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Antidepressants differentially modify the extinction of an aversive memory task in female rats

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ABSTRACT

Treatment of major depression, posttraumatic stress disorder and other psychopathologies with antidepressants can be associated with improvement of the cognitive deficits related to these disorders. Although the mechanisms of these effects are not completely elucidated, alterations in the extinction of aversive memories are believed to play a role in these psychopathologies. We have recently verified that female rats present low levels of extinction when submitted to the plus-maze discriminative avoidance task. In the present study, female rats were treated long term with clinically used antidepressants (fluoxetine, nortriptyline or mirtazapine) and subjected to the plus-maze discriminative avoidance task to evaluate learning, memory, extinction and anxiety-related behaviors as well as behavioral despair in the forced swimming test. All groups learned the task and exhibited retrieval. Chronic treatment with fluoxetine (but not with the other antidepressants tested) increased extinction of the discriminative task. In the forced swimming test, the animals treated with fluoxetine and mirtazapine showed decreased immobility duration. In conclusion, fluoxetine potentiated extinction, while both fluoxetine and mirtazapine were effective in ameliorating depressive-like behavior in the forced swimming test, suggesting a possible dissociation between the effects on mood and the extinction of aversive memories in female rats.

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1. Introduction

Psychopathological conditions, such as mood and anxiety disorders, are frequently related to aversive emotional experiences (APA, 2000), and the reframing of the aversive memory is believed to be beneficial (Friedman, 2008). It has also been suggested that the cognitive deficits presented in these disorders are reversed by treatment with antidepressants (Austin et al., 2001; Castaneda et al., 2008), which are largely indicated for both anxiety and mood disorders (Brunello et al., 2001; Marks et al., 1998; Nandam et al., 2007). Several studies indicate that the improvement of these cognitive deficits is associated with hippocampal neurogenesis (Dranovsky and Hen, 2006; Duman and Monteggia, 2006; Nandam et al., 2007; Paizanis et al., 2007; Pittenger and Duman, 2008; Sahay and Hen, 2007; Santarelli et al., 2003). Despite memory deficits being common in many anxiety- and depression-related disorders, the relationship between the improvement of mood-related symptoms by antidepressants and their effects on memory remain to be elucidated (Austin et al., 2001).

Several animal studies have investigated the effects of antidepressants on memory (Austin et al., 2001; Castaneda et al., 2008; MacQueen et al., 2003; Marks et al., 1998; Nestler et al., 2002; Paizanis et al., 2007; Schulz et al., 2007). However, few studies have examined the effects of antidepressants specifically on the extinction of aversive memories. For example, previous studies in rats have investigated the effects of rolipram, which has both antidepressant and anxiolytic effects, on extinction. In these studies, decrements in extinction of conditioned fear and fear-potentiated startle paradigms were shown (Monti et al., 2006; Mueller et al., 2010). On the other hand, this drug has also impaired extinction of a passive avoidance task (Cheng et al., 2010). In another study, D-cycloserine, an adjuvant in antidepressant therapy, enhanced extinction in a conditioned fear task (Yamamoto et al., 2008).

We have recently verified that female rats present low levels of extinction when submitted to an aversively motivated task (Ribeiro et al., 2010). It was suggested that a diminished capacity for extinction of aversive memories could be related to the predominance of certain psychopathological disorders, including mood disorders, in females in general (Curtis, 2005; McLean and Anderson, 2009; Parker and Brotchie, 2010; Scott, 2011). In the present study, we investigated if the extinction of this aversive task would be improved after treatment with clinically effective antidepressants.

Abbreviations: PMDAT, plus-maze discriminative avoidance task; FST, forced swimming test; SSRI, seletive serotonin reuptake inhibitor; AV, aversive enclosed arm; NAV, non-aversive enclosed arm; %TAV, percentage of time in the aversive arm; %TOA, percentage of time in the open arms; %EOA, percentage of entries into the open arms; Vehi, vehicle; Fluox, fluoxetine; Nort, nortriptyline; Mir, mirtazapine.

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The effects of antidepressants from three different pharmacological groups (fluoxetine, nortriptyline and mirtazapine) on learning, memory and extinction of the plus-maze discriminative avoidance task (PMDAT) in female rats were determined. Fluoxetine is a selective serotonin reuptake inhibitor (SSRI), which is the most frequently prescribed class of antidepressants (Dunlop and Davis, 2008). Nortriptyline is a tricyclic antidepressant that acts by nonselective inhibition of monoaminergic reuptake. Although these compounds are no longer largely prescribed, they are still an option for individuals who are non-responsive to other antidepressants and for patients susceptible to serotonin syndrome (Dagtekin et al., 2011). Finally, the atypical antidepressant mirtazapine is used to treat both mood and anxiety disorders (Gambi et al., 2005; Rauggi et al., 2005) and enhances central noradrenergic and serotonergic neurotransmission through inhibition of the noradrenergic α2-autoreceptor and the α2-heteroreceptor in serotonergic synapses (Bengtsson et al., 2000; Gambi et al., 2005).

The PMDAT allows the concomitant evaluation of memory and anxiety-related behavior. Several studies performed with this task have shown its effectiveness in evaluating the effects of (1) memory-enhancing or amnestic drugs, (2) anxiolytic or anxiogenic procedures and (3) variations in locomotor activity (Silva and Frussa-Filho, 2000; Silva et al., 1997, 2002a,b). This paradigm is also useful for the evaluation of learning and extinction processes by the analysis of animal behavior during the training and test sessions, respectively (Ribeiro et al., 2010; Silva et al., 2004, see Methods). Additionally, the rats were also assessed using the forced swimming test (FST), a classical tool used to evaluate the effects of antidepressants (Porsolt et al., 1977, 1978).

2. Materials and methods

2.1. Animals

Three-month-old female Wistar rats (120–230 g) from our colony were housed in groups of 4–5 animals in plastic cages (30 × 37 × 16 cm) in a room with acoustic isolation and airflow as well as controlled temperature (24–26 °C), humidity and luminosity (12 h light:12 h dark, lights on 6:30 a.m.). Food and water were available ad libitum throughout the experiments. The rats were handled according to Brazilian law for the use of animals in scientific research (Law Number 11.794), and all procedures described here were approved by the local ethics committee (CEUA-UFRN). All efforts were made to minimize animal pain, suffering or discomfort as well as the number of animals used.

2.2. Treatment and general procedures

Animals were allocated to one of the following treatment groups (n = 8–10): (a) fluoxetine (20 mg/ml/kg daily; Medley, Brazil), (b) nortriptyline (20 mg/ml/kg daily; Novartis, Brazil), (c) mirtazapine (10 mg/ml/kg daily; Torrent, India), or (d) vehicle (physiological saline with Tween 20). All treatments were injected intraperitoneally once a day (from 6:00 to 6:30 p.m.) for 19 days. All drugs were diluted in physiological saline containing three drops of Tween 20 per 1 ml.

The duration of treatments and doses were chosen based on previous studies that demonstrated the efficacy of these antidepressants in rodents submitted to the FST. In these studies, treatment lengths varied from a single dose to repeated daily doses for 4 weeks, and the effective dose ranges were 5 to 30 mg/kg for fluoxetine (Carr et al., 2011; Cryan and Lucki, 2000; Djordjevic et al., 2012; Dulawa et al., 2004; Iñiguez et al., 2010; Miyamoto et al., 2010; Wang et al., 2011), 20 to 25 mg/kg for nortriptyline (Consoni et al., 2006; Mallei et al., 2011; Petersén et al., 2009), and 2.5 to 10 mg/kg for mirtazapine (Nowakowska et al., 1999; Rogóż, 2010; Szymańska et al., 2009).

Throughout the pharmacological treatment and behavioral experiments, the estrous cycle was monitored daily by vaginal smears. Immediately after injection or behavioral session, plastic pipettes were gently introduced in the vagina with distillated water (approximately 0.1 ml). Slight, brief pressure was applied to the bulb of the pipette to collect vaginal cells. The material collected was stained with methylene blue (5 mg/ml) and analyzed by optical microscope. All animals included in the study were cycling normally. No estrous cycle stage predominated among the experimental groups at the time of testing (2 to 3 animals per stage in each experimental group).

On the 17th day of treatment, the animals were submitted to a PMDAT training session, and a test session was held 24 h later (on the 18th day of treatment). On the 19th day of treatment, the animals were submitted to the first session of the FST. The second session was performed 24 h later. The behavioral tests were performed in such an order because the stressful nature of the FST can interfere with the other behavioral evaluations. All behavioral tests were started at 1:00 p.m., and injections and cycle monitoring were completed at 6:00 p.m. after the behavioral assessments.

2.3. Plus-maze discriminative avoidance task (PMDAT)

The apparatus employed for the PMDAT is a modified elevated wood plus-maze containing two enclosed arms (50 × 15 × 40 cm) opposite two open arms (50 × 15 cm). In the 10-minute training session, each rat was placed in the center of the apparatus, and every time the animal entered the aversive enclosed arm, the rat encountered an aversive situation that lasted until the animal left the arm. The aversive stimuli included a 100 W light (1500 lx at the maze floor level) and an 80 dB noise applied through a speaker placed over the aversive enclosed arm. In the test/extinction session held 24 h later, the rats were again placed in the apparatus for 10 min without receiving the aversive stimulation. The lamp and the speaker were still present over the aversive arm but were in the off position. Several studies performed with this behavioral model have demonstrated that the aversive stimuli used are effective. Indeed, when exposed to these stimuli in a certain arm of the maze, the animals progressively avoid the specific arm during a behavioral session (Munguba et al., 2011; Patti et al., 2006; Ribeiro et al., 2010, 2011; Silva et al., 2004).

Learning was evaluated by the percentage of time spent in the aversive enclosed arm (time spent in aversive enclosed arm/time spent in both enclosed arms) during the training session. Retrieval of the task was evaluated by the comparison of the time spent in the aversive (AV) and the non-aversive enclosed arms (NAV) in each group, while extinction of the task was assessed by the percentage of time in the aversive arm (%TAV) during the test session. Both classical (percentage of time spent in open arms, %TOA: time spent in open arms/time spent in both open and enclosed arms; percent age of entries into the open arms, %EOA: entries into open arms/entries into both open and enclosed arms) and non-classical anxiety-related measures (risk assessment and head dipping) were registered. The risk assessment behavior was defined by body stretching to look into the open arms of the apparatus before entering (or not). The head dipping was characterized by an attempt to look "under" the maze with the head pointing towards the floor. Increases in head dipping indicate less anxious behavior (Rodgers and Dalvi, 1997; Rodgers et al., 1997). Locomotor activity was evaluated by the distance traveled within the apparatus. The behavior of the animals was monitored and analyzed using the video-tracking software Anymaze (Stoelting, USA).

2.4. Forced Swimming Test (FST)

In this test rats were placed in a cylinder (40 cm high × 25 cm wide) with water (30 cm deep, 24–27 °C), for two consecutive days. On the first day, the animals were submitted to 15 min of forced

exposure (pre-test session). Twenty-four hours after this pre-test session, rats were again placed in the water tank (test session) for 5 min, and immobility duration, climbing behavior and the latency to engage in immobility were registered.

2.5. Statistical analyses

The Kolmogorov–Smirnov test of normality was applied to all data, and an overall normal distribution was observed. One-way analysis of variance (ANOVA) with treatment as the between subject factor and arm type as the within subject factor was applied for the time spent in the enclosed arms for the total length of each behavioral session. When arm type effects were detected, pair-wise comparisons between time spent in AV and time spent in NAV arms were performed using the paired-samples Student *t*-test. In addition to the total length of the sessions in the PMDAT, behavioral sessions were divided into 5 blocks of 2 min each. Comparisons of the percentage of time in the aversive arm among these blocks within a session were used to evaluate learning (training) or extinction (test) of the task and were conducted by ANOVA with repeated measures (blocks across the session). When time (session blocks) effects were detected, the paired-samples *t*-test was used for pair-wise comparisons within sessions. Data from other behavioral parameters were analyzed with one-way ANOVA with Bonferroni's tests for post-hoc comparisons. Differences were considered significant at $p<0.05$ except when corrections for multiple *t*-tests comparisons were applied and the significance level was set at $p<0.01$.

3. Results

3.1. Plus-maze discriminative avoidance task: memory

In the training session, only a significant effect for arm type (NAV vs. AV) was found [$F(1,31)=236.68$, $p<0.001$]. The paired-samples *t*-tests revealed that all groups spent less time in the aversive enclosed arm in comparison with the non-aversive enclosed arm [vehicle ($t=7.23$, $p<0.001$), fluoxetine ($t=9.21$, $p<0.001$), nortriptyline ($t=7.63$, $p<0.001$) and mirtazapine ($t=7.36$, $p<0.001$)] (Fig. 1A). This result was confirmed by the analysis of the percentage of time in the aversive arm across the training session that revealed time (2-minute session blocks) [$F(4,124)=20.80$, $p<0.001$] but not treatment [$F(3,31)=0.73$, $p=0.54$] or time × treatment interaction effects [$F(12,124)=1.34$, $p=0.22$], indicating that all the groups had learned the task (Fig. 1B).

In the test session, once again, only a significant effect for arm type (NAV vs. AV) was found [$F(1,31)=99.42$, $p<0.001$]. The paired-samples *t*-tests showed that all groups spent more time in the non-aversive enclosed arm [vehicle ($t=9.08$, $p<0.001$), fluoxetine ($t=4.09$, $p=0.003$), nortriptyline ($t=3.86$, $p=0.008$) and mirtazapine ($t=4.54$, $p=0.003$)] (Fig. 1C). This result indicates that all the groups presented similar behaviors in the test session. However, the analysis of the percentage of time spent in the aversive arm throughout the test session revealed time [$F(4,124)=3.90$, $p=0.01$], treatment [$F(12,124)=3.48$, $p=0.02$] and time × treatment interaction [$F(3,31)=2.08$, $p=0.04$] effects (Fig. 1D). Pair-wise comparisons between the block 1 and all other blocks (2 to 5) showed that the vehicle-treated animals presented significant increases in aversive arm exploration only in block 5 ($t=3.74$, $p=0.005$), indicating extinction of the task only by the end of the session. On the other hand, this increase in aversive arm exploration was found earlier in the session for the fluoxetine-treated group (Block 3, $t=3.22$, $p=0.01$ and block 5, $t=3.28$, $p=0.01$). Conversely, the nortriptyline- and mirtazapine-treated animals did not modify aversive arm exploration across the sessions, indicating the absence of extinction. Additionally, as a significant effect of treatment was found across the sessions, separate ANOVAs were conducted for each session block. A significant effect of treatment was found in the block 3 [$F(3,31)=7.77$, $p=0.001$], and Bonferroni's post-hoc

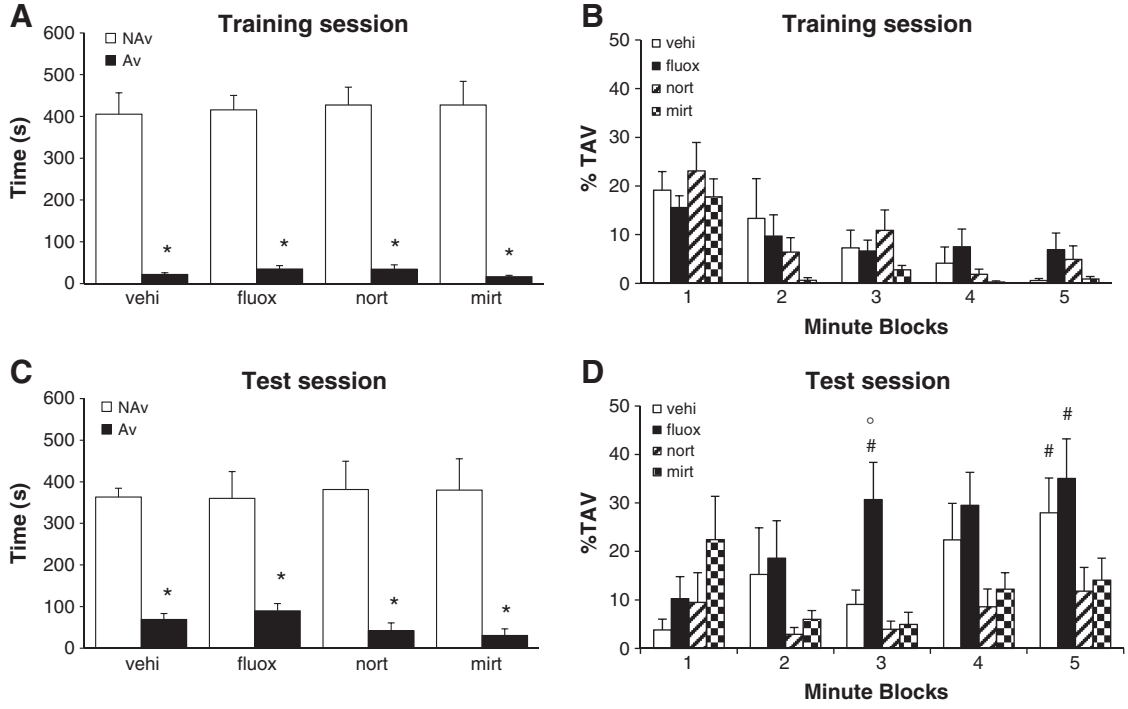

Fig. 1. Mean + S.E. of time (s) spent in aversive (Av) and non-aversive (NAv) enclosed arms (A,C) and percentage of time spent in the aversive arm (%TAV) in blocks of two minutes (B,D) in the training (A, B) and test (C, D) sessions of female rats repeatedly treated with vehicle (vehi), 20 mg/kg fluoxetine (fluox), 20 mg/kg nortriptyline (nort) or 10 mg/kg mirtazapine (mirt). (A) and (C): *$p<0.01$ compared to time in non-aversive arm (Two-way ANOVA and paired samples *t*-test). (B): Significant effect of time and (D) significant effects of time, treatment and time × treatment interaction (ANOVA with repeated measures); #$p<0.01$ compared to first block (paired-samples *t*-Student test); °$p<0.05$ compared to all other groups in the same block (ANOVA and Bonferroni post hoc).

analysis revealed that the fluoxetine group presented with an increased percentage of time in the aversive arm when compared with all other groups in this block.

3.2. Plus-maze discriminative avoidance task: anxiety

No significant effects of treatment were found for the percentage of time (Fig. 2A and B) or entries (Fig. 2C and D) into the open arms, indicating the absence of effects on anxiety-related behavior. Similarly, no differences were found in the time spent on head dipping or in risk assessment behaviors (Table 1).

3.3. Plus-maze discriminative avoidance task: locomotion

ANOVA revealed an effect of treatment in the distance traveled in the apparatus in the training [$F(3,31)=4.56$, $p<0.01$] and test [$F(3,31)=3.86$, $p<0.05$] sessions. Post hoc analysis with Bonferroni's test showed that the animals treated with mirtazapine exhibited significantly decreased locomotor activity compared with the other groups in both sessions (Table 2).

3.4. Forced swimming test

ANOVA revealed an effect of treatment in immobility duration [$F(3,30)=13.64$, $p<0.001$]. Post hoc analysis with Bonferroni's test showed that the animals treated with fluoxetine and mirtazapine had decreased immobility duration compared with the control and nortriptyline groups (Fig. 3A). Moreover, ANOVA revealed a treatment effect on climbing behavior [$F(3,30)=4.17$, $p=0.01$]. Post-hoc analysis with Bonferroni's test showed that the fluoxetine group had increased time spent in climbing behavior compared with the nortriptyline group (Fig. 3B). The latency to start immobility was not different among the groups (data not shown).

4. Discussion

In summary, our data showed that antidepressants can influence learning processes and more specifically, the extinction of an aversive task. Indeed, no treatment effects were observed in relation to the acquisition or retrieval of the task. The extinction of the task was evaluated by the progressive increase of the aversive arm exploration during the test session when the aversive stimuli were no longer present. During this session, vehicle and fluoxetine groups (but not the nortriptyline- or mirtazapine-treated animals) showed extinction of the task, i.e., significant increases in aversive arm exploration across the session blocks. However, the data suggest that treatment with fluoxetine was capable of improving the process of extinction (see Fig. 1D) because fluoxetine-treated rats showed increased aversive arm exploration earlier in the test/extinction session. Additionally, specifically regarding the antidepressant effects on mood, the results obtained in the FST showed that treatment with fluoxetine and mirtazapine reduced rat immobility time (see Fig. 3A), which is consistent with previous studies (Carr et al., 2011; Cryan and Lucki, 2000; Djordjevic et al., 2012; Dulawa et al., 2004; Iñiguez et al., 2010; Miyamoto et al., 2010; Nowakowska et al., 1999; Rogóż, 2010; Szymańska et al., 2009; Wang et al., 2011).

Table 1
Mean ± S.E. for time (s) spent in head dipping and risk assessment behavior in the training and test sessions presented by female rats repeatedly treated with vehicle, fluoxetine (20 mg/kg), nortriptyline (20 mg/kg) or mirtazapine (10 mg/kg).

	Head dipping (s)		Risk assessment (s)	
Treatment	Training	Test	Training	Test
Vehicle	38.3 ± 12.8	42.6 ± 7.3	25.8 ± 6.9	14.7 ± 3.5
Fluoxetine	28.8 ± 7.7	41.0 ± 6.3	24.4 ± 5.9	11.0 ± 3.0
Nortriptyline	30.4 ± 7.8	41.7 ± 11.3	19.5 ± 3.2	6.3 ± 1.9
Mirtazapine	19.9 ± 4.4	16.0 ± 6.3	18.6 ± 4.1	9.7 ± 2.7

ANOVA did not reveal significant effects of the treatment.

4.1. Extinction of the aversive task: fluoxetine

As mentioned above, the analysis of the aversive arm exploration throughout the test session of the PMDAT provides indication of both task retrieval (beginning of session) and extinction (subsequent session blocks) (Ribeiro et al., 2010). Indeed, with the aversive stimuli no longer present, the animals avoid the aversive arm at first but eventually visit the arm, which would now be considered safe. Animals then progressively increase exploration of this arm, reaching the amount they would explore a regular enclosed arm by chance (or even more because this arm is mostly unfamiliar for them in the

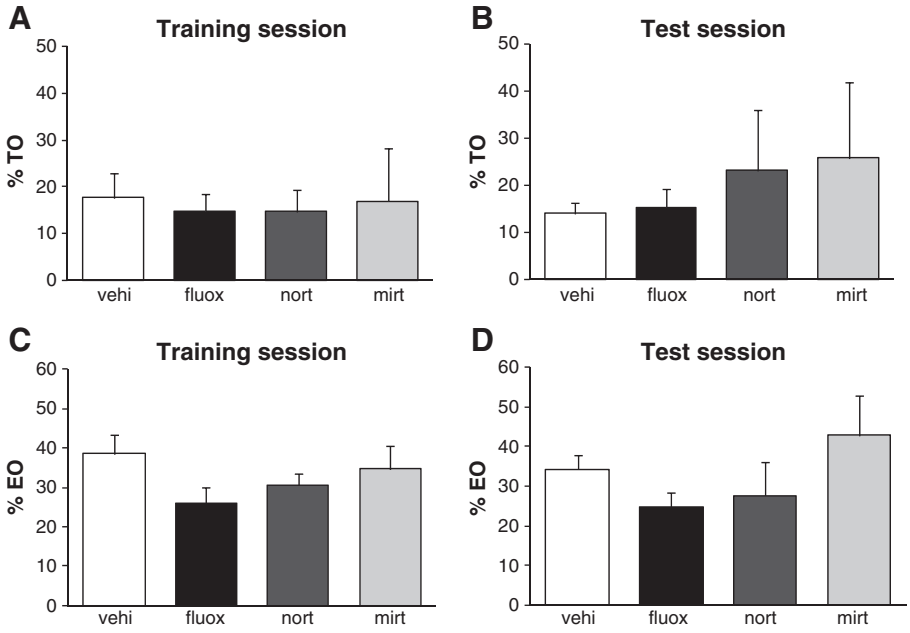

Fig. 2. Mean + S.E. for percentage of time (%TO — A, B) and entries (%EO — C, D) in the open arms in training (A, C) and test (B, D) sessions presented by female rats repeatedly treated with vehicle (vehi), 20 mg/kg fluoxetine (fluox), 20 mg/kg nortriptyline (nort) or 10 mg/kg mirtazapine (mirt). ANOVA did not reveal significant effects of the treatment.

Table 2
Mean ± S.E. of distance traveled (m) by female rats repeatedly treated with vehicle, fluoxetine (20 mg/kg), nortriptyline (20 mg/kg) or mirtazapine (10 mg/kg).

Treatment	Training session	Test session
Vehicle	19.0 ± 2.4	21.1 ± 1.9
Fluoxetine	20.0 ± 3.0	21.6 ± 4.4
Nortriptyline	22.9 ± 3.1	16.8 ± 4.4
Mirtazapine	10.4 ± 0.5*	8.5 ± 2.3*

* $p<0.05$ compared to all other groups (ANOVA followed by Bonferroni post hoc).

test session). This pattern is usually observed in males. However, in a previous study, we have shown that female rats kept avoiding the aversive arm despite the absence of aversive stimuli (Ribeiro et al., 2010). It has been suggested that this lack of extinction is similar to what occurs in some anxiety disorders, such as posttraumatic stress disorder (PTSD), and could be related to the greater prevalence of mood and anxiety disorders in females (Bekker and van Mens-Verhulst, 2007; Kendler et al., 2001; Mahendran and Yap, 2005; Rauch et al., 2006).

The data presented here indicated that the SSRI fluoxetine was capable of increasing or accelerating the process of extinction in females. Although speculative, one might hypothesize that the therapeutic action of fluoxetine could be related to the extinction of aversive memories. In line with this reasoning, this drug has shown potential benefits in the treatment of PTSD, which has a close relationship with deficits in the extinction of traumatic memories (APA, 2000; Quirk et al., 2006; Yamamoto et al., 2008, 2009).

The SSRIs are among the most commonly prescribed drugs for treating mood and anxiety disorders (Brunello et al., 2001; Marks et al., 1998; Nandam et al., 2007). It has been suggested that one of their effects is an improvement of possible cognitive deficits related to these disorders (Austin et al., 2001; Castaneda et al., 2008), particularly through the stimulation of neurogenesis (Dranovsky and Hen, 2006; Duman and Monteggia, 2006; Nandam et al., 2007; Paizanis et al., 2007; Pittenger and Duman, 2008; Sahay and Hen, 2007). In this respect, neurogenesis also plays a role in extinction processes (Gabriele and Packard, 2006). Further, treatment with fluoxetine also induces an increase in the release of brain-derived neurotrophic factor (BDNF), which is related to mechanisms of neuronal plasticity (Castrén and Rantamäki, 2010). In addition, another study has shown that BDNF is necessary for memory extinction (Gabriele and Packard, 2006). However, the role of BDNF in depression remains unclear because antidepressant effects of nortriptyline and escitalopram, for example, seem to be unrelated to hippocampal BDNF expression in female rats (Hansson et al., 2011).

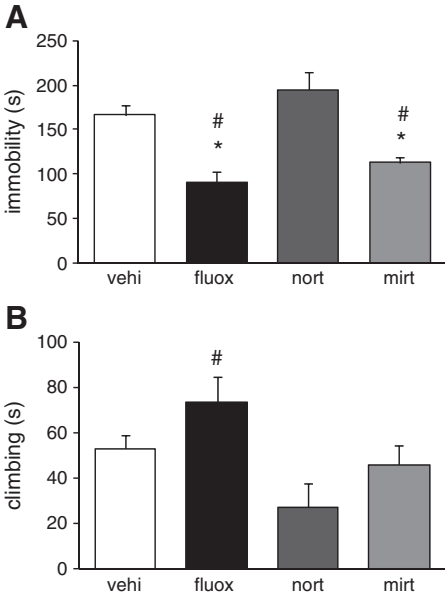

Fig. 3. Mean + S.E. for time (s) spent in immobility (A) and in climbing behavior (B) of female rats repeatedly treated with vehicle (vehi), 20 mg/kg fluoxetine (fluox), 20 mg/kg nortriptyline (nort) or 10 mg/kg mirtazapine (mirt) in the forced swimming test. *$p<0.05$ compared to vehi; #$p<0.05$ compared to nort (ANOVA followed by Bonferroni post hoc).

Relevant to the context of aversive memory extinction, previous studies have demonstrated that antidepressants may induce synaptic reorganization in the amygdala (McEwen and Chattarji, 2004). For example, fluoxetine chronically administered to adult rats reduces, in the amygdala, the polysialylated form of the neural cell adhesion molecule (PSA-NCAM), a molecule involved in synaptogenesis (Homberg et al., 2011; Varea et al., 2007). These plasticity-related mechanisms could underlie modifications of the experience of an aversive event, which is in line with the results shown in the present study. In summary, it seems plausible that the actions of antidepressants could be related to a modulation of plastic processes related to the extinction of aversive memories. Corroborating this hypothesis, the animals treated with fluoxetine showed decreased behavioral despair (evaluated by the forced swimming test, see Fig. 3), concomitant with improvement in aversive memory extinction. Finally, although these results seem relevant to understanding of the effects of antidepressants, it is important to mention that the rats used in this study were not submitted to any depression-inducing procedures. Thus, it is clear that more studies are needed to investigate the clinical relevance of these findings.

4.2. Extinction of the aversive task: mirtazapine and nortriptyline

In the present study, neither the mirtazapine nor the nortriptyline groups were able to extinguish the task. The nortriptyline-treated animals also failed to respond in the FST, which is in line with the assumption that alterations in extinction would play a role in the antidepressant effects of the treatment. On the other hand, mirtazapine treatment did induce an antidepressant effect in the FST (see Fig. 3) despite the lack of extinction improvement.

Clinical studies with mirtazapine administered acutely show decreased neural responses to fear and increased perception of happy faces in healthy volunteers (Rawlings et al., 2010). On the other hand, it has also been shown that mirtazapine decreases the perception of both aversive and rewarding stimuli (Harmer et al., 2006; McCabe et al., 2010). Nevertheless, there is a lack of information concerning the effects of long-term mirtazapine administration on emotional memory and/or upon confrontation with aversive situations. In the present study, during the course of the long-term mirtazapine treatment, the mood-related antidepressant effects could have appeared first (inducing modifications on the FST, see Fig. 3), while possible emotional memory changes under aversive situations could have appeared afterwards if a longer treatment period was used. Further studies are needed to investigate this issue.

The hypolocomotor effects observed in females treated with mirtazapine (see Table 2) have already been reported in a previous study (Reneric et al., 2002). In the present study, the hypolocomotor effects were specifically found in the PMDAT, i.e., they were not present in the FST. It is known that mirtazapine enhances both 5-HT and NA neurotransmissions, but differently from monoamine reuptake inhibitors, its antidepressant effects are mediated through the direct antagonism of both α2 and 5-HT2C receptors (de Boer et al., 1996; Haddjeri et al., 1996). In addition, mirtazapine also induces blockage of histamine-H_1 receptors, which is another possible mechanism that may produce sedation and decrease the exploratory activity (Gambi et al., 2005; Schüle et al., 2003, 2006).

4.3. Differential effects of the three drugs on extinction

Despite the fact that all drugs used in the present study are clinically effective antidepressants, they show differences in their mechanisms of action (see Introduction). Regarding target neurotransmitter systems of the three drugs, while fluoxetine is a selective serotonin reuptake inhibitor, both mirtazapine and nortriptyline have mechanisms of action related to the noradrenergic function. In this respect, it has been shown that noradrenergic activation impairs extinction learning (Soeter and Kindt, 2011), although other results have not corroborated this finding (Beralu and McGaugh, 2006).

From another standpoint, the differences in the mechanisms of action could lead to diverse effects not directly related to the antidepressant action per se. These effects could explain why only fluoxetine was effective in modifying extinction of the aversive task. It has been shown that mirtazapine and nortriptyline decrease hypothalamic–pituitary–adrenal (HPA) axis activity (Schüle et al., 2002; 2006). In this respect, an increase in HPA axis activity could impair the extinction of an inhibitory avoidance task (Cassol-Jr et al., 2010), which is opposite to the results obtained here. On the other hand, it is known that the higher the sensitivity to cortisol, the higher the amygdala response to stressful events (Morgan and LeDoux, 1995) and the lower the activation of hippocampus and prefrontal cortex (Lebrón et al., 2004). Due to the role of these brain regions on aversive memory extinction (Lebrón et al., 2004; Morgan and LeDoux, 1995; Quirk et al., 2006), these effects could explain the decreased extinction shown by the mirtazapine- and nortriptyline-treated animals when compared with controls. In particular, studies in our laboratory have shown that pharmacological manipulations within the amygdala modify extinction of the PMDAT (Ferreira et al., unpublished results).

4.4. Anxiety-related behavior

Usually, clinical treatment with antidepressants has an overall anxiolytic effect, although it can possibly induce an increase in anxiety at the beginning of treatment (for a review, see Borsini et al., 2002; Drapier et al., 2007). Animal studies have also shown that antidepressants can have anxiolytic effects when given acutely (Drapier et al., 2007; Holmes and Rodgers, 2003; Kurt et al., 2000; Silva et al., 1999). Herein, the treatment with antidepressants did not modify the anxiety-related behaviors in any of the parameters evaluated (see Fig. 2 and Table 1). To our knowledge, there have been few studies evaluating the effects of chronic treatment with nortriptyline in behavioral tests related to anxiety. For example, Brocco et al. (2002) showed that mice exposed to a novel environment failed to elevate locomotion, suggesting the absence of an anxiolytic effect. Conversely, the effects of chronic fluoxetine treatment on anxiety have been previously investigated, with some studies demonstrating anxiolytic (Griebel et al., 1995; Mirza et al., 2007) and others showing anxiogenic effects (File et al., 1999; Robert et al., 2011). In summary, it seems that the effects of antidepressants on animal models of anxiety are still controversial.

From another standpoint, despite most anxiety and mood disorders occurring twice as frequently in women (Bekker and van Mens-Verhulst, 2007), there is a strong bias towards the use of male animals in studying these phenotypes (Wald and Wu, 2010; Zucker and Beery, 2010). Indeed, most of the commonly used behavioral apparatuses were developed using male rats, including the PMDAT (Silva and Frussa-Filho, 2000). Although a previous study using this task did not show differences in open-arm exploration between untreated males and females (Ribeiro et al., 2010), studies using the conventional version of the plus-maze (from which the apparatus used here was adapted) have shown a difference in the minimal effective dose of the classical anxiolytic diazepam between male and female rodents (Basso et al., 2011; Nishino et al., 2008; Rodgers and Shepherd, 1993). Although speculative, this finding might suggest that females could differentially interpret and, more importantly, react differently when exposed to the assumed anxiety-evaluating situation in the plus-maze (i.e., the conflict between motivation to explore and fear of the open spaces). In this respect, it has been shown that female rats show different behavioral and hormonal reactions to both natural and laboratory aversive situations when compared with males (Astur et al., 2004; Perrot-Sinal et al., 2004; Ribeiro et al., 2010; Taylor et al., 2000).

4.5. Forced swimming test

The forced swimming test is usually applied in the screening of antidepressant drugs. In this test, immobility duration is believed to measure the levels of "behavioral despair" (Borsini and Meli, 1988). Moreover, the immobility presented by the animal is reversed by repeated antidepressant treatment, and for this reason, it is used as an index of a "depressive-like" state (Cryan et al., 2005; Porsolt et al., 1978). In the present study, the animals treated with fluoxetine and mirtazapine showed decreased immobility duration in the FST, as expected. However, rats treated with nortriptyline at a dose that was previously shown to be effective when administered acutely did not exhibit this decrease (Consoni et al., 2006). These rats also showed less climbing behavior than the fluoxetine-treated animals, suggesting that the nortriptyline group was less active in this behavioral test. However, the possibility that these effects were due to alterations in locomotion seems unlikely because in the PMDAT, there were no significant decreases in ambulation of the nortriptyline-treated animals.

5. Conclusion

Data reported in the present study showed that treatment with three antidepressant drugs differentially modified extinction of an aversive task in female rats. The modifications on memory extinction induced by antidepressants do not seem to be related to their effects on the FST, a classical test for mood-related antidepressant action. These data contribute to the understanding of the behavioral effects of antidepressant therapy, especially concerning the cognitive aspects of the treatment.

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