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

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Abstract

When a behaving system 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 a range of learning systems, but has not been well studied in the social domain in which the stimulus an agent investigates, another individual, is reactive to the agent's behaviors. We examined the effects of social novelty on interaction variability in laboratory-reared, female degus, known to readily express affiliative behaviors with initially unfamiliar, unrelated 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 dyads could be distinguished from one-another by their interactive behaviors, suggesting dyad-specific social relationships. Following the first session, stranger dyads were unexpectedly easier to differentiate than cagemates due to a combination of higher diversity of behavior between dyads and, in some cases, lower variability within dyads. Some evidence could be found for higher variability in stranger interactions within the first two exposures, though within-session variability increased in cagemates across reunions, ultimately exceeding levels in strangers. We also observed that while strangers interacted more than cagemates, this could be traced to only 30% of the animals and the higher interaction levels did not attenuate over sessions or after co-housing the animals. No strong differences were observed in the temporal structure of social behavior between the two groups. Results reveal that new relationships in adult, female degus are more diverse but not more variable compared with established relationships, particularly after the first social exposure. Given known tendencies of female degus to form and maintain new relationships, these findings are consistent with the notion that higher interaction variability may be maladaptive to building social coordination and trust.

Introduction

Novelty evokes exploratory behaviors in animals ranging from invertebrates to mammals (Berlyne, 1966; Hills et al., 2015; Hughes, 1997). Although exploration can take more systematic (directed) or random (undirected) procedures (Thrun, 1992), 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 decreasing the range of movements used. Taking spatial navigation as an example: as animals learn about their environment, they may decrease the scope of foraging in favor of goal-directed routes between locations. Eventually, these behavioral patterns can even become narrow stimulus-response contingencies and stereotypies.

It is well known that in many species, social novelty can increase social investigation—i.e., that animals explore new individuals. However, it's not always clear how this exploration affects the diversity 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. 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). Such changes in social interaction offer a window into how animal social relationships develop and change.

But social novelty differs from other forms of novelty, in that the stimulus an agent is exploring is highly sensitive and reactive to the agent's own behavior. In many cases, stranger interactions are characterized by relatively low variability. Ritualized greeting behaviors between initial strangers have been particularly well characterized in primates (Aureli & Schaffner, 2007; Dias et al., 2008; Kutsukake et al., 2006; Smuts & Watanabe, 1990; Whitham & Maestripieri, 2003). This is useful because investigating unfamiliar individuals is risky: other individuals are inherently unpredictable and, therefore, potentially

threatening. By displaying highly predictable behaviors and behavioral sequences, individuals can signal that their intentions are benign. The bias for or against variable social behaviors likely changes as relationships form, and may depend on contextual factors—such as hierarchy, species, developmental stage, and ecological conditions—in ways that are still poorly understood.

The present study was designed to examine the effects of social novelty on interaction variability in a rodent species that may be predisposed to form and maintain new peer relationships. We sought to test the hypotheses that 1) variability of interactions between unfamiliar adults is initially higher than between familiar individuals—i.e., when individuals are exploring one-another, and 2) increased behavioral variability between strangers will decrease as the animals become familiar with one-another. Female degus were chosen based on previous work showing that initially unfamiliar, adult peers can quickly become affiliative with one-another, using allogrooming and huddling behaviors (Insel et al., 2020; Lidhar et al., 2021). This is consistent with observation of degu behavior in natural environments, where females are known to form constructive, communal nesting relationships with unrelated peers (L. Ebensperger et al., 2002; L. A. Ebensperger et al., 2012, 2014; LuisA. Ebensperger et al., 2004; Hayes et al., 2009). The formation of stranger relationships may also fit evolutionary pressures, given that degu groups have high turnover over years (L. A. Ebensperger et al., 2009; Quirici et al., 2011), and larger, more stable group structures confer fitness benefits (L. A. Ebensperger et al., 2016; Hayes et al., 2009). By studying the dynamics of relationship formation between female degus in the laboratory setting, we hope to gain insight into how constructive peer relationships form in other species and situations.

One central question of the experiment is whether degus exhibit dyad-specific social relationships, as measured by the consistency of behavioral profiles within dyads from one social exposure to the next, relative to the differences in the behavioral profiles between dyads. For example: in one dyad an individual may frequently allogroom its partner while its partner tends to initiate agonistic behaviors, in another dyad both members may be prone to face-to-face contact. Based on the hypothesis that new relationships are characterized by less stable (more variable) patterns of behavior, and that long-term cagemates have

correspondingly more consistent relationships over time, we predict that cagemate dyads are more distinguishable from one-another by their behavioral profiles than initial strangers. While the use of only ethogram-based measures has some limitations, an underlying goal of the study is to advance generalizable methods to assess interaction patterns across measurement systems, species, and setting.

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 (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 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 and 2 that were not 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 (Logitech; Lausanne, Switzerland) and recorded at a sampling rate of 30 frames/s (audio at 48 kHz). Recording chamber was a 50 x 50 x 50 cm wooden box, the walls painted blue to enhance contrast for tracking and sealed to prevent fluid absorption. 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.

During the week prior to testing, each animal was pre-exposed to the testing chamber for at least five-minutes each day over five days. Pre-exposures to the recording chamber were performed without other animals present.

Testing

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 during scoring (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 cagemate and were left undisturbed for 24 hours. At this point, this cycle of co-housing, isolation, and testing would repeat, with exposures to strangers and cagemates interleaved in a consistent, pseudo-randomized order for a total of 5 cagemate and 5 stranger sessions (counterbalancing stranger-first and cagemate-first across dyads; **Figure 1A**).

To establish whether any observed differences between strangers and cagemates were due to familiarity, strangers were re-tested after being co-housed for 24 hours (20 of 26 dyads) and then again after co-housing for 7 days (14 of 26 dyads). To confirm that observed changes in stranger interactions were due to familiarity with the specific individual, rather

than general acclimation to the recording chamber and testing protocol, an additional one (all dyads) or two (20 of 26 dyads) testing sessions were performed with a new stranger. Each of the 52 degus therefore received between 11 and 16 sessions (the range due to the experimental protocol being updated to include more control sessions, e.g., first one day, then one week of co-housing). To prevent sampling biases, all comparisons that included the additional control conditions only included sessions from dyads that performed the controls.

Behavioral Scoring

Software: Scoring of physical behavior was performed using BORIS (Behavioral Observation Interactive Research Software; Torino, Italy CITE), which allows users to log events during video playback. All analyses were custom-written in MATLAB and are made freely available on Github.

Behavioral Scoring: filenames for video recordings were changed prior to scoring to ensure raters were blinded to conditions. Raters coded the start and end times of each observed behavior, the type of behavior, and the animal that initiated the behavior. 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. In females it was extremely rare to observe alternate forms of aggression. Sessions were said to start when the second degu was introduced into the chamber and to end when a degu was removed. Potential errors in the scoring of agonistic, allogrooming, rear-sniffing, and face-to-face behaviors were reduced by setting a threshold of 6 s as the longest any one of these interactions could last (determined by visual inspection of the distributions of all interactive behaviors across all sessions in each of the four categories).

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. Examination of total interaction time across sessions revealed a highly tailed distribution that could be normalized using a cube root transformation (**Supplementary Figure 1**). To ensure validity of parametric statistical tests and distance metrics, a cubed root transform was applied to all "interaction time" measures before performing statistical tests.

The primary dependent variable in the present study was interaction variability, though other measures were necessary to fully characterize behavior and are discussed further below. Since "interaction variability" is loosely defined, multiple measures were used that separately examined variability of behavior between and within sessions.

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 session time that each animal spent using a particular type of interactive behavior during a session. Types of interactions included 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, since face-to-face contact is at least partly an interaction performed mutually by both degus, the two face-to-face values were combined, yielding a vector of 9 elements. We then computed the distance between interaction vectors. For simplicity, we chose to use Euclidean distance on the cube-root transformed vectors. To account for the effect of total interaction levels, we also applied Euclidean distance on normalized interaction vectors (computed by dividing by the vector sum). Both within-dyad and between-dyad interaction vector distances were computed.

Dyad classification success was a secondary measure of interaction variability. This made use of a binary, support vector machine (SVM) classifier (implemented using Matlab's

fitcsvm method). SVM classifiers work 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, and classification is made on the remaining, repeated for all vectors from the two data sets. 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. Initially, both linear and radial basis function kernels were used; however, we found the latter to be susceptible to ceiling effects that poorly discriminated between conditions. Only results using a linear kernel are reported (i.e., only linear combinations of interaction vector elements were used for classification).

Within-session variability was estimated using distances between time- and count-based interaction vectors.

Time-based interaction vectors were computed in the same way as is described above for estimating between-session variability, except using time bins within a session rather than whole-sessions. 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. A regression was used to establish overall differences across bins, then follow-up analyses selected one bin from this range.

Count-based interaction vectors were computed by finding the number of interactions of each type within a block of interactions. For example: when using 10 interaction block sizes, the vector described how many of 10 sequential interactions were rear-sniffing events performed by degu A, allogrooming events performed by degu B, face-to-face bouts, etc. As with interaction vector distances, we had no a priori estimation of how many interactions should be considered within a vector, and so the tests were again run 30 times, using a range of 1 to 30 interactions per vector. Once again, a regression was used to establish overall differences across bins, and then follow-up analyses selected one bin from this

range. Notably, some sessions lack sufficiently high numbers of interactions, but by the same token within-session interaction variability is only meaningful to measure in sessions with many interactions. The number of sessions without sufficient interaction numbers for each block size is reported with analyses.

Variability in the temporal structure of interactions was estimated by Local Variance of intervals within interaction types, and by transition matrices 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 = $1/(n-1)\sum_{i=1}^{n-1} 3(T_i - T_{i+1})^2/(T_i + T_{i+1})^2$

Note that the spacing of stochastic events should fall into a Poisson or gamma distribution (Lienhard & Meyer, 1967; Ord, 1967), but empirical evaluation of the IBIs indicate distributions conformed more closely to an exponential (**Supplementary Figure 2A-C**). IBIs could represent a significant portion of the session time, so an increase in the number of events, even if stochastic, cause overall reductions in variance by splitting the session into more intervals of necessarily more similar lengths. While the variance of an exponential can be calculated as the square of the mean, LV had the advantage that the relationship with event numbers was not as strong (**Supplementary Figure 2D & E**).

Transition matrices between interaction types described the probability that an interaction of one type would lead to an interaction of another type within a 5 s period. Separate, independent transition matrices were computed for interactions performed by the same individual and those performed by the other individual; i.e., if one animal initiates a rear-sniffing behavior, the “self” transition matrix estimated the probability that the same animal would subsequently perform a body-sniffing, allogrooming, or another behavior, while the “other” transition matrix estimated the probability that the other individual would subsequently initiate an interaction of one type or another. Transition matrix probabilities

were not normalized by chance-expected probabilities, given the baseline levels of interaction; however, follow-up analyses using cross correlograms did take these into account. More complex transition analysis, which described the probability of interaction based on the joint behavior of both animals, were not presently investigated. 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. Finally, for the purposes of the present analyses, transition matrices did not include probabilities of “no-interaction” (including absence of interaction during a 5 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 first eigenvalue of the transition matrix was operationally used to estimate overall strength (magnitude and width) of the distribution of transition probabilities.

Cross correlations allowed more precise examination of temporal relationships between interactions. 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 was 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. 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.

Interaction time (time budget analysis) was also used as a descriptive measure.

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. In calculating interaction time, it was considered that during a given moment, interactive behavior may be performed by either one or both degus, so if summed together the values could, potentially, exceed total session time. To avoid this, the interaction time measure considered only time during which either one or the other degu

was interacting (i.e., the union of time, rather than the sum). As noted above, for statistical comparisons interaction time was always transformed by computing the cube root.

Statistics

Null-hypothesis significance testing was used to evaluate differences between cagemate and strangers, and assess changes over sessions. Use of ANOVA and Student's t-test were only done after data were inspected visually and recognized to approximate gaussian distributions. Although multiple tests were performed, most were directed at independent questions; however, in several cases multiple comparisons had to be taken into account and corrected for. This includes when testing differences between strangers and cagemates across each individual interaction type (5 types tested), and when testing significance of temporal relationships between interactions (20 types tested). In theses cases, significance values are corrected using the Benjamini and Hochberg false discovery rate procedure (Benjamini & Hochberg, 1995).

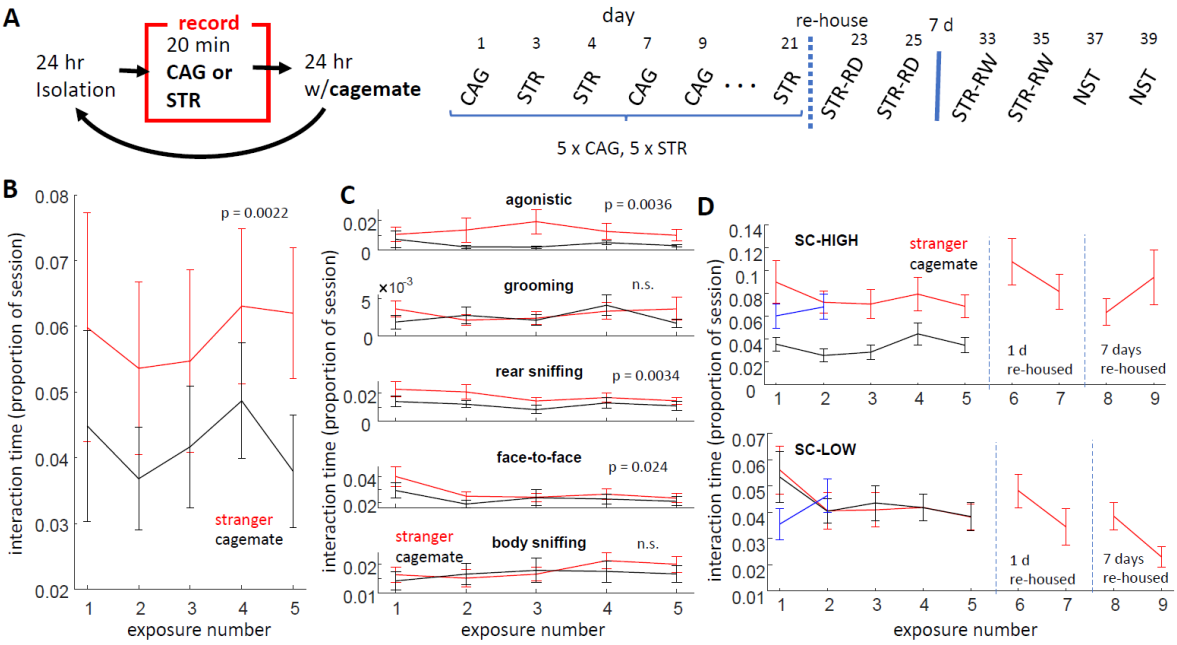
Ethical Note

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.

Results

Part 1: 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 evaluate how interaction levels changed as familiarity increased. Degus were separated from a cagemate for 24 hours and then reunited with either a cagemate or stranger for 20 minutes while video was collected. This was repeated 5 times each for cagemates and strangers in an interleaved way, counterbalancing the order (e.g., stranger-first versus cagemate-first, **Figure 1A**) between dyads. As predicted, strangers interacted more than cagemates (2-way ANOVA, effect of stranger/cagemate: $F(1,250) = 9.61$, $p = 0.0022$, effect of day: $F(4,250) = 0.51$, $p = 0.73$, stranger/cagemate x day: $F(4,250) = 0.11$, $p = 0.98$; **Figure 1B**). With respect to individual types of interactive behaviors, differences were significantly higher in strangers (false discovery rate correction) for agonistic ($F(1,250) = 8.64$, $p = 0.0036$), rear-sniffing ($F(1,250) = 8.75$, $p = 0.0034$), and face-to-face activity ($F(1,250) = 5.14$, $p = 0.024$; **Figure 1C**). The results replicated data from our previous study (Lidhar et al., 2021).



<u>Figure 1. Levels of social interaction over days.</u> A) Experimental design. Left-side depicts the repeated sequence of 24 hr isolation, 20 minute reunion recording sessions, and 24 hr co-habitation with cagemates. CAG = cagemate reunion, STR = stranger. Right-side

illustrates a sequence of recording sessions over days, interleaving cagemate and stranger reunions. Following 5 cagemate and 5 stranger reunions, degus were housed with the initial strangers for one day (STR-RD). Following two, post-rehousing recording sessions, the rehoused animals were kept together for 7 days, after which 2 more sessions were recorded. Finally, two recording sessions were performed with new stranger pairings. B) Total interaction levels for both stranger (red) and cagemate (black) dyads over the 5 initial days, p-value at the top reflects overall stranger-cagemate differences (re-pairing and new stranger sessions included in panel D, since not all dyads were recorded for all sessions). C) Breakdown of interaction levels across different types of interactions. Higher levels of agonistic, rear-sniffing, and face-to-face levels were observed in strangers (as shown by p-values). Changes across days were not significant. D) 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 day 1 through 5 strangers.

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 x 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 cagemte 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, distributed across 12 cagemate and 15 stranger dyads, and the remaining 36 degus (70%) as SC-LOW over (14 cagemate and 11 stranger dyads had only SC-LOW degus).

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, first, examining interaction levels over the 5 days of exposures and, second, by assessing changes after strangers were co-housed for either 1 day or 1 week ("new cagemates"). To control for internal validity threats, such as, experience with the

418 protocol itself, we performed a final set of 1 or 2 reunions in which degus were exposed to a
419 new stranger.
420
421 In contrast with predictions, we observed no effect of day nor an interaction between the
422 stranger/cagemate and day ($p >> 0.05$, reported above). This was true for total interaction
423 time and across all five types of interactions. To follow-up on this result, we created
424 separate regression models for SC-HIGH and SC-LOW degus. Among SC-HIGH degus, there
425 was, by design, an effect of stranger versus cagemate on interaction levels
426 ($beta_{stranger/cagemate} = 0.15$, 95% CI = 0.08 to 0.22); however, we observed no change over
427 days either when the strangers and cagemates were combined ($\beta_{day} = 0.0096$, CI = -0.0049
428 0.024) or considered separately ($\beta_{days\ x\ stranger/cagemate} = -0.014$, 95% CI = -0.034 to 0.007). In
429 the SC-LOW group, no effect of stranger/cagemate, day, nor interaction of
430 stranger/cagemate and day, were observed (95% CI = -0.047 to 0.048, -0.015 to 0.005, and -
431 0.014 to 0.014 respectively; **Figure 1D**).
432
433 One reason interaction levels may have stayed high in strangers is that the 5, 20 minute
434 reunion epochs provided insufficient time for partners to become fully familiarized. In 20
435 dyads, initial strangers were subsequently co-housed for 1 day and then tested twice in the
436 reunion; in 14 of these dyads, strangers were further co-housed for 1 week and again tested
437 twice. Surprisingly, interactions between SC-HIGH strangers remained higher than
438 cagemates following 24 hrs of co-housing (paired t-test, averaging all cagemate sessions and
439 both 24-hr co-housed stranger sessions, $t_8 = 4.86$, $p = 0.0013$) and following the 7 days of co-
440 housing ($t_6 = 2.50$, $p = 0.047$). Correspondingly, no significant differences were detected
441 between interaction levels in strangers before compared with after the one day and one
442 week of co-housing ($t_8 = 0.50$, $p = 0.63$ and $t_6 = 0.85$, $p = 0.43$). When degus were introduced
443 to new strangers after all other reunion sessions were complete, the SC-HIGH animals still
444 exhibited higher interactions compared with when interacting with cagemates ($t_{15} = 4.23$, p
445 $= 7.22 \times 10^{-4}$); thus higher interaction levels with strangers was not due only to the specific
446 stranger pairings.
447
448 In summary, strangers exhibited higher between-dyad diversity of interaction levels, largely
449 due to approximately 30% of female degus greatly increasing their levels of agonistic and

investigative 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), 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).

Part 2: 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 begin testing 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 (**Figure 2A**). “Interaction vector”, i.e., the dyad’s time budget, was the nine-element vector describing levels of agonistic, allogrooming, rear-sniffing, body-sniffing, and face-to-face in each degu. Interaction vector distances estimated differences in the time budget across sessions within and between dyads. Classification success estimated evaluated whether dyads could be discriminated from one-another, by measuring the success rates of SVM classifiers trained and cross-validated on pairs of dyads. 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 2B**.

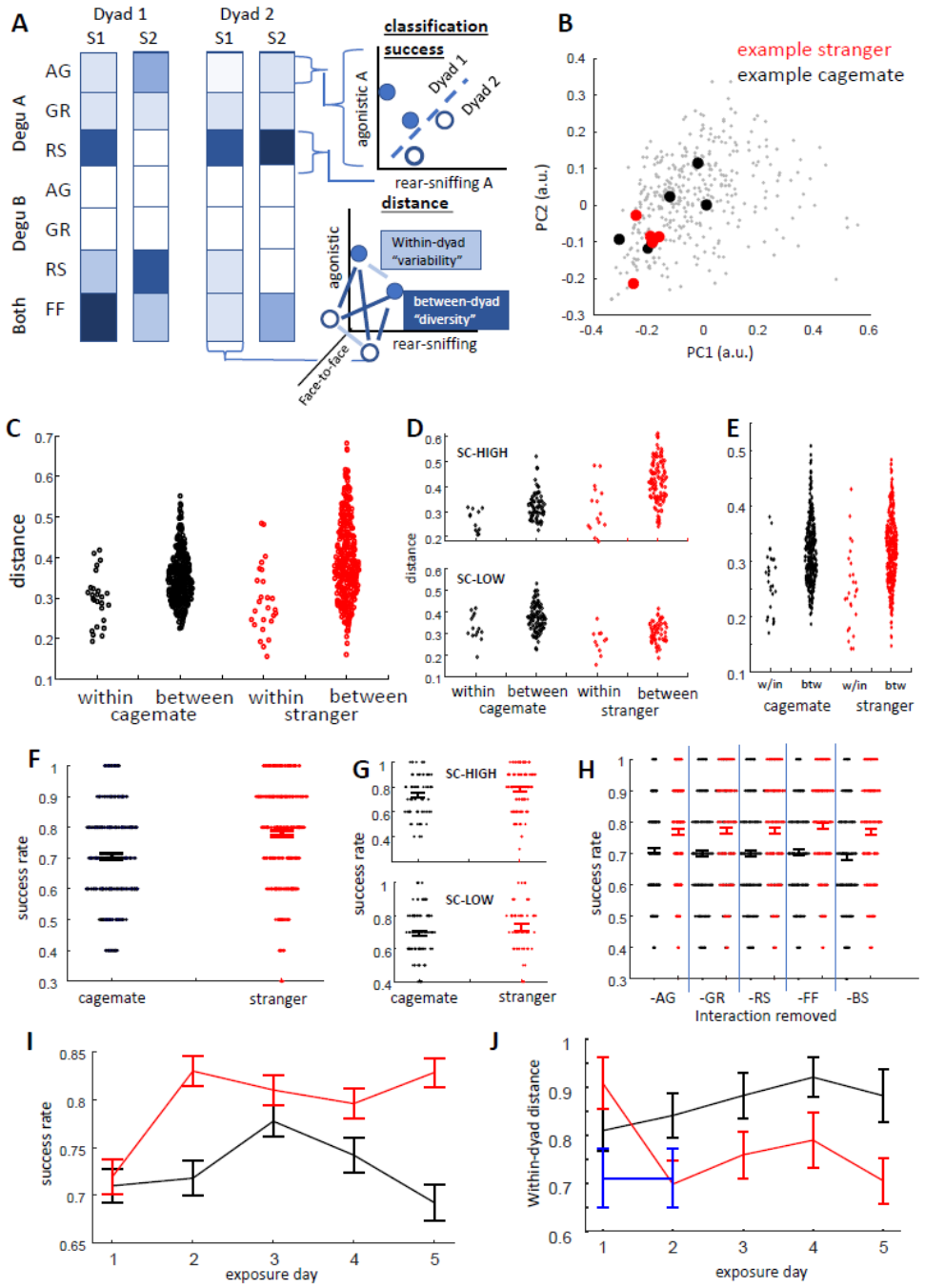


Figure 2. Variability and diversity across sessions. A) Diagram illustrating analysis methods. Each column of boxes represents the interaction vector from a single session (AG = levels of agonistic behavior, GR grooming, RS rear-sniffing, FF face-to-face, BS, body-sniffing, is left off). To the right of the vectors, vectors are plotted in 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 place 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 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 intriguingly dyad variability is particularly low among SC-LOW dyads. E) Removing agonistic interactions appeared to eliminate differences between strangers and cagemates. F) Success rate of SVM classifiers discriminating pairs of dyads among cagemates and strangers. 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 across sessions ±SEM. Pattern across days parallels that seen in classification success.

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 (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 x within/between-dyads: $F(1,698) = 3.39$, $p = 0.066$; **Figure 2C**). To control for non-specific effects, such as increased levels of activity in stranger dyads, we compared the ANOVA model model with those in two sets of shuffled-control datasets. One set of 1000 shuffled datasets was created by reassigning the interaction type category randomly in each session, a control 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 F statistic of within- versus between-dyad difference in the original model far exceeded the 99.9 percentile levels in the control cases. Similarly, the effect was preserved after normalizing interaction vectors prior to computing distances (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 x within/between: $F(1,698) = 1.14$, $p = 0.29$). The difference between within- and between-dyad distances could also be found in both the 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: $F(1,167) = 27.64$, $p = 4.43$; within/between: $F(1,167) = 12.76$, $p = 4.6 \times 10^{-4}$, interaction: $F(1,167) = 0.013$, $p = 0.91$; **Figure 2D**).

While both cagemates and strangers showed dyad-specificity, there was evidence that specificity was stronger, overall, in strangers compared with cagemates. This was initially hinted at by the statistical trend for differentially higher, between-dyad distances in strangers (ANOVA reported above, $p = 0.066$). Power in this test was weak, however, due to the very high variance in between-dyad distances (two-sample F-test between stranger and cagemate, between-dyad distances: $F(324,324) = 0.46$, $p = 3.75 \times 10^{-12}$). As illustrated by the statistical tests reported above, between-dyad diversity could be largely attributed to the higher overall levels of interaction in some degus (SC-HIGH). It is worth noting, however, that when distance measures were computed from normalized vectors, variability in stranger interactions decreased, preserving a trend for lower ratios of within versus between-dyad distances (non-normalized ratio of within and between: 0.85 cagemate, 0.77 stranger, two-sample t-test $t(49) = 1.9$, $p = 0.066$; normalized ratio of within and between: 0.84 cagemate, 0.74 stranger, $t(49) = 1.75$, $p = 0.086$). Higher diversity in strangers depended on differing levels of agonistic interactions between the dyads, as cagemate-stranger differences were lost when agonistic interactions were removed (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 2E**). Interaction vector distances therefore showed that strangers may have higher between-dyad diversity, primarily due to differences in levels of agonistic behavior, but not higher within-dyad variability when compared with cagemates.

Supervised learning methods reinforced the finding that dyads could be distinguished from one-another by their behavioral patterns, and revealed that strangers were more distinguishable than cagemates even beyond differing agonistic levels. For every pair of

561 dyads, a linear kernel SVM model was trained to discriminate the five sessions of one pair
562 with those of the other. The mean success rates for distinguishing dyads exceeded chance
563 for both cagemates and strangers (the binomial probability distribution predicts roughly 5%
564 probability of success classifying at least 7 of 10 sessions, mean rates for cagemates = 7.1,
565 mean for strangers = 7.8; **Figure 2F**). Differences between cagemates and strangers were
566 statistically significant (two-sample t-test, $t(648) = 5.84$, $p = 8.4 \times 10^{-9}$). To confirm that total
567 interaction levels were not affecting results, the method was repeated using normalized
568 interaction vectors (of 10 sessions, mean success in cagemates = 6.8, strangers = 7.5, $t(648)$
569 $= 6.5$, $p = 1.4 \times 10^{-10}$). Both stranger and cagemate classification success appeared to be
570 lower in dyads with only SC-LOW degus, though overall differences between strangers and
571 cagemates were still apparent (2-way ANOVA, cagemate/stranger: $F(1,313) = 5.43$, $p =$
572 0.020, SC-HIGH vs. SC-LOW: $F(1,313) = 6.45$, $p = 0.012$, cagemate/stranger x SC-HIGH/LOW:
573 $F(1,313) = 0.11$, $p = 0.74$; **Figure 2G**). Furthermore, in no case did removal of any one type of
574 interaction, including agonistic behaviors, result in a loss of cagemate versus stranger
575 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,
576 allogrooming, rear-sniffing, face-to-face, and body-sniffing were removed respectively;
577 **Figure 2H**). Overall, classification results confirmed that dyads had distinguishable
578 behavioral patterns from one session to the next, and also revealed that differences
579 between dyads tended to be stronger in strangers.
580
581 The higher discriminability of strangers relative to cagemates did not attenuate over days; in
582 fact, these differences were only apparent after day 1. This was evident by the cross
583 validation success rates when the "leave-one-out" algorithm was required to classify a first-
584 exposure session compared with when it classified day 2, 3, 4, or 5 sessions. Classification
585 success between stranger dyads was lowest on day 1, matching the generally lower values
586 of cagemates (**Figure 2I**). Evidence that strangers exhibited differential changes between
587 day 1 and 2 was also hinted at by within-dyad interaction vector distances. For each
588 exposure day, the median difference from all other exposure days was computed for that
589 dyad. Medians were found to be overall lower in strangers (2-way ANOVA, $F(1,250) = 4.21$, p
590 $= 0.041$; **Figure 2J**) while no significance differences were observed across days ($F(4,250) =$
591 1.02, $p = 0.40$, interaction between stranger/cagemate and day: $F(4,250) = 0.79$, $p = 0.53$).
592 However, averages exhibited patterns consistent with those observed with classification

success, with strangers initially showing higher within-dyad variance which subsequently dropped to low levels, while distances in cagemates remained relatively higher.

In summary, individual dyads interacted in ways that were relatively consistent across sessions, with stranger dyads being even more distinguishable from one-another than cagemates. The exception to this was during the first exposure, in which the behavior of strangers may have differed from behavior on subsequent days, making these sessions more difficult to classify. 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 less consistent social interactions.

Part 3: Within-session variability was higher in strangers on the first few days, but higher in cagemates on the last exposure day

Did strangers exhibit higher variability in the sets of interactions used over the course of a session? Two methods were used to examine this question, both involving a comparison of interaction vector distances. In the first, interaction vectors were computed for bins of session time. Rather than theorizing an “optimal” bin size, we examined the effect of bin size on within- versus between-dyad distances, and the effect of bin size on stranger-cagemate differences (from 20 to 600 s, multiple comparisons accounted for statistically as described in Methods). To decrease noise, all bins with zero interactions were removed, and to prevent overall interaction levels of from impacting results, all time-based interaction vectors were normalized by dividing by the sum. The second, count-based method generated interaction vectors based on the number of interactions, with each vector containing the same number of interactions. As with repeated testing of bin sizes, vectors with varying numbers of interactions were tested (block sizes of 1 to 30). The two methods differed in the meaning of “variance”: while time-based distances detected changes in interaction types used during different phases of a session (e.g., the first 5 minutes relative

to the final 5 minutes), count-based vector distances ignored time, reflecting changes between one series of interactions and another.

Differences in variability within sessions were observed between the stranger and cagemate groups using both the time-based and count-based interaction vector measures, though were generally stronger in the latter. An initial pass through the data revealed that the ratio of within- versus between-dyad distances reached a minimum of 0.81 using time-based vectors (with 500 s bins) and 0.62 (with 28-interaction blocks) using count-based vectors (**Supplementary Figure 4**). These values are comparable to the session-by-session ratios reported in Results Part 2. Stranger-cagemate differences could be observed across a wide range of bin sizes and interaction numbers, as demonstrated statistically using regression models that included both bin sizes (or number of interactions) and stranger versus cagemate terms (day 5 data, time-based vectors: β(stranger/cagemate x bin size) = -0.0053, CI: -0.0079 to -0.0026; count-based vectors, β(cagemate/stranger) = -0.040, CI: -0.078 to -0.002; **Supplementary Figure 5**). To examine these effects more closely, a bin size of 320 s was chosen for time-based vectors and block size of 11 interactions for count-based vectors. Time-based vectors revealed relatively minor differences between stranger and cagemate groups across days, the overall significant effects being primarily driven by an upward increase in cagemate variability on 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: $F(4,215) = 3.1$, $p = 0.017$; **Figure 3A**), combined with a tendency for lower variability in strangers on days 2, 4, and 5. Count-based vectors showed stronger differences between the groups across days, though much of the variance was still carried by changes taking place in cagemates (cagemate/stranger: $F(1,124) = 0.47$, $p = 0.50$, days: $F(4,124) = 1.82$, $p = 0.13$, cagemate/stranger x days: $F(4,124) = 4.55$, $p = 0.0018$; **Figure 3B**). Splitting dyads into SC-HIGH and SC-LOW groups revealed that the SC-HIGH group primarily carried the observed stranger/cagemate differences over days (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 3C & D**). All together, the data suggest that among sessions with at least 22 interactions (65% of all sessions), and particularly in those dyads that included the SC-HIGH degus, there was a tendency for interactions to be more variable in strangers during the first few social exposures, and then more variable in cagemates by the final exposure.

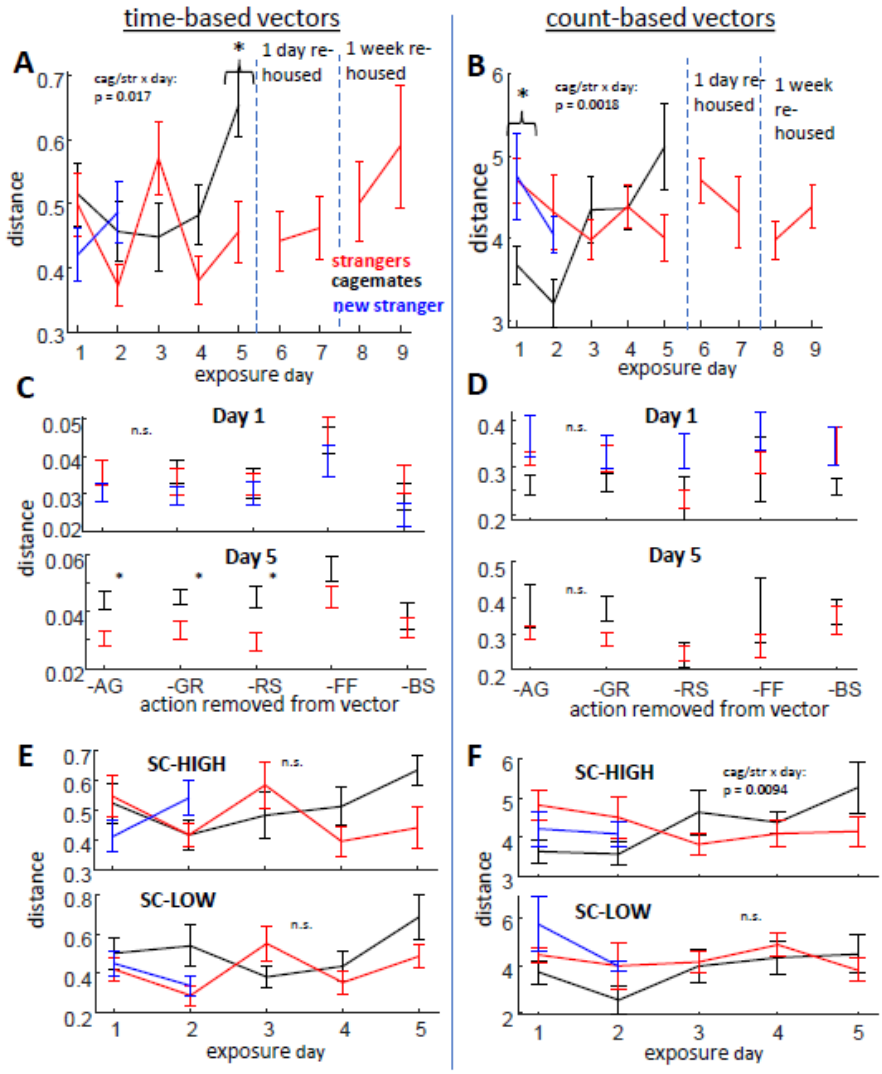


Figure 3. Within-session interaction variability. A) Mean interaction vector distances across days. Here, "time-based" interaction vectors were used, computed as the proportion of a time window that each animal spent using a particular interaction type (bin = 320 s). B) Mean interaction vector distances across days using "count-based" vectors, computed as the number of interactions of each type used by each animal across a set number (in this case, 11 interactions). Variability appeared to be higher in strangers on the first two days but subsequently decreased, while increasing in cagemates. C) Within-session vector distances computed after removing particular types of interactions to assess the relative contribution of those types to stranger-cagemate differences (AG agonistic, GR allogrooming, RS rear-sniffing, FF face-to-face, BS body sniffing). Face-to-face interactions appeared to have a stabilizing effect on bins over session time, the removal of which increased both cagemate and stranger variability and many differences between them. D) Same as part C, but for count-based interaction vectors. Rear-sniffing events appeared to increase variability and contributed to cagemate-stranger differences. E) Individual-differences in how much degus interacted with strangers (SC-HIGH versus SC-

LOW) did not appear to explain patterns across days using time-based vector distances; however, F) the SC-HIGH group did seem to carry the across-day changes when count-based distances were used, potentially suggesting that the higher levels of interactions helped resolve stranger relationships while causing cagemate interactions to be destabilized.

To examine which types of behaviors were involved in cagemate versus stranger variability differences, distances were re-calculated on interaction vectors in which specific interaction types had been removed (first vectors without agonistic interactions, then those without allogrooming, etc.). In time-based vectors, variability increased when face-to-face interactions were removed, and differences between cagemate and stranger dyads were lost in the absence of either face-to-face or body-sniffing (two-sample t-tests using false discovery rate correction for multiple comparisons, removing agonistic: $t(40)=2.94$, $p = 0.018$, allogrooming: $t(40) = 2.46$, $p = 0.031$, rear-sniffing: $t(41) = 2.82$, $p = 0.018$, face-to-face: $t(38) = 1.43$, $p = 0.20$, body-sniffing: $t(41) = 0.68$, $p = 0.50$; **Figure 3E**). This is most likely a reflection of degus spending more time using face-to-face and rear-sniffing behaviors throughout a recording session (cumulative distributions of interaction types are presented in **Supplementary Figure 5**). In count-based vectors, cagemate/stranger differences were lost with the removal of each interactive behavior (FDR-corrected p-values > 0.05). Reduced variability was particularly notable in cagemates when rear-sniffing bouts were removed (**Figure 3F**) suggesting that rear-sniffing events may have clustered together, increasing variability scores in strangers on early sessions, and on cagemates on later sessions.

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 3A & B**). These sessions did not significantly differ from either cagemate or stranger sessions before re-housing.

In summary, within-session variability, as measured by count-based vectors, appeared higher in strangers during the initial exposures, and higher in cagemates by day 5. This result was primarily carried by dyads with SC-HIGH degus, and differences were also sensitive to removal of individual interaction types.

Part 4:Temporal relationships between interactions did not strongly differ between strangers and cagemates.

To determine whether social novelty affected the temporal structure of interactions during a session, both the intervals between interactions of the same type, within the same individual, and the temporal relationships between interaction types, or between individuals, were examined.

Variability between interactions of the same type offered insight into how often each individual returned to a particular behavior. If face-to-face interactions, for example, are a way for the two individuals to “check-in” after a period of time had elapsed, these episodes 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 (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 >> 0.05; **Figure 5A**). The same was true when the analysis was repeated for only the first day of interaction.4Thus, 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 more consistent.

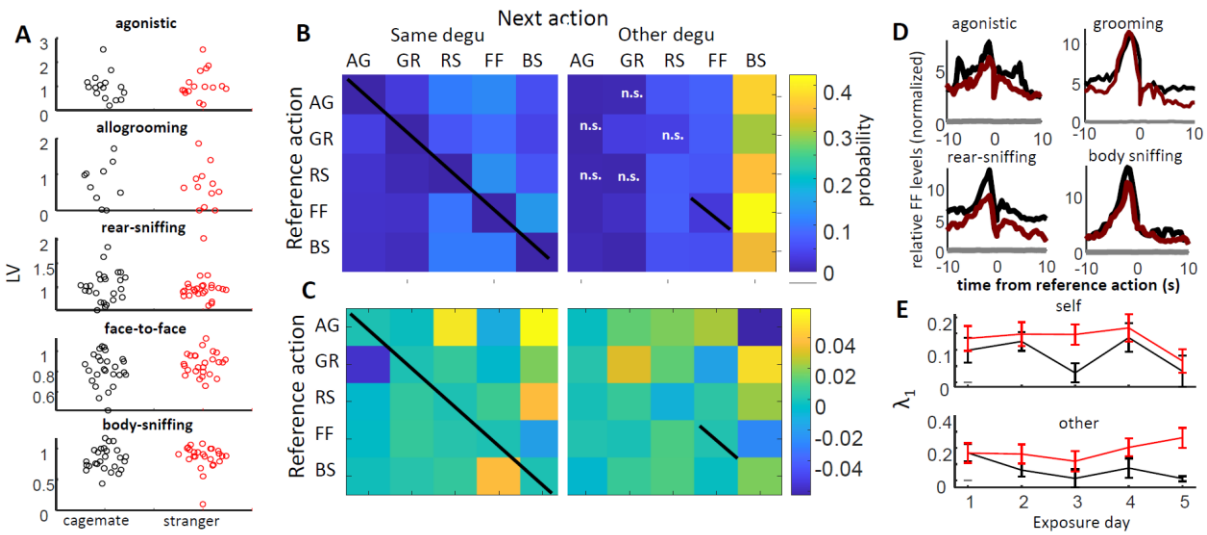


Figure 4. Variability in temporal relationships within and between interactions. A) Local Variance (LV) of IBIs was computed for each behavior, for each session. No differences were observed between cagemate and stranger groups. B) Transition matrix between interaction types, averaged across all degus. 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. C) The difference between the averaged transition matrices created for strangers and those for cagemates. Magnitude of differences were low (less than 0.06) and no difference was found to be statistically significant. D) Cross correlograms between interaction types further illustrate the absence of difference between cagemates and strangers. Face-to-face interaction level, normalized by total levels of face-to-face interaction, are shown relative to four different reference actions, in each case face-to-face was highly likely to precede other interaction types, but this did not differ between stranger and cagemates. E) The first eigenvalues of the transition matrices are averaged within stranger and cagemate groups to explore changes in transition matrices over exposure sessions. Higher levels in strangers can be traced to overall higher baseline probabilities of interaction types, apparent divergence between stranger and cagemate "other" transition matrices over days is inconsistent with the hypothesis that strangers become more like cagemates over days.

We next turned our attention to whether strangers were more variable with respect to the transitions between interactions. To first gain a sense of how different types of interactive behaviors were related to one-another, we computed transition matrices between interaction types for each session, separately considering transitions in the same animal and those between individuals (**Figure 4B,** upper panels). 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 5 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. To put another way: 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. Group differences were evaluated for each pair of interactions (2-sample t tests, $p >> 0.05$. For illustration, **Figure 4C** shows the magnitude of transition matrix differences after subtracting cagemate from strangers. It could be further illustrated and tested using the cross correlations, which also took into account baseline probabilities of each interaction type, and further showed that the timing of interactions relative to one-another was conserved 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 4D** (others in **Supplementary Figure 7**). Since transition matrix probabilities were not normalized by baseline interaction probabilities, some net increase of interaction probabilities should be apparent in the stranger transition matrices. We found that this was reflected in the first (highest) eigenvalue, computed for data exploration purposes. In cagemates, the first eigenvalue of the "other" transition matrices appeared to decrease over exposure sessions (**Figure 4E**). While transition matrix eigenvectors are difficult to interpret without additional, supporting analyses, the general pattern was not consistent with the hypothesis that strangers become more like cagemates with experience.

Discussion

We tested the hypothesis that adult female degus, known to be highly social and gregarious with strangers, become less variable and more consistent in their interactions as new relationships become more familiar. In a few ways, the results from the study are consistent with our initial hypothesis. In both strangers and cagemates, the relative levels of different types of behaviors (the time budgets) were more consistent within dyads than between dyads, which we interpret to reflect dyadic social relationships. As predicted, strangers showed more consistency in their day-to-day behavior after the first exposure day (**Figures 2 I&J**), and there was also evidence for higher within-session variability relative to cagemates on the first two days (measured using count-based vectors, comparing blocks of 10 or more interactions; **Figure 3B**). However, several unexpected patterns in the data suggested the story is more complex. The first unexpected results had to do with interaction levels. Increased interaction levels with strangers, which has been observed in female degus in

other studies (Insel et al., 2020; Lidhar et al., 2021; Villavicencio et al., 2009) did not attenuate to cagemate levels as social familiarity increased, even after a week of co-housing. This may suggest that, at least in our population of socially restricted animals, there are fundamental differences in new relationships formed by adults compared with conspecifics reared together. The second unexpected observation was the relatively higher levels of variability of behavior in cagemates, observed session-to-session and also, by the final session, within-sessions. This raises questions about whether degus were simply less constrained with cagemates, resulting in behaviors that depended more on day-to-day circumstantial factors. A third key unexpected observation, and perhaps the most salient message of the present study, was the generally high similarity observed between stranger and cagemate dyads—including an absence of statistical differences in the behavioral transition matrices. Although not initially anticipated, the high similarity between groups makes sense if the goal of interactions is to promote constructive, cooperative behavior.

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.

One question raised by the results is why higher levels of interaction between strangers—specifically those in the SC-HIGH group—did not attenuate with familiarity, even after one day and one week of co-housing. This was unexpected because we began with the premise that female degus are gregarious with unrelated and initially unfamiliar peers. This has been studied extensively both in and outside of the lab. In the wild, degu burrows are found near one-another, and animals of different burrows greet one-another with frequency (LuisA. Ebensperger et al., 2004; Fulk, 1976). Females also appear to communally nest with and nurse the pups of unrelated individuals (L. Ebensperger et al., 2002; L. A. Ebensperger et al., 2012, 2014; Hayes et al., 2009). In the lab, female degus show high sociality with strangers. They investigate unrelated conspecifics reared apart more than those reared together (Villavicencio et al., 2009). They engaging in more frequent rear-sniffing and agonistic behaviors with strangers compared with cagemates (Lidhar et al., 2021). And, when given a choice between cagemate and strangers during a 3 hour partner preference test, many female degus will spend most of their time with the strangers, even huddling with the new individual (a train that is unusual for laboratory rodents; Beery & Shambaugh, 2021; Insel et al., 2020). On closer look at the literature, however, there may be clues that degus do not necessarily form peer bonds as immediately as these data imply. Even in the wild, female degus have been reported to show preferences for members of their own burrow groups during daytime foraging and social greetings (LuisA. Ebensperger et al., 2004). And in the lab, genetic kinship appears to have some influence on behavior—just not as strong as familiarity (Villavicencio et al., 2009). The present study may be the first time that new social relationships in adult females of this species have been tracked over an extended timecourse, and the results suggest that while strangers may behave cooperatively, some of their relationships remain fundamentally different from those of cagemates reared

together. This lasts at least one week of co-housing, but could extend for months, years, or permanently. The results may be specific to the present dataset—given the specifical social history of the animals (most having experience exclusively with siblings) and lack of proximate ecological factors for group formation, like predator avoidance. Alternatively, it could be that there is a critical developmental window for forming the types of relationships observed in cagemates, suggesting that if degus become acquainted before reaching a particular age, their relationships have more potential to stabilize, in the form of reduced agonistic, face-to-face, and rear-sniffing behaviors, in adulthood.

One of the overarching hypotheses of this study was that stranger dyads would be more variable than cagemates from one day to the next, as relationships are established. There was some evidence supporting this hypothesis when looking within sessions: during the first two days, the composition of blocks of sequential interactions showed moderately higher variance in strangers on the first two days. More generally, however, cagemate behavior was more variable session-to-session and also, by the last exposure day, within sessions. There may not be a single reason why stranger dyads showed relatively higher behavioral stability. In SC-HIGH degus, a relative increase in investigative and agonistic behaviors may have driven more consistency in the overall structure of behavior, while SC-LOW degus may have been more wary of strangers, and therefore more deliberate and consistent in the specific interactive behaviors they engaged in. Cagemates, meanwhile, may have been relatively more comfortable and their behaviors may have been more subject to varying circumstantial variables, such as mood, ambient odors, etc. The lack of higher interaction variability in strangers is consistent with the idea that being unpredictable may be less conducive to forming cooperative relationships. Many primates use specific sets of vocal and physical interaction behaviors when encountering strangers, often in stereotyped and ritual sequences. The circumstances in which these behaviors are more likely to happen, such as between similar-ranking males, younger individuals greeting older, greetings with pregnant females, etc., suggest that the greetings function to reduce uncertainty and tension between the individuals involved (Aureli & Schaffner, 2007; Dias et al., 2008; Kutsukake et al., 2006; Smuts & Watanabe, 1990; Whitham & Maestripieri, 2003). To put another way, the consequence of increased consistence is increased predictability, which may help avert high-cost aggressive or competitive interactions. It is hypothesized,

therefore, that female degus are predisposed to show reduced variability with strangers due to their predisposition to form cooperative relationships with stranger peers. This predicts that the same patterns would not be true of adult male degus, which are not believed gain the same benefits from peer friendships and larger group structures (L. A. Ebensperger et al., 2016).

Temporal relationships within and between interactions did not appear to differ in any systematic way between cagemate and strangers. This was measured both by the variance in inter-behavior-intervals of the same type of interaction, and in the transition between interactions—within ("self" transitions) or between ("other" transitions) the two members of a dyad. The common structure of behavior in novel and familiar individuals may simply be due to the physics of interaction—e.g., degus attentive to one-another are more likely to encounter one-another's faces before body or tail. Or it may be due to the logic of investigative order—e.g., 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. Several papers have looked in detail at the effects of juvenile social isolation on adult social behavior 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)). Interestingly, in spite of many effects of early social deprivation on social motivation, types of interactions used, and tendencies toward aggression, sequencing between interactions has been found to be relatively resilient to early-life social isolation ((Van Den Berg et al., 1999)). Still, lack of early-life social experience may have more subtle effects on the timing of interactions that are used, not to mention vocalizations that are used (Keesom et al., 2017), that ultimately affect their social predictability, potentially 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.

While the present project focused its examination on social behavior and the development of social relationships in female degus, an underlying goal was to advance methods that

could generalize across species and behavioral measurement methods—relying on analyses that did not depend on the specific behaviors used by a particular animal or species. This paper is not unique in this effort, and joins a wider community of researchers applying standardized metrics to describing and analyzing animal relationships (e.g., see (Silk et al., 2013)). An important message of the present work is that even if distance metrics and classifiers can be used to examine social relationships, they can not be taken in isolation as an index of social relationship strength or history—after all long-term cagemates were, in fact, more variable than strangers. But the measures should be useful when complemented with other information about the animals being studied, or more hypothesis-directed choices on the specific set of ethogram behaviors being examined. While some hypotheses were confirmed, the construct of "social behavioral variance" may require further development to fully capture both the ways in which social exploration takes place, and the means by which relationships develop over a dyad's interaction history.

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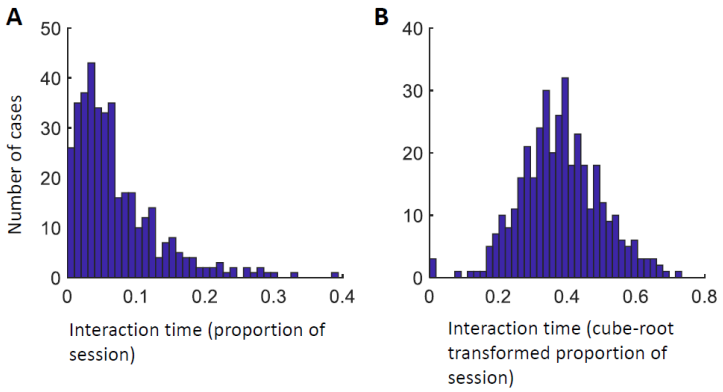
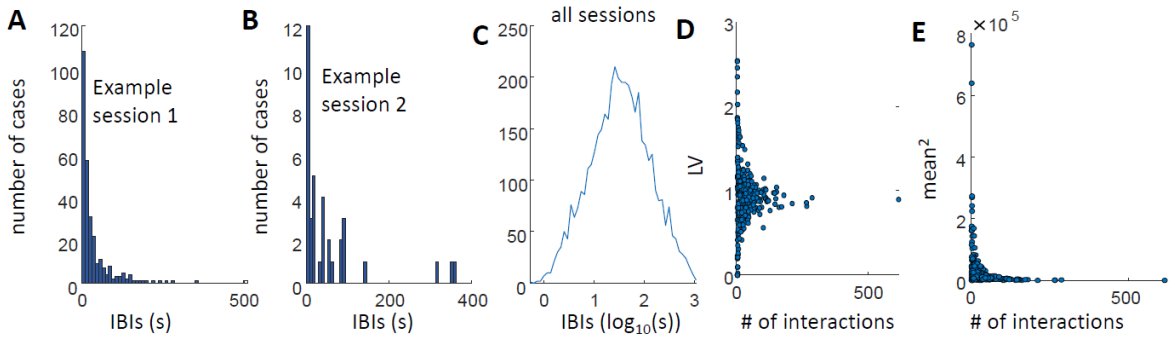
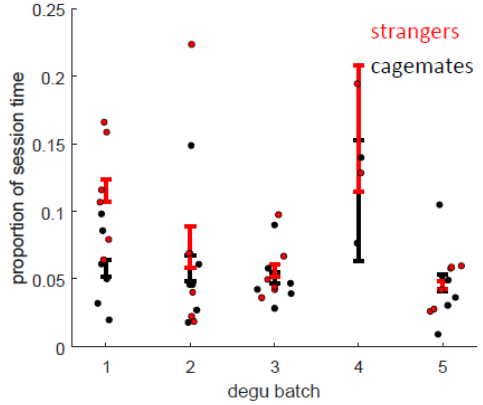
Supplementary Figures



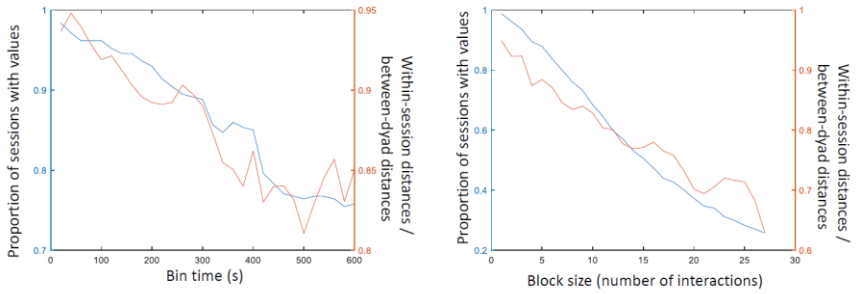
Supplementary Figure 1. Histograms of interaction time. A) Actual time spent interacting. B) Cube-root transformed interaction time. Interaction time was measured as a proportion of total session time, to account for slight variations in total time around 20 minutes. The transformation normalized data, aiding in analysis and interpretation.



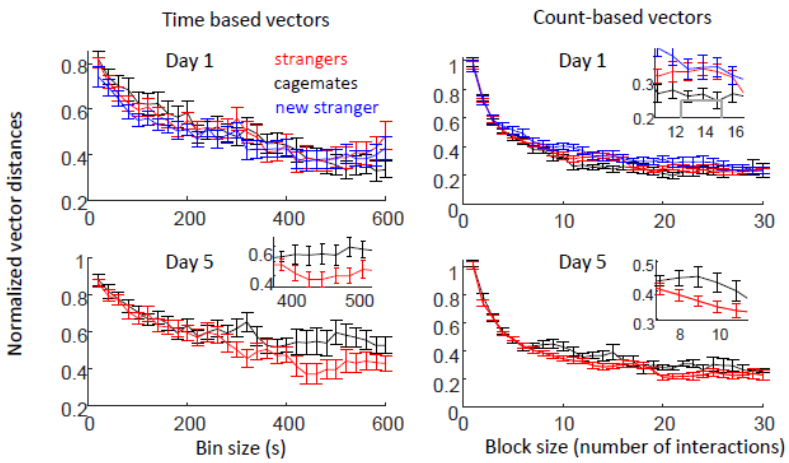
Supplementary Figure 2. Illustration of inter-behavior-intervals. A) Histogram of the intervals between face-to-face interactions in one example session. Distribution suggests the interval vary exponentially. B) Histogram of intervals between face-to-face interactions in another example session with far fewer interactions. Decreasing the number of interactions does not strongly impact the exponential fit of the curve. C) All inter- face-to-face intervals plotted on a log scale. Approximate gaussian distribution suggests that a log-normal fit. D) Local variance measure applied to all interaction types, across all sessions reveals that higher numbers of interactions results in smaller deviations from LV = 1. E) When computing the mean-squared of the inter-behavior intervals—the variance of the exponential distribution—a very strong relationship between number of interactions and variance is observed.



Supplementary Figure 3. Relationship between reunion “batch” across the 2 year experiment and the amount of interactions animals performed with strangers and cagemates. Differences in interaction levels may have been due to incidental variables such as experimenter, season, colony factors, etc.

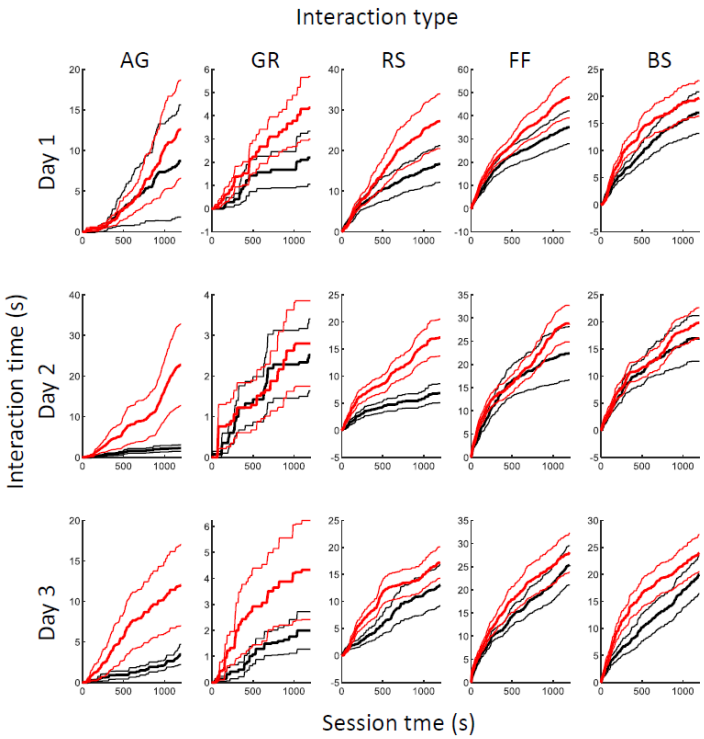


Supplementary Figure 4 Effects of varying bin size in time-based interaction vectors (left) and block size in count-based interaction vectors (right) on the number of total sessions with a minimum number of non-zero vectors (blue traces) as well as the ratio of within- versus between-dyad distances (red traces). Generally, increasing the bin and block sizes increased the dyad-specificity, but also decreased the number of valid sessions that could be used.



Supplementary Figure 5. Effects of varying bin size (left panels) and block size (right panels)

on stranger-cagemate differences. Differences between strangers and cagemates could be observed in time-based vectors on exposure day 5 (bottom) but not exposure day 1 (top) across bins approximately 300 s (5 min) and larger. Stranger-cagemate differences could also be observed using count-based vectors on day 1 for block sizes of at least 11 interactions, and day 5 for similar block sizes.



Supplementary Figure 6. Cumulative distributions of interaction levels over session time. Columns show five types of interactions, rows show exposure days 1, 3, and 5, traces show average plus or minus the standard error of interaction levels across 2 s bins of session time (stranger = red, cagemate = black). Distributions show a flattening-out during the second half of the session, indicating fewer interactions and driving-up time-based, within-session variance measures.