Orbitofrontal Cortex Inactivation Impairs Early Reversal Learning in Male Rats During a Sexually Motivated Task

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Abstract

This study analyzes whether inactivation of the orbitofrontal cortex (OFC) affects early discrimination or reversal learning during a T maze motivated task. Male rats received saline solution or one doses of tetrodotoxin (TTX) bilaterally into the OFC, and were permitted to have an intromission with a receptive female to induce a sexually motivated state. Discrimination and reversal sessions consisted of seven trials each to accomplish the non-overtrained condition. Each arm of the T maze was associated to different external cues. Subjects were sexually reinforced whenever they reached the receptive female box, and returned to the start-box if not. Spontaneous motor activity was not altered. Rats with OFC inactivated did not present alteration during discrimination. Males with higher doses of TTX had a deficit in the number of correct responses and increased number of trials without response during reversal learning. These data agrees with other studies and indicates that an intact OFC is essential for the adequate manifestation of reversal learning during its early phase in motivated tasks. However, disagrees with other findings about early perseverative responses, pointing out to a critical role of this structure in enhancing performance through incentive value re-assignment of predicted outcome cues.

Key words: orbitofrontal cortex, reversal learning, sexual motivation, T maze, tetrodotoxin.

Resumen

El objetivo de este estudio fue investigar si la inactivación de la corteza orbitofrontal (COF) afecta el aprendizaje de discriminación y/o de inversión, en su etapa temprana, durante la resolución de un laberinto T por ratas macho, utilizando como incentivo una interacción copulatoria con una hembra receptiva. Las ratas experimentales fueron infundidas con solución salina o una dosis de tetrodotoxina (TTX) bilateralmente en la COF. Luego de esto, se les permitió tener una intromisión con una hembra receptiva para inducirles un estado sexualmente motivado. Las sesiones de discriminación y de inversión consistieron en siete ensayos cada una para mantener al sujeto en un estado motivado y en la condición de aprendizaje inicial. Cada brazo del laberinto fue asociado a diferentes señales externas al mismo. Los sujetos fueron reforzados con una interacción sexual cada vez que llegaban al compartimento donde se encontraba una hembra receptiva, y eran retornados a la caja inicial cuando no lo hacían. Los sujetos que recibieron TTX no presentaron alteración en la evaluación de la conducta motora espontánea en campo abierto. Tampoco se encontró alteración significativa en las respuestas correctas durante la fase de discriminación. Las ratas que recibieron la dosis más alta de TTX presentaron un deterioro en el número de respuestas

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correctas y un incremento significativo en el número de ensayos sin respuesta durante la fase de inversión. Estos datos coinciden con los de estudios que señalan que una COF intacta es crítica para la adecuada ejecución durante la fase inicial del aprendizaje de inversión en tareas motivadas, aunque difieren de la mayoría de informes que encuentran un aumento de la respuesta perseverativa durante la etapa inicial de la inversión. Estos hallazgos parecen apoyar el probable papel crítico que juega la COF en la reasignación de valor incentivo de los estímulos que sirven de señal para la correcta ejecución de tareas motivadas.

Palabras clave: corteza orbitofrontal, aprendizaje de inversión, motivación sexual tetrodotoxina, laberinto T.

The prefrontal cortex can be defined as the cortical region where projections from the mediodorsal nucleus of the thalamus are received (Rose & Woolsey, 1948; Uylings & Van Eden, 1990). The orbitofrontal cortex (OFC) is a prefrontal subregion located in the orbital (ventral) surface of the prefrontal cortex. This area acts as a site for the convergence and integration of information received from visual, somatosensory, taste, olfactory, gustatory, and auditory cortices (Rolls & Treeves, 2001; Zald & Kim, 1996). The OFC receives strong inputs from the amygdale (AMG) (Armony, Servan-Schreiber & LeDoux, 1997; Balkenius & Morén, 2001), from the medial temporal structures such as the hippocampus, and also from the thalamic nuclei (Jowaisas, Taylor, Dewbury & Malagodi, 1971). The OFC projects back to different parts of the brain including temporal lobe areas, thalamic nuclei, hypothalamus, brain stem, basal ganglia, and other cortical regions such as the posterior parietal lobe (Kolb, 1990; Uylings & Van Eden, 1990; Uylings, Groenewegen, & Kolb, 2003). Through these widespread connections, the OFC influences a variety of autonomic, motivational, emotional and mnemonic processes in human and non-human primates (Barbas, 2000; Clark, Cools, & Robbins, 2004; Rolls, 2004; Rolls & Treeves, 2001).

The OFC plays a main role in the learning process. OFC neurons fire selectively during olfactory discrimination learning and this activity encodes specific aspects of the olfactory cues (Yonemori, Nishijo, Uwano, Tamura, Furuta, Kawasaki, Takashima, & Ono, 2000). Rats with lesions in the OFC continue to respond to stimuli, even when this behavior has not been reinforced (Clark, Cools, & Robbins, 2004; Rolls, 2000, 2004; Winstanley, Theobald, Cardinal, & Robbins, 2004), they commit more errors during discrimination tasks in the phase of extinction (Rolls, 2000, 2004) and also show a preference for immediate reinforcing experiences over longer, but delayed reinforcing experiences (Mobini, Body, Ho, Bradshaw, Szabadi, Deakin, & Anderson, 2002). It has also been observed that a lesion in the OFC alters the processes involved in reversal learning, i.e., the capacity of organisms to behave in a flexible way enabling them to identify the moment when a stimulus loses relevance and, contrastingly, where one, which was previously of no relevance, acquires it (Chudasama & Robbins, 2003; McAlonan & Brown, 2003; Schoenbaum, Setlow, Nugent, Saddoris, & Gallagher, 2003a; Schoenbaum, Setlow, & Ramus, 2003b).

Schoenbaum, Chiba, & Gallagher (2000), have reported that firing rate of OFC neurons is incremented on late phases and even more enhanced once the rat have achieved the number of correct responses criterion in discrimination learning. They

found also an increase in correlated activity of OFC neurons in reversal phase. On these basis authors propose that OFC activity is specifically related to the improving accuracy in task performance. In contrast, Rolls and Treeves (2001), have propose that OFC could be involved since early phases of learning, by contributing to the acquisition of reward value of stimulus through its connections with amygdala. These different approaches could be framed in Balleine and Dickinson (1998) hypothesis about two different mechanisms of instrumental motivated behavior, both implying prefrontal cortex. One mechanism, called stimulus-response habits, is more related with over-training based on instrumental contingency between response and reward and could be mediated by prefrontal-striatum circuits (Borchgrave, Rawlins, Dickinson, & Balleine, 2002). The other mechanism, named goal-directed process, implicate the acquisition of incentive value and could be mediated by limbic-prefrontal circuits, including amygdala (Hall, Parkinson, Connor, Dickinson, & Everitt, 2001).

Various experimental designs have been developed in order to demonstrate the role played by OFC in this type of learning processes, using incentives such as food or drink as primary reinforcing experiences (Chudasama & Robbins, 2003; Rolls, 2000; Schoenbaum, Chiba, & Gallagher, 2000; Schoenbaum, Setlow, Nugent, Saddoris, & Gallagher, 2003a; Schoenbaum, Setlow, & Ramus, 2003b).

On the other hand, sex has also been reported as an efficient incentive (Denniston, 1954; Sheffield, Wulff, & Backer, 1951; Matthews, Grigore, Tang, Doat, Lee-Ming, & Pfaff, 1997), and has been used as a reward in typical operant tasks such as mazes and Skinner boxes (Beach & Jordan, 1956; Everitt, Fray, Kostarczyk, Taylor & Stacey, 1987; Gemmel, Anderson & O'Mara, 2002; Hernández González, Prieto Beracoechea, Arteaga Silva, & Guevara, 2007; Karnath, Wallesch, & Zimmerman, 1991; Whalen, Beach, & Kuehn, 1961). The sexual behaviour of the rat not only depends on hormonal status and neurochemical changes, but also on their capacity to identify external stimuli which allow them to predict the whereabouts of potential sex partners (Sheffield, Wulff, & Backer, 1951; Sheffield, Roby, & Campbell, 1954; Toates, 1986) and to seek out, solicit, court, or make other efforts in order to obtain these sex partners, as well as to distinguish external cues and behavioral patterns of potential sex partners from those not sexually receptive (Agmo, 2002; Agmo, 1999; Kagan, 1955; Meisel & Sachs, 1994; Pfaff & Agmo, 2002). Neural mechanisms exist which allow the stimulation received during sexual contact to be perceived as rewarding. These rewards alter subsequent behavior, for example, by contributing to the formation of preferences for salient stimuli associated with positive sexual reinforcement.

Various studies have shown the importance of the prefrontal cortex in motivational aspects of sexual behavior (Agmo, Villalpando, Picker, & Fernández, 1995; Granon & Poucet, 1995; Hernández González, Guevara, Moralí, & Corsi Cabrera, 1998; Hernández González, Prieto Beracoechea, Arteaga Silva, & Guevara, 2007) as well as for processing the sexual stimulus incentives which are emitted from the receptive female.

Considering the major role that has been assigned to the OFC concerning the learning process involved in making an association with the incentive value of stimuli in early phases of discriminations and reversals, as well as its involvement in the motivo-emotional processes, the aim of this study was to determine if temporal inactivation

of the OFC modifies the performance of male rats during early discrimination and reversal learning phases in a T maze using a sexual stimulus, i.e. a receptive female, to provide the reinforcing experience.

Methods

Subjects

The subjects were 85-90 day old, male, Wistar rats, obtained from a colony bred in the Institute of Neurosciences, University of Guadalajara. All males were maintained in a room at 22-24ºC, with a 12:12 hr reversed light/dark cycle (lights on from 2000 to 0800h) and were selected on the basis of their high copulatory performance in preliminary mating tests. Purina Lab Chow and drinking water were constantly available in the home cage, where the animals were individually housed. Estrus was induced in females by s.c. injection of 5 µg estradiol valerate and 500 µg progesterone per animal, 48 and 4 h before testing, respectively. Animal care and all other procedures involving animals were approved by our Institutional Animal Care and Use Committee which is in accordance with NIH specifications.

Apparatus

To evaluate discrimination and reversal learning in a sexually-motivated condition, we used a T maze shaped apparatus, constructed of polyurethane-sealed wood. The maze consisted of a brown, wooden stem runway with a start box at one end and two goal boxes at the other. The start box (21x21 cm) was separated from the stem runway by a wooden guillotine door that prevented the rat from moving down the stem runway until the training started. This stem runway was 52 cm in length. The two arms extended 45 cm from the point of intersection. All the alleys were 9.0 cm in width and the walls were 10.5 cm in height. At the end of each arm there was a small goal box (21x21 cm) with a manual removable wooden guillotine door which had five small orifices and through these the male could hear, see, and smell the females but no copulatory interaction was possible.

Learning sessions

Two hours prior to the start of the learning sessions, rats were allowed to walk in the T maze for one session of 30 min, in order to become habituated. The rats were allowed to move freely inside the T maze in standard conditions (red light illuminated at a 20-25ºC temperature).

Before the learning sessions, each male rat was allowed an intromission with a receptive female inside a transparent acrylic box, in order to generate a sexually motivated state in the male, as reported in other studies (Hernández González, Guevara, Moralí, & Corsi Cabrera, 1998; Hernández González, Prieto Beracoechea, Arteaga Silva, & Guevara, 2007). In each session, a receptive and a non-receptive female were placed

in each one of the goal boxes. Immediately subsequent to the males' intromission, they were placed in the start box of the T maze and tested in two different learning sessions, both on the same day. The first session was a discrimination task where the male rats had to solve the T maze in order to choose between a receptive and a non-receptive female, each one placed in one of the goal-boxes in the T maze. The second session was a reversal learning task, where the position of the goal boxes with the females inside was changed to the other arm of the T maze, making it necessary for the male to re-locate and therefore re-learn the spatial location of the receptive female. If an animal entered the correct arm (where the receptive female was to be found) the door of the goal box was opened and the animal was permitted one intromission as a reinforcing experience. If the incorrect arm was selected (where the non receptive female was to be found) the animal was allowed to change its decision for 5 sec and then returned to the start box. The only difference between the two learning sessions was the reversal of the position of the goal boxes with the females inside and therefore the external cues associated with each T maze arm. The right T maze arm was close to a silver polygraph, while the left arm was close to a brown, wooden door. The initial position of the goal (receptive female) was assigned randomly, so that for half of the discrimination and reversal learning sessions the receptive female was placed in the right arm and for the other half, in the left one.

The recorded parameters in both sessions were: the time taken from when the start box door was opened until the rat entered the stem of the T maze; the time during which the rat walked in the maze stem until it arrived at the intersection of the arms; number of correct and incorrect trials and number of trials in which no response was registered (when the male did not leave the start box or when it left the start box but did not cross the T maze stem entirely, and therefore did not select a goal box with a female inside within a 5 min period).

EXPERIMENT 1

A number of studies have indicated that sex is an effective incentive and it has been used as a reward in classical operant tasks such as mazes and Skinner boxes. On the other hand, it has also been demonstrated that the motivational state of the male is different before ejaculation than after it, so that characteristic pre-ejaculatory arousal decreases temporally after ejaculation, and likewise the motivation. Because we required in this experiment that the male should perform the T maze task when in a motivated state, we needed to find out how many trials were necessary for the male to learn the task, without reaching ejaculation. Hence in this experiment, 10 male rats were set to resolve the T maze during discriminative and reversal learning sessions; each one consisting of 5, 7 or 9 trials, with an intertrial interval of 1-2 min and with an intersession interval of 30 min.

RESULTS

Sexually motivated rats showed a major preference for selecting the arm where the receptive female was placed, particularly in the discrimination session. During the

sessions consisting of 5 trials, it was observed that although the males demonstrated good discrimination learning, the reversal learning was deficient, as only 6 out of 10 males demonstrated adequate reversal learning (Table 1). In the learning sessions consisting of 7 trials, 9 out of 10 males showed an adequate discrimination and reversal learning, whereas in the learning sessions with 9 trials, 8 out of 10 males ejaculated before completing the sessions. Hence, it was decided that each learning session should consist of 7 trials (which was the maximum number of trials where males were found to participate, without reaching ejaculation). This number of trials was considered enough to evaluate effects of OFC inactivation specifically on early discrimination and reversal phase and to test the proposal of its possible involvement in the incentive value re-assignation through the OFC-Amygdala circuit, excluding OFC contribution into other circuits activated by overtraining.

EXPERIMENT 2

Table 1. Medians + interquartile ranges for correct, incorrect and non-response trials shown by the intact male rats (n= 10), during their performance in the T maze in discrimination and reversal learning sessions (5 trials).

Learning	Correct trials	Incorrect trials	Non-response trials
Discrimination	3.0 ± 1.0	1.5 ± 1.0	0.0 ± 0.0
Reversal	2.0 ± 3.0	3.0 ± 1.0	0.0 ± 2.0

In the previous experiment it was confirmed that a receptive female is an adequate reinforcing stimulus for a male, making it able to resolve the T maze task, and that 7 trials were sufficient for males to reach an incremented accurate performance in discrimination and reversal phases of the task. Thus, the aim of this experiment was to investigate whether temporal inactivation of OFC modifies the discrimination and reversal learning in a T maze, using a sexual stimulus, i.e. a receptive female to provide a reinforcing experience.

Subjects

The subjects were thirty-six 85-95 day old, male, Wistar rats which were obtained from a colony bred in the Institute of Neurosciences. All males were maintained in a room at 22-24ºC, under a 12:12 hr reversed light/dark cycle (lights on from 2000 to 0800h) and selected on the basis of their high copulatory performance in preliminary mating tests. Purina Lab Chow and drinking water were constantly available in the home cage, in which the animals were individually housed.

Surgical procedure

Approximately 7-10 days prior to the behavioral experiments, the rats were anesthetized with sodium pentobarbital (0.5 mg/kg) by intraperitoneal injection. The rats were fixed in a stereotaxic apparatus, and a midline incision was made in the skin

of the cranial region. The skull was dried and cleaned of fascias. Two slits were drilled bilaterally over the frontal region and two permanent stainless steel guide cannulae (23 gauge, 11 mm) were aimed 1 mm above the OFC at the following coordinates relative to bregma: AP + 3.7 mm; L + 2 mm (midline); DV - 3.5 mm from dura; with nose bar -3.30 mm below the inter-aural lines (Paxinos & Watson, 1997). The cannulae were fixed to the skull with dental acrylic; stylets were inserted into the cannulae to keep them patent. Adequate care was taken to minimize any pain or discomfort experienced by the animals throughout the experiment.

Reversible inactivation procedure

Tetrodotoxin (TTX, Sigma Co.) was used to temporarily inactivate the OFC, Two different doses of TTX were injected into the cannulae bilaterally, using injection needles (30 gauge, 12 mm) attached to a 10 µl Hamilton syringe via polyethylene tubing. These were either a lower dose (2.5 ng/0.5 µl) for the group labeled TTX1 (n= 12), or a higher dose (5ng/0.5 µl) for the group labeled TTX2 (n= 12), or saline solution for the group labeled SS (n= 12). The infusion was delivered at a rate of 0.5 µl/min during 1 min. The injection needles remained in the cannulae for 1 min following the infusion in order to maximize diffusion away from the needle tip and to minimize dorsal diffusion. It has been shown that the maximum effect of TTX can be reached in a short time (15 to 20 min) and that this inactivation is maintained at similar levels for about 4 hours (Ali-Vafaei & Rashidy-Pour, 2004; Quiroz *et al.*, 2003). Thus, each rat was bilaterally infused with saline or one dose of TTX, and thirty minutes after infusion was placed in the square plastic box for a period of thirty minutes in order to evaluate spontaneous motor activity. Subsequently, the rats were introduced into the T maze to evaluate discrimination and reversal learning.

Behavioral tests

To evaluate spontaneous motor activity in the male rat a square plastic box was used (55x55 cm) which had the floor divided into 25 squares (11x11 cm each). The interior color of the box was grey and the division lines were black. The walls measured 14 cm in height. Following the evaluation of motor activity (duration 30 min), the males were placed with a receptive female intending that the male should achieve one intromission (in order to increase its sexual motivation) and immediately after this, the rats from the three groups were tested in terms of their performance in the T maze learning sessions. The first session consisted of a discrimination task, whereas the second session was a reversal learning task. Each learning session consisted of 7 trials, with an intertrial interval of 1-2 min and with an intersession interval (ISI) of 30 min. The three rats (male and females) were maintained in their corresponding boxes during the ISI. This would continue to stimulate the learning of the position of the female by detection of olfactory cues from its same position and hence their association with the external cues. At the end of the ISI (for the reversal learning), the boxes in which females were placed were changed to the other arm, so that the cues from the receptive

female would not agree with its anterior external stimulus-associations. Both learning sessions were performed in a similar way to the method described for the first experiment. The initial position of the receptive female was counterbalanced throughout the trials, so that for half of the discrimination and reversal learning sessions she was placed in the right arm and for the other half, in the left one.

Histology

At the end of each experiment, the animals were deeply anesthetized using sodium pentobarbital. Intracardial infusion of isotonic saline (0.9%) followed by 5.0% buffered paraformaldehyde solution was used to fix the brain, which was subsequently removed and stored in formalin for at least two weeks; sections of 50μ thickness were sliced using a microtome and stained with cresyl violet. Inspection under a stereoscopic microscope following the stereotaxic coordinates allowed the path followed by the cannulae to be reconstructed. Only the behavior of subjects whose cannulae were correctly positioned over the OFC was included in the data analysis.

Statistics

A one-factor analysis of variance (ANOVA) was used to compare the number of crossed lines in a spontaneous motor activity task for the three groups. Two-way ANOVA for repeated measurements was used to analyze the interaction between the two variables (dose and condition) for the number of tests corresponding to correct, incorrect and non-responses. The temporal parameters referring to latency in going out and the time taken to walk through the maze stem on the part of the three different groups, were compared test by test under each condition using a non-parametric Kruskal-Wallis because those subjects who did not respond were assigned 200 sec as their temporal parameter in the corresponding test. Posthoc comparisons for parametric results were made using Tukey's test. Posthoc comparisons for non-parametric results were made using the Mann Whitney U test. Significance for all tests were set at $p< 0.05$ (two-tailed).

RESULTS

Histology. The tips of the cannulae were located in the 36 animals, specifically in the regions of interest. Tips over the OFC were bilaterally located between 4.1 and 3.2 mm anterior to bregma (Figure 1).

Spontaneous motor activity. The spontaneous motor activity manifested by subjects from the different groups was similar, so that no significant differences were observed ($F(2,27)= 0.33$; $p= 0.7202$) (Figure 2).

Discrimination and reversal learning. It was founded that a session with 7 trails was sufficient to the subjects learning the task in the T maze without reaching the ejaculation. The effect of the experience on the behavioral performance of the subjects of the three groups is showed in the Figure 3. In case of discrimination learning, despite

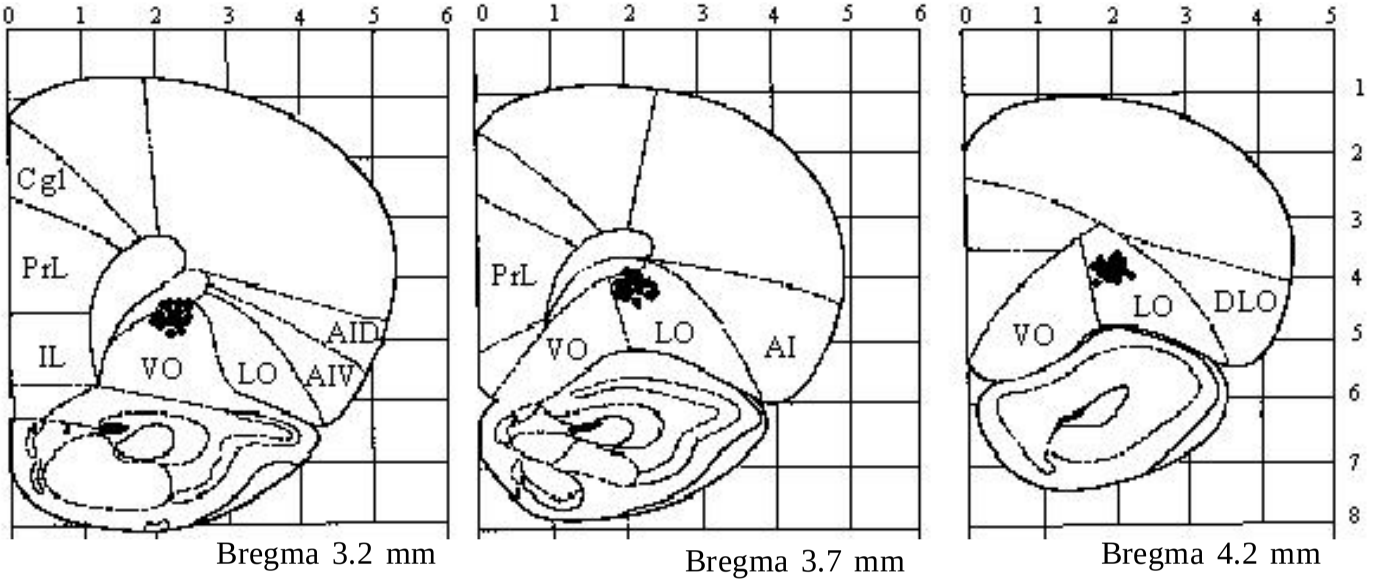

Figure 1. Schematic representation of needle tip placements in OFC (n= 12). PrL, prelimbic cortex; Cg1, cingulated cortex area; DLO, dorsolateral orbital cortex; LO, lateral orbital cortex; VO, ventral orbital cortex; AID, agranular insular dorsal cortex; AIV, agranular insular ventral cortex Anterior-posterior coordinates are given with respect to bregma.

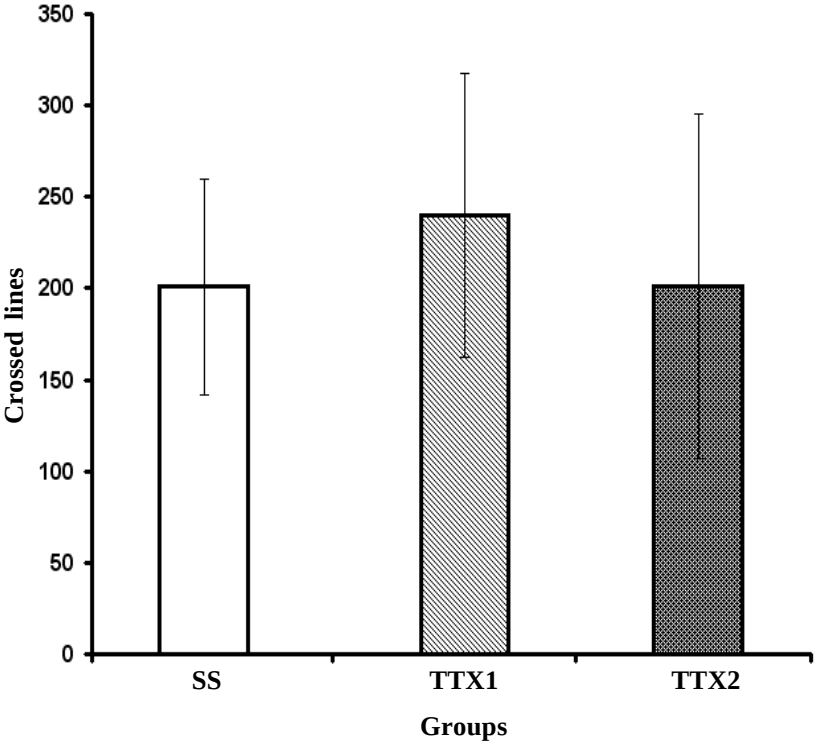

Figure 2. Mean ± SE of the number of crossed squares for the subjects from the three groups during the open field test (30 minutes).

of the fluctuations in both, correct and incorrect lines, the linear separation between them is not only preserved but tends to increase. For reversal learning, even though differences between correct an incorrect lines for SS and TTX1 groups are not as high as in discrimination session, linear separation remains from trial 4 to 7.

A significant difference was found between groups concerning the correct number of trials using a split fields ANOVA (FAXB (3, 2, 33)= 3.59; $p<0.01$). To determine for which groups and in which session a significant difference was evident, a Tukey-Kramer analysis was applied. The analysis revealed that neither of the doses of TTX produced changes concerning performance in discrimination learning. Only subjects which received higher doses of TTX (TTX2) manifested a lesser number of correct trials during the reversal learning session as compared to the discrimination learning session (q(2, 33)= 2.334; $p<0.01$) and also when they were compared to those from the reversal learning session of the SS (q(2, 33)= 2.417; $p<0.01$) and TTX1 (q(2, 33)= 1.75; $p<0.05$) groups (Figure 4A).

No differences were observed concerning the number of incorrect trials when the subjects of the three groups were compared (Figure 4B), however during the reversal learning session, a significant increase in the number of trials where no response was

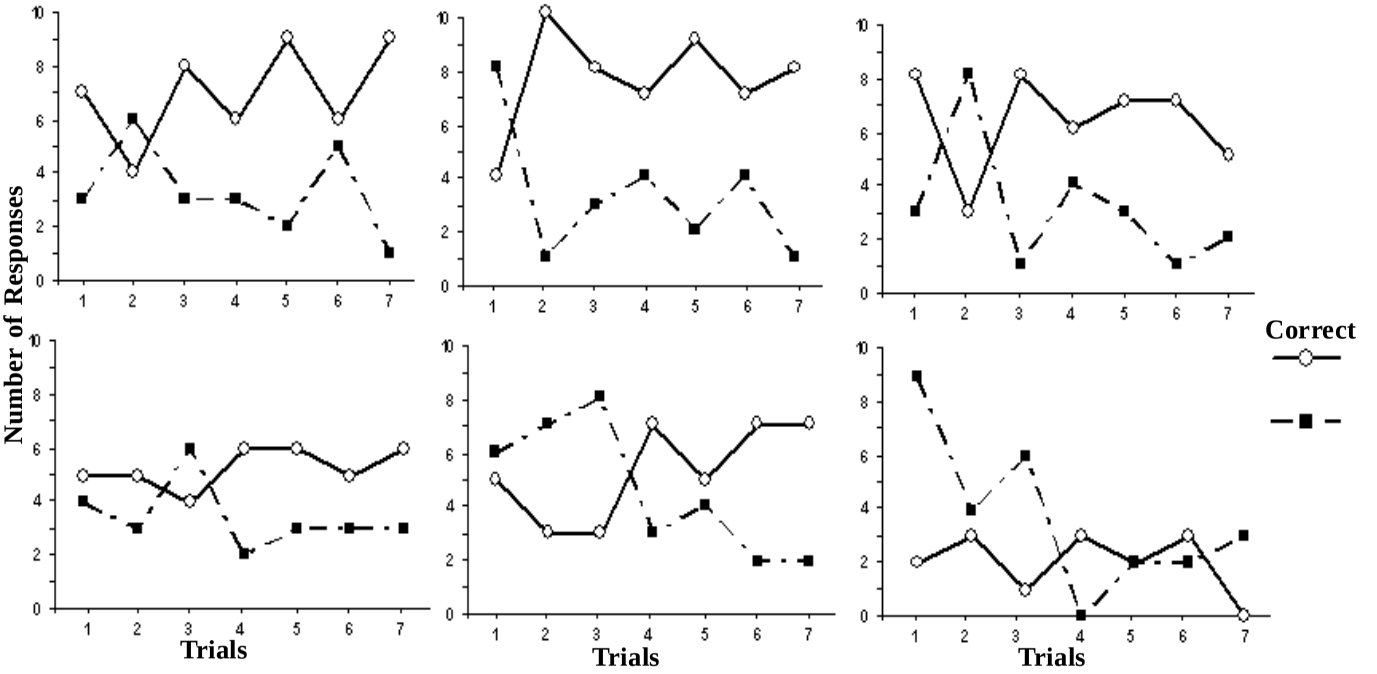

Figure 3. Total number of responses (correct and incorrect) performed by the subjects of the three groups during the seven trials of each discrimination and reversal learning. The three upper graphics correspond to the discrimination phases (from left to right) of saline solution, lower doses of TTX and higher doses of TTX. As shown, in the discrimination phase, the total number of correct responses is higher than the number of incorrect responses from the second trial and it remains so until the total number of incorrect responses descends in trial number seven. The lower graphics represent the performance of the three groups during the seven trials of reversal learning. It should be noted that this reversal learning seems to be more difficult to achieve for both, controls and lower TTX dose groups. The rats treated with higher doses of TTX were unable to achieve this.

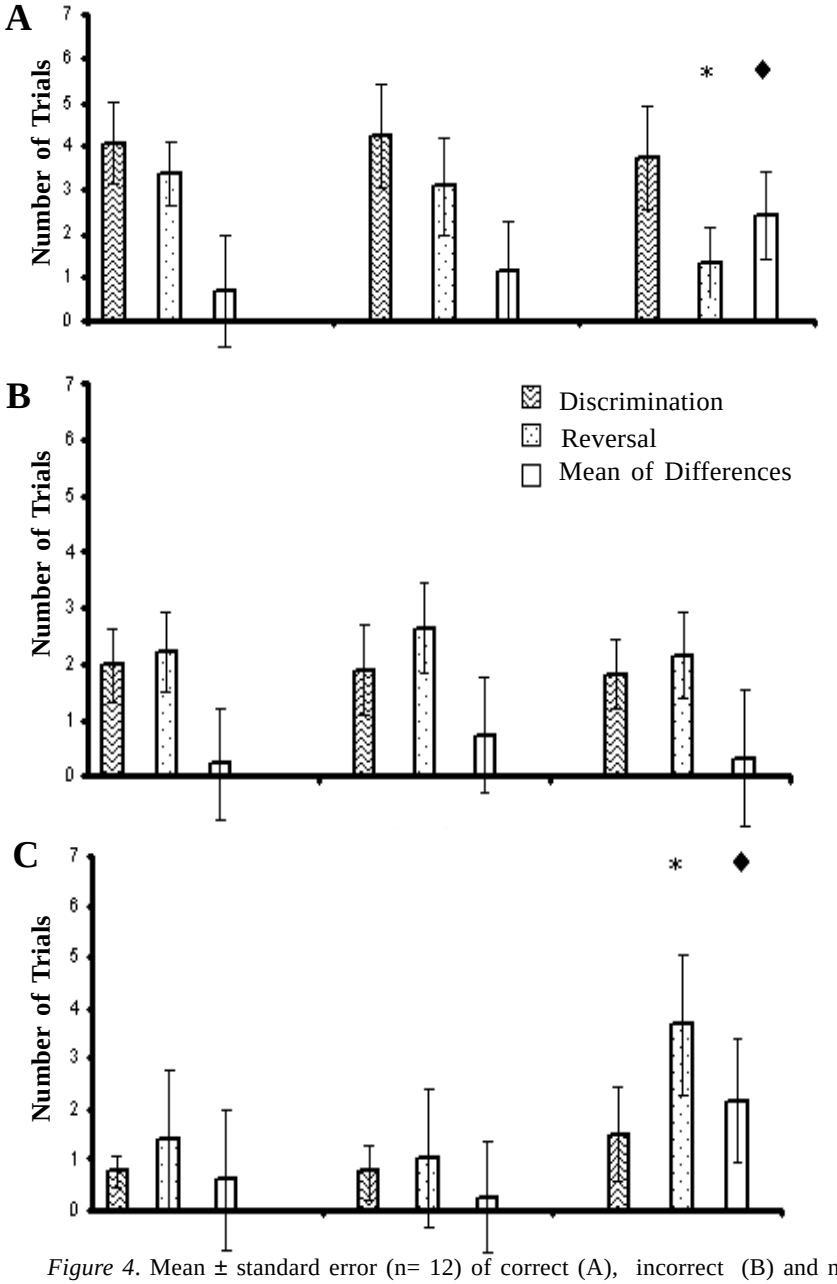

Figure 4. Mean ± standard error (n= 12) of correct (A), incorrect (B) and non response (C) trials for groups in each learning session. Two-way split fields ANOVA and Tukey test.

* $p < 0.05$ as compared to the discrimination and reversal learning sessions of the SS and TTX1 groups.

◆ $p < 0.05$ mean of differences between the discrimination and reversal learning sessions within the same group.

Differences in the time taken to cross the stem runway were found between groups in the third (H=7.98; *p*= 0.0185), fourth (H= 10.35; *p*= 0.0057) and fifth (H=9.62; *p*= 0.0082) trials. These were between TTX2 and TTX1 (U= 28; p= 0.0011) in the third trial; between TTX2 and SS (U= 31; *p*= 0.0124), TTX2 and TTX1 (U= 22; *p*= 0.0026) in the fourth trial; and, finally, between TTX2 and SS (U=29; *p*= 0.0109); and TTX2 and TTX1 (U= 27; *p*= 0.0077) in the fifth trial (Table 3).

Discussion

The present studies address the question of whether the rat's OFC plays a role in discrimination and reversal learning in a sexually-motivated task. High doses of TTX induced reversible inactivation of the OFC before the learning sessions, and disrupted only the reversal learning in the T maze, when a receptive female was used as a source of incentive stimuli.

This is the first study in which was evaluated the reversal learning using sexual stimuli to reinforce the behavioural response of a task. The sexual motivation of male rats has been reported to change along the sexual interaction, and genital stimulation received during intromission is necessary to increase sexual arousal and in order to reach the ejaculatory threshold. Following ejaculation, the sexual motivation decreases temporarily. In the present learning sessions, the experimental male rats had to be sexually motivated in order to perform; hence, sexual motivation of the subjects had to be maintained throughout the tests, and ejaculation should not be reached. Our data showed that the maximum number of trials that the male could perform without ejaculating was seven. This number of trials seemed adequate for the male to execute a reliable discrimination, and allowed us to conclude that all subjects passed the early phase of learning and to avoid overtraining.

Reversible inactivation procedure: Effect of TTX. TTX blocks voltage-dependent sodium channels and is capable of preventing impulse generation and conduction (Boehnke & Rasmusson, 2001). Based on experiments that quantify the time course and spatial spread of TTX (Ali-Vafaei & Rashidy-Pour, 2004; Boehnke & Rasmusson, 2001), our inactivation should have affected a spherical region measuring about 1 mm in diameter, which approximately corresponds to the diameter of LO and VO. The effect derived from applying TTX reaches a maximum value after 30-40 at a concentration and volume similar to those used in this work (Ali-Vafaei & Rashidy-Pour, 2004). Moreover, it is known that this effect maintains the same level of inactivation for about 4 hours (Quiroz, Martínez, Quirarte, Morales, Díaz Cintra, & Prado Alcalá, 2003). Thus, in this work, the OFC was temporarily inactive at a constant level throughout both learning sessions: discrimination and reversal.

The OFC contribution to early discrimination learning. Spatial navigation is the principal modality of goal-directed action and OFC is part of a network of areas implicated in spatio-motor processing. OFC is directly connected to a number of areas concerned with spatial information processing, including the posterior parietal cortex, medial agranular cortex (Fr2) (Gemmel, Anderson, & O'Mara, 2002) and the medial prefrontal cortex.

Various reports have implicated the OFC in the performance of tasks that involve spatial navigation associated with reward. For example, Poucet and Benhamou (1997) found that OFC neurons in rats encode learned associations between odors and locations. Similarly, Feierstein, Quirk, Uchida, Sosulki and Mainen (2006) using an odor-cued two-alternative choice task requiring orientation and approach to spatial goal parts, showed that the neuronal activity in rat OFCs encodes information relating both, the motivational significance and spatiomotor variables needed to define specific behavioral goals. Discrimination learning in the T maze task involves, besides learning and spatial navigation, the spatial location of the receptive and non-receptive females, learning how to perform the task, making a choice between the two female stimuli, and associating the external cues with attaining the reward.

TTX injected into the OFC, in higher or lower doses, prior to the discrimination learning session did not prevent spatial navigation or the association of the external cues with the reward in this sexually-motivated T maze task. The fact that the temporary lesion in the OFC did not affect discrimination learning agrees with other studies where rats associated a light stimulus with a subsequent reward consisting of food (Schoenbaum & Setlow, 2001) or a specific odor with the subsequent delivery of a sucrose solution (Schoenbaum, Setlow, 2001; Schoenbaum, Setlow, Nugent, Saddoris, & Gallagher, 2003a). In both studies, even when discrimination learning was not affected, reversal learning was highly impaired. Rolls (2000; 2004; see also Rolls & Treeves, 2001) proposed that an associative circuit between the OFC and the AMG participates in discrimination learning, where the AMG increases the incentive value of a novel neutral stimulus and the OFC confirms or updates this value in the case of known stimulus. In addition, Schoenbaum, Setlow, Saddoris and Gallagher (2003c) indicate that, during this discrimination phase, the OFC codes for an association between a cue-predicted outcome and the correct response, where the assignation of the incentive value is incoming from other structures such as the AMG. Taking these observations into account, the discrimination sessions in this study could not have been affected by an OFC lesion because the assignation of the stimuli incentive values (cues emitted by the female, external stimuli, and internal spatial bias) could be processed in the AMG, as proposed by Rolls (2000; 2004; see also Rolls & Treeves, 2001) and Schoenbaum, Setlow, Saddoris and Gallagher (2003c).

OFC contribution to early reversal learning. During the reversal learning session the rat is required to re-learn the spatial location if it aims to reach the receptive female box, so that external cues associated with the prior location of the receptive female and internal spatial bias associated with the propioceptive stimuli related to the instrumental oriented response, are now incorrect and must therefore be devalued as predictors of outcome cues. On the other hand, stimuli which were not relevant for the correct performance of the task have become associated with the new location of the receptive female as predictors of outcome cues. As described in other studies (Schoenbaum, Setlow, 2001; Schoenbaum, Setlow, Nugent, Saddoris, & Gallagher, 2003a), any procedure affecting the functionality of the OFC seems to impair abilities required during reversal learning. In this study, no differences were found between the group injected with lower doses of TTX and controls, only those subjects injected with higher doses showed

a reduction in the number of correct trials during the reversal learning. Thus, the effects of TTX on performance in these tasks appear to be due to a specific effect on reversal learning. This finding agrees with the hypothesis of Rolls (2000; 2001; 2004), which suggests that neurons in the OFC participate in the re-assignation of the incentive value of the environmental signals in a determining way, allowing those external stimuli that were not relevant to the correct performance of the task to become associated with the reward. This encoding in the OFC constitutes an internal representation of the association between the incentive value related to an environmental stimulus and an effective or adaptive behavior, which permits problem solving during future attempts (Balkenius & Morén, 2001; Morén, 2002). In this study, the reduction of correct responses in rats with an inactivated OFC may be explained by the absence of an internal representation of previous associative learning, as the experimental subject would not have access to an adequate guide once the incentive value of the stimuli was modified, as reported in studies using monkeys (Baxter, Parker, Lindner, Izquierdo, & Murray, 2000). The loss of an effective behavioral guide could be due to the inactivation of the OFC as damage there would make it impossible to modify previous associations of the external stimuli with the reward (Schoenbaum, Setlow, Saddoris, & Gallagher, 2003c; Balleine & Dickinson, 1998).

During the reversal learning session, a significant increase in the number of trials without responses was obtained only in the case of the TTX2 group, so that differences between groups concerning the number of correct trials were due to an increase in trials without response, i.e. although the males went out from the start box and traversed the stem runway, when they arrived to the intersection they preferred not to enter into the arms of the T maze, as if in conflict to select a specific arm. Although other reports claim that animals with an OFC lesion have shown evident perseverance (Ali-Vafaei & Rashidy-Pour, 2004; Chudasama & Robbins, 2004; Rolls 2000, 2004; Rolls & Treeves, 2001; Winstanley, Theobaild, Cardinal, & Robbins, 2004), this particular result is interesting and agrees with studies which show that OFC lesions can result in less impulsive or persevering behavior. For example, Chudasama & Robbins (2003) trained rhesus monkeys with OFC lesions in acquiring the reversal reward contingency task, and found that lesioned monkeys learned as quickly as controls to inhibit their impulsive/prepotent responses and selected 1 half peanut in order to receive 4. Thus, in the context of these and other findings (Baxter, Parker, Lindner, Izquierdo, & Murray, 2000; Eichenbaum, Shedlack, & Eckmann, 1980), the absence of response during reversal learning is not as surprising as it might first appear. In addition, the OFC in neurological patients is not activated when there is high degree of conflict between predicted reward magnitude and outcome probability (Clark, Cools, & Robbins, 2004). In the present study, the fact that several males with OFC inactivated left the start box and walked along the stem runway, but instead of taking a choice remained in the intersection after the first few reversal trials, could be explained as a conflict between deflected values of cues associated to the receptive female in discrimination session and low values of the rest of stimuli perceived in the reversal session, so that the males preferred not to respond and returned to the start box (i.e., the perseverance behaviors were no longer sustained). Moreover, the fact that males showed a similar

motor activity to that shown during discrimination learning and that of the control subjects, precludes the possibility that these effects on reversal learning could be a result of a low motor activity. Thus, these findings agree with other works in which the inactivation effect of TTX was evaluated for a period as long as 2 or 3 h after intracerebral administration (Ali-Vafaei & Rashidy-Pour, 2004; Boehnke & Rasmusson, 2001; Quiroz, Martínez, Quirarte, Morales, Díaz Cintra, & Prado Alcalá, 2003), and are contrary to the notion that OFC damage invariably results in impulsive decision-making.

Taken together, these findings indicate that a functionally intact OFC appears to be essential for adequate manifestation of reversal learning during sexually-motivated tasks applied to male rats. The crucial role that this prefrontal area plays in reversal learning may result from its influence over the flexibility of response when new signals are established based upon its participation in reassigning incentive value to the stimuli to adequately perform in reversal learning.

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