Female Rats Tend to be More Pro-social with Acquaintances than with Strangers[1,2]

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Abstract

One experiment explored the impacts of familiarity between female rats on the pro-social behavior. We trained pairs of Wistar rats in the Pro-social Choice Task. All pairs of rats had an actor and a partner rat that were placed within a double T-maze. Throughout the sessions, actors decided between two options only differing in the food delivered to the partner. In the *selfish option* only the actor received food, while the *pro-social option* produced additional food for a partner rat or an inanimate toy. Half of the actors lived in the same cage with their partner rats during the experiment (Familiar Group), whereas the other half only had contact in the double T-maze (Stranger Group). We found that actor rats in the Familiar Group performed higher rates of the pro-social choice in the condition of the partner rats with respect to the toy; while the actor rats in the Stranger Group showed a similar levels of pro-social choices regardless of whether the partner was a rat or a toy.

Key words: *Familiarity, pro-social responses, rats, social behavior*

Resumen

Un experimento exploró los impactos de la familiaridad entre ratas hembras en la conducta pro-social. Ratas de la cepa Wistar fueron entrenadas en parejas en la Tarea de Elección Pro-social. Todas las parejas estaban compuestas por un actor y un compañero que fueron colocados dentro de un laberinto en forma de doble T. A lo largo de las sesiones, los actores decidieron entre dos opciones para entregar alimento a su compañero. En la *opción egoísta* sólo el actor recibió alimento, mientras que la *opción pro-social* producía alimento adicional para el compañero o para un juguete en forma de rata. La mitad de los actors vivieron en la misma caja con sus compañeros rata durante el experimento (Grupo Familiar), mientras que la otra mitad únicamente tuvo contacto en el laberinto doble T (Grupo Extraño). Encontramos que las ratas actor

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en el Grupo Familiar mostraron niveles más altos de elecciones pro-sociales en la condición en la que estaban con su compañero, en comparación en la condición en presencia de un juguete; mientras que las ratas actor en el Grupo Extraño mostraron niveles similares de elección pro-social tanto en presencia de su compañero como en presencia del juguete-

Palabras clave: Conducta social, familiaridad, ratas, respuestas pro-sociales.

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Social behavior is defined by any interaction that an animal displays towards another, and results in relations with different durations (Blumstein, et al., 2010). Pro-social behavior is part of social behavior and is defined as any behavior that increase the welfare of one of the individuals of the interaction and does not imply any disadvantage to the individual that displays the pro-social behavior (Batson, & Powell, 2003; Wittek, & Bekkers, 2015). An important difference of pro-social behavioral with altruism is related to the advantages and disadvantages (Reynoso-Cruz, & Bernal-Gamboa, 2019). Altruism has a cost (energy or time) and could put in danger the survival of and animal that performs the altruistic behavior, while prosociality does not have any cost or the cost is really small for the individual that displays the behavior (Wittek, & Bekkers, 2015).

Since complex social behavior like pro-sociality needs a complex cognitive system to process the information needed to behave pro-social towards another subject, this ability was considered exclusive of humans and their primates relatives like bonobos and chimpanzees (Paul, 2000; Angantyr, et al., 2011; Jensen, et al., 2014). However in the last decade a series of experiments have suggested that complex social behavior may be shared by all social species, implying that pro-sociality might be found in rodents (Grenier, & Lüthi, 2010; Bartal, et al. , 2011; Watanabe, 2011; Meyza et al., 2017; Ueno, et al., 2019).

In 2011 an experiment showed that rats were able to rescue a companion in need (Bartal, et al., 2011). In this experiment a rat was place inside a tubular container that restrict the rat movement and could be open only from outside. The trap was placed in an arena with a free companion that after some days learn how to set-free the trap rat. This behavior was considered pro-social since the energetic cost for helping their companion was low (Wittek, & Bekkers, 2015). One concern of these experiments was the impact of distress in the trapped and free rats as a trigger of helping behavior. To avoid the problems associated with the task and the trap, a new task previously used with chimpanzees was adapted to rats (Horner, et al., 2011).

In this new task a double T-maze made of clear acrylic was used, thus, rats were able to see, smell and hear between them. The Ts were placed one opposite to the other with the terminal arms one in front of the other (see Hernandez-Lallement,van Wingerden, Marx,Srejic & Kalenscher, 2015). A pair or rats (unknown to each other) were placed inside this double T-maze, one rat played the role of *actor* and the other one was the *partner*. The actor had two choices, she could go to the pro-social option (producing food to the actor and the partner) or to the selfish option (producing food only to the actor). Note, that this paradigm fits to the definition of pro-sociality since the actor's choice does not involve any cost (i. e., she always receives food).

Hernandez-Lallement et al., found that most of the actor rats prefers the pro-social option (both actor and partner receive food) and these choices increase over time. Those authors ran a control group that involved the actor rats with a toy-rat (with the same size and color of a real rat), and they reported that the actor rats were indifferent between the pro-social option and the selfish option, implying that the result in their experimental group may be considered as a pro-social behavior that is performed only in the presence of a real rat, and is not just an exploratory behavior (Hernandez-Lallement, et al., 2015).

Notably, in the same year another research group found similar results using an automatized version of the double T-maze (Marques, Rennie, Costa & Moita, 2015). Marques et al., reported that rats that knew each other preferred the pro-social arm more frequently when they were with a rat than with a toy-rat.

Both above-mentioned findings show a clear effect of pro-social behavior in rats. However, the mechanisms that underlies that behavior are not quite understood. Some authors have proposed that pro-social behaviors may be motivated by empathy (Bartal & Mason, 2018; Bernal-Gamboa, 2017). In particular, the Russian doll model of empathy (see de Waal & Preston, 2017) have straight implications that can be experimentally tested. According to this model, empathic abilities are not exclusive to humans, but shared by different animals, and these abilities are divided in three levels of empathy: emotional contagion, concern for others and perspective taking. All three levels are mediated by a Perception-Action Mechanism (PAM) that is activated when the observer perceives (and in some cases shares) the affective state of the observed. Since, it has been noted that the PAM activates more easily between known or familiar subjects, the main goal of the present experiment was to evaluate whether rats that lived together show higher rates of pro-social choices, than rats that lived in different cages.

Method

Subjects

Twenty-four female Wistar rats from the animal facilities of the Faculty of Psychology of the National University of Mexico were used. They were about six and a half months old and experimentally naïve at the beginning of the experiment. They were housed in groups of four in methracrylate cages (21 x 94 x 46 cm, height x width x depth) inside a room maintained on a 12-12 hr light-dark cycle (07:00 onset and 19:00 offset of lights). The temperature of the colony room ranged between 20–25 °C, while the humidity value was 45–60%. All subjects were maintained with ad libitum access to water but were food-deprived to 85% of their initial body weights throughout the experiment.

Material and apparatus

We used a double T-maze (see, Figure 1). The Ts were divided by a transparent wall of 5 mm made from Plexiglas. This wall was cover with 1.5 mm holes that allows rats to hear and smell between them. The lateral walls and the start arm were made from foam PVC and the floor and the lid of the apparatus were made of transparent plexiglas. Since the floor was transparent, we use a white background during all the experiment. Plastic tubes were place in the terminal arms and served as pellet dispenser. Purina pellets of 0.45 g from Bio Serv were used as reward.

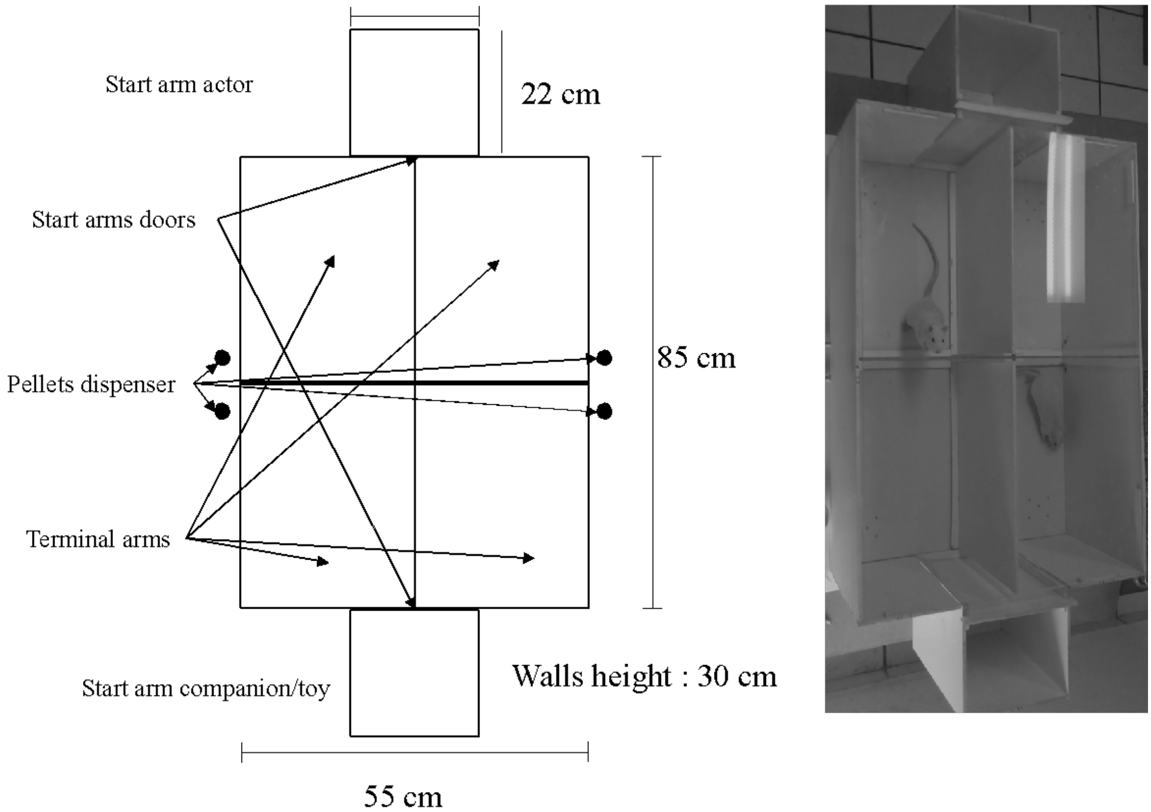

Figure 1. Design and measures of the double T-maze. Left panel show a general scheme of the apparatus, measures and disposition of the arms. Right panel shows a real picture of the apparatus with a pair rats during training.

Procedure

The present experimental protocol was conducted under strict agreement of the guidelines established by the Ethical Committee of the Faculty of Psychology of the National University of Mexico. The rats were trained for seven days to move from the start arm to the terminal arms and to come back the end of the trial. The objective was to reduce the contact of the researchers with the animals. The first day of training the rats were placed in the maze to move freely in the apparatus for 20 minutes. Every rat of a pair was set in one of the start arms, and the doors of this arms were removed. The second day the rats were placed in their respective start arms without doors and two pellets were delivered every four minutes in every terminal arm (20 pellets per subject).

In the third day of training the doors of the start arms were set and two pellets were placed inside the start arms. After the individuals consume the pellets the door was open and the individual could choose between the terminal arms, once the individual move to one of the terminal arms the doors were closed, two pellets were set inside the start arm, and two pellets were delivered in the terminal arm that the subject chose. Once the individual ate the pellets in the terminal arm, the doors were open, and the individual could return to the start arm and eat the pellets and start again. This session was run for 20 minutes. For the next two days the same procedure from day 3 was conducted of day three but the pellets in the start arm were delivered only in half of the trials. During day six the pellets in the arms were delivered only in a quarter of the trial and finally during day seven, no pellets were delivered in the start arm. If the individuals choose the same arm three consecutive times, we blocked that terminal arm, and we forced the rats to go in three consecutive trials to the opposite arm.

The objective of this pre-training during day three was to teach the animals how the reward would be delivered and how they can return to the start arm to begin a new trial. If the individual did not come back to the start arm in 35 seconds, they were gently carried back to the start arm. The individuals that eat more rewards since day three were chosen as the actors and their pairs were chosen as the partners. During the training none of the individuals developed a bias.

Experiment

The experiment was divided in two conditions of 10 sessions each. During the first condition the actors and their partner rats worked together, and during the second condition the actors worked with a toy-rat. Each session consisted in 10 forced trial and 15 free choice trials. The terminal arms were called pro-social and selfish. In the pro-social arm, the actors and their partners received two purina pellets as reward for each trial. In the selfish arm only the actors received the pellets, while their partners could see and heard the actors eating the pellets. The arms were randomly selected every day to avoid spatial biases. All the trials lasted 45 s. During the 10 first forced trials the rats learn the daily position of the arm and the visits were ordered randomly (five trials to the selfish arm and five trials to the prosocial arm).

Trial structure

A trial started with the actor and the partner in their correspondent start arm. The door of the actor was open, allowing to the actor entered in one of the terminal arms. After 10s, the door of the partner was open and the rat was directed to the same side the actor choice (e. g., if the actor was in the right arm the companion was directed to their left arm). Fifteen seconds after the trial started, both rats (or the toy) received food, as long as the actor choice was the pro-social arm, but if the actor choice was the selfish arm the reward was delivered only to the actor. 30s later the doors were open again, allowing for the rats to return to the start. If a rat refuses to come back, they were gently carried by the researches to the start. Rats were weighted at the beginning and at the end of the sessions to control their weight (see Figure 2 for a detailed example of the trial structure).

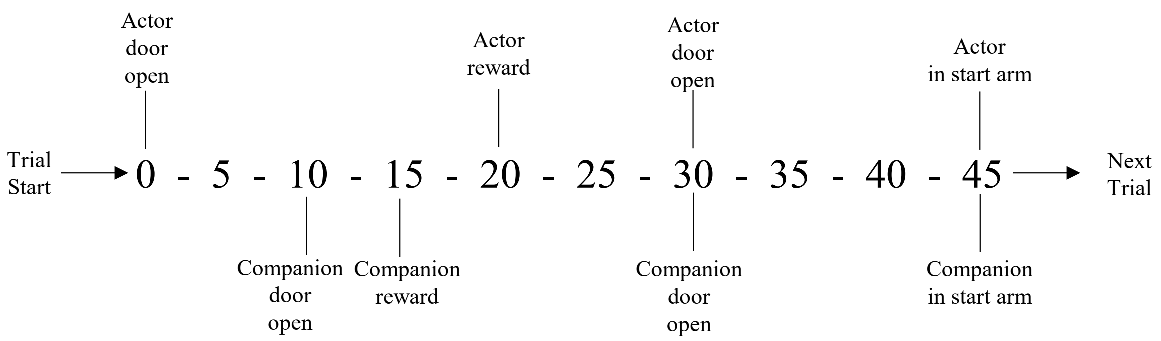

Figure 2. Trial structure and schedule of the events used during the force and free choice trials. The same structure and schedule were used in the block with the toy.

Statistical Analysis

For all the experiments presented, mean pro-social choices per group were compared using analyses of variance (ANOVA). The rejection criteria were set at $p<.05$, and effect sizes were reported using partial eta-squared (η_p^2).

Results

Figure 3 shows the percentage of pro-social choices performed by rats in the Strange Group. The mean percentage of the pro-social choices towards a partner rat was 54.11; whereas the mean pro-social choices towards a toy-rat was 51.77%. A 2 "Choice" (Selfish vs Pro-social) x 2 "Companion" (Partner vs Toy) x 10 (Session) only found a significant main effect of Choice factor $F(1, 19) = 17.83$, p=.0004, η_p^2 = .49. However, the main factor Companion $F(1, 19) = .01$, p=.92, nor the main factor Session $F(9, 171) = .02$, p= .99 were significant. In addition, the Choice x Companion interaction $F(1, 19)= 3.00$, p= .09 and the triple Choice x Companion x Session interaction did not reach significance $F(9, 171) = 1.16$, p= .32. Those findings indicate that rats in the Strange Group performed a similar number of pro-social choices regardless of whether the companion was a partner rat or a toy.

It is important to note that in this experimental task pro-sociality is estimated by the ability of the actor rat to distinguish and behave differently among conditions, thereby an actor rat shows pro-sociality when choices the pro-social option only towards a partner rat and not to a toy-rat. Thus, if an actor chooses the pro-social option the same amount of times during the toy-rat and the partner rat condition it's consider not pro-social, since it's not capable to act pro-social when is needed.

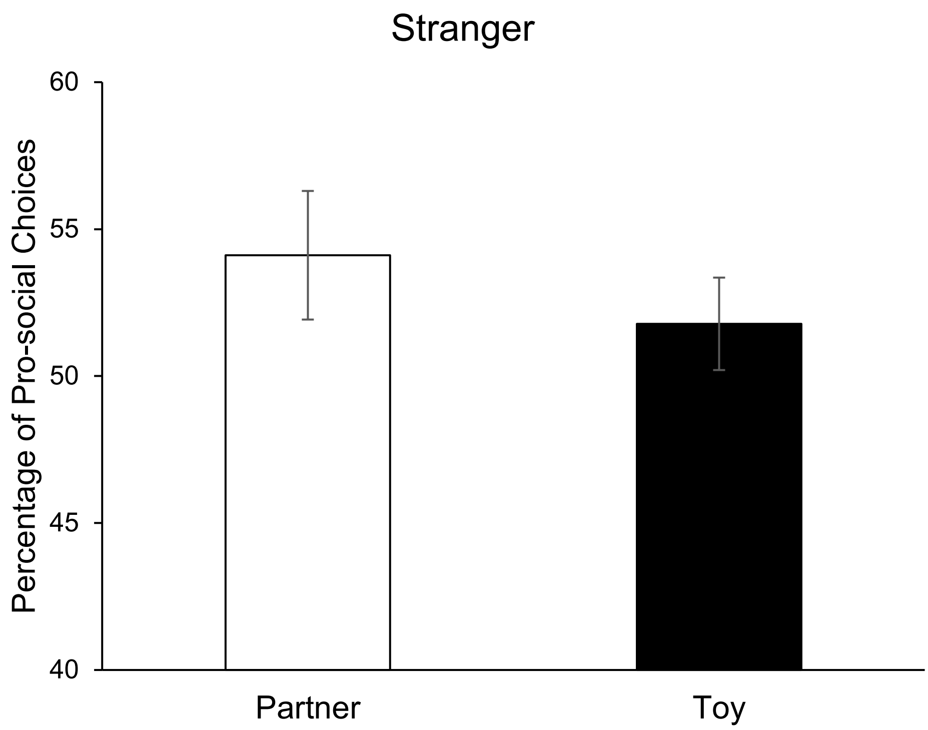

Figure 3. Mean percentages pro-social choices in the Strange Group towards a partner rat and a toy rat.

Figure 4 shows the percentage of pro-social choices performed by rats in the Familiar Group. The mean percentage of the pro-social choices towards a partner rat was 54.33; whereas the mean pro-social choices towards a toy-rat was 50.22%. A 2 "Choice" (Selfish vs Pro-social) x 2 "Companion" (Partner vs Toy) x 10 (Session) found a significant main effect of Choice $F(1, 19) = 29.37$, p=.0002, $\eta_p^2 = .53$, and Companion F(1, 19) = 9.25, p=.006, $\eta_p^2 = .32$. The main factor of Session was not significant, F<1. More importantly, In addition, the Choice x Companion interaction F(1, 19)= 7.53, p = .01, $\eta_p^2 = .27$ and the triple Choice x Companion x Session interaction were significant F(9, 171) = 3.22, p= .001, $\eta_p^2 = .13$. Subsequent planned comparisons exploring the triple interaction shows that rats in the Familiar Group performed higher levels of pro-social choices towards a partner rat, F(1, 19) = 6.95, p= .01, $\eta_p^2 = .19$. Moreover, those choices were performed consistently throughout the experiment, smallest F = 39.22, p= .001, $\eta_p^2 = .68$.

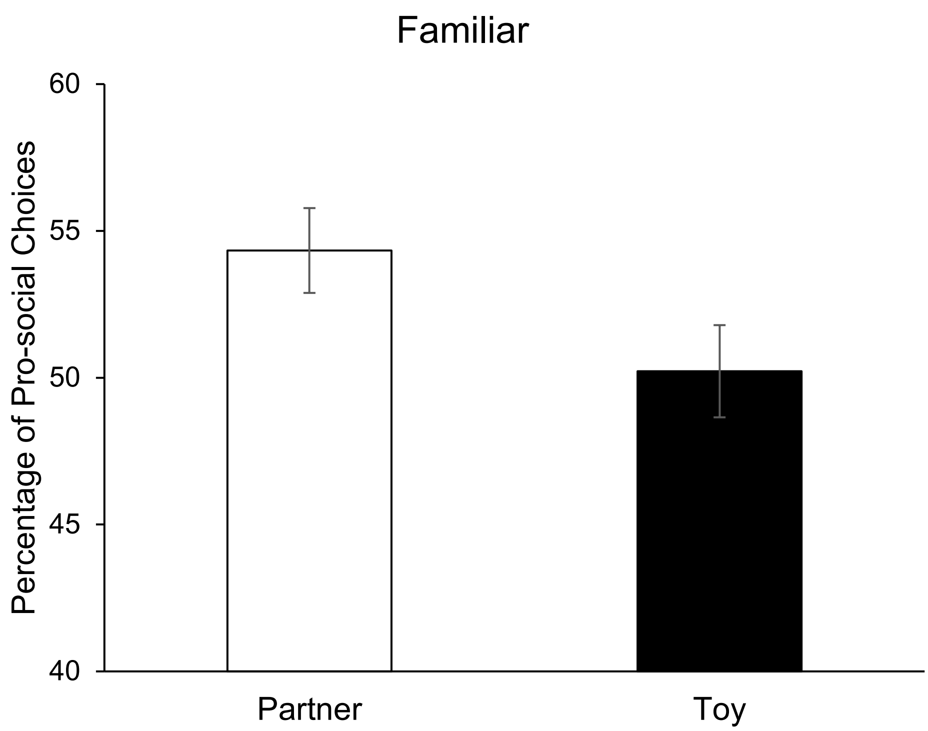

Figure 4. Mean percentages pro-social choices in the Familiar Group towards a partner rat and a toy rat.

Figure 5 shows the accumulated choices to both pro-social (positive values) and selfish (negative values) options towards a partner rat or a toy-rat for Stranger and Familiar Groups. The data show an increase tendency of the actors in the Familiar Group to choose the pro-social option more often towards a partner rat almost since the beginning, and increased as the experiment progressed, while the selfish option was chosen in more occasion when the companion was a toy-rat. The actor rats in the Stranger Group, show a similar level of pro-social choices towards a partner rat and a toy-rat. Note that actor rats in the Strange Group started to perform higher levels of pro-social choices towards a partner rat almost at the end of the experiment (after 125 trials elapsed).

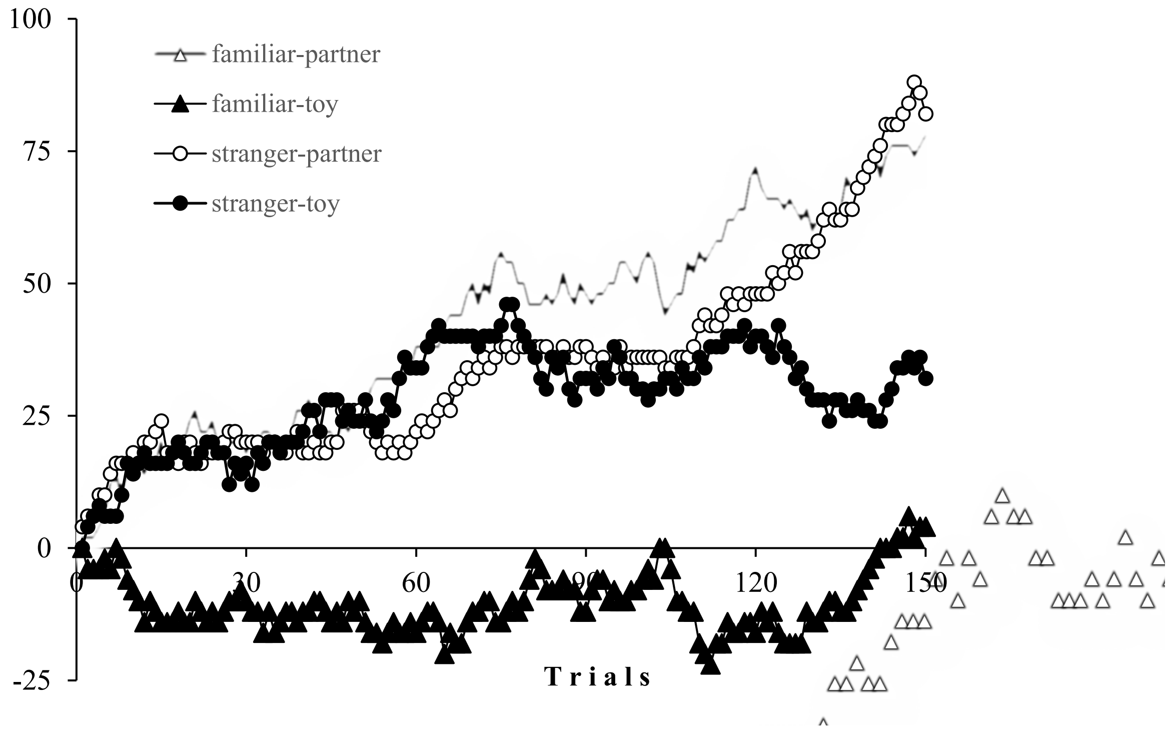

Figure 5. Cumulative trials for pro-social choices (positive values) and selfish choices (negative values) for all Strange and Familiar Groups.

Discussion

One experiment with rats showed that familiarity (understood as a coexistence within the same homecage) might be a variable that facilitates pro-social behavior, given that actor rats in the Familiar Group shows higher levels of pro-social choices toward a partner rat than a toy-rat compared with the actor rats in the Stranger Group. Nevertheless, it is important to note that the data in the cumulative analysis shows that the actor rats in the Strangers Group increase their pro-social choices towards a partner rat rather a toy-rat in the last 25 trials which suggests that after all the interaction within the double T-maze with a stranger partner rat, actor rats could develop a certain bond of familiarity with their partner rats, which is consistent with our suggestion about the importance of the familiarity as a factor that modulates the pro-social behavior.

Our results are consistent with previous findings that suggests that familiarity also play a role in helping behavior (Bartal et al., 2014). In those experiments where rats learn to release another rat trapped in a tubular restrainer, it has been noted that helping behavior may be facilitated if rats received 14 days of pre-exposure (in our experiment, actor rats in the Stranger Group started to behave pro-socially after 7 days of exposure to the partner rat).

Even that one experiment is not enough to elucidate the mechanism underlying the pro-social behavior in rats, the present data indicates that the PAM may play a key role in how the rats display pro-social behavior towards others, because actor rats that lived in the same homecage with its partner rats, shows pro-sociality since the beginning to the experiment, supporting the idea that the PAM is easily activated between acquaintances (De Waal, & Preston, 2017).

Although our finding is consistent with the PAM proposed by the Russian doll model of empathy, it is important to note that there are other possible accounts for our data. For example, some authors have proposed for other pro-social experiments with rats that these behaviors might be explained by argued that subjects changes its behavior to increase the contact with other subjects (Silberberg et al., 2014), thereby, this hypothesis would predict a similar levels of choices in the selfish and pro-social arms (because, the relevant factor for the actor rat would be close to the partner rat), however, our data indicates a preference for the pro-social arm, indicating that social contact is not enough to produce the pro-sociality.

The positive reinforcement hypothesis is based in the idea that rats increase their choices to the pro-social arm to see their companions eating (Epstein et al., 2007). The problem with this hypothesis is that predicts a similar level of pro-social choices between groups (Familiar/Strangers), nevertheless, our data show a difference in the number of pro-social choices between groups. A third account may be comprised by the negative reinforcement hypothesis that proposes that individuals displays specific ultrasonic vocalization during aversive situations (Takahashi, et al, 2010). The prediction of this hypothesis would be that the actors chose the pro-social option to decrease the aversive vocalizations of the partner (this should occur even in the Strangers Group). However, our data were inconsistent with this hypothesis since the familiarity had an effect in the number of pro-social choices.

Even that our experiment was based in a previous work of Hernandez-Lallement, et al., (2015) there was a series of differences in our procedure (i. e., size of our sample, sex of our rats and the strain of the rats) that is worth to mention. The size of the sample increases the reliability of the data as is an importance factor in the size of the effect. The original study worked with 68 rats compared with the 24 rats used in our study. In this sense our experiment show small and medium size of effects in some of our statistical analysis and a bigger sample could increase this value of our effect size.

Previous work in a double T-maze procedure was able to test pro-social behavior in Sprague Dawley (Marquez et al., 2015) and Long Evans (Hernandez-Lallement, et al., 2015; Hernandez-Lallement, et al., 2016; Oberliessen, et al., 2016). Testing different strains of rats is important for experimental procedures since the strains could response different to the conditions of our experiment. In a previous work of our research group, we showed that Wistar rats were not so sensitive to help a companion in a water tramp without training (Bernal-Gamboa et al., in press), even when a previous study with Sprague-Dawley was able to produce this behavior without training (Sato, et al., 2015). Given that some authors have reported behavioral differences of different rat's strains in relation to distress situations and response to anxiolytic treatment (Rex etal., 1996; Rex et al., 2004; Nosek et al., 2008), it will be important to explored to test the generality of our finding across different strains and tasks.

It has been argued that sex plays an important role in empathy and pro-social behavior. Previous experiments in rats had shown that females are more able to help a trapped companion compared with males (Bartal, et al., 2011). In experiments related to emotional contagion females where more sensitive than males (Langford, et al., 2010). In this sense we suggest for future research to considered females rats as adequate subjects (i. e., it is easier to detect) to test pro-sociality and empathy in laboratory models.

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