion session. After the completion of this reunion, the "new cagemate" dyad was housed together for another 24-hour period followed by isolation and another testing session. The purpose of creating the "new cagemates" was to maximize familiarity between the initial strangers, with the expectation that behavioral patterns (variability and levels of interaction) would become like those of cagemates if they had not already. Following the 24-hour co-housing procedure, adult female stranger dyads were co-house for an additional 7 days and then were tested twice more (with the 24-isolation period occurring prior to the reunion session).

Following re-housing reunion sessions in the females and the 10 reunion sessions in the males, all animals were subsequently returned to their original cagemates and then, following 24 hours of isolation, tested with a new stranger. This procedure was done twice. The purpose of the "new stranger" reunions was to establish whether changes in stranger behavior across repeated exposures were due to increased social familiarity, rather than acclimation to the recording chamber or testing protocol.

Behavioral Scoring

Software. Scoring of physical behavior was performed using BORIS (Behavioral Observation Interactive Research Software; Friard & Gamba, 2016)), which allows users to log events during video playback. All analyses were custom written in MATLAB and will be made freely available.

Behavioral Scoring. Filenames for video recordings were changed prior to scoring to ensure raters were blinded to conditions. Raters coded the start and end times of each observed behavior, the type of behavior, and the animal that initiated the behavior. The following behaviors were scored: agonistic (mounting, biting, wrestling, boxing, marking, rear-push, tail shaking), allogrooming (sniffing of neck or body with small, repetitive movements), rear-sniffing (anogenital sniffing), face-to-face (nose-to-nose and nose-to-mouth sniffing/contact), and body sniffing (sniffing toward neck and body; see Table 1 for more detailed descriptions of each behavior). Sessions were said to start when the second degu was introduced into the chamber and to end when a degu was removed. Occasionally there were accidental omissions of behavior "end" times; to ensure these did not impact results, these interactions were said to end when the same degu initiated a new behavior, or after a time-out threshold of 6 s (based on the distribution of all interactive behaviors across all sessions).

Measures

For simplicity and consistency with prior work (Lidhar et al., 2021; Thatcher & Insel 2021) the 17 behaviors listed on the ethogram were collapsed into five behavioral categories: Agonistic (mounting, biting, wrestling, boxing, marking, rear-push, tail shaking), allogroom (sniffing of neck or body with small repetitive movements), rear-sniffing (anogenital sniffing), face-to-face (nose-to-nose and nose-to-mouth sniffing and/or contact), and body sniffing (sniffing of neck and body). As almost all instances of agonistic type behaviors in juvenile and adolescent animals can be classified as play behaviors, as they rarely escalate into serious fights, and so we will therefore consider agonistic encounters observed here as play behaviors and will refer to them as play from this point on (Pellis et al., 2010). All measures were computed from ethogram-based scoring of behavior, performed manually in BORIS. The majority of the measures were based on the time animals spent using different behaviors during each session, or during part of a session (i.e., time budget analysis). Examination of total interaction time across sessions revealed a tailed distribution that closely resembled a gaussian distribution following a cubed root transformation. This transformation was applied to all measures of interaction time prior to statistical analyses.

The first measure created and used for analyses was total interaction time (or time budget analysis). This variable was computed as a proportion of session time, with total time spent interacting created through the summation of all behavior types (total time spent interacting divided by total session time). Interaction vectors were then created for each behavioral category and used for a more detailed analysis of social interactions across sessions. Interaction vectors were also computed as a proportion, but of total interaction time rather than of session time (i.e., total time interacting agonistically divided by the total amount of time spent interacting).

The variability of social interactions was compared between sessions through two measures, "interaction vector distances" and "dyad classification success". Interaction vector distances were created by computing the distance between the vectors associated with different sessions within the same individual. This yields a vector of 10 elements (5 interaction types by each degu in the dyad). For the purposes of this study, however, face-to-face interactions of both individuals were combined as it is a mutual interaction resulting in a vector of 9 elements for this study. Cosine distances were used to compute the distances between interaction vectors. Cosine distance has many advantages over the other options, such as Euclidean distance which can be affected by the number of interactions, while cosine distances do not. Pearson correlations also are not affected by differences in the length of vectors would ignore changes that are common across all interaction types. This would therefore not capture the full variability of interactions across sessions.

Dyad classification success was computed by finding the degree to which a group of interaction vectors could be discriminated from another group. Classification success combines the variables of within-dyad variability, and between-dyad diversity. The advantage of dyad classification success compared with interaction vector distances is that, after a model is trained, it should ignore types of social interactions that are not useful for the discrimination between two dyads. It is also able to emphasize the interactions, or combinations of interactions that do have a large influence in the discrimination of dyads from one another. Classification success was determined through the use of a binary support vector machine classifier (SVM). This method identifies the boundaries (or hyperplanes) through n-dimensional space that best differentiate between two sets of data. Support vectors are the data points closest to the boundaries which separate data into two categories. This allows for us to estimate common patterns between the two sets of data based on how successful the model is in its classification. A leave-one-out cross-validation method which uses all observations (in this case of the 9 interaction vectors, the model is trained on 8), except one, for training, then the model is used to predict the value of the one observation left out. This process is repeated for each observation. This training method is less biased than using a single test/training.

Statistical Analyses

Null-hypothesis significance testing was used to evaluate differences between strangers and cagemates, age groups, sessions, interaction variability across sessions and age groups and classification success. To investigate overall interactions levels between strangers, cagemate, juveniles and adolescents across session days a 3-way mixed effect ANOVA was run with stranger/cagemate and session day as repeated measures and age as the between subjects' measure. To assess the effects of exposure day and stranger/cagemate for each behavior a separate 2-way mixed effects ANOVA was conducted. Due to the multiple comparisons, significance values are corrected using the Benjamin & Hochberg (1995) false discovery rate procedure.

Results

The effects of stranger/cagemate, sex, and familiarity on general interaction levels

In order to explore the general effects of familiarity on social interactions of degus we first examined how the total interactions times differed between strangers and cagemates, and how this differed between the sexes. In line with our hypothesis and previous findings, degus interacted more with strangers than with their cagemates (3-factor mixed ANOVA with exposure day and stranger/cagemate as repeated measures and sex as between-subject; effect of stranger/cagemate: $F(1,94) = 34.18$, $p = 7.2 \times 10^{-8}$, $\eta^2 = 0.003$; Figure 2A). Both stranger and cagemate male degus interacted more than females (effect of sex: $F(1,94) = 12.47$, $p = 6.4 \times 10^{-4}$, $\eta^2 = 0.004$). A significant interaction between stranger/cagemate and exposure day indicated that the amount of time stranger and cagemate dyads spent interacting decreased over time (interaction between stranger/cagemate and exposure: $F(4,376) = 3.16$, $p = 0.014$, $\eta^2 = 0.001$). This effect was driven by the males as female dyad interaction levels did not change over exposure day ($F(1,4) = 0.48$, $p = 0.75$, $\eta^2 = 0.001$).

To investigate the effects of familiarity on specific behavior types we divided the interactions into five types: agonistic, allogrooming, rear-sniffing, face-to-face, and body-sniffing. Both male and female degus had more agonistic (primarily mounting) interactions with strangers than with cagemates (2-factor mixed ANOVA, repeated measures over days, false discovery rate correction for 5 tests, female agonistic: $F(1,51) = 8.70$, $p = 0.001$, $\eta^2 = 0.02$; male agonistic: ($F(1,43) = 17.44$, $p = 1.4 \times 10^{-4}$, $\eta^2 = 0.02$; Figure 2C, right panels). Female stranger dyads also demonstrated more rear-sniffing and face-to-face interactions than cagemates (rear-sniffing: $F(1,51) = 12.37$, $p = 9.3 \times 10^{-4}$, η^2

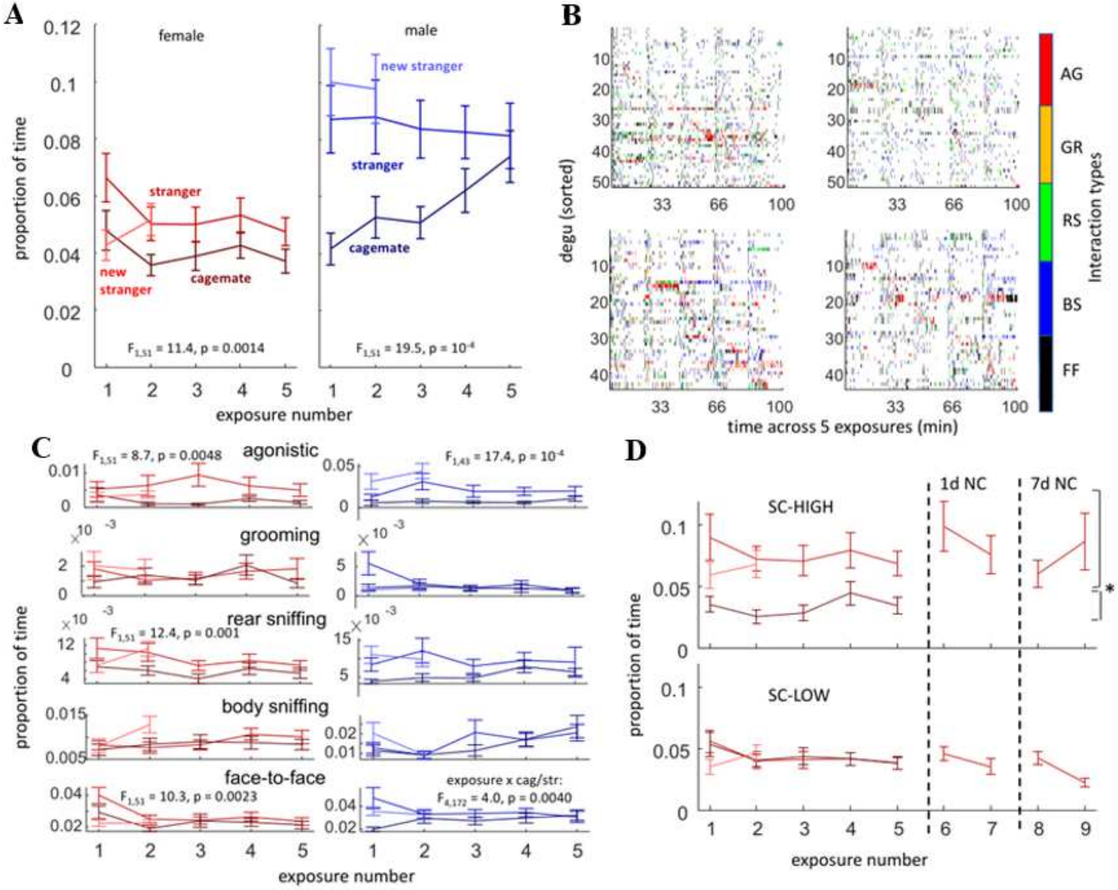
= 0.01, and face-to-face: $F(1,51) = 3.56$, $p = 0.01$, $\eta^2 = 0.002$; Figure 2E, left panels). Changes in stranger versus cagemate interaction levels across days could only be detected in face-to-face interactions in female degus ($F(1,51) = 10.32$, $p = 0.002$, $\eta^2 = 0.002$; all other individual interaction types: $p > 0.05$). Males decreased face-to-face interactions over time, more so in strangers than cagemates ($F(4,172) = 4.00$, $p = 0.01$, $\eta^2 = 0.002$; Figure 2C). Trends for other behavior types indicated a decrease in interactions across exposure days only in males (e.g., higher rear-sniffing over the first few days, session x stranger/cagemate interaction: $F(4,172) = 2.36$, $p = 0.056$, $\eta^2 =0.01$).

As we did not observe any significant changes in the interaction levels of female stranger or cagemate dyads, we wanted to further explore whether dyads with overall low levels of interaction were impacting these results. We therefore divided female dyads into those that showed significantly higher levels of interaction with strangers ("SC-HIGH", 16 of 52 degus, determined using a paired t-test on each animal across 5 stranger, 5 cagemate sessions, $p < 0.1$) and those that did not ("SC-LOW", 36 of 52 degus). We then ran a 2-factor repeated measures ANOVA for each group. There was no evidence that stranger-cagemate interaction levels changed over days in either the SC-HIGH animal (2-factor repeated-measures ANOVA stranger/cagemate: $F(1,15) = 83.40$, $p = 1.63 \times 10^{-7}$, $\eta^2 = 0.02$ day: $F(4,60) = 0.89$, $p = 0.47$, $\eta^2 = 0.001$; stranger/cagemate x day: $F(4,140) = 0.20$, $p = 0.94$, $\eta^2 = 0.001$) or the SC-LOW animals (stranger/cagemate: $F(1,35) = 8.00 \times 10^{-4}$, $p = 0.98$, $\eta^2 = 0.00$; day: $F(4,140) = 1.49$, $p = 0.22$, $\eta^2 = 0.001$; stranger/cagemate x day: $F(4,140) = 0.20$, $p = 0.94$, $\eta^2 = 8.72 \times 10^{-5}$; Figure 2C). Even after co-housing the stranger dyads for a period of 24 hours and 7 days interaction levels of these dyads remained higher than long-term cagemates (paired t-test, averaging all cagemate sessions and both 24-hr co-housed stranger sessions, $t_9 = 5.50$, $p = 3.80 \times 10^{-4}$; 7 days of co-housing ($t_7 = 2.98$, $p = 0.021$)).

In order to investigate the potential effects of habituation to the procedure on the observed changes in interaction levels over time. In both males and SC-HIGH females, levels of interaction with new strangers were higher than interactions with cagemates (paired t-test between within-animal averages in cagemates and new-strangers, males: $t_{43} = 5.13$, $p = 6.66 \times 10^{-6}$, SC-HIGH females: $t_{15} = 4.17$, $p = 8.18 \times 10^{-4}$) and were not lower than interactions with the original strangers (males: $t_{43} = 1.76$, $p = 0.086$ with new stranger > old stranger; SC-HIGH females: $t_{15} = 1.13$, $p = 0.28$, Figure 2D).

Figure 2

The Effects of Sex and Familiarity on Interaction Levels



Note: A) Total interaction levels for females (left panel) and males (right panel) across the 5 initial exposures (errorbars are mean ± SEM; statistics are cagemate/stranger factors in 2-way ANOVA). B) Colormaps of interaction types for each degu in 2 s bins across the five exposures helps illustrate the variance within and between animals. Individual degus (rows) are sorted according to their peak levels of agonistic interactions (AG, red; other abbreviations: GR = allogrooming, RS = rear-sniffing, BS = body sniffing, FF = face-to-face). More interactions were observed when degus interacted with strangers (left panels), with particularly high variance in males (bottom panels). C) Breakdown of interaction levels across different types of interactions. Higher levels of agonistic, rear-sniffing, and face-to-face were observed in female strangers relative to cagemates (left panels). Among males, some interaction types decreased and others increased over days (right panels). D) Interaction levels over days in female degus that interacted more with strangers (SC-HIGH, top) and those that did not (SC-LOW, bottom). Levels of social interaction between new cagemates (exposures 6 through 9), as well as levels in new strangers (blue), were consistent with those observed in initial strangers on exposures 1 through 5.

The effects of stranger/cagemate, sex, and familiarity on session-to-session variability

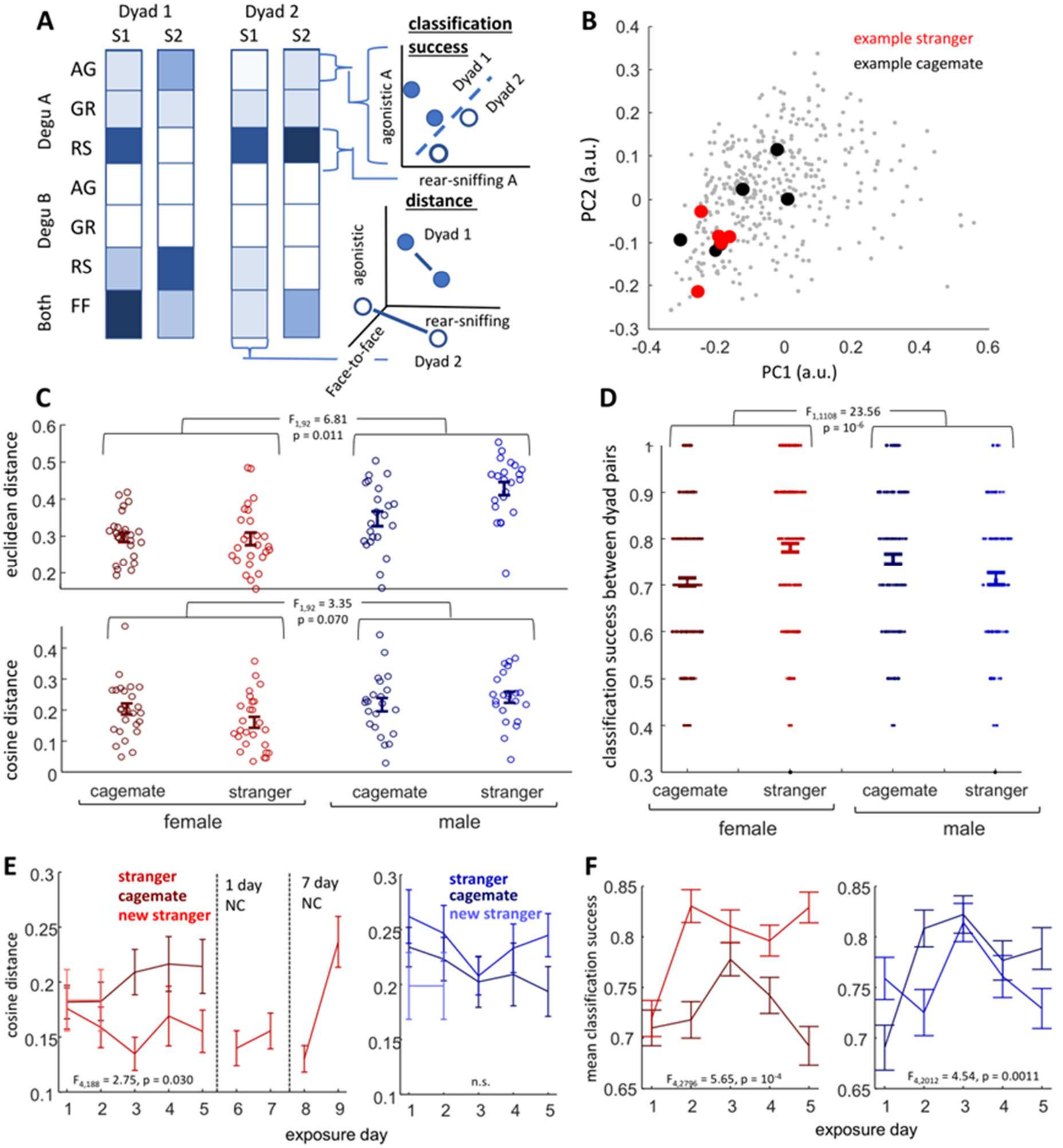
The next set of analyses focus on the variability of social interactions. To measure this, we first computed “interaction vectors”: social interaction time budgets across each of 5 interaction types (agonistic, allogrooming, rear-sniffing, body-sniffing, and face-to-face), in each of the two individuals in a dyad. We then computed the distances (dissimilarity) between interaction vectors to observe the overall variability within each dyad. We found that the cosine distances between interaction vectors was significantly higher in cagemates than in strangers ($F(1,92) = 32.09$, $p = 1.7 \times 10^{-7}$, $\eta^2 = 0.02$). This observed difference was driven by the females, as subsequent analyses did not show any significant differences in males (cagemate/stranger: $F(1,41) = 1.85$, $p = 0.18$, $\eta^2 = 0.01$; exposure day: $F(4, 164) = 1.91$, $p = 0.46$, $\eta^2 = 0.002$). A significant increase in female stranger-cagemate differences was observed with variability in stranger dyads decreasing after the first exposure day (2-way mixed ANOVA, repeated measures over days, cosine distance: cagemate-stranger: $F(1,47) = 2.19$, $p = 0.14$, $\eta^2 = 0.01$, exposure day *F*(4,188) = 1.9, $p = 0.10$, $\eta^2 = 0.002$, cagemate-stranger x exposure day: F(4,188) = 2.75, $p = 0.030$, $\eta^2 = 0.003$)).

We then used a classifier-based method to establish whether dyads could be distinguished from one another using unique combinations of interaction vector elements (illustrated in Figures 3A&B). This classifier method measures “discriminability”, which evaluates within-dyad variability relative to differences between dyads. This relative measure of variability is useful in that it allows specific interaction behaviors (and combinations) to be ignored if a dyad is inconsistent in that behavior, effectively zeroing in on behaviors that more selectively define the social relationship. Dyad classification success was evaluated using SVM models trained to discriminate the five, full-session interaction vectors of one dyad from the five interaction vectors of another dyad within the same group (e.g., a given SVM model was trained to classify one female cagemate dyad from another). Each model was repeatedly trained and tested using a leave-one-out cross validation method, yielding a success rate between 0 and 1 where a success rate of 0.5 would be exactly the probability expected by chance. Female cagemate and stranger groups each included 325 SVM models (number of unique pairings between 26 dyads) and male cagemate and stranger groups each included 231 models (pairings between 22 dyads). The average success rate in each of these groups was higher than chance, suggesting that in spite of session-to-session variability, individual dyads expressed behavioral patterns that were distinguishable from other dyads (binomial probability distribution predicts ~5% probability of success classifying at least 7 of 10 sessions, mean rates for female cagemates = 7.1, female strangers = 7.8, male cagemates = 7.6, male stranger = 7.3). Classification success was significantly higher in female strangers than cagemates. In males, however, stranger dyads had significantly lower classification success than cagemates (2-way ANOVA effect of stranger-cagemate: $F(1,1108) = 7.11$, $p = 0.0078$, $\eta^2 = 0.01$; effect of sex: $F(1,1108) = 0.03$, $p = 0.86$, $\eta^2 = 2.64 \times 10^{-5}$; stranger-cagemate x sex interaction: $F(1,1108) = 23.56$, $p = 1.4 \times 10^{-6}$, $\eta^2 = 0.02$; Figure 3D). The effects were not due to any one interaction type, as the sex difference remained even after the removal of any specific type (agonistic, allogrooming, rear-sniffing, face-to-face, or body-sniffing) from the interaction vectors (stranger-cagemate x sex interaction for each: $p < 0.01$).

We then further investigated the effects of familiarity on inter-session variability by comparing dyad classification success across each of the five social exposures/reunion sessions. Consistent with the results seen using the cosine distance metric, the variability of female stranger dyads decreased over (2-way mixed-effects ANOVA, repeated measures over days, cagemate-stranger: $F(1,699) = 29.6$, $p = 7.4 \times 10^{-8}$, $\eta^2 = 0.002$; exposure day: $F(4,2796) = 6.65$, $p = 2.53 \times 10^{-5}$, $\eta^2 = 0.001$; cagemate-stranger x exposure day: $F(4,2796) = 5.65$, $p = 1.6 \times 10^{-4}$, $\eta^2 = 0.001$; Figures 3E&F, left panels). In males, changes over days were less apparent, though classification success rates showed signs of increasing in cagemates after the first exposure, while strangers showed less evidence of change (2-way mixed ANOVA, stranger-cagemate: $F(1,503) = 1.5$, $p = 0.22$, $\eta^2 = 0.0001$; exposure day: $F(4,2012) = 6.05$, $p = 7.74 \times 10^{-5}$, $\eta^2 = 0.001$; stranger-cagemate x exposure day: $F(4,2012) = 4.54$, $p = 0.001$, $\eta^2 = 0.001$; Figures 3E&F, right panels).

Figure 3

Variability Across Sessions and the Effects of Sex and Familiarity



Notes. A) Diagram illustrating analysis methods. Each column of boxes represents the interaction vector from a single session (abbreviations same as in Figure 1). To the right, vectors are plotted in cartesian coordinates to illustrate measures of variability. The top right shows SVM-based classification success, with the dashed line representing a hyperplane separating groups in the relevant feature space. The bottom panel shows interaction vector distances, with points placed in 3 dimensions to emphasize that even non-relevant features contribute to the measure. Lines between points depict within- (light colored) and between- (dark colored) dyad distances. B) Plot of all female dyad interaction vectors using the first two principal components of the vector feature space. Dark black dots show all 5 sessions from one example cagemate dyad, red dots show all 5 sessions from one stranger dyad. In this example, the first two principal components reveal higher clustering (less session-to-session variance) in the stranger relative to cagemate. C) Swarm plots showing the distribution of within-dyad interaction vector distances (operationally "variability"; top: Euclidean, bottom: cosine) in cagemate and stranger dyads of both sexes. F and P values refer to interaction between cagemate/stranger and sex. D) Success rate of SVM classifiers discriminating pairs of dyads among within each of the four groups (error bars are mean ± SEM). E) Within-dyad interaction vector distances after separating-out each exposure, e.g., "exposure 1" only includes distances between exposure 1 vectors and other days—it would not include distances between exposures 2 and 3, or 3 and 4. Values are across-dyad averages of within-dyad medians, ±SEM, F and P values refer to interaction between exposure day and cagemate/stranger. F) Relative success rates for different days, e.g., "exposure 1" only includes models trained on exposures 2 through 5, with accuracy tested for the exposure 1 vector.

Discussion

The main goal of this research was to identify whether the variability of social interactions differed between familiar and unfamiliar individuals and measure these differences over time and between males and females. The overall variability of social interactions, as measured by cosine distances, were found to differ between strangers and cagemates in both males and female, but not in the direction we predicted. We predicted that social interactions would be more variable in initially unfamiliar (new) relationships than in established (familiar) relationships independent of sex. Surprisingly we found that female stranger interactions were more stable than those of cagemates. This result was unexpected, but the results of our dyad classification provide further support for this finding. We also predicted that the variability of interactions would decrease (interactions would become more stable) over time. This prediction was partially supported by our data with female strangers, male strangers, and male cagemates all showing decreased interaction variability across exposure days.

While we did not find results to support our hypothesis that these measures of variability could be used to identify social relationships independent of the type of relationship formed, we did observe several unexpected but interesting results. The first unexpected result was that 30% of degus that interacted more with stranger peers than with familiar cagemates did not decrease interactions to cagemate levels even after becoming highly familiar with their new partners. The second was that female cagemates showed relatively higher levels of variability in their interactions than strangers and cagemate interaction variability increased over time.

Previous work has also shown that adult female stranger degus displaying higher interaction levels compared to cagemate dyads (Insel et al., 2020; Lidhar et al., 2021; Villavicencio et al., 2009) and is supported by previous work reporting a preference for and increased behavioral response to novel social stimuli (Cavigelli et al., 2011; Moy et al., 2004). The highly social nature of this species in the wild also lends its support to this finding as females degus are known to form close associations with other female group members, nest with unrelated females and nurse each other's pups (Ebensperger et al., 2004; Fulk, 1976). The high turnover rates experienced by these groups in the wild, also is indicative of a motivation to interact with and form relationships with new, unfamiliar others (Davis et al., 2016). These natural behaviors also imply, that these animals would be driven to form stable, cooperative relationships with new individuals. Our results, however, indicate that this process may not occur as it is implied. We observed that interaction levels of initial strangers were initially high, but did not decrease over time as the individuals began to familiarize themselves with one another. Even after a period of

24 hours of cohabitation and a period of 7 days of cohabitation did not decrease the interactions levels to those seen in long-term cagemates.

One potential explanation for this effect is the genetic relatedness of the long-term cagemates compared to the non-sibling relationships between our strangers. It may be that the relationship formed between the long-term full sibling cagemates early in their development is fundamentally different from relationships (non-sibling or sibling) formed during adulthood. Many species do display a kinship bias and display differential interactions in relationships with siblings compared to non-siblings (Gauffre et al., 2009; Lee, 1987). There is little to no research on the differences of social interactions between siblings and non-sibling pairs over time, however, so it is unclear whether this would explain our results. Further support for the potential effects of genetic relatedness on social interactions has been seen in degus. While in the wild, degus do not display a kinship bias, laboratory based research has shown that genetic kinship can influence social behavior, just not as strongly as familiarity (Quirici et al., 2011; Villavicencio et al., 2009). Further research with non-sibling cagemates should be completed in order to determine the true effects of kinship on the social relationships of captive degus.

We also observed that, contrary to our predictions, female cagemate dyads exhibited high levels of variability relative to strangers. Previous findings suggested that established relationships were less variable in their interactions (or more stable) than new, not yet established relationships, making our results much more surprising to us (Carter et al, 2020; Lee et al., 2019). As our established relationships were formed months prior to testing, it may be that these relationships when newly established were stable, but over time the individuals became much more relaxed and comfortable. This more relaxed state may have then allowed for the emergence of more variable behaviors that are dependent on day-to-day circumstantial factors (e.g., hormonal fluctuations, ambient odors, etc.). Another potential explanation for these results is that stranger dyads may be more wary, and therefore less likely to engage in more variable, and therefore less predictable behaviors. Research does tend to support the idea that being unpredictable with strangers is potentially dangerous and not conducive to the formation of any form of affiliative relationship. Many primates, for example use sets or rituals of specific vocalizations and physical interactions when they encounter strangers. These behaviors often occur between similar-ranking males or when younger individuals greet older conspecifics and signify that these behaviors signal predictability and function to reduce uncertainty and tension (Aureli & Schaffner, 2007; Dias et al., 2008; Kutsukake et al., 2006; Smuts & Watanabe, 1990; Whitham & Maestripieri, 2003). According to this explanation, it could by hypothesized that female degus are predisposed for lower variability social interactions with strangers as part of their natural motivation to form cooperative relationships with new individuals (Ebensperger et al., 2004).

Several potential methodological factors must be considered when interpreting these results. The first is the novelty of the testing environment. While all animals were individually exposed to the chamber for five days prior to the beginning of the experiment, they may not have fully habituated to the chamber, room, or the overall procedure. This was observed through the low interaction levels between degus, especially in the females (averaging around 4 and 6% of the session for cagemate and stranger dyads respectively, or just over 8% in SC-HIGH animals). Previous work with rats demonstrated similar effects of environmental novelty on social behaviors

(Varlinskaya, 2008). A similar testing procedure done with adult degus in the home cage setting report much higher levels of interactions and while these results are not directly comparable, when taken in combination with previous work in rats it does suggest that the novel environment could have impacted the social interactions of these animals (Lidhar et al., 2021). Another potential methodological factor that may have influence the results is the categories of social behaviors used. Many of the categories, such as rear-sniffing includes several distinctive behaviors such as anal sniffing, genital sniffing, and rump sniffing. It may be that these behaviors should have been separated out rather than combined into one category. Furthermore, there are several behaviors that were left out of these analyses that may have provided a more detailed and in-depth depiction of the degu's relationships. For example, behavior such as in-proximity, while not an active interaction, is still a common marker of affiliative relationships in primates (Silk et al., 2013). It is also important to note, however, that these behavioral categories were created to provide a more general description of social interactions. They also allowed us to remove specific behavior types to investigate whether any behaviors were driving the differences we observed.

The overall purpose of this paper was to investigate the effects of sex, familiarity, and time on the behavioral variability of degu social interactions. In order to do this, we developed a new methodology (distance metrics and classifiers) in an attempt to directly measure this variability in a way that could be compared between the sexes, across species, and throughout development. Our results suggest that the methods used to measure and compare interaction variability were successful, just not in the way we initially predicted. We did identify clear differences in the variability of interactions between cagemates, strangers, males, and females and while differences did not change significantly over time, it still suggests that these measures could be useful in describing relationships. Further research needs to be conducted comparing these results to other species, however, to fully determine the usefulness of these measures. Furthermore, given the unexpected nature of several of our results it may be that these measures are not able to fully capture the full picture of relationship formation, and should be further investigated with the inclusion of other generalizable methods.

Chapter 6: Relationship Formation Across the Lifespan in Female Octodon Degus

Introduction

Social relationships serve many important functions across the lifespan of animals. In many species, adults receive increased reproductive fitness and life expectancy with increasing number of social relationships (Brent et al., 2017; McFarland & Majolo, 2013). In younger animals, however, the function of social relationships differs. The function of social relationships for younger animals has instead been attributed to learning appropriate social responses, the development of muscles, motor control, neurological pathways, and even normal cognitive development (Blakemore, 2008; Pellis et al., 2010; Shimada & Sueur, 2018; more details on the differences in social behaviors across development can be found in chapter two). As the function of relationships during the early developmental periods is in part to learn and test out different behavioral strategies, it could be that the social interactions that occur during these stages are more variable in order to increase learning. Previous research has shown that the more variability that an individual encounters when learning certain tasks, improves their performance on those tasks in later testing, across multiple disciplines, but whether this is true for social behaviors is still not well known (Raviv et al., 2022).

The present study, therefore, aims to describe the social interactions of juvenile and adolescent female degus and examine whether the variability of interactions differs between familiar and unfamiliar individuals, and over time. We chose to examine female degus because adults of this sex and species have been shown to become quickly affiliative with strangers, and in natural environments, unrelated adult females form cooperative interactions. We hypothesized that juvenile and adolescent female degus will have high variability in their interactions regardless of the dyad's familiarity level. Previous research has indicated that the main function of social interactions in early development is learning, and increased variability of situations and behaviors enhances learning. We therefore predicted that the variability of social interactions will not be significantly different between familiar (cagemates) and unfamiliar (strangers) for either age group. We also predicted that the variability of the interactions will not decrease over time. To test these predictions, we repeatedly exposed juvenile and adolescent female degus to a cagemate and stranger same-sex peer and allowed them to interact freely for 10, 20-minute sessions. The total time spent interacting and the time spent performing specific behavior types was recorded and compared. Then, using two distance metrics compared the variability of interactions between and within dyads over time.

Methods

Subjects

24 juvenile female degus aged 28 to 35 days (mean = 1.36 mo, mode = 1.3 mo) and 24 adolescent female degus aged 56 to 112 days (mean = 2.49, mode = 2.13) were used. As the procedure chosen included a 24-hour period of isolation prior to testing, juvenile degus only entered the procedure once weaning had occurred. This was done in order to keep testing procedures consistent between adults and juvenile animals and so juveniles were not deprived of their mother's milk too soon. All degus had been weaned from parents and separated from male siblings between postnatal days 20 and 45 (M = 30.36 days). After weaning all animals were monitored for evidence of vaginal openings, noting any changes in coloration of the vaginal region and the opening of the vaginal membrane three times a week (Mahoney et al., 2011). The first day of complete vaginal

opening was defined as the onset of puberty (mean age of onset of puberty = 61.97 days, min = 55, max = 94).

All animals were housed in the same conditions described in chapter 5. Cagemate dyads were created by pairing two same-sex individuals together at the time of weaning, resulting in 11 cagemate dyads that were full siblings and 1 that was not related. Unfamiliar “strangers” were created using age-matched individuals with no history of interaction or genetic relatedness. Olfactory, auditory, and visual contact were possible prior to testing as all animals inhabited the same vivarium; however, in no cases had strangers been in physical contact with one another (consistent with methods described in Insel et al., 2020; Lidhar et al., 2021).

Apparatus

All sessions recorded video and audio using a Logitech HD Pro Webcam C920 USB2 camera and recorded at a sampling rate of 30 frames/s. The recording chamber was a 50 x 50 x 50 cm wooden box (Figure 1B). Following each social exposure, the enclosure was cleaned using 70% ethanol and then dried to prevent residual olfactory cues from affecting the behavior of subsequent animal pairs.

Testing

During the week prior to testing, each animal was pre-exposed to the testing chamber for at least 5 minutes each day over five days. Pre-exposures to the recording chamber were performed without other animals present. Twenty-four hours prior to the first testing session, all individuals were separated into individual cages within the same vivarium room. Testing began after degus were transported to the testing room and the backside of one degu was marked for identification (Pet Paint; Camarillo, CA). The painted degu was then placed in the chamber with an unpainted conspecific, either its cagemate or a stranger. The pair were allowed to interact freely in the recording chamber for 20 minutes. Once animals completed their 20-minute session they were returned to their home cage with their cagemate and were left undisturbed for 24 hours. Animals were placed back with their cagemate following each reunion session to allow the dyad to "recalibrate" their relationships, preserving their dyadic interactions over the weeks and minimizing the impact of the stranger exposures. At this point, this cycle of co-housing, isolation, and testing would repeat, with exposures to strangers and cagemates interleaved in a consistent, pseudo-randomized order for a total of 5 cagemate and 5 stranger sessions (counterbalancing stranger-first and cagemate-first across dyads).

Following 10 reunion sessions, juvenile and adolescent stranger dyads were co-housed together for a period of 24-hours there were again isolated for 24-hours and finally tested in an additional reunion session. After the completion of this reunion, the "new cagemate" dyad was housed together for another 24-hour period followed by isolation and another testing session. The purpose of creating the "new cagemates" was to maximize familiarity between the initial strangers, with the expectation that behavioral patterns (variability and levels of interaction) would become like those of cagemates if they had not already. Following the 24-hour co-housing procedure, adolescent stranger dyads were co-house for an additional 7 days and then were tested twice more (with the 24-isolation period occurring prior to the reunion session). Due to the typical length of the juvenile developmental period in this species, no juveniles were run through the 7-day re-housing paradigm. The 7-day co-housing paradigm was completed with adolescents as previous work with adult female degus has shown that 5, 20-minute sessions was an insufficient amount of time for unfamiliar dyads to establish a relationship (Chapter 5).

Following re-housing reunion sessions, degus were subsequently returned to their original cagemates and then, following 24 hours of isolation, tested with a new stranger. This procedure was done twice. The purpose of the “new stranger” reunions was to establish whether changes in stranger behavior across repeated exposures were due to increased social familiarity, rather than acclimation to the recording chamber or testing protocol.

The software, behavioral scoring, and statistical analyses sections of these methods are the same as described in chapter five.

Results

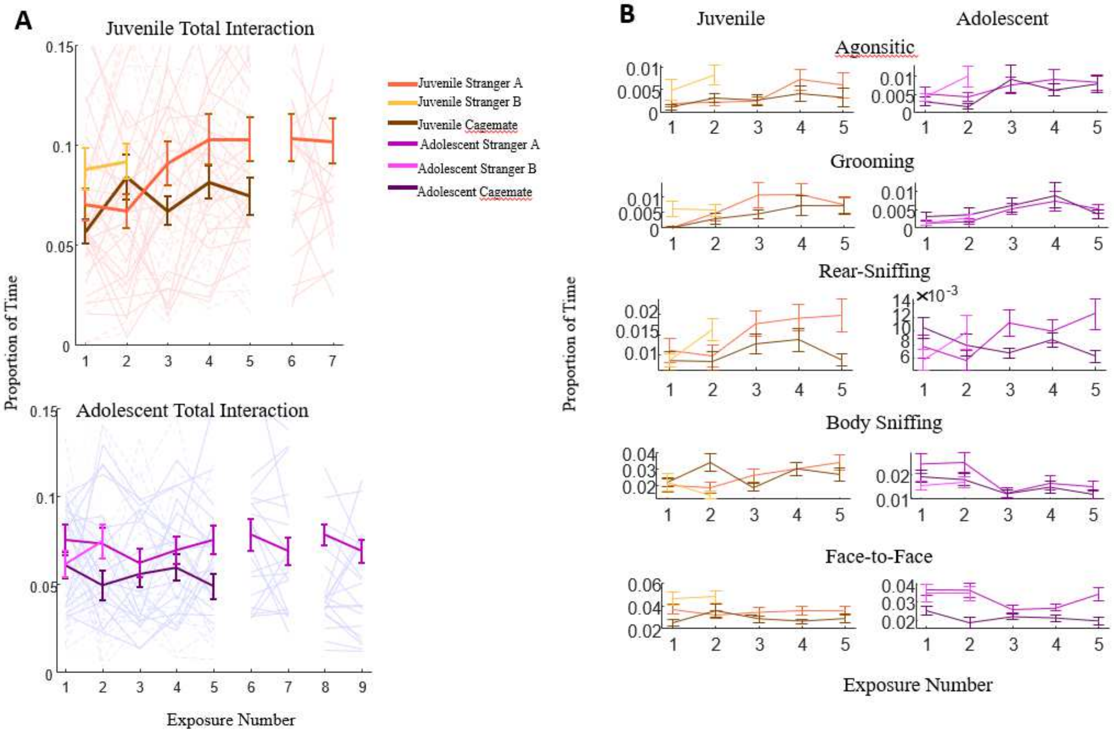
To order to assess the general differences in interactions between juvenile and adolescent female degus we first evaluated the effects of social novelty on total interaction time. We discovered that adolescent stranger dyads interact more than cagemate dyads (3-way mixed effects ANOVA, with cagemate/stranger and session day as repeated measures and age as the between-subjects variable, interaction of age, exposure day, and stranger/cagemate, $F(4,168) = 3.31$, $p = 0.01$, $\eta^2 = 0.001$; Figure4A). Juveniles, on the other hand do not initially differ in their interaction levels between stranger and cagemate dyads. Over time juvenile stranger dyads slowly increase the amount of time that they spend interacting, and by day 5, the interaction levels of stranger dyads are significantly higher than that of cagemate dyads (2-way repeated measures

ANOVA; interaction effect of stranger/cagemate and exposure day: $F(4,92) = 4.85$, $p = 0.01$, $\eta^2 = 0.01$).

To identify which behaviors accounted for the differences between strangers and cagemates we compared the interaction vectors of specific behaviors: play, allogrooming, rear-sniffing, face-to-face, and body sniffing (2-way mixed effects ANOVA for each behavior in each age group, applying the Benjamin & Hochberg (1995) false discovery rate procedure; Figure4B). Juvenile stranger dyads were found to increase their rates of allogrooming and rear-sniffing behaviors over days (allogrooming: $F(4, 230) = 9.13$, $p < 0.001$, $\eta^2 = 0.12$; rear-sniffing: $F(4, 230) = 3.45$, $p < 0.01$, $\eta^2 = 0.05$). Juveniles also demonstrated a trend of increasing play behaviors and body sniffing over time (play: $F(4, 230) = 2.94$, $p = 0.02$, $\eta^2 = 0.05$; body sniffing: $F(4, 230) = 2.90$, $p = 0.02$, $\eta^2 = 0.04$). Juveniles overall had more face-to-face interactions in stranger dyads than in cagemates ($F(4, 230) = 8.67$, $p = 0.003$, $\eta^2 = 0.03$). Adolescent female degus increased allogrooming and body sniffing across exposure days (allogrooming; $F(4, 190) = 3.63$, $p = 0.01$, $\eta^2 = 0.07$; body sniffing: $F(4, 190) = 3.47$, $p = 0.01$, $\eta^2 = 0.06$). Adolescents also demonstrated more face-to-face interactions in stranger dyads than in cagemate dyads ($F(4, 190) = 32.72$, $p < 0.001$, $\eta^2 = 0.12$). Indicating that the specific behaviors that are used during interactions differ between the age groups. The exposure day seems to affect the prevalence of behaviors most with stranger/cagemate differences only appearing in face-to-face interactions.

Figure 4

The Effects of Age and Familiarity on Interaction Levels



Note. A) Total interaction levels for juveniles (top) and adolescents (bottom) across the 5 initial exposures, the 24-hour co-housing tests, and the 7-day co-housing tests (7-day test only completed with adolescents; error bars are mean ± SEM; statistics are cagemate/stranger factors in 2-way ANOVA). B) Breakdown of interaction levels across each behavior type. Higher levels of grooming, rear-sniffing, and body sniffing were observed in juveniles across session days (left panels). Among adolescents, agonistic and grooming behaviors increased over sessions (right panels).

The effects of stranger/cagemate, age, and familiarity on session-to-session variability

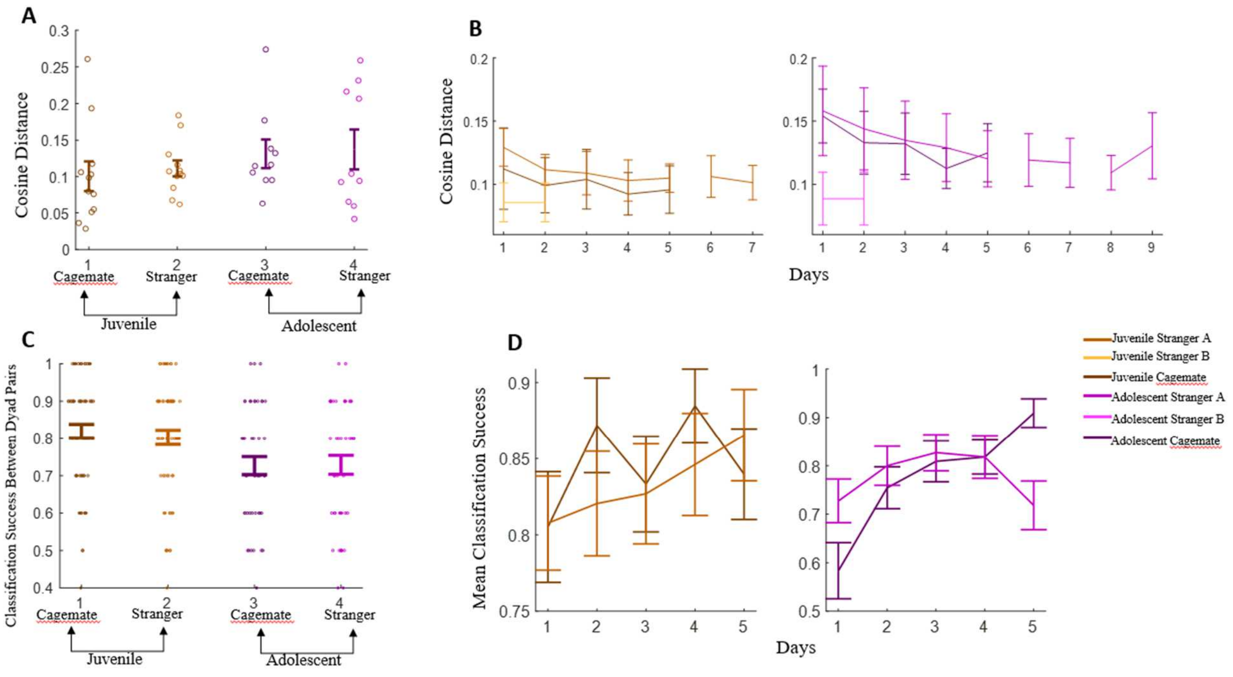
The main goal of this research was to investigate the variability of juvenile and adolescent social interactions. To estimate the differences in the variability of behavior between sessions within each dyad we calculated the cosine distances between interaction vectors. There was no statistically significant difference in the interaction variability of juveniles compared to those of adolescents ($F(1,40) = 2.24$, $p = 0.14$, $\eta^2 = 0.01$). In line with our predictions we did not observe a significant difference in the variability of stranger dyads compared with cagemate dyads in either age group (2-way mixed effects

ANOVA; effect of stranger/cagemate: $F(1,40) = 0.12$, $p = 0.73$, $\eta^2 = 7.07 \times 10^{-5}$; Figure5A). Interestingly, we did visually observe a bimodal distribution in adolescent stranger dyads with about half of the dyads seemingly to fall well below the mean of cagemate variability and half falling well above the mean, however none of the analyses demonstrated this effect statistically. This may indicate that some of the adolescent females are beginning to display patterns seen in adult female degus (Chapter 5). Both adolescent and juvenile dyads, regardless of whether they were strangers or cagemates, had decreasing levels of variability over time ($F(4, 160) = 3.78$, $p = 0.005$, $\eta^2 = 0.005$; Figure5B). This pattern was in contrast with the prediction that variability would remain high across sessions, independent of their level of familiarity. This result suggests that the relationships became more stable across sessions.

The dyad classification method was then used to investigate to what extent juvenile and adolescent relationships are distinguishable from one another within each group (cagemate vs. stranger). A benefit of using the support vector machine (SVM) method of classification is that it can effectively perform classifications in high-dimensional spaces even when the number of dimensions (i.e., interaction vectors) is greater than the number of samples (i.e. dyads). Another advantage of SVMs is that they are able to handle classification even when the data is not linearly separable. It can therefore identify non-linear boundaries that effectively separate the classes. The rates of dyad classification success were not significantly different in cagemates and strangers (2-way mixed effects ANOVA; effect of stranger/cagemate: $F(1,218) = 0.11$, $p = 0.74$, $\eta^2 = 0.001$; Figure5C). Interestingly, juvenile degus were more easy to classify than adolescent degus ($F(1,218) = 15.31$, $p < 0.001$, $\eta^2 = 0.06$). These findings partially support our prediction that juvenile and adolescent animals do not seem to form individual specific relationships based on familiarity, but contrary to what we predicted, juveniles do seem to form more unique and therefore distinguishable relationships compared to adolescents. This finding is also inconsistent with the results seen using the cosine distance metrics. The variability of social interactions of juveniles was not significantly different from adolescents on any exposure day. Dyad classification success rates increased over session days in adolescent female dyads, which is consistent with the finding of decreased variability over time ($F(4,428) = 5.74$, $p < 0.001$, $\eta^2 = 0.01$; Figure5D). This increase may indicate that as they age and become more familiar with their environment and their peers they begin to form more distinct relationships, specifically for stranger dyads (cagemate/stranger and exposure day $F(4,428) = 4.43$, $p = 0.002$, $\eta^2 = 0.004$).

Figure 5

Variability Across Sessions and the Effects of Age and Familiarity



Note. A) Swarm plot showing the distribution of within-dyad interaction vector distances (operationally "variability") in cagemate and stranger dyads of both age groups. There were no significant differences between strangers and cagemates for with age group. Adolescent strangers do display a bimodal distribution. B) Within-dyad interaction vector distances after separating-out each exposure, e.g., "exposure 1" only includes distances between exposure 1 vectors and other days—it would not include distances between exposures 2 and 3, or 3 and 4. Values are across-dyad averages of within-dyad medians, ±SEM in juveniles (left) and adolescents (right). C) Success rate of SVM classifiers discriminating pairs of dyads among within each of the four groups (error bars are mean ± SEM). D) Relative success rates for different days, e.g., "exposure 1" only includes models trained on exposures 2 through 5, with accuracy tested for the exposure 1 vector in juveniles (left) and adolescents (right).

Discussion

As the function and the specific behaviors observed during social interactions has been found in previous literature to differ across the lifespan, we first investigated the total interaction time and of specific behaviors in juveniles and adolescents. We also explored whether these differences changed over time. In line with our predictions, interactions were found to differ between juveniles and adolescents both in the amount and type of behaviors observed. Much of the previous literature on the effects of familiarity on the social interaction of juveniles, however, indicated that play and social interaction levels were higher with familiar individuals than unfamiliar individuals (Argue & McCarthy, 2015; Cox & Rissman, 2011; Terranova et al., 1993). These findings are in contrast to what our study shows that juvenile female degus spend similar amounts of time interacting with familiar and unfamiliar individuals. This effect was constant across the first three or four sessions, but on the fourth or fifth exposure day they began to interact more with strangers than cagemates. We posit that the differences seen in interactions between strangers and cagemates in the last few sessions are not due to familiarity, but instead due to the age of the individuals. We observed that adolescent animals do in fact interact differently due to familiarity, with more interactions between stranger dyads than cagemates. As the end of the juvenile experiments often overlapped with the degus transition from the juvenile phase to the adolescent phase, these effects are likely due to this transition. The lack of differences in interactions due to familiarity of the individuals further suggests that unlike juvenile rats and mice, juvenile degus do not interact differently with familiar and unfamiliar individuals. This may speak to species differences in the function of relationships at this stage of development.

The main purpose of this project was to test whether interaction variability as measured by a distance metric and classification success would differ between juvenile and adolescent female degus. The variability of social interactions was not significantly different between strangers and cagemates, supporting our prediction. There were several unexpected results, however, that were not in line with what was predicted. The first unexpected result was that the variability of interactions decreased over time in both juvenile and adolescents. The second unexpected observation regarded the dyad classification success rates. We observed more successful classification rates in juveniles than in adolescents and this result was not consistent with the variability observed using the cosine distance method. The final unexpected observation was that adolescent's classification rates became more successful across sessions, especially in stranger dyads.

While there is not much research on the stability of social interactions in juvenile and adolescent animals, previous research does suggest that relationships are more variable (and therefore less stable) than those of adults (Argue & McCarthy, 2015; Borgeaud et al., 2016; Terranova et al., 1993). The interaction variability of adult relationships, however, is greatly affected by the familiarity level of the dyad (Lee et al., 2019). The results this study indicate a very different pattern to those suggested in previous work. We found that the variability of social interactions does not differ with level of familiarity in either juveniles or in adolescents, but they do become more stable (less variable) across sessions. The lack of differences in the variability of social interactions due to familiarity suggests interactions between familiar and unfamiliar dyads are overall more alike than they are different. This finding is further supported by the results of the dyad classification success rates as this method also did not reveal a significant difference between stranger and cagemate dyads. In other words, for juvenile and adolescent female degus, whether the dyads are familiar with one another or not does not influence the variability of their interactions. Our prediction that the variability of interactions would not change was influenced by the function relationships have at these stages of development, learning appropriate social behaviors and testing the behavioral responses of others. Our result of less variability (more stable) across sessions may suggest that juvenile and adolescent relationships take much longer to form and stabilize and are therefore more variable initially. Another potential interpretation of this result regards the novelty of the environment. Exposure to new environments is known to increase stress levels and alter the number of social interactions that occur (Varlinskaya & Spear, 2008). While this study reports a decrease in the number of social behaviors that occur when in a novel environment, and even our own results show that social interactions increase across sessions these interactions may still be more variable initially and decrease with familiarity of the environment.

The final two unexpected observations are closely related, and therefore will be discussed in conjunction with one another. The observation that juvenile relationships, independent of their familiarity had been more easily classified than adolescent relationships was surprising, even more so when looking at the cosine distance measure of variability. We did not see any statistically significant difference in the variability of juvenile interactions compared to those of adolescents. This may suggest that measures of variability may not be useful for detecting relationships within or between age groups. It may be that the SVM dyad classification method used something other than variability to identify a dyad, or it used variability in conjunction with a specific behavior. In other words, variability cannot be used alone as a marker of relationship formation, however further investigation should be done to identify what made juvenile relationships easier to classify than adolescents. Another surprising result was that adolescent cagemate and stranger interactions became more stable over time. This was observed through both the decreased variability and increased classification success across sessions. The increase in classification success over time was more apparent in adolescent strangers, an effect that was also seen in adult female degus. Adult female stranger dyads were more variable and more easily classified than cagemate dyads. This similarity may suggest that as the adolescents begin to reach the end of the adolescent developmental phase, they begin to interact more like adult females do. The change we observed over sessions, if truly due to the aging of the animals, implies that there are distinct differences in the social interactions of adolescent and adult female degus, and also the function of these interactions. Adolescent social interactions were initially more variable, suggesting that they may be sampling different methods of interacting with peers, and therefore learning appropriate social behaviors. As they age, the variability decreases, the interactions become more stable, and the function may be to form new bonds and maintain group cohesion.

There are several limitations of these methods that may have impacted the results observed in this study. First, we must consider the potential confounding effects of stress. The novel environmental setting in addition to the stress associated with post-weaning isolation (for the juveniles specifically) may have contributed to the relatively low interaction levels we observed. In both mice and rats, prolonged social isolation post-weaning increases cortisol responses and abnormal social behavior, and these effects last into adulthood (Toth et al., 2011; Walker et al., 2019). While our animals did not experience more than 24hours of isolation at a time, the repeated nature of the isolation may have produced similar effects. Another limitation of this study are the limited behaviors included in our ethogram. Previous work with juvenile and adolescent male degus, for example, have identified many more nuanced behaviors that are involved in play and play-fighting in degus that we did not include here (Pellis et al., 2010). Furthermore, previous work suggests that measures of "in-proximity" greatly influence measures of affiliative behavior in adults, as well as juvenile and adolescent animals (Brent et al., 2014; Kulik et al., 2015). Inclusion of these behaviors may have altered the results observed in this study and therefore the impact of the findings as well. A final limitation of this study regards the isolation procedure. Due to the reliance on their mother's milk until at least four weeks of age, we were not able to begin the juvenile phase of this study until after the pups were weaned. This often resulted in juveniles reaching the adolescent phase of their development prior to the end of their juvenile reunion sessions and could have influenced the effects seen in juvenile social interactions, specifically the effects seen in the last few interaction sessions.

Though the results of the measures of variability were unclear with the juvenile degus, the results still provided some useful observations. We were able to demonstrate that measures of variability alone were not sufficient markers of relationship formation. The decreasing variability of juveniles and adolescent dyads, regardless of their familiarity also suggests that their relationships are changing. This change may be due to the increasing age, or it may be due to the formation of more established relationships. The result from our adolescent degus further supports the possibility that age is influencing their relationships. The decrease of variability over time may indeed be suggestive that as the degus age the function of social interactions changes from learning to one of social cohesion. Additionally, the results of this study greatly add to the body of literature on social interactions in juvenile and adolescent rodents through the introduction of a new species, and the addition of multiple interactions. There is very little research on the social behavior of degus, and even less about juvenile and adolescent behaviors. This research not only adds to our knowledge of the ontogeny of social behaviors in the degu, but also furthers our understanding of the formation of social relationships over time during early development.

Chapter 7: Summary and Future Directions

It was the goal of the current project was to identify a method of measuring social relationship formation that could be generalizable across ontogeny and species. Our results suggest that the methods used to measure and compare interaction variability were valuable and provided us with important information but cannot be used in isolation to describe social relationships and their formations. We did identify clear differences in the variability of interactions between cagemates, strangers, males, and females and across development. The variability of interactions was highest in adult males and lowest in juvenile females with adult females and adolescent females resting in between them. These differences do suggest that variability should be included in future studies in addition to other more general methods of measuring relationships.

In adult degus, while the results were not as we predicted still provided information needed for future research. Our finding that female strangers were more variable than cagemates suggest that in female degus, new relationships are initially stable, which may be an attempt to reduce uncertainty and increase feelings of safety while getting to know one another, as has been seen in other species (Aureli & Schaffner, 2007; Dias et al., 2008; Kutsukake et al., 2006; Smuts & Watanabe, 1990; Whitham & Maestripieri, 2003). These findings also suggest that there may be distinct differences in the relationships between siblings and non-siblings in the laboratory setting. As many young degus emigrate to other groups in wild degu populations long-term relationships between adult female siblings has not been documented and may not be a common occurrence. This interpretation is also supported through laboratory-based research which has shown that genetic kinship can influence social behavior, just not as strongly as familiarity (Quirici et al., 2011; Villavicencio et al., 2009). As these dyads were highly familiar with one another (many being housed together since their weaning) and their genetic relatedness they may have more unique relationships that are not easily formed with unfamiliar peers.

The results of our developmental study, when compared with our adult females have provided useful insight into the ontogeny of social relationships. The decreasing variability of juveniles and adolescent dyads, regardless of their familiarity indicate that as the degus age the function of social interactions may be changing from learning to social cohesion. It has been found in previous work that increased variability benefits learning across many disciplines and our work adds to this concept in the social realm (Raviv et al., 2022). This is further evidenced through the classification success results in adolescents. The increase in classification success over time was more apparent in adolescent strangers, an effect that was also seen in adult female degus. Adult female stranger dyads were more variable and more easily classified than cagemate dyads. This similarity may suggest that as the adolescents begin to reach the end of the adolescent developmental phase, they begin to interact more like adult females do.

The results of this project highlight the usefulness of methods such as these and warrant further investigation. Furthermore, while our results of how this variability changed over time were not what we predicted, they still provided a useful initial glance into the connection between the variability of interactions and social relationships. Additionally, the results of this project have added to the limited body of research on how social relationships are formed, and the ontogeny of this process. Further research needs to be conducted comparing these results to other species, however, to fully determine the usefulness of these measures. Given the unexpected nature of several of our results it may be that these measures are not able to fully capture the full picture of relationship formation and should be further investigated with the inclusion of other generalizable methods.

References

Argue, K. J., & McCarthy, M. M. (2015). Characterization of juvenile play in rats: Importance of sex of self and sex of partner. *Biology of Sex Differences*, *6*(1), 16. https://doi.org/10.1186/s13293-015-0034-x

Armstrong, R. A. (2014). When to use the Bonferroni correction. *Ophthalmic & Physiological Optics: The Journal of the British College of Ophthalmic Opticians (Optometrists)*, *34*(5), 502–508. https://doi.org/10.1111/opo.12131

Asgari, P., McKinney, G., Hodges, T. E., & McCormick, C. M. (2021). Social Instability Stress in Adolescence and Social Interaction in Female Rats. *Neuroscience*, *477*, 1–13. https://doi.org/10.1016/j.neuroscience.2021.09.022

Aureli, F., & Schaffner, C. M. (2007). Aggression and conflict management at fusion in spider monkeys. *Biology Letters*, *3*(2), 147–149. https://doi.org/10.1098/rsbl.2007.0041

Barale, C. L., Rubenstein, D. I., & Beehner, J. C. (2015). Juvenile social relationships reflect adult patterns of behavior in wild geladas. *American Journal of Primatology*, *77*(10), 1086–1096. https://doi.org/10.1002/ajp.22443

Barrett, L., & Henzi, S. P. (2002). Constraints on Relationship Formation among Female Primates. *Behaviour*, *139*(2/3), 263–289.

Beery, A. K., Christensen, J. D., Lee, N. S., & Blandino, K. L. (2018). Specificity in Sociality: Mice and Prairie Voles Exhibit Different Patterns of Peer Affiliation. *Frontiers in Behavioral Neuroscience*, *12*. https://doi.org/10.3389/fnbeh.2018.00050

Beery, A. K., & Shambaugh, K. L. (2021). Comparative Assessment of

Familiarity/Novelty Preferences in Rodents. *Frontiers in Behavioral Neuroscience*, *15*. https://doi.org/10.3389/fnbeh.2021.648830

Bekoff, M. (1972). The Development of Social Interaction, Play, and Metacommunication in Mammals: An Ethological Perspective. *The Quarterly Review of Biology*, *47*(4), 412–434. JSTOR.

Bell, H. C., Pellis, S. M., & Kolb, B. (2010). Juvenile peer play experience and the development of the orbitofrontal and medial prefrontal cortices. *Behavioural Brain Research*, *207*(1), 7–13. https://doi.org/10.1016/j.bbr.2009.09.029

Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, *57*(1), 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Blakemore, S.-J. (2008). Development of the Social Brain during Adolescence. *Quarterly Journal of Experimental Psychology*, *61*(1), 40–49. https://doi.org/10.1080/17470210701508715

Borgeaud, C., Sosa, S., Bshary, R., Sueur, C., & van de Waal, E. (2016). Intergroup Variation of Social Relationships in Wild Vervet Monkeys: A Dynamic Network Approach. *Frontiers in Psychology*, *7*. https://www.frontiersin.org/articles/10.3389/fpsyg.2016.00915

Bowker, J., & Weingarten, J. (2022). Temporal approaches to the study of friendship: Understanding the developmental significance of friendship change during childhood and adolescence. In *Advances in Child Development and Behavior* (Vol. 63). https://doi.org/10.1016/bs.acdb.2022.04.005

Brent, L. J. N., Chang, S. W. C., Gariépy, J.-F., & Platt, M. L. (2014). The neuroethology of friendship. *Annals of the New York Academy of Sciences*, *1316*(1), 1–17. https://doi.org/10.1111/nyas.12315

Brent, L. J. N., Ruiz-Lambides, A., & Platt, M. L. (2017). Family network size and survival across the lifespan of female macaques. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1854). https://doi.org/10.1098/rspb.2017.0515

Bukowski, W. M., Newcomb, A. F., & Hartup, W. W. (1998). *The Company They Keep: Friendships in Childhood and Adolescence*. Cambridge University Press.

Carter, G. G., Farine, D. R., Crisp, R. J., Vrtilek, J. K., Ripperger, S. P., & Page, R. A. (2020). Development of New Food-Sharing Relationships in Vampire Bats. *Current Biology*, *30*(7), 1275-1279.e3. https://doi.org/10.1016/j.cub.2020.01.055

Cavigelli, S. A., Michael, K. C., West, S. G., & Klein, L. C. (2011). Behavioral responses to physical vs. Social novelty in male and female laboratory rats. *Behavioural Processes*, *88*(1), 56–59. https://doi.org/10.1016/j.beproc.2011.06.006

Cheney, D. L., Silk, J. B., & Seyfarth, R. M. (2016). Network connections, dyadic bonds and fitness in wild female baboons. *Royal Society Open Science*, *3*(7). https://doi.org/10.1098/rsos.160255

Cirulli, F., Terranova, M. L., & Laviola, G. (1996). Affiliation in periadolescent rats: Behavioral and corticosterone response to social reunion with familiar or unfamiliar partners. *Pharmacology Biochemistry and Behavior*, *54*(1), 99–105. https://doi.org/10.1016/0091-3057(95)02169-8

Colonnello, V., Iacobucci, P., Fuchs, T., Newberry, R. C., & Panksepp, J. (2011). Octodon degus. A useful animal model for social-affective neuroscience research:

Basic description of separation distress, social attachments and play. *Neuroscience & Biobehavioral Reviews*, *35*(9), 1854–1863. https://doi.org/10.1016/j.neubiorev.2011.03.014

Corrêa, S. A., Fernandes, M. O., Iseki, K. K., & Negrão, J. A. (2003). Effect of the establishment of dominance relationships on cortisol and other metabolic parameters in Nile tilapia (Oreochromis niloticus). *Brazilian Journal of Medical and Biological Research*, *36*(12), 1725–1731. https://doi.org/10.1590/S0100-879X2003001200015

Cox, K. H., & Rissman, E. F. (2011). Sex differences in juvenile mouse social behavior are influenced by sex chromosomes and social context. *Genes, Brain and Behavior*, *10*(4), 465–472. https://doi.org/10.1111/j.1601-183X.2011.00688.x

Curtis, T. M., Knowles, R. J., & Crowell-Davis, S. L. (2003). Influence of familiarity and relatedness on proximity and allogrooming in domestic cats (Felis catus). *American Journal of Veterinary Research*, *64*(9), 1151–1154. https://doi.org/10.2460/ajvr.2003.64.1151

Davis, G. T., Vásquez, R. A., Poulin, E., Oda, E., Bazán-León, E. A., Ebensperger, L. A., & Hayes, L. D. (2016). Octodon degus kin and social structure. *Journal of Mammalogy*, *97*(2), 361–372. https://doi.org/10.1093/jmammal/gyv182

Davis, T. M. (1975). Effects of familiarity on agonistic encounter behavior in male degus (Octodon degus). *Behavioral Biology*, *14*(4), 511–517. https://doi.org/10.1016/S0091-6773(75)90696-3

Dias, P. A. D., Luna, E. R., & Espinosa, D. C. (2008). The functions of the “Greeting Ceremony” among male mantled howlers (Alouatta palliata) on Agaltepec Island, Mexico. *American Journal of Primatology*, *70*(7), 621–628. https://doi.org/10.1002/ajp.20535

Ebensperger, L. A., Aracena, S., Avendaño, N., Toro, A., León, C., Ramírez-Estrada, J., & Abades, S. (2017). Social instability decreases alloparental care and quality of weaned offspring in a communally rearing rodent. *Animal Behaviour*, *133*, 195–205. https://doi.org/10.1016/j.anbehav.2017.09.021

Ebensperger, L. A., Chesh, A. S., Castro, R. A., Tolhuysen, L. O., Quirici, V., Burger, J. R., & Hayes, L. D. (2009). Instability Rules Social Groups in the Communal Breeder Rodent *Octodon degus*. *Ethology*, *115*(6), 540–554. https://doi.org/10.1111/j.1439-0310.2009.01635.x

Ebensperger, L. A., Correa, L. A., León, C., Ramírez-Estrada, J., Abades, S., Villegas, Á., & Hayes, L. D. (2016). The modulating role of group stability on fitness effects of group size is different in females and males of a communally rearing rodent. *Journal of Animal Ecology*, *85*(6), 1502–1515. https://doi.org/10.1111/1365-2656.12566

Ebensperger, L. A., & Hurtado, M. J. (2005). Seasonal Changes in the Time Budget of Degus, Octodon degus. *Behaviour*, *142*(1), 91–112.

Ebensperger, L. A., Hurtado, M. J., & Ramos□Jiliberto, R. (2006). Vigilance and Collective Detection of Predators in Degus (Octodon degus). *Ethology*, *112*(9), 879–887. https://doi.org/10.1111/j.1439-0310.2006.01242.x

Ebensperger, L. A., Hurtado, M. J., Soto-Gamboa, M., Lacey, E. A., & Chang, A. T. (2004). Communal nesting and kinship in degus (Octodon degus). *Naturwissenschaften*, *91*(8), 391–395. https://doi.org/10.1007/s00114-004-0545-5

Ebensperger, L. A., Sobrero, R., Quirici, V., Castro, R. A., Tolhuysen, L. O., Vargas, F., Burger, J. R., Quispe, R., Villavicencio, C. P., Vásquez, R. A., & Hayes, L. D. (2012). Ecological drivers of group living in two populations of the communally rearing rodent, Octodon degus. *Behavioral Ecology and Sociobiology*, *66*(2), 261–274. https://doi.org/10.1007/s00265-011-1274-3

Ebensperger, L. A., Veloso, C., & Wallem, P. (2002). Do female degus communally nest and nurse their pups? *Journal of Ethology*, *20*(2), 143–146. https://doi.org/10.1007/s10164-002-0063-x

Ebensperger, L. A., Villegas, Á., Abades, S., & Hayes, L. D. (2014). Mean ecological conditions modulate the effects of group living and communal rearing on offspring production and survival. *Behavioral Ecology*, *25*(4), 862–870. https://doi.org/10.1093/beheco/aru061

Ebensperger, L., & Hayes, L. (2016). *An integrative view of caviomorph social behavior* (pp. 326–355). https://doi.org/10.1002/9781118846506.ch13

Ellis, S., Franks, D. W., Nattrass, S., Cant, M. A., Weiss, M. N., Giles, D., Balcomb, K. C., & Croft, D. P. (2017). Mortality risk and social network position in resident killer whales: Sex differences and the importance of resource abundance. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1865). https://doi.org/10.1098/rspb.2017.1313

Fischer, R. B., & Meunier, G. F. (1985). Responses to conspecifics' urine by the degu (Octodon degus). *Physiology & Behavior*, *34*(6), 999–1001. https://doi.org/10.1016/0031-9384(85)90027-7

Fischer, R. B., Smith, S. L., White, P. J., & Meunier, G. F. (1986). Sex differences during initial social contact in the degu (Octodondegus). *Behavioural Processes*, *12*(1), 67–76. https://doi.org/10.1016/0376-6357(86)90072-0

Friard, O., & Gamba, M. (2016). BORIS: A free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, *7*(11), 1325–1330. https://doi.org/10.1111/2041-210X.12584

Fulk, G. W. (1976). Notes on the Activity, Reproduction, and Social Behavior of Octodon degus. *Journal of Mammalogy*, *57*(3), 495–505. JSTOR. https://doi.org/10.2307/1379298

Gauffre, B., Petit, E., Brodier, S., Bretagnolle, V., & Cosson, J. F. (2009). Sex-biased dispersal patterns depend on the spatial scale in a social rodent. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1672), 3487–3494. https://doi.org/10.1098/rspb.2009.0881

Gherardi, F., Cenni, F., Parisi, G., & Aquiloni, L. (2010). Visual recognition of conspecifics in the American lobster, Homarus americanus. *Animal Behaviour*, *80*(4), 713–719. https://doi.org/10.1016/j.anbehav.2010.07.008

Goldstein, S., Field, T., & Healy, B. (1989). Concordance of play behavior and physiology in preschool friends. *Journal of Applied Developmental Psychology*, *10*(3), 337–351. https://doi.org/10.1016/0193-3973(89)90034-8

Gottman, J., Gonso, J., & Rasmussen, B. (1975). *Social Interaction, Social Competence, and Friendship in Children*. 11.

Graham, M. E., & Herberholz, J. (2009). Stability of dominance relationships in crayfish depends on social context. *Animal Behaviour*, *77*(1), 195–199. https://doi.org/10.1016/j.anbehav.2008.09.027

Hallgren, K. A. (2012). Computing Inter-Rater Reliability for Observational Data: An Overview and Tutorial. *Tutorials in Quantitative Methods for Psychology*, *8*(1), 23–34.

Hayes, L. D., Chesh, A. S., Castro, R. A., Tolhuysen, L. O., Burger, J. R., Bhattacharjee, J., & Ebensperger, L. A. (2009). Fitness consequences of group living in the degu Octodon degus, a plural breeder rodent with communal care. *Animal Behaviour*, *78*(1), 131–139. https://doi.org/10.1016/j.anbehav.2009.03.022

Hinde. (1976). Interactions, Relationships and Social Structure. *Man*, *11*(1), 1–17. JSTOR. https://doi.org/10.2307/2800384

Hinde. (1979). *Towards understanding relationships*. Published in cooperation with European Association of Experimental Social Psychology by Academic Press.

Hodges, T. E., Baumbach, J. L., Marcolin, M. L., Bredewold, R., Veenema, A. H., & McCormick, C. M. (2017). Social instability stress in adolescent male rats reduces social interaction and social recognition performance and increases oxytocin receptor binding. *Neuroscience*, *359*, 172–182. https://doi.org/10.1016/j.neuroscience.2017.07.032

Holekamp, K. E., Sakai, S. T., & Lundrigan, B. L. (2007). Social intelligence in the spotted hyena (*Crocuta crocuta*). *Philosophical Transactions of the Royal Society B: Biological Sciences*, *362*(1480), 523–538. https://doi.org/10.1098/rstb.2006.1993

Insel, N., Shambaugh, K. L., & Beery, A. K. (2020). Female degus show high sociality but no preference for familiar peers. *Behavioural Processes*, *174*, 104102. https://doi.org/10.1016/j.beproc.2020.104102

Jäger, H. Y., Han, C. S., & Dingemanse, N. J. (2019). Social experiences shape behavioral individuality and within-individual stability. *Behavioral Ecology*, *30*(4), 1012–1019. https://doi.org/10.1093/beheco/arz042

King, J. A. (1956). Social Relations of the Domestic Guinea Pig Living under Semi-Natural Conditions. *Ecology*, *37*(2), 221–228. https://doi.org/10.2307/1933134

Kondrakiewicz, K., Kostecki, M., Szadzinska, W., & Knapska, E. (2018). Ecological validity of social interaction tests in rats and mice. *Genes, Brain and Behavior*. https://doi.org/10.1111/gbb.12525

Koski, S. E., de Vries, H., van de Kraats, A., & Sterck, E. H. M. (2012). Stability and Change of Social Relationship Quality in Captive Chimpanzees (Pan troglodytes). *International Journal of Primatology*, *33*(4), 905–921. https://doi.org/10.1007/s10764-012-9623-2

Kulik, L., Amici, F., Langos, D., & Widdig, A. (2015). Sex Differences in the Development of Social Relationships in Rhesus Macaques (Macaca mulatta). *International Journal of Primatology*, *36*(2), 353–376. https://doi.org/10.1007/s10764-015-9826-4

Kummer, H. (1978). On the value of social relationships to nonhuman primates: A heuristic scheme. *Social Science Information*, *17*(4–5), 687–705. https://doi.org/10.1177/053901847801700418

Kutsukake, N., Suetsugu, N., & Hasegawa, T. (2006). Pattern, Distribution, and Function of Greeting Behavior Among Black-and-White Colobus. *International Journal of Primatology*, *27*(5), 1271–1291. https://doi.org/10.1007/s10764-006-9072-x

Lee, P. C. (1987). Sibships: Cooperation and competition among immature vervet monkeys. *Primates*, *28*(1), 47–59. https://doi.org/10.1007/BF02382182

Lee, W., Fu, J., Bouwman, N., Farago, P., & Curley, J. P. (2019). Temporal microstructure of dyadic social behavior during relationship formation in mice. *PLOS ONE*, *14*(12), e0220596. https://doi.org/10.1371/journal.pone.0220596

Lidhar, N. K., Thakur, A., David, A.-J., Takehara-Nishiuchi, K., & Insel, N. (2021). Multiple dimensions of social motivation in adult female degus. *PLOS ONE*, *16*(4), e0250219. https://doi.org/10.1371/journal.pone.0250219

Livia Terranova, M., Cirulli, F., & Laviola, G. (1999). Behavioral and hormonal effects of partner familiarity in periadolescent rat pairs upon novelty exposure. *Psychoneuroendocrinology*, *24*(6), 639–656. https://doi.org/10.1016/S0306-4530(99)00019-0

Mahoney, M. M., Rossi, B. V., Hagenauer, M. H., & Lee, T. M. (2011). Characterization of the Estrous Cycle in Octodon degus. *Biology of Reproduction*, *84*(4), 664–671. https://doi.org/10.1095/biolreprod.110.087403

Majolo, B., de Bortoli Vizioli, A., & Schino, G. (2008). Costs and benefits of group living in primates: Group size effects on behaviour and demography. *Animal Behaviour*, *76*(4), 1235–1247. https://doi.org/10.1016/j.anbehav.2008.06.008

Massen, J. J. M., & Koski, S. E. (2014). Chimps of a feather sit together: Chimpanzee friendships are based on homophily in personality. *Evolution and Human Behavior*, *35*(1), 1–8. https://doi.org/10.1016/j.evolhumbehav.2013.08.008

Massen, J. J. M., & Sterck, E. H. M. (2013). Stability and Durability of Intra- and Intersex Social Bonds of Captive Rhesus Macaques (Macaca mulatta). *International Journal of Primatology*, *34*(4), 770–791.

https://doi.org/10.1007/s10764-013-9695-7

McCormick, C. M., Merrick, A., Secen, J., & Helmreich, D. L. (2007). Social instability in adolescence alters the central and peripheral hypothalamic-pituitary-adrenal responses to a repeated homotypic stressor in male and female rats. *Journal of Neuroendocrinology*, *19*(2), 116–126. https://doi.org/10.1111/j.1365-2826.2006.01515.x

McFarland, R., & Majolo, B. (2013). Coping with the cold: Predictors of survival in wild Barbary macaques, Macaca sylvanus. *Biology Letters*, *9*(4), 20130428. https://doi.org/10.1098/rsbl.2013.0428

McGraw, K. O., & Wong, S. P. (1996). Forming inferences about some intraclass correlation coefficients. *Psychological Methods*, *1*, 30–46. https://doi.org/10.1037/1082-989X.1.1.30

Meaney, M. J., & Stewart, J. (1979). Environmental factors influencing the affiliative behavior of male and female rats (Rattus norvegicus). *Animal Learning & Behavior*, *7*(3), 397–405. https://doi.org/10.3758/BF03209692

Meaney, M. J., & Stewart, J. (1981). A descriptive study of social development in the rat (Rattus norvegicus). *Animal Behaviour*, *29*(1), 34–45. https://doi.org/10.1016/S0003-3472(81)80149-2

Moy, S. S., Nadler, J. J., Perez, A., Barbaro, R. P., Johns, J. M., Magnuson, T. R., Piven, J., & Crawley, J. N. (2004). Sociability and preference for social novelty in five inbred strains: An approach to assess autistic-like behavior in mice. *Genes, Brain and Behavior*, *3*(5), 287–302. https://doi.org/10.1111/j.1601-1848.2004.00076.x

Nunes, S., Weidenbach, J. N., Lafler, M. R., & Dever, J. A. (2015). Sibling relatedness and social play in juvenile ground squirrels. *Behavioral Ecology and Sociobiology*, *69*(3), 357–369. https://doi.org/10.1007/s00265-014-1848-y

O'Connor, C., & Joffe, H. (2020). Intercoder Reliability in Qualitative Research: Debates and Practical Guidelines. *International Journal of Qualitative Methods*, *19*, 1609406919899220. https://doi.org/10.1177/1609406919899220

Overli, O., Harris, C. A., & Winberg, S. (1999). Short-term effects of fights for social dominance and the establishment of dominant-subordinate relationships on brain monoamines and cortisol in rainbow trout. *Brain, Behavior and Evolution; Basel*, *54*(5), 263–275.

Palagi, E. (2018). Not just for fun! Social playas a springboard for adult social competence in human and non-humanprimates. *Behavioral Ecology and Sociobiology*, *72*(6), 90. https://doi.org/10.1007/s00265-018-2506-6

Pellis, S. M., & Pellis, V. C. (2007). Rough-and-Tumble Play and the Development of the Social Brain. *Current Directions in Psychological Science*, *16*(2), 95–98. https://doi.org/10.1111/j.1467-8721.2007.00483.x

Pellis, S. M., Pellis, V. C., & Bell, H. C. (2010). The Function of Play in the Development of the Social Brain. *American Journal of Play*, *2*(3), 278–296.

Pellis, S. M., Pellis, V. C., & Foroud, A. (2005). Play Fighting: Aggression, Affiliation, and the Development of Nuanced Social Skills. In *Developmental Origins of Aggression* (pp. 47–63). Guilford Press.

Quirici, V., Faugeron, S., Hayes, L. D., & Ebensperger, L. A. (2011). Absence of kin structure in a population of the group-living rodent Octodon degus. *Behavioral Ecology*, *22*(2), 248–254. https://doi.org/10.1093/beheco/arq196

Raviv, L., Lupyan, G., & Green, S. C. (2022). How variability shapes learning and generalization. *Trends in Cognitive Sciences*, *26*(6), 462–483. https://doi.org/10.1016/j.tics.2022.03.007

Sapolsky, R. M. (1990). Adrenocortical function, social rank, and personality among wild baboons. *Biological Psychiatry*, *28*(10), 862–878. https://doi.org/10.1016/0006-3223(90)90568-M

Shimada, M., & Sueur, C. (2018). Social play among juvenile wild Japanese macaques (Macaca fuscata) strengthens their social bonds. *American Journal of Primatology*, *80*(1), e22728. https://doi.org/10.1002/ajp.22728

Silk, J., Cheney, D., & Seyfarth, R. (2013). A practical guide to the study of social relationships. *Evolutionary Anthropology: Issues, News, and Reviews*, *22*(5), 213–225. https://doi.org/10.1002/evan.21367

Smuts, B. B., & Watanabe, J. M. (1990). Social relationships and ritualized greetings in adult male baboons (Papio cynocephalus anubis). *International Journal of Primatology*, *11*(2), 147–172. https://doi.org/10.1007/BF02192786

Soto-Gamboa, M., Villalón, M., & Bozinovic, F. (2005). Social cues and hormone levels in male Octodon degus (Rodentia): A field test of the Challenge Hypothesis. *Hormones and Behavior*, *47*(3), 311–318. https://doi.org/10.1016/j.yhbeh.2004.11.010

Terranova, M. L., Laviola, G., & Alleva, E. (1993). Ontogeny of amicable social behavior in the mouse: Gender differences and ongoing isolation outcomes. *Developmental Psychobiology*, *26*(8), 467–481. https://doi.org/10.1002/dev.420260805

Toth, M., Mikics, E., Tulogdi, A., Aliczki, M., & Haller, J. (2011). Post-weaning social isolation induces abnormal forms of aggression in conjunction with increased glucocorticoid and autonomic stress responses. *Hormones and Behavior*, *60*(1), 28–36. https://doi.org/10.1016/j.yhbeh.2011.02.003

Tyack, P. L. (2008). Convergence of calls as animals form social bonds, active compensation for noisy communication channels, and the evolution of vocal learning in mammals. *Journal of Comparative Psychology*, *122*(3), 319–331. https://doi.org/10.1037/a0013087

Uekita, T., & Okanoya, K. (2011). Hippocampus lesions induced deficits in social and spatial recognition in Octodon degus. *Behavioural Brain Research*, *219*(2), 302–309. https://doi.org/10.1016/j.bbr.2011.01.042

Vandeleest, J. J., Capitanio, J. P., Hamel, A., Meyer, J., Novak, M., Mendoza, S. P., & McCowan, B. (2019). Social stability influences the association between adrenal responsiveness and hair cortisol concentrations in rhesus macaques. *Psychoneuroendocrinology*, *100*, 164–171. https://doi.org/10.1016/j.psyneuen.2018.10.008

Varlinskaya, E. (2008). Social interactions in adolescent and adult Sprague–Dawley rats: Impact of social deprivation and test context familiarity. *Behavioural Brain Research*, *188*(2), 398–405. https://doi.org/10.1016/j.bbr.2007.11.024

Varlinskaya, E. I., & Spear, L. P. (2008). Social interactions in adolescent and adult Sprague–Dawley rats: Impact of social deprivation and test context familiarity. *Behavioural Brain Research*, *188*(2), 398–405. https://doi.org/10.1016/j.bbr.2007.11.024

Varlinskaya, E. I., Vogt, B. A., & Spear, L. P. (2012). Social context induces two unique patterns of c-Fos expression in adolescent and adult rats. *Developmental Psychobiology*, n/a-n/a. https://doi.org/10.1002/dev.21064

Villavicencio, C. P., Márquez, I. N., Quispe, R., & Vásquez, R. A. (2009). Familiarity and phenotypic similarity influence kin discrimination in the social rodent Octodon degus. *Animal Behaviour*, *78*(2), 377–384. https://doi.org/10.1016/j.anbehav.2009.04.026

Walker, D. M., Cunningham, A. M., Gregory, J. K., & Nestler, E. J. (2019). Long-Term Behavioral Effects of Post-weaning Social Isolation in Males and Females. *Frontiers in Behavioral Neuroscience*, *13*. https://www.frontiersin.org/article/10.3389/fnbeh.2019.00066

Wang, Y., Palonen, T., Hurme, T.-R., & Kinos, J. (2019). Do you want to play with me today? Friendship stability among preschool children. *European Early Childhood Education Research Journal*, *27*(2), 170–184. https://doi.org/10.1080/1350293X.2019.1579545

Whitehead, H., & Dufault, S. (1999). Techniques for analyzing vertebrate social structure using identified individuals. *Adv Stud Behav*, *28*, 3–74.

Whitham, J. C., & Maestripieri, D. (2003). Primate Rituals: The Function of Greetings between Male Guinea Baboons. *Ethology*, *109*(10), 847–859. https://doi.org/10.1046/j.0179-1613.2003.00922.x

Winslow, J. T. (2003). Mouse Social Recognition and Preference. *Current Protocols in Neuroscience*, *22*(1). https://doi.org/10.1002/0471142301.ns0816s22

Yamada-Haga, Y. (2002). Characteristics of social interaction between unfamiliar male rats (Rattus norvegicus): Comparison of juvenile and adult stages. *Journal of Ethology*, *20*(1), 55–62. https://doi.org/10.1007/s10164-002-0054-y

Table 1

Ethogram with Descriptions of Each Behavior

Behavior Type	Behavior	Description
Agonistic	Wrestling	rapid movement of both bodies and usually accompanied by other actions such as punching, biting and body-slams; often visible hair loss or blood
	Biting	mouth/head of one degu making contact with any part of the body of the other, paired with a vocalization and/or rapid movement of the degu being bitten
	Rear Push	one animal approaching the other and swiping their rear end against the other animal or using their rear end to push the other animal
	Marking	any time an animal lifted their rear leg close to any area of the other animal’s body
	Boxing	both animals rearing on their hind legs and pushing each other with their forepaws
	Tail Shake	rapid movement of the degu’s tail side to side
	Avoid	One degu runs from another as they approach or during an interaction
	Mounting	one degu placing its front paws above or on top of the other animal

Affiliative	Huddling	both animals sitting next to the other with some part of their body touching
	Grooming	nose/face of one degu touching any area of the conspecific paired with rapid head or mouth movements
	In-Proximity	both animals sitting, inactive within one quadrant of the enclosure, neither animal is touching or interacting with the other
Investigative	Face-to-face	both faces of the degus are touching or close to touching
	Rear-Sniffing	one degu's nose close to the rear and/or tail of the other animal
	Body-Sniffing	nose to the any area of the body between the ears and hind-leg