Variability of social interactions in new and established relationships in female degus

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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 develop a set of generalizable measures of interaction variability to test the hypothesis that social relationship formation is associated with initially high variability, followed by stabilization of dyadic interaction patterns. We focus these tests on female degus (*Octodon degus*), rodents predisposed to form cooperative peer relationships. 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 degu pairs could be distinguished from one-another by their session-to-session interactive behaviors, suggesting dyad-specific social relationships. Consistent with predictions, stranger interactions changed from the first to second exposure, after which they became less variable across days. Unexpectedly, however, long-term cagemates showed overall more interaction variability across sessions than strangers. Taking into account all interaction variability measures, differences between strangers and cagemates were only observed when considering many (> 10) interactions, or longer (>5 min, or session-to-session) time periods. Shorter scales, including interaction transitions, showed meaningful patterns but no reliable group differences. The results suggest that while variability of social behavior over minutes or across sessions may change as relationships develop, interactions between unfamiliar adult female degus are not systematically more variable than those of cagemates. Given known ecological pressures on female degus to form large, stable social networks, these patterns are consistent with the notion that interaction variability may be maladaptive for social coordination.

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). The process of sampling an environment or stimulus normally requires behavior to vary across its dimensions--e.g., eye movements around a new scene, or body movements through a new place. Assuming stability in the structure and values of a space, associative learning should subsequently decrease this sampling process, and correspondingly decrease the range of movements used. A demonstrative example of this is spatial navigation: as an animal learns about a new environment, its scope of foraging decreases in favor of goal-directed routes between locations, which in turn may eventually converge to narrow stimulus-response contingencies and stereotypies.

Animals of many species are prone to explore new individuals. In murine rodents, this phenomenon is so well established that it is used as the basis for tests of social recognition (Crawley, 2007; Thor & Holloway, 1981; Winslow, 2003). It is not clear, however, how this exploration affects the patterns of animals' interactive behavior. By analogy with exploration of space or other stimuli, behaviors between strangers may be initially more variable, but this variability may subsequently decrease as animals learn how best to respond to one-another. Specific examples of this can be found even in fruit flies: after losing fights with a particular individual, flies will narrow the type of fighting movements used with that conspecific in the future (Yurkovic et al., 2006). Similarly, in male mice, the establishment of a dominance relationship can be observed by an increasingly stable asymmetry within the dyad, one member using only aggressive behaviors, and the other only subordinate (W. Lee et al., 2019). Such changes in social interaction offer a window into how animal social relationships develop. The focus of these particular examples is on competitive relationships, and it is not clear whether the same principles apply to cooperative social relationships. The parallels and differences between relationship types will become more clear when we have generalizable metrics that can be applied across species and social contexts.

The present study was designed to examine the effects of social novelty on interaction variability in female degus, a rodent predisposed to form and maintain new, cooperative peer relationships. Female degus, like murine rodents, are known to interact more with a stranger compared with familiar peer (Lidhar et al., 2021), even when they have the option to choose between the two (Insel et al., 2020). In contrast with most data in murines, degus also quickly become affiliative with stranger peers, using allogrooming and huddling behaviors (Insel et al., 2020; Lidhar et al., 2021). This is consistent the animals' behavior in the wild, where females are known to form constructive, communal nesting relationships with unrelated peers (L. Ebensperger et al., 2002; L. A. Ebensperger et al., 2004, 2012, 2014; Hayes et al., 2009). The formation of stranger relationships may also fit evolutionary pressures: for female degus larger, more stable group structures confer fitness benefits (L. A. Ebensperger et al., 2016; Hayes et al., 2009), but larger groups of only related, familiar individuals may be unsustainable in the face of high predation rates (L. A. Ebensperger et al., 2009; Quirici et al., 2011). By studying the dynamics of relationship formation between female degus in the laboratory setting, we may gain insight into how peer relationships form in other species and situations.

We hypothesize that, just as animals vary their behavior to explore new environments and stimuli, female degus also explore novel conspecifics through more variable interactions, and this variability decreases as long-term relationships are established. In the process of addressing this hypothesis, we can examine the sources of variability, including whether interactions change similarly across dyads or are more individualized. It may be, for example, that all dyads show a decrease in agonistic behavior with familiarity, or it may be that one dyad converges to frequent face-to-face contact, while another to high allogrooming and low rear sniffing (illustrated in **Figure 1A**). Use of multiple metrics will also allow us to identify the temporal scales of interaction variability, including timescales as long as one 20 min session to the next, and as short as one interaction to the next. We have designed a set of different, interaction variability measures to test the prediction that variability in the interactions of initial strangers decrease with familiarity. As a control, we will examine the interactions of already-familiar, cagemate dyads over the same time period, in the same setting, in "reunion" social exposure sessions interleaved with those of

strangers. We predict that that cagemate dyads will exhibit lower within-dyad variability and be more easily discriminated from one-another than strangers.

Figure 1 Experimental design. A) Illustration of expected changes in interaction variability and dyad specificity over session. Arrows reflect hypothetical changes in interaction levels from one social exposure to the next. Hypotheses predict that in strangers, variability will decrease over exposures (shorter arrows) and that dyads will converge to specific interaction patterns (clusters of arrows in right panel). Interaction levels may, also or alternatively, change in a similar way across dyads (right panel). B) Recordings took place in a 50 x 50 cm square chamber. C) 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. D) Sequence of recording sessions over days. Cagemate and stranger reunions were interleaved. Degus were subsequently housed with the initial strangers instead of with cagemates, tested after one day (STR-RD) and then after one week (STR-RW). In two final reunion sessions they were presented with a new stranger (NST).

Methods

Subjects

Fifty-two adult female degus aged seven to twenty-nine months (mean = 11.9 mo, median = 11 mo) were used. 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 (T. M. Lee,

2004). Age effects are not reported due to uneven sampling and potential history threats; e.g., the two oldest dyads had been transferred from another colony. All degus had been weaned from parents and separated from male siblings between post natal day 30 (4 degus) and 60 (5 degus)(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 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 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., 2019; 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 (**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..

166

167 *Testing*

168

169 During the week prior to testing, each animal was pre-exposed to the testing chamber for at
170 least 5 minutes and no longer than 7 minutes each day over five days (variation due only to
171 experimenter scheduling, and not changed according to the animals' behavior). Pre-
172 exposures to the recording chamber were performed without other animals present, were
173 not recorded, and all animals received the same treatment independent of behavior during
174 the exposures.

175 Twenty-four hours before the first testing session, cagemates were separated into individual
176 cages within the same vivarium room. Testing began after degus were transported to the
177 testing room and the backside of one degu was marked for identification (Pet Paint;
178 Camarillo, CA). The painted degu was then placed in the chamber with an unpainted
179 conspecific, either its prior cagemate or a stranger. The pair was allowed to interact freely in
180 the recording chamber for 20 minutes. Once animals completed their 20 minute session
181 they were returned to their home cage with their previous cagemate and were left
182 undisturbed for 24 hours (**Figure 1C**). By placing cagemates together following each reunion
183 session, we hoped to allow the dyad to "recalibrate" their relationships, preserving their
184 dyadic interactions over the weeks and minimizing impact of the stranger exposures. At this
185 point, this cycle of co-housing, isolation, and testing would repeat, with exposures to
186 strangers and cagemates interleaved in a pseudo-randomized order (i.e., alternating
187 partners after either one or two reunions with the same partner) for a total of 5 cagemate
188 and 5 stranger sessions (counterbalancing stranger-first and cagemate-first across dyads;
189 **Figure 1D**). Degus always spent 24 hours with their original cagemate and then 24 hours in
190 isolation before returning to the recording chamber.

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192 Following 10 reunion sessions, initial stranger dyads were co-housed together and re-tested
193 four times. 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. The re-housed 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 (of 26 total dyads, 20 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).

Following re-housing reunion sessions, degus were subsequently returned to their original cagemates and then, following 24 hours of isolation, tested either once (all dyads) or twice (20 of 26 dyads) 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.

When taking into account all re-housed (“new cagemate”) and new stranger reunions, each of the 52 degus received between 11 and 16 testing sessions. The range was due to updates made to the experimental protocol to include more control sessions (e.g., first one day, then one week of co-housing after initial examination revealed that interaction levels remained high in the strangers across days). To prevent sampling biases, all comparisons that included these additional control sessions only included dyads that performed the controls.

Behavioral Scoring

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 are made freely available on Github.

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. It was extremely rare to observe alternate forms of aggression or play-fighting in this dataset of adult interactions. Sessions were said to start when the second degu was introduced into the chamber and to end when a degu was removed. During post-processing it was found that rare errors were 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. Inter-rater differences appeared to contribute significantly to the variance (noise) in the dependent variables (e.g., total interaction levels varied up to 20% between raters), but not to general effects, 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 1). 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. Interaction time was computed for each individual degu in each cagemate, stranger, and “new stranger” sessions. As just noted, for statistical comparisons interaction time was always transformed by computing the cube root.

We applied a large set of interaction variability metrics to to test how variability across timescales changes with social familiarity

“Interaction variability” is loosely defined, and multiple measures were used that separately examined variability of behavior *between sessions*, at different timescales *within sessions*, and with respect to *temporal dynamics* within and between interaction types. Across all measures, we predicted that strangers would initially show higher within-dyad variability, and lower between-dyad differences, than cagemates, but that variability in initial strangers would gradually come to resemble cagemates as the animals became more familiar with one-another.

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. Variability within a dyad could be measured by estimating distances

between a dyad's reunion sessions, while differences ("diversity") between dyads could be measured as distances between reunion sessions of different dyads.

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. To further confirm that total interaction levels were not responsible for results, analyses were also performed on unit-normalized interaction vectors (i.e., values were divided by the vector sum). As a final control, the results of the distance metrics were compared with those of two sets of "shuffle control" surrogate datasets. One set of 1000 shuffled datasets was created by reassigning the interaction type category randomly in each session, which preserved total levels of interaction within each session. Another set of 1000 datasets was created by reassigning the dyad labels across sessions, thereby scrambling all within- and between-dyad differences. The effect sizes of the original dataset was compared against shuffle controlled to assess the probability that results were obtained due to chance.

Dyad classification success was a secondary measure of interaction variability, computed by finding the degree to which a group of interaction vectors could be discriminated from another group. It is important to note that dyad classification success conflated two variables: within-dyad variability, and between-dyad differences (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, “fitsvm” 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 based on 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 using three measures: 1) distances between time-based interaction vectors, 2) distances between count-based interaction vectors, and 3) “sequence randomness”—related to the predictability of interactions, given the immediately preceding interactions.

Time-based interaction vectors were computed in the same way as session interaction vectors, except using time bins within a session rather than whole-sessions. This measure helped reveal the degree to which dyads changed their behavior over the 20 minutes of a session. To avoid assumptions about how long the time intervals should be, we ran tests using 30 different bin sizes, between 20 s and 600 s, varying by 20 s increments. Once overall differences between the cagemate and stranger groups were established across bin sizes, through use of a regression model, follow-up analyses were performed using a single bin size.

Count-based interaction vectors were computed by finding the number of interactions of each type within a block of interactions. For example: when using block sizes of 10

interactions, 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. Once again, a regression analysis first established whether there were cagemate versus stranger differences across block sizes (1 to 30 interactions per vector), and then follow-up analyses were performed using a single block size.

One caveat to the time- and count-based vector distances is that 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 size are reported for each analysis.

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. We chose 10 s as the cutoff based on temporal patterns observed in the interaction cross correlograms (next section). Actions sequenced by a single individual have a fundamentally different meaning from responses of one animal to another; therefore, separate, 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:

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

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 the temporal structure of interactions was estimated by Local Variance of intervals within interaction types, and cross correlations between interaction types.

Local variance (LV) was used to compute inter-behavior-interval (IBI) variance, which has been used more generally for gamma distributed neuron spike times (Shinomoto et al., 2005). LV was defined as

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

Where T_i is the time at which the i th interaction took place and “n” the number of spikes.

Local variance helped show whether strangers and cagemates differed with respect to variability in the pacing of their behaviors. Local variance was used because it makes no assumptions about the distribution of time intervals between interactions. We initially assumed that the event times would be roughly stochastic, and the intervals would therefore fit Poisson or gamma distribution (Lienhard & Meyer, 1967; Ord, 1967). Empirical evaluation of the IBIs indicate distributions conformed more closely to an exponential (**Supplementary Figure 2A-C**); i.e., behaviors often come in bursts, similar to what has been described in previous reports (W. 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 2D & E**).

Cross correlations were used to examine interaction transitions with higher temporal precision than transition matrices. Where transition matrices offer a “big picture” illustration of the relationship between interaction types, the cross correlograms provide a more temporally precise assessment of those relationships. The 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 the y-axis were, therefore, not standard correlation coefficients, but normalized rates.

Statistics

We used null-hypothesis significance testing to evaluate differences between when degus interacted with 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 done after data were inspected and recognized to approximate gaussian distributions. In analysis of day-by-day changes, repeated measures and mixed ANOVA models were used. Specifically, when assessing interaction levels in each degu, a 2-way repeated measures ANOVA was used (exposure day and cagemate/stranger conditions). When assessing interaction variability changes across days, each dyad was considered independent, and mixed models were used (exposure day as a within-dyad

repeated measure variable, and cagemate/stranger as a between-subjects variable). The exception to this was when estimating the time- and count-based interaction vector distance, within-session variability across exposure days. In these cases there was a relatively high frequency of sessions that lacked sufficient numbers of interactions, creating many cells with “missing data” that, when applying repeated measure models, resulted in non-representatively small sample sizes. In these cases, standard, 2-way ANOVA models were used.

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 & 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. 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). 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 subsequently used for other research.

Although the female degus tested in this study did not show highly aggressive fight behavior, the possibility was prepared for in advance. The experimenter remained in the recording room, and in the event of a “scramble” (rapid, erratic movements occasionally observed in adult male strangers, in which degus moved fast to tackle, bite, and body-slam one-another) animals were immediately separated using a cardboard partition and the

session was ended prematurely. None of these potentially injurious, aggressive behaviors were observed in the present experiment.

Results

Stranger dyads interact more than cagemates, but this was heterogeneous across dyads and did not attenuate with increased familiarity.

The first set of analyses were designed to confirm that strangers interacted more than did cagemates, as has been previously reported in laboratory settings (Villavicencio et al., 2009; Insel et al., 2020; Lidhar et al., 2021), and to evaluate whether interaction levels changed as familiarity increased. Consistent with prior results, more interactions were observed between strangers than between cagemates (2-factor repeated measures ANOVA: effect of stranger/cagemate: $F(1,51) = 12.06$, $p = 0.0011$, effect of day: $F(4,204) = 1.71$, $p = 0.15$, stranger/cagemate x day: $F(4,204) = 0.62$, $p = 0.65$; **Figure 2A**). Across specific interaction types, differences were significantly higher in strangers (2-factor repeated measures ANOVA with false discovery rate correction) for 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 activity ($F(1,51) = 10.32$, $p = 0.0023$; **Figure 2B**). These also replicated results from our prior study, which measured behavior during only a single reunion session (Lidhar et al., 2021)

Figure 2. Levels of social interaction over days. A) Total interaction levels for both stranger (red) and cagemate (black) individuals over the 5 initial days (errorbars are mean $\pm$ SEM). P-value reflects stranger versus cagemate difference. B) Breakdown of interaction levels across different types of interactions. Higher levels of agonistic, rear-sniffing, and face-to-face levels were observed in strangers. P-values show stranger versus cagemate differences, changes across days were not significant. C) Interaction levels over days in SC-HIGH (top) and SC-LOW (bottom) degus. Stranger-cagemate differences were heterogeneous across degus. Levels of social interaction between former strangers after re-housing (days 6 through 9), as well as levels in new strangers (blue), were consistent with those observed in initial strangers on exposures 1 through 5.

Although cagemates and strangers differed statistically, there was high heterogeneity between dyads. Sources of variation were unclear, though seemed to involve variation across the four “batches” of animals run over approximately 2 years of data collection levels (all sessions averaged within a dyad: 2-way ANOVA, stranger/cagemate: $F(1,42) = 3.46$, $p = 0.07$, batch: $F(4,42) = 4.32$, $p = 0.0051$, stranger/cagemate $\times$ batch: $F(4,42) = 0.88$, $p = 0.48$; **Supplementary Figure 3**). This could have been due to minor procedural changes, differences in experimenter, seasonal effects, or unknown housing/colony changes. This variance was subsequently taken into account by separating degus into those with significant stranger-cagemate differences (“SC-HIGH”, determined using a paired t-test on each animal across 5 stranger, 5 cagemate sessions, $p < 0.1$) and those that showed no systematic differences (“SC-LOW”, $p \geq 0.1$). Of the 52 degus used for the experiment, 16 (30%) were classified as SC-HIGH and the remaining 36 (70%) as SC-LOW. For the purposes

of the dyad-based analyses in subsequent sections, we distinguished between dyads with only SC-LOW degus (14 cagemate and 11 stranger) and those with at least one SC-HIGH animal (12 cagemate and 15 stranger).

We predicted that the difference in interaction levels between strangers and cagemates would diminish as the new individuals became more familiar and their social relationship formed. This was tested by examining interaction levels over the 5 days of exposures. To control for internal validity threats, such as experience with the protocol itself, we performed a final set of 1 or 2 reunions with new stranger dyads, with the expectation that decreased interaction levels among the initial strangers would not generalize to the new strangers. In contrast with predictions, we observed no effect of day, nor an interaction between the stranger/cagemate and day ($p \gg 0.05$, reported above). This was true for total interaction time and across all five types of interactions. To follow-up on this result, we separately tested changes over days in SC-HIGH and SC-LOW degus (2-factor repeated-measures ANOVA). We found no evidence that interaction levels changed over days in either the SC-HIGH (stranger/cagemate: $F(1,15) = 83.40$, $p = 1.63 \times 10^{-7}$, day: $F(4,60) = 0.89$, $p = 0.47$, stranger/cagemate $\times$ day: $F(4,140) = 0.20$, $p = 0.94$) or 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 $\times$ day: $F(4,140) = 0.20$, $p = 0.94$; **Figure 2C**).

One reason interaction levels may have stayed high in strangers is that the 5, 20 minute reunion epochs provided insufficient time for partners to become fully familiarized. In 20 dyads, initial strangers were subsequently co-housed for 1 day and then tested twice in the reunion; in 14 of these dyads, strangers were further co-housed for 1 week and again tested twice. Surprisingly, interactions between SC-HIGH strangers remained higher than long-term cagemates following 24 hrs of co-housing (paired t-test, averaging all cagemate sessions and both 24-hr co-housed stranger sessions, $t_8 = 4.86$, $p = 0.0013$) and following the 7 days of co-housing ($t_6 = 2.50$, $p = 0.047$). Correspondingly, no significant differences were detected between interaction levels in strangers before compared with after the one day and one week of co-housing ($t_8 = 0.50$, $p = 0.63$ and $t_6 = 0.85$, $p = 0.43$). When degus were introduced to new strangers after all other reunion sessions were complete, the SC-HIGH animals still exhibited higher interactions compared with when interacting with cagemates

($t_{15} = 4.23$, $p = 7.22 \times 10^{-4}$); thus higher interaction levels with strangers was not due only to the specific stranger pairings.

In summary, female degus showed a diversity of responses when exposed to strangers, with 30% greatly increasing their levels of agonistic, investigative (anogenital sniffing), and greeting (face-to-face) behaviors with animals they were not reared with--even after those individuals became more familiar over time. One question this raises is whether the difference is due to different genetic relatedness between strangers and cagemates. Of the two non-sibling cagemate dyads, both members of one dyad interacted more with strangers (i.e., were also SC-HIGH animals) while the members of the other dyad did not (i.e., were SC-LOW animals), suggesting that even if genetic relatedness played a role, it could not fully account for the differences. This is consistent with research from other labs (Villavicencio et al., 2009).

Stranger dyads were more distinct from one-another than cagemates, but only after their first social exposure

The overarching hypothesis of this study is that social interactions are more variable in novel social contexts, before relationships have formed. To test this, we examined whether initial strangers show more session-to-session variability in the types of interactions they engaged in. Two complementary measures were used: interaction vector distances and dyad classification success, illustrated in **Figure 3A**. The distance-based method allowed observation of overall variability within dyads and the diversity between them, while the classifier-based method could establish whether there were unique combinations of interaction types that distinguished between dyads. An example of how interaction vectors from a cagemate and stranger dyad distribute in feature space is illustrated in **Figure 3B**.

Figure 3. Variability and diversity across sessions. A) Diagram illustrating analysis methods. Each column of boxes represents the interaction vector from a single session (AG = levels of agonistic behavior, GR grooming, RS rear-sniffing, FF face-to-face, BS, body-sniffing, is left off). To the right, vectors are plotted in cartesian coordinates to illustrate measures of variability. Top panel shows SVM-based classification success, with dashed line representing a hyperplane oriented in the classification-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 interaction vectors from the experiment using the first two principle 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 principle components do not very well distinguish the two dyads, but do show higher

clustering among the stranger points. C) Distribution of within- and between-dyad Euclidean distances among cagemates (black, left side) and strangers (red, right side), illustrated using swarm plots. Inter-dyad distances were both more variable and overall higher in strangers, while within-dyad distances were not. D) The same swarm plots as in panel C, separating-out dyads with SC-HIGH degus (top) and those with only SC-LOW degus (bottom). Much of the inter-dyad diversity arises from SC-HIGH animals, though dyad variability is particularly low among SC-LOW dyads. E) Same as part C after removing agonistic interactions, effectively eliminating differences between strangers and cagemates. F) Success rate of SVM classifiers discriminating pairs of dyads among cagemates and strangers (errorbars are mean $\pm$ SEM). G) As in part F, but separating out dyads with SC-HIGH degus and those with only SC-LOW degus. Improved classification in strangers was preserved in both sets of dyads. H) As in part F, but classifications were performed on reduced interaction vectors lacking specific interaction types (-AG = vectors lacking agonistic interactions from both degus). Abbreviations are the same as in part A. I) Relative success rates for different days (model training performed on all days other than the one tested). Classification rates in strangers (red) were generally higher than 80%, except for exposure day 1 when success rate in strangers was lower and matched that of cagemates (black). J) Within-dyad distances across exposure days 1 in strangers (red), new strangers (blue) and cagemates (black). Values are medians $\pm$ SEM.

There was strong evidence of dyad specificity in both cagemate and stranger interactions over sessions. This could be assessed by comparing interaction vector distances within- versus between-dyads in the two groups (results for Euclidean distances: 2-way ANOVA, effect of cagemate/stranger: $F(1,698) = 2.20$, $p = 0.14$; within/between-dyads: $F(1,698) = 42.04$, $p = 1.7 \times 10^{-10}$; cagemate/stranger $\times$ within/between-dyads: $F(1,698) = 3.39$, $p = 0.066$; **Figure 3C**). Similar effect sizes were observed for cosine distance, which is less sensitive to overall levels of activity (cagemate/stranger: $F(1,698) = 0.73$, $p = 0.39$; within/between-dyads: $F(1,698) = 47.24$; cagemate/stranger $\times$ within/between-dyads: $F(1,698) = 2.42$, $p = 0.12$; **Supplementary Figure 4A**). To further rule-out the effect of overall interaction levels, statistical tests were repeated after normalizing interaction vectors, yielding similar results (cagemate/stranger: $F(1,698) = 10.85$, $p = 0.0010$, within/between: $F(1,698) = 18.17$, $p = 2.3 \times 10^{-5}$, cagemate/stranger $\times$ within/between: $F(1,698) = 1.14$, $p = 0.29$). Interaction levels and other non-specific effects could be further ruled-out by comparing the strength of the differences with those of surrogate datasets in which either interaction types or dyad identities were randomly shuffled; the F statistic of within- versus between-dyad difference in the original model far exceeded the 99.9 percentile levels in both cases. Finally, the difference between within- and between-dyad distances was found to be present in both SC-HIGH and SC-LOW dyad groups (SC-HIGH: stranger/cagemate:

$F(1,194) = 22.95$, $p = 3.30 \times 10^{-6}$, within/between: $F(1,194) = 30.02$, $p = 1.3 \times 10^{-7}$;
interaction: $F(1,194) = 2.07$, $p = 0.15$; SC-LOW: stranger/cagemate: $F(1,167) = 27.64$, $p =$
 0.43 ; within/between: $F(1,167) = 12.76$, $p = 4.6 \times 10^{-4}$, interaction: $F(1,167) = 0.013$, $p = 0.91$;
Figure 3D; once again replicated using Cosine distances, SC-HIGH: stranger/cagemate:
 $F(1,194) = 0.70$, $p = 0.40$, within/between: $F(1,194) = 19.99$, $p = 1.3 \times 10^{-5}$,
stranger/cagemate x within/between: $F(1,194) = 0.92$, $p = 0.34$; SC-LOW:
stranger/cagemate: $F(1,194) = 4.91$, $p = 0.028$, within/between: $F(1,194) = 9.41$, $p = 0.0025$,
stranger/cagemate x within/between: $F(1,194) = 0.40$, $p = 0.53$; **Supplementary Figure 4B**).

While both cagemates and strangers showed dyad-specificity in their interactive behaviors,
there was some evidence that specificity was higher in stranger dyads relative to cagemates.
The cagemate/stranger difference was initially hinted at by the statistical trend for
differentially higher, between-dyad Euclidean distances in strangers (ANOVA reported
above, $p = 0.066$), although was largely masked by different variances between the groups
(two-sample F-test between stranger and cagemate, between-dyad distances: $F(324,324) =$
 0.46 , $p = 3.75 \times 10^{-12}$). The trend for higher specificity in strangers was largely due to
between-dyad diversity in their agonistic behaviors, as the effect was lost after removing
this interaction type (cagemate/stranger, $F(1,698) = 0.54$, $p = 0.46$; within/between, $F(1,698)$
 $= 50.74$, $p = 2.6 \times 10^{-12}$, interaction: $F(1,698) = 0.75$, $p = 0.39$; **Figure 3E**). When controlling for
overall interaction levels using either unit-normalized vectors or cosine distance, the higher
specificity in strangers was lost (test statistics reported above), though in these cases
strangers often showed more generalized reductions in variability. Interaction vector
distances therefore showed that, contrary to predictions, strangers were not more variable
day-to-day than cagemates; if anything, strangers could be considered less variable
(normalized vectors and cosine distance), or to have similar variability but with higher
between-dyad diversity (Euclidean distance).

Supervised learning methods strengthened the observation that dyads could be behaviorally
distinguished from one-another, and supported the notion that strangers were more
distinguishable than cagemates. A linear kernel SVM model was trained to discriminate
between pairs of dyads. Mean success rates exceeded chance for both cagemates and
strangers, indicating that interactions between the animals of one dyad were more similar

across reunion sessions than when compared with the interactions of other dyads (the binomial probability distribution predicts roughly 5% probability of success classifying at least 7 of 10 sessions, mean rates for cagemates = 7.1, mean for strangers = 7.8; **Figure 3F**). Strangers dyads were more distinguishable than cagemates (two-sample t-test, $t(648) = 5.84$, $p = 8.4 \times 10^{-9}$). Once again, unit-normalized interaction vectors were used to confirm that the result was not an artifact of higher overall interaction levels in the stranger degus (of 10 sessions, mean success in cagemates = 6.8, strangers = 7.5, $t(648) = 6.5$, $p = 1.4 \times 10^{-10}$). Dyads with SC-HIGH degus did show stronger dyad specificity compared those with only SC-LOW degus; however, the overall effect was observed in both groups (2-way ANOVA, cagemate/stranger: $F(1,313) = 5.43$, $p = 0.020$, SC-HIGH vs. SC-LOW: $F(1,313) = 6.45$, $p = 0.012$, cagemate/stranger $\times$ SC-HIGH/LOW: $F(1,313) = 0.11$, $p = 0.74$; **Figure 3G**). In no case did removal of any one type of interaction, including agonistic behaviors, result in a loss of cagemate versus stranger differences ($p = 1.1 \times 10^{-5}$, 6.2×10^{-8} , 6.2×10^{-8} , 4.2×10^{-12} , 1.1×10^{-10} when agonistic, allogrooming, rear-sniffing, face-to-face, and body-sniffing were removed respectively; **Figure 3H**). Overall, classification results suggested that the day-to-day variability of behavior within-dyads, relative to differences between dyads, was lower in strangers.

The higher discriminability of strangers relative to cagemates did not attenuate over days; in fact, differences between the two groups were only apparent after the first exposure. Classification success between stranger dyads was lowest on the first exposure day, indicating that behavior on that first day could not be as accurately predicted based on behavior on subsequent days (**Figure 3I**). This change in behavior between the first and second exposures was also apparent by the within-dyad interaction vector distances across exposure day (Euclidean: mixed ANOVA, between-subjects stranger vs. cagemate: $F(1,50) = 0.041$, $p = 0.84$; within-subjects exposure day: $F(4,200) = 3.49$, $p = 0.0088$; stranger/cagemate $\times$ exposure: $F(4,200) = 2.90$, $p = 0.023$; **Figure 3J**; cosine: mixed ANOVA, between-subjects stranger vs. cagemate: $F(1,47) = 2.19$, $p = 0.15$; within-subjects exposure day: $F(4,188) = 1.95$, $p = 0.10$; stranger/cagemate $\times$ exposure: $F(4,188) = 2.75$, $p = 0.030$, **Supplementary Figure 4C**).

One explanation for changes between exposure sessions is that the degus altered their behavior when exposed to a different individual during the time between reunions. Although the experiment was designed to minimize these effects, by placing degus with their original cagemates for 24 hours after each reunion, it is also likely that animals treated the experimental context differently, and previous social experience in that context might therefore influence subsequent behavior. To address this, we compared interaction vectors distances when there was an intervening reunion with another dyad (e.g., cagemate-stranger-cagemate, “C-S-C”, or cagemate-stranger-stranger-cagemate, “C-S-S-C”) with those that did not (e.g., cagemate-cagemate, “C-C”). Euclidean distances were higher between cagemate sessions in C-S-C (or C-S-S-C) relative to C-C sequences, but not between stranger sessions in S-C-S (or S-C-C-S) relative to S-S sequences (mixed ANOVA, repeated measures between reunion sequence: $F(1,102) = 8.51$, $p = 0.0044$, between-dyad cagemate versus stranger: $F(1,102) = 0.82$, $p = 0.037$, reunion sequence $\times$ cagemate/stranger: $F(1,102) = 4.30$, $p = 0.040$; **Supplementary Figure 5A**). Differences were not observed using cosine distance, suggesting they were largely related to changes in total interaction levels (repeated measures between reunion sequence: $F(1,102) = 1.62$, $p = 0.21$, between-dyad cagemate versus stranger: $F(1,102) = 8.60$, $p = 0.0042$, reunion sequence $\times$ cagemate/stranger: $F(1,102) = 1.80$, $p = 0.18$; **Supplementary Figure 5B**). Although intriguing, a notable confound is that the cagemate reunions in the C-S-C/C-S-S-C sequences take place 4 or 6 days apart and only 2 days apart in C-C the reunions.

In summary, the interactions within a dyad were relatively consistent across sessions, with stranger dyads being even more distinguishable from one-another than cagemates. The exception was during the first exposure, when the behavior of strangers tended to differ more from behavior on subsequent days. The results could not be explained either by the generally higher interaction levels, nor by any one type of behavior--such as the increased agonistic interactions among strangers. The results suggest that, at least after an initial social exposure, new relationships in female degus are not associated with more variable social interactions.

Within-session variability was initially higher in strangers, but increased in cagemates over exposure days

Did strangers exhibit higher variability in the sets of interactions used over the course of a session? Changes in interactions within a session were estimated using both a time-based (interactions during one interval of time compared with the next) and count-based (the first “n” interactions compared with the next) method. The ratio of within- versus between-dyad distances using time-based vectors reached a minimum of 0.81 (Euclidean), 0.71 (cosine) using 500 s bins, or 0.62 (Euclidean), 0.48 (cosine) with 28-interaction blocks (**Supplementary Figure 6**). These values are comparable to the session-by-session ratios reported earlier.

Differences in variability within sessions were observed between stranger and cagemate groups using both time- and count-based methods, but were stronger in the latter. Overall differences across bin and block sizes were estimated using regression models. Stranger-cagemate differences on the fifth day were observed independent of the measure used (Euclidean distance, time-based, 95% confidence intervals of regression coefficients: $\beta(\text{stranger/cagemate} \times \text{bin size}) = -0.0079$ to -0.0026 ; count-based, $\beta(\text{cagemate/stranger}) = -0.078$ to -0.002 ; **Supplementary Figure 7**; cosine distance, time-based vectors: $\beta(\text{cagemate/stranger} \times \text{bin size}) = -0.0071$ to -0.0019 , count-based: $\beta(\text{cagemate/stranger}) = -0.015$ to -0.011). To examine whether these effects generalized over days, a bin size of 320 s was chosen for time-based vectors and block size of 14 interactions for count-based vectors, both selected based on the strength of the group differences observed on exposure day 5. Using count-based vectors, a shift over days was observed, with initially higher variability in strangers, following increases in variability among cagemates (in this case, statistical models with repeated measures were not used due to most dyads having at least one session without a two, 14-interaction block minimum; 2-way ANOVA: cagemate/stranger: $F(1,124) = 0.47$, $p = 0.50$, days: $F(4,124) = 1.82$, $p = 0.13$, cagemate/stranger $\times$ days: $F(4,124) = 4.55$, $p = 0.0018$; **Figure 4A**, post-hoc comparisons show group differences only significant on the first exposure day, $p = 0.0074$; cosine distance: cagemate/stranger: $F(1,124) = 4.60$, $p = 0.034$, days: $F(4,124) = 1.51$, $p = 0.20$, cagemate/stranger $\times$ days: $F(4,124) = 3.21$, $p = 0.015$, post-hoc comparisons show significant group differences on the first two days: $p = 0.0068$, p

= 0.018; **Supplementary Figure 8A**). Notably, and unexpectedly, much of the variance was carried by changes taking place in the cagemates rather than in strangers, suggesting that within-session variability increased in cagemates as the dyads either became more familiar with the experimental chambers, or gained more experience with strangers (considered in more depth below). Time-based vectors showed minor differences between stranger and cagemate groups across days, and those that were present were again primarily driven by increased cagemate variability on exposure day 5 (2-way ANOVA, cagemate/stranger: $F(1,215) = 3.5$, $p = 0.063$, exposure day: $F(4,215) = 3.19$, $p = 0.014$, cagemate/stranger x day: $F(4,215) = 3.1$, $p = 0.017$, **Supplementary Figure 8B**; cosine distance: cagemate/stranger: $F(1,215) = 2.14$, $p = 0.16$, exposure day: $F(4,215) = 2.95$, $p = 0.021$, cagemate/stranger x day: $F(4,215) = 3.15$, $p = 0.015$, **Supplementary Figure 8C**). Splitting dyads into those with SC-HIGH and SC-LOW animals revealed that the SC-HIGH group primarily carried the interaction effect between stranger/cagemate and exposure day (2-way ANOVA, stranger/cagemate x day F-values, in SC-HIGH: $F(4,74) = 3.64$, $p = 0.0094$; SC-LOW: $F(4,40) = 0.84$, $p = 0.51$, **Figure 4C & D**; cosine distance: SC-HIGH: $F(4,74) = 2.45$, $p = 0.054$; SC-LOW: $F(1,4) = 7.35$, $p = 0.010$, $F(4,40) = 0.77$, $p = 0.55$; **Supplementary Figure 7C & D**). Within-session interaction distance scores were also examined in strangers after re-housing them together for one day or one week (exposure days 6 through 9 on **Figures 4A & B**). These sessions did not significantly differ from either cagemate or stranger sessions before re-housing.

Figure 4. Within-session interaction variability. A) Mean interaction vector distances across days ($\pm$ SEM). Here, “count based” interaction vectors, with blocks of 14 interactions compared against one-another. Variability appeared to be higher in strangers on the first two days but subsequently decreased, while increasing in cagemates. B) Within-session variability in cagemates following a cagemate (C-C) or stranger (S-C) session (black trace, left) and in strangers following a stranger (S-S) or cagemate (C-S) session (red trace, right). Variability may have been higher in strangers when sessions followed cagemate reunions—i.e., when the strangers had not seen one-another for 4 or 6 days, compared with only two days. C) Comparison of within-session interaction variability in dyads with SC-HIGH versus SC-LOW degus. The SC-HIGH group appeared to carry the across-day changes. D) Transition matrix between interaction types, averaged across all degus. Left matrix shows probability of “next action” (columns) given a reference action (rows) performed by the same degu, right matrix 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. E) Sequence randomness of interactions in stranger (red) and cagemate (black) dyads. No significant changes were observed over days, nor differences observed between dyad groups.

To examine whether previous experience in the experimental chamber with a different dyad impacted day-to-day changes in within-session variability, count-based interaction vector distances that were preceded by a same-dyad reunion session were compared against those

preceded by a different-dyad reunion (as a reminder: the prior session was always two days earlier, separated by 24 hours with a cagemate and another 24 hours of isolation). No differences in variability were observed in cagemate reunions preceded by cagemate (same dyad) or stranger (different dyad) sessions (Euclidean: $t(29) = 0.32$, $p = 0.75$; cosine: $t(29) = 0.18$, $p = 0.86$; **Figure 4B, black traces**; the low degrees of freedom reflects that there were only 13 cagemate dyads with at least 28 interactions in C-C sessions, and 18 dyads with at least 28 interactions in S-C sessions). There was evidence that variability was higher when stranger reunions were preceded by cagemate (different dyad) compared with stranger (same dyad) sessions (Euclidean: $t(33) = 2.29$, $p = 0.028$; **Figure 4B, red traces**) although the result was not significant using cosine distance ($t(33) = 1.22$, $p = 0.23$). In other words, stranger interactions might have been overall more variability across blocks of interactions if the animals had not seen one-another for 4 or 6 days, compared with when they had seen one-another only two days prior.

The third measures used to examine within-session variability was a “sequence randomness” score, inversely related to how predictable one interaction type is, given the previous interaction. As a first step, transition matrices between interaction types were generated for each session, separately considering transitions in the same animal and those between individuals (**Figure 4D**). Before computing sequence randomness we established that different interactive behaviors were significantly related to one another. All within-individual interaction transitions showed higher probability than expected by chance (where chance was determined by baseline probabilities of each action, one-sample t-test with false discovery rate correction). In other words: if one animal performed an action toward another, there was a higher-than chance probability it would perform another, different interaction within the following 10 seconds. Transitions between individuals were also typically found more frequently than chance, with several exceptions: agonistic and allogrooming behaviors did not reciprocate one-another, and no significance was observed for transitions between allogrooming and rear-sniffing, or rear-sniffing-to-agonistic interactions; i.e., if one individual behaved agonistically, the other was unlikely to respond by grooming the first—and vice versa. Strangers and cagemates did not differ from one-another in their transition probabilities (evaluated for each pair of interactions; 2-sample t tests, $p \gg 0.05$, examined further using cross correlograms, next section).

836

837 Just as individual transition probabilities did not appear to differ between stranger and
838 cagemates, the overall predictability of interaction types, given a preceding interaction
839 (estimated using sequence randomness) also failed to show differences between groups
840 (SELF transitions: mixed ANOVA, repeated measures across exposures: $F(4,128) = 1.17$, $p =$
841 0.33 , between-subjects cagemate/stranger: $F(1,128) = 0.33$, $p = 0.57$, exposures x
842 cagemate/stranger: $F(4,128) = 0.79$, $p = 0.53$; OTHER transitions: exposures: $F(4,168) = 0.69$,
843 $p = 0.60$, cagemate/stranger: $F(1,168) = 1.74$, $p = 0.19$, interaction: $F(4,168) = 1.36$, $p = 0.25$).
844 This is consistent with the above observation that count-based, interaction vector distances
845 with only two or three interactions showed no stranger versus cagemate differences, nor
846 did time-based interaction vector distances of only 5 or 10 s.

847

848 In summary, within-session variability, as measured by count-based vectors, appeared
849 higher in strangers during the initial exposures, and higher in cagemates by day 5. Changes
850 across days seemed to primarily be due to increased variability in the cagemate group,
851 though there was also evidence that within-session variability between strangers increased
852 if the animals had not encountered one-another for 4 or 6 days, relative to only two days.
853 Stranger-cagemate differences were primarily carried by dyads with SC-HIGH degus.

854

855

856 *Temporal relationships between interactions did not strongly differ between strangers and*
857 *cagemates.*

858

859 To determine whether social novelty affected the temporal structure of interactions during
860 a session the intervals within and between interaction types were examined using local
861 variance and normalized cross correlograms respectively.

862

863 Variability between interactions of the same type offered insight into how often each
864 individual returned to a particular behavior. If face-to-face interactions, for example, are a
865 way for the two individuals to “check-in” after a period of time had elapsed, these episodes
866 may be more consistently repeated between those that know one-another compared with
867 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 (agonistic, allogrooming, rear-sniffing, face-to-face, and body-sniffing; detailed in Methods). There was no indication of stranger/cagemate differences in IBI variance for any of the 5 interaction types (two-sample t-tests, false-discovery rate correction, $p \gg 0.05$; **Figure 5A**). The same was true when the analysis was repeated for only the first day of interaction. Thus, although the overall frequency of certain behaviors, such as rear-sniffing, was higher in strangers, there was no evidence to suggest that the intervals between interactions were less consistent.

Figure 5. Variability in temporal relationships within and between interactions. A) Local Variance (LV) of IBIs was computed for each behavior, for each session. No differences were observed between cagemate and stranger groups. D) Example cross correlograms between interaction types, further illustrating the absence of difference between cagemates and strangers. Relative levels of face-to-face interaction before and after other actions, normalized by total levels of face-to-face interaction, are shown across four different reference interaction types. In each case face-to-face was highly likely to precede other interactions, but this did not differ between stranger and cagemates.

To examine temporal variability between interactions, we followed-up on the transition matrix analysis described in the previous section by creating time-lagged, cross-

correlograms for each pair of interaction types. As with the transition matrices, there was no evidence that the temporal relationship between interaction types differed between cagemate and stranger dyads (2-sample t-tests, p-values did not meet false discovery rate criteria). Examples of cross correlograms showing face-to-face contact relative to the start and end times of other interaction types are presented in **Figure 5B** (others in **Supplementary Figure 9**).

Discussion

The present studies were designed to test whether social exploration between female degu peers is associated with more variable interactive behaviors. Interactions between initial strangers were found to change after an initial social exposure, then became more consistent from one exposure session to the next. This was measured in the form of interaction vector distances, and in the ability of a machine learning classifier to distinguish between dyads. While this result was consistent with predictions, three unexpected observations stood out. First, the 30% of degus that interacted more with stranger peers than with familiar cagemates (the “SC-HIGH” animals) did not decrease interactions to cagemate levels even after becoming highly familiar with their new partners. The second unexpected observation is that cagemates showed relatively higher levels of variability in their interactions than strangers. This was apparent in the session-to-session interaction vector distances, but also more prominently in the failures of a machine learning classifier to tell cagemate dyads apart. Additionally, interaction variability within sessions was initially lower in cagemates relative to strangers, but unexpectedly increased across exposures. The third unexpected observation was that in spite of the initially high variability in strangers between the first two sessions, there was little evidence that interactions were more variable than those of cagemates on shorter timescales. This includes measures of interaction transitions, vector distances between groups of several interactions, or vector distances between time bins of a few seconds or minutes. Each of these observations is discussed in turn below.

Higher levels of interaction between adult female, stranger degus has been reported previously (Insel et al., 2020; Lidhar et al., 2021; Villavicencio et al., 2009), and fits with the well-known tendency of animals to approach novelty (Berlyne, 1966; Hills et al., 2015; Hughes, 1997). This behavior is also unsurprising given the species' gregarious behavior in natural habitats. Degu burrows are found near one-another, and animals of different burrows greet one-another frequently (L. A. Ebensperger et al., 2004; Fulk, 1976). Females also nest with unrelated individuals, and nurse one-another's pups (L. Ebensperger et al., 2002; L. A. Ebensperger et al., 2004, 2012, 2014; Hayes et al., 2009). But if degus form stable, cooperative peer relationships with new individuals, then what may explain why animals in the present study continued to treat new relationships differently from those with prior cagemates? We found that interaction levels remained high in initial strangers even after the degus were co-housed over days. The patterns suggest that there are fundamental differences in non-sibling relationships formed in adulthood compared with conspecifics reared together. There are hints of this from other studies of degus in both natural and laboratory habitats. Females in the wild have been reported to show preferences for members of their own burrow groups during daytime foraging and social greetings (L. A. Ebensperger et al., 2004). In the lab, genetic kinship can influence social behavior—just not as strongly as familiarity (Villavicencio et al., 2009). The present study may be the first time that adult female social relationships have been tracked in a controlled setting over an extended timeline, and the results suggest that while strangers typically do refrain from high levels of aggression, some of the relationships remain different from those of cagemates reared together. This could be due to contextual factors, such as the limited social history of the laboratory animals, or absence of proximate ecological pressures such as predators. Alternatively, it may be that there are critical developmental windows for establishing certain types of peer relationships, a hypothesis that could be addressed by performing similar studies in juvenile animals.

Cagemate dyads exhibited unexpectedly high levels of variability relative to strangers. This was observed most when measuring variability across sessions using cosine distance of interaction vectors, and by the difficulties a machine learning classifier had differentiating between dyads based on their session-to-session behavior. It was also apparent when measuring interaction vector distances within the final (fifth) exposure session. One

explanation for this phenomenon is that individual degus felt less constrained when placed with a cagemate, resulting in behaviors that dependent more on day-to-day circumstantial factors (e.g., hormonal fluctuations, ambient odors, experimental room temperature, etc.). But there may not be a single reason why cagemates showed higher variability than strangers. It may be, for example, that strangers dyads with SC-HIGH degus were less variable across days because they were more motivated to maintain levels of agonistic or investigative behaviors, while dyads with SC-LOW degus may have been more wary of strangers, and therefore less likely to engage in unpredictable social interactions. This latter explanation is consistent with the idea that being unpredictable is less conducive to forming cooperative relationships. Many primates will use specific sets of vocal and physical interactions when encountering strangers, often in stereotyped and ritual sequences. The circumstances in which these behaviors take place—e.g., between similar-ranking males, younger individuals greeting older, and when approaching pregnant females—suggest that the 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 & Maestriperi, 2003). To put another way, reduced variability may help avert high-cost aggressive or competitive interactions. It can be hypothesized, therefore, that female degus are predisposed for lower variability interactions with strangers as part of their predisposition to form cooperative relationships with new individuals. This predicts that the same patterns would not be found in adult male degus, which are not believed to benefit as much from large, stable social groups (L. A. Ebensperger et al., 2016).

The final main surprise in the data was that strangers showed many of the same social interaction patterns as cagemates. There was no evidence for differences in variability from one interaction to the next, as measured using sequence variance and interaction cross correlograms, or over groups of only several (fewer than 10) interactions within sessions. The common structure of behavior in novel and familiar individuals may simply 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 4 and 6% of the session for cagemate and stranger dyads respectively, or just over 8% in SC-HIGH animals) may indicate that the environmental context was not of optimal 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 female 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 female degus 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 many 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.

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