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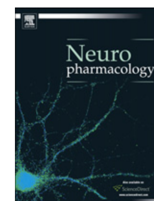

Impairment of endocannabinoids activity in the dorsolateral striatum delays extinction of behavior in a procedural memory task in rats

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ABSTRACT

The dorsolateral striatum (DLS) has been implicated in the learning of habits and procedural memories. Extinction of this kind of memories has been poorly studied. The DLS expresses high levels of the cannabinergic receptor one (CB1), and, lately, it has been suggested that the activation of CB1 in this structure is indispensable for long-term depression (LTD) development. We performed experiments in a T-maze and evaluated the effects of intrastriatal and intrahipocampal administration of the CB1 antagonist AM251 on extinction and on c-Fos expression. We also administered anandamide to evaluate if an artificial increase of endocannabinoids facilitates extinction. Our results indicate clearly a dose–response blockade of extinction induced by AM251 injected into the striatum but a facilitation of extinction when administered into the hippocampus. Anandamide did not induce any observable changes. AM251 effects were accompanied by an increase in c-Fos immunoreactivity in the DLS and its decrease in the hippocampal region, suggesting that the activation of CB1 in the striatum is necessary for the extinction of procedural memories. These findings could be important in some neurological conditions, such as obsessive–compulsive disorder in which striatal activity seems to be abnormal.

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1. Introduction

The existence of different memory systems has long been recognized (Milner et al., 1998; Packard and Cahill, 2001; White and McDonald, 2002; Squire, 2004). Among the brain systems mostly studied in relation to memory are the hippocampal and striatal memory systems, responsible for spatial and procedural memories, respectively (Packard et al., 1989; McDonald and White, 1993), observing an interaction between these two systems. For example, the hippocampus is responsible for the expression of a spatial strategy during the first stages of training. Later on, it is substituted presumably by the striatum when the spatial strategy is switched to a procedural strategy (Packard et al., 1989; McDonald and White, 1993; McIntyre et al., 2003; Chang and Gold, 2003; Colombo et al., 2003). In this study we decided to evaluate the role of endocannabinoids in the dorsolateral striatum in the procedural memory and in the process of extinction.

The high expression of cannabinergic system components, such as receptors and ligands, in structures related to memory, like the hippocampus, amygdale, and basal ganglia (Tsuo et al., 1998; Pettit et al., 1998; Egertova and Elphik, 2000) has made this system a critical target for memory studies (Hampson and Deadwyler, 1998; Murillo-Rodriguez et al., 2001; Rueda-Orozco et al., 2007; for a review see Castellano et al., 2003). Important advances have been achieved in relation to the cannabinergic system and hippocampal (Deadwyler et al., 1996; Sullivan, 1999; Wilson and Nicoll, 2001; Robbe et al., 2006), amygdala (Katona et al., 2001; Marsicano et al., 2002; Azad et al., 2005; Zhu and Lovinger, 2005), and striatal activity (Gerdeman et al., 2002; Kreitzer and Malenka, 2007). Aside from the literature concerning memory and cannabinoids, a growing body of evidence suggests that one of the important functions of the cannabinergic system is its role in the process of extinction.

Extinction of memories has been largely studied in fear conditioning paradigms (for a review see Barad et al., 2006). Extinction has been considered to be a specific inhibitory learning that prevents the expression of a specific behavior without deleting it (for reviews see Rescorla, 2001; Eisenhardt and Menzel, 2007). Recently, the cannabinoid receptor one (CB1) has been implicated in the extinction of fear (Marsicano et al., 2002) and spatial memories (Pamplona et al., 2006; Varvel et al., 2007). One of the structures where the cannabinergic system might be exerting its effects in extinction processes is the striatum, in which CB1 is highly expressed (Tsuo et al., 1998; Pettit et al., 1998; Egertova and Elphik, 2000; Köfalvi et al., 2005). For example, it has been demonstrated that CB1 receptor activation is necessary for striatal LTD (Gerdeman et al., 2002; Robbe et al., 2002) and its failure to produce LTD may

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account for the development of neuropsychiatric conditions, such as Parkinson disease (Kreitzer and Malenka, 2007). In this context, we have decided to explore the potential role of the striatal cannabinergic system in the extinction of procedural memories.

2. Methods

2.1. Animals

Wistar albino male rats (weight 250–300 g at the beginning of the experiment) were used for all experiments. They were housed individually in a temperature-controlled environment with a 12-h light/dark cycle (lights on at 8:00 A.M.) and ad libitum access to water. Rats were housed individually 5 days before experimental procedures started.

2.2. Drugs

The CB1R antagonist AM251 was obtained from Cayman Chemical, anandamide was obtained from Sigma–Aldrich. The AM251 concentrations were 0.4 μg (AM251 – 0.4), 1.6 μg (AM251 – 1.6), and 3.2 μg (AM251 – 3.2) dissolved in dimethyl sulfoxide (30%DMSO) in phosphate-buffer saline (PBS). Anandamide concentration was 1.0 μg in the same vehicle. Final volume for all injections was 1 μl. The concentration of anandamide was selected on the base of our previous study were this concentration was effective to impair the consolidation of memory in a different task (Rueda-Orozco et al., 2007).

2.3. Surgery

Ten days before starting the experimental procedures, rats were implanted bilaterally under anesthesia (cocktail: ketamine 66 mg/kg + xylazine 0.26 mg/kg + acepromazine 1.3 mg/kg) with a couple of guide cannulae (23 gauge) directed to either the dorsolateral striatum ($A = 0.2$, $L = 3.0$, $V = 3.0$, Fig. 4b) or the hippocampus ($P = 4$, $L = 2.5$, $V = 2.2$, Fig. 4a). The injector protruded 1 mm from the tip of the guide cannula. Three supporting stainless steel screws were implanted into the skull. Once the experiment was finished, brains were prepared for histological analysis with cresyl violet staining to verify the correct placement of the injector (Fig. 1a and b).

2.4. Behavioral training

The behavioral apparatus was a home-made adaptation of the plus maze described in Packard and McGaugh (1996). The maze consisted of four identical arms (length × width × height, 60 × 12 × 15 cm) containing food dispensers at their ends; the center of the maze was a platform measuring 12 × 12 cm. The maze was elevated 90 cm above the floor and located in the center of a testing room that contained extramaze cues. Cocoa-Krispies (Kellogg's) were used as reinforcement.

Ten days after the surgery rats were reduced to 85% of their free-feeding weight over 5 days and maintained at this weight throughout the experiment. Five days before starting the training, rats were manipulated and habituated to the maze (10 min per day). Approximately 4 g/day of food reward was delivered inside the maze during the habituation process. The next day, after attaining habituation, rats were trained to turn right or left (50% of the rats were trained to the left and 50% to the right) to obtain food reward located at the end of the arm (approx. 0.4 g). In this type of training, rats can use either a spatial strategy (go to the arm in a fixed position of the room) or a procedural strategy (turn right or left according to the situation) to obtain the reward (Packard and McGaugh, 1996; Chang and Gold, 2003; McIntyre et al., 2003). Each trial started with the rat confined into the start box at the extreme of the start arm for 10 s. Once this time elapsed, the rat was released and allowed to explore the three arms until it found the reward or after 2 min had elapsed. Once the rat reached the end of the arm with the reward, the rat was confined until consuming it or after 45 s had elapsed. Entering into the unrewarded arm of the maze was considered as an incorrect trial. When rats entered into the incorrect arm, they were allowed to trace back to the reinforced correct arm and then they were confined there and allowed to consume the reward. Ten trials per session (five sessions of training, one session per day), were performed for each rat, an approximate 35-s interval was allowed between trials. During this time, the rats were returned to their home cages and the maze was cleaned with chlorine solution to prevent odor cues.

At the end of the training, rats were subjected to two sessions of 10 extinction trials. In these trials, rats were placed into the start arm used during training but the reward was provided at the arm opposite to the one rewarded during training (for example, training: start arm north, rewarded arm west; extinction: start arm north, rewarded arm east). As in the training phase, trials were considered as incorrect when rats entered the unrewarded arm (rewarded arm during training) and they were also allowed to trace back to the rewarded arm (unrewarded arm during training). A schematic view of the training and extinction protocols can be seen in Fig. 1c and d. Vehicle or AM251 was given 5 min before starting both extinction sessions to affect cannabinergic transmission specifically during the extinction process. Finally, in order to insure the integrity of the motor control, the day after the last session of extinction, 15 rats were randomly selected to integrate three independent groups. Animals were trained to run for food reward into a corridor of 140 cm length and 12 cm width allowing the rats to run without turns. Eight trials per rat were performed while animals were under vehicle or AM251 (3.2 μg) or ANA (1 μg).

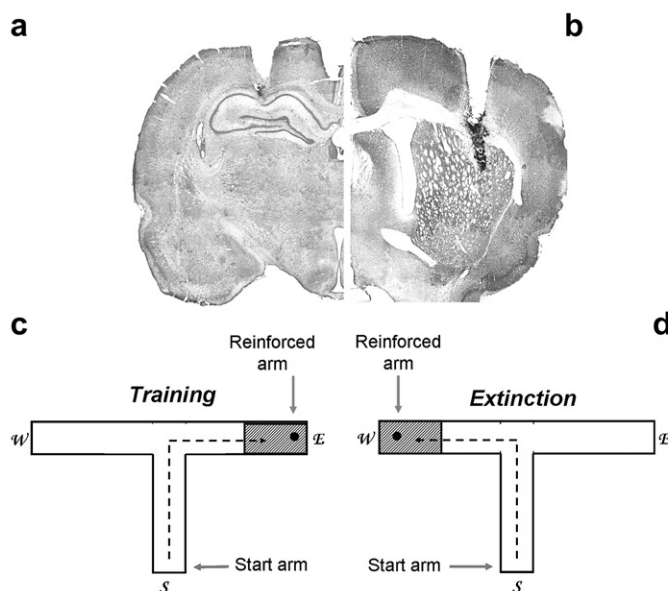

Fig. 1. Photomicrographic representation of the injection sites in the hippocampus (a) and striatum (b). Schematic representation of training (c) and extinction (d). During training, rats are released from "S" and reinforced in "E", during extinction rats are released again from "S" but are reinforced in "W".

2.5. c-Fos immunohistochemistry

Five groups of rats ($n = 5$ per group) were used for immunohistochemistry. Groups were assigned as follows: group of food deprivation (85% of weight during 5 days, Control-0), group of first day of training (10 trials, Training-1) group of fifth day of training (50 trials of training in 5 days, Training-5), group of extinction (50 trials of training and five trials of extinction, Extinction), and group of extinction under AM251 (50 trials of training and 5 trials of extinction, AM-Extinction). Ninety minutes after the last trial in each group, rats were anaesthetized with sodium pentobarbital and perfused transcardially with 200 ml PBS and 200 ml 4% PFH. The moment selected to kill the animals and collecting the brains was determined according to previous literature showing that maximum peak of c-Fos expression is reached between 60 and 120 min after an event (Morgan and Curran, 1989). The brains were removed and processed for immunohistochemistry. Coronal sections (50 μm) were obtained by means of a cryostat. Sections were collected, one out of five, and were selected with reference to the Paxinos and Watson atlas (1986). Immunohistochemistry was developed with the peroxidase-diaminobenzidine reaction. Briefly, tissue sections were incubated at room temperature with 0.075% H_2O_2 in PBS for 20 min to block the endogenous peroxidase. Then, sections were blocked in 5% normal goat serum/PBS with 0.3% Triton X-100 (TPBS). Upon the completion of this part of the procedure, sections were incubated for 24 h at 4 °C with c-Fos antibody (Santa Cruz, CA, USA) (1:1000 in TPBS). Bound antibodies were revealed by using the avidin–biotin peroxidase complex method (Vector Laboratories). Sections were observed under a light microscope (Olympus BX41). Images were captured on a 12-bit digital camera (Evolution VF; MediaCybernetics) at a magnification of 10× for cell-nuclei counting. Analysis of the c-Fos immunoreactivity was performed in the dorsolateral region of the striatum and in the granular layer of the dentate gyrus (DG), CA1 and CA3 hippocampal regions. Images were displayed on a computer screen using the Image-Pro Plus software (MediaCybernetics), which allowed us to mark individual c-Fos immunoreactive (F-ir) cells. Counts of F-ir cells were obtained bilaterally from three brain sections for each region and averaged for subsequent analysis. Data on the analysis of the sub-regions of the hippocampus was performed as percentage of change with respect to the total number of F-ir cells of the hippocampus.

All behavioral training and killing of animals for c-Fos immunohistochemistry were performed between 12:00 and 15:00 (corresponding to the light phase of the cycle).

2.6. Statistical analyses

For the performance on the plus maze an index of correct responses was elaborated fort each rat (correct trials/total trials). Index of correct responses were analyzed by means of a two-way ANOVA test (treatment × session of training); Bonferroni t-test was used to conduct multiple comparisons among groups.

Significant differences for c-Fos immunohistochemistry and running speed were obtained by using a one-way ANOVA test and a Bonferroni post hoc test. Results are displayed as means ± s.e.m. Additional Pearson correlation analysis was used to detect interactions between-structures and intra-structures. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Control group in the T-maze

The first experiment was performed with the control group with no implant. This group would set the standard parameters of normal behavior and c-Fos activity of the rats acquiring and extinguishing the behavior. Repeated measures and the one-way ANOVA analysis revealed significant changes depending on the day of training, clearly delineating a learning curve, and indicating that rats improve their performance as the training progresses. In brief, session 1 was significantly different from the rest of sessions, including extinction 1 ($P < 0.001$ in all cases except against extinction 2$P = 0.004$, and extinction 3$P = 0.035$). No significant differences were observed between sessions 2 and 5, indicating that rats learned the task relatively fast. Data from extinction sessions were divided in blocks of five trials to better appreciate the trial in which the behavior becomes extinct. Extinction 1 (first five trials) was significantly different from all other sessions including training and extinction ($P > 0.001$ in all cases). These data indicate that the first five trials of extinction are critical since rats take only those trials to reach a high level of correct responses. We observed that, the first five trials of extinction are the ones in which the rats "realize" that the reinforced arm has changed. In the second five trials, the level of accuracy went back above the 0.8 index and no significant differences were observed when compared against sessions 2, 3, 4, or 5. After extinction 2, the high level of accuracy remained unchanged in extinction 3 and 4, indicating that extinction had been achieved (Fig. 2a).

3.2. Control group c-Fos

The immunohistochemical assays reveal a significant increase in immunoreactive cells in the dorsolateral striatum as a result of training. As shown in Fig. 2, there is a significant difference between day-0 and day-1 ($P = 0.028$), and extinction session ($P = 0.048$) but not with regard to day 5 (Fig. 2b). Analysis of the hippocampus (CA1, CA3, and DG together, Fig. 2c) indicates that there is also a significant increase in the number of positive cells, between day-0 and day-1, day-5 and the extinction session ($P < 0.001$ in all cases). Significant differences also emerged between day-1 and day-5, and extinction session ($P < 0.001$ in both cases). Comparison between day-5 and extinction session was also significant ($P < 0.001$). The analysis of the hippocampal sub-regions failed to show significant differences in the DG among groups (Fig. 2d). On the other hand, CA3 (Fig. 2e) and CA1 (Fig. 2f) regions show significant differences between day-0 and day-5 (CA3 $P = 0.001$; CA1 $P < 0.001$), between day-0 and extinction session (CA3 $P < 0.001$; CA1 $P < 0.001$), and between day-0 and day-1 (CA1 $P = 0.19$). A correlation analysis was performed considering the striatum, general activity of the hippocampus, CA1, CA3, and DG. The number of c-Fos-reactive cells corresponding to the first day of training reveals a significant correlation between CA1 and CA3 hippocampal regions. Interestingly, after the fifth day of training and besides the general increase of hippocampal activity no correlation between CA1 and CA3 was observed. After the first block of extinction trials, the correlation between CA1 and CA3 was regained. No correlation between the hippocampus and the striatum was observed. All correlations are summarized in Table 1.

3.3. Striatal administration of AM251

Four groups of rats ($n = 10$ per group) were used to construct a dose–response curve of AM251 administered in the striatum. As shown in Fig. 3a, no differences among groups were observed during the 5 days of training in which no drugs were given; nevertheless significant differences emerged during the extinction sessions. For a better appreciation of these differences we plotted the last training session and the extinction sessions on a different graph (Fig. 3b). As aforementioned, no differences were observed among groups during training session 5. On extinction 1, no differences between control group, AM-0.4 and AM-1.6 were observed, nevertheless AM-3.2 was significantly different from the control group ($P = 0.042$) and AM-0.4 ($P > 0.001$). AM-0-04 was also significantly different from AM-1.6 ($P = 0.018$).

On extinction 2, AM-1.6 and AM-3.2 were significantly different from the control group ($P < 0.001$ in both cases). AM-3.2 was also significantly different from AM-0.4 ($P = 0.002$). On extinction 3, only AM-3.2 remained significantly different ($P = 0.002$) from the control group. These data demonstrate a clear dose–response curve for AM251, since the 3.2-μg dose showed effects in three of the four sessions of extinction, whereas the 1.4-μg dose exerted effects only on two sessions, and the 0.4-μg dose had no effects.

3.4. c-Fos and striatal administration of AM251

Intrastriatal administration of AM251 produced a clear increase in the number of c-Fos immunoreactive cells in the dorsolateral striatum as compared with the control group and day-5 of training ($P < 0.001$ in both cases; Fig. 3c). In contrast, a significant decrease was observed in the hippocampus ($P < 0.001$; Fig. 3d). Further analysis of the hippocampus revealed a significant decrease in the number of c-Fos immunoreactive cells in the CA1 region ($P < 0.001$; Fig. 3g) whereas CA3 (Fig. 3e) and DG (Fig. 3f) remained unchanged. The correlation between CA and CA3 observed during extinction was lost under AM251 (Table 1).

3.5. Hippocampal administration of AM251

Hippocampal administration of AM251 during extinction was performed to assess the specificity of the striatum on this kind of extinction. As depicted in Fig. 4a, hippocampal administration of the most effective dose of AM251 in the striatum (3.2 μg) did not block extinction rather facilitated it significantly in the first session ($P = 0.014$). No differences as compared to the control group were observed in extinction sessions 2, 3, and 4. When compared against AM251 – 3.2 μg in the striatum, the effect was significantly different in extinction session 1 ($P < 0.001$), session 2 ($P < 0.001$), and session 3 ($P = 0.006$). Similarly to the striatum groups, hippocampal group did not show differences with regard to the control group during the training sessions.

3.6. Striatal administration of anandamide

The administration of ANA, a natural agonist of the CB1, into the striatum during extinction was performed to assess the specificity of the CB1 blockade in the striatum on this kind of extinction. As depicted in Fig. 4a, 1 μg of ANA in the striatum did not block or facilitates extinction. No differences as compared to the control group were observed in extinction sessions 1, 2, 3, and 4. Similarly to the rest of the groups in the striatum and hippocampus, ANA group did not show differences with regard to the control group during the training sessions.

Finally, rats under the highest dose of AM251 or ANA showed a running speed similar to control groups. Additionally, all rats in all training, extinction and motor control sessions consumed the

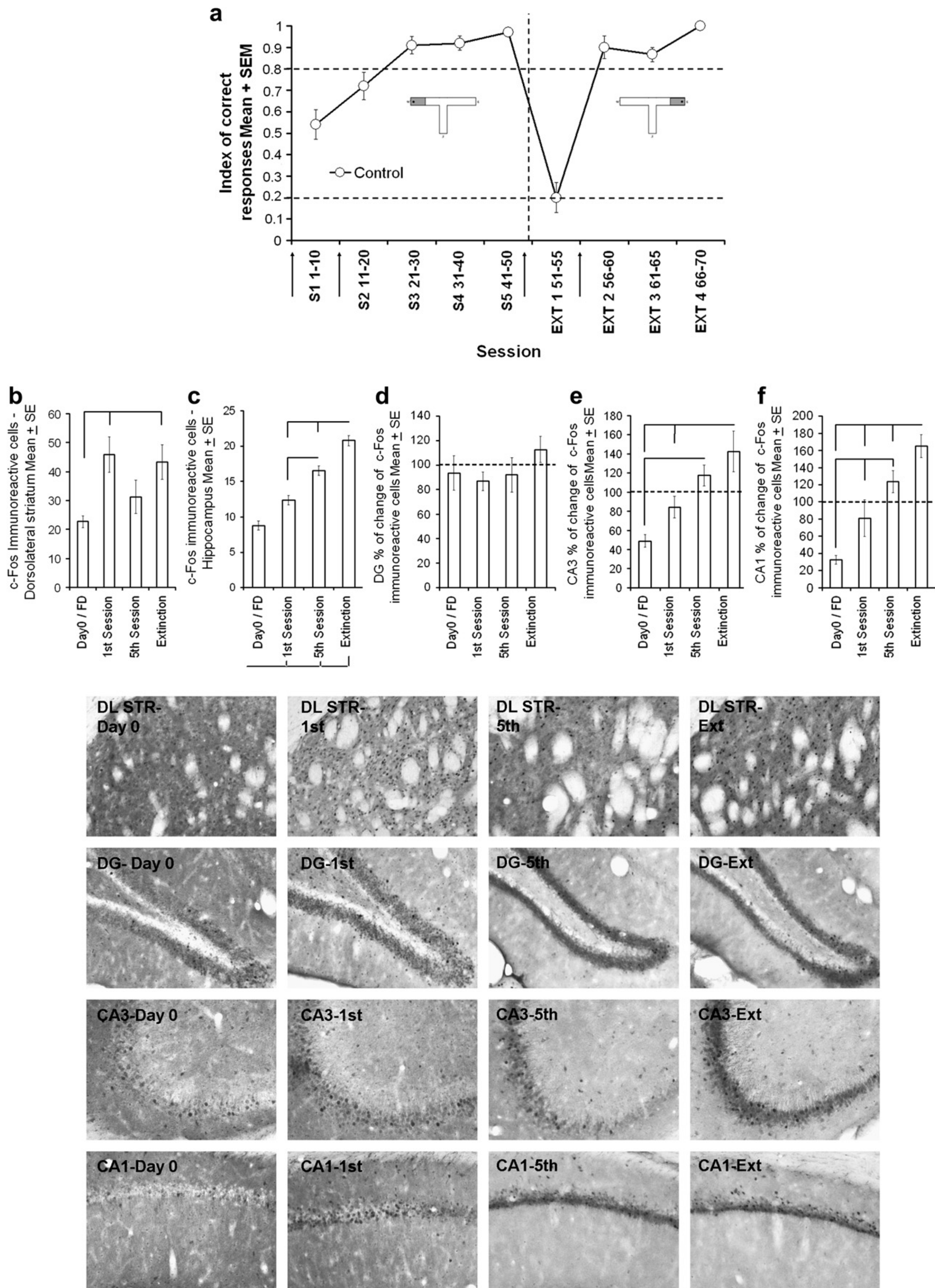

Fig. 2. Control group. (a) Performance of rats with no implants during training and extinction sessions. Data are presented as the mean ± SEM of the index of correct responses (correct trials/total trials). The extinction sessions are presented after vertical doted line (dashed area on inset maze represents the place where the reinforcer was given during training and extinction sessions), arrows on the *x*-axis indicate the moments at which samples for c-Fos were collected. Horizontal dotted line in 0.8 indicates high preference for the reinforced arm, while doted line in 0.2 indicates preference for the unreinforced arm. Histograms for c-Fos immunoreactive cells during day-0/FD, first and fifth training session and extinction session on the dorsolateral striatum (b), hippocampal formation (c) and DG (d), CA3 (e) and CA1 (f) hippocampal sub-regions, significant differences are represented by lines between corresponding conditions. Bottom panels show representative micrographs of the dorsolateral striatum (DL STR), dentate gyrus (DG), CA3 (CA3) and CA1 (CA1) on day 0 (Day-0), training session one (first) and five (fifth), and extinction session (Ext).

Table 1
Correlations between dorsolateral striatum, hippocampus and the different regions of hippocampus

	CA1	GD	CA3	Hippocampus
Day-0/FD				
DL striatum	0.655; $P=0.230$	0.878; $P=0.0501$	0.881; $P=0.0486$	0.755; $P=0.140$
CA1		0.900; $P=0.0372$	0.882; $P=0.0477$	0.968; $P=0.0068$
GD			0.961; $P=0.0091$	0.965; $P=0.0076$
CA3				0.955; $P=0.0112$
Day-1				
DL striatum	0.0461; $P=0.941$	−0.448; $P=0.450$	−0.124; $P=0.843$	0.200; $P=0.747$
CA1		0.626; $P=0.259$	0.878; $P=0.0499$	0.966; $P=0.00740$
GD			0.373; $P=0.537$	0.488; $P=0.405$
CA3				0.886; $P=0.0457$
Day-5				
DL striatum	0.727; $P=0.164$	−0.309; $P=0.613$	0.847; $P=0.702$	0.729; $P=0.162$
CA1		−0.695; $P=0.192$	0.385; $P=0.523$	0.282; $P=0.646$
GD			−0.0521; $P=934$	0.364; $P=0.547$
CA3				0.831; $P=0.815$
Extinction				
DL striatum	0.148; $P=0.812$	0.492; $P=400$	0.191; $P=0.758$	0.215; $P=0.728$
CA1		0.334; $P=0.583$	0.877; $P=0.0479$	0.984; $P=0.0024$
GD			−0.109; $P=0.861$	0.320; $P=0.600$
CA3				0.885; $P=0.0463$
Extinction-AM				
DL striatum	−0.102; $P=0.870$	0.048; $P=0.938$	0.139; $P=0.823$	0.0280; $P=0.964$
CA1		0.799; $P=0.105$	0.556; $P=0.330$	0.947: $P=0.0144$
GD			0.252; $P=0.682$	0.876; $P=0.0512$
CA3				0.656; $P=0.229$

totality of the rewards indicating that the results on extinction are not related to motor or motivational alterations (Fig. 4b).

4. Discussion

Our data indicate that rats learn this task relatively fast, reaching a stable performance in session 3 and maintaining their proficiency through session 5. The c-Fos activity during these stages indicates that the striatum increases its activity on day-1 but not on day-5 when compared with the activity observed in non-trained and food-deprived groups. We discard that the increase in the activity during the first day was caused by the motor activity performed by the rat, since during the fifth day of training, motor activity is also performed and c-Fos immunoreactivity decreases to non significant levels. This fact suggests that the increase during the first day is related with something more than motor activity. After the fifth day of training, during the extinction session, rats show a fast rate of extinction; only five trials are required to learn the new location of the reinforcer, regaining proficiency to a similar level to that exhibited on the fifth day of training. Interestingly, c-Fos activity increases to levels similar to those of day-1 of training, suggesting that the increase during day-1 is also related with something relevant to the task and the new location of the reinforcer.

Analysis of the general hippocampal activity (not divided by regions) reveals a significant increase in the number of c-Fos positive cells during days 1, 5, and during the extinction session, when compared with day-0. The correlation analysis revealed that CA1 and CA3 are correlated on day-1. On the other hand, and besides the significant increase in general hippocampal activity on day-5, no correlation was observed between CA1 and CA3, finally during the extinction session the correlation between CA1 and CA3 is recovered.

These data indicate that day-1 and day-5 are functionally different, in the sense that when rats are exposed for the first time to the task and the learning process is starting (day-1), a correlation between hippocampal regions is required; whereas during day-5 when the task is well learned and proficiency is high this correlation does not seem to be required anymore. Concurrently, striatal activity is reduced on the fifth day as compared with the first day of training. This is consistent with the literature to certain extent. For example, in different studies, the higher activity of the hippocampus has been related with early stages of learning (McIntyre et al., 2003). Here we show an increase in hippocampal activity during the first and last day of training but the correlation of hippocampal sub-regions changed, suggesting that during the last day of training no hippocampal correlation is necessary for solving the task. On the other hand, an increase in striatal activity seems to be necessary on the first day but not on the fifth day.

Based on c-Fos expression, a clear difference in activity of these brain structures is observed depending on the stages of training. Interestingly, during the extinction session when experimental conditions have changed, striatal activity increases a new exhibiting the significant increase lost on the fifth day of training. In addition, the hippocampal activity reaches its highest number of c-Fos-reactive cells and, more importantly, the correlation between CA1 and CA3 activity is observed again. The activity during extinction in the hippocampus and striatum is similar to the activity observed during the first day of training when learning is at its beginning. Hence, we believe this kind of activity reflects the hippocampal and striatal encoding of the conditions to be learned in the task, either on the first day of training, when a specific location of the reinforcer must be learned or during the extinction session, when a relocation of the reinforcer must be learned.

The main finding of our experiments is that CB1 receptor activation in the dorsolateral striatum seems to be necessary for the extinction of this particular kind of memory, since its acute blockade prevents extinction (see Fig. 3a and b). For rats under AM251 – 3.2 μg at least 15 trials were required to recover the high level of proficiency; whereas the control (vehicle) group required only five trials. According to our data, pharmacological activation of the CB1 in the dorsolateral striatum does not induce any effect on extinction rates (Fig. 4a), indicating that the effects induced by AM251 are

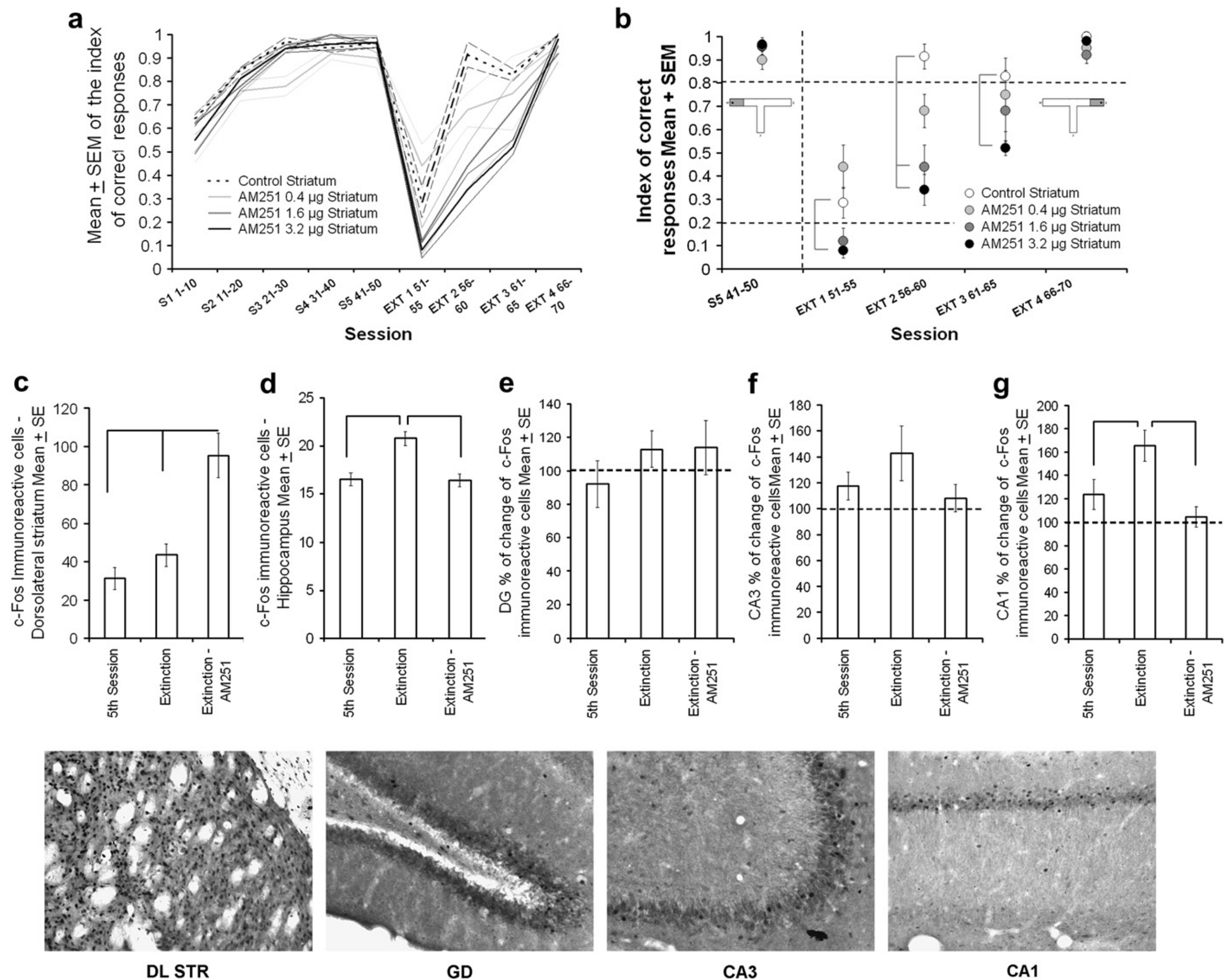

Fig. 3. Dose–response curve of AM251 in the dorsolateral striatum. (a) Performance of control and experimental groups during training and extinction. Note that no significant differences were obtained among groups during training sessions when no treatment was given; differences emerged during extinction sessions when treatments were given. (b) A closer view of the last day of training (data before vertical doted line; no differences among groups) and extinction sessions. Note the clear dose–response effect on extinction, with the highest dose been the most effective for delaying extinction; significant differences are marked with lines between specific treatments. Horizontal dotted line in 0.8 indicates high preference for the reinforced arm, while dotted line in 0.2 indicates preference for the unreinforced arm. Histograms for c-Fos immunoreactive cells during the fifth control session and extinction sessions under 3.2 μg of AM251 (most effective dose on behavior) applied to the dorsolateral striatum (c), hippocampal formation (d), and DG (e), CA3 (f) and CA1 (g) hippocampal sub-regions; significant differences are represented by lines between the corresponding conditions. Bottom panels show representative micrographs of dorsolateral striatum (DL STR), dentate gyrus (DG), and CA3 (CA3) and CA1 (CA1) extinction session under 3.2 μg AM251. Data from day-0/FD and day-1 of training is presented in Fig. 2. Data from the fifth and control extinction sessions are presented again for comparison against AM251 treatments.

indeed related to the blocked of CB1. Also, the running speed of the animals under the different treatments into the striatum does not change with respect to control groups, discarding any effect on motor activity (Fig. 4b). The blockade of extinction caused by AM251 was accompanied by a significant increase in c-Fos activity in the dorsolateral striatum as compared with the control group in the same condition. This activity was the highest activity observed in all conditions, suggesting an over-activation of the striatum. On the other hand, the hippocampal activity was reduced as compared with the control group in the same condition. The sub-region analysis of the hippocampus reveals that CA1 activity was significantly decreased and the correlation between CA1 and CA3 was lost. This kind of hippocampal activity is very similar to that observed during the fifth day of training and is consistent with the rat going to the T-maze arm where the reinforcer was originally located. Summarizing, the delay of extinction caused by intrastriatal blockade of CB1 was accompanied by an over-activation of the dorsolateral striatum and similar hippocampal activity to that observed on the fifth day of training.

The specificity of the relevance of the striatal CB1 activation was proven by using the most effective dose of AM251 in the hippocampus. This manipulation resulted in no blockade but rather a facilitation of extinction as observed in the first block of extinction trials. These results are consistent with the proposal that, at the beginning of the training, hippocampal activity is more important for solving the task. At the end of the training, when the task has become a habit, the striatum is more important. However, when conditions change during extinction, the hippocampus is reactivated, presumably to re-evaluate the task conditions as at the beginning of the training (Packard et al., 1989; Packard and McGaugh, 1996; Chang and Gold, 2003; McIntyre et al., 2003). It is feasible that the blockade of CB1 in the hippocampus in this phase may facilitate hippocampal participation and hence extinction. This is also consistent with previous reports showing that CB1 blockade

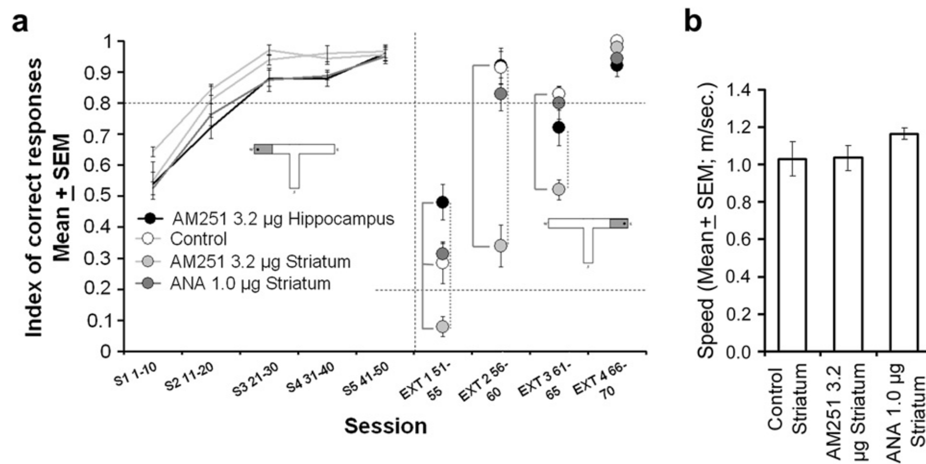

Fig. 4. Intrahippocampal administration of AM251 and intrastriatal administration of anandamide. (a) Performance of rats during training (before vertical doted line) and during extinction sessions under control treatment (open circles), and with 3.2 µg of AM251 into the hippocampus (black circles) and into the striatum (clear gray circles) and 1.0 µg of anandamide into the striatum (dark gray circles). Note that hippocampal administration does not induce blockade of extinction, it rather facilitates it during the first block of extinction. Also, intrastriatal administration of ANA does not induce any change in extinction rates. Significant differences against control group are indicated by solid lines whereas significant differences between hippocampal and striatal administration of AM251 are indicated by doted lines. Horizontal dotted line in 0.8 indicates high preference for the reinforced arm, while doted line in 0.2 indicates preference for the unreinforced arm. (b) Running speed (m/s) of groups under treatments into the striatum, note that the most effective dose of AM251 and the dose of ANA does not affect running speed indicating no effect on motor activity.

in the hippocampus facilitates learning in hippocampal related tasks (Rueda-Orozco et al., 2007; Pamplona et al., 2006; Varvel et al., 2007). Nevertheless, the relationship between hippocampus and striatum and how it is modified by extinction has not been studied. However, our data suggest that, during extinction, the activity of the brain may be similar to the first stages of learning. This is only natural, since the rat is substituting a strategy previously learned but no longer effective with a new more efficient one. Along these lines, previous reports have shown that in different paradigms studied, the extinction process is dependent on protein synthesis, following the same time course as the one occurring in the first acquisition (Inda et al., 2005; Santini et al., 2004). It is noteworthy to mention, that the behavior of the rats, perseverating on the previous location of the reinforcer, indicates that CB1 blockade in the striatum does not affect the ability to recall, since our rats were recalling the previous position very effectively, but prevents the repression of this kind of memory. The exhibition of this behavior also discards possible effects on motor activity.

The blockade of extinction by AM251 in the striatum results in a delayed ability to re-learn the new location of the reward; hence, leading to perseveration on the old position. These results suggest that the cannabinergic modulation of glutamatergic terminals is prevented by AM251, as has been suggested by Köfalvi et al. (2005). Thus, the lack of cannabinergic modulation on glutamatergic terminals may result in hyperactivity of the striatum, as can be seen in the immunohistochemical assays. This hyperactivity may cause the impossibility of extinguishing memories regulated by the striatum. Our data are consistent with previous reports showing that the cannabinergic system is necessary for the extinction of memories (Marsicano et al., 2002; Pamplona et al., 2006; Varvel et al., 2007). Further experiments should be performed to establish the exact role of the CB1 on striatal glutamatergic and GABAergic terminals in the extinction of memories and to determine what kind of cells have been activated.

On the other hand, our data might shed some light on psychiatric conditions, such as obsessive–compulsive disorder, in which abnormal activity of the basal ganglia has been suggested as a contributing factor in humans. This condition is characterized by the incapability of extinguishing certain thoughts or actions with the undesirable result of perseverating on a specific action or thought (for a review, see Tekin and Cummings, 2002; Friedlander and Desrocher, 2006). This is similar to the behavior exhibited by our rats under AM251, where perseveration on the previous reinforcer position was observed. Nevertheless, more experiments must be performed to be able to establish an obsessive–compulsive disorder model.

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