Behavioural Brain Research 247 (2013) 92–100

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

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

Research report

Repetitive fluoxetine treatment affects long-term memories but not learning

Estibaliz Ampuero[a], Jimmy Stehberg[b], Daniela Gonzalez[a], Nicolas Besser[a], Monica Ferrero[a], Gabriela Diaz-Veliz[c], Ursula Wyneken[a], Francisco Javier Rubio[a,*]

[a] Laboratorio de Neurociencias, Universidad de los Andes, Chile
[b] Laboratorio de Neurobiologia, Universidad Andres Bello, Santiago, Chile
[c] Laboratorio Neurofarmacologia del Comportamiento, Universidad de Chile, Santiago, Chile

HIGHLIGHTS

- Fluoxetine treatment specifically impairs long-term memory.
- Fluoxetine treatment does not affect learning.
- Fluoxetine withdrawal restores impairment in spatial memory, but not in recognition memory.

ARTICLE INFO

Article history:
Received 10 November 2012
Received in revised form 28 February 2013
Accepted 4 March 2013
Available online 16 March 2013

Keywords:
Antidepressants
Hippocampus
Cortex
Explicit memory

ABSTRACT

Fluoxetine is currently being administered for long-term maintenance and for prophylactic reasons following the remission of depressive symptoms and several other psychiatric and neurological conditions. We have previously found that in naïve adult male rats, repetitive administration of fluoxetine induced maturation of telencephalic dendritic spines. This finding was associated with the presence of a higher proportion of GluA2- and GluN2A-containing glutamate receptors. To gain further insight into the possible consequences of such synaptic re-organization on learning and memory processes, we evaluated hippocampal- and non-hippocampal-dependent memories following administration of 0.7 mg/kg fluoxetine for four weeks. Standard behavioral tasks were used: the Morris Water Maze (MWM) and Object Location Memory (OLM) tasks to assess spatial memory and the Novel Object Recognition (NOR) task to assess recognition memory. We found that treated rats showed normal learning and short-term memory (1 h post-learning). However, either recent (24 h) or remote (17 days) memories were impaired depending upon the task. Interestingly, spatial memory impairment spontaneously reverted after 6 weeks of fluoxetine withdrawal.

1. Introduction

Fluoxetine was the first selective serotonin reuptake inhibitor (SSRI) approved to treat depression in humans, and it is one of the most widely prescribed antidepressant drugs [1], modulating long-term neuronal function in brain structures involved in mood control [2]. Fluoxetine is administered in a prolonged prophylactic manner after the remission of depressive symptoms, and it is prescribed for multiple additional psychiatric and neurological conditions beyond major depression [3,4]. Fluoxetine affects brain structures involved in the regulation of both emotional and cognitive behaviors, including the hippocampus and cerebral cortex [5,6]. Therefore, besides positively modulating mood, fluoxetine might affect learning and memory. The medial temporal lobe memory system, including the hippocampal formation and the perirhinal cortex, plays an essential role in declarative memory [7,8]. At the functional level, both hippocampal- and non-hippocampal-dependent memories can be evaluated using several validated tasks. In spatial memory tasks such as the Morris Water Maze (MWM) task and the Object Location Memory (OLM) task, the hippocampus is crucial in the acquisition phase [9,10] as well as in the initial consolidation phase from short- to long-term memory within 4–24 h post-learning (so-called recent memory) [11]. Additional cortical areas, such as the retrosplenial cortex and mediotemporal lobe cortical structures, then become essential in the storage of permanent memory traces of remote spatial memory [12–15]. In turn, recognition memory, as assessed by the Novel Object Recognition (NOR) task, depends mainly on an intact perirhinal cortex for both acquisition and consolidation [16,17] and although still under debate, has been shown not to be dependent directly on the hippocampus [18]. We have previously found

* Corresponding author at: Laboratorio de Neurociencias, Universidad de los Andes, San Carlos de Apoquindo, 2200, Las Condes 762001, Santiago, Chile. Tel.: +56 2 6181353; fax: +56 2 2149259.
E-mail addresses: fjrubiog@gmail.com, jrubio@uandes.cl (F.J. Rubio).

0166-4328/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbr.2013.03.011

that repeated administration of low doses of fluoxetine (0.7 mg/kg) reaches clinically relevant plasma levels [19] and decreases depressive-like symptoms in adult rats, inducing adaptive changes in glutamate neurotransmission in the telencephalon [5]. Thus, here we focus on the effect of repeated fluoxetine administration on memory functions that requires hippocampal and/or cortical structures. Whereas previous studies in rats have shown normal learning and spatial memory when tested 24 h post-learning in the MWM task, recognition memory in the NOR task has been reported to be impaired over the same interval [20,21]. To gain insight into the effects of repetitive fluoxetine treatment on different stages of hippocampal and non-hippocampal-dependent memories, we used two spatial memory tasks, the MWM and OLM, and the memory recognition task, NOR. We then tested whether the adverse effects of fluoxetine on memory processes could be reversed after discontinuation of the drug. We found that learning and short-term memories were unaffected by repeated fluoxetine treatment. However, long-term memory at 24 h was impaired specifically in the OLM and NOR tasks. In addition, a selective impairment in remote, but not recent, memory was found in the MWM task, impairment that could be reversed after 6 weeks of fluoxetine discontinuation.

2. Materials and methods

2.1. Animals and drug administration

Adult male Sprague-Dawley rats (250–300 g) at the beginning of the treatment were housed in groups of four animals with a controlled 12 h light/dark cycle and ad libitum food and water. Either saline (0.9% NaCl) or 0.7 mg/kg fluoxetine (Ely-Lilly Co., Indianapolis, USA) dissolved in saline was administered daily via i.p. injection between 9:00 and 10:00 a.m. for 28 days. Additionally, a second control group of rats received a single fluoxetine injection. All experiments were performed in accordance with the Universidad de los Andes Bioethical Committee and the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health.

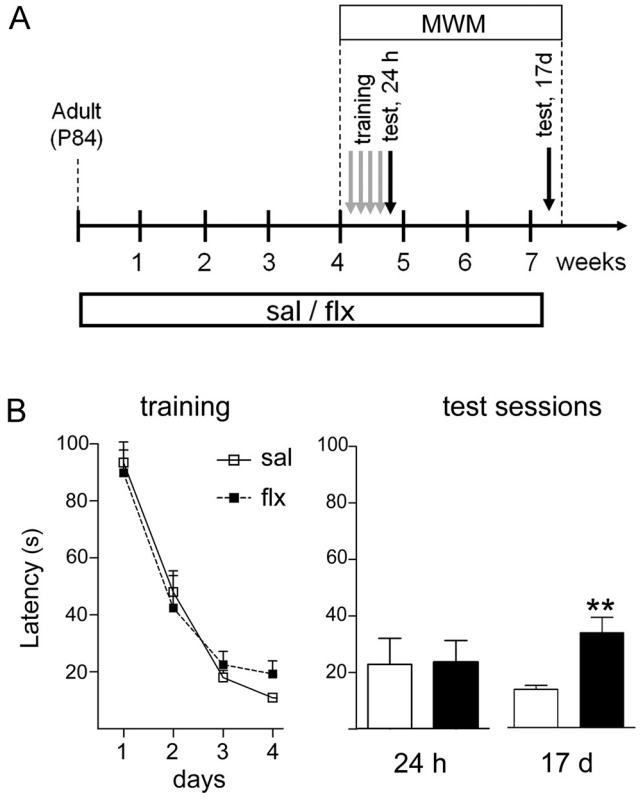

Fig. 1. Rats exhibited impairment in remote spatial memory, but not learning, after repetitive fluoxetine administration. (A) Experimental design: fluoxetine (flx) or saline (sal) were administered in total for 7 weeks. Rats were trained in the MWM task following the first 4 weeks of treatment. At twenty-four hours and seventeen days post-training, the corresponding test session was performed to evaluate recent and remote long-term memory, respectively. (B) Left graphic: the mean time to reach the platform was plotted over four consecutive days (5 trials/day). A mixed model analysis of the learning curves revealed no differences between saline- and fluoxetine-treated rats. Right graphic: the mean latencies to reach the platform 24 h and 17 d after the learning phase were plotted. Only 17 d post-learning was detected a significant difference (**$p < 0.01$, sal: $n = 12$; flx: $n = 11$; Mann–Whitney U-test).

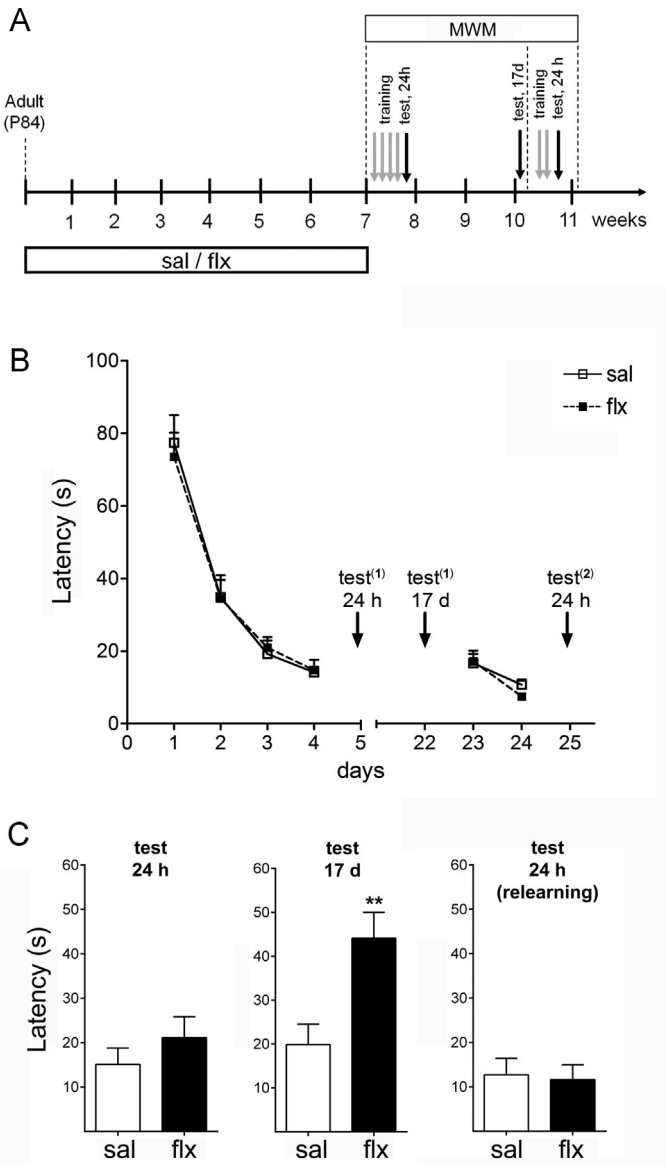

Fig. 2. Remote spatial memory, but not learning or relearning, remained impaired after cessation of fluoxetine administration during the MWM task. (A) Experimental design: adult rats received fluoxetine (flx) or saline (sal) for 7 weeks. After completion of treatment, rats were trained in the MWM task. Twenty-four hours and 17 days post-training, a probe test was performed to evaluate recent and remote memory, respectively. Then, the platform was relocated to perform a new training (reversal learning, i.e., five trials of 2 min each) and tested 24 h later. (B) The mean time to reach the platform was plotted for each experimental group. Arrows indicate the days after learning in which memory was assessed. The superscript number (1) indicates that the memory test was referred to learning on days 1–4, while superscript (2) indicates that the memory test was referred to relearning on days 23–24. (C) The bar graphics show: left, the latency to reach the platform on day 5; middle, the latency for the first platform crossing on day 22; and right, the latency for the first platform crossing on day 25 (**$p < 0.01$, sal: $n = 10$; flx: $n = 10$; Mann–Whitney U-test).

2.2. Morris Water Maze (MWM) task

In the MWM task, rats learned to find a hidden platform in a swimming pool based upon visual cues. This task was developed to assess spatial learning and memory in rodents [22]. The apparatus consisted of a circular pool of water (200 cm

diameter, 50 cm depth) that contained water (22 ± 1 °C) to 20 cm below the rim. The pool was divided into 4 quadrants. A circular Plexiglas platform (17 cm diameter) was located 2 cm below the water surface in the middle of the North-West quadrant. This platform was not visible to the rat, as it was the same color as the swimming pool. Spatial cues were located on the walls of the room, and the observer was located at a fixed place within the room. Rats were moved to the room 1 h before testing. Daily training consisted of five trials limited to 2 min (~45–60 min of inter-trial intervals), in which the sequence of starting quadrants was counterbalanced every day. This was conducted for 4 consecutive days, after which the rats had learned to reach the platform in a minimum amount of time. The latency of escape onto the platform was recorded. Each rat was placed on the platform for 30 s after the end of each trial. Reference memory was assessed at 24 h and 17 days post-training.

Three experimental groups were designed using the MWM task. In the first group, the learning phase was initiated after 4 weeks of fluoxetine administration, and fluoxetine treatment continued until the end of the experimental design, including an injection on the test days, i.e. day 17 after learning (Fig. 1A). In the second group, the learning phase was initiated after completion of the treatment (49 days in total, Fig. 2A). The third group was used to evaluate the long-lasting effects of fluoxetine in rats after a 6 week period of withdrawal after 4 weeks of antidepressant administration (Fig. 5A). For testing memory 17 days post-learning, we recorded the latency to the first platform crossing or the number of platform-site crossovers after removing the platform (in a pre-defined annulus 10 cm larger than the target) during the first minute in the pool. In addition, the second group of rats was exposed to a new training paradigm in which the platform was relocated to the opposite quadrant to assess reverse learning. The second test was performed 24 h after two days of training (5 trials per day) in which rats were able to learn the new location.

2.3. Object Location Memory (OLM) and Novel Object Recognition (NOR) tasks

The OLM and NOR tasks were developed to evaluate memory based upon spontaneous object exploration behaviors [10,23]. The OLM task was used to assess spatial memory in a less aversive manner than in the MWM task. Differently shaped objects of the same material were used, and objects were of similar weight and size. Objects were validated using an additional control group of rats. Each rat was subjected to the same task, which was always presented sequentially to evaluate memory at 24 h and 1 h. In turn, each rat was subjected to only one task, either the OLM or the NOR. In one experimental design, animals were treated for 4 weeks with either saline or fluoxetine (Figs. 3A and 4A). For both tasks, fluoxetine or saline were administered 4 weeks prior to the beginning of the 24 h-task and during 10 additional days up to the end of the 1 h-task. When tasks were performed during fluoxetine treatment, the drug was discontinued between the training session and the test session to avoid possible acute effects of fluoxetine on memory. In a second experimental group, memory after fluoxetine discontinuation was assessed. For this, memory was evaluated in the same rats at two different time-points: (1) after 4 weeks of either saline or fluoxetine treatment (experimental designs identical as in Figs. 3A and 4A) and (2) following a fluoxetine withdrawal period of 6 weeks (Fig. 5A).

For both tests, a rectangular plastic open field 60 × 40 × 40 cm (length × width × height) was divided into 24 identical squares of 10 × 10 cm, and two identical objects were located on the floor near the shorter side of the field, 10 cm away from the walls. The OLM task consisted of 3 steps: habituation to allow accommodation to the open field (3 trials on 2 consecutive days, 10 min/trial), a training session 24 h later to allow exploration of 2 identical objects (1 trial, 3 min) followed by a test session performed 24 h post-training, in which one of the two objects was randomly moved to a new location (1 trial, 3 min). The discrimination capacity was represented by the time that rats took to explore the relocated object compared to the familiar one. After 4 days, a second task with different objects was performed, and rats were tested 1 h after training (Fig. 3A). The arena and the objects were thoroughly cleaned between trials with 5% ethanol. Exploration time was recorded and was defined as time spent sniffing or touching the object with the nose and/or forepaws. To control locomotor activity and other motivation and anxiety-related behaviors, three parameters were recorded during the first 3 min in the first habituation phase: number of squares explored as indicative of locomotor activity and number of rearings and time spent in the center as indicative of anxiety-related

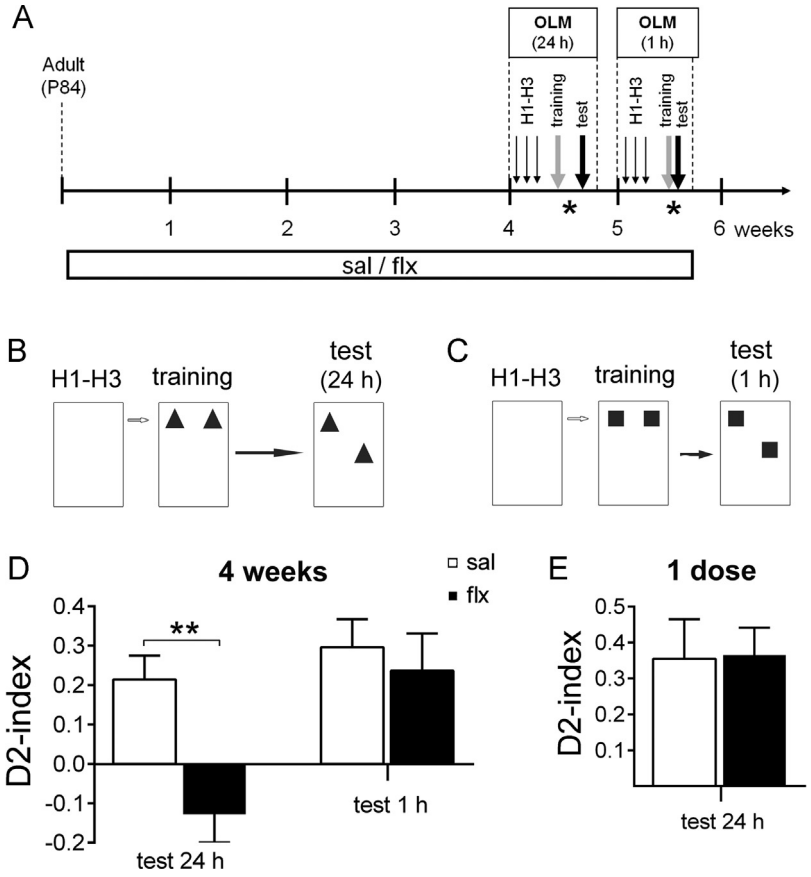

Fig. 3. Long-term, but not short-term, spatial memory was impaired after 4 weeks of repetitive fluoxetine administration in the OLM task. (A) Experimental design: two OLM tasks were performed in a sequential manner, the first at 24 h and the second at 1 h post training. (B-C) Diagrams illustrate the consecutive sessions of the OLM task: three habituation sessions (H1-H3), one training session (with two identical objects located in a fixed position) and one test session (with one of the objects moved to a new location). Notice that two different pairs of objects for each time point were used to allow testing the same rats but repeating the task at 24 h and 1 h, consecutively. (D) Graphic shows the ability of rats to discriminate the relocated object at 24 h and 1 h post-training after repeated saline or fluoxetine administration, expressed by the D2-index according to Ennaceur and Delacour (1988) (**$p < 0.01$, sal: $n = 18$; flx: $n = 17$; Mann–Whitney U-test). (E) Graphic shows the D2-index after a single injection of either saline or fluoxetine performed in an independent group of animals at 24 h post-training (sal: $n = 6$; flx: $n = 8$).

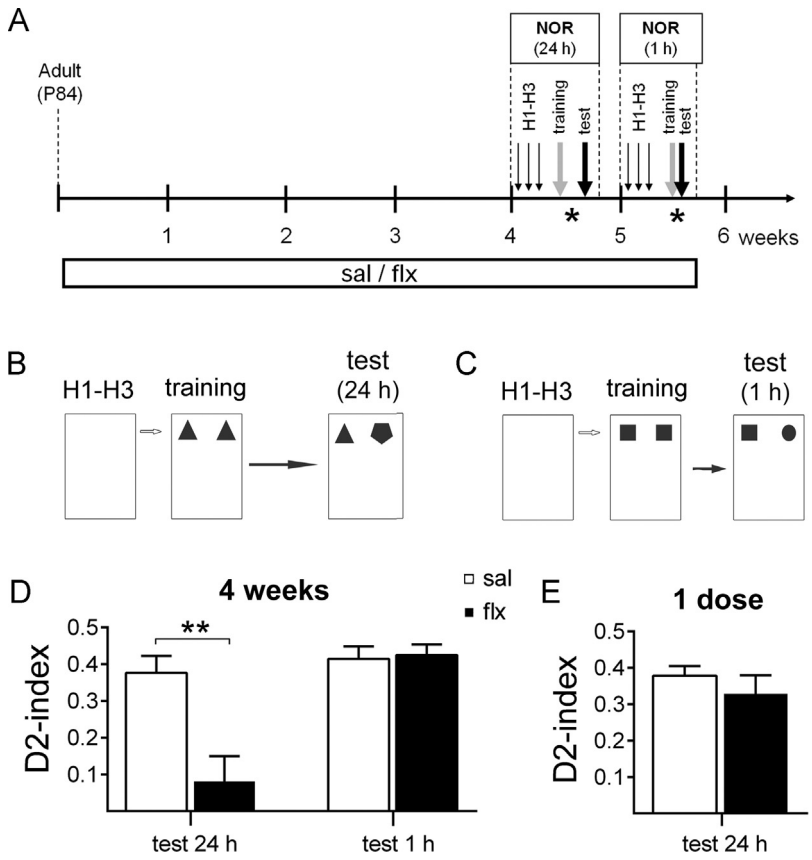

Fig. 4. Long-term, but not short-term, recognition memory was impaired after 4 weeks of repetitive fluoxetine administration in the NOR task. (A) Experimental design: two NOR tasks were performed in a sequential manner, the first at 24 h and the second at 1 h post training. (B–C) Diagrams illustrate the consecutive sessions of the NOR task: three habituation sessions (H1-H3), one training session (with two identical objects) and one test session (with one objects replaced by a novel object). Notice that different familiar and novel objects were used for each training and test sessions, respectively, to allow testing the same rats but repeating the task at 24 h and 1 h, consecutively. (D) Graphic shows the ability of rats to discriminate the novel object at 24 h and 1 h post-training after repeated saline or fluoxetine administration, expressed by the D2-index according to Ennaceur and Delacour (1988) (**$p < 0.01$, sal: $n = 16$; flx: $n = 18$; Mann–Whitney U-test). (E) Graphic shows the D2-index after a single injection of either saline or fluoxetine performed in an independent group of animals at 24 h post-training ($n = 7$ animals per condition).

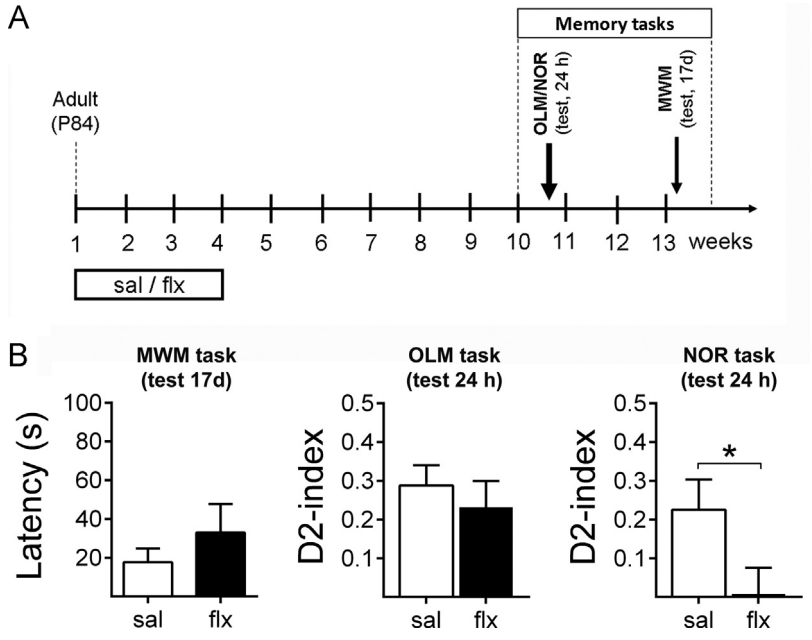

Fig. 5. Fluoxetine withdrawal following antidepressant treatment led to the recovery of spatial memory but not recognition memory. (A) Experimental design: adult rats were treated with fluoxetine (flx) or saline (sal) for 4 weeks. Six weeks after the end of treatment, 3 independent groups of rats were trained in the MWM, OLM or NOR tasks and then memory was tested at the same time points used in Figs. 1A, 3A and 4A, at which memories had been impaired after repeated fluoxetine administration. (B) Left graphic: the latency to find the platform 17 days post-learning in the MWM task was plotted; middle graphic: the D2-index for the OLM task 24 h post-training is shown; and right graphic shows the D2-index in the NOR task for both groups of rats (*$p < 0.05$, sal: $n = 8$; flx: $n = 9$; Mann–Whitney U-test).

Table 1
Locomotor/spontaneous activity in the OLM and NOR tasks after 4 weeks of repetitive fluoxetine administration. Data are expressed as mean ± SEM.

OLM		
Habituation	sal (n = 10)	flx (n = 10)
Locomotor activity (number of squares)	106.2 ± 7.3	103.0 ± 10.2
Rearing number	21.2 ± 1.5	20.8 ± 2.9
Time center (s)	5.4 ± 1.6	5.8 ± 2.7
NOR		
Habituation	sal (n = 10)	flx (n = 10)
Locomotor activity (number of squares)	70.6 ± 6.0	58 ± 6.2
Rearing number	8.7 ± 2.1	11.6 ± 2.0
Time center (s)	9.5 ± 2.6	8.6 ± 2.8

Table 2
Exploratory activity of rats treated for 4 weeks with either saline or fluoxetine. Data from the training and test sessions during the OLM and NOR tasks.

OLM task		
Training	sal (n = 18)	flx (n = 17)
Object A1	11.9 ± 0.6	10.7 ± 1.1
Object A2	12.2 ± 1.0	14.1 ± 1.7
E1-index[a]	24.1 ± 1.1	24.9 ± 2.3
Test, 24 h		
Familiar object	4.4 ± 0.4	6.3 ± 0.6
Relocated object	8.0 ± 1.2**	5.1 ± 0.6
E2-index[b]	12.3 ± 1.5	11.4 ± 0.9
NOR task		
Training	sal (n = 16)	flx (n = 18)
Object B1	11.0 ± 0.9	8.6 ± 1.1
Object B2	11.6 ± 0.9	8.2 ± 0.8
E1-index[c]	22.6 ± 1.5	16.8 ± 1.9
Test, 24 h		
Familiar object	4.4 ± 0.6	6.0 ± 0.8
Novel object	9.5 ± 1.2***	7.0 ± 0.7
E2-index[d]	17.5 ± 2.1	12.6 ± 1.6

Data are expressed as mean ± SEM.
**p < 0.01 (two-tailed unpaired t-test) Familiar vs. Relocated.
***p < 0.001 (two-tailed unpaired t-test) Familiar vs. Novel.
[a] Total exploration time (s): A1 + A2.
[b] Total exploration time (s): Familiar + Relocated.
[c] Total exploration time (s): B1 + B2.
[d] Total exploration time (s): Familiar + Novel.

behaviors (see Table 1). In addition, the total exploration time between the experimental groups was controlled to demonstrate that treatment did not affect the exploration ability of the rats. Furthermore, available exploration time did not differ between pairs of identical objects during the training sessions (Table 2).

In the NOR task, instead of location changes, one of the familiar objects was randomly changed to a novel object. For validation, the same additional measurements as for the OLM task were performed (Table 2).

2.4. Statistical analysis

We used GraphPad PRISM 4.0 software to analyze all data, with the exception of the learning phase of the MWM task, which was analyzed with STATA 9.0 software. The data are presented as the mean ± SEM. For the learning period of the MWM task, a mixed model analysis was applied with 'treatment' as a between-group factor and 'days' as a within-group factor. For memory data a two-tailed unpaired Student's t-test was applied to compare differences between groups for each of the tasks used. For intra-group comparisons between object exploration in both training and test sessions in the NOR and OLM tasks, we used two-tailed paired t-tests.

3. Results

3.1. Repetitive fluoxetine administration led to impairment of long-term spatial memory

To evaluate the effects of fluoxetine on hippocampal-dependent memory in adult rats, we administered either fluoxetine or saline for 4 weeks and performed widely used spatial MWM task (Fig. 1A). Rats displayed normal learning and memory during the 4 days of training and after 24 h post-learning, respectively. However, in fluoxetine-treated rats, the latency to reach the platform significantly increased when memory was tested 17 days after the learning phase (sal: 13.5 ± 0.4 s, n = 12, flx: 33.6 ± 5.5 s, n = 11, p < 0.01; Fig. 1B). As fluoxetine had been continuously administered during the experimental design (7 weeks in total), the memory impairment at the 17th day post-learning could indicate an accumulative adverse effect due to exposure to fluoxetine between the training and the test session. Therefore, we modified the previous experimental design to administrate fluoxetine for 7 weeks before training (Fig. 2A). Then, fluoxetine was discontinued thereby ensuring that fluoxetine would not directly act on the memory consolidation process. The new two groups of rats showed identical learning curves (Fig. 2B). Twenty-four hours post-learning, recent memory was unaffected (Fig. 2C, left graphic). However remote memory was still impaired 17 days after training (Fig. 2C, middle graphic). In this case, the platform was removed to ensure learning specificity and that rats were not reaching the platform in a casual manner. We quantified the latency to the first platform crossing and the number of target platform crossings as other commonly used parameters [24]. Fluoxetine induced both a longer latency (sal: 19.9 ± 4.6 s, n = 10, flx: 44.1 ± 5.9 s, n = 10, p < 0.01; Fig. 2B, middle graphic) and a 3-fold decrease in the number of target crossovers (sal: 3.2 ± 0.4, flx: 1.2 ± 0.3, p < 0.001, n = 10 per group). To assure a specific adverse effect on remote memory, but not learning, a second/reversal learning was performed during the two posterior days (at days 23 and 24 after the first learning phase) by placing the platform in the opposite quadrant (Fig. 2A and B). Similar to the first learning protocol, fluoxetine-treated rats did not differ in their latency to reach the platform during the training phase when compared with the saline-treated group. Furthermore, both experimental groups had similar performance when memory was tested 24 h later by measuring the latency to the first platform crossing (Fig. 2E) and the number of crossings (sal: 3.8 ± 0.7, flx: 3.5 ± 0.3, p = 0.422, n = 10 per group). Thus, data from this experiment indicate that repeated fluoxetine administration induced a selective deficiency in remote spatial memory assessed in the MWM task but did not alter learning nor recent memory consolidation.

To assess learning and spatial memory in a non-aversive paradigm, we used the OLM task (see experimental design in Fig. 3A). The ability to recognize the newly located object was tested 24 h (Fig. 3B) and 1 h (Fig. 3C) after a single training session. After 4 weeks of treatment fluoxetine-treated rats spent similar time exploring the familiar or relocated object at 24 h post-training (familiar: 6.3 ± 0.6 s, relocated: 5.1 ± 0.6 s, p = 0.277, n = 17, not shown) whereas saline-treated rats explored the relocated object for more time, as expected (familiar: 4.4 ± 0.4 s, vs. relocated: 8.0 ± 1.2 s, p < 0.01, n = 18, not shown). The discrimination D2-index following Ennaceur and Delacour was also calculated to represent memory performance [23], i.e. the exploration time of the relocated object in reference to the total exploration time (Fig. 3D–E). While a similar D2-index at 1 h post-learning revealed no differences among groups, this index decreased after an interval of 24 h indicating no memory retention (Fig. 3D). However, a single administration of fluoxetine 24 h before testing (n = 8) had no effect on memory performance (Fig. 3E). In addition, the total exploration time for the training and test sessions as the E1- and E2-indexes, respectively, were calculated. Similar E1-indexes indicated that exploration ability of rats did not depend on the treatment. Table 2 also reveals that there was not a left/right side-preference for the identical objects (A1 and A2) in the training session (Table 2). Additionally, the relocated object was counterbalanced in the test session.

3.2. Repetitive fluoxetine administration led to impairment of long-term recognition memory

The NOR task is a learning paradigm similar to the OLM task, but it is used to assess non-hippocampal-dependent memory such as recognition memory that depends primarily on cortical regions [25]. After 4 weeks of treatment, two consecutive tasks were performed to assess recognition memory 24 h and 1 h post-training (Fig. 4A–C). Similarly to the OLM task, 1 h after the training session, the performance of the fluoxetine-treated rats was indistinguishable from the control group in the NOR task (showed as D2-index in Fig. 4D). However, at 24 h post-training, only saline-treated animals explore the novel object for more time (sal group, familiar: 4.4 ± 0.6 s, novel: 9.5 ± 1.2 s, $p < 0.001$, $n = 16$; flx group, familiar: 6.0 ± 0.8 s, novel: 7.0 ± 0.7 s, $n = 18$, not shown). We also used E1- and E2-indexes (Table 2), and the D2-index (Fig. 4D–E) to analyze the NOR data [23]. Importantly, both groups of rats showed similar exploration time with each of the two objects (B1 and B2) in the training session, indicating not left/right side preference (see the E1 index, Table 2).

Additionally, the novel object was counterbalanced in the test session. As for the OLM analysis detailed above, we found exclusively differences in the D2-index at a 24 h memory retention interval among groups in the NOR analysis (Fig. 4D) while a single fluoxetine dose revealed no differences (Fig. 4E, $n = 7$ animals per group).

Spontaneous motor behaviors in turn were undistinguishable during the first habituation session of the OLM and NOR tasks and, therefore, showed no treatment effects (see data of locomotor activity, number of rearings and time spent in the center in Table 1).

3.3. Effects of fluoxetine withdrawal on memory

Three additional groups of rats were used to evaluate in the 3 memory tasks the long-lasting behavioral effects of fluoxetine, tested at the same time points in which retention had been shown impairment. For this, fluoxetine was administered during 4 weeks, and memory was tested 6 weeks after cessation of treatment (Fig. 5A). In contrast to the previous findings, remote spatial memory, assessed 17 days post-training, was not different among groups ($n = 9$ per group, Fig. 5B, left graphic). This indicates that the effect of chronic fluoxetine treatment on remote memory in the MWM task is reversible. In turn, the OLM data also showed a spontaneous recovery of recent memory in fluoxetine-treated rats ($n = 9$) 24 h post-training (compare middle graphic of Fig. 5B with Fig. 3D). However, fluoxetine-treated animals ($n = 10$) still displayed a memory impairment in the NOR task after 6 weeks of withdrawal (right panel of Fig. 5B). As expected, after fluoxetine withdrawal, rats showed normal learning and memory at 24 h post-learning in the MWM task as well as in both the OLM and NOR tasks 1 h post-learning (data not shown).

Additionally, we tested depressive-like behaviors after 6 weeks of withdrawal and found no differences among groups (Supplementary Fig. 1). This suggests that the antidepressant effects observed immediately after 4 weeks of fluoxetine treatment [5] was not present after the withdrawal period.

4. Discussion

The effects of repetitive fluoxetine administration on learning that is primarily hippocampus- (MWM and OLM) or non-hippocampus- (NOR) dependent were investigated. The main finding of our present paper is that chronic fluoxetine treatment did not affect learning but specifically impaired long-term memories with a time scale that was task-dependent. Recent or remote memories were impaired in the one-trial or multi-trial learning-paradigms, respectively. These data indicate that memory storage may be affected, a phenomenon that recruits cortical structures [8], including areas in which we had previously shown that repeated fluoxetine administration induced extensive synaptic re-arrangements.

4.1. Repetitive fluoxetine administration did not affect learning

Consistent with previous findings, both spatial and recognition learning did not differ between saline- and fluoxetine-treated animals [20,21,26]. Additionally, we found that animals with impaired remote memory could re-learn using a newly located platform in the MWM task. These findings are consistent with normal hippocampal function, which is critically important for the OLM [10] and MWM tasks [27], while hippocampal participation in the NOR task, which depends largely on the perirhinal cortex, is only partial if existent [16,17,28].

Several studies converge on an essential role of the dentate gyrus (DG) in spatial learning [29–31]. In that hippocampal region, repetitive fluoxetine treatment increases the neurogenesis that underlies antidepressant-like effects [32,33]. Furthermore, fluoxetine treatment promotes neurogenesis in the DG and also is directly related to an LTP increase in the medial perforant path-DG synapses [34,35]. This positive effect on synaptic plasticity in hippocampal circuits might explain why the encoding of new information was not affected after chronic fluoxetine treatment [36–39]. However, in telencephalic regions, devoid of neurogenesis, the putative plasticity-limiting adaptations that we have described at glutamatergic synapses induced by fluoxetine might have a functional relevance in memory consolidation [5]. These adaptations include a higher proportion of large, more stable mushroom-type spines associated to increased calcium-impermeable glutamate receptor subtypes.

4.2. Effect of fluoxetine on recent or remote spatial memories

In previous rodent studies using the MWM, normal recent memory was found in fluoxetine-treated animals when tested 24 h post-learning [20,21], irrespective of whether fluoxetine was administered during the training and test sessions. In those studies, doses of 1 or 5 mg/kg, respectively, were used for 2 weeks, and longer retention intervals post-learning were not tested. In the first MWM protocol used by us (Fig. 1), detrimental effects on remote memory at 17 days post-learning could depend on continued fluoxetine affecting the consolidation process, and not on plastic changes induced by a more permanent manner by repeated fluoxetine treatment. For this reason, a second group of rats, in which fluoxetine had been discontinued to avoid a possible post-learning effect, was subjected to the MWM task. In this case, fluoxetine affected remote memory, but no effects on learning or on recent memory at 24 h post-learning were observed in the MWM task. This suggests that long-lasting adaptive changes occur in brain areas involved in remote memory consolidation/storage.

A detrimental effect of fluoxetine on spatial memory was also revealed in the OLM task, in which failures in performance were detected in this task at 24 h post-learning (recent memory). Different outcomes in both tests were also found when hippocampal neurogenesis was inhibited [40]. This could be due to the differential participation or time course of brain structures involved in memory consolidation in these two paradigms. For instance, two comparative studies testing spatial memory have shown that at the same post-learning time, the hippocampus was crucial for remote spatial memory in the MWM task [41], but not in the five-arms maze, which requires the activation of cortical regions such as the retrosplenial cortex [42]. Differences in task aversiveness used in

assessing hippocampal-dependent memories and therefore, corticosterone levels [43,44], the number of trials required to learn and/or the requirement of habituation prior to the task [45] could influence the performance of the animals. Indeed, while the MWM task is a stressful task in which rats are forced to swim, the OLM task is performed in an open field arena where animals have been previously habituated. Perhaps the difference might also be explained by modulation of spatial consolidation by stress hormones in the aversive MWM task, that is particularly mediated by corticosterone [46,47], but does not affect memory when rats are pre-habituated to the context of the OLM task, similar to the findings described by Okuda and collaborators in the NOR task [48].

4.3. Specific long-, but not short-term, memory impairment of object recognition memory

Similarly to spatial learning test outcomes in the OLM task, we found impairments in long-term memory tested at 24 h, but not 1 h post-learning, in the NOR task. This is consistent with studies showing that chronic fluoxetine treatment impaired recognition memory at 24 h [20,49], but not at 1 h [26]. Interestingly, fluoxetine improved the D2-index 2 h after a single fluoxetine administration in the isolated rat model, used to induce depressive-like symptoms. However, this beneficial effect disappeared after a longer treatment of 7 total days [50]. In this paper, NOR memory was tested 1 h post-training. In agreement with that result, we did not find any effect of repeated fluoxetine administration at that time point.

Therefore, we could now clarify that, using the same animals to test memory at different time points, the different outcomes are likely due to fluoxetine-induced impairments in the natural consolidation process from short-term (1 h) to recent (24 h) memory. An important point is that only a minority of the studies performed in rodents have used comparable fluoxetine doses during chronic treatment (0.7–2.0 mg/kg per day) [5,21,51,52], highlighting the fact that even low doses may present adverse effects in naïve animals.

4.4. Possible hippocampal and cortical areas and mechanisms involved in the effect of fluoxetine on memory

The consolidation of explicit memories involves the gradual reorganization of brain regions that support specific types of memory [11,53]. Thus, there are changes within the medial temporal lobe system from the hippocampus to cortical regions to form or store recent or remote memories [12–14]. Contrary to the general rule that explicit memories become independent of the hippocampus with time, this structure contributes to both recent and remote memory in the MWM task [9,30,41,54]. One possible explanation is that different hippocampal sub-regions are recruited in learning vs. memory storage or retrieval in the MWM task. In this way, the inactivation of CA3 prior to learning affects acquisition but not memory retrieval [55], while the importance of CA1 pyramidal neurons in the consolidation/storage of spatial memory has been demonstrated [56]. In addition, the hippocampal CA3 region is involved in the consolidation of memory in the OLM task but not in the NOR task [57]. Furthermore, whereas hippocampus participates in short-term memory at 1 h post-training [58], the role of the perirhinal cortex is essential for consolidation of long-term memory in the NOR task [17], but also the activity of this cortical region has been associated to a good performance in the OLM task [59,60]. Therefore, the perirhinal cortex might be involved in decreased NOR and OLM long-term memory performance. Interestingly, 6 weeks of fluoxetine withdrawal is enough to recover the loss of spatial memory function both in the hippocampal-dependent MWM as well as in the OLM task, but not in the non hippocampal-dependent NOR task.

From data presented herein, we hypothesize that possible fluoxetine-induced changes affecting the hippocampus might be partially mitigated by the activation of alternative hippocampal circuits. In contrast, fluoxetine-induced plasticity in cortical structures involved in explicit memory processes may not be functionally compensated.

It is accepted that the mechanism of action of fluoxetine involves the up-regulation of trophic factors [19,61]. For example, 2 or 6 weeks of 0.7 mg/kg fluoxetine increased brain-derived neurotrophic factor (BDNF) and its receptor TrkB proteins at glutamatergic forebrain synapses [19]. Interestingly, the BDNF protein elevation in the hippocampus preceded increases of its mRNA, favoring plastic adaptations induced by this neurotrophic factor [62]. A consequence of plasticity-inducing extracellular signals could converge on the cytoskeletal re-organization leading to the observed changes in spine morphology already reported [5,63,64].

In a different study, the potential consequences of fluoxetine-induced plasticity on the molecular composition of glutamatergic synapses was studied. Increased spine density and of large, mushroom-type spines was associated to increased levels in GluA2-containing AMPA-receptors as well as GluN2A-containing NMDA-receptors after 4 weeks of fluoxetine administration [5]. This subunit composition of AMPA- and NMDA receptors leads to decreased calcium influx during periods of activity. Therefore, in the long term, activity-dependent synaptic plasticity such as LTP and LTD, which contribute to memory consolidation in cortical regions, might be altered because of reduced calcium-permeable glutamate receptors, leading to a deficiency of down-stream mechanisms that have been described elsewhere with details [17,65,66]. In such a way, calcium-activated protein kinases and nuclear signaling that leads to synthesis of new proteins might be affected, a possibility that should be investigated at the cellular level in the future.

Taken together, the growing idea that the glutamatergic system is targeted by antidepressants suggests that the re-organization of glutamatergic networks may lead to adverse effects on memory consolidation. Therefore, the use of cognitive enhancers that act on glutamatergic synapses might be potentially effective to overcome memory impairment when co-administered with long-term fluoxetine [67].

5. Conclusions

The most relevant finding of the present study is that repetitive, but not a single, administration of low-dose fluoxetine had detrimental effects on long-term hippocampal- and non-hippocampal-dependent memories in adult naïve rats, whereas learning was unaffected. Furthermore, after 6 weeks of fluoxetine withdrawal, spatial memory, but not recognition memory, was restored. The behavioral data indicates that chronic fluoxetine treatment specifically affects the formation/storage of enduring memories, and also suggests that long-lasting effects of fluoxetine is likely related to the processes in which cortical areas play a greater role.

Acknowledgements

The authors thank Nataly Quezada for taking care of the animals. Funding for this study was provided by grants to U.W. from Conicyt (Anillo ACT09_2006) and by Universidad de los Andes (MED-001-09) to F.J.R.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbr.2013.03.011.

References

[1] Wong DT, Perry KW, Bymaster FP. Case history: the discovery of fluoxetine hydrochloride (Prozac). Nature Reviews Drug Discovery 2005;4:764–74.
[2] Krishnan V, Nestler EJ. Linking molecules to mood: new insight into the biology of depression. The American Journal of Psychiatry 2010;167:1305–20.
[3] Mostert JP, Koch MW, Heerings M, Heersema DJ, De Keyser J. Therapeutic potential of fluoxetine in neurological disorders. CNS Neuroscience & Therapeutics 2008;14:153–64.
[4] Barondes SH. Thinking about Prozac. Science 1994;263:1102–3.
[5] Ampuero E, Rubio FJ, Falcon R, Sandoval M, Diaz-Veliz G, Gonzalez RE, et al. Chronic fluoxetine treatment induces structural plasticity and selective changes in glutamate receptor subunits in the rat cerebral cortex. Neuroscience 2010;169:98–108.
[6] Bannerman DM, Rawlins JN, McHugh SB, Deacon RM, Yee BK, Bast T, et al. Regional dissociations within the hippocampus – memory and anxiety. Neuroscience and Biobehavioral Reviews 2004;28:273–83.
[7] Squire LR, Zola-Morgan S. The medial temporal lobe memory system. Science 1991;253:1380–6.
[8] Squire LR, Zola SM. Structure and function of declarative and nondeclarative memory systems. Proceedings of the National Academy of Sciences of the United States of America 1996;93:13515–22.
[9] Moser E, Moser MB, Andersen P. Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience 1993;13:3916–25.
[10] Ennaceur A, Neave N, Aggleton JP. Spontaneous object recognition and object location memory in rats: the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. Experimental Brain Research Experimentelle Hirnforschung Experimentation cerebrale 1997;113:509–19.
[11] Squire LR, Alvarez P. Retrograde amnesia and memory consolidation: a neurobiological perspective. Current Opinion in Neurobiology 1995;5:169–77.
[12] Wiltgen BJ, Brown RA, Talton LE, Silva AJ. New circuits for old memories: the role of the neocortex in consolidation. Neuron 2004;44:101–8.
[13] Frankland PW, Bontempi B. The organization of recent and remote memories. Nature Reviews Neuroscience 2005;6:119–30.
[14] Squire LR, Bayley PJ. The neuroscience of remote memory. Current Opinion in Neurobiology 2007;17:185–96.
[15] Vann SD, Aggleton JP, Maguire EA. What does the retrosplenial cortex do? Nature Reviews Neuroscience 2009;10:792–802.
[16] Winters BD, Bussey TJ. Transient inactivation of perirhinal cortex disrupts encoding, retrieval, and consolidation of object recognition memory. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience 2005;25:52–61.
[17] Winters BD, Saksida LM, Bussey TJ. Object recognition memory: neurobiological mechanisms of encoding, consolidation and retrieval. Neuroscience and Biobehavioral Reviews 2008;32:1055–70.
[18] Winters BD, Forwood SE, Cowell RA, Saksida LM, Bussey TJ. Double dissociation between the effects of peri-postrhinal cortex and hippocampal lesions on tests of object recognition and spatial memory: heterogeneity of function within the temporal lobe. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience 2004;24:5901–8.
[19] Wyneken U, Sandoval M, Sandoval S, Jorquera F, Gonzalez I, Vargas F, et al. Clinically relevant doses of fluoxetine and reboxetine induce changes in the TrkB content of central excitatory synapses. Neuropsychopharmacology 2006;31:2415–23.
[20] Valluzzi JA, Chan K. Effects of fluoxetine on hippocampal-dependent and hippocampal-independent learning tasks. Behavioural Pharmacology 2007;18:507–13.
[21] Stewart CA, Reid IC. Repeated ECS and fluoxetine administration have equivalent effects on hippocampal synaptic plasticity. Psychopharmacology 2000;148:217–23.
[22] Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. Journal of Neuroscience Methods 1984;11:47–60.
[23] Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behavioural Brain Research 1988;31:47–59.
[24] Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nature Protocols 2006;1:848–58.
[25] Squire LR, Wixted JT, Clark RE. Recognition memory and the medial temporal lobe: a new perspective. Nature Reviews Neuroscience 2007;8:872–83.
[26] Bianchi M, Fone KC, Shah AJ, Atkins AR, Dawson LA, Heidbreder CA, et al. Chronic fluoxetine differentially modulates the hippocampal microtubular and serotonergic system in grouped and isolation reared rats. European Neuropsychopharmacology: The Journal of the European College of Neuropsychopharmacology 2009;19:778–90.
[27] Mumby DG, Gaskin S, Glenn MJ, Schramek TE, Lehmann H. Hippocampal damage and exploratory preferences in rats: memory for objects, places, and contexts. Learning & Memory 2002;9:49–57.
[28] Barker GR, Warburton EC. When is the hippocampus involved in recognition memory? The Journal of Neuroscience: The Official Journal of the Society for Neuroscience 2011;31:10721–31.
[29] Barnes CA, Jung MW, McNaughton BL, Korol DL, Andreasson K, Worley PF. LTP saturation and spatial learning disruption: effects of task variables and saturation levels. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience 1994;14:5793–806.
[30] Sutherland RJ, Weisend MP, Mumby D, Astur RS, Hanlon FM, Koerner A, et al. Retrograde amnesia after hippocampal damage: recent vs. remote memories in two tasks. Hippocampus 2001;11:27–42.
[31] Morris RG, Garrud P, Rawlins JN, O'Keefe J. Place navigation impaired in rats with hippocampal lesions. Nature 1982;297:681–3.
[32] Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience 2000;20:9104–10.
[33] Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, et al. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 2003;301:805–9.
[34] Wang JW, David DJ, Monckton JE, Battaglia F, Hen R. Chronic fluoxetine stimulates maturation and synaptic plasticity of adult-born hippocampal granule cells. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience 2008;28:1374–84.
[35] Bath KG, Jing DQ, Dincheva I, Neeb CC, Pattwell SS, Chao MV, et al. BDNF Val66Met impairs fluoxetine-induced enhancement of adult hippocampus plasticity. Neuropsychopharmacology 2012;37:1297–304.
[36] Maya Vetencourt JF, Sale A, Viegi A, Baroncelli L, De Pasquale R, O'Leary OF, et al. The antidepressant fluoxetine restores plasticity in the adult visual cortex. Science 2008;320:385–8.
[37] Karpova NN, Pickenhagen A, Lindholm J, Tiraboschi E, Kulesskaya N, Agustsdottir A, et al. Fear erasure in mice requires synergy between antidepressant drugs and extinction training. Science 2011;334:1731–4.
[38] Maya Vetencourt JF, Tiraboschi E, Spolidoro M, Castren E, Maffei L. Serotonin triggers a transient epigenetic mechanism that reinstates adult visual cortex plasticity in rats. The European Journal of Neuroscience 2011;33:49–57.
[39] Maya-Vetencourt JF, Tiraboschi E, Greco D, Restani L, Cerri C, Auvinen P, et al. Experience-dependent expression of NPAS4 regulates plasticity in adult visual cortex. The Journal of Physiology 2012;590:4777–87.
[40] Goodman T, Trouche S, Massou I, Verret L, Zerwas M, Roullet P, et al. Young hippocampal neurons are critical for recent and remote spatial memory in adult mice. Neuroscience 2010;171:769–78.
[41] Broadbent NJ, Squire LR, Clark RE. Reversible hippocampal lesions disrupt water maze performance during both recent and remote memory tests. Learning & Memory 2006;13:187–91.
[42] Maviel T, Durkin TP, Menzaghi F, Bontempi B. Sites of neocortical reorganization critical for remote spatial memory. Science 2004;305:96–9.
[43] Conrad CD. The relationship between acute glucocorticoid levels and hippocampal function depends upon task aversiveness and memory processing stage. Nonlinearity in Biology, Toxicology, Medicine 2005;3:57–78.
[44] Segev A, Ramot A, Akirav I. Stress hormones receptors in the amygdala mediate the effects of stress on the consolidation, but not the retrieval, of a non aversive spatial task. PloS One 2012;7:e29988.
[45] Broadbent NJ, Squire LR, Clark RE. Rats depend on habit memory for discrimination learning and retention. Learning & Memory 2007;14:145–51.
[46] Lozano YR, Serafin N, Prado-Alcala RA, Roozendaal B, Quirarte GL. Glucocorticoids in the dorsomedial striatum modulate the consolidation of spatial but not procedural memory. Neurobiology of Learning and Memory 2013;101. C:55-64.
[47] de Quervain DJ, Roozendaal B, McGaugh JL. Stress and glucocorticoids impair retrieval of long-term spatial memory. Nature 1998;394:787–90.
[48] Okuda S, Roozendaal B, McGaugh JL. Glucocorticoid effects on object recognition memory require training-associated emotional arousal. Proceedings of the National Academy of Sciences of the United States of America 2004;101:853–8.
[49] Carlini VP, Poretti MB, Rask-Andersen M, Chavan RA, Ponzio MF, Sawant RS, et al. Differential effects of fluoxetine and venlafaxine on memory recognition: possible mechanisms of action. Progress in Neuro-psychopharmacology & Biological Psychiatry 2012;38:159–67.
[50] Bianchi M, Baulieu EE. 3beta-Methoxy-pregnenolone (MAP4343) as an innovative therapeutic approach for depressive disorders. Proceedings of the National Academy of Sciences of the United States of America 2012;109:1713–8.
[51] Rodriguez-Landa JF, Contreras CM, Gutierrez-Garcia AG, Bernal-Morales B. Chronic, but not acute, clomipramine or fluoxetine treatment reduces the spontaneous firing rate in the mesoaccumbens neurons of the rat. Neuropsychobiology 2003;48:116–23.
[52] Contreras CM, Rodriguez-Landa JF, Gutierrez-Garcia AG, Bernal-Morales B. The lowest effective dose of fluoxetine in the forced swim test significantly affects the firing rate of lateral septal nucleus neurones in the rat. Journal of Psychopharmacology 2001;15:231–6.
[53] McGaugh JL. Time-dependent processes in memory storage. Science 1966;153:1351–8.
[54] Clark RE, Broadbent NJ, Squire LR. Hippocampus and remote spatial memory in rats. Hippocampus 2005;15:260–72.
[55] Florian C, Roullet P. Hippocampal CA3-region is crucial for acquisition and memory consolidation in Morris water maze task in mice. Behavioural Brain Research 2004;154:365–74.
[56] Shimizu E, Tang YP, Rampon C, Tsien JZ. NMDA receptor-dependent synaptic reinforcement as a crucial process for memory consolidation. Science 2000;290:1170–4.
[57] Stupien G, Florian C, Roullet P. Involvement of the hippocampal CA3-region in acquisition and in memory consolidation of spatial but not in object information in mice. Neurobiology of Learning and Memory 2003;80:32–41.

[58] Gould TJ, Rowe WB, Heman KL, Mesches MH, Young DA, Rose GM, et al. Effects of hippocampal lesions on patterned motor learning in the rat. Brain Research Bulletin 2002;58:581–6.
[59] Ennaceur A, Aggleton JP. Spontaneous recognition of object configurations in rats: effects of fornix lesions. Experimental Brain Research Experimentelle Hirnforschung Experimentation cerebrale 1994;100:85–92.
[60] Melichercik AM, Elliott KS, Bianchi C, Ernst SM, Winters BD. Nicotinic receptor activation in perirhinal cortex and hippocampus enhances object memory in rats. Neuropharmacology 2012;62:2096–105.
[61] Calabrese F, Molteni R, Racagni G, Riva MA. Neuronal plasticity: a link between stress and mood disorders. Psychoneuroendocrinology 2009;34(Suppl. 1):S208–16.
[62] Musazzi L, Cattaneo A, Tardito D, Barbon A, Gennarelli M, Barlati S, et al. Early raise of BDNF in hippocampus suggests induction of posttranscriptional mechanisms by antidepressants. BMC Neuroscience 2009;10:48.
[63] Hajszan T, MacLusky NJ, Leranth C. Short-term treatment with the antidepressant fluoxetine triggers pyramidal dendritic spine synapse formation in rat hippocampus. The European Journal of Neuroscience 2005;21: 1299–303.
[64] Bianchi M, Shah AJ, Fone KC, Atkins AR, Dawson LA, Heidbreder CA, et al. Fluoxetine administration modulates the cytoskeletal microtubular system in the rat hippocampus. Synapse 2009;63:359–64.
[65] Krugers HJ, Hoogenraad CC, Groc L. Stress hormones and AMPA receptor trafficking in synaptic plasticity and memory. Nature Reviews Neuroscience 2010;11:675–81.
[66] Wang H, Hu Y, Tsien JZ. Molecular and systems mechanisms of memory consolidation and storage. Progress in Neurobiology 2006;79:123–35.
[67] Cheung ZH, Ip NY. From understanding synaptic plasticity to the development of cognitive enhancers. The International Journal of Neuropsychopharmacology/Official Scientific Journal of the Collegium Internationale Neuropsychopharmacologicum 2011;14:1247–56.