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Environmental Enrichment and Prosocial Behavior in Wistar Rats: an Exploratory Study

Enriquecimento Ambiental e Comportamento Pró-social em Ratos Wistar: um Estudo Exploratório
Enriquecimiento Ambiental y Conducta Prosocial en Ratas Wistar: un Estudio Exploratorio

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Abstract

This research aimed to experimentally explore how rats reared in environmental enrichment (EE) and in isolation (ISO) behave in the prosocial behavior liberation task. The subjects were reared in two conditions: EE and ISO, and then submitted to the task. The results suggests that EE subjects spent more time near the restrainer, displayed faster door-opening behavior but interacted less with the released conspecific task compared with the ISO subjects. Our preliminary findings suggest that the rearing conditions may affect prosocial behavior, and that EE may increase the probability of displaying prosocial behavior. We discussed the need of more studies about the effect of rearing conditions and how it may impact motivations associated with the prosocial behavior and its consequences.

Key words: rats, environmental enrichment, prosocial behavior, helping behavior.

Resumo

O presente estudo teve como objetivo explorar experimentalmente como ratas criadas em uma condição de Enriquecimento Ambiental (EE) ou em Isolamento (ISO) pode afetar o comportamento pró-social, utilizando a tarefa de liberação. Para isso, os sujeitos foram expostos a duas condições: EE e ISSO, e então foram submetidos à tarefa de liberação. Os resultados apontam que os sujeitos criados na condição EE tendem a passar mais tempo próximo do restritor, liberar mais rápido, porém interagir menos com o coespecífico liberado em comparação com os animais criados em ISO. Se discute a necessidade de estudos sobre o efeito das condições de crescimento no comportamento prósocial, nas motivações associadas a este comportamento e as suas implicações.

Palavras-chave: ratos, enriquecimento ambiental, comportamento prosocial, comportamento de ajuda.

Resumen

El presente estudio tuvo como objetivo explorar experimentalmente como ratas criadas en una condición de enriquecimiento ambiental (EE) o en aislamiento puede afectar la conducta prosocial utilizando la tarea de liberación. Para esto los sujetos fueron creados en dos condiciones: EE y ISO, y entonces expuestos a la tarea. Los resultados apuntan que los sujetos criados en EE tienden a pasar más tiempo cerca al restrictor, liberar más rápido, pero interactuar menos con el coespecífico liberado en comparación con los animales criado en ISO. Fue discutida la necesidad de estudios sobre como las condiciones de crianza pueden afectar la conducta prosocial, las motivaciones asociadas a la misma y sus consecuencias.

Palabras clave: ratas, enriquecimiento ambiental, conducta prosocial, comportamiento de ayuda.

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This research aimed to experimentally explore how rats reared in environmental enrichment (EE) and in isolation (ISO) behave in the prosocial behavior liberation task. The subjects were reared in two conditions: EE and ISO, and then submitted to the task. The results suggests that EE subjects spent more time near the restrainer, displayed faster door-opening behavior but interacted less with the released conspecific task compared with the ISO subjects. Our preliminary findings suggest that the rearing conditions may affect prosocial behavior, and that EE may increase the probability of displaying prosocial behavior. We discussed the need of more studies about the effect of rearing conditions and how it may impact motivations associated with the prosocial behavior and its consequences.

Palavras-chave: rats, environmental enrichment, prosocial behavior, helping behavior.

Prosocial behavior refers to any action voluntarily performed by an individual that may increase the probability of ensuring the welfare of conspecifics; it requires a behavioral cost for a donor and a -mandatory- benefit for a recipient (Anacker & Beery, 2013; Cronin, 2012; Dovidio et al., 2017; Rault, 2019).

Studies with animals have shown that prosocial behavior is not a unique characteristic of humans. For instance, researchers have extensively observed the expression of prosocial behavior in primates (see Colman et al., 1969; De Waal & Preston, 2017). Recently, the development of models that study prosocial behaviors under laboratory conditions have shown that rodents also express these behaviors (e.g Bartal et al., 2011, 2014, 2016; Hiura et al., 2018; Sato et al., 2015; for a review see Mason, 2021) moreover, it has allowed the exploration of variables that may expose the evolutionary roots of prosocial behaviors (Meyza et al., 2017; Schroeder & Graziano, 2015).

In order to observe the prosocial behavioral in rats, Bartal et al., (2011) designed a task in which a rat is trapped into a restrainer and assessed whether a free rat would learn to release the trapped conspecific. Using this model, Bartal et al. found that (1) rats learn to open a door to release a trapped rat without prior training, (2) the door-opening behavior is maintained by its consequences because the probability of such behavior increases with the sessions once the subject learned to release the conspecific and (3) rats seems to discriminate whether the restrainer contained a conspecific, since the subjects did not open empty restrainers or one containing a toy rat.

Two main explanations have been presented to the behavior observed using the trapped rat task. The first one asserts that the prosocial behavior is motivated by an emotional contagion process from the stress induced to the trapped by the restrained condition (Emotional Contagion Hypoteses; Bartal et al., 2011; Bartal et al., 2016). So from this perspective prosocial behavior is a behavior mantained by negative reinforcement result of the elimination of the emotional stress of both rats. The second hypothesis is that prosocial behavior is maintained by the positive reinforcement effect of social interaction (Evans et al., 1994; Silberberg et al., 2014; Hiura et al., 2018).

Both hypotheses have been supported by the literature, from one side, the restraining procedure is well known as a stressor that inducing specific autonomic responses (e.g. ultrasonic pain vocalizations or pheromones) that produces emotional contagion in rats (see review by Keysers et al., 2022) and the prosocial behavior is reduced when an anxiolytic is administered to the trapped rat (Bartal et al., 2016). By the other side, Hiura et al., (2018) have demonstrated that social behavior has been maintained by the reinforcing properties of social interaction (see also Evans et al., 1994 and Silberberg et al., 2014).

Despite of the underline hypothesis, in the last ten years, some researchers have used the trapped rat task to assess the variables that may influence the appearance of prosocial behavior (Hachiga et al., 2018, 2020; Silberberg et al., 2014). For instance, in a subsequent study Bartal et al., (2014) found that free rats released strangers of their own strain, but they avoided doing so with strangers of an unfamiliar strain. However, after two weeks of pair-housing with a member of the other strain, the experimental subjects started to release strangers of that strain. The researchers also fostered pups with litters of another strain and observed that the rats displayed helping behavior towards strangers of their adoptive strain, but not to stranger of their own strain. These findings suggest that prosocial behavior may depend on the individual's social experience and social context.

Even though social experience modulates the emission of prosocial behavior in the trapped rat task, as demonstrated by Bartal et al., (2011), it is still unclear how physical and social characteristics of the development environments may affect the prosocial behavior under the helping behavior paradigm. Neal et al. (2018) conducted a study that answers partially this question. They evaluated the relationship between rearing conditions and social interest behaviors by comparing the approaches of rats reared in different environmental conditions toward a trapped rat (i.e., social investigation task). In this study, the subjects were reared in an environmental enrichment (EE) condition - animals housed in groups with physical and sensorial stimulation-, social control –animals housed in groups but without any kind of physical and sensorial stimulation- and isolation (ISO); and finally, the subjects were exposed to the trapped rat. They considered latency of approaching to the tube and behaviors related to the tube such as digging and sniffing around, climbing on top and touching or biting as indicators of social interest (i.e., free subjects had no possibility of helping or displaying prosocial behavior). The researchers found no difference on latency of approaching; EE animals exhibit more digging bouts directed toward the trapped conspecific and displayed more exploratory behaviors related to the conspecific than to the environment. The authors discussed the data as a possible influence of EE by facilitating social behaviors as the result of the early exposure of the subjects to complex environments in the rearing.

These results suggested EE as a related variable of prosocial behaviors that may help to clear the pathways of prosociality. Therefore, the exploration of the interaction between prosociality and the development environments would help us to understand better the modulating variables of prosocial behavior. Considering that this, we propose an exploratory study in which we seek to explore if the rearing conditions produced some tendencies in the prosocial behavior, but also to evaluate if the parameters used in our laboratory would allow us an explanatory approach in the future.

An approach from an experimental exploratory perspective enables researchers to manipulate the conditions to pursue a non-theory driven hypothesis (i.e., even though exploration is always attached to theory); its value relies in the nature of discovery itself, it helps to structure empirical regularities in order to expand the understanding scope of a behavioral phenomenon (Franklin, 2005; Stojanovic, 2013; Waters, 2007). We designed an exploratory study to evaluate whether the rearing conditions may affect the prosocial behavior in rats. To do so, we carried out a study that aimed to evaluate how rats reared in EE and ISO condition could behave toward a trapped conspecific, using Bartal et al., (2011) trapped rat task.

Different from the original studies, that allowed only one door-opening per session (e.g. Bartal et al., 2011), we modified their original task to let the subjects display the door-opening behavior three times per experimental session with the purpose of allowing them to interact more with the task and therefore facilitate the acquisition of the door-opening prosocial behavior. Once the rat presented the door-opening behavior, they were allowed to interact for a few minutes and the experimenter proceeded to restrain the rat again and a new trial began. As to the original studies, in our experiment the only explicit consequences planned to the door-opening was to release the other subject (see procedural details in methods section).

To evaluate how the rearing conditions might modify the interactions between subjects once the prosocial behavior was emitted, we also analyzed as a dependent variable: the social contact between conspecifics during the experimental sessions. We measured then: the social interactions of free rats towards the trapped rats before door-opening behavior, the latency of trapped rat releases and the social interactions of the rats after the door-opening behavior.

Method

Subjects and Rearing Conditions

Sixteen naive male Wistar rats were obtained from Colombian National Institute of Health. Rats with twenty-one days old at arrival in our laboratory, were immediately assigned randomly to one of two rearing conditions: EE or ISO (see Figure 1). ISO subjects were housed individually in polypropylene cages (40 x 27 x 20 cm). EE subjects were housed in cages (60 x 35 x 32cm) with social interaction and physical stimulation. For social interaction the EE animals were house in groups of four subjects and for physical stimulation different objects were used. The objects were replaced within the cage every three days and were divided into four intended functions: shelter objects, climbing objects, manipulation objects and escape objects (see Supplementary Material 1 with photo and list of the objects per category). The cages had four objects -one object per function- and sixteen objects were used to cover all functions. Animals were kept on a12-hr light/dark cycle with food and water provided ad libitum (i.e. no food or water deprivation), humidity (40-70%) and temperature (18-26 °C) was kept constant under the standards of the colony room. The subjects that were part of the experimental dyads had no previous interaction before the experimental phase; each group condition had four experimental dyads. The EE subjects were housed so that a door-opener rat released a trapped rat housed in the other cage, in order to avoid further interactions between dyad after sessions. Experimental sessions were conducted during the light cycle. All protocols are in accordance with Colombian legislation of animal research and were approved by the Ethical Committee at San Buenaventura's University (001-2019).

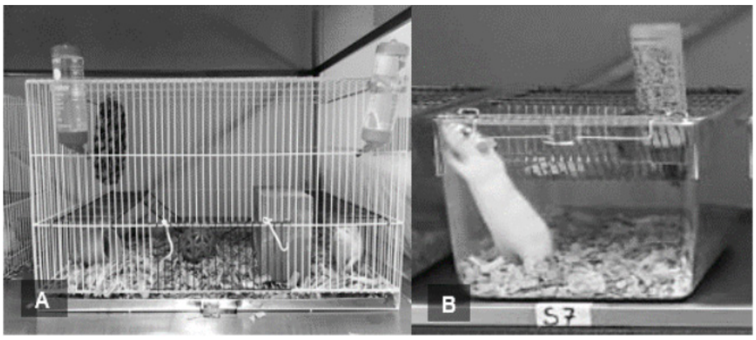

Figure 1. Examples of rearing conditions for each group. Panel A: EE subjects were housed in groups of four animals per cage with four stimulation objects. Panel B: ISO subjects were housed individually per cage with no stimulation objects.

Apparatus

An acrylic restrainer tube (22 x 8 x 8 cm; see Figure 2) was adapted from Bartal et al. (2011). The restrainer had holes located on the lateral and posterior side that allows the rats to have olfactory, auditory, and tactile interaction between them. The restrainer door was inserted in one of the six slots located at the front part of the restrainer, it was designed to be opened only from the outside by the free rat either with its snout or with its paws. The door was devised with two panels attached to each other; the front panel was shorter to allow the free rat to open the door with its snout. To reduce the response cost of door-opening behavior, a metallic weight (45,4 g) was supported by a shape "L" screw located in the upper right corner of the door. A testing arena made of black acrylic with 16 quadrants (60 x 60 x 30 cm) was used for the experimental session (see Figure 2). All experimental sessions were recorded with a video camera to later behavioral analysis (see Supplemental Material 2 with a video).

Liberation Task

All experimental sessions consisted of a maximum three trials. In beginning of each trial, a rat was trapped into the movement restrainer, and it was placed in the center of the testing arena. Then, a free rat was placed in the corner marked of the arena to start one discrete trial. The free rat was allowed to explore the testing arena and interact with the trapped conspecific. If the free rat released the trapped one, by opening the door using its paws or its nose, the rats were allowed to behave freely for 3 minutes, and then other trial was initiated if the free rat did not display the door-opening behavior the rat remaining in the arena until de end of the session. Once the experimental session ended, the rats were removed, and the testing arena and the restrainer were cleaned. The experimental sessions finished if a rat released three times the trapped rat in the same period or if the maximum length of 30 minutes was reached. If the rat displayed no door-opening behavior within the first 25 mins, the researcher partially opened the door by 90° o decrease the response cost and facilitate door-opening behavior by the rat. In case that the trapped rats learned how to open the door from the inside, a blocker (an acrylic alike the second panel of the door) was implemented until the end of the experiment to prevent escape behaviors.

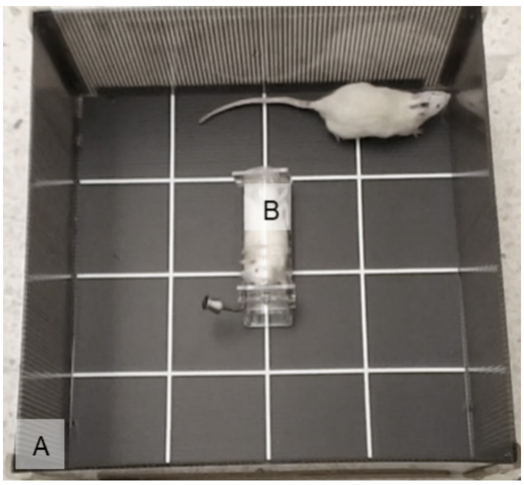

Figure 2. Experimental apparatus used for the liberation task: (A) the testing arena and (B) the restrainer adapted from the description of Bartal et al., (2011).

Procedure

Rearing Phase. Subjects were randomly assigned to one of the rearing conditions (EE or Isolation); the rearing phase took from postnatal (PN) day 21 to postnatal day 78. No experimental sessions were carried out in this phase. Researchers weighted and daily handled the subjects.

Experimental Phase. This phase was carried out the next day after the end of the rearing phase and consisted in four experimental manipulations: habituation to the experimental arena, time-out, restrainer habituation and liberation task. The habituation was carried out on PN day 79 and 80, the subjects were placed individually into the testing arena for 30 minutes to decrease exploration behaviors for preserving the novelty of the situation during the liberation task. To assign the subjects to the role of trapped or door-opener subject, a time-out test was carried out on PN day 81. During the time-out test each subject was individually located into a polypropylene cage and the upper grid was removed; immediately after the removal, the latency of placing one of its paws on the edge of the cage was measured. The fastest subjects were assigned as door-openers. Restrainer habituation was carried out on PN days 82-86, the experimental dyads were located for 30 minutes in the testing arena with the restrainer opened with no trapped rat to decrease exploratory behaviors toward the restrainer *per se*. Finally, the liberation task was carried out on PN days 87 to 97.

The experimental sessions were conducted during ten consecutive days at approximately the same time each day (from 8:00am to 2:00pm; see the Procedure Timeline in Figure 3).

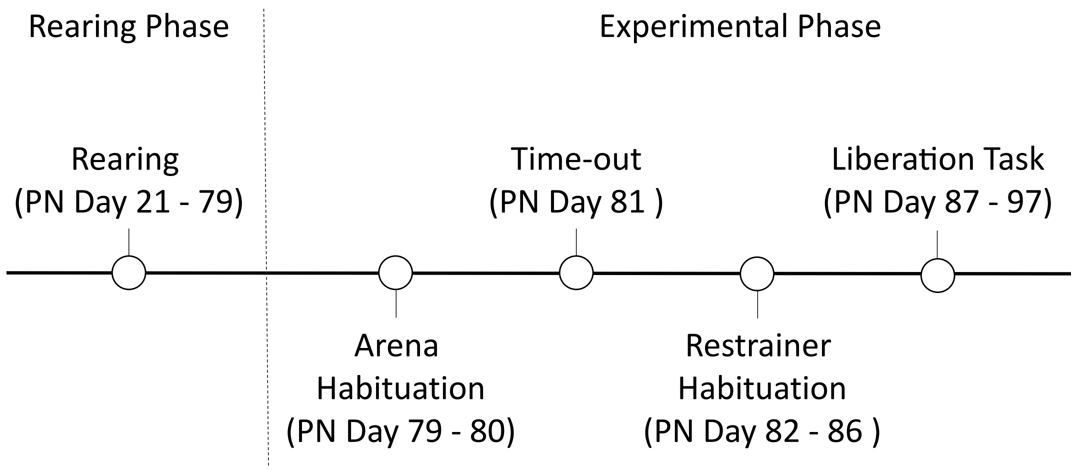

Figure 3. Timeline of the Experimental Procedure.

Measures and data analysis

All experimental sessions were recorder for analyzing three categories: before door-opening behaviors, prosocial door-opening behavior and after door-opening behaviors. *Before door-opening behaviors* had two categories operationally defined as: 1) the latency (in seconds) of approaching the restrainer with the trapped conspecific in the first trial of every experimental session and 2) the percentage of time per session that the door-opener spent near the restrainer, in the center of the arena.

Door-opening prosocial behavior was measured as the latency (in seconds) of the first door-opening per experimental session. *After door-opening behaviors* were operationally defined as: 1) frequency and 2) length of social interactions between animals per session. Due to the exploratory nature of the study and the small number of subjects in each condition, the behavioral measures were analyzed only descriptively (i.e., individual data, range, median (*Mdn*) central tendency, mean ($\bar{X}$) central tendency and standard deviation) to understand and possibly identify tendencies in the data between groups.

Results

Before Door-Opening Behaviors

The latency of approaching the trapped rat showed a tendency of the subjects to behave similarly. The EE subjects (*Mdn* = 5.2, $\bar{X}$ = 5.5, SD = 1.5) took between 4 and 7 secs (Range) to approach the trapped rat while the ISO subjects (*Mdn* = 5.4, $\bar{X}$ = 5.6, SD = 1.1) approached to the trapped rat ranging between 5 secs to 7 secs (Figure 4A). However, the percentage of the time near the restrainer shows that the EE subjects (*Mdn* = 29, $\bar{X}$ = 29.5, SD = 11.7) spent more time near the trapped rat before releasing compared with the ISO subjects (*Mdn* = 18, $\bar{X}$ = 19.1, SD = 5.6; Figure 4B). The EE subject that spent most of their time sessions behaving near the trapped rat spent 42%, meanwhile the ISO subject spent 27% of their time sessions.

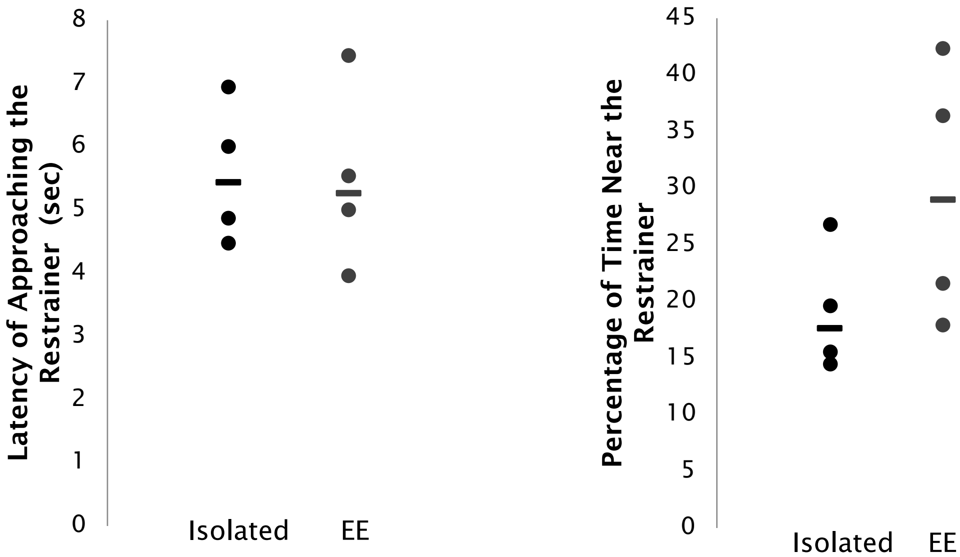

Figure 4. Social Interactions Before Door-Opening Behavior. The dots represent the median (*Mdn*) of all liberation sessions of each subject and the lines the median of the group. Panel A: Median latency (in seconds) of approaching to the trapped rat for each experimental subject, EE subjects *Mdn* = 5.2; ISO subjects *Mdn* = 5.4. Panel B: Median percentage of time near the restrainer interacting with the trapped rat for each experimental subject, EE subjects *Mdn* = 29.0; ISO subjects *Mdn* = 18.0.

Door-Opening Prosocial Behavior

The latency of the first door-opening behavior of each experimental session suggests that the EE subjects (*Mdn* = 292.3, $\bar{X}$ = 311.5, SD = 224.3) seemed to open faster the restrainer than the ISO subjects (*Mdn* = 656.5, $\bar{X}$ = 598.8, SD = 377.4; Figure 5). The EE subjects opened the restrainer between 60 secs to 601 secs while the ISO subjects opened the restrainer between 113 secs to 969 secs. Also, it was observed that the EE subjects displayed the door-opening prosocial behavior 90 out of 120 possibilities while the ISO subjects did it 66 out of 120 possibilities. Regarding the days that the subjects took to start displaying the door-opening behavior, we observed that in the first experimental session all the subjects opened the door at least one time, however for the third experimental session all four subjects of the EE group achieved the criterion for the termination of the experimental session; meaning that they released three times the trapped rat, whereas only two subjects of the ISO group were able to achieve this.

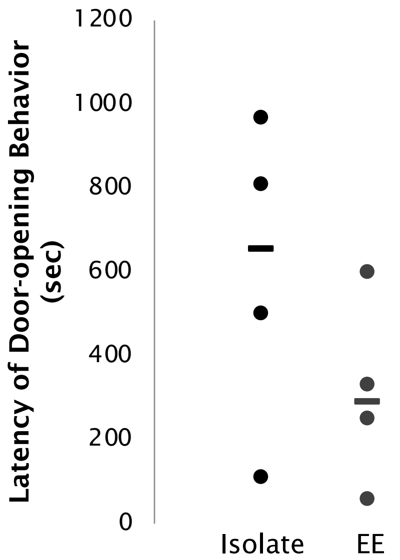

Figure 5. Latency of door-opening prosocial behavior. The dots represent the median (*Mdn*) of *all liberation sessions for* each subject and the lines the median of the group. Median latency (in seconds) of door-opening behavior for each subject in the first trial of each experimental session, EE subjects *Mdn* = 292.3 and ISO subjects *Mdn* = 656.5.

After Door-Opening Behavior

The time and frequency of interactions after the door-opening behavior pointed out a tendency of the ISO subjects to have higher frequency and higher length interactions in time with the released conspecifics. The EE subjects (*Mdn* = 20.2, $\bar{X}$ = 23.1, SD = 15.2) seemed to initiate fewer interactions with the released conspecific since they had between 8 to 44 interactions, on the contrary, the ISO subjects (*Mdn* = 39.8, $\bar{X}$ = 40.5, SD = 27.5) initiated between 8 to 74 interactions (Figure 6A). Regarding the length of the interactions, the EE subjects (*Mdn* = 13.8, $\bar{X}$ = 17.9, SD = 14.4) seemed to spend less time interacting between 5 secs to 39 secs with the released conspecific compared to the ISO subjects (*Mdn* = 37.4, $\bar{X}$ = 34.9, SD = 23.8) that interacted between 5 secs to 60 secs with the released conspecific (Figure 6B). The EE dyad of subject 12 interacted 44 times and spent 39 secs interacting while the ISO dyad of subject 9 interacted 74 times and spent 60 secs interacting.

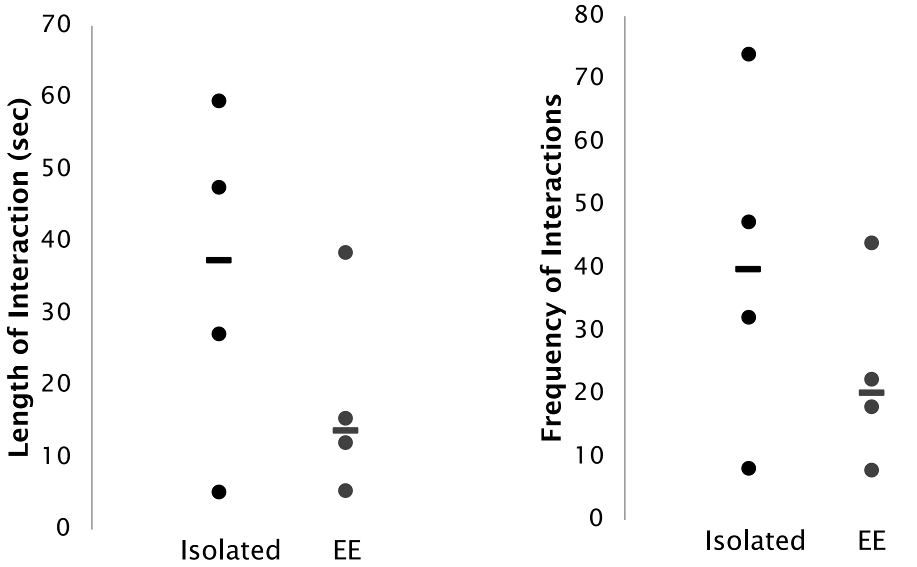

Figure 6. Social Interaction After Door-opening Behavior. The dots represent the median (*Mdn*) of all liberation sessions of each subject and the lines the median of the group. Panel A: Median of the frequency of interactions for each subject with the released conspecific, EE subjects *Mdn* = 20.2 and ISO subjects *Mdn* = 39.8. Panel B: Median of the length (in seconds) of the interactions for each subject with the released conspecific, EE subjects *Mdn* = 13.8 secs and ISO subjects *Mdn* = 37.4.

Discussion

The aim of this study was to explore whether early rearing conditions may affect the emission of prosocial behavior. To accomplish this, rats were reared since postnatal day 21 in two different conditions: environmental enrichment or isolation. Then in adulthood, they were tested for prosocial behavior using the trapped rat task (Bartal et al., 2011). It is important to highlight the fact that this is an exploratory study with a small sample size which seek to explore some tendencies related to the prosocial behavior of rats, in order to be used as a basis for bigger confirmatory studies (as recommended by Arain et al., 2010 and Thabane et al., 2010).

The protocol that was used, allowed us to replicate some of the essential findings of Bartal et al., (2011): 1) most of the subjects learned to release an unknown trapped conspecific without prior training, 2) the subjects released the trapped rat in the first experimental session and 3) the door-opening behavior was maintained by its consequences. In our study all the subjects (n = 8) displayed at least one door-opening behavior in the first experimental session and five of the subjects displayed door-opening behavior more than once. Our findings differ from what it is commonly observed in the literature, for example, in the study of Havlik et al., (2020) only five of forty-eight subjects opened the restrainer in the first session. Furthermore, the inclusion of three opportunities of displaying the door-opening behavior facilitated its acquisition. As it happens in our study seven of eight subjects achieved the learning criterion proposed by Bartal et al. (2014) where the subjects that performed the door-opening behavior at least three times in a row were named "openers". We observed that most of our animals displayed three opening behaviors by the second day while in Bartal's study animals took around 7 days to achieve the criterion.

The main findings of this study were: when compared to ISO animals, subjects reared in EE appeared to spend more time near the restrainer before releasing the trapped rat, plus they took less time to perform the door-opening behavior, interacted less and with lower frequency with the trapped conspecific after the releasing. These results suggest that the EE rearing may influence the emissions of prosocial behavior and the interactions with the trapped rat before and after the door-opening behavior.

The interactions before the door-opening behavior point out that the EE subjects displayed more exploratory and social interest behaviors toward the trapped rat compared to the ISO subjects. These results are in accordance with the observed by Neal et al., (2018). In their social investigation task, Neal et al found that EE animals displayed more behaviors related to social contact; the EE subjects tended to exhibit more social interest toward the trapped conspecific than toward the habitat which is explained by a possible influence of the early social engagement in EE. The authors suggest that the EE rearing could increase the attentiveness to the behavioral responses of conspecifics because of the complex social environment, where thanks to the interactions, the animals learned to direct their attention to conspecifics and respond to their behavior. Another possible explanation for these results, is that the EE could affect the novel object exploration of the restrainer because the experimental sessions provided a new situation to the EE subjects -a conspecific inside the restrainer. For instance, Zimmermann et al., (2001) also observed that EE animals emitted more behaviors related to the exploration of novel objects compared to ISO and social control animals. They explained that the EE rearing improves spatial abilities that are related to situations where they can explore novel objects. Although, in this study, the animals were habituated to the empty restrainer, meaning it could be possible that the novelty of the trapped rat inside the restrainer took an important role in the social interactions before the door opening. Future studies might consider to include control conditions similar than the ones used by Bartall et al., (2014) in which subjects had to choose between liberate the trapped rat or to explore new objects or other kind of relevant items (e.g. food) inside of another restrainer.

In relation to the door-opening behavior latency, we observed that the latency of EE subjects was apparently different from the one in ISO animals. The EE subjects tended to take less time to perform the prosocial behavior compared with the ISO animals. According to these results, the physical and social interactions that the EE provided may facilitate the development of the door-opening behavior. On possible explanation to these results may be related with the consequences of the rearing conditions on brain development of rats. For example, Brenes et al. (2016) conducted a study to assess the effect of social and physical enrichment on brain plasticity and ultrasonic communication in rats. They found that the animals reared in physical EE (but not social enrichment) showed greater brain plasticity, but they showed a deficit in prosocial ultrasonic emissions and a decrease in social approach behaviors in response to them. On the other hand, the social EE (physical and social enrichment) animals showed minor effects on neuronal plasticity but an increase in the rates of prosocial ultrasonic emissions and greater social approach behaviors in responses to them. These tendencies observed with the social EE animals may help to understand the apparently differences between latency of door-opening behavior and social enrichment in EE which might cause the subjects to be more responsive toward the trapped conspecific by displaying the door-opening behavior. Additionally to Brenes et al, Neal et al. (2018) observed that the social stimulation present in the EE rearing enhanced the oxytocin immunoreactive responsiveness which is an hormone closely related to social behaviors and bonding. It would be expected then that the EE rearing may help with the interactions with conspecifics thanks to the oxytocin which in turn would be translated into higher probabilities of displaying the door-opening behavior with shorter latencies when compared with the ISO subjects.

Although the EE subjects seemed to perform faster the door-opening behavior, the results pointed out that once they release the trapped rat, they interacted less in terms of frequency and length of interaction compared with the ISO animals. The fact that the social housed rats interact less with a conspecific than the ISO animals has been previously reported. Varlinskaya et al. (1999) observed that, when assessed in a social interaction test, rats housed in social groups interacted less with a conspecific compared to subjects housed in ISO conditions. Also, Douglas et al. (2004) compared the rewarding value of the social interaction of animals housed in social groups or individually by means of the place preference conditioning paradigm, they found that individually housed rats tend to prefer places associated with the previous social interaction with a conspecific. These results shown in literature makes us think that the rearing conditions may alter the motivations of prosocial behavior and that the animals rewarding value of the social interactions after the door-opening behavior was different for both groups.

Having in mind that rats housed in EE tended to interact less with the released conspecific, we hypothesized that this behavior may not be maintained by the positive reinforcement effects of the social interaction. Thus, we considered that for EE subjects this behavior was probably maintained by negative reinforcement, i.e., escaping from a stressful situation induced by another animal in distress (Emotional contagion hypothesis;

Bartal et al., 2011; 2014; Sato et al., 2015). Nevertheless, for the ISO subjects the case might be different, they took longer to emit the door-opening behavior but once they freed the trapped rat, they spent more time interacting with the social partner. These findings point that for the ISO subjects, the social interaction apparently helps to induce and maintain the door-opening behavior. In accordance with Hiura et al. (2018) suggestion, where the social contact deprivation may increase the probability of performing the door-opening behavior as a result of pursuing social contact. Taken together, these results suggest that different reinforcement contingencies are involved in prosocial behavior in rats under differential rearing conditions and that observing the interactions after the social behavior may inform about the contingencies that maintain it.

After analyzing our data, we observed some important behavioral tendencies in the study, like the fact that the probability of displaying the prosocial behavior may depend on the early social experience and the rearing conditions, but also, that the contingencies of reinforcement that may maintain the prosocial behavior could be different for both groups.

Considering that this is an exploratory study, we highlight the importance of conducting a confirmatory study. Moreover, we observed three important limitations in our experimental design that future studies might have to consider for amplifying the scope of the investigation. The first limitation is related with the number of animals in each group, we consider that by expanding the groups size it would be possible to evaluate more precisely the differences between groups. The second limitation concerns to the control of the rearing variables, specifically in the control group, we compared subjects reared in EE versus subjects reared in isolation (ISO). It is essential to include a social control group (i.e. subjects housed in groups but without physical stimulation) to observe how subjects would display the door-opening behavior and interact with the released conspecific when they had had a history of social interactions (see, Varlinskaya & Spear, 2008). As we know, rodents are highly social animals, in natural conditions they interact with both a physical environment and a social environment, hence, it is not natural to maintain a rat in isolation its whole life (Balcombe, 2010; Baumans, 2005; Hutchinson et al., 2005), that is why, future investigations should compare the emission of prosocial behavior of rats reared in EE, social control (dyads) and isolation in order to level the group comparison.

The third limitation refers to the exploration of the restrainer, the EE subjects had a history of object manipulation since their cages had four objects that allowed them to display some of their natural exploration repertory. We hypothesized that the exploration of the restrainer *per se* could have been a variable that might interfered with door-opening helping behavior (Hachiga et al., 2020; Ueno et al., 2019). In order to clarify this limitation, we suggest that future studies include a choice task -similar to Hachiga et al., (2018)- in which the experimental subjects where presented simultaneously with both an empty restrainer and a trapped conspecific.

This would help to determine whether the subjects are displaying the door-opening behavior to release the trapped conspecific or because they can explore the restrainer.

We gather that the exploration of the physical and social characteristics of the development environments in this exploratory study gave us evidence regarding the nature of the prosocial behavior. We observed some preliminary data about the possible influence of the rearing in EE on the prosocial behavior of rats; also, we were able to analyze the social interactions after the door-opening behavior, something that is not commonly described in this kind of researches. Even if our results are not conclusive, this fact does not take away the value of the experimental exploratory researches; we emphasize on exploring the elements of the environmental rearing and the prosocial behavior -viewed from the helping behavior parading- since the trapped rat task procedure is quite resent (Bartal et al., 2011, 2014, 2016) and the information regarding the social interaction of subjects reared in EE need to be broadly examined (Renner & Rosenzweig, 1986; Rosenzweig et al., 1978). This alternative to theory-driven experimentation allowed us to approach this behavioral phenomenon from an inquisitive perspective by selecting a factor to measure (i.e., prosocial behavior) and two conditions as samples (i.e., EE-ISO) in order to observe how would they interact with each other (Franklin, 2005; Stojanovic, 2013).

The importance of the current exploratory study relies in the fact that we are providing initial evidence concerning the possible role that early exposure to complex environments plays on the development of behaviors that may increase the welfare of a conspecific. We hypothesize that the early exposure to EE may increase the probability of displaying prosocial behavior toward a trapped rat, but it may also affect the contingency of reinforcement associated with the prosocial behavior and its consequences.

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