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Social interaction and conditional self-discrimination under a paradigm of avoidance and positive reinforcement in Wistar rats

Julio C. Penagos-Corzo*[1], Andrés M. Pérez-Acosta[2] and Ingrid Hernández[1]

[1]*Universidad de las Américas Puebla, Cholula, Puebla, México.*

[2]*Universidad del Rosario, Bogotá, Colombia.*

The experiment reported here uses a conditional self-discrimination task to examine the influence of social interaction on the facilitation of self-discrimination in rats. The study is based on a previous report (Penagos-Corzo et al., 2011) showing positive evidence of such facilitation, but extending the exposition to social interaction conditions prior to training. Specifically, rats were assigned to three conditions with different levels of social interaction and exposed to two conditional self-discrimination tasks, under an avoidance and positive reinforcement paradigm, where they had to discriminate their own internal state (with or without methylphenidate). Our results indicate that conditional self-discrimination was higher in groups with higher social interaction than in those with lower interaction and support the conclusion that self-discrimination learning curves for different degrees of social interaction positively increased with number of sessions and level of interaction.

Conditional self-discrimination is a form of learning in which the behavior of each individual is discriminated socially, with the intervention of the contingencies of reinforcement (Dymond & Barnes, 1997). The study of this process has its roots in the work of Beninger, Kendal and Vanderwolf (1974) with rats. Even in the eighties it was widely believed that self-discrimination was a uniquely human phenomenon tied to language or verbalization (Pérez-Acosta, Benjumea Rodríguez & Navarro Guzmán, 2001); however, it is possible to state that verbal language is not a

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* Correspondence: Julio César Penagos-Corzo. Universidad de las Américas Puebla. Department of Psychology. Ex Hacienda Sta Catarina Mártir. 72810, Puebla. México. Tel +52 222 229 2055- Fax +52 222 229 2634. E-mail: julioc.penagos@udlap.mx

determinant factor in the consciousness of a subject (Parr, Waller & Furgate, 2005). Consciousness may present itself in its basic form, self-awareness, a form of self-discrimination in different vertebrate species (Pérez-Acosta, *et al.* 2001). Self-discrimination refers to stimuli control (Pérez-Acosta, *et al.* 2001) and may include processes such as discrimination of one's behavior in its spatial dimension (García García & Benjumea Rodríguez, 2006), "metacognition" as described by Shimp (1982), discrimination of changes in the intensity of thirst associated with specific responses (Penagos-Corzo & Aguilar, 2009), among others.

To study self-discrimination capabilities, a procedure consisting in a task where the organism has to discriminate a feature of its own state, in order to receive reinforcement, is used. It is therefore necessary to induce a different state to contrast with the original state of the organism. This can be achieved through the use of drugs (Lubinski & Thompson, 1987; Lubinski & Thompson, 1993) such as methylphenidate. The properties of this drug allow its use as an interoceptive discriminative stimulus (Emmett-Oglesby, Wurst & Lal, 1983; Wood & Emmett-Oglesby, 1988). A variable that can enhance the ability of self-discrimination is social interaction or social behavior (Penagos-Corzo, *et al.*, 2011).

The social behavior of animals appears to be governed by a communication procedure known as key-lock that is subject to the internal state of the animal and the right environmental conditions (Gahagan, 1981). This interaction drives learning itself (Pedro Arriaga-Ramírez *et al.*, 2006) and may be mediated by physiological variables. For example, there is evidence that shows that vasopressin and oxytocin influence social behavior and cognitive processes such as learning and memory (Ophir, Zheng, Eans & Phelps, 2009). Such social behavior goes beyond a simple interaction, and may include clear aspects of cooperation in lower mammals (Łopuch & Popik, 2011; Segura & Gutiérrez, 2006).

Social interaction is a form of interaction between the external and internal environment. From the first early experiences, these interactions will be crucial in shaping neural connections and the cognitive capacities of such connections: synapses that receive constant stimulation will be retained and the others will be eliminated (Cziko, 1996). It is known that parental care and maternal or reciprocal stimulation have a close relationship with social interaction (Klopfer, 1976) and that the involvement of epigenetic factors from the mother and littermates has effects on the behavior of both of the mother and the offspring (Melo & Fleming, 2006). Since these interactions seem to have a positive impact on behavioral performance, and since self-discrimination is a clear example of environment-organism interaction, it is likely that self-discrimination is related to the degree of social interaction. In this sense, Penagos-Corzo *et al.* (2011) found a qualitative trend in the positive relationship between the different levels of social interaction and conditional self-discrimination, indicating that a longer exposure to such interactions increases the ability of self-discrimination. However, the observed trend is an interpretation that requires quantitative confirmation or rebuttal, with longer exposure to the above mentioned social interaction conditions and the improvement of some controls.

The exposure time is relevant because in the conclusions of the work cited above the need to increase the interaction time (number of sessions) is highlighted, as the observed qualitative differences show a trend that a greater number of sessions could confirm. On the subject of greater control, it is interesting to note that Penagos-Corzo *et al.* (2011) had a substantial amount of interaction with their subjects, i.e. the rats had to return to the initial position every time they made a mistake. These interactions can and must be avoided because the handling of the researchers may be functioning as a discriminative stimulus. Also, the intensity of the electric shocks (6.3 mA) administered to the animals can be significantly decreased. On the other hand, it is important to obtain additional data to gain more certainty about the relationship between social interaction and conditional self-discrimination. The only work that studies this relationship is the one cited above.

Therefore, this study investigates the facilitation of self-discrimination in rats by the amount of early social interaction. It is based on a previous report (Penagos-Corzo et al., 2011), with an improvement on the controls and with extended social interaction conditions prior to training. It is hypothesized that rats with increased social interaction will have a better performance in self-discrimination than rats with less interaction.

METHOD

Subjects. 24 female Wistar rats[1] were randomly selected from 14 pregnant rats from the vivarium Claude Bernard at the Universidad

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[1] Female rats were chosen because, as noted above, this work is a replica, and except for the variables of interest all the other variables were kept similar to the original work. Furthermore, females compared to males experience a lower reduction in their motor activity when exposed to aversive stimulation situations (Heisnbroek, van Haaren & van de Poll, 1988) or exploring (van Haaren & Heinsbroek van Hest, 1990), both relevant in this study.

Autónoma de Puebla. The 24 female rats were assigned as follows: Eight pregnant rats were assigned to the "isolated" experimental group (Is), four of them were assigned to the "couples" experimental group (Cs) and two were selected for the "quartets" group (Qs). At birth, the rats were kept for a week with their littermates and mothers. Later, the offspring were separated according to the group they would be assigned to: a female and her mother for the Is experimental group, four couples and mothers for the Cs experimental group and two sets of four females and mothers for the Qs experimental group. All groups were kept under managed care for three additional weeks. After four weeks, weaning and separation from the mother followed. Work with the rats began once they turned 30 days old. All animals were treated and cared for in accordance with the ethical standards of the Code of Ethics of Psychologists (*Sociedad Mexicana de Psicología*, 2007) and in compliance with the Mexican Official Standard NOM-062-ZOO-1999 (*Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación*, 2001).

Materials. *Rodent Chow* (Purina®) was used for animal feed. To trigger the difference in the state of the organism weighted doses of methylphenidate (Ritalin®) were used and administered orally 30 minutes prior to conditioning sessions. The drug was diluted in water and artificial flavoring at 1 mg methylphenidate per ml of water. The dose administered to the rats was 10.0 mg/kg. This dose is similar to the one used by Hermosillo, Penagos-Corzo and Pérez-Acosta (2011) to induce self-discrimination tasks in rats. Its properties were also tested in rats as interoceptive discriminative stimuli (Emmett-Oglesby *et al.*, 1983, Wood & Emmett-Oglesby, 1988). An Escape-Exploration / Restricted Field (EERF) box© (Figure 1), similar to that used by Penagos-Corzo, *et al.* (2011) was used, equipped with an electric shock device adjusted to 0.6 mA. The box has an electrified grid that is activated, in some conditions, when the organism leaves the center of the box. It is delimited by transparent acrylic with four doors that allow the passage of the rat. The corners of the box have receptacles that allow the placement of reinforcements. Rats were exposed to 24 30-minute daily interventions, with two non-intervention days every five days. Therefore, the duration of the whole experimental series was 32 days, the age of the rats at the end of the experimental series was 8 weeks.

Procedure. The procedure was similar to that carried out by Penagos-Corzo *et al.* (2011) regarding the allocation of conditions and discrimination tasks. Overall, there was a task under positive reinforcement and one under aversive stimulation. The target behavior was staying in one place or leaving that place depending on the state each rat was experiencing (with or without methylphenidate).

The experimental groups were divided into two subgroups (A and B). During the first session, in the “leaving” condition, the rats in the "A" groups were administered methylphenidate orally, previously diluted in flavored water, 30 minutes before the session. In this "leaving" condition, animals were allowed access to water if they approached the corner and crossed Area 1 of the EERF box. In the next session, "staying" in Area 1 condition, rats were given only flavored water; they were allowed 30 minutes of inactivity as in the first session and then were placed in Area 1 of the EERF box. If the animal moved toward the exits and tried to leave Area 1, it received an electric shock. In the first five sessions a transparent cover that fully blocked the free space of the corners between the acrylic walls was implemented to prevent the rats from escaping the administration of the electric shock; in the following five the exit was partially hindered; and finally the cover was taken off. The “leaving” and “staying” sessions were interspersed daily to reach the 24 interventions. The same procedure was followed for the "B" groups, except that in the “leaving” condition they were given water and in the “staying” condition methylphenidate was administered.

Behavioral measurement. The hits and misses of the target behaviors (leaving or staying in the EERF box) and the time the rats spent in the reinforcement and avoidance areas were analyzed. Conditional self-discrimination was measured by the hits being made by the rat in accordance with the target behavior. To this end, during the final phase the number of times subjects crossed Area 1 and the times they approached the corners were measured. It was considered a hit or a miss if the conduct occurred or did not occur in a three second period. The same method of quantification was used in the "staying" conduct. In this case if the rat remained three seconds in Area 1 it was considered a hit, otherwise it was considered a miss.

For the final phase the rats were subjected to the same conditions of drug and flavored water, but the aversive stimulation and positive reinforcement were eliminated. These sessions were conducted to measure the self-discrimination of the rats. The rats were subjected to the behavioral task during 14 minutes. In this period they were placed inside the box for two minutes, taken out for a minute and went back again, until five inclusions in the box were reached.

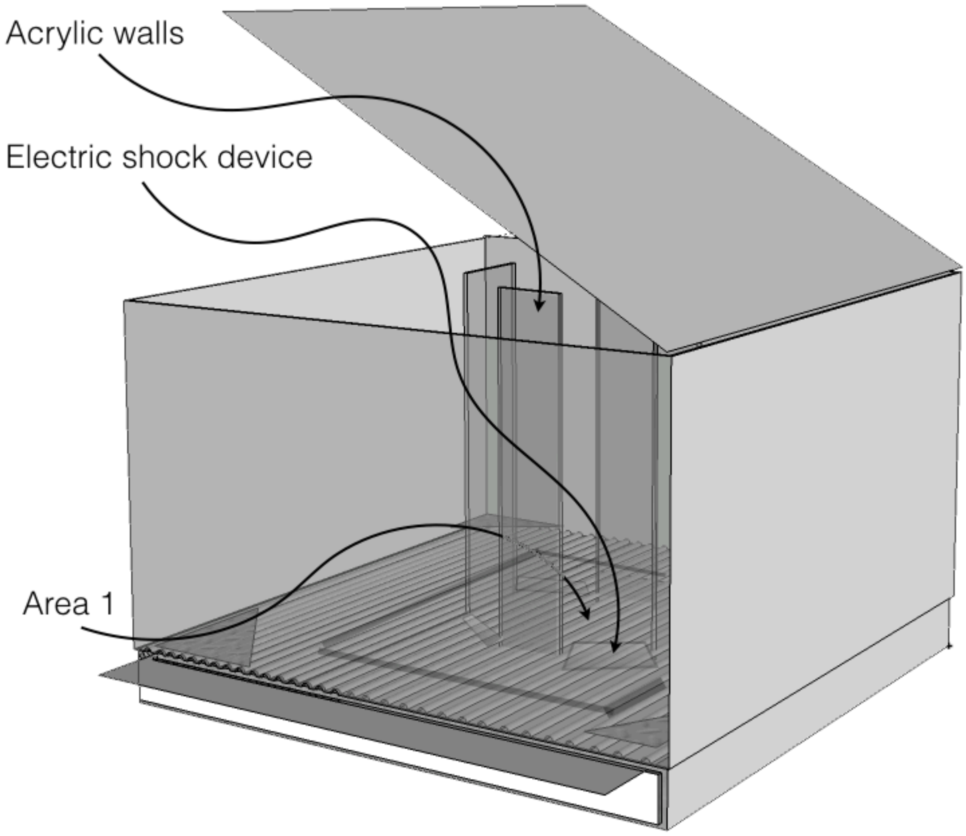

Figure 1. Escape-Exploration / Restricted Field (EERF) box.

RESULTS

Learning curves for target behavior are shown in Figure 2. The equation for the slope of the line for the studied conditions indicates a steeper slope for hits in the Qs condition ($y = 27.697x - 60{,}831$) than in Cs ($y = 20.812x - 50{,}097$) and in Cs more than Is ($y = 18.762x - 43{,}443$). The analysis of covariance indicated that the differences between the conditions, considering the number of sessions as a covariate variable, are significant for both hits $F_{(2, 66)} = 86.28$, $p < .001$ η $p^2 = .72$, and misses $F_{(2,66)} = 37.16$, $p < .001$ η $p^2 = .53$.

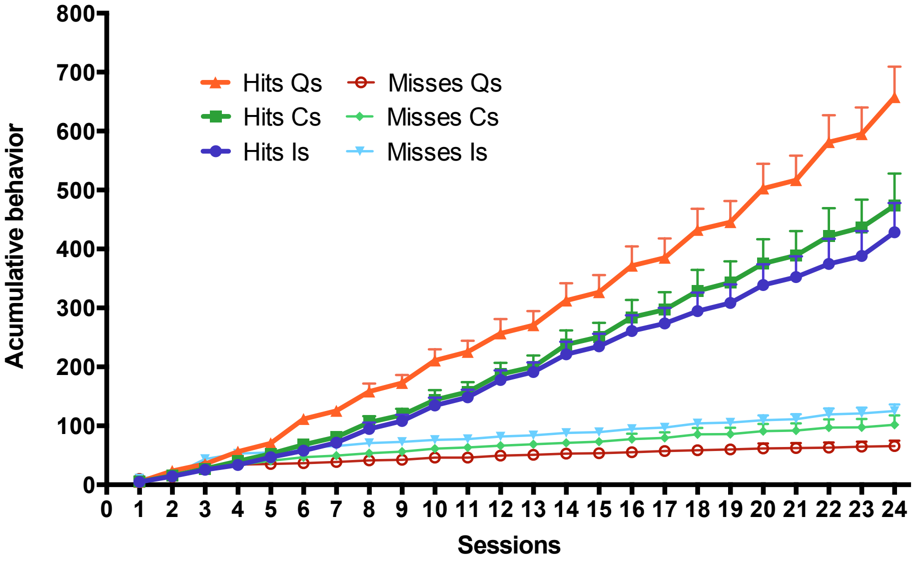

Figure 2. Learning curve for the three conditions of social interaction. Is: Isolated. Cs: Couples. Qs: Quartets. The frequencies refer to the cumulative data of the average of hits, recorded during the 24 sessions. For the "leaving" condition, the hits are the number of times the animal crossed Area 1 and approached a corner, while in the "staying" condition a hit corresponds to one minute the rat stayed in Area 1. The misses correspond to the opposite behavior: leaving when the condition was staying and vice versa.

In the analysis of interactions between self-discrimination training and the level of social interaction, significant differences were observed by a two-way mixed ANOVA in self-discrimination learning (hits vs. misses) $F_{(1, 21)} = 13.84$, $p = .001$ and in self-discrimination x social interaction $F_{(2, 21)} = 7.62$, $p = .003$. No significant differences were found in the levels of social interaction $F_{(2, 21)} = 1.00$, $p = .385$. The effect of the interactions of the variables can be clearly seen in Figure 3, in the reverse behavior of hits and misses during the conditions of social interaction.

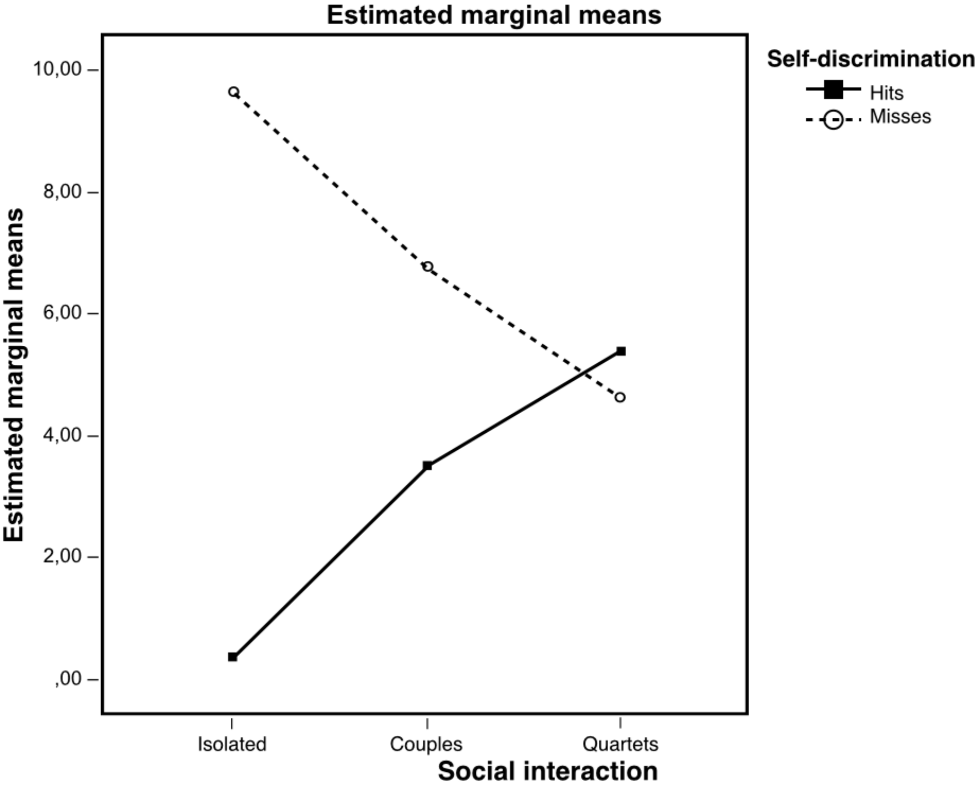

Figure 3. Estimated marginal means. Effects of conditional self-discrimination regarding social interaction.

As can be noted, the hits are increased according to the social interaction while the misses decrease accordingly. Thus, these data tend to have comparable averages and summations, and explain the value of F in social interaction in the two-way ANOVA. Hence, it was appropriate to conduct an additional one-way ANOVA in which only hits or misses were considered.

The one-way ANOVA performed for the "leaving" condition showed significant differences between the conditions of social interaction $F_{(2, 23)} = 7.46$, $p = .004$. These significant differences called for a post hoc analysis with the Tukey test. This analysis indicated a significant difference between the Is condition of social interaction and the Qs condition ($p = .003$), but not in the other comparisons. The ANOVA for the "staying" condition did not show significant differences between the conditions of social interaction $F_{(2,23)} = 2.54, p = 0.102$.

DISCUSSION

The data provided by this study, in addition to showing that the subjects had the ability to discriminate their internal states, support the conclusion that self-discrimination learning curves for different degrees of social interaction positively increased with number of sessions and level of interaction. In this sense, there is a steeper slope for the "Quartets" condition when compared to the other conditions, especially after the sixth session. The difference found in the effects of social interaction, specifically for Is vs. Qs comparisons implies that the number of littermates is relevant, also confirming the trend found by Penagos-Corzo *et al.* (2011). Note that the average of each rat offspring ranges between 8 and 12, so the difference between the stimulation that a single offspring can provide vs. no offspring does not allow a significant discrimination gradient. This implies that litter size is important, not stimulation per se, as the offspring with less littermates has similar or greater maternal stimulation. Studies report that maternal care is relevant and that it mediates the effects of aversive stimulation (Meaney, 2001; Parent, Del Corpo, Cameron & Meaney, 2013). However, there is evidence that the number of littermates has an important contribution in behavior, even in the adult life of the rat (Dimitsantos, Escorihuela, Fuentes, Wardrobe & Nadal, 2007). In this regard, it has been suggested that differences in behavior such as an increased social play, competition and strategies for getting milk can be related to a bigger litter size (Dimitsantos *et al.*, 2007). This may contribute to explain the found differences, as the possibility of competition for milk will be little or none for Is and Cs conditions. Although the quartets condition does not involve high competition, it is more likely to happen than for other conditions, and allows greater opportunity for social play. Furthermore, it is known that the size of the group impacts the animal cognitive processes, particularly those related to socially mediated learning (Croney & Newberry, 2007), as might be the case of social play.

In relation to litter size, there is some evidence that the substitution of parental care by that provided by littermates results in a reversal of the effects of isolation (Francis, Diorio, Plotsky & Meaney, 2002; Lévy, Melo, Galef Jr., Madden & Fleming, 2003; Melo *et al.*, 2006). If the incorporation of peers influences the effects of isolation, it is to be expected as well that a greater number will trigger differential effects, as it was the case of the subjects of this study. An enhanced ability in conditional self-discrimination in the rats in quartets, and a less ability in isolated rats, could imply that reduced social interactions such as early social deprivation could be harmful. This can be supported by other studies in which it was noted that early social deprivation disrupts inhibitory control in attentional selection, leading to slower learning in isolated subjects (Schrijver & Wurbel, 2001).

The manipulation of the social interaction resulted in a greater or lesser amount of stimulation of the rats. Therefore, the information from other studies on the physiological changes resulting from isolation and sensory deprivation in rats is considered relevant, as these changes may be mediating the self-discrimination capabilities. In this sense, it is quite accepted that the interaction with the environment has both behavioral and biological effects (Domjan, 2010; Francis *et al*, 2002; Gluck, Mercado, Myers, 2009; Marcus, 2008; Mieu *et al*. 2006). For example, deprivation of sensory information on neonatal rats, by cutting off their whiskers, changes their behavior; and there is a permanent impact to the sensory cortex despite the subsequent growth of the whiskers (Chu, Yen & Lee, 2013; Simons & Land, 1987). Also, it is reported that in isolated rats biochemical changes occur in relation to memory and spatial discrimination (Schrijver, Pallier, Brown & Würbel, 2004). In the same way, glutamate subunits GluR1 and NR1 are reduced, causing a profound deregulation in synapse-related proteins and behavior, marked deficits in social interaction behavior and an increased anxiety in the open field (Hermes, Li, Duman & Duman, 2011).

Anxiety in the open field can be an important variable that may have mediated in exploring or staying vs. leaving behaviors. If the level of social interaction has an effect in anxiety, perhaps it contributes to the explanation of why differences were found between the variations of social interaction in the "leaving" condition and not in the "staying" condition. The latter condition was associated with the presence of aversive stimulation (electric shocks), which naturally led to increased anxiety or stress in rats. It would be expected, according to the findings by Hermes et al. (2011), that there would be some impact on behavior due the number of littermates. If this impact was not present in the "staying" condition, it is likely that differences were not noticeable because of the impact of the aversive stimulus in the rats, so that generally the subjects didn't make mistakes. In this sense, the results show that. During the learning sessions, subjects from all the social interaction conditions showed a decrease in the number of misses and an increase in the hits in conditional self-discrimination.

It seems clear that social contact has an effect on several variables, and to some extent it may be relevant to self-discrimination capabilities. In fact, the work of Hermosillo *et al*. (2011) illustrates that genetically similar strains of rats show differences in social self-discrimination if a strain exercised better parental care and had a higher quality social activity.

Due to the fact that the data in this study suggest that the number of littermates is relevant and that other studies indicate that it is possible to reverse the harmful effects of isolation, it is possible to suggest that future studies should examine what is the number of littermates that produces significant changes in the ability of conditional self-discrimination and if rats that lived in isolation can increase their self-discrimination when placed in groups of rats. It may also be interesting to assess if there are differences in task performance between male and female rats. Female rats were used because there is evidence indicating a better performance in tasks involving aversive stimulation. However, sex differences can be studied in relation to the possible effect of the estrous cycle. In this sense, the evidence appears to be contradictory. There is evidence that negates the effect of the estrous cycle in tasks of aversive stimulation (Rubio et al., 1999) as well as evidence that shows the opposite (Beatty, 1992). It is also noted that the estrous cycle of rats can interfere with latent inhibition, a task involving discrimination (Quinlan, Duncan, Loiselle, Graffe & Brake, 2010). Moreover, the interaction of methylphenidate with the estrous cycle appears to have no direct link. However, because on the one hand the main mechanism of action of methylphenidate includes blocking dopamine and norepinephrine transporters (Solanto, 1998), and on the other hand the stimulation of D1 dopamine receptors improves learning related with passive avoidance during estrous cycle in rats (Fedotova & Sapronov, 2012), it is likely that there may be a relationship in the estrous cycle -task-methylphenidate interaction that can be studied in the future. Because the relationship is indirect, the authors of this study believe that it is unlikely to impact the self-discrimination task here studied.

The data obtained in this work provide additional findings on the variables that may be involved in conditional self-discrimination, i.e. social interaction, which as crucial in this case. This may help shed some light on how social interactions become an independent variable in individual learning.

RESUMEN

Interacción social y auto-discriminación condicional bajo un paradigma de reforzamiento positivo y evitación en ratas Wistar. El experimento aquí reportado usa una tarea de auto-discriminación condicional para examinar la influencia de la interacción social sobre la facilitación de la auto-discriminación en ratas. El estudio está basado en un reporte previo (Penagos-Corzo et al., 2011) que señala evidencia positiva, pero aquí se extiende la exposición a las condiciones de interacción social previas al entrenamiento. Concretamente, las ratas fueron asignadas a tres condiciones con diferentes niveles de interacción social y expuestas a dos tareas de auto-discriminación condicional, bajo un paradigma de evitación y de refuerzo positivo, donde tenían que discriminar su propio estado interno (con o sin metilfenidato). Nuestros resultados indican que la auto-discriminación condicional fue mayor en los grupos con mayor interacción social que en aquellos con menor interacción y apoyan la conclusión que indica que las curvas de aprendizaje de auto-discriminación para los diferentes grados de interacción social aumentaron positivamente con el número de sesiones y el nivel de interacción.

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