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ACTIVE AVOIDANCE CONDITIONING IN RATS: ABSENCE OF SEX DIFFERENCE AND ESTROUS EFFECT

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In this paper, possible differences between male and female rats (*Rattus norvegicus*) in the acquisition and consolidation of 2-way active avoidance learning are studied. In addition to monitoring behavioural parameters (escape and avoidance), the phase of the estrous cycle of the female rats was also recorded in order to establish whether this physiological phase affects the animals' performance. Other authors reported differential behaviour between the sexes and a modulatory effect of the estrous cycle. In contrast, in our study we did not find any differences in the escape and avoidance responses between the sexes and no influence of the estrous cycle. It was only observed a greater male than female intertrial activity. This contradicts the hypothesis about an organisational or activational effect of the sex hormones on shuttle-box learning.

Condicionamiento de evitación activa en ratas: ausencia de efecto del sexo y ciclo estral. En este artículo se estudian las posibles diferencias entre las ratas macho y hembra *(Rattus norvegicus)* en la adquisición y consolidación del aprendizaje de evitación de 2 vías. Además de monitorizar los parámetros conductuales (escapes y evitaciones), se determinó la fase del ciclo estral en que se encontraban diariamente las ratas hembras para establecer si el momento del ciclo afecta a la ejecución de los animales. Otros autores han señalado diferencias conductuales entre los sexos y un efecto modulador del ciclo estral. En contraste con estos datos, en nuestro estudio no encontramos ninguna diferencia en las respuestas de evitación y escape entre los sexos y tampoco ninguna influencia del ciclo estral sobre las mismas. Tan sólo observamos una mayor actividad entre ensayos de los machos frente a las hembras. Estos resultados contradicen las hipótesis previas sobre un efecto organizacional o activacional de las hormonas sexuales en el aprendizaje de evitación activa.

Sex differences between male and female rats in reproductive and non-reproductive behaviors have been reported in several studies. This behavioral dimorphism has been registered in running wheel activity, reactivity to footshock, intracranial self stimulation (Diaz-Veliz et al., 1989), spatial learning (Warren et al., 1990), lever-pressing (Van Haaren et al., 1990) and classical conditioning (Wood and Shors 1998). However, other works have discarded the existence of behavioral dimorphism in some of these

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tasks, such as the Morris water maze (Berry et al., 1997) or studies of operant conditioning (Van Haaren et al., 1990). In avoidance conditioning tasks, females avoid more shocks than males (Beatty and Beatty, 1970), acquire avoidance response more quickly and have a slower extinction of the response (Van Haaren et al., 1990). Differences in the active avoidance conditioning according to the estrous cycle phase have also been found (Diaz-Veliz et al., 1989; Sfikakis et al., 1978). However, not all of the studies corroborate these results (Denti and Epstein, 1972).

The aim of this work was to determine whether sex differences exist in the acquisition and consolidation of the active avoidance response and if the estrous cycle of the rat influences avoidance behavior during learning. We used a two-way shuttle-box active avoidance procedure that made it possible to acquire the response in only one day. In this way, we were able to analyze the influence of gender and cycle on acquisition in spite of the shortness of the cycle phases. Once the avoidance response had been acquired, the task was prolonged for 4 more days to observe the possible influences of the fluctuating hormones on the consolidation of the task.

This behavioral analysis provided us with information on the performance of the females in different phases on the same day of learning. In addition, we were able to compare the performance of the same animal during its cycle since, in the final days, the avoidance response was totally consolidated and potential variations in the avoidance response could only be attributed to fluctuations of the gonadal hormones.

Materials and methods

Subjects

Ten male and ten female, three month old Wistar rats (weighing 250 ± 50 g) from the University of Oviedo vivarium were used. The animals were kept in independent cages and were maintained on a 12-hour light-dark cycle (8:00-20:00) at a constant temperature (21 ± 1 ºC) and with free access to food and water. Care of the animals was in strict accordance with current guidelines on the care and use of experimental animals established by the A.P.A. on the 2nd of August, 1985.

Estrous Cycle Determination

Daily vaginal epithelium samples were taken from each female rat before behavioral testing following the Feder method in order to determine the state at estrous cycle (Feder, 1981). The rats were classified according to whether they were in one of the four phases corresponding to the estrous cycle: proestrus, estrus, metestrus and diestrus.

Apparatus

The learning test consisted of a two-way active avoidance task performed in a shuttle-box (Letica Scientific Instruments, Spain). The box (53 cm x 71 cm) was divided into two compartments, and a light and a sound source could be used jointly or separately in each of the compartments. Furthermore, the floor grid could be electrified independently in each compartment (see illustration I). The learning program carried

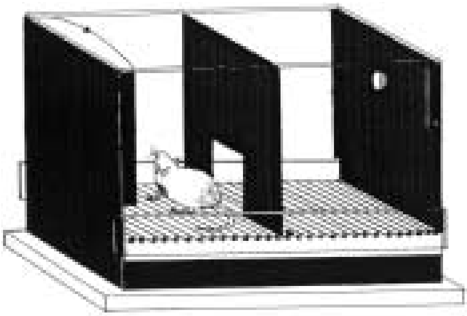

Illustration I. Shuttle-box.

out in the box was controlled by an associated module. The shuttle-box was covered by a black curtain when the animals were introduced into it. By this way the animals are kept in semi-darkness during the habituation and learning period.

Procedure

The male and female animals, were handled prior to the experiment. The learning process took place during the light period between 9:00-12:00 A.M. over 6 days, the first day of which was for habituation. The habituation consisted of maintaining the animal inside the shuttle-box for 15 minutes during which period it could freely explore. The schedule for the remaining days was as follows: the animals were kept in semi-darkness and in a soundproof area for 30 seconds (intertrial interval) after which they were subjected to a sound stimulus (80dB, 1400Hz) (Conditioned Stimulus -CS). During this second period, which lasted for 5 seconds, while the tone is present the animal had to change of chamber to avoid an electric footshock (0.8mA) (Unconditioned Stimulus -US). If the animals did not change of chamber (avoidance response) the electric shock was maintained during 5 secs. or until the rat escape to the other chamber (see table I). The animal, therefore, learned that the sound predicted the shock. There were 50 trials daily. The number of responses of each animal recorded in the intertrial period was considered as an index of exploratory activity. Three variables were recorded daily: intertrial responses (A), number of changes of chamber per animal during the intertrial interval; avoidances (B), changes of chamber during the presentation of the tone; and escapes (C), changes of chamber during the presentation of the shock.

Results

Analysis of variance (two-way ANOVA with repeated measures) was used to compare the differences between the sexes of each variable. The independent variable was sex (male or female) and the dependent variable corresponded to the different measurements (A,B,C) recorded on each learning day. The post hoc test used was the Tukey HSD (honestly significant difference) test. There were performed three independent analysis for each variable.

The number of intertrial responses differed significantly between sexes ($F(1,4)= 9.67$, $p<0.0l$) and between the days ($F(1,4)= 5.75$, $p<0.01$). There were more intertrial responses on day 3 than on the first and last day ($p<0.01$), since the responses of the males increased until this day ($p<0.01$) and decreased from then on (see fig. 2).

Table I
Protocol of learning

Phase of experiment	*Habituation*	*Acquisition*		*Consolidation*		
Days of learning	0	1	2	3	4	5
Time	1 day (15 minutes)			50 trials/day (50 min. aprox.)		
		Phase 1		Phase 2	Phase 3	
Stimulus	No CS-US	No CS-US (30 sec.)		US (Shock -0.8mA, 5sec.)	CS (Tone -80db, 5 sec.)	
Behavior	Free exploration	Intertrial Responses (A)		Avoidances (B)	Escapes (C)	

There were no significant differences in the avoidances or escapes between the male and female rats. However, differences were found between the days (F(1,4)= 31.4, *p*<0.01). The level of avoidance responses increased after the first day (*p*<0.01) (see fig. 1).

The estrous cycle analysis involved comparison of the females on each of the learning days. In this way, possible differences between the four phases of the estrous cycle were determined on each day. This analysis was, therefore, repeated 5 times, once on each learning day. The Kruskal-Wallis test showed no differences between the different phases of the estrous cycle in any of the three parameters (see fig. 3).

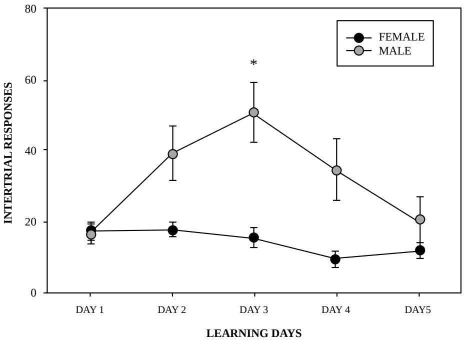

*Fig.1. Effects of sex in intertrial activity. Each bar represents the mean ±SEM of the intertrial responses given on each day of learning. Comparisons were made by using two-way ANOVA followed by the Tukey HSD test (* = significantly different, p< 0.01).*

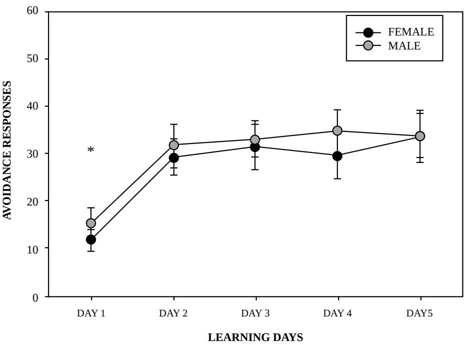

*Fig.2. Effects of the sex in acquisition of conditioned avoidance responses. Each bar represents the mean ± SEM of the avoidance responses recorded on each learning day. Comparisons were made by using two-way ANOVA followed by the Tukey HSD test (* = significantly different, p< 0.01).*

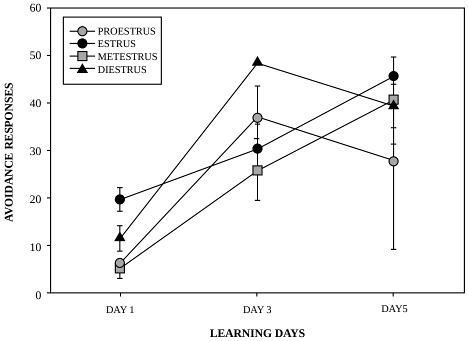

Fig.3. Effects of the estrous cycle in acquisition of conditioned avoidance responses. Each bar represents the mean ± SEM of the avoidance responses given during days 1, 3, and 5 of learning. Comparisons were made by using Kruskal-Wallis test.

Discussion

The present study showed no sex differences in active avoidance conditioning. Both sexes rapidly acquired avoidance behavior and an optimum level of avoidance response was observed on day 2, this level was maintained during the learning period. These data coincide with those of Brush et al. (1985) who did not find any sex differences either in high-avoidance line and low-avoidance line rats. By the other hand, Beatty et al. (1970) observed a worse performance in the males, regardless of the shock intensity, while on the other hand. They find that the sexual hormones can have hight activational effects on avoidance acquisition in both sexes (Beatty, 1992). However, discrepancies between the results of the different studies can be explained by the fact that experimental procedures do not use either the same shock intensity or duration, the same conditioned stimulus (CS) or even the same number of trials or strain, and the data are, therefore, not comparable.

In our study, consolidation and acquisition of the avoidance response, and the intertrial responses were examined in both sexes and during the estrous cycle and no differences were found. Furthermore, other studies did not detect any relationship between the estrous cycle and activity level or the avoidance conditioning reached (Denti and Epstein, 1972; Kristal et al., 1978). Some authors found estrous cycle interference on learning, although no agreement has been reached on the phases in which facilitation or deterioration of the response in the two-way avoidance learning is produced. Sfikakis et al. (1978) report facilitation during proestrus and impairment during the diestrus and estrus phases. Later studies, found facilitation of acquisition during diestrus and impairment in the proestrus and estrus phases (Diaz-Veliz et al., 1989). In order to explain this controversy, we must, once again, look at the variability of the procedures used for this type of learning and memory studies. Thus, Sfikakis et al. (1978) used an avoidance task of one single day, using light and 45v electric shock as the CS. In another work, Diaz-Veliz et al. (1989) performed a learning test in which a discharge of 0.2 mA was presented after a 5-second sound. If the animal did not avoid the shock, it was maintained until it escaped. Both procedures differ substantially from that used in this work, which could explain the differences found.

On the other hand, it has been demonstrated that both the amygdala as well as the hippocampus show certain plasticity in response to fluctuations of the circulating gonadal hormones in female rats. The amygdala is related to fear conditioning and the hippocampus is necessary for complex data processing, such as the details of the spatial environment (LeDoux, 1992). As avoidance behavior requires the participation of both structures, it could be affected by these variations (LeDoux, 1992)

Variations in synaptic pattern of dendrite spine synapses (Nishizuka and Arai 1983) and in serotonin receptor density (Biegon and McEwen, 1982) have been found in the amygdala of female rats. Such changes seem to be related with the regulation of estrous rhythm in rats (Chateau et al., 1984) and with the control that serotonin exerts on the release of prolactin, LH and FSH during the estrous cycle (Becu de Villalobos et al., 1984). Different changes have also been found in the hippocampus, among them a decrease in the GABA and glutamate levels, facilitation of the long-term potentiation (LTP) during proestrus (Warren et al., 1995) and a 30% decrease in the density of the hippocampal dendritic spines in CA1 during estrus (Woolley and McEwen, 1992). Rats with an inborn high (HP) learning capacity to perform in a shuttle-box avoidance paradigm present a lower threshold for inducing long-term potentiation (LTP) (Keller et al., 1992). However, it has recently been pointed out that the experience dependent increase in the synapses is beneficial for the learning capacity in the spatial hippocampal task, but that caused by the gonadal hormones has no beneficial effect on these (Warren and Juraska, 1997). We were also unable to verify that these changes are relevant for active avoidance conditioning. However, morphological alterations in the hippocampus of the female rats have been detected in Sprague-Dawley rats (Warren et al, 1995), a different strain from that used in this study.

Our data reveal sex differences in the number of intertrial responses. Female activity was more constant over the learning days whereas the males showed more variable activity. We could think that this greater variability exists because the males are more sensitive to the electrical shock. However, Beatty et al. (1970) did not find any sex differences in the intertrial responses during the avoidance test although they did record differences in the sensitivity threshold to electrical shock (1mA). Brush et al. (1985) did not find any relationship between the intertrial responses and sensitivity to pain either since the low-avoidance line rats had fewer responses than the high-avoidance line rats. However, neither rat differed in regards to the electrical sensitivity thresholds. In our case, it is also possible that the variability in intertrial responses was not influenced by a different sensitivity to shock.

In other anxiety models, aversion provokes different levels of activity between male and female rats, although these variations seem to depend on the procedure used. Thus, in the Vogel punished drinking test, it could be concluded that the males are less anxious than the females (Johnston and File, 1991), but in the elevated plus-maze test, the opposite occurs (Johnston and File, 1991). In all these models, the behavioral differences between sexes are generally described in terms of the presence or absence of behavioral inhibition in response to aversive stimulation (Van Haaren et al, 1990). In our work, neither of the sexes presented behavioral inhibition since both emitted responses without interference of the variable activity presented by the males in the intertrial period. The intensity of the aversive stimulus was 0.8 mA and this did not provoke paralysation and when it was associated with the sound, avoidance responses were possible.

Since the greater exploratory activity of the males can not be explained by differences in anxiety or in behavioral inhibition it could possibly reflect a search for other response strategies. Thus, the increase in male activity is greater than the increase in female activity during the intertrial period. Once learning is consolidated, the activity is reduced to the same level as the females. The animals have learnt that only the responses given fortuitously with the sound (CS) are effective at avoiding the shock.

In conclusion, our results imply that no differences exist between male and female rats in the acquisition and maintenance of active avoidance learning, although the number of intertrial responses varies between sexes, possibly due to a greater exploratory activity of the males. In addition, it was also impossible to establish that the estrous cycle exerts an influence on two-way shuttle-box active avoidance conditioning.

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