New relationships are not necessarily more variable: female degus are more consistent with strangers, male degus are less

Short title: Variability of interactions in new peer relationships

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Abstract

When an organism explores a new environment or stimulus it varies its behavior to ensure proper sampling. As contingencies are learned, behavioral variance can give-way to routines and stereotypies. This phenomenon is common across animal species and non-biological learning systems but has not been well studied in the social domain, in which the stimulus an animal investigates, another individual, may react negatively to unexpected behaviors. Here we investigate the effects of social familiarity on variability of social interactions in degus, female members of which have been shown to be predisposed to form relationships with new, same-sex individuals. Degus were presented with a series of 20 minute, dyadic "reunion" sessions across days, interleaving exposures to familiar and unfamiliar same-sex conspecifics. We found that session-to-session variability in males was higher between strangers compared with cagemates, suggesting that males may establish relationships by testing different social roles across encounters. In contrast, following an initial social exposure, female strangers showed lower session-to-session change compared with cagemates, consistent with the possibility that females establish new relationships by maintaining behavioral norms across encounters. Social novelty had only subtle effects on the structure of social behavior within a reunion session, and did not appear to affect variability in the relative timing of interactions. Given known ecological pressures on female degus to form large, stable social networks, these data are consistent with the notion that higher behavioral variability across social encounters may be maladaptive for establishing cooperative peer relationships.

Keywords

degu, exploration, learning, peer, relationship, social

Introduction

Novelty evokes exploratory behaviors in animals ranging from invertebrates to mammals (Berlyne, 1966; Hills et al., 2015; Hughes, 1997). During exploration, animals vary their behavior to sample across the dimensions of a stimulus or environment: hands are moved around to rotate and grip new objects, variable eye movements help view a new scenes, and bodies are moved through the landmarks and edges of a new place. As the structure and values of a stimulus space become learned, behavioral variability decreases, and movements become more restricted to sample only the relevant features. With further experience the movement sequences themselves can become stereotyped habits.

Social novelty can also increase investigative behavior, but the effects of social novelty on behavioral variability have not been well studied. By analogy with exploration of space or other stimuli, strangers may initially try-out different behaviors, later converging to particular sets and sequences of interactions as animals learn how best to respond to one-another. There is indirect evidence that interaction variability decreases as relationships formed across animal taxa. When fruit flies lose a fight with a particular conspecific, for example, they alter the types of fighting movements used with that individual in the future (Yurkovic et al., 2006). Similarly, the formation of dominance relationships in male mice can be observed as an increasingly stable asymmetry of aggressive versus subordinate behaviors within the dyad (Lee et al., 2019). These particular examples involve competitive relationships, raising the question of whether social familiarity is also associated with reduced variability in cooperative social relationships. Directly comparing competitive and cooperative relationships can be difficult, in that traditional methods used to investigate relationship formation rely on measures specific to the type of relationship and species studied. It is therefore important to construct metrics that generalize across relationship types, allowing for comparisons between them.

The present study was designed to examine the effects of social novelty on interaction variability in degus (*Octodon degus*), female members of which are predisposed to form and maintain new, cooperative peer relationships. Unrelated female degus nest together and nurse

one-another's young (Ebensperger et al., 2014, 2012, 2004, 2002; Hayes et al., 2009), and reproductive success is associated with larger, more stable social groups (Ebensperger et al., 2016; Hayes et al., 2009). Unrelated male degus are, in contrast, less likely to cohabitate, and reproductive success is not as tightly linked to group size (Ebensperger et al., 2016). In laboratory settings, female degus interact with strangers more than familiar peers (Lidhar et al., 2021), even when they have the option to choose between the two (Insel et al., 2020). They also quickly become affiliative with stranger peers in ways that have not been observed in other laboratory rodents (Beery and Shambaugh, 2021; Insel et al., 2020; Lidhar et al., 2021). Degus therefore offer a unique case study to investigate behavioral patterns associated with the formation of relationships, one that allows a direct contrast between potentially more competitive relationship types in males and the more cooperative predispositions of females.

The principal hypothesis of the present work is that degus are more variable in their social interactions when exploring novel conspecifics, and that this holds irrespective of the nature of their relationship. That is, we predict that both males and females, with potentially more competitive and cooperative relationships respectively, both show decreases in variability as long-term relationships are established. To assess this, we expose same-sex strangers to one-another multiple times over 20 to 40 days and apply multiple, complimentary measures of interaction variability within and between the 20 minute social ("reunion") exposures. For controls, we also examine the interactions of already-familiar cagemate dyads over the same time period, and subsequently "new cagemate" and "new stranger" dyads. Our results suggest that differences in interaction variability between cagemate and stranger dyads are particularly evident between sessions, rather than across time or interaction-transitions within sessions. Contrary to expectations, we find that cagemate/stranger differences are in the opposite direction in female compared with male degus, suggesting that variability between encounters depends on the nature of an animal's relationship. To build on traditional approaches, the present study focuses on ethogram-based classification of social interactions; however, one goal of this work has been to establish measures that can be flexibly applied across modes of behavioral observation, species, and setting.

Results

Degus interact more with strangers

To help establish validity of the dataset and assess general effects of social novelty, we first tested whether degus interact more with strangers (Insel et al., 2020; Lidhar et al., 2021; Villavicencio et al., 2009) Degus were separated from a cagemate partner for 24 hours and then united with either a cagemate or stranger in a 50 x 50 cm experimental recording chamber for 20 minutes while video was collected (a "reunion" session, **Figure 1A**). The procedure was repeated 5 times each for cagemates and strangers in an interleaved way, counterbalancing the order between dyads (e.g., stranger-first versus cagemate-first, **Figure 1B**). Consistent with previous findings, degus interacted more during exposures with strangers than with cagemates (3-factor mixed ANOVA across individuals with exposure day and strangers/cagemate as repeated measures, sex as between-subject; effect of stranger/cagemate: $F(1,94) = 34.18$, $p = 7.2 \times 10^{-8}$; **Figure 1C**). Interaction levels were also found to be higher in males across both stranger and cagemate groups (effect of sex: $F(1,94) = 12.47$, $p = 6.4 \times 10^{-4}$). Interactions were subdivided into five types: agonistic, allogrooming, rear-sniffing, face-to-face, and body-sniffing. There was relatively high variability in the levels and types of interactions both within and across animals over the five cagemate and stranger exposures, illustrated in **Figure 1D**. However, across the five exposure sessions female degus showed overall higher levels of agonistic interactions, rear-sniffing, and face-to-face with strangers relative to cagemates (2-factor mixed ANOVA, repeated measures over days, false discovery rate correction for 5 tests, agonistic: $F(1,51) = 8.70$, $p = 0.0048$, rear-sniffing: $F(1,51) = 12.37$, $p = 9.3 \times 10^{-4}$, and face-to-face: $F(1,51) = 10.32$, $p = 0.0023$; **Figure 1E**, left panels). In males, only agonistic behaviors distinguished strangers and cagemates across the five exposures ($F(1,43) = 17.44$, $p = 1.4 \times 10^{-4}$, **Figure 1E**, right panels).

<FIGURE 1 HERE>

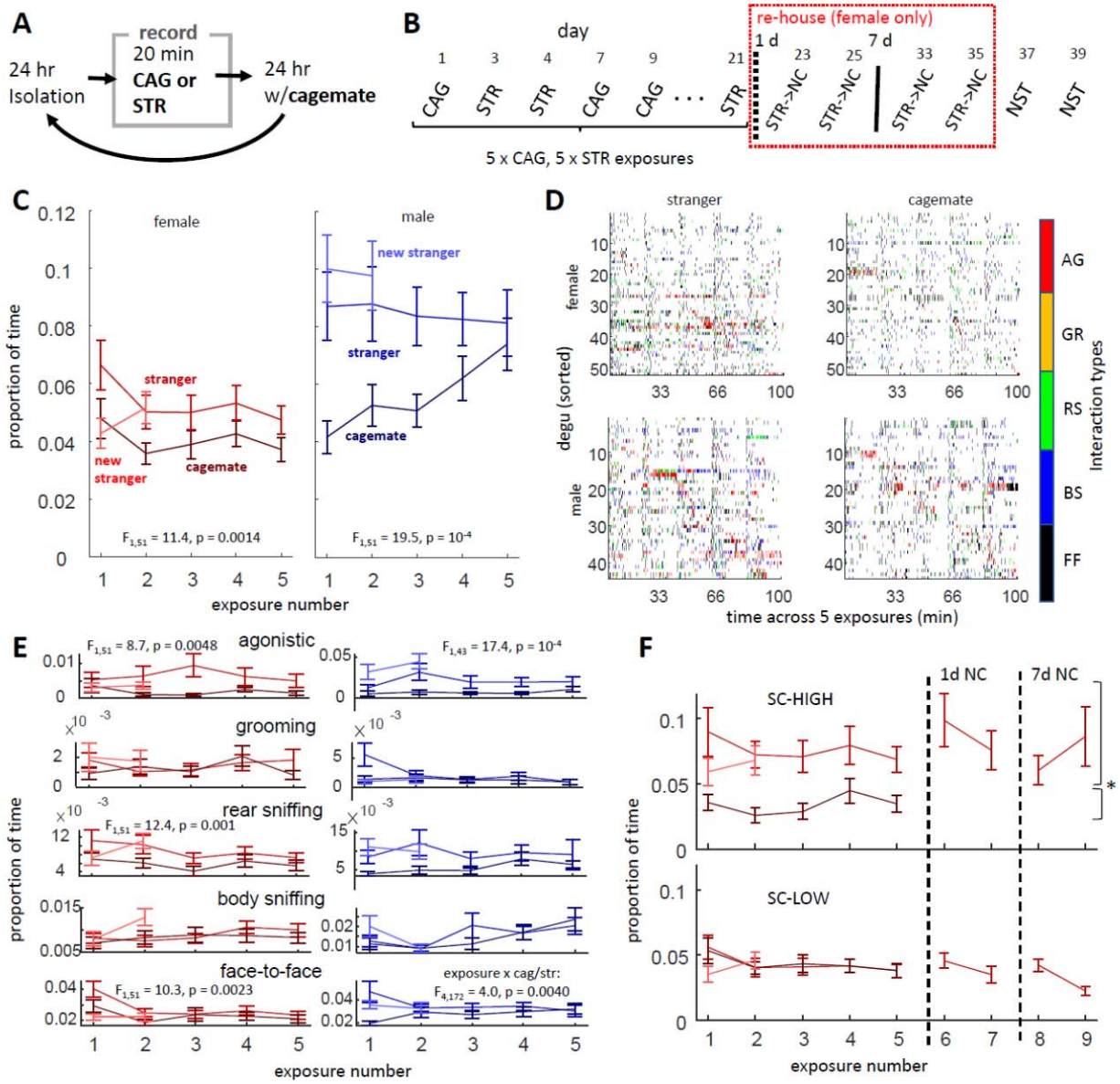

Figure 1. Design and interaction levels. A) Degus received a repeated sequence of 24 hr isolation, 20 minute reunion recording sessions, and 24 hr co-habitation with cagemates. CAG = cagemate reunion, STR = stranger. B) Sequence of recording sessions over days. Cagemate and stranger reunions were interleaved. 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). C) 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). D) 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). Higher concentration of

face-to-face at session starts help demarcate the 5 sessions. More interactions were observed when degus interacted with strangers (left panels), with particularly high variance in males (bottom panels). E) 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 (lower panels), some interaction types decreased and others increased over days. F) 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.

We predicted that the difference in interaction levels between strangers and cagemates would diminish as the initial strangers became more familiar. This prediction was confirmed by a statistical interaction between stranger/cagemate and exposure day (interaction between stranger/cagemate and exposure: $F(4,376) = 3.16$, $p = 0.014$). The pattern was particularly apparent in males degus, in which face-to-face interactions were more prevalent between strangers during initial exposures (interaction between stranger/cagemate and exposure number, false discovery correction for 5 behaviors: $F(4,172) = 4.00$, $p = 0.0040$, **Figure 1E**), with other, non-agonistic behaviors showing similar trends (e.g., higher rear-sniffing over the first few days, session x stranger/cagemate interaction: $F(4,172) = 2.36$, $p = 0.056$). Female degus, in contrast, did not show evidence of diminishing stranger-cagemate differences across days (all interactions: $F(4,204) = 0.48$, $p = 0.75$; individual interaction types: $p >> 0.05$).

To further examine the absence of familiarity effects in females, we subdivided female degus 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). 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: $F(1,15) = 83.40$, $p = 1.63 \times 10^{-7}$, day: $F(4,60) = 0.89$, $p = 0.47$, stranger/cagemate x day: $F(4,140) = 0.20$, $p = 0.94$) or the SC-LOW animals (stranger/cagemate: $F(1,35) = 8.00 \times 10^{-4}$, $p = 0.98$, day: $F(4,140) = 1.49$, $p = 0.22$, stranger/cagemate x day: $F(4,140) = 0.20$, $p = 0.94$; **Figure 1E**). A possible explanation for this

result is that the 20 minute reunion sessions provided insufficient time for partners to become fully familiarized with one-another. To test this, a subgroup of 40 female degus were co-housed with their stranger partners for 1 day, then tested twice more in the reunion session, creating "new cagemates". A smaller subgroup of female degus was co-housed for an additional 7 days. Unexpectedly, interactions between SC-HIGH strangers remained higher than long-term cagemates following either 24 hrs of co-housing (paired t-test, averaging all cagemate sessions and both 24-hr co-housed stranger sessions, $t_9 = 5.50$, $p = 3.80 \times 10^{-4}$) or 7 days of co-housing ($t_7 = 2.98$, $p = 0.021$). To investigate whether interaction levels were due to genetic relatedness, we compared sibling versus non-sibling pairs. Due to low sampling (only two of the cagemate dyads were non-siblings) this could not be evaluated statistically; however, both members of one, non-sibling cagemate dyad interacted more with strangers (i.e., they were also SC-HIGH animals) while the members of the other dyad did not (were SC-LOW animals). Overall, therefore, female degus exhibited higher interaction levels with strangers than cagemates, but there was no evidence that these attenuated with familiarity and, consistent with research from other labs (Villavicencio et al., 2009), this was unlikely to be due exclusively to genetic relatedness.

In males, the differences between interactions with strangers versus cagemates did decrease with familiarity, though this pattern could have resulted from other testing or history effects, including familiarity with the protocol and experimental apparatus. As a final control condition, degus were exposed to a new, unfamiliar conspecific ("new stranger"). 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 1F**).

In summary, degus tended to interact more with strangers than with cagemates, though in females this was only the case for around one-third of the animals (SC-HIGH group), and the SC-HIGH females continued to interact more even after the strangers were co-housed to become new cagemates. Both male degus and SC-HIGH females also interacted more with new strangers after all other behavioral sessions were complete.

In males, strangers have higher session-to-session variability that cagemates; in females, strangers have lower session-to-session variability

The overarching hypothesis of this study is that social interactions are more variable between unfamiliar individuals. Variability can be assessed at multiple timescales, and we began by examining the consistency of a dyad's interactive behavior between 20 minute reunion sessions. To measure this, we 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 two, complementary measures: distances (dissimilarity) between interaction vectors, and dyad classification success (the ability of a support vector machine, SVM, to correctly identify which dyad an interaction vector belongs to). While the distance-based method allowed observation of overall variability within a dyad, the classifier-based method established whether dyads could be distinguished from one another using unique combinations of interaction vector elements (illustrated in **Figures 2A&B**). Notably, the classifier method measures "discriminability", which evaluates within-dyad variability relative to differences between dyads. The measure is useful in that it allows specific interaction behaviors (and combinations) to be ignored if a dyad is inconsistent in that behavior, honing-in on behaviors that more selectively define the social relationship.

<FIGURE 2 HERE>

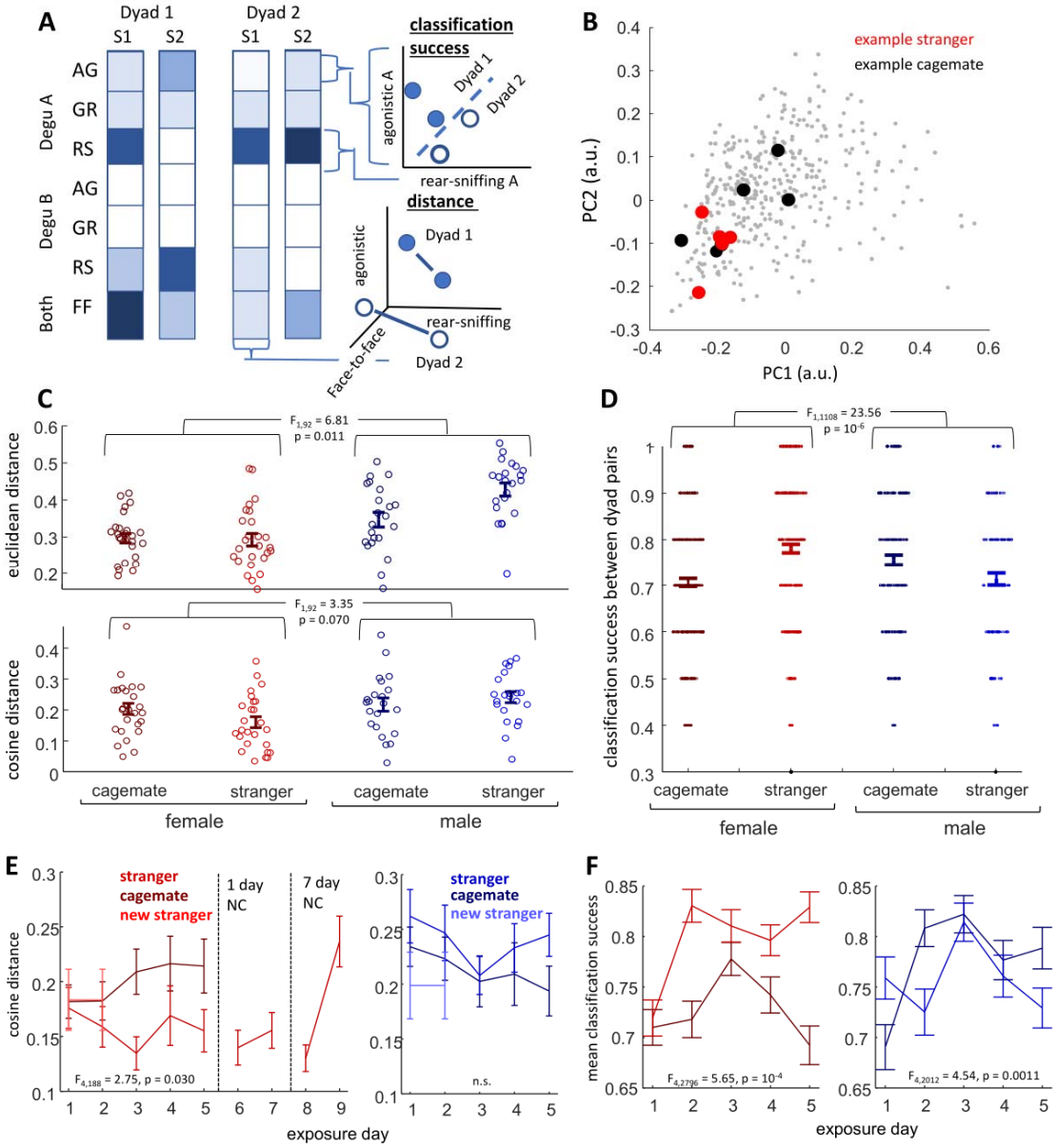

Figure 2. Variability across sessions. A) Diagram illustrating analysis methods. Each column of boxes represents the interaction vector from a single session (abbreviations same as in Figure 1D, body-sniffing omitted for illustration purposes). To the right, vectors are plotted in cartesian coordinates to illustrate measures of variability. Top right shows SVM-based classification success, with dashed line representing a hyperplane separating groups in the relevant feature-space. 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 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 (errorbars 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.

Across-session variability was found to differ between cagemates and strangers, but the direction of this difference depended on sex. This could be observed using both interaction vector distances and classifier success rates. Within-dyad distances tended to be lower than between-dyad distances (within- versus between-dyad ratio of 0.73 and 0.69 for females and males respectively), suggesting that individual dyads exhibited unique profiles. The within-dyad, Euclidean distances between interaction vectors were found to be significantly higher in male degus relative to females (2-way ANOVA, effect of sex: $F(1,92) = 5.53$, $p = 0.021$) significantly higher in strangers compared with cagemates (effect of stranger/cagemate: $F(1,92) = 32.09$, $p = 1.7 \times 10^{-7}$) but relatively higher in male strangers compared with female stranger (sex x stranger/cagemate interaction: $F(1,92) = 6.81$, $p = 0.011$; **Figure 2C, top**). A concern with using Euclidean distances is its sensitivity to total interaction levels; therefore, a second measure, cosine distance, was also used. Cosine interaction vector distances showed differences between males and females (2-way ANOVA, effect of sex: $F(1,92) = 6.6$, $p = 0.012$) and a statistical trend for stranger-cagemate differences to differ between males and females (sex x stranger/cagemate interaction: $F(1,92) = 3.35$, $p = 0.070$; **Figure 2C, bottom**). Contrasting with hypotheses, female strangers appeared to show less variability (more consistency) across sessions relative to female cagemates, male cagemates, or male strangers. As described below, the stranger-cagemate differences in females became more apparent when considering exposure number. There was no evidence that this effect in females was restricted to only dyads with SC-HIGH or only SC-LOW degus (**Supplementary Figure 1A&B**). The statistical

interaction between stranger-cagemate and depended most on allogrooming and rear-sniffing, as effects decreased when removing these interactions, but increased when other interaction types were removed (stranger/cagemate x sex, cosine distances after removing agonistic: $F(1,92) = 4.3$, $p = 0.040$; allogrooming: $F(1,92) = 2.1$, $p = 0.15$; rear-sniffing: $F(1,92) = 1.65$, $p = 0.20$; face-to-face: $F(1,92) = 4.52$, $p = 0.036$; body-sniffing, $F(1,92) = 6.2$, $p = 0.015$; **Supplementary Figure 2A**). Overall variability differences between males and females appeared to depend more on face-to-face and body-sniffing (effect of sex after removing agonistic: $F(1,92) = 7.2$, $p = 0.0085$; allogrooming, $F(1,92) = 8.3$, $p = 0.0050$; rear-sniffing, $F(1,92) = 7.3$, $p = 0.0083$; face-to-face: $F(1,92) = 3.0$, $p = 0.086$; body-sniffing: $F(1,92) = 2.7$, $p = 0.10$).

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). Rates significantly differed between groups, with classification success higher in female strangers than cagemates, but lower in male strangers than male cagemates (2-way ANOVA effect of stranger-cagemate: $F(1,1108) = 7.11$, $p = 0.0078$, effect of sex: $F(1,1108) = 0.03$, $p = 0.86$, stranger-cagemate x sex interaction: $F(1,1108) = 23.56$, $p = 1.4 \times 10^{-6}$; **Figure 2D**). The effects were not due to any one interaction type, as the sex difference remained even after removal of any specific type from the interaction vectors (stranger-cagemate x sex interaction for each: $F > 20$, $p << 0.01$,

Supplementary Figure 2B). Higher classification success rates in female strangers was also observed when classifying only between dyads with SC-HIGH degus, or between those with only SC-LOW degus (2-way ANOVA, effect of stranger/cagemate: $F(1,313) = 6.2$, $p = 0.012$, effect of SC-HIGH vs. SC-LOW: $F(1,313) = 5.4$, $p = 0.020$, stranger/cagemate x SC-HIGH/LOW interaction: $F(1,313) = 0.11$, $p = 0.74$; **Supplementary Figure 1C&D**).

We next asked how variability changed over exposure sessions. In females, there was a significant increase in stranger-cagemate differences over days, with variability in stranger dyads decreasing after the first exposure (2-way mixed ANOVA, repeated measures over days, cosine distance: cagemate-stranger: $F(1,47) = 2.19$, $p = 0.14$, exposure day $F(4,188) = 1.9$, $p = 0.10$, cagemate-stranger x exposure day: $F(4,188) = 2.75$, $p = 0.030$); classification success: cagemate-stranger: $F(1,699) = 29.6$, $p = 7.4 \times 10^{-8}$, exposure day: $F(4,2796) = 6.65$, $p = 2.53 \times 10^{-5}$, cagemate-stranger x exposure day: $F(4,2796) = 5.65$, $p = 1.6 \times 10^{-4}$; **Figures 2E&F, left panels**). In males, classification success rates appeared to increase in cagemates after the first exposure, while strangers showed less evidence of change (2-way mixed ANOVA, stranger-cagemate: $F(1,503) = 1.5$, $p = 0.22$, exposure day: $F(4,2012) = 6.05$, $p = 7.74 \times 10^{-5}$, stranger-cagemate x exposure day: $F(4,2012) = 4.54$, $p = 0.0011$; **Figures 2E&F, right panels**). These patterns were counterintuitive, in that strangers did not converge to become more like cagemates with familiarity; instead, after an initial social exposure in the experimental chamber both female strangers and male cagemates began interacting similarly across days, while female cagemates and male strangers either remained or became less consistent in their day-to-day interactions

Within-sessions, interaction patterns were not strongly affected by social context

There are no standard measures for variability of social interaction. We selected four measures that provide estimates of variation in the sequencing of actions, the timing between actions, and in blocks of interactions within sessions.

To assess sequencing between actions, we computed transition matrices describing the probability that a "reference" interaction type will be followed by another interaction type. The majority of interactions showed statistically significant relationships (t-test across sessions of probabilities minus chance, baseline probabilities, Benjamini & Hochberg false-discovery correction across 20 comparisons for same-degu interaction pairings and 25 between-degu interaction pairings; **Figure 3A**). The most notable exception was an absence of association between agonistic in one individual with allogrooming in the other. In males, there were also no observable pairings between rear-sniffing and agonistic behaviors across two members of a dyad.

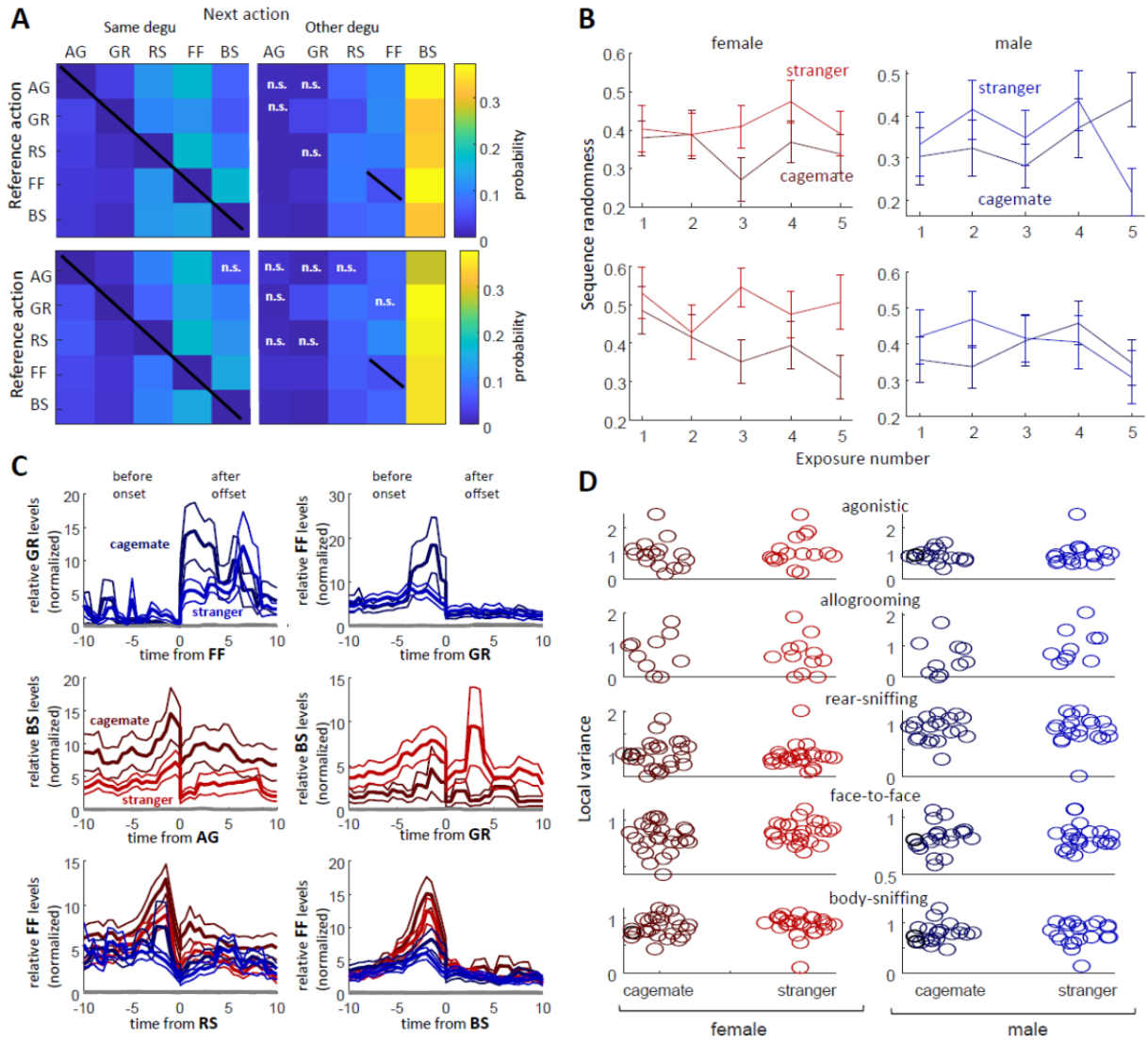

Figure 3. A) Transition matrices between interaction types, averaged across all degus (top: female, bottom: male). Left matrices show probability of "next action" (columns) given a reference action (rows) performed by the same degu, right matrices shows probability that the other degu will perform an action, given a reference action in the first degu (matrices computed independently). All cells showed a significant, non-zero probability aside from those labeled "n.s." Diagonal black lines indicate same-action transitions that were not included. Interaction acronyms are the same as used in Figure 2. B) Sequence randomness of interactions across exposures. No significant changes were observed over exposures, nor differences observed between dyad groups in either female (left) or male (right) dyads. C) Example interaction cross-correlograms, the top four selected to emphasize rare cases of potential difference between cagemate and stranger dyads (thicker lines are averages across dyads, thinner lines SEM). Top two panels show male levels of allogrooming before and after face-to-face (left) and male levels of face-to-face before and after allogrooming (right). Allogrooming happened only after face-to-face and a statistical trend suggested was more often after face-to-face interactions in cagemates ($p = 0.02$). Middle panels show female levels of body-sniffing, with cagemates potentially more likely to body-sniff before and after agonistic encounters (left) and less likely than strangers before and after allogrooming (right; $p < 0.05$). Bottom two panels show levels of face-to-face interactions around rear-sniffing (left) and body-sniffing (right) in both male (blue) and female (red) dyads. D) Local variance measures examining the consistency of temporal intervals between specific interaction types. No differences between strangers and cagemates were detected.

Although the transition matrices revealed structure in the sequencing of interactions, there was no compelling evidence for overall variability differences between cagemate and stranger groups. This was measured using a sequence randomness score, an index into the overall degree to which behaviors can be predicted given a current behavioral state. Sequence randomness was evaluated across exposures for both same-degu and between-degu transition matrices of both sexes. No apparent effects of cagemate/stranger or exposure were observed (mixed 2-way ANOVA, between-subject variability of cagemate/stranger dyad, repeated measures over exposures; female same-degu transitions, stranger/cagemate: $F(1,4) = 0.32$, p – 0.57, stranger/cagemate x exposure: $F(4,128) = 0.79$, $p = 0.53$; female between-degu transitions, effect of stranger/cagemate: $F(1,42) = 1.74$, $p = 0.19$, stranger/cagemate x exposure: $F(4,168) = 1.36$, $p = 0.25$; **Figure 3B**). When female degus were split into pairs

containing SC-HIGH animals and those with only SC-LOW animals, a trend was observed in SC-HIGH for increased, between-degu sequence randomness among strangers ($F(1,84) = 3.40$, $p = 0.080$; **Supplementary Figure 3**). While this hinted at possibly higher, action-to-action variability, consistent with hypotheses, the low effect size and lack of generalizability across sexes, or across all females, emphasized that any differences that may exist are very subtle.

In addition to the absence of overall cagemate-stranger differences in variability, there was also little evidence that stranger and cagemate groups systematically differed in specific interaction transitions (two-sample t-tests, false discovery corrections as above, corrected p-values > 0.05). These results did not change when only the first exposure day was considered, nor were differences found in the second-order transition matrix (i.e., the probabilities that one interaction type leads to another, subsequent to the next). Cross-correlations were used to more precisely evaluate the interaction pairings. As with the transition matrices, and as previously observed (Lidhar et al., 2021) most pairs of actions showed temporal relationships; however, differences between strangers and cagemates did not exceed threshold for statistical significance (**Supplementary Figure 4**). It is possible that the correction for multiple comparisons occluded subtle differences between cagemate and stranger interaction sequencing. Taking a data exploration (hypothesis-generation) angle, a few specific interaction pairings may have been less likely in strangers. First, male strangers may have been less likely to follow face-to-face encounters with allogrooming (two-sample t-test, $p = 0.020$; **Figure 3C**, top-panels), which would be consistent with observations made in females in a prior study ("nuzzling after grooming", (Lidhar et al., 2021). Male strangers also may have been less likely to precede agonistic behaviors with body-sniffing ($p = 0.008$). Similarly, female strangers may have been less likely to body-sniff before and after agonistic encounters ($p = 0.038$ and $p = 0.030$), but more likely than cagemates to body-sniff before and after allogrooming ($p = 0.021$ and $p = 0.025$). Cross correlograms offered no evidence of higher overall variability in the timing or sequencing of interactions in stranger compared with cagemate groups.

A separate analysis was performed to assess variability in the intervals between interactions of the same type, indicating how often each individual returned to a particular behavior. If face-to-face interactions, for example, are a way for two individuals to “check-in” after a period of time had elapsed, these interactions may be more consistently repeated between those that know one-another compared with those that do not. This was measured as the Local Variance of inter-behavior-intervals (IBI’s) for each of the 5 main interaction types There was no evidence for differences in the Local Variance for any type of interaction (two-sample t-tests, false-discovery rate correction, $p >> 0.05$; **Figure 3D**). The same was true when the analysis was repeated for only the first day of interaction.

A set of final tests examined within-session variability in sets of interactions, by measuring interaction vector distances across blocks of “n” interactions (where n was varied from 1 to 30). As illustrated by **Supplementary Figure 5**, the analysis had fundamental limitations by inflating distance scores for sessions with overall more interactions. When attempting to controlling for these limitations, the data offered no evidence for higher levels of variability in strangers compared with cagemates for either male or female dyads.

Discussion

The present studies were designed to test whether relationship formation involves an initial period of higher variability in the interactions between two individuals. Previous evidence suggests that male and female degus are predisposed to different types of peer relationships, offering an opportunity to compare variability patterns associated with the formation of different types of relationships. Although we found that interaction variability differed between new and established relationships, the differences were not consistent between male and female degu groups. Male stranger dyads showed evidence for higher variability across reunions compared with cagemates—particularly when estimated using dyad classification success. Female stranger dyads, however, appeared to be less variable than cagemates across reunions, a reduction that became apparent after either the first exposure or second exposure

443 session when measured using dyad classification success and interaction vector distance
444 respectively. Analyses examining behavior at shorter timescales, within sessions, revealed a
445 structure in the transition between interactions that is consistent with previous observations
446 (Lidhar et al., 2021); however, only subtle differences were observed between cagemate and
447 stranger groups, with no compelling evidence for higher variability, as measured using a
448 sequence randomness score. As discussed below, degus did appear to interact more with
449 strangers than with cagemates. This suggests that social exploration may not be expressed as
450 much by behavioral variability, but by higher levels of engagement, in ways that conform to a
451 familiar behavioral structure.
452
453 A primary question raised by the results of this study is why, after an initial social exposure,
454 female cagemate dyads exhibited higher levels of session-to-session variability than female
455 strangers. It is tempting to speculate that animals were explicitly motivated to maintain
456 consistency with strangers, either to establish their role in the new relationship or avoid conflict
457 altogether. Conversely, it may be that female degus felt less constrained or attentive to the
458 social situation when placed with a cagemate, resulting in behavior that depended more on
459 day-to-day circumstantial factors (e.g., hormonal fluctuations, ambient odors, experimental
460 room temperature, etc.). These explanations are also consistent with the notion that being
461 unpredictable is less conducive to forming cooperative relationships. Many primates use
462 specific sets of vocal and physical interactions when encountering strangers, often in
463 stereotyped and ritual sequences. The circumstances in which these behaviors take place—e.g.,
464 between similar-ranking males, younger individuals greeting older, and when approaching
465 pregnant females—suggest that the behaviors signal predictability, and function to reduce
466 uncertainty and tension (Aureli and Schaffner, 2007; Dias et al., 2008; Kutsukake et al., 2006;
467 Smuts and Watanabe, 1990; Whitham and Maestripieri, 2003). To put another way, reduced
468 variability may help avert high-cost aggressive or competitive interactions. It can be
469 hypothesized that any predisposition females have for more consistent interactions with
470 strangers goes hand-in-hand with their predisposition to form cooperative relationships with
471 new individuals. This may also explain why male stranger dyads did not appear to be less

variable than cagemates, as degu males are not believed to benefit as much from large, stable social groups (Ebensperger et al., 2016).

One way in which female and male degus were similar is that both interacted more with strangers than with cagemates; however, this increase took-on different patterns. Male strangers showed more greeting (face-to-face) interactions, and a trend for increased investigation (rear-sniffing) during the first few exposures that subsequently attenuated (agonistic interactions with strangers notably became more common after the first social exposure). In female strangers, higher face-to-face, rear-sniffing, and agonistic encounters persisted across reunions (agonistic again being more evident after the first day). Higher levels of interaction with strangers is not surprising, and has been previously observed in our lab and others (Insel et al., 2020; Lidhar et al., 2021; Villavicencio et al., 2009). It is also unsurprising given the species' gregarious behavior in natural habitats: degu burrows are found near one-another, and members of different burrows greet one-another frequently (Ebensperger et al., 2004; Fulk, 1976). But if female degus form stable, cooperative peer relationships with unrelated individuals, as ecological data suggest (Ebensperger et al., 2014, 2012, 2004, 2002; Hayes et al., 2009), it is difficult to understand why the animals in the present study continued to treat new relationships differently from those with prior cagemates. We found that interaction levels between female strangers remained high even after they had been co-housed to become "new cagemates". We cannot rule-out the possibility that the structure of the protocol played a role; e.g., extended time away from the original cagemates may have sensitized effects of the 24 hour isolation period. It is also possible, however, that degus form fundamentally different bonds with non-siblings encountered in adulthood compared with conspecifics reared together. There are some hints of this from other studies. Females in the wild have been reported to show preferences for members of their own burrow groups during daytime foraging and social greetings (Ebensperger et al., 2004). In the lab, genetic kinship can influence social behavior—just not as strongly as familiarity (Villavicencio et al., 2009). Future investigation using similar protocols in young animals may help establish whether there are critical periods of development in which certain types of peer bonds can take place.

Another interesting feature of the present data is that in both males and females, the structure of social behavior was largely preserved between cagemates and strangers. There was no strong evidence for differences in variability in sequencing or temporal relationships of interactions within sessions. Some of the common structure of behavior between familiar and relatively unfamiliar individuals may be due to the physics of interaction—e.g., two socially attentive degus are more likely to encounter one-another's faces before body or tail. Or it may be due to the logic of investigative order: the mouth may offer the most important information about another individual, the anogenital region the next most, etc. It also seems possible that the sequencing and timing of behaviors are supported by genetic and early-life learning to ensure coordinated activity between individuals of a species. The effects of juvenile social isolation on adult social behavior has been looked at in detail in other species (e.g., chimpanzees: (Kalcher-Sommersguter et al., 2011; Turner et al., 1969), rats: (Himmler et al., 2016; Van Den Berg et al., 1999)). While early social deprivation has been shown to have effects on social motivation, the types of interactions used, and an animal's tendency toward aggression, the sequencing between interactions appears to be relatively resilient to early-life social isolation (Van Den Berg et al., 1999). Early-life social deprivation may still have subtle effects on the timing of interactions, as well as how vocalizations are used (Keesom et al., 2017), which may make an animal less predictable and thereby destabilizing relationships to greater escalation of aggressive/agonistic encounters (Himmler et al., 2016). Further work examining interaction sequencing and the effect of early-life isolation in female degus will be valuable for understanding the animals' abilities to engage constructively with strangers.

Several methodological factors may be critical to interpreting the present results. One of these is the relatively "strange" setting that the experimental recordings took place in. Although degus were individually habituated to the experimental chamber over five days prior to the start of the experiment, the room and chamber were still relatively novel, and relatively low levels of interaction between the degus (averaging around 5% in females and below 10% in males) may indicate that the environmental context was not optimal for comfort . Similar

effects of testing context have been observed in rats (Varlinskaya, 2008). The patterns of interaction levels and interaction variance with a new individual should therefore be considered within the backdrop of environmental uncertainty. A second, potentially critical factor for understanding the results is the relatively few ethogram categories that were used to track degu interactions. Face-to-face, for example, comprises both nose-to-nose and nose-to-mouth contact, while rear-sniffing includes both anal and genital sniffing. Social interactions can include a high dimensionality of subtle but relevant movements, such as flattening or raising of ears, immobility versus rapid escape, and hesitant versus unrestrained approach. Measuring these additional variables could provide a richer understanding of social dynamics and moment-to-moment metrics of variability. On the other hand, the behaviors that are measured—agonistic (primarily mounting), allogrooming, rear-sniffing, face-to-face, and body-sniffing interactions—represent specific physical interactions that likely have distinct social/ethological relevance for the animals. These categories may serve as "anchor point" clusters in the multidimensional spectrum of social activity. The categories also made it possible to selectively remove action types to identify which factors were responsible for the results. Thus, while the ethogram measures fail to capture the depth of social interaction patterns, they may have helped streamline identification of the similarities and differences between dyads.

While the present project focused its examination on the development of social relationships in degus, an underlying goal was to advance methods that could generalize across species and behavioral measurement tools. In some cases, it may only be possible to interpret an animal's behavior through its specific ethogram. But while degu are unique in their sociality and peer structures, the hope is that these same methods can be applied to better understand the formation of cooperative social relationships across species, sexes, and developmental stages. In this way, the study of interaction variability joins a wider movement to apply standardized metrics toward analysis of animal social behavior (e.g., see (Silk et al., 2013)). Given the unexpected results of the present work, a key message of this research is that while distance metrics and classifiers may be useful in articulating how relationships develop over time, they cannot be taken in isolation.

Materials and Methods

Subjects

Fifty-two adult female degus aged seven to twenty-nine months (mean = 11.9 mo, median = 11 mo) and 44 male degus aged six to twenty months (mean = 10.4, median = 10) were used. A higher number of female degus were used than males due to a late addition of a new experimental condition in only females (the "new cagemate" groups, see Testing). Degus reach developmental maturity by 3.5 months (Hummer et al., 2007; Mahoney et al., 2011) and can live in laboratory settings up to 8 years (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 weened from parents and separated from opposite-sex siblings between postnatal day 30 and 60 (mean = 42 days).

All animals were housed in same-sex pairs 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 mixure of chinchilla and guinea pig "Teklad" feeds (Envigo; Indianapolis, IN). Animals were housed on a 12:12 h light/dark cycle, with all tests occuring 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 with an attempt to keep animals matched for age, but were otherwise random. Strangers inhabited the same vivarium, so olfactory, auditory, and visual contact were 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. Recording chamber was a 50 x 50 x 50 cm wooden box. Following each social exposure, the enclosure was cleaned using 70% ethanol and then dried to minimize residual scents.

Testing

During the week prior to testing, each animal was pre-exposed to the testing chamber for at least 5 minutes and no longer than 7 minutes each day over five days (variation due only to experimenter scheduling, and not changed according to the animals' behavior). Pre-exposures to the recording chamber were performed without other animals present, were not recorded, and all animals received the same treatment independent of behavior during the exposures. Twenty-four hours before the first testing session, cagemates 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 prior cagemate or a stranger. The pair was 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 previous cagemate and were left undisturbed for 24 hours (**Figure 1A**). By placing cagemates together following each reunion session, we hoped to allow the dyad to "recalibrate" their relationships, preserving their dyadic interactions over the weeks and minimizing 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 pseudo-randomized order (alternating partners after either one or two reunions with the same partner)

for a total of 5 cagemate and 5 stranger sessions (counterbalancing stranger-first and cagemate-first across dyads; **Figure 1B**). Degus always spent 24 hours with their original cagemate and then 24 hours in isolation before returning to the recording chamber.

Following 10 reunion sessions, female stranger dyads were co-housed together to create "new cagemates" and re-tested four times. This was not performed with males to avoid aggression-related injury. New cagemate degus were tested after one 24 hour period of co-housing and 24 hours of isolation, again after a second 48 hour cycle of co-housing and isolation (20 of 26 female dyads were re-tested after 1 and 2 cycles of re-housing), a third time after 7 days of co-housing, followed by 24 hours of isolation, and again after a final 48 hour period of co-housing and isolation (14 of 26 dyads were re-tested after the full week of re-housing). To prevent sampling bias, statistical comparisons that included the control sessions only included dyads that performed the controls.

Following re-housing reunion sessions in females, and the initial 10 reunion sessions in males, degus were subsequently returned to their original cagemates and then, following 24 hours of isolation, tested with a new stranger. 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

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

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. Although the ethogram comprised 17 individual behaviors, for simplicity and consistency with our prior work (Lidhar et al., 2021), analyses collapsed behavior types into the following categories: 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). Mounting behaviors were classified as agonistic for consistency with past work and because the behavior normally included one animal attempting to avoid or escape mounting attempts by the other individual. Sessions were said to start when the second degu was introduced into the chamber and to end when a degu was removed. Rare errors were sometimes made when a rater noted the initiation but not the termination of a behavior. To minimize the effect of these errors, an interaction was said to terminate either when the same degu initiated a new interaction, or after a time-out threshold of 6 s (determined as the longest reasonable interaction interval, based on the distribution of all interactive behaviors across all sessions). Approximately 52% of sessions were scored by author AT and the remainder by 7 undergraduate students all directly trained by AT. Inter-rater reliability (IRR) was estimated using 6 sessions scored both by AT and another student, applying the same transformations and measures used for the primary analyses. Total interaction levels could vary up to 20% between raters, but this did not appear to contribute to overall results as 1) sessions were distributed randomly across students and 2) the magnitude of inter-rater error scaled with interaction levels, and analysis controls were used to ensure that interaction levels could not account for interaction variability scores.

Measures

All measures were computed from ethogram-based scoring of behavior, performed manually in BORIS. Most measures were based on the time animals spent using different behaviors during a 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 cube root transformation (**Supplementary Figure 7**). To ensure validity of

parametric statistical tests and distance metrics, a cube root transform was applied to all "interaction time" measures before performing statistical tests.

Interaction time (time budget analysis) allowed comparison with previous datasets and assessment of overall interaction level changes across days. Interaction time was computed as a proportion of session time, and was considered both as a sum total across behavioral types (agonistic, face-to-face, etc.), and separately between types of behaviors.

Between-session variability was estimated using two measures: "interaction vector distances" and "dyad classification success".

Interaction vector distances were computed by first calculating the proportion of time that each animal spent using a particular type of interactive behavior during a session (an "interaction vector") and then computing the distance between the vectors associated with different sessions. The types of interactions used for interaction vectors were agonistic, allogrooming, rear-sniffing, body-sniffing, and face-to-face contact. In principle this yields a 10 element vector (5 interaction types x 2 degus); however, it could be argued that face-to-face contact is a mutual interaction, so face-to-face values were combined yielding a vector of 9 elements. To compute distances between the interaction vectors, both Euclidean and cosine distance were used. Euclidean distance takes into account total interaction levels, while cosine distance does not (As noted above, a cubed-root transformation was performed to normalize data before distance metrics were computed.) Euclidean and cosine distance are relatively simple, but have advantages over alternatives: Jensen-Shannon distance (based on Kullback-Leibler divergence) ignores zero elements, which would have misrepresented distances between vectors; the Pearson correlation is, like cosine distance, scale invariant, and likely would yield similar values, though would also ignore changes common across all interaction types.

Dyad classification success was computed by finding the degree to which a group of interaction vectors could be discriminated from another group. Notably, classification success conflates

two variables: within-dyad variability, and between-dyad diversity. The advantage of dyad classification success relative to interaction vector distances is that, after a model is trained, it should ignore types of social interactions that are not useful for discriminating between two dyads while emphasizing those interactions--or combinations of interactions--that are helpful for discrimination. Classification success made use of a binary support vector machine classifier (SVM, "fitcsvm" in Matlab). SVM works by finding boundaries (i.e., hyperplanes) through n-dimensional space that best differentiate between two sets of data. It is possible to estimate common patterns between two sets of data based on how successful the model is in classifying each vector within a set. We used a leave-one-out cross validation method, in which the model is trained on all vectors but one (i.e., of 10 interaction vectors, the model is trained on 9), and classification is made on the remaining, repeated for each of the ten interaction vectors across the two dyads. A linear kernel was used in favor of a radial basis function because the latter yielded ceiling effects that failed to differentiate conditions. Thus, all results are based on a classifier using linear combinations of interaction vector elements. SVM classifiers were used in favor of neural network classifiers due to the algorithm's relative tolerance for small training sets.

Within-session variability was estimated by examining action-to-action transitions and timing, as well as by comparing interaction vector distances across sets of actions.

Action-to-action transition variability was computed using "sequence randomness". Sequence randomness is inversely related to how well an animal's next action can be predicted, given its previous action. To compute sequence randomness, it was necessary to first compute an interaction transition matrix. This was the probability that an interaction of one type would lead to one of another type within a 10 s interval. The 10 s cutoff was chosen based on temporal patterns observed in the interaction cross correlograms. Actions sequenced by a single individual have a fundamentally different meaning from responses of one animal to another; therefore, independent transition matrices were computed for interactions performed by the same individual and those performed by the other individual. For example, if one animal begins

to sniff the other animal's rear, the "self" transition matrix estimated the probability that the same animal would subsequently perform a body-sniff, allogroom, or another behavior, while the "other" transition matrix estimated the probability that the other individual would subsequently perform a body-sniff, allogroom, or other interaction type. Higher-order transition matrices that estimated the probability of future interactions (beyond the action that immediately followed) were not reported, as probabilities degraded to chance levels relatively quickly. For the purposes for computing sequence randomness, transition matrices did not include probabilities of "no-interaction" (including absence of interaction during a 10 s period resulting in an interaction type, or an interaction type followed by the absence of an action); thus, the probabilities of the matrix did not sum to 1.

The Shannon entropy was computed for each row (preceding action) of a transition matrix, then averaged across rows, as follows:

$$\mathrm{SR} = 1/n \sum_{p=1}^{n} \sum_{a=1}^{n} -P\left(x_a | y_p\right) log P\left(x_a | y_p\right)$$

Where x_a is interaction type "a", y_p is the immediately preceding interaction type, and "n" is the number of interaction types.

Variability in action-to-action timing was estimated using Local Variance and cross correlations. *Local variance* (LV) was used to compute inter-behavior-interval (IBI) variance, a measure more commonly used to estimate variance in neuron spike intervals (Shinomoto et al., 2005). LV was defined as

$$\mathrm{LV} = 1/(n-1) \sum_{i=1}^{n-1} 3(T_i - T_{i+1})^2 / (T_i + T_{i+1})^2$$

Where T_i is the time at which the ith interaction took place and "n" the number of spikes.

Local variance makes no assumptions about the distribution of time intervals between interactions. We initially assumed that event times would be roughly stochastic, and the intervals would therefore fit Poisson or gamma distribution (Lienhard and Meyer, 1967; Ord, 1967). Empirical evaluation of the IBIs indicate distributions conformed more closely to an exponential (**Supplementary Figure 7A-C**); i.e., behaviors often come in bursts, similar to what has been described in previous reports (Lee et al., 2019). While the variance of an exponential can be calculated as the square of the mean, LV showed less dependence on total event numbers (**Supplementary Figure 7D & E**).

Action-to-action interactions and timing were also evaluated using cross correlations. Cross-correlations can be visualized as a histogram, where times preceding zero (left side of the x-axis) showed the incidence of a target action relative to the initiation of another, reference action, and times following zero (right side of the x-axis) showed the incidence of the target relative to reference action termination. For example, when examining the cross correlogram with face-to-face as the reference, and agonistic behavior as the target, a high value at -3 s indicates high incidence of agonistic behaviors 3 s prior to the initiation of face-to-face interactions, and a low value at 1 s means low incidence of agonistic behavior 1 s following the completion of face-to-face behaviors. To make the cross-correlations more interpretable, values were normalized by dividing by the proportion of time during the session that a target action took place. In other words, values of 1 in the cross correlation meant that there was no observable temporal relationship between the two interaction types. Units on cross-correlogram y-axis were, therefore, not standard correlation coefficients, but normalized rates.

Within-session, interaction vector distances were computed using count-based interaction vectors, where each vector element was the sum of interactions of each interaction type over a block of n interactions. If block size n = 10, for example, the vector described how many of 10 consecutive interactions were rear-sniffing events performed by degu A, allogrooming events performed by degu B, face-to-face bouts, etc. This measure helped identify the degree to which dyads changed the set of interactions they were using after a number of behaviors had been

performed. Exploratory methods were used to investigate cagemate versus stranger differences across block sizes (1 to 30 interactions per vector), across days. Some sessions lacked sufficiently high numbers of interactions to yield even a single distance value; within-session interaction variability therefore emphasized differences between the cagemate and stranger groups specifically for sessions with multiple interactions. Sample sizes are reported for each analysis.

Statistics

Null-hypothesis significance testing was frequently used, e.g., to evaluate interaction levels in cagemates versus strangers, to compare interaction variability between cagemate and stranger dyads, and to assess changes over sessions. Use of ANOVA and Student's t-test were only performed after data were inspected and recognized to approximate gaussian distributions. When assessing interaction levels in each degu across exposures, a 2-way repeated measures ANOVA was used (exposure number and cagemate/stranger conditions). When assessing interaction variability changes across exposures, each dyad was considered independent, and mixed models were used (exposure number as a within-dyad repeated measure variable, and cagemate/stranger as a between-subjects variable).

In several cases, multiple comparisons had to be taken into account and corrected for. This includes tests that separately examined each interaction type (5 types tested), and when testing significance of temporal relationships between interactions (20 types tested). Significance values are corrected using the Benjamini and Hochberg false discovery rate procedure (Benjamini and Hochberg, 1995).

Ethical Note

All protocols were approved by the Institutional Animal Care and Use Committee at University of Montana (protocol number 036-18NIPSYC-061918). Degus were housed with extra space and

enrichment (see *Subjects*, above), and no invasive procedures were performed prior to or during the course of the experiment. Original sample sizes were determined by power analysis performed in G*Power (Faul et al., 2007) and were based on preliminary effect sizes obtained from within-dyad behavioral patterns across conditions in a prior experiment (Lidhar et al., 2021). (As noted above, female sample sizes were further increased to account for the "new cagemate" condition, added after data collection began.) Following the experiment, degus were transferred to other behavioral and, in some cases, neural experimental protocols. When retired from the laboratory, animals were transferred either to other labs, adopted locally, or humanely euthanized, with post-mortem tissue sometimes used for other research.

Degus tested in this study typically did not show levels of aggression high enough to cause injury. To prevent potential injuries, the experimenter remained in the recording room, and in the event of a "scramble" (rapid, erratic movements in which degus moved fast to tackle, bite, and body-slam one-another) animals were separated using a cardboard partition and the session was ended.

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