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Reciprocity in Dyads and Triads: Female Rats Alter Their Prosocial Behavior According to the Social Context

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Abstract – Recognizing and appropriately responding to others' helpful actions are critical components of social cognition and reciprocity. This ability has been well-documented in various species where animals differentiate between experimenters who are “unwilling” versus “unable” to provide help, but it is unclear if this ability extends to rats. The present studies investigate the prosocial decision-making behavior of female rats in response to the prior actions of another rat in both triadic and dyadic social contexts. In Experiment One, an "actor" rat repeatedly pressed a lever to open a restrainer door, enabling a trapped conspecific to access food. Consistent with reciprocity, when the roles were reversed, the previously trapped conspecific helped the actor. In Experiments Two and Three, the actor rat's ability to open the door was manipulated, with some trials where the door was blocked and the actor was unable to open it and some trials where the actor was able to, but rarely opened the door. In the triadic context, the previously trapped rat then had the opportunity to help the actor, a neutral control, both, or neither (Experiment Two). In the dyadic context, the previously trapped rat could then help the actor (Experiment Three). Reciprocity was *not* observed in the triadic context. However, unexpectedly, relative to a neutral control conspecific, rats showed a stronger preference for the less helpful actor compared to the helpful or unable actor. In contrast, in the dyadic context, rats *did* show reciprocity and displayed a higher propensity to help the helpful or unable actor compared to the less helpful actor. These findings shed light on rats' complex social behavior and highlight the context-dependent nature of their helping behavior.

Keywords – Rat, Reciprocity, Helping behavior, Social triads, Social decision making, Prosociality

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Prosocial behaviors, behaviors that benefit others, manifest in different forms across a range of species (Pfattheicher et al., 2022). Common examples of prosocial behavior include food sharing, consolation, alleviating the distress of another individual, enabling access to food, or assisting a conspecific in achieving a goal (Cronin, 2012).Variations of these behaviors have been seen in multiple species including humans, chimpanzees (Yamamoto et al., 2012), bonobos (Tan & Hare, 2013), vampire bats (Wilkinson, 1984), dolphins (Kuczaj et al., 2015), elephants (Douglas-Hamilton et al., 2006), and various rodent species (Burkett et al., 2016; Lalot et al., 2021; Wrighten & Hall, 2016). Although controversial (see Stevens & Hauser, 2004), behaviors that appear prosocial have been observed across multiple taxa; therefore, it is critical to apply careful experimentation to better understand the underlying biological, affective, and cognitive processes that facilitate such behaviors.

Given their natural sociality, group-living nature, and prevalence in research settings, rats are an ideal model organism with which to examine prosocial behavior (see Schweinfurth, 2020 for review). Moreover, a better understanding of their social and emotional cognition affords more refined models for use in biomedical and psychological research (Mogil, 2019), and increases our general understanding of social cognition across the animal kingdom. In naturalistic settings, rats recognize each other by odors and engage in multiple social behaviors such as joint huddling, food sharing, and allogrooming (Baenninger, 1970; Barnett, 1958; Barnett & Spencer, 1951; Gheusi et al., 1994; Schweinfurth, 2020). Various laboratory experiments have leveraged the natural sociability of rats to study their prosocial behavior. For example, rats will work to free a trapped rat from a restrainer, and this behavior is influenced by familiarity (Ben-Ami Bartal et al., 2011, 2014). Additionally, rats will open a door to help a soaked cagemate in distress escape from water (Sato et al., 2015). A distressed conspecific is not necessary to elicit prosocial behavior. For example, in a choice task, rats dependably make a prosocial choice that delivers food rewards to a partner rat (Hernandez-Lallement et al., 2015; Márquez et al., 2015). Although the motivational mechanisms underlying these behaviors are debated (see Blystad, 2021; Silberberg et al., 2014 for examples), in most cases, the acting rat does not directly benefit from this prosocial behavior. In rats and other species, prosocial behaviors can be costly; thus, there are multiple hypotheses to explain the prevalence of prosociality.

Notably, Trivers (1971) proposed the idea of reciprocal altruism. By this mechanism, individuals base their helpful decisions on past experiences and/or anticipated future interactions. For humans, this is ubiquitous and plays a critical role in the maintenance and stability of social systems (Fehr & Fischbacher, 2003; Gouldner, 1960; Trivers, 1971). Reciprocity has also been seen in various animal species, where it has been observed that individuals are more inclined to help or cooperate with others who have previously shown helpful behavior. Examples of such species include vampire bats (Wilkinson, 1984), parrots (Brucks & von Bayern, 2020), capybaras (Lalot et al., 2021), capuchin monkeys (Leimgruber et al., 2014), tamarin monkeys (Hauser et al., 2003), chimpanzees, bonobos (Jaeggi et al., 2013; Schino, 2007; Schino & Aureli, 2010), and rats (see Schweinfurth, 2020 for review).

Many studies investigating reciprocity in rats have focused on wild-type Norway rats (*Rattus norvegicus*) using a bar-pulling paradigm (Schweinfurth, 2020). In this experimental setup, one rat is placed in a chamber with a rope attached to a movable platform containing food, and a partner rat is in an adjacent chamber. The helpful rat can pull the rope which moves the platform and allows the partner rat to access the food. This paradigm has greatly contributed to our understanding of the intricate nature of rat reciprocity. For example, Rutte and Taborsky (2007) demonstrated generalized reciprocity (i.e., extending help to anyone after receiving help), where female Norway rats provided more food to a *novel* conspecific if they had previously received food from a *different* conspecific. Furthermore, rats exhibit direct reciprocity, showing a preference for previously helpful conspecifics over unhelpful individuals (Rutte & Taborsky, 2008). Kettler et al. (2021) used this setup to demonstrate rats' ability to remember previous interactions with different cooperative and non-cooperative partners, as the rats continued to help those who had previously cooperated. This finding suggests that rats can track the behavior of multiple partners and appropriately respond based on their prior actions.

Further investigations using the bar-pulling paradigm have revealed various social factors that influence reciprocity in female Norway rats. These factors include the effort required to provide help (Schneeberger et al., 2012), the aggression displayed by the social partner (Dolivo & Taborsky, 2015a), the quality of help received in previous interactions (Dolivo & Taborsky, 2015b), the quality of social interactions (Stieger et al., 2017), the intensity of need displayed by the food recipient (Schweinfurth & Taborsky, 2018a), and the relatedness of the cooperating individuals (Schweinfurth & Taborsky, 2018c). Moreover, this paradigm has allowed researchers to uncover some underlying rules of rat reciprocity. For example, Schweinfurth et al. (2019) demonstrated that female Norway rats engage in both generalized and direct reciprocity, whereas males primarily display only direct reciprocity. Additionally, Schweinfurth and Taborsky (2020) found that rats base their helping behavior on their partner's most recent behavior rather than their overall prosocial behavior in prior interactions.

Multiple experiments have also employed the bar-pulling paradigm to explore the sensory cues involved in reciprocal helping in rats. Notably, rats do not require visual information to exhibit reciprocity (Dolivo & Taborsky, 2015a), but olfaction plays a crucial role in prosocial behavior, as olfactory cues from a social partner acting prosocially can cause other conspecifics to behave prosocially (Gerber et al., 2020). Furthermore, researchers have explored alternative explanations beyond reciprocity that could account for rats' helping behaviors using this paradigm. Schmid et al. (2017) found that reciprocity was not driven by positive associations with a social partner but rather by the partner's specific behaviors. In a more recent study, Engelhardt and Taborsky (2022) examined whether imitation, rather than reciprocity, could explain rats' helping behavior. They exposed subject rats to a helpful partner that provided food to the subject rat and a partner that only provided food to itself. Despite identical behavior from the partner in both conditions, the subject rats subsequently displayed higher levels of assistance towards the previously helpful partner, aligning with a reciprocity explanation.

Although reciprocity has predominantly been studied using the bar-pulling mechanism with Norway rats, multiple studies have explored this phenomenon with other paradigms and strains. For example, male Sprague-Dawley rats (*Rattus norvegicus domestica*) sustained cooperation in an iterated Prisoner's Dilemma paradigm (iPD) using a T-maze apparatus, and the physical proximity of the partner influenced their decisions (Simones, 2007). In other iPD paradigms, rats adjusted their strategies based on their opponent's strategy (Viana et al., 2010), exhibited fewer cooperative responses towards non-cooperative partners compared to cooperative ones (Wood et al., 2016), and were generally able to achieve high levels of cooperation, but their cooperation rates changed as the temptation to defect increased (Delmas et al., 2019). Additionally, female Norway rats reciprocate allogrooming, the licking and careful nibbling and grooming behavior of one individual by another conspecific, and will reciprocally exchange allogrooming for food and food for allogrooming (Schweinfurth et al., 2017; Schweinfurth & Taborsky, 2018b; Spruijt et al., 1992). Reciprocity has also been associated with rats' long-term health outcomes: reciprocal allogrooming during a brief stressor was correlated with tumor development, physiological stress responses to restraint, and longevity in female Sprague-Dawley rats (Yee et al., 2008).

Whereas there is ample evidence that rats engage in reciprocal helping behavior, the motivation behind this behavior is still unclear. It is uncertain whether rats reciprocate because they have received help or because of their partner's cooperative behavior. Schweinfurth (2021) aimed to explore this question by investigating whether Norway rats reciprocate helpful behavior based on a partner's attempted but failed help (i.e., "intentions" to help) using the bar-pulling paradigm. The rats experienced two conditions with the same partner: one in which the partner was "able" to move the platform and deliver food to the subject rat, and another in which the partner tried but was "unable" to help due to a visible piece of rope blocking the platform. Subsequently, the subject rat had the opportunity to help the partner, and the number of pulls was recorded. The data were compared to archival data where the same subject underwent the experiment with a non-helpful conspecific that did not attempt to pull the rope ("unwilling" partner). Schweinfurth (2021) found that rats provided more food to the partner when she had been “able” to help, but the rats did not differentiate between when the partner was “unable” or “unwilling,” suggesting that female Norway rats’ reciprocal helping is unaffected by others’ prior intentions. These results are in contrast to studies showing that various species including chimpanzees (Call et al., 2004), capuchin monkeys (Phillips et al., 2009), Tonkean macaques (Canteloup & Meunier, 2017), African gray parrots (Péron et al., 2010), horses (Trösch et al., 2020), and dogs (Völter et al., 2023) show an understanding of intentional actions by differentiating between experimenters who are “unwilling” versus “unable” to provide them with food. Whereas Schweinfurth (2021) suggests that rats do not reciprocate help based on a partner’s prior intentions, there are likely social and experimental contexts in which rats perceive and/or utilize others’ intentions.

Overall, our current understanding of reciprocity in rats has been limited to primarily dyadic interactions. Although Quinn et al. (2018) provided evidence that rats show reciprocal helping behavior when interacting with two robotic rats, few, if any, studies have explored rats’ reciprocal helping behaviors in triadic contexts with more than two rats present. However, rats are a group-living species; thus, individuals will likely affiliate themselves with some group members more than others (Massen et al., 2010). Therefore, in naturalistic contexts, if rats exhibit reciprocity, they should help a previously helpful partner more *relative* to other group members. From the vast empirical studies, it is evident that rats engage in reciprocal helping, but there is limited understanding of how reciprocity varies according to others' specific behaviors and the social context.

Here, we employed a novel paradigm and used female Sprague-Dawley rats to investigate reciprocity and the understanding of intentionality in rats across different social and decision-making contexts. We implemented a multi-phase procedure that utilized a three-chambered apparatus (Figure 1) that allowed rats in adjacent chambers to interact through a clear perforated barrier. In the first phase, the habituation phase, rats in the separate chambers could interact with each other and explore the apparatus. Next, in the experience phase, a restrainer was placed in one of the side chambers, and the subject rat was trapped inside. An "actor" rat in the center chamber could forcefully press a lever protruding into the center to open the restrainer and allow the subject rat to exit and get a food reward. Once the subject rat got her food, she went back into the restrainer, the door was closed, and the food was replaced. Then, the actor could press the lever to allow the subject rat to access the food again. In the subsequent phase, the test phase, the roles were reversed, and the subject rat was in the center while the actor rat was trapped. The propensity to help the actor rat was assessed with the amount of help that the subject rat subsequently provided.

Importantly, in this set-up, helping behavior was operationalized as pressing the lever to allow a trapped rat to access food. While the use of a restrainer follows Ben-Ami Bartal et al. (2011), the present design utilized a larger restrainer that aimed to minimize distress for the rats by allowing them to move about easily. Trapped rats did not display signs of high distress (e.g., defecation, prolonged immobility), and after getting their food reward, rats frequently returned to the restrainer, suggesting that the restrainer itself was not aversive and may have had reinforcing properties (Hachiga et al., 2020). Together, this bolsters the presumption that the helpful behavior, the lever pressing, was to provide access to the food, not relieve the distress of the trapped animal. Moreover, there was always a divider present, so the rats were not opening the restrainer in order to engage in direct social contact (see Hachiga et al., 2018; Heslin & Brown, 2021; Hiura et al., 2018; Silberberg et al., 2014) or enter the restrainer themselves (Hachiga et al., 2020).

We used this setup to investigate the impact of social context and the actor rat's initial behavior on reciprocal helping in rats. To change the social context, sometimes a third rat, known as the "neutral control" rat, was present during the habituation phase. The control rat was neutral with respect to helping behavior as she was not present during the experience phase, where helping did or did not occur. During the test phase, the neutral control rat was present again and trapped in a separate restrainer from the actor rat. The subject rat could help the actor, the control, both, or neither during the test phase. In this case, the subject rat's preference for the actor was evaluated by comparing the propensity to help the actor *relative* to the control. Additionally, the behavior of the actor rat varied in the experience phase. The actor either helped the subject rat multiple times (*repeat help* condition), pressed the lever once or avoided pressing (*rare press* condition), or repeatedly pressed the lever without the restrainer opening (*repeat attempt* condition).

In our first experiment, we validated the utility of a novel decision-making set-up using female Sprague-Dawley rats by performing a conceptual replication of the general finding that rats reciprocate help. This conceptual replication (Schmidt, 2009; Stroebe & Strack, 2014) strengthens the notion that rats exhibit reciprocity by showing that it is not contingent on specific variables or rat strains (Experiment One). Experiment Two had two primary aims: (1) to assess reciprocity in a triadic context by comparing the propensity to help a previously helpful conspecific versus a neutral control and (2) to examine how the helpful actions (or inactions) of a partner, regardless of success, influence the recipient's prosocial preferences. The actor in the experience phase exhibited one of three possible behaviors: repeatedly successfully helped (*repeat help* condition), rarely (0-1 times) helped (*rare press* condition), or repeatedly and unsuccessfully attempted to help (*repeat attempt* condition) the trapped subject rat (Experiment Two). In the final experiment, we explored reciprocity and the understanding of helpful actions by repeating the experimental protocol of Experiment Two but in a strictly dyadic context. We provided evidence that reciprocity in rats differs in triads and dyads (Experiment Three). By implementing a novel set-up with both dyadic and triadic contexts to investigate how rats differentially help a conspecific who repeatedly provided help, rarely provided help, or repeatedly attempted to provide help, this experiment sheds light on the complexity of rats' understanding of others' actions and highlights the importance of the social context in which rats are tested.

Experiment One:
Do Female Sprague-Dawley Rats Show Reciprocity in a Novel Decision-Making Setup?

The aim of Experiment One was to use a new experimental environment to conceptually replicate the general finding that rats show reciprocity. We further examined the robustness of this general finding by testing the extent to which this phenomenon is generalizable across rat strains and contexts. To assess this, we used a task wherein a free rat could press a lever to release a trapped rat from a large restrainer. We examined if female Sprague-Dawley rats demonstrate an increased propensity to help a partner that had previously been helpful, compared to one that had not.

Experiment One: Method

Ethics Statement

All animal procedures were performed in accordance with National Institutes of Health (NIH) and local Institutional Animal Care and Use (IACUC) ethical guidelines (UC San Diego Protocol Number: S04172) in an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International) accredited facility.

Subjects

Ten female Sprague-Dawley rats, Envigo (Harlan), took part in the experiment. Female rats were chosen based on their propensity to show both generalized and direct reciprocity in previous studies (Schweinfurth et al., 2019). The rats were approximately three months old at the start of testing and approximately five months old at the conclusion of data collection. Prior to testing, all animals were acclimated to the experimenters and handled daily for four weeks. The rats did not show signs of distress (e.g., abnormal weight fluctuations, porphyrin excretion, piloerection, or defecation/urination with handling). While the rats had the same date of birth, the researchers did not have access to information about their relatedness.

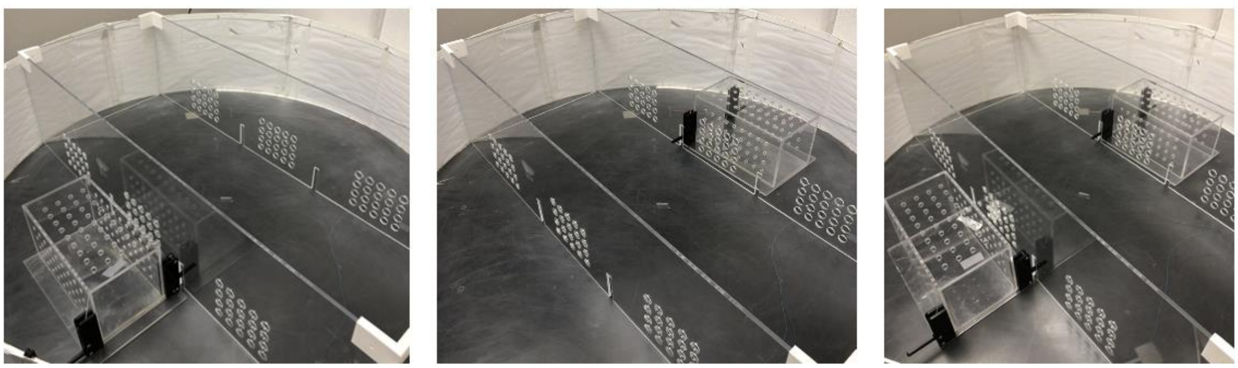
Rats were housed in pairs in individually ventilated 10.5 in X 19 in X 8 in high-temp polycarbonate cages. Each rat remained with the same cagemate for the duration of the experiment. The rats were numbered 1-10 for identification with each cage containing rats with adjacent numbers (e.g., EW1 housed with EW2, EW3 housed with EW4, etc.). Each cage contained Envigo 7099 TEK-Fresh laboratory animal bedding with a 4 in-diameter PVC pipe and a Nyla bone (Power Chew Durable Dog Chew Toy, x-small) for in-cage enrichment. The vivarium was maintained on a 12-hr light/dark cycle at 68-72 degrees Fahrenheit and 30-70% humidity. Testing occurred during the light cycle. Animals were food restricted to 25 g rat chow (Envigo Teklad 22/5 Rodent Diet) per cage per day, and the rats were weighed weekly to ensure no significant changes in weight. Animals were fed at the same time daily. Water was provided *ad libitum*. At least twice a week, each pair of cagemate rats were placed in a circular enriched environment (48 in diameter) for 20 min. The enriched environment contained toys such as a rodent running wheel, plastic enclosures, wooden chew toys, transparent plastic tubes, plastic balls, a wooden ladder, and other miscellaneous items. To provide greater levels of enrichment, the experimenters varied which toys were present and changed the placement of the items in the environment with each session. All experimenters were female.

Apparatus

The apparatus, shown in Figure 1, contained four walls made from 0.25 in clear acrylic. The two short walls served to stabilize the walls separating the central and outside chambers. The separating walls contained three sets of 15 1 in diameter holes to allow the rats in the apparatus to see, hear, and smell each other. In each side chamber, there was a restrainer measuring 6.02 in X 11.50 in X 6.02 in The dimensions of the restrainers allowed the rat to move freely inside. The restrainer walls also contained 36 holes on three sides to allow for visual, auditory, and olfactory information to be transmitted between a rat on the inside and a rat on the outside. Each restrainer had a lever that protruded into the center chamber. The restrainer door could be opened by forcefully pressing the lever (Video S1). Once the restrainer door was opened, the trapped rat could exit and get food placed ~ 6 in away.

Figure 1

Apparatus Used for the Helping Behavior



Note. A restrainer could be placed on either or both sides of the apparatus. The lever protruded into the center chamber.

Demonstration Phase

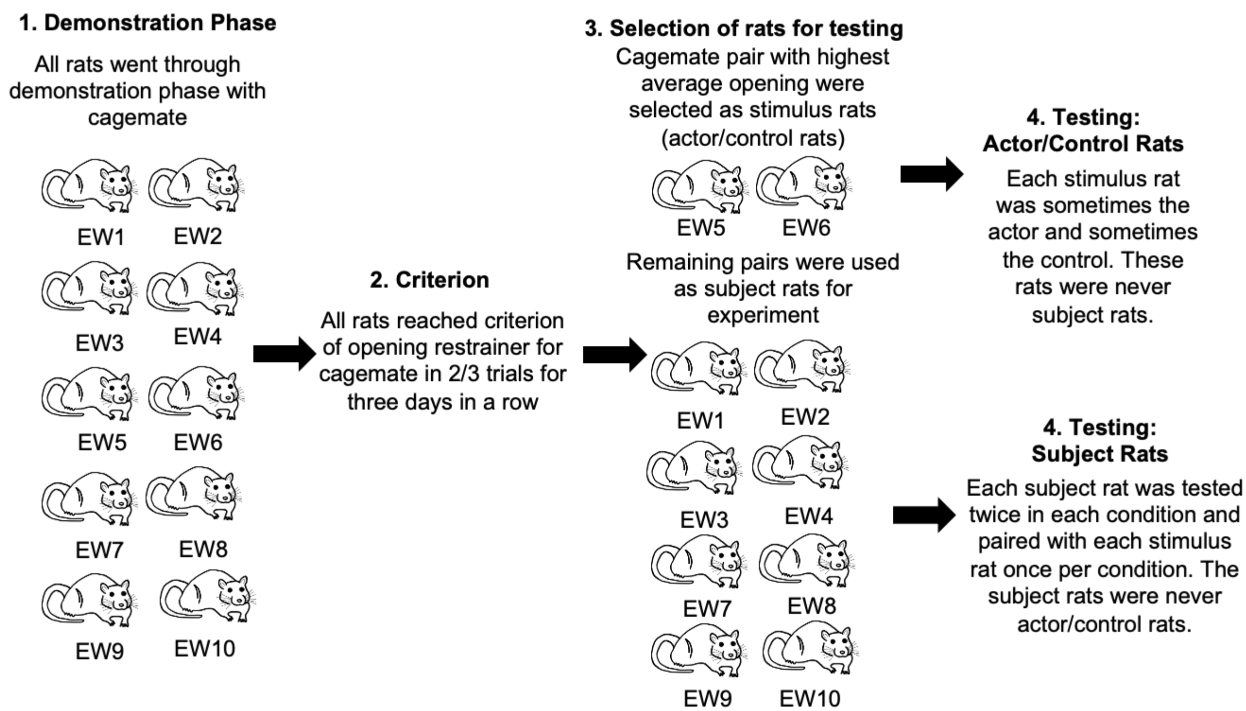
All ten rats first underwent a demonstration phase to ensure that they could operate the restrainer. All rats were paired with their cagemate, and the restrainers were always operable. To start, one cagemate was placed in a restrainer on one side, an empty restrainer was placed on the opposite side, and the other cagemate was placed in the center chamber. The rat in the center was allowed five minutes to open the restrainers. If she did not do so in the allotted time, the experimenter reached in and "showed" the rat how to open the cagemate's restrainer by poking the lever with a long stick. The experimenter's hands were not visible to the rat. Once the restrainer door was open, either by the experimenter after five minutes or by the rat in the center, the rat in the restrainer exited and obtained her food, an oat flake and mini chocolate chip, placed ~ 6 in. away. The rats remained in the apparatus for one more minute to increase familiarity with the testing environment. If the rat in the center opened the empty restrainer, the food reward was removed, and the trial continued. Importantly, the rat in the center was not directly reinforced for lever pressing as she never obtained food for herself by pressing.

The roles were then reversed such that the rat that had been in the restrainer was then placed in the center. Every day, each rat was in the restrainer three times and provided help three times. The side in which the restrainer with the cagemate was placed was counterbalanced across all trials and days of the demonstration phase. A rat was considered to have learned the task if she opened the restrainer two out of three times for three days in a row. All rats reached this criterion within six days.

At the end of the demonstration phase, one pair of cagemates was chosen to be the stimulus rats by acting as the actor/control rats. This pair exhibited the highest average opening behavior among all pairs, and *both* rats in the pair consistently opened the restrainer. During testing, the roles of the stimulus rats were alternated, such that sometimes a specific rat was the actor and sometimes that rat was the control. The stimulus rats were never subject rats. The remaining eight rats exclusively served as test subjects and were never the actor or control. Using cagemate rats as stimulus rats offered several advantages. First, testing a subject rat with her own cagemate as the actor/control could introduce bias due to increased familiarity. Therefore, using stimulus rats from the same cage allowed *all* the subject rats to be tested with *both* stimulus rats, maximizing the number of subjects. Second, due to olfactory familiarity from the cage placement in the vivarium, using cagemate rats as the stimulus rats also ensured that the subject rat had equal olfactory familiarity with both actor/control rats. The process of exposing the rats to the demonstration phase and subsequently selecting them as stimulus or subject rats is depicted in Figure 2.

Figure 2

Experience of the Rats and Selection to be Subject or Stimulus Rats



Note. All ten rats first went through the demonstration phase where they alternated between being trapped and opening the restrainer for her cagemate. Once all rats reached the criterion, the cagemate pair with the highest average opening where both rats opened a similar number of times was selected to be stimulus rats and serve as the helpful actor or neutral control rat depending on the condition. The remaining eight rats were used as subject rats and were each tested twice in each condition. Each subject rat was paired with each stimulus rat once per condition.

Experimental Design

We used a three-chambered apparatus (Figure 1) and implemented a three-phase protocol with a habituation, an experience, and a test phase (Figure 3). Rats were briefly returned to their home cage between phases. There were two conditions: the *helping* condition (Figure 3: column 1), and the *neutral control* condition (Figure 3: column 2).

For both conditions, each trial began with a two-minute habituation period where the subject rat could interact with an unfamiliar conspecific through a clear perforated barrier (Figure 3: 1A, 2A). The conditions differed in the next phase, the experience phase. In the experience phase of the first condition, the *helping* condition, the subject rat was placed in a restrainer on one side, and an empty restrainer was placed on the opposite side (Figure 3: 1B). The *helping* actor rat was placed in the center and could forcefully press a lever to open the restrainer to enable the subject rat to exit and get a food reward: an oat flake and mini chocolate chip. The subject rat was then placed back in the restrainer, and the food reward was replaced. If the *helping* actor opened the empty restrainer, the food was removed and replaced. Lever pressing of either or both restrainers occurred repeatedly over a seven-minute period. In the *neutral control* condition, the subject rat was placed in the restrainer for seven minutes, and an empty restrainer was placed on the opposite side, but there was no actor rat in the center chamber (Figure 3: 2B). At the end of the seven minutes, the experimenter opened the restrainer and allowed the subject rat to access the food. This ensured that differences in the subject rat's behavior across the conditions could not be attributed to receiving or not receiving a treat. Lastly, in the test phase, the actor rat (*helping* condition) or the control rat (*neutral control* condition) was placed in one restrainer, and an empty restrainer was placed in the opposite side. The subject rat could press the lever to open the restrainer for the actor/control (Figure 3: 1C and Figure 3: 2C, respectively) and/or the empty restrainer. Alternatively, the subject rat could abstain from pressing either lever.

The timestamp of every opening was recorded during the seven-minute timeframe. The subject rat was not constrained in the number or sequence of opening either restrainer. This meant that the subject rat could open one restrainer multiple times before opening the other, alternate between the two, or even refrain from opening either restrainer. This flexibility applied to both conditions, and the experimenter never provided reinforcement for pressing either lever.

We employed a within-subject design such that each subject rat went through both conditions twice (four trials total per rat). Each subject rat encountered each stimulus rat twice, one time as the *helpful* actor and one time as the *neutral control* conspecific. The order that the subject rat went through the conditions and which of the stimulus rats the subject was paired with was counterbalanced.

Statistical Analysis

All data analysis and visualization were carried out in R (version 4.3.0; R Core Team, 2023) within RStudio using the packages 'lme4' and 'ggplot2.'

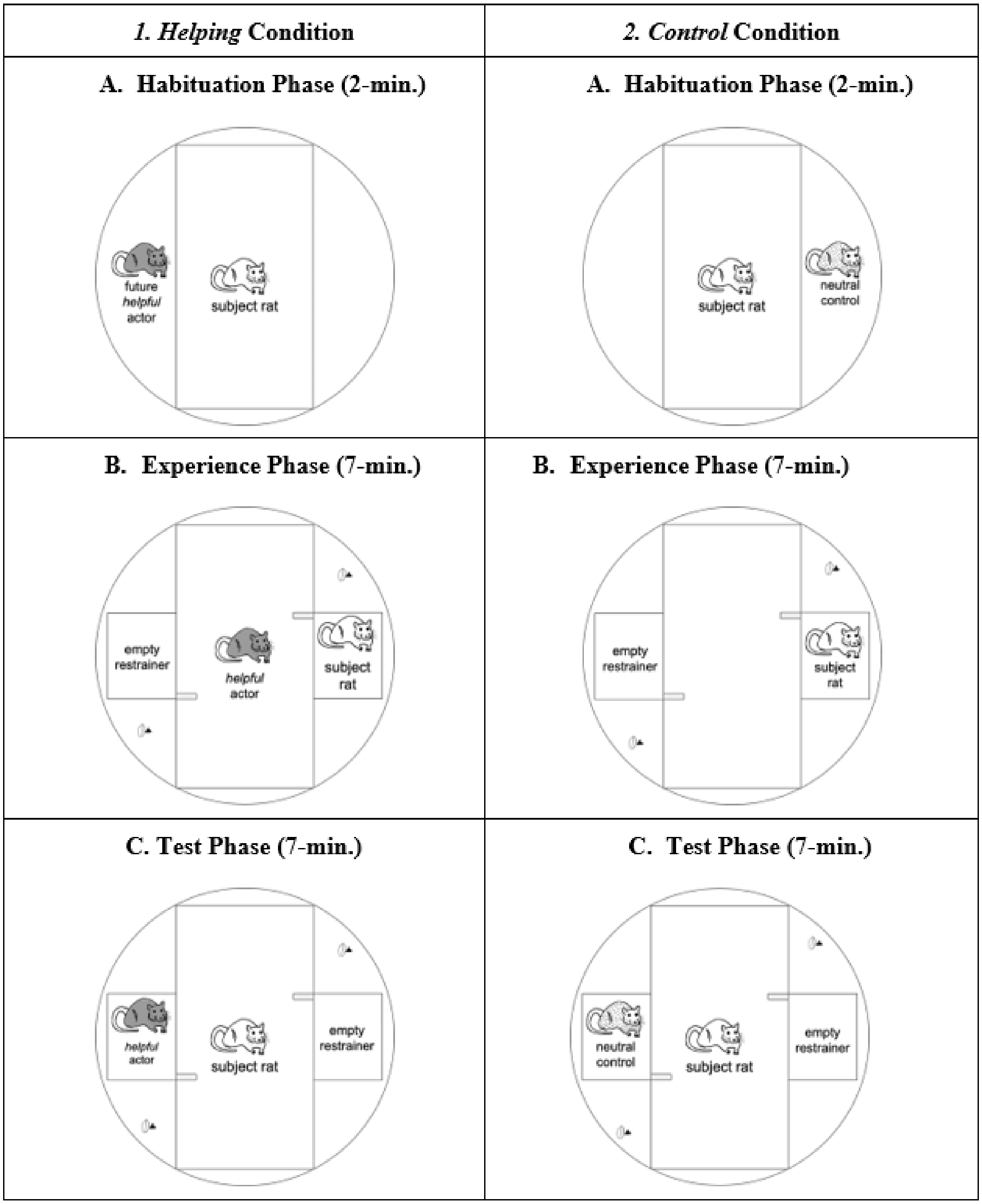
We operationally defined reciprocity as showing a stronger preference for helping the helpful actor compared to the neutral control conspecific during the test phase. This was assessed using the proportion of a subject rat's overall lever presses (within each trial) that were for the conspecific with the following formula:

$$\frac{\text{\# of lever presses for conspecific}}{\text{\# of lever presses for conspecific} + \text{\# of lever presses for empty restrainer}}$$

Using a linear mixed effects model (LMM), the above-defined proportion was then included as the dependent variable in a model with the condition as the independent variable and the test and actor rats' identities as random effects. If the rat did not open either restrainer, the proportion was entered as zero for analysis. In all the LMM analyses, to determine if a fixed effect was statistically significant, likelihood ratio tests (LRT) were performed to evaluate if a model with the fixed effect explained significantly more variance in the dependent variable than a null model with no fixed effect. Across all analyses, the alpha level for significance was set at .05. A p-value less than .10 was considered a trend, and post-hoc pairwise comparisons were conducted.

Figure 3

Order and Timing of Experimental Phases



Note. In each diagram above, the medium sized rectangles represent the restrainers with the lever sticking out into the center. The food reward, an oat flake and a mini chocolate chip, was placed ~6 in from the restrainer opening. The subject rat is shown in white, the actor is shown in gray, and the control rat is dotted.

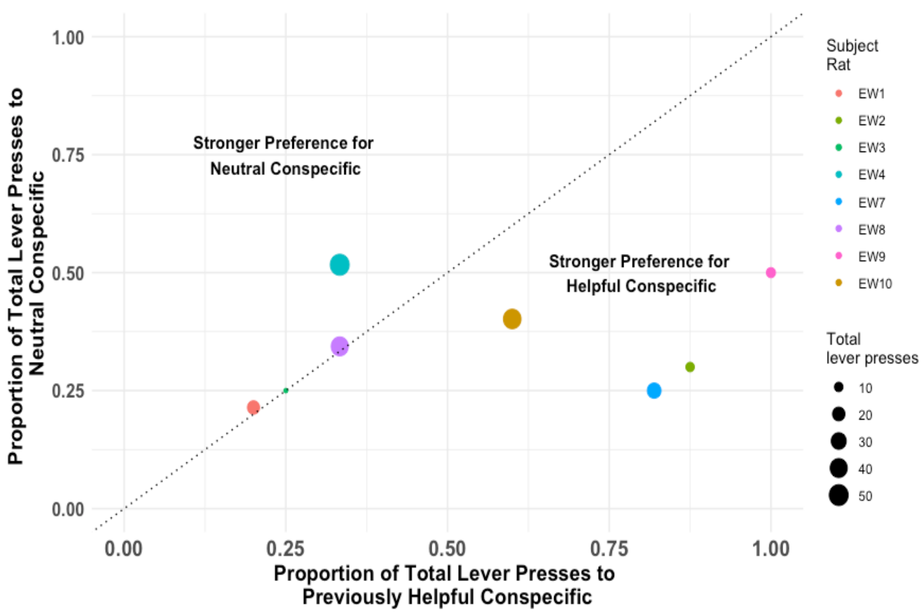
Experiment One: Results

As a metric of prosocial preference, we analyzed the proportion of a rat's total lever presses within a trial that were specifically directed to the conspecific. Overall, the rats showed a stronger preference for the previously helpful conspecific relative to the empty restrainer than the neutral conspecific compared to the empty restrainer (LRT: N = 8, X^2= 4.83, *p* = .03, 95% CI [0.03, 0.38], Figure 4). On average, with a previously helpful conspecific in one of the restrainers, 55.1% of the subject rats' lever presses were for the conspecific (number of lever presses for conspecific: *M* = 3.25, *SD* = 2.46). In contrast, in the neutral control condition, the rats directed an average of 34.7% of their help to the conspecific (number of lever presses for conspecific: *M* = 2.75, *SD* = 2.59).

Overall, relative to the empty restrainer, the rats showed a stronger preference for the helpful conspecific than the neutral conspecific, but there was a lot of individual variability in the subject rats' overall lever pressing and prosocial preferences. The total number of times the subject rats pressed the lever across the experiment ranged from 6-43 times total (*M* = 25.63, *SD* = 17.07). The total number of lever presses each subject rat performed is represented by the dot size in Figure 4. Based on the raw proportions, one subject rat showed a stronger preference for the neutral conspecific (Figure 4, above diagonal line), and three subject rats showed approximately equal preferences (Figure 4, on the diagonal line). Four of the eight subject rats showed a stronger preference for the helpful conspecific, and the strength of their preference (indicated by distance from the diagonal line) was higher than that of the single subject rat with a preference for the neutral conspecific (Figure 4, below diagonal line). On average, despite the individual variability, the relative preference for the conspecific was different across the two conditions. However, the *total* number of lever presses the rats performed was not significantly different across the two conditions (LRT: N = 8, X^2= 0.10, *p* = .75, 95% CI [-2.35, 3.23]; Total lever presses across all trials: *M* = 6.41, *SD* = 5.34).

Figure 4

Distribution of Lever Pressing



Note. Each point represents a single test rat. The size of each dot represents the total number of lever presses performed by the specific subject rat across the entire experiment. Larger dots indicate that the subject rat pressed the lever more frequently. On the x-axis is the average proportion of help that was directed to the previously helpful conspecific by each subject rat, while on the y-axis is the average proportion of help that was directed to the neutral conspecific by each subject rat. The diagonal line represents equal preference for the conspecific in both conditions, below the line indicates that the preference for the conspecific was higher when the conspecific had been helpful, and above the line indicates that the preference for the neutral conspecific was higher.

Experiment One: Discussion

The results of Experiment One show that in a decision-making context where the rat can repeatedly open a restrainer door for a conspecific or for an empty restrainer, the rats' preference for helping the conspecific was stronger when the conspecific had previously provided help. Whereas many of the rats' preferences did not show this pattern, the overall data suggests that reciprocity in rats extends to a novel lever-pressing context and female Sprague-Dawley rats. Even when the rats had options of where to direct their helping behavior (i.e., a conspecific and/or an empty restrainer), across the rats, the overall preference for helping a conspecific was stronger when the conspecific had been helpful.

Experiment Two: Do Rats Show Reciprocity in a Triadic Context, and Do Rats Show Preferences in their Helping Behavior Depending on the Prior Helpful Actions of a Social Partner?

In Experiment One, rats demonstrated reciprocity when tested individually with a previously helpful or neutral partner. However, it remains uncertain whether rats exhibit reciprocity when both a previously helpful partner and a neutral partner are presented simultaneously. This was investigated in the first condition of Experiment Two, known as the *repeat help* condition. During the habituation phase, a subject rat interacted with two novel rats who were cagemates. In the experience phase, one of the novel rats became the actor and repeatedly pressed the lever to grant the subject rat access to food. The other novel rat, the control, was not present during the experience phase. In the subsequent test phase, the subject rat had the opportunity to help the actor, the control, both, or neither. If rats display reciprocity in a triadic context, they should show a higher propensity to help the previous actor rat compared to the control rat. The inclusion of a neutral control in this triadic context offers a closer approximation to rats' natural social decision-making where rats must recognize and match multiple conspecifics with their past behaviors.

By the definition of reciprocity, after receiving help, an individual is more likely to give help in the future. However, sometimes an individual can have a helpful intention and behave prosocially but be unsuccessful in achieving the desired outcome. Multiple studies indicate that various non-human species can differentiate between an experimenter who unsuccessfully attempted to help and one who refrained from helping altogether (Call et al., 2004; Canteloup & Meunier, 2017; Péron et al., 2010; Phillips et al., 2009; Trösch et al., 2020; Völter et al., 2023). This distinction has also been investigated in Norway rats. In Schweinfurth (2021), the rats increased their helping behavior for a partner who was previously capable of providing help ("able" condition). However, they did not exhibit a higher propensity to help a partner who attempted to help ("unable" condition) compared to one who did not provide help ("unwilling" condition). Notably, these three conditions were examined in separate experiments rather than within the same experiment (Schweinfurth, 2021).

Here, Experiment Two included three conditions to investigate whether rats modify their reciprocal helping behavior based on the actor rat's repeated unsuccessful attempts to provide help. For all three conditions, a trial started with a habituation phase where the subject rat met two cagemate conspecifics. Then, the conditions varied in the experience phase. In the *repeat help* condition, as previously described, the actor rat pressed a lever to allow the trapped rat to exit and get a food reward. Once the subject rat returned to the restrainer, the actor could press the lever again. In the *rare press* condition, the actor rat either helped the subject rat once or did not help the subject rat, thereby demonstrating a lower willingness to help. In the *repeat attempt* condition, the actor rat repeatedly pressed the lever, although the restrainer door was blocked. Presumably the *repeat attempt* actor rat had a robust helping "intention," as she still attempted to perform the helping behavior and continued to press the lever even after an unsuccessful initial attempt. The term "intention" assumes that the actor rat had a goal to help, as demonstrated by its actions, regardless of the outcome. In the subsequent phase, the test phase, the subject rat was placed in the center chamber and had the opportunity to help the previous actor and/or the neutral control rat. While the control rat was present during the habituation phase and again during the test phase, she was not present during the manipulation of the experience phase and was neutral with respect to her helping behavior. These conditions parallel the "unwilling vs unable" studies conducted in other species, but here, action-based terminology is used to describe the rats' behaviors instead of referencing their unobservable mental states.

We compared the subject rat's reciprocal helping behavior by assessing the relative amount of help given to the prior actor and the neutral control. This allowed us to determine if rats modify their prosocial preferences based on a conspecific's helpful actions regardless of the outcome. If reciprocity depends on outcomes rather than actions, rats will show a stronger preference for the actor that repeatedly helped them (*repeat help*) compared to the one that seldom helped (*rare press*) or repeatedly attempted to help (*repeat attempt*). Conversely, if rats adjust their behavior based on a partner's helpful actions, there will be no significant difference in helping preference between a helpful and successful actor (*repeat help*) and a helpful but unsuccessful actor (*repeat attempt*). However, in this case, rats would have a lower preference for helping an unhelpful partner compared to a helpful but unsuccessful partner. It is also possible that rats do not exhibit reciprocity in this triadic context. However, rats may still exhibit differences in their helping behavior towards the actor relative to the control across conditions.

To rule out the possibility that subtle behavioral differences in the rats' behavior could be driving our results, frame-by-frame video analysis was conducted to evaluate the social interactions, exploratory behaviors, and self-grooming behaviors of the rats in all phases. By implementing a novel set-up with a triadic context to investigate how rats differentially help a conspecific who repeatedly provided help, repeatedly attempted to provide help, or rarely performed the helpful behavior, this experiment sheds light on the complexity of rats' understanding of others' actions.

Experiment Two: Method

Ethics Statement

All animal procedures were performed in accordance with National Institutes of Health (NIH) and local Institutional Animal Care and Use (IACUC) ethical guidelines (UC San Diego Protocol Number: S04172) in an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International) accredited facility.

Subjects

A new cohort of 20 female Sprague-Dawley rats, Envigo (Harlan), were used for this experiment. The animals were approximately four months old when testing began and approximately six months old when data collection ended. The relatedness of the individual rats was not provided by the breeder. Animals were acclimated to all experimenters and handled daily for a month prior to experimentation. No animals showed signs of distress.

Rats were housed in pairs. All the rats were numbered 1-20, and each cage contained rat pairs with sequential numbers (e.g., EL1 was housed with EL2, EL3 was housed with EL4, etc.). The cage set up, vivarium conditions, time of testing, food and water access, enriched environment experience, and the experimenters' sex was the same as in Experiment One.

Apparatus

The apparatus was the same as previously described in Experiment One (Figure 1).

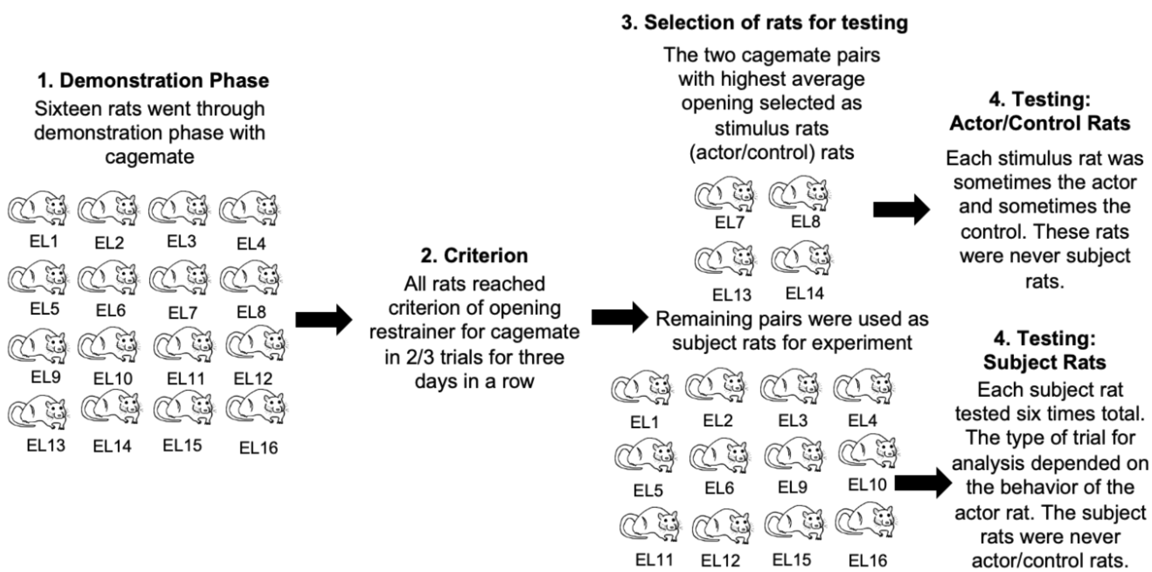
Demonstration Phase

Sixteen of the 20 rats were randomly selected to undergo the demonstration phase. The four remaining rats underwent conditioning to avoid the lever (see *Lever-avoidance conditioning* section for description). Except for two changes, the demonstration phase was executed as described in Experiment One. The first change was that the food reward was changed from an oat flake and mini chocolate chip to a ¼ piece of Cinnamon Toast Crunch cereal. This change was maintained for all of Experiment Two as, compared to the oat flake and chocolate, the cereal has a stronger smell and makes an audible sound when the rat eats it. This increases the salience of the fact that the previously trapped rat was getting food. The second change was that there was no longer an empty restrainer option during the demonstration phase. However, the side on which the trapped rat was placed still varied from trial to trial to ensure that the subject rat had equal experience opening the restrainer on both sides.

As with Experiment One, a rat reached the learning criterion if she opened the restrainer for her cagemate two out of three times for three days in a row. Seven out of the eight pairs (14/16 rats) reached this criterion within five days of exposure to the apparatus, but one pair required an additional day. At the end of the demonstration phase, the two cagemate pairs (four rats total) with the highest number of openings where *both* rats also independently had a high number of openings were selected to serve as actor and control rats for the experiment. Each of these four rats were sometimes the actor and sometimes the control, but their specific role depended on the trial. If in one trial one cagemate was the actor, the other cagemate was the control rat and vice versa. The rats that were actor/control rats were never subject rats, and the subject rats were never actor/control rats. The specific rats that went through the demonstration phase and the selection of specific rats to be actor/control rats or subject rats is represented in Figure 5.

Figure 5

Experience of the Rats and Selection to be Test or Actor/Control Rats



Note. Sixteen rats went through the demonstration phase. Rats alternated between opening the restrainer for her trapped cagemate and being trapped. After all rats reached criterion, the two cagemate pairs with the highest average opening where both rats opened a similar number of times were selected to be actor/control rats for testing. The remaining twelve rats were used as subject rats.

Experimental Design

We used the same three-chambered apparatus as described in Experiment One (Figure 1) and implemented a similar three-phase protocol that had a habituation, an experience, and a test phase. As with the previous experiment, rats were briefly placed in their home cages between phases. There were three conditions: the *repeat help* condition (Figure. 6: column 1), the *rare press* condition (Figure 6: column 2), and the *repeat attempt* condition (Figure 6: column 3). All conditions began with a two-minute habituation period where the subject rat could interact through a clear perforated barrier with two unfamiliar rats known as the future “actor” and the future “control.” By design, the two unfamiliar rats were cagemates with one another (Figure 6: 1A, 2A, and 3A).

The three conditions differed from each other in the experience phase. In the *repeat help* and *rare press* condition, the subject rat was placed in a restrainer on one side of the apparatus, and the actor rat was placed in the center. The actor could forcefully press a lever to open the restrainer to enable the subject rat to exit and get a food reward, a ¼ piece of Cinnamon Toast Crunch cereal. The subject rat was then placed back in the restrainer, and the food reward was replaced (Video S1). If the actor rat pressed the lever again, the trial was labeled as *repeat help* for analysis (Figure 6: 1B). However, if the actor rat pressed the lever only once or abstained from pressing the lever, the trial was labeled as a *rare press* trial for analysis (Fig. 6: 2B). The set up for the third condition, the *repeat attempt* condition, was identical, but a piece of clear tape was placed over the top of the door to block the restrainer from opening (Figure 6: 2C). Thus, the actor could press the lever multiple times but was unsuccessful in providing help (Video S2). The conditions, their dependence on the actor rat's behavior and restrainer operability are described in Supplemental Table S1A. For all conditions, the experience phase lasted four minutes. This duration is shorter than in Experiment One (seven minutes) because it was observed that, in Experiment One, the rats showed more escape-like behavior and performed very few lever presses during the last three minutes.

Lastly, in the test phase, the actor and control rats were placed in separate restrainers, and the subject rat could repeatedly open the restrainer to allow the previous actor, the control, both, or neither to access food (Figure 6: 3A, 3B, and 3C). The timestamp of every opening was recorded. As with Experiment One, within the four minutes, a subject rat was not limited in the number of times or the pattern in which it opened either restrainer, and the subject rat was never reinforced by the experimenter for pressing either lever. Like the experience phase, the test phase was shortened to four minutes (Video S3).

Lever-avoidance Conditioning

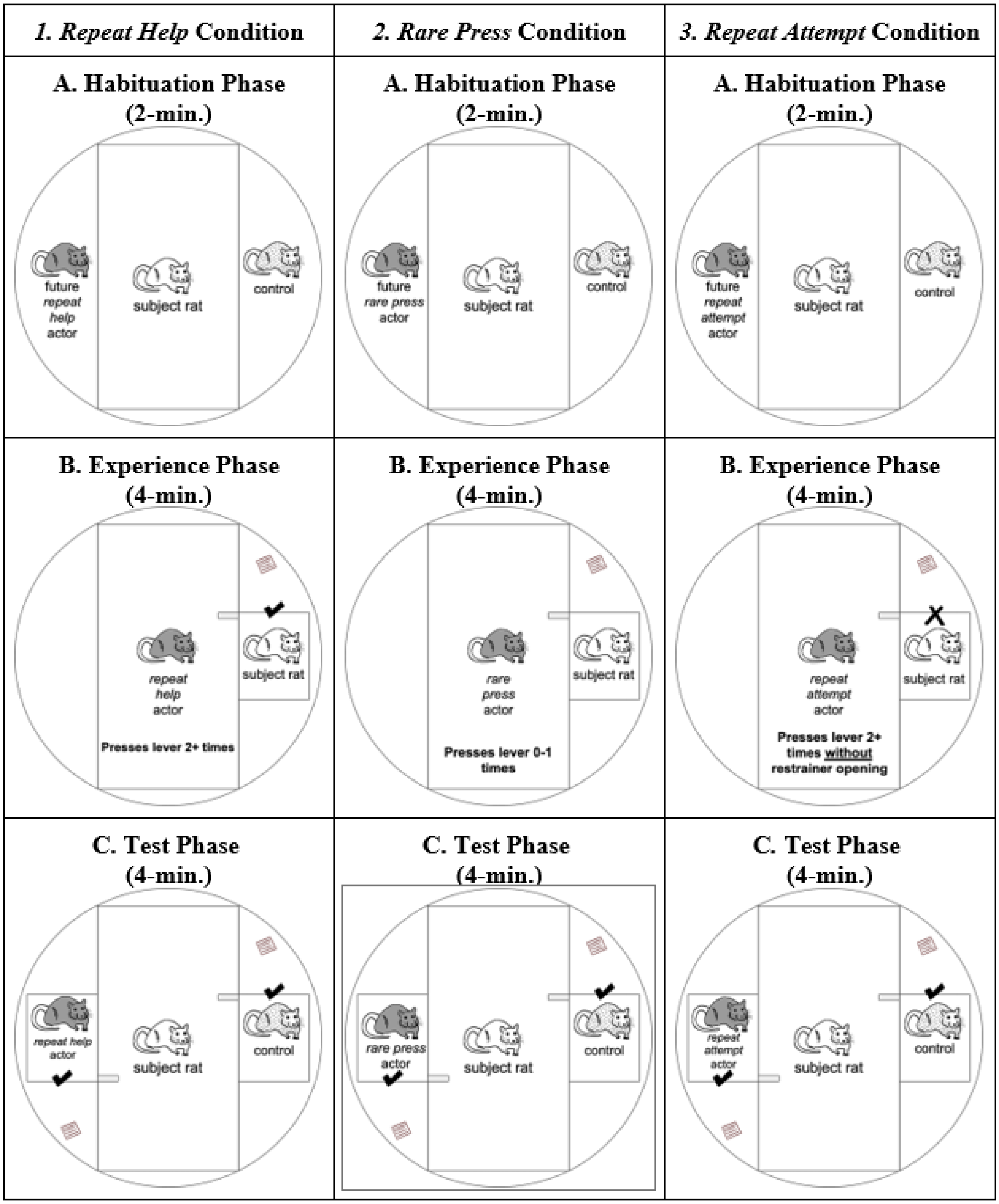
Four rats (two cagemate pairs) were excluded from the demonstration phase and were instead conditioned to avoid the lever. For this, the naïve rat was placed in the center chamber for five minutes. An empty restrainer with a lever protruding into the center was placed on one side. Any time the rat got within approximately ~1 in. of the lever, a baby nasal aspirator was used to give the rat an aversive mild air puff to her face. This occurred for three consecutive days before experimentation began. On two occasions, the conditioning procedure was also repeated immediately before testing to increase the likelihood that the rat would avoid the lever. If in testing, the conditioned rat pressed the lever more than once, the trial was labeled *repeat help* for analysis. If she pressed the lever once or did not press the lever, the trial was labeled *rare press* for analysis.

Exposure to Conditions and Counterbalancing

Each subject rat was tested six times—four in which the restrainer was operable and two in which the restrainer was taped closed. Regardless of whether the restrainer was operable or inoperable, the actor rat's number of lever presses determined how a given trial was categorized for analysis. If the restrainer was operable and the actor rat pressed the lever two or more times, the trial was labeled as *repeat help* for analysis. If the restrainer was operable and the actor rat did not press the lever or pressed the lever just once, the trial was labeled as *rare press*. Lastly, if the restrainer was blocked from opening and the actor rat pressed the lever more than once, the trial was labeled as a *repeat attempt* trial for analysis. Supplemental Table S1A describes how the condition label depended on the actor rat's behavior and the restrainer operability. The number of observations of each condition is also described. In total, there were 48 trials (4 per subject rat) where the restrainer was operable. In 32 of these trials, the actor rat pressed the lever two or more times, and in the remaining 16 trials, the actor rat pressed the lever once (N = 5) or did not press the lever (N = 11). There were 24 trials where the restrainer was blocked from opening. In one trial, the actor did not press the lever, and the trial was labeled as a *rare press* trial for analysis. In the remaining 23 trials, the actor pressed the lever two or more times, and the trial was labeled *repeat attempt*. The number of times that each specific subject rat experienced each condition is described in Supplemental Table S1B, and the number of times each actor rat was used for each condition as well as her conditioning history is described in Supplemental Table S1C.

Figure 6

Order and Timing of Phases in All Conditions



Note. In each diagram above, the medium sized rectangle represents the restrainer with the lever sticking out into the center. If it is possible for the rat in the center to open the restrainer, there is a checkmark. If the restrainer is blocked from opening, there is an X. The food reward, a ¼ piece of Cinnamon Toast Crunch cereal, is represented by the brown square with lines. The side on which the subject rat was placed in the experience phase and the sides of the actor and control in the test phase were counterbalanced. The subject rat is shown in white, the actor is shown in gray, and the control rat is dotted.

Counterbalancing measures were also taken to ensure that prosocial behavior was not dependent on side biases. During the experience phase, each subject rat was in a restrainer on the left side for half of the trials and in a restrainer on the right side for the remaining trials. In the test phase, the placement of the actor rat was counterbalanced such that half the time the actor was in a restrainer on the same side that the subject rat had been on previously and half the time the subject rat was in a restrainer on the opposite side. This minimizes the possibility that the subject rat helped the actor more than the control rat because the actor was on the same side in which the subject rat had previously received food (i.e., place preference) or a novelty bias towards the side where there previously had not been a restrainer. Additionally, we counterbalanced which subject rat was paired with which actor rat, such that a subject rat was not paired with the same actor rat twice. However, later in the experiment, a given subject rat would meet a previous actor rat again but as the control rat instead (and the control rat's cagemate was the actor rat). Unfortunately, due to experimenter error, there was one instance where a subject rat, EL9, was paired with the actor rat, EL8, twice.

The experiment occurred over the course of 28 days total. The first day of testing occurred three days after the conclusion of the demonstration phase. After 12 days of testing, the rats underwent four days of the preference tests. Then, the rats went through 12 more days of testing. Each rat was tested every 3-6 days. See Supplemental Table S2 for a trial-by-trial description of the days each subject rat was used, the condition she experienced on each day she was tested, the actor rat with whom she was paired, and the control rat that was present during the habituation and test phases.

Behavioral Coding

The timestamps of each lever press during the experience and test phases were recorded live by the experimenter during testing, and all trials were video recorded for later analysis.

Videos were hand-coded using ELAN (version 5.9). For both the habituation and test phases, coders were blind to which rat was the actor rat and which one was the control rat. As a measure of social interaction, coders recorded the time that the subject rat spent interacting with the actor and control rats during the habituation phase. To count as an interaction, both the subject rat in the center and the rat on the side had to simultaneously have their noses in the same or adjacent holes in the wall.

During the experience phase, as a measure of anxiety-like behavior, the time the actor rat spent self-grooming was coded. A rat was considered self-grooming when she was in a hunched position and using her front paws to repeatedly stroke her head or when the rat appeared to be scratching herself. To measure exploratory behavior, the time in which the rat in the center was sniffing various locations in the apparatus was coded during both the experience and test phases. For example, when the rat had her nose in the section of holes adjacent to the food, the behavior was coded as "sniffing the food area." When the actor was sniffing the holes adjacent to the restrainer, the behavior was coded as "sniffing at the restrainer." Coders also indicated when the actor rat was "interacting with the lever." This included sniffing at and whisking next to the lever, chewing on the gauze placed over the lever, climbing over the lever, sniffing immediately above or below the lever, or pressing the lever. During the test phase, coders also specified on which side the center rat was performing the behavior of interest.

Interrater reliability was very high among the four raters based on a randomized subset of the data. Cohen's Kappa values were calculated after coders two, three, and four each checked ⅓ of the videos that coder one analyzed, and coder one checked ⅓ of the videos that each of the other three coders analyzed. Each phase (habituation, experience, and test) was evaluated separately. All Cohen's Kappa values were greater than 0.925, indicating excellent interrater reliability.

For analysis, the total duration of each behavior was calculated and normalized by the duration of the phase. Thus, the total duration that each behavior occurred was normalized to reflect the percentage of time the rat(s) was engaged in the behavior of interest during the phase.

Statistical Analyses

Analysis of the subject rats' helping behavior was performed similarly to the analysis used in Experiment One. The only difference was the way in which the prosocial preference (the dependent variable) was calculated. For each trial, we calculated the proportion of a subject rat's overall help that was directed to the actor with the following formula:

$$\frac{\text{\# of lever presses for the actor}}{\text{\# of lever presses for the actor} + \text{\# of lever presses for the control}}$$

We ran a full model with all three conditions then conducted planned pairwise comparisons between each condition.

We also analyzed the behavior of the rats in the habituation and experience phases. We used a paired t-test to compare the percent of time the subject rat interacted with the future actor and the percent of time the subject rat interacted with the future control rat during habituation. This was independent of the condition. Next, we calculated the ratio of interaction with the future actor versus the future control by the following formula:

$$\frac{\text{\% of time interacting with future actor}}{\text{\% of time interacting with future actor} + \text{\% of time interacting with future control}}$$

In our analysis, this ratio was included into a LMM as the dependent variable, and the independent variable was the condition. The test and actor rats' identities were included as random effects.

In the next set of analyses, the number of lever presses that a subject rat experienced in the experience phase of the *repeat help* condition and the *repeat attempt* condition was compared using an LMM with the number of lever presses as the dependent variable, the condition as the independent variable, and the identity of the subject rat as a random effect.

We also used four additional separate models to analyze the actor rats' interaction with the lever, sniffing of the restrainer, sniffing of the food area, and self-grooming behavior. To assess if the subject rat's experience varied across the conditions, the identity of the subject rat was included as a random effect, the condition was the independent variable, and each of the behavioral variables were included as the dependent variable. If there was a significant difference between the three conditions, post-hoc pairwise comparisons of the conditions were conducted.

To assess non-helping behaviors that the subject rat performed during the test phase, we evaluated the proportion of time the subject rat was engaged in the behavior of interest on the side with the actor out of the total time the rat was engaged in the behavior. The behaviors we analyzed were the sniffing of the food and the sniffing of the restrainer. These behaviors were also evaluated using an LMM with the condition as the independent variable, the behavior as the dependent variable, and the identities of the test and actor rats as random effects.

Preference Test

Over the course of the experiment, each subject rat encountered each actor/control rat (stimulus rat) multiple times. As previously described, which subject rat encountered which actor/control rat was counterbalanced, such that a given subject rat would meet each actor/control rat once as the actor and then later as the control, or first as the control and then later as the actor. Because the stimulus rats were used multiple times and in multiple roles, we wanted to ensure that no stimulus rat was systematically preferred compared to her partner. Additionally, we wanted to ensure that subject rats did not exhibit a significant preference for the previous actor or control rat that carried over from the prior testing in any of the conditions when pooled together or in the conditions independently. Lastly, we wanted to ensure that the preference for the actor relative to the control did not vary significantly by condition.

The preference test was performed following the first half of the experiment. The preference test setup was a shortened version of the test phase, and each subject rat underwent a preference test with the same conspecifics it had met in the *repeat help, rare press*, and *repeat attempt* conditions. The subject rat was placed in the center chamber, and the previously experienced actor and control rats were placed in the restrainers on the same sides as the subject rat had experienced in the test condition. For two minutes, the subject rat could open the restrainer for the previous actor and/or the control rat, and the timestamp of every opening was recorded. There was no experience phase during the preference tests. Each subject rat underwent the preference test three times- once with each pair of actor/control rats it had encountered during the first half of the experiment.

The preference test analyses used the amount of help, either in raw counts or proportion of total help directed to the actor. The data from the preference tests were primarily analyzed using Wilcoxon signed-rank tests. Specifically, this analysis was used to determine if the number of times the subject rat opened the restrainer for the actor was different from the number of times the subject rat opened the restrainer for the control rat in the conditions, altogether and independently. Additionally, data from the preference tests was used to assess whether the subject rats preferred one specific rat in a cagemate pair. To do this, a paired Wilcoxon signed-rank test was used to evaluate if the number of times a stimulus rat received help was different than the number of times her cagemate received help. Finally, an LMM was used to evaluate if the preference for the actor relative to the control varied across conditions. Here, the proportion of a subject rat's total help that was specifically directed to the prior actor during the shortened preference test was the dependent variable, and the condition was the independent variable. The identity of the subject rat and the actor were included as random effects.

Experiment Two: Results

Reciprocity in a Triadic Context

The test phase was the final phase of each condition, and this was the phase in which the helping preferences of the subject rats were evaluated. In this phase, the subject rat was in the center and the actor and control rats were in separate restrainers on opposite sides. The subject rat could open the restrainer for the actor, the control, both, or neither (Video S3). For analysis, the preference for the actor was assessed by the number of times the subject rat pressed the lever to help both rats.

Here, we define reciprocity in a triadic context as a preference for the previously helpful actor compared to the control rat when both conspecifics are presented simultaneously. Only the data from the *repeat help* condition were used for this analysis. In these trials, there was no significant difference in the number of times the subject rat helped the actor versus the number of times the subject rat helped the control (LRT: N = 12, X^2= 0.60, p = .44, 95% CI [-0.42, 0.18]). On average, the subject rat helped the actor 2.78 times (SD = 1.96) and the control rat 2.47 times (SD = 2.21). There was also no significant correlation between the number of times that the subject rat was helped and the number of times that she subsequently helped the actor rat (LRT: N = 12, X^2= 1.66, p = .20, 95% CI [-0.02, 0.10]) nor the subsequent preference for the actor (LRT: N = 12, X^2= 0.06, p = .81, 95% CI [-0.03, 0.02]). These data suggest that female Sprague-Dawley rats do not exhibit reciprocity in a triadic context.

Prosocial Preference

The present experiment also examined how rats' helping propensity is affected by others' helpful actions and the outcomes of such actions. Because the set-up provided the subject rat with two simultaneous options of who to help, it was critical to evaluate helping propensity by the amount of help given to the actor *relative* to the control. The proportion of help directed to the actor within a trial was calculated to provide a metric to analyze the distribution of help to the two conspecifics. Across the three conditions, there was a significant difference in the distribution of help given to the actor relative to the control (LRT: N = 12, X^2 = 8.41, p = .02; Figure 7). On average, in the *repeat help* and *repeat attempt* conditions, only

51.3% (*M* = 2.78 presses, *SD* = 1.96 presses) and 47.2% (*M* = 2.87 presses, *SD* = 1.89 presses) of the subject rat's help was towards the actor in each condition, respectively. However, in the *rare press* condition, on average, 70.8% (*M* = 3.76 presses, *SD* = 3.63 presses) of the subject rat's help was to the actor.

Post-hoc pairwise comparisons of each condition indicated that, across trials where the restrainer was operable (*repeat help* and *rare press* conditions), subject rats gave a greater proportion of their help to the previously rare pressing actor than the previously repeat helping actor (LRT: N = 12, X^2= 5.43, p = .020, 95% CI [-0.36, -0.32]; Figure 7A). While there was a significant difference in the way the rats distributed their help to the actor versus the control across the two conditions, there was also a lot of variability in the individual rat's behavior. Across these two conditions, relative to the control, six out of 11 (~55%) of the rats demonstrated a stronger preference for the *rare press* actor (Figure 7A, above diagonal line) while five out of 11 (~45%) showed a stronger preference for the *repeat help* actor (Figure 7A, below the diagonal line). Three rats, EL6, EL12, and EL15. showed a very strong preference (> 0.5 difference) for the *rare press* actor. There was no significant difference in how the subject rats distributed their help to the actor that had attempted to help (*repeat attempt* actor) and one that had repeatedly helped (*repeat help* actor) (LRT: N = 12, X^2= 0.31, p = .58, 95% CI [-0.10, 0.18]; Figure 7B). Lastly, the rats gave a greater proportion of their help to the previous *rare press* actor than the previous *repeat attempt* actor (LRT: N = 12, X^2= 8.50, p = .004, 95% CI [0.08, 0.40]; Figure 7C). Again, there was a lot of individual variability with eight out of 11 rats (~73%) of the rats demonstrating a preference for the *rare press* actor (Figure 7C, above diagonal line) and three out of eleven rats (~27%) showing a stronger preference for the *repeat help* actor relative to the control (Figure 7C, below diagonal line).

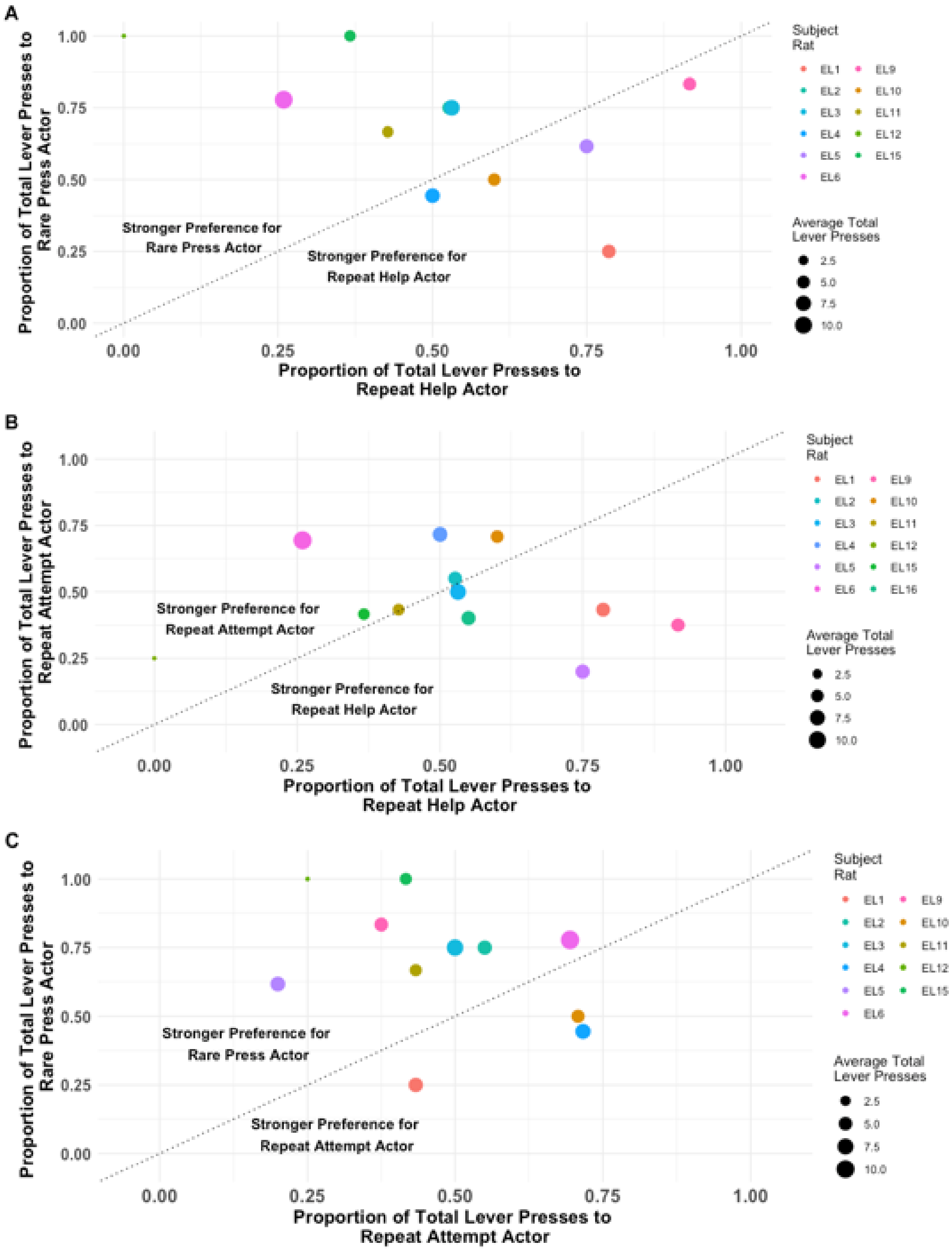
Finally, there was a lot of general individual variability in the propensity of individual rats to press the levers. To quantify the variability in the general amount of help each subject rat gave within each trial, the average amount of help each subject gave was calculated for all the trials of each condition. Then, the averages from all three conditions were averaged. Based on this metric, the average total lever presses of individual rats ranged from 0.83 to 11.06 lever presses per trial (average lever presses per trial: *M* = 5.70, *SD* = 2.51; Figure 7 dot size). Although the way that the help was distributed differed by condition, the total number of times that the subject rats provided help (actor + control) did not significantly differ across conditions: *repeat help* (*M* = 5.25, *SD* = 2.99), *rare press* (*M*= 5.47, *SD* = 4.57), and *repeat attempt* (*M* = 5.83, *SD* = 2.87) (LRT: N = 12, X^2= 1.15, p = .56). Together, these data indicate that the general helping propensity was not affected by the condition, but the prosocial preferences of the rats changed significantly across conditions. Based on the way the subject rats distribute their help, there is evidence that, relative to a control conspecific, female Sprague-Dawley rats have an increased tendency to help a conspecific that had previously provided little help or abstained from providing help compared to ones that had repeatedly provided or attempted to provide help.

Non-helping Behaviors During the Test Phase

Although the way the help was distributed to the actor versus the control differed across conditions, other metrics of the subject rat's behavior towards the actor rat relative to the control rat did not vary by the condition. For example, the amount that the subject rat sniffed the food on the side of the actor relative to the control did not significantly differ across conditions (LRT: N = 12, X^2= 0.21, p = .90). Additionally, there is no evidence that the subject rats' interaction with the actor and control levers significantly differed across conditions (LRT: N = 12, X^2= 2.43, p = .30). Lastly, it does not appear that the subject rat's sniffing behavior towards the restrainer with the actor and control differed significantly by condition (LRT: N = 12, X^2=0.50, p = .78). Together, these data support the notion that the increased propensity to help the actor relative to the control in the *rare press* condition was not a byproduct of other salient exploratory behaviors.

Figure 7

Distribution of Lever Pressing



Note. Each point represents a single subject rat. For the average total lever presses, the number of lever presses performed in each condition was averaged for each subject rat. These three averages were then averaged. Larger dots indicate a higher average number of lever presses that the subject rat performed during a trial. On the x-axis is the average proportion of help that was directed to the *repeat help* actor (A and B) or the *repeat attempt* actor (C) for each subject rat. On the y-axis is the average proportion of help that was directed to the *rare press* actor (A and C) or the *repeat attempt* actor (B). The diagonal line represents equal preference for the actor in both conditions. Above the line indicates that preference for the actor was higher in the condition on the y-axis while below the line indicates that the preference for the actor was higher in the condition on the x-axis.

Baseline Social Interactions with Actor and Control Rats

Each condition began with a two-minute habituation phase where the subject rat was in the center chamber, and the actor and control rats were in separate chambers on opposite sides. The subject rat was considered to be "interacting" with one of the conspecifics when the subject rat and the actor or control had their noses within 3 in. of each other, through the perforations in the wall. Independent of condition, there was no significant difference in the proportion of time that the subject rat interacted with the actor (M = 5.04, SD = 4.43) or the proportion of time she interacted with the control rat (M = 5.66, SD = 4.53, $t(7)$ = -0.80, p = .42, 95% CI [-2.14, 0.91]). The amount of interaction with the actor relative to the control did not differ significantly across conditions (LRT: N = 12, X^2= 2.56, p = .28). Thus, there is no evidence that there were baseline differences in interaction with the actor and control rats, and this difference did not vary by condition.

Behavior of the Actor Rats During the Experience Phase

The conditions differed from each other in the experience phase. In the *repeat help* condition, the actor rat pressed the lever, the restrainer opened, and the subject rat was able to exit and get her food. This happened multiple times. In the *rare press* condition, the actor either pressed the lever once or did not press the lever at all. Lastly, in the *repeat attempt* condition, the actor repeatedly pressed the lever, but the restrainer did not open. Given that there was no overt task, and the subject rat was free to take any actions, we examined various micro-behaviors of the actor rats to ensure that the helping behaviors observed during the test phase were not a result of the actor rat's previous exploratory or anxiety-like behaviors.

The main manipulation of this experiment was in the actor rat's helping actions during the experience phase, and consistent with this, the number of times the actor rat pressed the lever varied across conditions (LRT: N = 12, X^2= 66.4, p < .001). Across conditions, there were significant differences in the percent of time the actor rat spent interacting with the lever (LRT: N = 12, X^2= 13.63, p = .003). Post-hoc pairwise comparisons revealed that there was a trend towards the *repeat help* actors interacting with the lever significantly more than the *rare press* actor rats (LRT: N = 12, X^2= 3.03, p = .082, 95% CI [-0.47, 12.38]). However, the *repeat help* and *repeat attempt* actors did not display significantly different amounts of interaction with the lever (LRT: N = 12, X^2= 2.62, p = .11, 95% CI [-0.72, 8.72]), and there was no significant difference between the *repeat attempt* and *rare press* conditions in the percent of time that the actor rat interacted with the lever (LRT: N = 12, X^2= 1.04, p = .31, 95% CI [-10.82, 3.57]). Thus, even though the *rare press* actor rats pressed the lever significantly less, they did not avoid the lever altogether. Therefore, differences in the subject rat's subsequent helping behavior are unlikely attributable to the actor rat's lever exploration.

In addition to interacting with the lever, the actor rats also frequently poked their nose through the holes closest to the restrainer and appeared to sniff at the subject rat in the restrainer. The apparatus did not allow direct contact between the animals during this phase; thus, sniffing at the restrainer through the closest holes to the subject rat appeared to be an exploratory social behavior. There was a trend towards a difference in the percentage of the time that the actor rats sniffed at the rat in the restrainer across the *repeat help* (M = 4.56, SD = 3.07), *rare press* (M = 5.34, SD = 3.07), and *repeat attempt* (M = 6.44, SD = 3.12) conditions, (LRT: N = 12, X^2= 5.03, p = .081). Post-hoc pairwise comparisons showed that there was a difference in the percentage of time the *repeat help* and *repeat attempt* actors spent sniffing at the restrainer (LRT: N = 12, X^2= 4.91, p = .03, 95% CI [-3.53, -0.22]), and a trend towards a significant difference between the percentage of time the actors in the *rare press* and *repeat attempt* conditions sniffed at the restrainer (LRT: N = 12, X^2= 3.55, p = .060, 95% CI [-2.97, 0.064]). However, there was no significant difference in the amount of time the *repeat help* and *rare* press actors spent sniffing the restrainer (LRT: N = 12, X^2= 0.74, p = .39, 95% CI [-2.66, 1.02]). Given that the *rare press* actors did not differ from the *repeat help* actors in the percentage of time they spent sniffing the restrainer, it appears that the *rare press* actor did not ignore the subject rat. As an additional measure of exploratory behavior and interest in the food, we also recorded the amount of time that the actor rat spent sniffing at the food area, indicated by putting her nose in the holes adjacent to the food. There was no significant difference in the percentage of time the actor rat sniffed at the food across the *repeat help* (M = 8.35, SD = 4.89), rare *press* (M = 6.18, SD = 5.42), and *repeat attempt* (M = 6.71, SD = 5.83) conditions (LRT: N = 12, X^2= 3.33, p = .19).

In rodents, self-grooming can be used as a metric to assess distress. Rodent models of anxiety show increased self-grooming, and self-grooming behavior can be increased by acute distress (see Kalueff et al., 2016 for review). There was no significant difference in the percent of time the actor rats spent self-grooming across conditions: *repeat help* (M = 2.16, SD = 2.30), *repeat attempt* (M = 2.87, SD = 2.53), *rare press* (M = 2.59, SD = 2.42), (LRT: N = 12, X^2= 1.88, p = .39). Together, these data suggest that the behavior of the actor rat towards the lever including if the lever was pressed and if the restrainer opened was the defining difference across conditions. None of the other behaviors including sniffing at the rat in the restrainer, sniffing at the area containing the food, and self-grooming behavior differed significantly across conditions.

Evaluation of the Effect of Conditioning History

A subset of actor/control rats (EL17, EL18, EL19, and EL20) underwent lever-avoidance conditioning prior to the experiment. The goal of the conditioning was to increase the probability that these rats, when in the role of the actor rat, would press the lever less (or not at all), thus creating the *rare press* condition. This manipulation resulted in actor rats that had a conditioning history pressing the lever significantly less than those that did not have the history (LRT: N = 8, X^2= 6.83, p = .009, 95% CI [-2.29, -0.45]). Moreover, because the conditioning history could have resulted in higher anxiety levels, we examined if there was a difference in self-grooming behavior of the conditioned versus non-conditioned actor rats, independent of their lever pressing behavior. Based on self-grooming behavior, there was no evidence that the conditioned rats were significantly more stressed during the experience phase (LRT: N = 8, X^2= 0.33, p = .86).

During the test phase, it is plausible that the subject rats' differential helping behavior across conditions was in response to the trapped rats’ conditioning history, rather than the actor rat’s prior helpful actions. Overall, the conditioning history had no significant effect on the proportion of help that the subject rat directed to the actor relative to the control (LRT: N = 12, X^2= 1.00, p = .32). Thus, there is no evidence that the conditioning history drove the prosocial preferences displayed by the subject rats across conditions. Importantly, the design of the experiment was such that, in all conditions, the actor and control rat that were simultaneously presented to the subject rat were cagemates and always had the same conditioning history.

Preference Test

Over the course of the experiment, the subject rats met the same actor/control rats multiple times, but, in each meeting, the specific rat that was the actor and the specific one that was the control were different. Prior to the second time that a specific subject rat met a specific stimulus rat pair, preference tests were conducted to ensure that there was no preference for a specific rat in the cagemate pair, no systematic preference for the prior actor/control rat regardless of the condition, and no preference for the actor that varied by condition.

To determine if a rat within a stimulus rat pair received more help, regardless of their prior role as actor/control, each pair of stimulus rats was analyzed separately. During the preference test, there was no significant difference in the amount of help that EL7 received compared to the amount that her cagemate, EL8, received (Wilcoxon Signed-Rank test: V = 49.50, p = .56). Similarly, across the other cagemate pairs that served as actor/control rats, there was no significant difference in the amount of help received during the preference tests: EL13 and EL14 (Wilcoxon Signed-Rank test: V = 22.50, p = .63), EL17 and EL18 (Wilcoxon Signed-Rank test: V = 5, p = .14), and EL19 and EL20 (Wilcoxon Signed-Rank test: V = 26.5, p = .96). See Supplemental Table S3 for the number of times each rat was helped in the preference tests.

Independent of condition, the previous actor rat (M = 0.82, SD = 1.07) did not receive significantly more help than the previous control rat (M = 0.99, SD = 1.22) (Wilcoxon Signed-Rank test: V = 384, p = .39). In preference test trials from the previous *repeat help* condition, there was a trend towards the subject rats giving more help to the previous control (M = 1.44, SD = 1.25) than the previous actor rat (M = 0.89, SD = 1.18) (Wilcoxon Signed-Rank test: V = 15, p = .05). However, there was no significant difference in amount of help the previous actor (M = 1.43, SD = 1.62) and previous control (M = 0.57, SD = 0.53) received in trials from the previous *rare press condition* (Wilcoxon Signed-Rank test: V = 4, p = .20) nor in the previous *repeat attempt* condition (Wilcoxon Signed-Rank test: V = 10.5, p = .60; Actor: M = 1.09, SD = 1.22, Control: M = 1.45, SD = 1.37). Importantly, the preference for the previous actor relative to the previous control did not vary significantly by condition (LRT: N = 12, X^2= 2.49, p = .29) suggesting that the actor rat's earlier behavior in the experience phase before did not carry over when tested days later.

Experiment Two: Discussion

Experiment One demonstrated that female Sprague-Dawley rats show reciprocity in a novel dyadic helping context. In Experiment Two, we extended this to examine if rats show reciprocity in a triadic context and if rats alter their helping propensity based on a partner's prior helpful actions. Rats did not show reciprocity in a triadic context, and rats' prosocial preference was stronger for a partner that had previously been less helpful. These results suggest that reciprocity differs in dyadic and triadic social contexts, but the disparate results of Experiments One and Two could have been instead due to methodological changes. A third experiment was conducted to assess these possibilities.

Experiment Three: Do Rats Show Changes in their Helping Behavior Based on Others' Prior Actions in a Dyadic Context?

The aim of Experiment Three was to reconcile the results of the previous two experiments, determining whether the differences in results were due to methodological differences (i.e., time of trial, food used, the conditions that were tested) or the social context (dyadic versus triadic). Therefore, for Experiment Three, for testing, we precisely replicated the methodology of Experiment Two but instead utilized a dyadic context. This allowed us to assess whether rats change their reciprocal helping behavior based on the previous helpful or non-helpful actions of a conspecific differentially in dyadic and triadic social contexts. Altogether, by comparing how rats alter their reciprocal helping behavior based on others' prior helpful intentions in both triadic and dyadic contexts, we gain a greater understanding of the way in which the social context of testing affects their reciprocity.

Experiment Three: Method

Ethics Statement

All animal procedures were performed in accordance with National Institutes of Health (NIH) and local Institutional Animal Care and Use (IACUC) ethical guidelines (UC San Diego Protocol Number: S04172) in an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International) accredited facility.

Subjects

A new cohort of 21 female Sprague-Dawley rats (Charles River) were used for this experiment. The animals were aged approximately 18 months at the time of testing. The relatedness of the individual animals was unknown. All animals had been used in a prior experiment and were accustomed to daily handling. Rats were housed in pairs. One of the rats was housed with a partner that, due to health concerns, was never used in testing. Housing conditions, vivarium conditions, time of testing, food and water access, and the enriched environment experience were the same as in Experiments One and Two. The experimenter was female.

Apparatus

The same apparatus that was used in Experiments One and Two was used in Experiment Three (Figure 1).

Experimental Design

As with Experiments One and Two, Experiment Three employed a three-phase protocol with a habituation, experience, and test phase (Figure 8). Rats were placed in empty cages by themselves between phases. There were the same three conditions as in Experiment Two: the *repeat help* condition (Figure 8: column 1), the *rare press* condition (Figure 8: column 2) and the *repeat attempt* condition (Figure 8: column 3). The predominant difference between Experiments Two and Three was that the third experiment was carried out in a strictly dyadic context with no control rat present.

Each condition began with a two-minute habituation phase. The subject rat was in the center chamber, and one conspecific, the future actor, was placed in one of the side chambers. The side on which the future actor was placed was always the same (Figure 8: 1A, 2A, and 3A). The next phase, the experience phase, occurred as previously described in Experiment Two. The actor rat was placed in the center chamber, and the subject rat was placed in a restrainer on the side. As with Experiment Two, the condition that a trial was labeled for analysis depended on the actor rat's behavior during the experience phase and the operability of the restrainer. If the actor rat pressed the lever multiple times, the trial was coded as *repeat help* for analysis (Figure 8: 1B). However, if the actor rat pressed just once or did not press at all, the condition was analyzed as *rare press* (Figure 8: 2B). Lastly, in the *repeat attempt* condition, a piece of tape was placed over the restrainer to stop it from opening. If the actor rat pressed the lever repeatedly, the trial was labeled as a *repeat attempt* trial for analysis (Figure 8: 3B). The experience phase was four minutes.

In the final phase, the test phase, the actor rat was placed in the restrainer where the subject rat had previously been, and the subject rat was placed in the center. Here, the subject rat could press the lever to allow the actor rat to access food (Figure 8: 1C, 2C, and 3C). The number of times and the timestamp of each lever press were recorded. This phase lasted four minutes.

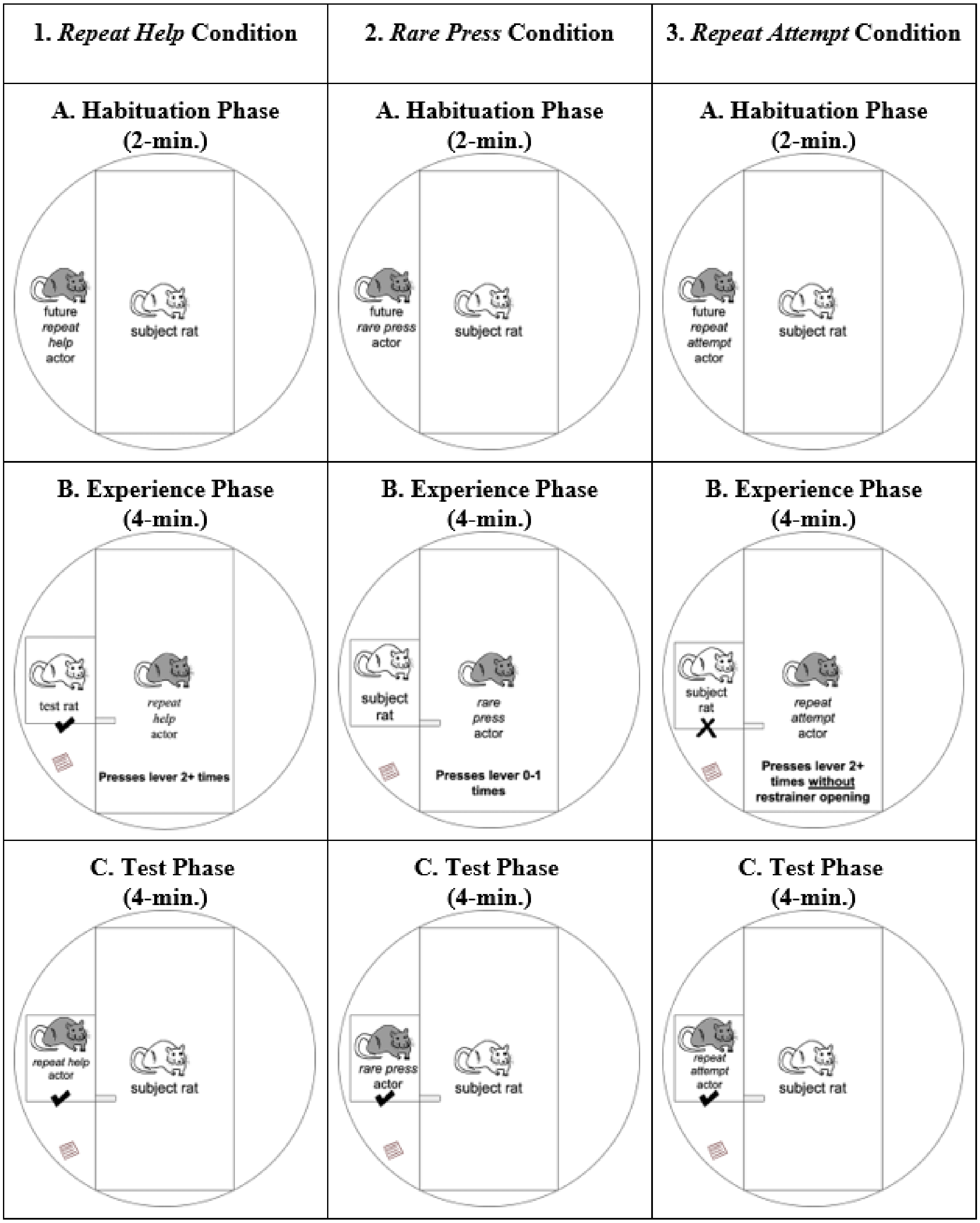
Selection of Actor and Subject Rats

There was no demonstration phase in Experiment Three. Previously, all experimental rats had been used in a study with the same apparatus. All rats underwent 40 trials in which they could release their cagemate. Based on their lever pressing behavior over those trials, rats were classified as high openers (n = 6), intermediate openers (n = 8), and low openers (n = 7). The intermediate openers were used as subject rats. The subject rats were never actor rats, and the actor rats were never subject rats.

The high opener rats were selected to be actor rats, and given their prior behavior, they were likely to press the lever two or more times in the experience phase and thus be actors in the *repeat help* and *repeat attempt* conditions. The low openers were also selected to be actor rats, but they were more likely to press the lever once or not press at all (*rare press* condition). However, as with Experiment Two, a trial was labeled as a specific condition for analysis depending on the real-time lever pressing behavior of the actor rat; not our *a priori* predictions based on the actor rat's previous behavior.

Figure 8

Order and Timing of Phases in All Conditions



Note. In each diagram above, the medium sized rectangle represents the restrainer with the lever sticking out into the center. If it is possible for the rat in the center to open the restrainer, there is a checkmark. If the restrainer is blocked from opening, there is an X. The food reward, a ¼ piece of Cinnamon Toast Crunch cereal, is represented by the brown square with lines. The side on which the subject rat was placed in the experience phase and the sides of the actor and control in the test phase were counterbalanced. The subject rat is shown in white, and the actor is shown in gray.

Statistical Analyses

The number of times that the subject rat helped the actor rat in the test phase was used to assess reciprocal helping behavior. A generalized linear mixed effect model (GLMM) assuming a Poisson distribution was used to assess if the number of times the subject rat helped the actor varied by condition. The number of lever presses during the test phase was the dependent variable while the condition was the fixed effect. For both models, random effects of the subject rat's identity and the actor rat's identity were included. Likelihood ratio tests (LRT) were performed as previously described. The significance level was set as .05, but post-hoc pairwise comparisons were conducted for any p-value below .10.

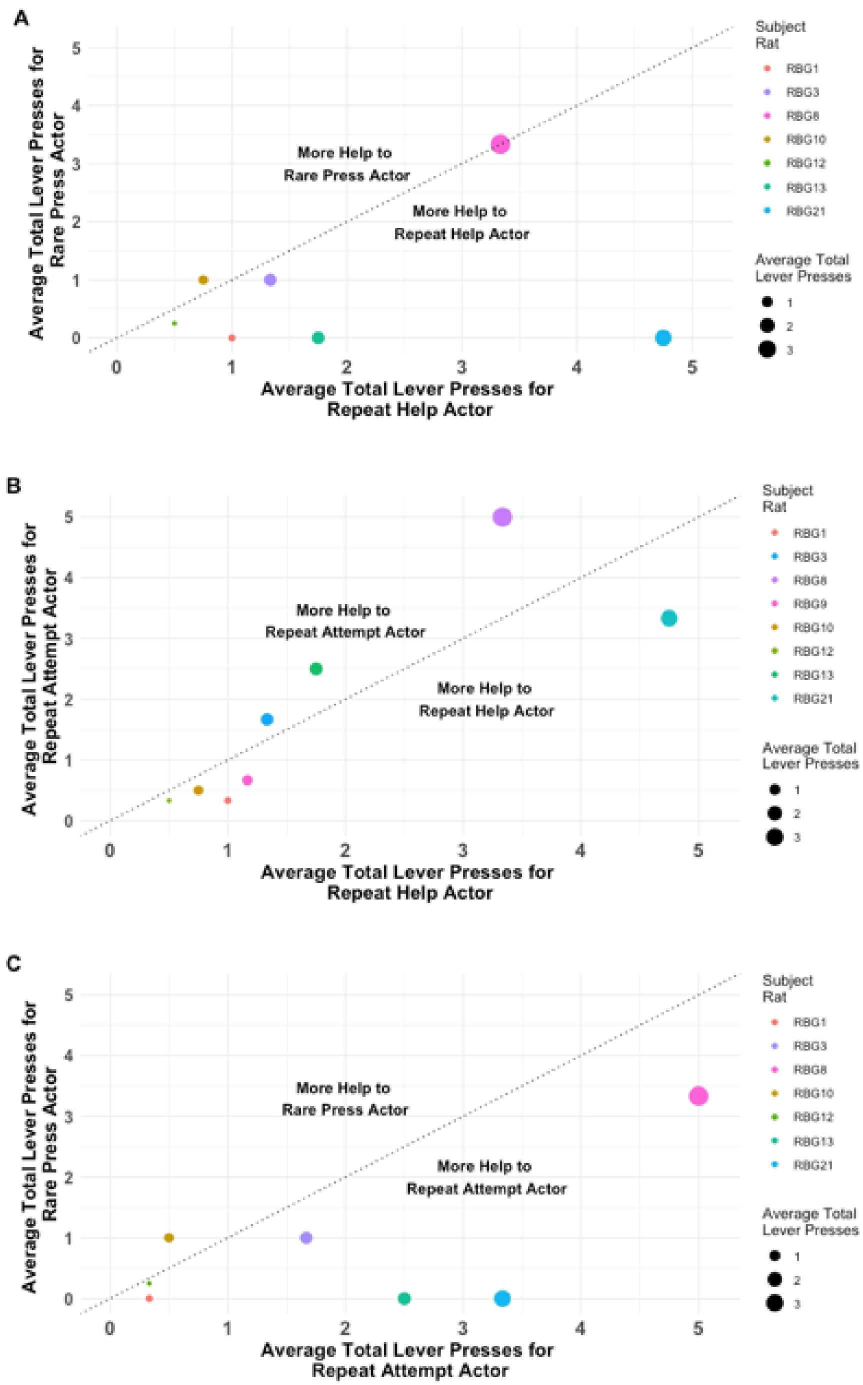
Experiment Three: Results

The number of times that the subject rat helped the actor rat varied significantly across conditions (LRT: N = 8, X^2= 8.87, p = .012, Figure 9). Post-hoc pairwise comparisons revealed that the subject rat helped the actor more in the *repeat help* condition compared to the *rare press* condition (LRT: N = 8, X^2= 5.80, p = .016, 95% CI [0.15, 1.34], Figure 9A), and she helped the *repeat attempt* actor more times than the *rare press* actor (LRT: N = 8, X^2= 7.54, p = .0063, 95% CI [-1.42, -0.21] Figure 9C). However, there was no significant difference in the number of times that the subject rat helped the *repeat help* actor compared to the *repeat attempt* actor (LRT: N = 8, X^2= 0.059, p = .81, 95% CI [-0.36, 0.47] Figure 9B).

As with Experiments One and Two, there was a lot of individual variability in both the subject rats' general helping propensity and the amount of help given to the actor across conditions. The average number of lever presses performed by each subject rat was calculated for each condition first, and then the averages from each condition were averaged together. For the individual rats, this average ranged from 0.36 to 3.89 lever presses per trial (M = 1.48, SD = 1.22). The general opening propensity of each subject rat, based on this calculation, is represented by the dot size in Figure 9. There was also a lot of variability in the amount of help given to the actor across conditions. Between the *repeat help* and *rare press* conditions, five subject rats showed a stronger preference for the *repeat help* actor (Figure 9A, below the diagonal line) while one subject rat showed a preference for the *rare press* actor (Figure 9A, above the diagonal line), and one subject rat showed an equal preference. Across the *repeat help* and *repeat attempt* conditions, five subject rats showed a preference for the *repeat help* actor (Figure 9B, below the diagonal line), and three subject rats showed a preference for the *repeat attempt* actor (Figure 9B, above the diagonal line). Lastly, between the *repeat attempt* and *rare help* conditions, six subject rats showed a preference for the *repeat attempt* actor (Figure 9C, below the diagonal line), and one subject rat demonstrated a preference for the *rare press* actor. Overall, these data suggest that, while there was a lot of variability in the helping propensity of the subject rats, in a dyadic context, on average, rats demonstrated an increased propensity to help a conspecific that had previously repeatedly provided (*repeat help* condition) or attempted to provide help (*repeat attempt* condition) than a conspecific that seldom provided help (*rare press* condition).

Figure 9

Number of Lever Presses for Actor Rats



Note. Each point represents a single subject rat. For the average total lever presses, the number of lever presses performed in each condition was averaged for each subject rat. These three averages were then averaged. Larger dots indicate a higher average number of lever presses that the subject rat performed during a trial. On the x-axis is the average amount of help that each rat directed to the *repeat help* actor (A and B) or the *repeat attempt* actor (C). On the y-axis is the total amount of help that each subject rat gave to the *rare press* actor (A and C) or the *repeat attempt* actor (B). The diagonal line represents equal preference for the actor in both conditions. Above the line indicates that the number of lever presses for the actor was higher in the condition on the y-axis while below the line indicates that the number of lever presses for the actor was higher in the condition on the x-axis.

General Discussion

Our experiments revealed that reciprocity in rats is dependent on the social context. Further, they revealed that rats' helping preferences vary based on others' prior actions. Consistent with previous studies, in dyads, rats demonstrated a greater propensity to help a previously helpful conspecific. However, in triads, relative to a control, rats' helping preference was stronger when the conspecific had previously been *less* helpful. In both dyads and triads, rats' prosocial behavior towards a partner that successfully helped or attempted but failed to help did not differ significantly, suggesting that others' helpful actions, not the *outcomes* of the actions, were sufficient to influence helping behavior.

Our findings support and expand upon previous research on reciprocity in rats. Experiment One confirmed the robustness of reciprocity in a new dyadic experimental setup, where female Sprague-Dawley rats helped each other access food by pressing a lever. However, Experiment Two yielded unexpected results contrary to prior literature (e.g., Dolivo & Taborsky, 2015b; Rutte & Taborsky, 2008; Schneeberger et al., 2012). Surprisingly, rats did *not* exhibit a preference for helping a helpful rat over a neutral control, but they did show a preference for helping mostly *unhelpful* rats. Despite testing in a novel triadic context, we initially hypothesized that reciprocity would extend to this scenario based on existing literature and the social nature of rats.

In comparing Experiments One and Two, the primary distinction was the use of dyads versus triads. However, another crucial methodological difference was the inclusion of a condition where the actor rat repeatedly attempted but failed to provide help. This additional condition introduced complexity, as the actor rat's behavior could not be easily classified as purely "helpful" or "non-helpful." To address this discrepancy, Experiment Three directly addressed these varied results by incorporating all three conditions exclusively in a dyadic context.

In Experiment Three, rats provided more help to a partner that had previously helped (*repeat help* actor) or attempted to help (*repeat attempt* actor) than a partner that helped only once or did not help at all (*rare press* actor). Moreover, rats' reciprocal helping behavior did not differ depending on whether the partner was previously successful or unsuccessful in providing help. These results corroborate the finding from Experiment Two that rats' reciprocal helping behavior varies based on a partner's prior intention. However, in Experiment Two, the rats demonstrated an *increased* propensity to help a previously less helpful partner, but in Experiment Three, the results were in the opposite direction, and the rats showed a *decreased* propensity to help the less helpful partner. The results of Experiment Three provide additional evidence that rats show reciprocity, mirroring prior studies that utilized dyadic interactions. Therefore, it was likely the presence of a third conspecific in Experiment Two that resulted in a preference for less helpful partners.

Studies on rats (Havlik et al., 2020; Heslin & Brown, 2021) and other non-human species (Massen et al., 2010; Sabbatini et al., 2012) indicate that the presence of a third conspecific can influence behavior. Rats, for instance, demonstrated varying helping behavior depending on the presence and competence of a third rat (Havlik et al., 2020). Additionally, Heslin and Brown (2021) showed that rats' prosocial behavior changed when there was another conspecific present with whom the subject rat could interact. In multiple primate species, prosocial behavior, in the form of food provision, also changed based on the number of individuals present (Massen et al., 2010). In dyads of long-tailed macaques, relationship quality did not affect prosocial behavior. However, in triads, the test subject's prosocial behavior depended on the relative dominance ranking of the two social partners. In a partner-choice paradigm, capuchin monkeys' prosocial helping behavior was less dependent on short-term reciprocity when a third conspecific was present (Sabbatini et al., 2012). Computational models utilizing triadic interactions also yield novel insights into complex social behaviors, especially in the presence of dominance hierarchies and the formation of coalitions (Mesterton-Gibbons & Sherratt, 2010). Collectively, our data and the literature provide strong rationale for examining the impact of social context on social behavior.

In addition to showing that reciprocity differs in dyadic and triadic contexts, the results of Experiments Two and Three suggest that rats alter their reciprocal helping based on the prior helpful actions of others. Corroborating Schweinfurth (2021), in dyads, rats showed reciprocal helping behavior. Our data also demonstrated that female Sprague-Dawley rats extend their helping behavior to conspecifics that repeatedly unsuccessfully attempted to help. However, Schweinfurth (2021) found that female Norway rats did *not* extend their increased helping propensity to conspecifics that unsuccessfully attempted to help. In addition to using different strains of rats, there are other possible explanations for the different results. For example, Schweinfurth (2021) tested the rats with a helpful partner and a helpful but unsuccessful partner and compared these behaviors to archival data of the rat's behavior with a non-helpful partner. In contrast, in our studies, all three conditions were compared in the same experiment. The subject rats repeatedly experienced all three conditions in a random order within a four-week timeframe. It is possible that rats differentially value others' helping actions based on the other actions they are experiencing. Thus, when the rat experiences just two conditions (a helpful partner or a partner that attempts to help but is unsuccessful), the attempt has a lower relative value. However, when the rat experiences all three conditions (a helpful partner, a partner that attempts to help but is unsuccessful, or an unhelpful partner), attempting to help assumes a higher value. Rats are capable of positive-negative contrast (Gutman et al., 1975), and it is possible that this extends to social contexts.

Our results collectively show that rats demonstrate reciprocity in dyadic contexts (Experiments One and Three) but do not show reciprocity in a triadic context (Experiment Two). Moreover, in a triadic context, the rats demonstrated a stronger preference for the previously *less* helpful actor. Given prior literature in rats (see Schweinfurth, 2020 for review) and humans (Behne et al., 2005; Hamlin, 2013), these results were unexpected. There are multiple putative explanations that are worthy of future investigation. For example, it is possible that the triadic context presents a "noisier" situation. In the presence of uncertainty about a partner or their behaviors, cooperating even after a partner fails to cooperate can prevent an endless cycle of mutual defection (Fishman, 2006; Nowak & Sigmund, 1990). Excusing others' defection in uncertain situations can help sustain cooperation (Krams et al., 2013).

The rats may be employing a strategy to promote reciprocity by incrementally increasing their investment in successive interactions. Theoretical models suggest that this "raise-the-stakes" strategy can become dominant over generations in populations initially composed of non-altruistic individuals (Roberts & Sherratt, 1998). By demonstrating a higher propensity to help the actor that previously showed less helpful behavior, rats may be effectively implementing this strategy to encourage future cooperation. Additionally, the presence of the control rat and the altered relationship dynamics it brings could diminish the significance of the actor rat's prior lack of helping. Future studies systematically utilizing iterative interactions across various social contexts will yield greater insight into the long-term cooperative strategies and decision rules that rats apply in response to a conspecific that is less willing to help.

Limitations

The present experiments have several methodological limitations. Although the labeling of trials based on the actor rat's spontaneous behavior during the experience phase was a strength, it resulted in an imbalance in the number of times each subject rat experienced each condition in Experiments Two and Three. Additionally, the history of the rats varied across the studies, with Experiment Three involving older rats that had a history of lever pressing leading to social contact from use in a prior experiment, and a history of social contact could influence rats' prosocial behavior (Hiura et al., 2018). Nonetheless, the within-subject design used in each experiment allowed for each rat to serve as its own control, minimizing the impact of prior history on the main behaviors of interest: rats' differential helping behavior across conditions and social contexts. With our current data, we cannot rule out the possibility that the rats have a second-order association with the actor rat's lever press and are receiving the same amount of reinforcement in the *repeat help* and *repeat press* conditions. Furthermore, as with any laboratory experiment on social cognition, the results may not generalize to more naturalistic contexts.

Future Directions

The present studies raise many important questions to be assessed with future research. For example, utilizing the same rats in both dyadic and triadic contexts would elucidate if individual rats show context-specific behaviors. Additional work is also needed to directly examine rats' preference for helping when confronted simultaneously with a previously helpful and a previously non-helpful conspecific. To better understand the mechanisms of rats' prosocial behavior, future work can also examine individual differences in micro- and prosocial behaviors, use male rats and/or include additional electrophysiological recordings, pharmacological manipulations, analyses of ultrasonic vocalizations, and/or assessments of dominance.

Conclusion

Across three separate experiments, we show that female Sprague-Dawley rats reciprocate help in a novel experimental set-up (Experiments One and Three) but do not show reciprocity in a triadic context (Experiment Two). Additionally, in a triadic context, rats' reciprocal helping preferences are influenced by a partner's prior helpful intentions, and rats show a higher propensity to help a partner that has seldom provided help compared to those that have repeatedly provided or attempted to provide help. In a dyadic context, rats' reciprocal helping behavior also varies according to a partner's prior actions, but the rats show a higher propensity to help the previously helpful or helpful-acting conspecific (Experiment Three). These findings highlight the influence of social contexts on helping paradigms and suggest that understanding others' action-based intentions is widespread and extends to the genus *Rattus*. Overall, our results contribute to our understanding of the factors influencing social cognition and prosocial behavior across species.

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Author Contributions: EW conceived of the experiments, designed the experiments, collected the data, carried out statistical analysis, and drafted the manuscript; CS assisted with primary data collection, EL participated in experimental design, data collection, and manuscript editing, JC assisted with video analysis, SK assisted with data analysis and visualization, DS assisted with data collection, MAR designed and built the restrainers and walls, FR, LQ, and AC were critical in experiment design, data analysis, and the editing of the manuscript. All authors approved the manuscript for publication.

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Data Availability: The data that support the findings of this study are available on request from the corresponding author.

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Supplemental Videos and Tables

Video S1
Example of helping behavior (all experiments)
https://doi.org/10.6084/m9.figshare.23837283.v1

Video S2
Example of attempting to help (Experiments Two and Three)
https://doi.org/10.6084/m9.figshare.23837484.v1

Video S3
Example of test phase with multiple options of who to help (Experiment Two)
https://doi.org/10.6084/m9.figshare.23837565.v1

Table S1

Experiment Two- Number of Occurrences of Each Condition

A)

Actor rat's lever-pressing behavior	Restrainer operability		Total number of observations	Condition label for analysis
2+ lever presses	Operable		32	Repeat help condition
0-1 lever presses	Operable (N = 16)	Inoperable (N = 1)	17	Rare press condition
2+ lever presses	Inoperable		23	Repeat attempt condition

B)

Subject Rat	Repeat help	Rare press	Repeat attempt
EL1	3	1	2
EL2	2	2	2
EL3	3	2	1
EL4	3	1	2
EL5	2	2	2
EL6	3	1	2
EL9	2	2	2
EL10	2	2	2
EL11	3	1	2
EL12	2	2	2
EL15	3	1	2
EL16	4	0	2
Total	32	17	23

C)

Actor Rat	Conditioning history	Repeat help	Rare press	Repeat attempt	Total
EL7	No history	6	0	6	12
EL8	No history	6	0	7	13
EL13	No history	6	0	5	11
EL14	No history	4	3	5	12
EL17	Prior history	3	3	0	6
EL18	Prior history	0	6	0	6
EL19	Prior history	4	4	0	8
EL20	Prior history	3	1	0	4

Note. Frequency of conditions and experience in each condition for both subject and actor rats. A) Description of the behavior of the actor rat and the restrainer operability for each condition as well as the total number of observations for each condition. B) Number of times that each subject rat experienced each condition. C) Each actor rat's conditioning history, number of times that she was the actor for each condition, and total number of times that she was used as an actor rat.

Table S2

Experiment Two- Trial-by-Trial Information

Subject Rat	Day	Condition	Actor Rat	Control Rat
EL1	3	Repeat help	EL13	EL14
EL1	6	Repeat attempt	EL8	EL7
EL1	12	Rare press	EL18	EL17
EL1	15	Repeat attempt	EL14	EL13
EL1	21	Repeat help	EL7	EL8
EL1	24	Repeat help	EL19	EL20
EL2	3	Rare press	EL19	EL20
EL2	9	Repeat attempt	EL14	EL13
EL2	12	Repeat help	EL7	EL8
EL2	15	Rare press	EL18	EL17
EL2	18	Repeat help	EL13	EL14
EL2	24	Repeat attempt	EL8	EL7
EL3	2	Repeat help	EL7	EL8
EL3	5	Rare press	EL14	EL13
EL3	11	Repeat help	EL17	EL18
EL3	14	Repeat help	EL13	EL14
EL3	17	Rare press	EL20	EL19
EL3	20	Repeat attempt	EL8	EL7
EL4	2	Repeat help	EL20	EL19
EL4	8	Repeat attempt	EL8	EL7
EL4	11	Repeat help	EL13	EL14
EL4	17	Repeat attempt	EL14	EL13
EL4	20	Rare press	EL18	EL17
EL4	23	Repeat help	EL7	EL8
EL5	5	Rare press	EL19	EL20
EL5	8	Repeat help	EL14	EL13
EL5	11	Repeat attempt	EL7	EL8
EL5	14	Rare press	EL17	EL18
EL5	17	Repeat help	EL8	EL7
EL5	23	Repeat attempt	EL13	EL14
EL6	2	Repeat attempt	EL13	EL14
EL6	5	Repeat help	EL8	EL7
EL6	8	Rare press	EL18	EL17
EL6	14	Repeat attempt	EL7	EL8
EL6	20	Repeat help	EL14	EL13
EL6	23	Repeat help	EL19	EL20
EL9	1	Repeat help	EL8	EL7
EL9	4	Repeat help	EL17	EL18
EL9	7	Repeat attempt	EL7	EL8
EL9	13	Rare press	EL19	EL20
EL9	16	Rare press	EL14	EL13
EL9	22	Repeat attempt	EL8	EL7
EL10	1	Rare press	EL19	EL20
EL10	4	Repeat help	EL14	EL13
EL10	10	Repeat attempt	EL7	EL8
EL10	13	Repeat attempt	EL13	EL14
EL10	16	Rare press	EL17	EL18
EL10	19	Repeat help	EL8	EL7
EL11	6	Repeat help	EL20	EL19
EL11	9	Repeat help	EL7	EL8
EL11	12	Repeat attempt	EL13	EL14
EL11	18	Repeat attempt	EL8	EL7
EL11	21	Rare press	EL18	EL17
EL11	24	Repeat help	EL14	EL13
EL12	3	Repeat attempt	EL8	EL7
EL12	6	Rare press	EL14	EL13
EL12	9	Repeat help	EL19	EL20

Table S2 (cont.)

EL12	15	Repeat help	EL7	EL8
EL12	18	Rare press	EL17	EL18
EL12	21	Repeat attempt	EL13	EL14
EL15	4	Repeat attempt	EL7	EL8
EL15	7	Repeat help	EL13	EL14
EL15	10	Repeat help	EL20	EL19
EL15	13	Repeat help	EL8	EL7
EL15	19	Repeat attempt	EL14	EL13
EL15	22	Rare press	EL18	EL17
EL16	1	Repeat attempt	EL14	EL13
EL16	7	Repeat help	EL17	EL18
EL16	10	Repeat help	EL8	EL7
EL16	16	Repeat attempt	EL7	EL8
EL16	19	Repeat help	EL19	EL20
EL16	22	Repeat help	EL13	EL14

Note. Trial-by-trial experience of each subject rat in Experiment Two. The day that each subject rat was tested, the condition she experienced, the actor rat with whom she was paired, and the control rat that was present is shown.

Table S3

Experiment Two Preference Test Helping Behavior

Rat ID	Total Received	Cagemate ID	Cagemate Total Received	Paired Wilcoxon signed-rank tests of trial-by-trial amount of help received
EL7	22	EL8	28	V = 49.5, p = .56
EL13	13	EL14	15	V = 22.5, p = .63
EL17	8	EL18	17	V = 5, p = .14
EL19	13	EL20	14	V = 26.5, p = .96

Note. Number of times each stimulus rat was helped during the preference test. The ID of each stimulus rat, the number of times she was helped, her cagemate's ID, the number of times the cagemate was helped, and the statistical analysis of the amount of help each rat received compared to her cagemate is shown.

Table S4

Experiment Three Subject Rats

Subject Rat	Repeat help	Rare press	Repeat attempt
RBG1	5	1	3
RBG3	3	3	3
RBG8	3	3	3
RBG9	6	0	3
RBG10	4	3	2
RBG12	2	4	3
RBG13	4	3	2
RBG21	4	2	3
Total	31	19	22

Note. Experiment Three subject rats and exposure to conditions. The number of times that each subject rat experienced each condition is shown in the table.

Table S5

Experiment Three Trial-by-Trial Information

Subject Rat	Day	Condition	Actor Rat
RBG3	16	Repeat help	RBG22
RBG3	18	Repeat help	RBG17
RBG8	2	Repeat attempt	RBG19
RBG8	4	Repeat help	RBG4
RBG8	6	Rare press	RBG20
RBG8	8	Repeat attempt	RBG16
RBG8	10	Repeat help	RBG17
RBG8	12	Rare press	RBG11
RBG8	14	Repeat attempt	RBG14
RBG8	16	Rare press	RBG15
RBG8	18	Repeat help	RBG22
RBG9	1	Repeat help	RBG2
RBG9	3	Repeat attempt	RBG15
RBG9	5	Repeat help	RBG20
RBG9	7	Repeat attempt	RBG19
RBG9	9	Repeat help	RBG16
RBG9	11	Repeat help	RBG7
RBG9	13	Repeat help	RBG14
RBG9	15	Repeat help	RBG18
RBG9	17	Repeat attempt	RBG17
RBG10	2	Rare press	RBG2
RBG10	4	Repeat help	RBG16
RBG10	6	Repeat attempt	RBG17
RBG10	8	Rare press	RBG18
RBG10	10	Repeat attempt	RBG20
RBG10	12	Repeat help	RBG14
RBG10	14	Repeat help	RBG7
RBG10	16	Repeat help	RBG19
RBG10	18	Rare press	RBG15
RBG12	1	Repeat help	RBG20
RBG12	3	Repeat attempt	RBG17
RBG12	5	Rare press	RBG2
RBG12	7	Repeat help	RBG15
RBG12	9	Repeat attempt	RBG14
RBG12	11	Rare press	RBG18
RBG12	13	Rare press	RBG19
RBG12	15	Repeat attempt	RBG16
RBG12	17	Rare press	RBG7
RBG13	1	Rare press	RBG15
RBG13	3	Repeat help	RBG16
RBG13	5	Rare press	RBG11
RBG13	7	Repeat help	RBG22
RBG13	9	Repeat help	RBG19
RBG13	11	Repeat attempt	RBG20
RBG13	13	Rare press	RBG4
RBG13	15	Repeat press	RBG17
RBG13	17	Repeat attempt	RBG16
RBG21	2	Repeat help	RBG17
RBG21	4	Repeat attempt	RBG14
RBG21	6	Rare press	RBG2
RBG21	8	Rare press	RBG15
RBG21	10	Repeat help	RBG18
RBG21	12	Repeat attempt	RBG19
RBG21	14	Repeat help	RBG16
RBG21	16	Repeat help	RBG7
RBG21	18	Repeat attempt	RBG20

Note. Trial-by-trial experience of each subject rat in Experiment Three. The day that each subject rat was tested, the condition she experienced, and the actor rat with whom she was paired is shown.