Behavioural Brain Research 277 (2015) 193–203

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Dual role of serotonin in the acquisition and extinction of reward-driven learning: Involvement of 5-HT1A, 5-HT2A and 5-HT3 receptors

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HIGHLIGHTS

- We examine the role of serotonin in a reward dependent operant conditioning task.
- Serotonin levels modulated acquisition and extinction phases.
- Differential contribution of serotonin receptors was observed in both phases.

ARTICLE INFO

Article history:
Received 22 January 2014
Received in revised form 9 June 2014
Accepted 10 June 2014
Available online 17 June 2014

Keywords:
Operant conditioning
Serotonin
Extinction
Acquisition
Reward

ABSTRACT

Serotonin (5-HT) has been proposed as a possible encoder of reward. Nevertheless, the role of this neurotransmitter in reward-based tasks is not well understood. Given that the major serotonergic circuit in the rat brain comprises the dorsal raphe nuclei and the medial prefrontal cortex (mPFC), and because the latter structure is involved in the control of complex behaviors and expresses 1A (5-HT1A), 2A (5-HT2A), and 3 (5-HT3) receptors, the aim was to study the role of 5-HT and of these receptors in the acquisition and extinction of a reward-dependent operant conditioning task. Long Evans rats were trained in an operant conditioning task while receiving fluoxetine (serotonin reuptake inhibitor, 10 mg/kg), tianeptine (serotonin reuptake enhancer, 10 mg/kg), buspirone (5-HT1A partial agonist, 10 mg/kg), risperidone (5-HT2A antagonist, 1 mg/kg), ondansetron (5-HT3 antagonist, 2 mg/kg) or vehicle. Then, animals that acquired the operant conditioning without any treatment were trained to extinct the task in the presence of the pharmacological agents. Fluoxetine impaired acquisition but improved extinction. Tianeptine administration induced the opposite effects. Buspirone induced a mild deficit in acquisition and had no effects during the extinction phase. Risperidone administration resulted in learning deficits during the acquisition phase, although it promoted improved extinction. Ondansetron treatment showed a deleterious effect in the acquisition phase and an overall improvement in the extinction phase. These data showed a differential role of 5-HT in the acquisition and extinction of an operant conditioning task, suggesting that it may have a dual function in reward encoding.

1. Introduction

Dysregulation of serotonin (5-HT) has been widely associated with the pathophysiology of psychiatric disorders like depression and obsessive compulsive disorder [1]. The dorsal raphe nucleus (DRN) is the origin of the serotonergic system; projections from this area innervate multiple structures of the forebrain, including the prefrontal cortex (PFC) [2]. The PFC is a critical brain region in behavioral flexibility, decision making, and for learning operant conditioning tasks [3–8]. The PFC receives serotonergic projections from the DRN, where pyramidal and parvabulmin neurons are highly enriched in serotonin receptor types 1A (5-HT1A) and 2A (5-HT2A), whereas GABAergic interneurons also express the 3A subtype (5-HT3A) [9,10]. Pyramidal neurons from the PFC send projections back to the DRN and the anatomical characteristics of this circuit suggest a critical role of 5-HT in modulation

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http://dx.doi.org/10.1016/j.bbr.2014.06.025
0166-4328/

of the PFC cortical networks involved in learning and memory [10].

Behavioral experiments in humans and rodents have shown that the depletion of 5-HT impairs flexibility and rewards cue association [11–13]. Interestingly, animals with a genetic deletion of 5-HT have an impaired retrieval of spatial memory but have enhanced learning and memory in fear conditioning tasks [14]. Inhibition of the serotonin transporter (SERT) by the selective serotonin reuptake inhibitor (SSRI) fluoxetine has been classically used for the treatment of psychiatric illnesses [15–17], but the effects on reward-dependent learning have not been fully characterized. In this sense, it has been shown that SERT knockout (KO) rats had perseverative responses in Pavlovian conditioning tasks and advantageous decision-making [18,19]. Additionally, either fluoxetine administration or the deletion of SERT in mice reduces food responding behavior [76].

Although the serotonin receptor subtype 1A has been widely studied, contradictory evidence has been reported. Some works showed that the activation of this receptor improved memory [20], while others reported it to impair spatial learning, navigation, memory, and passive avoidance [21–24]. Although 5-HT3 receptors were less studied regarding learning and memory, it has been shown that 5-HT3 antagonism impairs fear conditioning but improves hippocampal-dependent learning [25,26]. A positive effect of the 5-HT3 blockade on cognition was also observed after the administration of a 5-HT3 antagonist to non-human primates [27]. Activation of 5-HT2A receptor facilitates the consolidation of an object memory whereas blockade of this receptor impaired the acquisition of fear memory extinction [28]. Additionally, 5-HT2A antagonism reduced premature responding and impulsivity in a 5-choice serial reaction time task (5-CSRTT) [29,30]. Collectively, the previous studies showed that manipulations of 5-HT levels or the blockade/activation of different subtypes of 5-HT receptors induce different effects on diverse behavioral paradigms. Despite the considerable amount of reports that highlighted an important role of 5-HT in different kinds of tasks, the effects of 5-HT in reward-dependent tasks like the operant conditioning task are still poorly understood.

Therefore, the aim of this manuscript was to study whether manipulation of the serotonergic circuit affects acquisition and/or extinction of an operant conditioning task. For this purpose, we studied the effects of fluoxetine and tianeptine on acquisition and extinction of the task. Cortical 5-HT levels were also measured in several stages of these phases. Next, we evaluated the different roles of the 5-HT receptors relevant to the mPFC, i.e., 5-HT1A, 5-HT2A, and 5-HT3, in both phases of an operant conditioning task.

2. Materials and methods

All experimental procedures were approved by the Ethics Committee of the IByME-CONICET (A2008) and were conducted according to the NIH Guide for Care and Use of Laboratory Animals.

2.1. Animals

Two-month-old Long Evans rats (250–300 g, IBYME-CONICET) were used for behavioral testing. The animals were housed in stainless-steel cages (40 cm × 22 cm × 20 cm, $L \times W \times H$) with sawdust as bedding and metal lids. Room conditions were of 21 ± 2 °C with a 12/12 h light/dark cycle (lights on at 8 a.m.). Rats were handled for at least 10 min every day and then weighed as a habituation routine to the operator. Experimental subjects were singly housed to maintain precise control of their body weight during behavioral testing and to avoid any male dominance issues that could lead to reduced food ingestion and fluctuations in body weight.

2.2. Sample preparation and 5-HT quantification by HPLC

We quantified the levels of 5-HT in the PFC by HPLC in different phases of the acquisition and extinction of the operant conditioning task. To achieve this, rats were sacrificed and the PFC was dissected in animals trained with the following schedule: naïve (untrained animals, $n = 8$); 1° acquisition session (coincident with ~50% correct responses, $n = 7$); 5° acquisition session (animals that completely acquired the task, $n = 8$); 1° extinction session ($n = 6$); 5° extinction session (animals that reached ~50% of responses, $n = 7$); and 10° extinction session (animals that reached almost complete extinction with residual response ~10–20%, $n = 6$). After the corresponding session, animals were killed by decapitation; PFC was immediately dissected and homogenized in perchloric acid 0.2 N. Quantification of 5-HT in these samples was carried out by means of high-performance liquid chromatography using a Phenomenex Luna 5-lm, C18, 150 × 4.60 mm column (Phenomenex, Torrance, CA, USA), and LC-4C electrochemical detector with glassy carbon electrode (BAS, West Lafayette, IN, USA). The working electrode was set at +0.80 V with respect to an Ag/AgCl reference electrode. The mobile phase contained 0.76 M NaH2PO4.H2O, 0.5 mM EDTA, 1.2 mM 1-octane sulfonic acid, and 15% acetonitrile, and pH was adjusted to 2.8. The limit of quantification of 5-HT was 1.2 ng/ml.

2.3. Experimental design and drug administration

Fluoxetine (10 mg/kg, Gador) and tianeptine (10 mg/kg, Servier) were dissolved in tap water and orally administered 1 h and 45 min before the initiation of experimental procedures. Risperidone (1 mg/kg, Gador) and buspirone (10 mg/kg, Raffo) were administered and dissolved in the same way but given 1 h prior to training sessions. Ondansetron (2 mg/kg, LKM) was intraperitoneally (I.P.) injected 30 min before starting behavioral tests. Control animals received the corresponding vehicle via the same route. The animals that received any of the drugs listed above during the phase of acquisition of the operant conditioning task were not used for further studies in the extinction protocol. The effects on the extinction of the pharmacological agents described above were assessed in a whole new set of animals (drug naive) that successfully acquired the operant conditioning task without any treatment. The rationale of this experimental design was to avoid carryover and sensitization effects that could be interfering with the true effects on the extinction of the administered drug. Experimental groups for the acquisition of an operant conditioning were as follows: control ($n = 21$), fluoxetine ($n = 16$), buspirone ($n = 14$), risperidone ($n = 12$), ondansetron ($n = 16$), and tianeptine ($n = 14$). The following experimental design was used for extinction experiments: control ($n = 31$), fluoxetine ($n = 6$), buspirone ($n = 7$), risperidone ($n = 6$), ondansetron ($n = 5$) and tianeptine ($n = 10$).

2.4. Acquisition and extinction of an operant conditioning task

All behavioral procedures were performed during the light phase of the light/dark cycle, using a standard operant chamber (MED associates Inc., St. Albans, VT, USA) equipped with an input (DIG 710/711) and output (DIG 720/721/722) card for data acquisition and processing; one automated retractable lever; white house light; contextual red light; white noise (random signal with a flat power spectral density); and an automated feeder. Rats were food restricted to maintain ~80% of their ad libitum body weight for 3 days before training and throughout the experiments. During the period of food restriction and experimental procedures, animals were fed with rat chow plus the 45 mg pellets dustless precision pellets (BioServ) used for training procedures to avoid palatability issues during the experiments.

Before the training, animals were subjected to one habituation session. The rats were placed in the operant chamber and were exposed to contextual red light and white noise and fed with 25 pellets given randomly by the automated feeder.

Before starting the training procedure, the operant chamber had the lever retracted, the house light on, white noise, and a red context light that remained on at all times. An operant conditioning task training session with a fixed ratio of 1 consisted of 25 trials. Each trial began with the delivery of the lever (60 s) and the house light was turned off. If the animal pressed the lever within 60 s, the lever retracted, and it received a pellet of 45 mg as a reward. A pellet was delivered within 1 s of pressing the lever; this was coupled with the activation of a white light inside the feeder for 2 s. When the trial finished, the white house light turned on and the lever remained retracted for 20 s. The action of pressing the lever was considered a correct response. It is important to remark that the animals were able to press the lever only once per trial. When the animals did not respond during the trial, no reward was given. The percentage of responses in a session was calculated by counting the total number of lever presses in a training session and then divided by 25 (maximum number of lever presses that can be performed in a session) and then multiplied by 100. The latency time of a response represents the time between the lever delivery and the lever press of the animal. If no response was performed during the trial, the latency time of response was considered 60 s. It is important to remark that for data analysis we only included animals that reached 100% in the last session of that phase. Animals received two training sessions per day: the first between 9 and 10 a.m. and the second between 3:30 and 5 p.m.

For the extinction phase, animals were first subjected to five training sessions; the animals that did not reach 100% correct responses were discarded. The structure of the trials was exactly the same as previously described for the acquisition phase, but the animals did not receive the pellet as a reward for pressing the lever. For this protocol, pressing the lever was computed as a response. Two extinction sessions were performed per day for 6 days; one in the morning (9–10 a.m.) and one in the afternoon (3:30–5 p.m.).

2.5. Statistical analysis

Statistical analysis was performed using GraphPad Prism software. Values are expressed as mean ± SEM and compared using one way or two-way repeated measures ANOVA followed by Bonferroni multiple-comparisons corrected post hoc tests (session as within, and treatment as between subject factors). Differences among experimental conditions were considered statistically significant if $p < 0.05$.

3. Results

3.1. Fluctuations of cortical 5-HT levels associated with "reward" and "no reward" encoding

First, we evaluated whether cortical 5-HT levels are modified through different stages of the acquisition and extinction of the operant conditioning.

In the acquisition, we evaluated 5-HT levels in the PFC in the first session (which also coincided with 50% correct response) and again after the rule was completely learned. One-way ANOVA showed that there was a statistically significant difference among the mean values of 5-HT levels in the PFC from the different experimental groups [$F_{2,20} = 3.708$, $p < 0.05$]. We found a transient increase in the levels of 5-HT in the PFC after the 1° session compared to untrained animals in the acquisition phase (Fig. 1A).

In the extinction phase, the quantification of 5-HT levels in the PFC revealed a progressive increase in this neurotransmitter, with a significant difference between the mean values of the different groups [$F_{3,32} = 3.265$, $p < 0.05$]. The increase found in the 10° extinction session was statistically significant compared to naïve animals (Fig. 1B).

3.2. Opposite effects of fluoxetine on acquisition and extinction of an operant conditioning

Next, we tested the ability of fluoxetine, a 5-HT reuptake inhibitor, to modulate performance in this reward-dependent task. Statistical analysis of the performance in the acquisition phase showed a significant effect associated with the treatment factor [$F_{1,175} = 15.82$, $p < 0.001$]. Besides, a significant effect was observed for the session factor [$F_{4,175} = 35.41$, $p < 0.001$] and an interaction between factors [$F_{4,175} = 2.89$, $p = 0.0239$]. Post hoc comparisons resulted in significant differences in the second and third sessions (Fig. 2A). The latency time analysis showed a significant effect for the factors treatment [$F_{1,175} = 25.66$, $p < 0.001$] and session [$F_{4,175} = 46.16$, $p < 0.001$]. No interaction was found between factors [$F_{4,175} = 1.31$, $p = 0.2698$]. Latency times were increased in the second and third session (Fig. 2B). Data analysis of the percentage of responses in the phase of extinction showed a significant effect of the factors treatment [$F_{1,389} = 41.86$, $p < 0.001$] and session [$F_{11,389} = 33.29$, $p < 0.001$], whereas there was no interaction between these factors [$F11,389 = 1.09$, $p = 0.3686$]. Animals treated with fluoxetine reached complete extinction faster than controls; this difference was statistically significant between the fifth and seventh sessions (Fig. 2C). There was no interaction when latency times were analyzed [$F_{11,389} = 0.76$, $p = 0.6788$], but the main effects were found for treatment [$F_{1,389} = 21.63$, $p < 0.001$] and session [$F_{11,389} = 35.85$, $p < 0.001$]. Although animals treated with fluoxetine tended to drift to higher latency times compared to controls, the post hoc test showed no significant differences across extinction sessions (Fig. 2D).

3.3. Tianeptine delays extinction but improves acquisition

We also evaluated the effect of tianeptine, a 5-HT reuptake enhancer, on the acquisition and extinction of this reward-dependent learning.

For acquisition, data analysis of the effects of tianeptine versus control groups in the percentage of responses showed a significant effect for the factor session [$F_{4,115} = 16.98$, $p < 0.001$] but not for treatment [$F_{1,115} = 3.20$, $p = 0.0764$]. Additionally, no significant interaction was found between factors [$F_{4,115} = 1.77$, $p = 0.1405$]. Tianeptine significantly increased the percentage of responses only in the second session (Fig. 3A). The statistical analysis of latency time resulted in a significant effect for treatment [$F_{1,115} = 7.12$, $p = 0.0087$] and session [$F_{4,115} = 29.81$, $p < 0.001$] factors. We did not find any interaction between these factors [$F_{4,115} = 1.97$, $p = 0.1043$]. Bonferroni's post hoc test indicated a difference only in the second session (Fig. 3B).

There was a significant effect of treatment on the percentage of responses in the extinction phase [$F_{1,441} = 45.24$, $p < 0.001$] and this effect was also observed for session [$F_{11,441} = 36.93$, $p < 0.001$]. There was no significant interaction between factors [$F_{11,441} = 1.14$, $p = 0.3308$]. We found that there was a difference between the animals from the control and tianeptine groups in the sixth, seventh, and eighth sessions (Fig. 3C). The analysis of latency time showed that there was no interaction between factors [$F_{11,441} = 0.99$, $p = 0.4544$], although significant effects were observed for treatment [$F_{1,441} = 42.93$, $p < 0.001$] and session [$F_{11,441} = 40.78$, $p < 0.001$]. Bonferroni's post hoc analysis

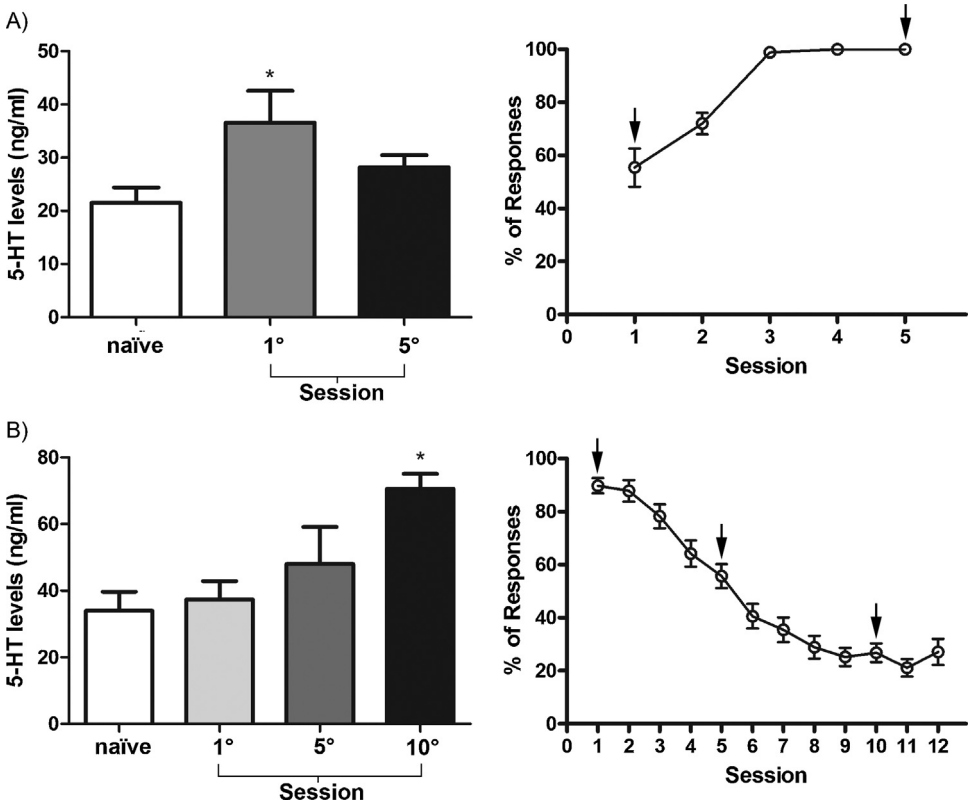

Fig. 1. Levels of 5-HT in the PFC in different time-points of the acquisition and extinction phases of an operant conditioning task. There was an increment in 5-HT levels in the first training session (panel A left). Learning performance of the acquisition phase is shown in panel (right). 5-HT levels were increased in the 10 session when animals successfully extinct the operant conditioning task (panel C left). The percentages of responses across sessions in the extinction phase are shown in panel B (right). Naïve (untrained animals, $n = 8$); 1° acquisition session (coincident with ~50% correct responses, $n = 7$); 5° acquisition session (animals that completely acquired the task, $n = 8$); 1° extinction session ($n = 6$); 5° extinction session (animals that reached ~50% of responses, $n = 7$); and 10° extinction session (animals that reached almost complete extinction with residual response ~10–20%, $n = 6$). Values are expressed as the mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. One-way ANOVA followed by Bonferroni's post hoc multiple comparison test.

resulted in differences only in the sixth and seventh sessions (Fig. 3D).

3.4. Buspirone administration only affects acquisition phase

The partial agonist of 5-HT1A receptors, buspirone, was also tested. Significant effects for the factors treatment [$F_{1,165} = 8.40$, $p = 0.0043$] and session [$F_{4,165} = 62.82$, $p < 0.001$] were found when the acquisition phase percentage of responses was analyzed. An interaction between these two factors was also detected [$F_{4,165} = 2.68$, $p = 0.0335$]. We found a significant difference between groups in the first session (Fig. 4A). Similarly, significant effects were found for treatment [$F_{1,165} = 5.82$, $p = 0.0169$] and session [$F_{4,165} = 84.04$, $p < 0.001$] after the analysis of latency times. No interaction was found between these factors [$F_{4,165} = 1.76$, $p = 0.1395$]. The post hoc test showed a difference between groups in the first session (Fig. 4B).

Analysis of the percentage of responses in the extinction phase resulted in significant effects for the factors session [$F_{11,405} = 21.21$, $p < 0.001$] and treatment [$F_{1,405} = 10.83$, $p < 0.001$], but without any interaction [$F_{11,405} = 0.31$, $p = 0.9835$]. No differences were found across training sessions between the control and buspirone groups (Fig. 4C). We found that analysis of latency time showed a significant effect in the factors treatment [$F_{1,405} = 10.58$, $p < 0.001$] and session [$F_{11,405} = 26.49$, $p < 0.001$], whereas there was no interaction between these factors [$F_{11,405} = 0.39$, $p = 0.9607$]. Further analysis according to Bonferroni's post hoc test showed no differences between animals treated with buspirone and control groups (Fig. 4D).

3.5. Risperidone improved extinction but induced severe learning deficits in the acquisition phase

Risperidone, a 5-HT2A antagonist, was also administered during the acquisition or extinction of the operant conditioning task. We found a significant effect for the factors treatment [$F_{1,155} = 0.442.02$, $p < 0.001$] and session [$F_{4,155} = 135.38$, $p < 0.001$] on the percentage of responses in the acquisition phase. There was an interaction between factors [$F_{4,155} = 44.16$, $p < 0.001$]. The post hoc test revealed a significant decrease in the percentage of responses of animals treated with risperidone between the first and fourth session (Fig. 5A). We additionally found a significant effect of treatment [$F_{1,155} = 311.47$, $p < 0.001$] and session [$F_{4,155} = 119.97$, $p < 0.001$] when latency times were analyzed. There was an interaction between the factors treatment and session [$F_{4,155} = 22.43$, $p = 0.001$]. Afterwards, we found an increase in the latency time of the risperidone group between the first and fourth sessions compared to the control group (Fig. 5B).

Interestingly, an analysis of the extinction phase showed significant effects for treatment [$F_{1,390} = 98.61$, $p = 0.001$] and session [$F_{11,390} = 17.80$, $p = 0.001$]. We also found an interaction between these factors [$F_{11,390} = 2.16$, $p = 0.0157$]. We observed that the risperidone group had an important reduction in the percentage of responses in the second, third, fourth, fifth and seventh sessions (Fig. 5C). We analyzed the latency times and identified a

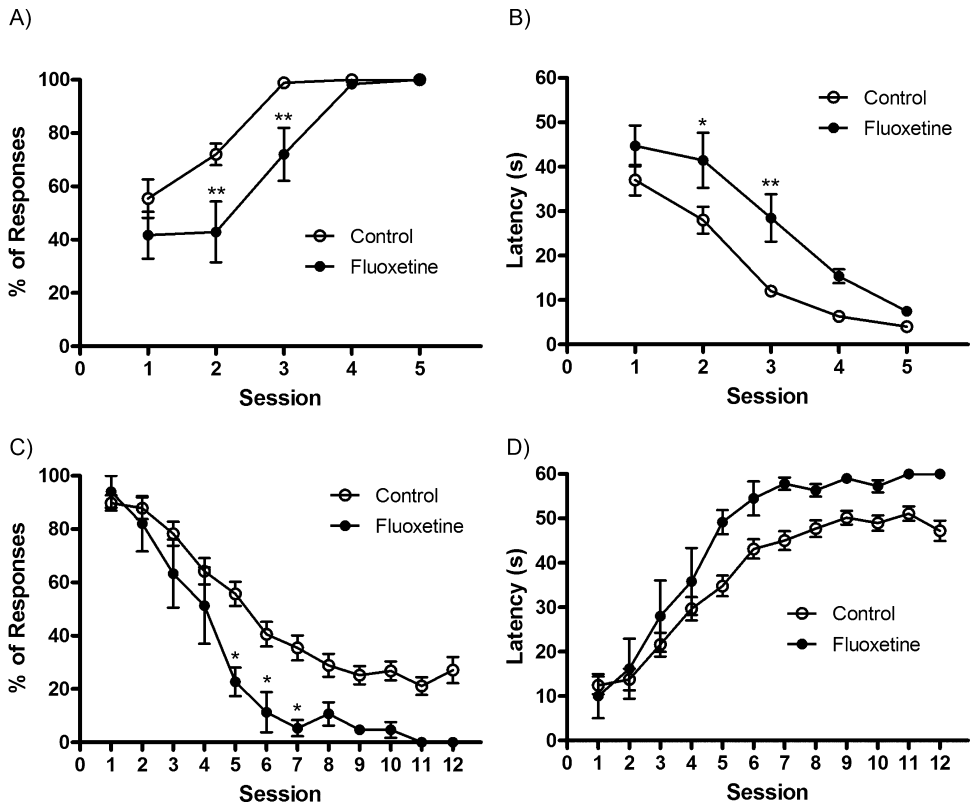

Fig. 2. Fluoxetine produces mild deficits in the acquisition phase but improves the extinction of an operant conditioning task. Learning performance during the acquisition phase is measured by the percentage of responses (panel A) and latency time (panel B). The percentage of responses (panel C) and latency time (panel D) during the extinction phase are shown in both panels. The percentage of responses is expressed as the mean ± SEM of the total of lever pressings in a training session of 25 trials. Latency time is expressed as the mean ± SEM of the time that elapses between presentation of the conditioned stimulus and occurrence of the lever pressing. Control (acquisition, $n = 21$); fluoxetine (acquisition, $n = 16$); control (extinction, $n = 31$) and fluoxetine (extinction, $n = 6$). $^{*}p < 0.05$, $^{**}p < 0.01$. Two-way repeated measures ANOVA followed by Bonferroni's multiple-comparisons corrected post hoc tests.

significant effect for session [$F_{11,390} = 19.26$, $p < 0.001$] and treatment [$F_{1,390} = 2.16$, $p < 0.001$]. Additionally, we found a significant interaction [$F_{11,390} = 2.70$, $p = 0.002$] regarding the administration of risperidone to animals, which increased the latency times between the second and fifth sessions (Fig. 5D).

3.6. Ondansetron induce learning deficits only in the acquisition phase

Finally, the 5-HT3 antagonist Ondansetron was evaluated in both phases of the task. Analysis of the percentage of responses during the acquisition phase resulted in significant effects due to treatment [$F_{1,175} = 23.75$, $p < 0.001$] and session [$F_{4,175} = 40.66$, $p < 0.001$]. There was no significant interaction between these groups [$F_{4,175} = 2.05$, $p = 0.0895$]. Animals treated with ondansetron showed a significant decrease in performance during the first, second, and third sessions (Fig. 6A). Treatment [$F_{1,175} = 28.36$, $p < 0.001$] and session [$F_{4,175} = 59.34$, $p < 0.001$] had a significant effect on latency time. We did not find any interaction between these factors [$F_{4,175} = 1.77$, $p = 0.1380$]. The administration of ondansetron increased latency time between the first and third sessions (Fig. 6B).

We found significant effects for treatment [$F_{1,369} = 19.39$, $p < 0.001$] and session [$F_{1,369} = 19.39$, $p < 0.001$] in the extinction phase. No interaction was detected between groups [$F_{11,369} = 0.27$, $p = 0.9912$]. However, Bonferroni's post hoc test showed no differences between animals from the ondansetron and control groups (Fig. 6C). Next, we found that there was a statistically significant effect for treatment [$F_{1,369} = 15.57$, $p < 0.001$] and session [$F_{11,369} = 16.07$, $p < 0.001$] when latency times were analyzed. Additionally, no interaction was found between the factors analyzed [$F_{11,369} = 0.22$, $p = 0.9959$]. Although animals treated with ondansetron showed higher latency times, these increases across training sessions were not significant (Fig. 6D).

It is important to remark that we did not observed a substantial and significant gain or loss of weight during training procedures between animals that received a pharmacological treatment or controls (Table 1). This is a critical factor to be considered since weight gain or loss could affect the performance in this reward-dependent task.

4. Discussion

The results presented in this manuscript suggest that 5-HT and its receptors play differential roles in the acquisition and extinction of operant conditioning tasks. Herein, we found that the levels of 5-HT fluctuated in the acquisition and extinction phases: 5-HT was significantly increased in the first session of acquisition (~50% correct responses) and was reversed after the learning. Taken together, these results imply that 5-HT in the PFC is involved in the acquisition and extinction of an operant conditioning task but with different roles. In this sense, it has been shown that in the analogous structure to the prefrontal cortex in pigeons, there are fluctuations in 5-HT levels when pigeons were taught delayed matching to samples [31]. Additionally, systemic and PFC depletion of 5-HT has been shown to produce deficits in operant and reversal learning, decision-making, and short term memory [32–35]. However, sub regions of the PFC like the orbitofrontal cortex (OFC) and mPFC are differentially modulated by 5-HT. Depletion of 5-HT in the OFC of rodents disrupts reinforcement devaluation but not extinction

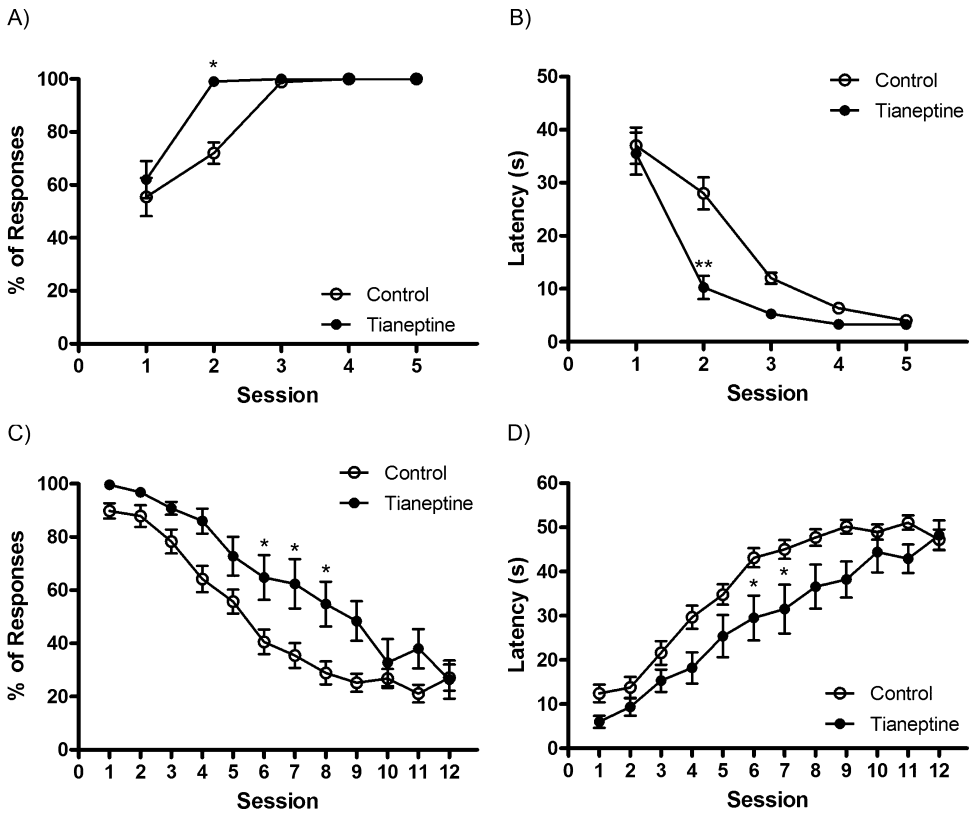

Fig. 3. Administration of tianeptine induces deficits in the extinction phase, but improves acquisition. Learning performance during the acquisition phase is measured by the percentage of responses (panel A) and latency time (B). The percentage of responses (panel C) and latency time (panel D) during the extinction phase is shown in both panels. The percentage of responses is expressed as the mean ± SEM of the total of lever pressings in a training session of 25 trials. Latency time is expressed as the mean ± SEM of the time that elapses between presentation of the conditioned stimulus and occurrence of the lever pressing. Control (acquisition, $n = 21$); tianeptine (acquisition, $n = 14$); control (extinction, $n = 31$) and tianeptine (extinction, $n = 10$). $^{*}p < 0.05$, $^{**}p < 0.01$. Two-way repeated measures ANOVA followed by Bonferroni's multiple-comparisons corrected post hoc tests.

[36], whereas a similar protocol in marmosets showed that 5-HT in the OFC is critical for preventing competing tasks regardless of the salient stimuli [37]. Additionally, there is solid evidence that increased levels of extracellular 5-HT OFC produce abnormal c-fos immunoreactivity, which has also been associated with persistent instrumental responsiveness [18]. Although the modulatory role of 5-HT in the mPFC is known, the dynamics of how this neurotransmitter can influence positive or negative learning and memory are still under investigation. It has been shown that a strong efflux of 5-HT occurs in the mPFC while animals are performing delay discount tasks [38]. On the contrary, tonically elevated 5-HT state in this brain region induces impulsive behavior [39]. Due to the multiple 5-HT receptors expressed in different localizations of pyramidal and parvabulmin neurons, sometimes it has been difficult to interpret the behavioral outputs of serotoninergic manipulations. Herein, although the 5-HT levels measured in the PFC are related to different phases of the acquisition and extinction of an operant conditioning task, we did not discriminate between different sub-regions of the PFC. We propose that both the mPFC and OFC are involved in the acquisition and extinction, although further studies will be necessary to establish the different contributions of these areas.

Pharmacological treatment with fluoxetine was shown to have two opposite effects in the acquisition and extinction phases. This SSRI improved the extinction of the task but produced a significant decrease in the performance in the acquisition phase. Similarly, Sanders et al. (2007) showed that, in mice, SSRI administration or genetic deletion of SERT reduced operant responding. On the contrary, the genetic deletion of SERT or the chronic administration of fluoxetine improved reversal learning [40]. Although reversal learning and extinction are substantially different tasks, we suggest that fluoxetine could exert its effects in both tasks through a similar mechanism. Fluoxetine is able to increase the levels of 5-HT in the PFC, and it has been shown that 5-HT reduces the excitability of pyramidal neurons through an increased firing rate of fast-spiking neurons in this area [41–43]. These findings are of particular interest because the operant conditioning task is a PFC-mediated task [3–7] and this could be one of the potential mechanisms involved in the effects observed herein.

Next, we found that tianeptine had the opposite effect when compared to animals that were treated with fluoxetine. This 5-HT reuptake enhancer improved the acquisition of an operant conditioning task but induced deficits in the extinction phase. Although it is possible that the effects observed by tianeptine or fluoxetine

Table 1
Body weight change of treated groups. The percentage of weight changes is expressed as the mean ± SEM of the body weight of treated animals with respect to the untreated group across the five days of experimental procedures.

Treatment	Buspirone	Risperidone	Ondansetron	Fluoxetine	Tianeptine
Percentage of weight modification with respect to control group	+5.1% ± 1.1	−3.4% ± 0.8	+5.7% ± 2	+4.1% ± 1.7	+3.9% ± 1.4

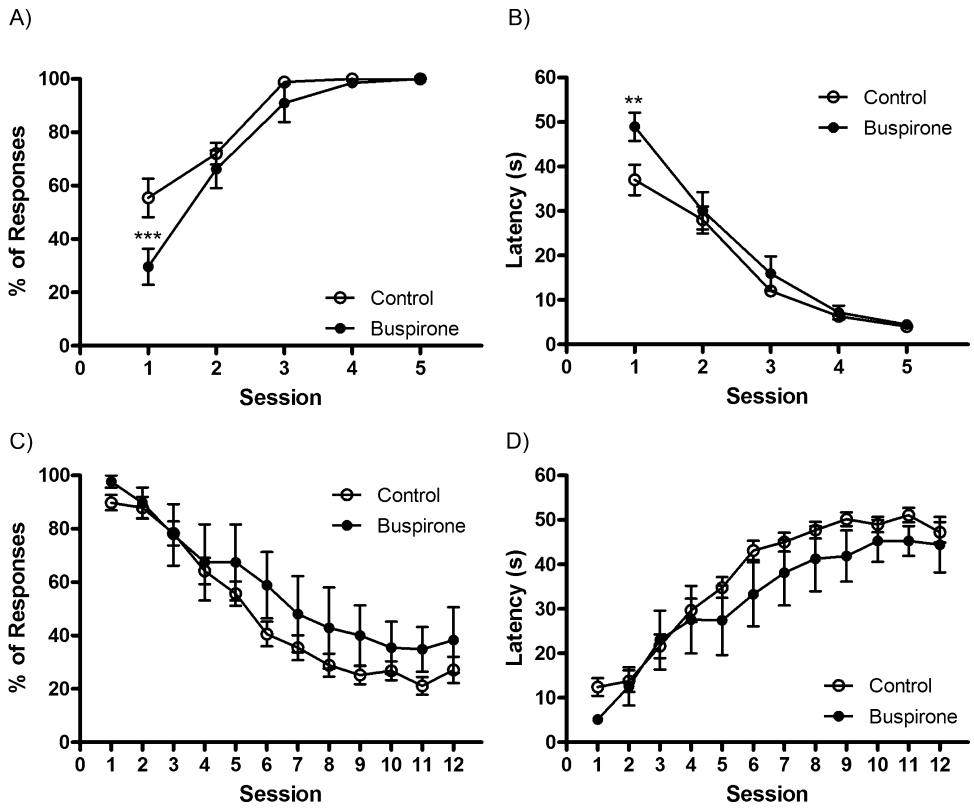

Fig. 4. Activation of 5-HT1A receptor produces mild deleterious effects only in the acquisition phase. Learning performance during the acquisition phase is measured by the percentage of responses (panel A) and latency time (panel B). The percentage of responses (panel C) and latency time (panel D) during the extinction phase are shown in both panels. The percentage of responses is expressed as the mean ± SEM of the total of lever pressings in a training session of 25 trials. Latency time is expressed as the mean ± SEM of the time that elapses between presentation of the conditioned stimulus and occurrence of the lever pressing. Control (acquisition, $n = 21$); buspirone (acquisition, $n = 14$); control (extinction, $n = 31$) and buspirone (extinction, $n = 7$). $^{**}p < 0.01$, $^{***}p < 0.001$. Two-way repeated measures ANOVA followed by Bonferroni's multiple-comparisons corrected post hoc tests.

could be due to an increment or decrement of locomotor activity, these doses have been shown to have no effect on this behavioral parameter [44,45]. Tianeptine has been shown to improve the ability to learn autoshaping instrumental tasks just as it affects fear conditioning and stimuli discrimination [46–48] MIRAR. Earlier works proposed that the role of 5-HT is related to the prediction of negative rewards or aversive stimuli [49,50]. Antidepressants like fluoxetine produce weight loss in humans due to a reduction in appetite [51], which is something that could be critical in a reward-dependent task like the operant conditioning task. Nevertheless, we did not observe a difference in body weight between animals treated with fluoxetine and the control group (Table 1); these results are in agreement with those observed by Sanders et al. (2007). The other antidepressant used here, tianeptine, did not produce any significant changes in body weight, which is supported by other reports that showed no effect of tianeptine on body weight and food intake [52,53]. Recently, a groundbreaking study by Miyazaki and colleagues [54] proved that 5-HT is more closely related to the behavioral withholding of an action to obtain future rewards than to avoid punishment or codification of an omitted reward. The results described above suggest that the serotoninergic circuit has a strong but different influence on the acquisition and extinction in a reward-dependent task like operant conditioning. It is possible that there is a relationship between 5-HT levels and performance: factors that increase 5-HT, impair acquisition and improve extinction. The opposite occurs when 5-HT levels are reduced.

Nevertheless, an important aspect to be discussed in the context of the present study is how 5-HT levels modulate impulsivity and decision making, especially considering the effects observed in the latency period due to the treatments used herein. The effects of fluoxetine and tianeptine on the latency period observed during the acquisition phase could be related to alterations in the structures that mediate this task, reward processing or both. These antidepressants generated the opposite effect for extinction, although the role of 5-HT in this scenario seems to be closely related to controlling the impulsive action of still pressing the lever (even when no reward is given under the new circumstances). Recently, Worbe et al. [55] showed that tryptophan depletion in humans augments impulsivity; this is of particular interest since these results parallel those obtained in rodents. It is important to remark that tryptophan depletion can affect withholding of the action but not the mechanisms necessary to stop an already initiated action [56]. On the contrary, fluoxetine administration in rats produces a decrease impulsivity and clock speed on an instrumental task [57]. Besides, this decrease in impulsivity after fluoxetine administration has also been observed in the 5-CSRTT test [58]. 5-HT also modulates reversal learning and higher cognitive functions like decision making [59]. In this sense, oral administration of 5-hydroxytryptophan in humans generates impairment in an Iowa gambling task [60]. Herein, we found that fluoxetine and tianeptine had an effect on latency time, but it is too complicated to draw a solid conclusion if the effects observed in the extinction phase are due to changes in impulsivity. However, the results presented here suggest that impulsive action in the extinction phase of an operant conditioning is increased or decreased when fluoxetine and tianeptine are administered. In this sense, our data are in agreement with previous findings that relate fluctuations of 5-HT levels to the control of action withholding.

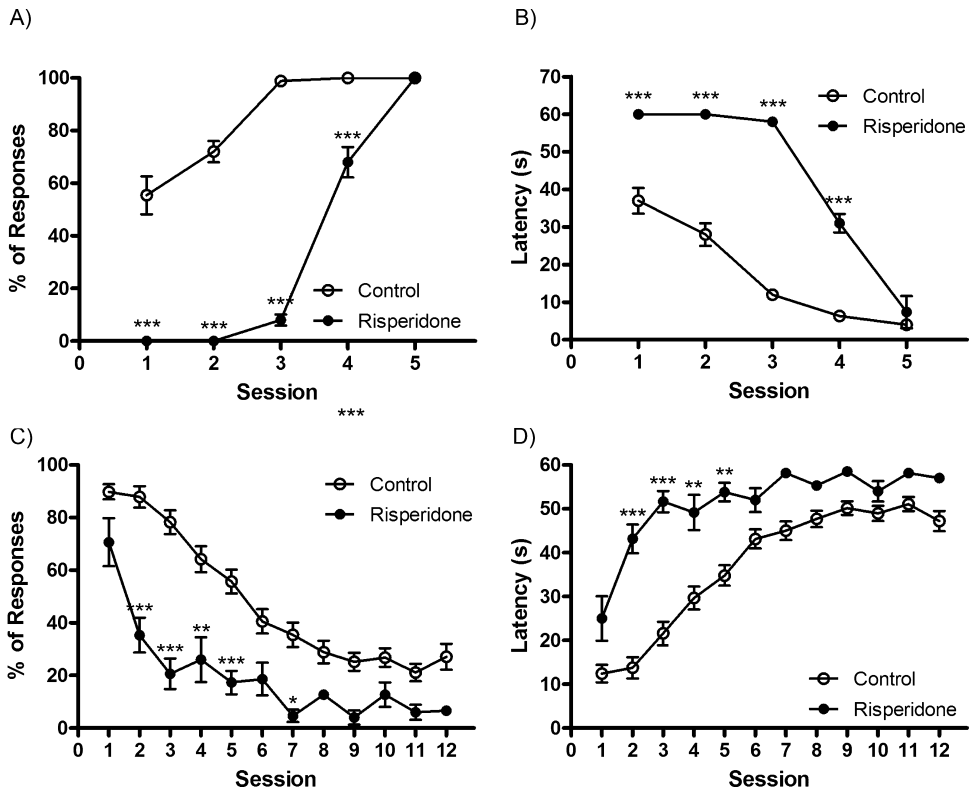

Fig. 5. Risperidone administration improves extinction of operant conditioning but induces severe learning deficits in the acquisition phase. Learning performance is shown during the acquisition phase measured by the percentage of responses (panel A) and latency time (panel B). The percentage of responses (panel C) and latency time (panel D) during the extinction phase is shown above. The percentage of responses is expressed as the mean ± SEM of the total number of lever pressings in a training session of 25 trials. Latency time is expressed as the mean ± SEM of the time that elapses between presentation of the conditioned stimulus and the occurrence of lever pressing. Control (acquisition, $n = 21$); risperidone (acquisition, $n = 12$); control (extinction, $n = 31$) and risperidone (extinction, $n = 6$). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Two-way repeated measures ANOVA followed by Bonferroni's multiple-comparisons corrected post hoc tests.

Then, we studied whether there was a differential contribution of 5-HT1A, 5-HT2A, and 5-HT3 receptors in the acquisition and extinction phases. Buspirone, a 5-HT1A partial agonist, generated a mild effect only in the acquisition phase and had no effect in the extinction phase. Additionally, buspirone induced a significant difference in the first training session; we believe that it must be related to the mild learning deficits observed in the first session. This drug is shown to have deleterious effects on multiple tasks like spatial learning and navigation, fear conditioning, and passive avoidance [23,24,61]. Also, there is striking evidence that supports a role of the 5-HT1A receptor in modulating the levels of impulsivity, particularly after buspirone administration [62,63]. We propose that 5-HT1A has a relevant role in the operant conditioning task but only during the early stages of the acquisition phase, through the effects observed in other tasks and impulsivity. It is important to remark that the dose of buspirone used in this manuscript did not produce alterations in locomotor activity [64].

The administration of risperidone in the acquisition phase resulted in severe learning deficits, whereas extinction phase performance was strongly improved in animals treated with this pharmacological agent. Risperidone is shown to have a differential effect in the acquisition and extinction phase. In this sense, it has been shown that risperidone has a negative effect on the performance of allothetic place and conditioned avoidance tasks [65,66]. Nevertheless, the data obtained here must be cautiously interpreted since risperidone is also a D2 antagonist. D2 antagonists have been shown to reduce operant responding [67,68] and to improve extinction [69]. However, the effects observed here cannot only be due to D2 blockade, since it has been previously observed that 5-HT2A blockade facilitate the acquisition of instrumental learning [70]. Although an acute administration of risperidone induced a small increase of locomotor activity [71], we propose that the effect observed here is not due to altered locomotor activity because it has been shown that repeated injections of this drug do not produce that kind of alteration [71]. The data obtained here showed that latency was oppositely affected in the acquisition and extinction phase. Taking previous data together with our results, the most suitable explanation for the increased latency time during the acquisition phase is because of a deleterious effect of risperidone on learning performance. On the contrary, latency times were strongly increased during the extinction phase after risperidone administration. Based on previous evidence, it seems possible that risperidone affects impulsivity since other antagonists of 5-HT2A receptor (M100907) significantly reduce impulsivity [30]; however, due to the effects of risperidone on the D2 receptor, the results obtained here are difficult to interpret.

The 5-HT3 receptor has not been extensively studied in the context of learning and memory, especially in reward-dependent tasks. We observed that blocking this receptor with ondansetron produced a strong decrease in learning performance during the acquisition phase and an overall improvement of the performance in the extinction phase. Also, this 5-HT3 antagonist induced a strong increment in latency during the acquisition but not in the extinction phase. Likewise, with buspirone and risperidone administration, we observed that the increased latency time found in the acquisition phase is more closely related to a decrement in the learning performance than to side effects on other behavioral parameters. Our results show that the effects observed in the acquisition phase are not related to an increase or decrease of locomotor activity, since higher and lower doses than those used here did not induce

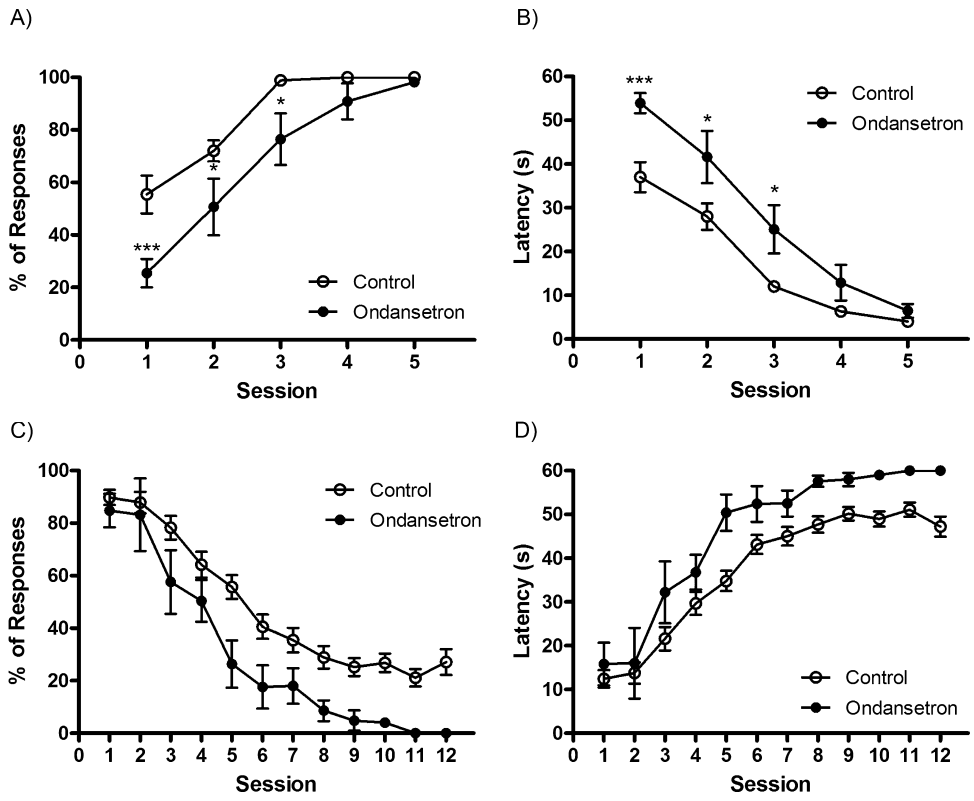

Fig. 6. Blockade of 5-HT3A receptor induce detrimental effects in the acquisition phase. Learning performance during the acquisition phase is measured by the percentage of responses (panel A) and latency time (panel B). The percentage of responses (panel C) and latency time (panel D) during the extinction phase is shown in both panels. The percentage of responses is expressed as the mean ± SEM of the total of lever pressings in a training session of 25 trials. Latency time is expressed as the mean ± SEM of the time that elapses between the presentation of the conditioned stimulus and the occurrence of the lever pressing. Control (acquisition, $n = 21$); ondansetron (acquisition, $n = 16$); Control (extinction, $n = 31$) and ondansetron (extinction, $n = 5$). $^{*}p < 0.05$, $^{***}p < 0.001$. Two-way repeated measures ANOVA followed by Bonferroni's multiple-comparisons corrected post hoc tests.

any kind of alteration [72]. In primates and rodents, ondansetron has been shown to improve reversal learning and a simplified version of the T-maze [73]. However, ondansetron did not affect basal performance in a spatial discrimination task [74]. Interestingly, overexpression of 5-HT3 receptor in mice produced an improvement in hippocampal-dependent learning tasks [26].

In conclusion, we found that 5-HT and its receptors had a different role depending on whether the animals were in the acquisition or extinction phase of an operant conditioning task. Our results indicate that 5-HT modulates the acquisition and extinction phases of a reward-dependent task in an opposite manner. We propose that 5-HT modulates withholding the action of pressing the lever when the reward is no longer given, but it is also able to influence obtaining the reward. The results presented here support the dual role of 5-HT in reward learning proposed by computational models [49,75] in which this neurotransmitter is a codifier for reward and the absence of it in the context of reinforcement learning. Besides, we found some tantalizing data that suggest an important role of 5-HT in modulating impulsive behavior in operant conditioning tasks. Further studies will be necessary to unravel the role of other 5-HT receptors in the acquisition and extinction of a reward-dependent operant conditioning task.

Conflicts of interest

The authors have declared that no competing interests exist.

Acknowledgments

This study was funded by Agencia Nacional de Promocion Cientifica y Tecnologica (PICT 1202 1519), Consejo Nacional de Investigaciones Cientificas y Tecnicas (PIP 112 201101 01054) and Universidad de Buenos Aires (UBACYT 200 20 100 100 978).

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