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A longitudinal study of 5-HT outflow during chronic fluoxetine treatment using a new technique of chronic microdialysis in a highly emotional mouse strain

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1. Introduction

Depression and anxiety are common psychiatric disorders in developed countries. Serotonin-selective reuptake inhibitors (SSRIs) are currently the most widely prescribed class of antidepressants to treat mood disorders including major depressive disorders. Interestingly, several weeks of sustained SSRIs treatment are required for measurable clinical benefits (Blier, 2003), indicating that adaptive mechanisms in the central 5-HT system and/or other systems are involved in the therapeutic effects. SSRIs rapidly block the neuronal plasma membrane serotonin (5-HT) transporter and, after oral intake in mice, then studying its relation with behavior, analyzed mainly with open field paradigm. One of the neural mechanisms underlying such delay has been proposed to be the functional status of 5-HT1A autoreceptors in raphe nuclei. Thus, we also assessed the degree of 5-HT1A autoreceptor desensitization by using a local infusion in the raphe of the antagonist, WAY 100635 via reverse microdialysis. We report that the anxiolytic-like effects of fluoxetine correlate in time and amplitude with 5-HT1A autoreceptor desensitization, but neither with the extracellular levels of 5-HT in the raphe nuclei, nor in the hippocampus. Our study suggests that the beneficial anxiolytic/antidepressant-like effects of chronic SSRI treatment indeed depend on 5-HT1A autoreceptor internalization, but do not require a sustained increase in extracellular 5-HT levels in a territory of 5-HT projection such as hippocampus.

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ABSTRACT

The onset of a therapeutic response to antidepressant treatment exhibits a delay of several weeks. The present study was designed to know whether extracellular serotonin (5-HT) levels need to be increased in territories of 5-HT innervation in order to obtain beneficial effects from a chronic treatment with a serotonin-selective reuptake inhibitor (SSRI). Thus, we performed a longitudinal study of a chronic fluoxetine treatment in a model of highly emotional mice (BALB/c). The function of the 5-HT system in the raphe nuclei and hippocampus, was assessed by using repeated in vivo microdialysis sessions in awake freely moving mice, then studying its relation with behavior, analyzed mainly with open field paradigm. One of the neural mechanisms underlying such delay has been proposed to be the functional status of 5-HT1A autoreceptors in raphe nuclei. Thus, we also assessed the degree of 5-HT1A autoreceptor desensitization by using a local infusion in the raphe of the antagonist, WAY 100635 via reverse microdialysis. We report that the anxiolytic-like effects of fluoxetine correlate in time and amplitude with 5-HT1A autoreceptor desensitization, but neither with the extracellular levels of 5-HT in the raphe nuclei, nor in the hippocampus. Our study suggests that the beneficial anxiolytic/antidepressant-like effects of chronic SSRI treatment indeed depend on 5-HT1A autoreceptor internalization, but do not require a sustained increase in extracellular 5-HT levels in a territory of 5-HT projection such as hippocampus.

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affected by chronic SSRI treatment (Duman et al., 2001). Such treatments induced changes in serotonergic afferent stimulation (Chaput et al., 1991), modulation of the 5-HT-induced potentiation of synaptic transmission (Kobayashi et al., 2008), downregulation of the 5-HT transporter (Benmansour et al., 2002) and enhanced tonic inhibition mediated by 5-HT1A receptors (Blier and de Montigny, 1987; Haddjeri et al., 1998; Blier and Ward, 2003).

However, by using intracerebral in vivo microdialysis, several groups have reported an increase in basal extracellular 5-HT levels in the hippocampus following chronic SSRI administration (Kreiss and Lucki, 1995; Gundlah et al., 1997; Hajas-Korcsok et al., 2000; Wegener et al., 2003), while others found no changes (Hjorth and Auerbach, 1994, 1999; Gardier et al., 2003; Keck et al., 2005). These discrepancies could be explained by the fact that each study used a different protocol in terms of drugs, dose range and rhythm of drug administration either in rats or mice. Moreover, in all these studies, the measure of 5-HT in dialysates was performed only at the end of the chronic SSRI treatment, and longitudinal effects of changes in dialysate 5-HT levels during this SSRI treatment and their correlation with the behavioral effects of the treatment have not been examined. In addition, preventing 5-HT1A autoreceptor activation with 5-HT1A receptor antagonists can enhance the effects of SSRIs on extracellular 5-HT levels (Artigas et al., 1994; Hjorth and Auerbach, 1994; Malagie et al., 2001; Artigas et al., 2006).

Here, we studied the function of the 5-HT system (raphe-hippocampus) and its relation with behavior during a chronic SSRI treatment. To analyze the time course of extracellular 5-HT levels by using multiple in vivo microdialysis sessions, we measured the effects of chronic fluoxetine treatment on extracellular 5-HT in the cell groups of origin (e.g. midbrain raphe) and territories of 5-HT innervation (e.g. hippocampus) (Toth, 2003). Microdialysis in the raphe and hippocampus brain regions allows to monitor 5-HT levels in the vicinity of cell bodies and nerve terminals of 5-HT neurons, respectively.

Thus, oral fluoxetine was chronically administered to mice that have been surgically prepared with guide cannula affixed to the skull to allow repeated microdialysis (one session every week for four weeks: see the protocol in Fig. 1) in the raphe and hippocampus in mice under waking conditions. Since SSRIs are known to induce a variety of extracellular 5-HT changes (Artigas et al., 2006; Hjorth and Auerbach, 1994; Malagie et al., 2001; Artigas et al., 2006), we chose to study the effects of SSRIs in a highly emotional mouse strain, the BALB/cJ. Indeed, these mice display high baseline 5-HT levels (Artigas et al., 2006).

2. Materials and methods

2.1. Animals

All experiments were performed on male BALB/cByJ mice (25–30 g, Janvier Breeding Laboratories). Mice were individually housed in a temperature-controlled room (23 ± 1 °C) under 12 h light/dark cycle (lights on at 7:00 A.M. to 7:00 P.M.), with food and water available ad libitum. After arrival, mice were allowed at least 2 weeks for habituation before surgery. All procedures involving animals and their care were conducted in conformity with the institutional guidelines and in compliance with national and international laws and policies in the Animal Care Facility of the ‘Institut Fédératif de Recherche–IFR141’ of the Paris-Sud University (Council directive # 87-848, October 19, 1987, Ministère de l’Agriculture et de la Foret, Service Vétérinaire de la Santé et de la Protection Animale, permissions # 92-196 to A.M.G.).

2.2. Drugs

Oral fluoxetine hydrochloride (Lilly Research Laboratories, Indianapolis) was chronically administered in the drinking water for 4 weeks (160 mg/l of water), corresponding to 18 mg/kg/day, changed weekly (Santarelli et al., 2003; Dulawa et al., 2004) to mice that have been surgically prepared with guide cannula affixed to the skull to allow repeated microdialysis sessions (each session lasted 2 h; one session every week, Fig. 1) in the raphe nuclei and hippocampus under waking conditions.

Local infusion of the selective 5-HT1A receptor antagonist WAY 100635 (Sigma-Aldrich, Saint Quentin Fallavier, France; 100 µM perfused at a 0.5 µl/min flow rate for 2 h) dissolved in 100 µl of aCSF, was performed by reverse dialysis infusion into the RN for 2 h, after basal 5-HT levels have been measured, during each microdialysis session.

2.3. Microdialysis studies

2.3.1. Implantation of guide cannula

Animals were anesthetized with a combination of ketamine (100 mg/kg i.p.) and xylazine (5 mg/kg i.p.). After loss of toe pad reflexes, mice were mounted in a Kopf stereotaxic frame in the flat skull position. CMA/7 cannulas (Carnegie Medicin, Stockholm, Sweden) were implanted to serve as guides for subsequent insertion of microdialysis probes (CMA/7) into the RN and the hippocampus. The coordinates for these sites were based on Hof mice brain atlas (Hoff et al., 2000; in mm from Bregma) for the raphe nuclei: anteroposterior, −4.5 mm, lateral 0, depth, −5.5 mm; hippocampus: anteroposterior, −3.4 mm, lateral +3.4 mm, depth, −4 mm.

The guide cannulas were anchored to the skull with superglue, super-bond and embedded in acrylic cement. After completion of the surgery, mice were housed individually and maintained under standard laboratory conditions. Microdialysis experiments started at least 1 week after the surgery.

At the end of the chronic experiment, placement of microdialysis probes was verified by histological examination according to the method set up by (Bert et al., 2004). When a probe was incorrectly placed, the corresponding results were discarded (4 of the 32 animals used in the study).

2.3.2. Microdialysis session

Mice were attached to a fluid swivel and microdialysis probes CMA/7 were inserted vertically through a guide cannula into each area 3 h prior to the experimental session. The length of the exchange surface of the dialysis membrane was 2 mm for the raphe nuclei and the hippocampus. Dialysis probes were perfused with artificial cerebrospinal fluid (aCSF) containing: 140 mM NaCl, 3 mM KCl, 1.5 mM CaCl₂, 1 mM MgCl₂, 1.2 mM NaH₂PO₄, 0.27 mM Na₂HPO₄, 8.1 mM glucose, pH 7.4. In the present study, a 7.5 ml/h perfusion rate was used.

Fig. 1. Experimental protocol. D, day; W, withdrawal; aCSF, artificial cerebrospinal fluid. Insert. Day protocol (performed on D0, D7, D14, D21, D28 and W14): Wash-out, 3.2 h of aCSF perfusion for wash-out of 5-HT originating from non-neural sources; baseline, 2h of aCSF; 5-HT1A receptor status, 2h of aCSF + raphé nuclei injection of the 5-HT1A receptor antagonist, the WAY 100635 (100 µM).
and pH was adjusted to 7.4. The aCSF was infused at 0.5 and 1.5 μl/min for RN and hippocampus, respectively, using a CMA/100 microdialysis pump (Carnegie Medicin, Stockholm, Sweden) as previously described (Guilloux et al., 2006). Infusion of aCSF started 3 h before experiments to allow sufficient time for wash-out of 5-HT originating from non-neuronal sources.

Microdialysis sessions and behavioral tests were performed at different time points before, during and after treatment: Day (D) 0, D7, D14, D21, D28, and Withdrawal (W) 14. For withdrawal, normal water was reintroduced in the drinking water for 14 more days. To test for the intensity of the effect of the SSRI regimen on 5-HT neurotransmission during chronic microdialysis method, a control group received the fluoxetine treatment in the same conditions, but was tested only on D28.

2.3.3. 5-HT sampling and analysis

Samples were collected at 30 or 15 min intervals for the hippocampus and raphe nuclei, respectively. They were analyzed by high performance liquid chromatography (HPLC, XL-ODS, 4.6×7.0 mm, particle size 3 μm; Beckman) coupled to an amperometric detection (1049A, Hewlett-Packard, Les Ulis, France) [limit of sensitivity for 5-HT – 0.5 fmol/sample (signal-to-noise ratio = 2)]. The amounts of 5-HT in dialysate samples were calculated by measurement of peak heights relative to known amounts in external standards (Guilloux et al., 2006).

2.4. Behavioral tests

Behavioral activities of chronic fluoxetine treatment were assessed by using animal models predictive of anxiolytic/antidepressant-like activity.

2.4.1. Open field

Mice were placed in the center of the open field and explored the open field for 30 min (Dulawa et al., 2004; Holick et al., 2008). 15 min was allowed for habituation; then mice were tested in the open field for 15 min. Locomotor activity was quantified in two Plexiglas open 43 × 43 cm² field boxes (MED Associates, Georgia, VT). Two sets of 16 pulse-modulated infrared photobeams were placed on opposite walls 2.5-cm apart to record x-y ambulatory movements. Activity chambers were computer interfaced for data sampling at 100-ms resolution. The computer defined grid lines that divided each open field into center and surround regions, with each of four lines being 11 cm from each wall. Dependent measures were the number of entries into the center, the distance travelled in the center, total time spent in the center, and distance travelled in the center divided by total distance travelled. Overall motor activity was quantified as the total distance travelled (cm).

2.4.2. Novelty suppressed feeding (NSF)

Mice were food deprived for 24 h prior to the test. Testing was performed in a 50 × 50 cm box covered with litter and illuminated by a 70 watt lamp. Pellets of food were placed in the center of a box, on top of a piece of white filter paper. The mice were tested individually by placing them in the box for a period of 5 min, and recording the latency to approach and start consuming the food. If the mice did not approach the food, they were excluded from analysis. Directly following the 5 min test, the mice were placed back in their home cage and the amount of food consumed was measured for 5 min (Santarelli et al., 2003; David et al., 2009). This test is very sensitive to chronic, but not acute, antidepressant treatment (Santarelli et al., 2003).

2.4.3. Tail suspension test (TST)

Mice were suspended by the tail with adhesive tape to a hook and were tested for a total of 6 min. The time of immobility was assessed as previously described (Alexandre et al., 2006). The TST is a paradigm of behavioral despair in the face of an inescapable stress, with the periods of immobility used to characterize depression-like behavior. The reduction in the immobility time in this test is also used to identify potential antidepressant properties of compounds (Steru et al., 1987).

2.5. Statistical analysis

Values in text and figures are mean ± standard error (SEM). ANOVAs and corresponding F-values had been indicated in the Results section. Repeated-measures ANOVA was performed for microdialysis studies and to assess the effect of fluoxetine treatments (with time, and treatment when required, as repeated factor(s)). Paired tests were used for repeated measures in post-hoc tests when no more than two groups had to be compared. Statistical significance was set at p < 0.05.

3. Results

3.1. Microdialysis studies

In awake, freely moving BALB/cJ mice, basal extracellular 5-HT levels in the raphe nuclei and hippocampus, were 4.3 ± 0.4 fmol/10 µl and 3.05 ± 0.30 fmol/20 µl, respectively (for 16 determinations per group).

3.1.1. Effects of chronic administration of fluoxetine on 5-HT levels

Chronic administration of the antidepressant fluoxetine (18 mg/kg/ day, for 28 days) in BALB/cJ mice induced a sustained significant increase in extracellular 5-HT levels during the treatment period in both the RN and the hippocampus compared to baseline determined the first day of dialysate collection (D0), and to a control group that had received vehicle in drinking water during the same period (Fig. 2, F(1,42) = 26.72, p = 0.0001 for the RN and F(1,42) = 18.44, p = 0.0007 for the hippocampus). This increase was larger in the raphe nuclei than in the hippocampus at all time points (F(4,42) = 5.65, p < 0.0001).

On withdrawal (W14), 5-HT levels returned to the baseline values in the raphe nuclei (p = 0.59) and hippocampus (p = 0.63), indicating that chronic SSRI treatment and repeated microdialysis sessions induced reversible alterations in central 5-HT neurotransmission.

To test whether the present results are confound by effects of repeated microdialysis assessments, we administrated fluoxetine orally in a new group of mice and drinking water in another and performed only one microdialysis session at the end of the treatment, on D28. Increases in 5-HT levels in the RN (control group, n = 4, fmol/10 µl: 3.45 ± 0.7; fluoxetine group, n = 4, fmol/10 µl: 11.94 ± 3.4, p = 0.04) and hippocampus (control group, n = 4, fmol/19 µl: 2.82 ± 0.3; fluoxetine group, n = 4, fmol/19 µl: 4.03 ± 0.6, p = 0.11) were similar after one or 5 sessions (p < 0.05 for all groups), demonstrating the feasibility of repeated microdialysis study in mice 5-HT system.

3.1.2. 5-HT1A receptor functional status

Administered after the baseline session, an intra-raphe perfusion of the 5-HT1A receptor antagonist, WAY100635 (100 μM) significantly potentiated the effects of fluoxetine on extracellular 5-HT levels in the raphe nuclei during the first 3 weeks of the treatment and in the hippocampus at D7 and D14 (Fig. 3). WAY100635 did not potentiate 5-HT levels anymore at D28 in the RN and at D21 in the hippocampus, suggesting that a desensitization of 5-HT1A autoreceptors occurred. It seems that such a desensitization of presynaptic 5-HT1A autoreceptors is sufficient enough at D28 to block the feedback control exerted by somatodendritic 5-HT1A autoreceptors on hippocampal 5-HT release at D21. Finally, no effect of WAY100635 on 5-HT levels in the vehicle-treated group was noticed.

3.2. Behavioral studies

3.2.1. Anxiolytic/antidepressant-like activity of chronic fluoxetine treatment

The behavioral effects of chronic fluoxetine treatment were assessed in the open field test, in which the time in the central area is used to
characterize the anxiolytic-like activity of the SSRI. Chronic administration of the antidepressant fluoxetine in BALBc mice decreased anxiolytic-like activity and increased exploration as measured by the open field (Fig. 4): increase number in center entries ($F_{(1,42)} = 11.86, p = 0.004$) and increase distance travelled in the center ($F_{(1,42)} = 12.38, p = 0.0035$), with an optimal effect at D28. These effects developed over one month of daily fluoxetine treatment (Fig. 4), a time course that is classically observed for the beneficial effect of antidepressants in patients. Finally, fluoxetine did not alter the total time spent in the center (session time center: $F_{(1,42)} = 0.14, p = 0.71$; ambulatory time center: $F_{(1,42)} = 0.56, p = 0.47$) nor the total locomotor activity in BALB/cj mice ($F_{(1,42)} = 2.74, p = 0.12$).

Then at D28, two different behavioral tests were performed. In the tail suspension test (TST) (Fig. 5A), chronic fluoxetine treatment induced a significant decrease in immobility duration compared to the vehicle-treated control group ($F_{(1,20)} = 13.65, p = 0.0014$), indicating the fluoxetine antidepressive-like activity of fluoxetine on D28. In the novelty suppressed feeding (NSF) test (Fig. 5B), chronic fluoxetine treatment induced a significant decrease in the latency to feed and in the food consumption compared to the control group receiving drinking water ($F_{(1,20)} = 6.04, p = 0.0232, F_{(1,20)} = 17.66, p = 0.0004$, respectively), indicating an anxiolytic-like activity of fluoxetine on D28. Overall, these results indicate anxiolytic/antidepressant-like activities of fluoxetine after 4 weeks of its administration, thus validating the fluoxetine behavioral benefits.

### 3.2.2. 5-HT1A receptor status and behavior

To determine whether behavioral effects of chronic fluoxetine treatment are correlated with the 5-HT1A autoreceptor functional status, we computed the correlations between behavioral measurements in the open field and extracellular 5-HT levels in the raphe nuclei and hippocampus during the whole treatment. Dialysate 5-HT levels were measured after WAY 100635 administration for individual subjects and expressed in fmol/sample. There was no relationship between extracellular 5-HT levels in both the raphe nuclei and hippocampus, and the measures in the center in open field during the first 3 weeks of treatment (data not shown). However, a strong negative correlation between the number of central entries and the amount of 5-HT in the RN was found on D28 after WAY 100635 administration (Fig. 6A; Pearson $r = −0.91, p < 0.001$), thus unveiling a strong relationship between the 5-HT1A autoreceptors desensitization and the behavioral effects of chronic fluoxetine treatment. In contrast, no relationship was observed for the control group in the open field (Fig. 6A: Pearson between the number of central entries and the amount of 5-HT in the RN on D28 after WAY 100635 administration in control group $r = −0.32, p = 0.05$), in the hippocampus (Fig. 6B: Pearson between the number of central entries and the amount of 5-HT on D28 after WAY 100635 administration in control group $r = 0.39, p = 0.05$ and in fluoxetine group $r = −0.1, p = 0.05$) or with TST and NSF.

No relationship was found between the amount of 5-HT in the RN or hippocampus and the ambulatory distance in the center/total ambulatory
distance: RN: Pearson = −0.08, \( p < 0.05 \) for fluoxetine group and = 0.46, \( p < 0.05 \) for the control group; hippocampus: = − 0.03, \( p < 0.05 \) for fluoxetine group and = 0.32, \( p < 0.05 \) for the control group.

4. Discussion

This is the first longitudinal study of the effects of chronic antidepressant treatment on raphe 5-HT1A receptor function, on cerebral extracellular 5-HT levels and on behavior. Our results demonstrate that, in an anxiolytic/antidepressant-responsive mouse strain, the SSRI treatment induces a rapid, long-lasting and reversible increase in raphe nuclei and hippocampus basal extracellular 5-HT levels, while the effects on behavior exhibit a slower onset over 4 weeks of fluoxetine treatment. The 5-HT1A autoreceptor desensitization in the raphe as evaluated by using intracerebral microdialysis appears only after 4 weeks of treatment. Moreover, the extent of desensitization on D28 correlates with the behavioral effects of the treatment in one parameter (i.e., center entries) of the open field test. These results are in agreement with the postulated role of serotonin autoreceptor desensitization in the therapeutic effects of SSRI.

4.1. The feasibility of repeated microdialysis in 5-HT system in mice

Most microdialysis studies are performed in an acute preparation with a between-subjects design. The protocol of repeated microdialysis that we setup in the present study allowed within-subject designs, in which each mouse is tested under chronic fluoxetine treatment and as its own control, under baseline and withdrawal conditions. We tested the feasibility of repeated in vivo microdialysis sessions in awake mice and we found that the integrity of the brain 5-HT system was maintained. First, the basal extracellular 5-HT levels in the RN and hippocampus were very stable in the control group across probe insertions. Second, the increase in 5-HT levels during the chronic fluoxetine treatment had an amplitude similar to the changes found in studies with between-subject design (Hrdina, 1987; Caccia et al., 1992; Durand et al., 1999; Nakayama et al., 2003). We found that these changes are reversible and 5-HT values returned to baseline at 14 days after the end of fluoxetine treatment. Third, the levels of 5-HT after multiple dialysis probe implantations were similar to those found in a control group that was submitted to only one session of dialysis on D28. In summary, we demonstrated the feasibility of repeated in vivo measurement of 5-HT by microdialysis in awake, freely moving mice.

4.2. A longitudinal study in serotonin-selective reuptake inhibitor responsive mice

While several attempts to study the desensitization of 5-HT1A autoreceptors and its effects following a chronic treatment (see Introduction) have been made, our work differs from these earlier studies on several aspects. First, our experiments were performed in the BALB/cJ mice strain. Chronic, but not subchronic, SSRI treatments have antidepressant/anxiolytic-like activities in these mice, as measured in forced swim test, novelty-induced hypophagia and open-field tests (Dulawa et al., 2004). The genetic factors responsible

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**Fig. 4.** Effect of chronic fluoxetine treatment on open field. The number of central entries (left) and the ratio of the distance travelled in the center on the total ambulatory distance were measured in the open field in BALBc mice receiving fluoxetine in drinking water (fluox, black, \( n = 8 \)) or plain water (control, white, \( n = 8 \)) in their bottle; the x-axis represents baseline (D0) and the days of tests (D7–D28) or of withdrawal (W14). Values plotted are mean±SEM. * \( p < 0.05 \); ** \( p < 0.01 \), significant difference between groups (comparison between fluoxetine and water group on the same day according to a one-way ANOVA followed by a post-hoc Student’s \( t \)-test). # \( p < 0.05 \), within-groups significant difference with D0 (i.e., no fluoxetine administration) (one-way ANOVA followed by a post-hoc Student’s \( t \)-paired test). The grey area underlines when the function of 5-HT1A autoreceptors was reduced, i.e., 28 days after the beginning of fluoxetine treatment.

**Fig. 5.** Depression and anxiety-related behavior after chronic fluoxetine treatment were measured on D28 in A. Tail suspension test and B. Novelty suppressed feeding. Values plotted are mean±SEM. * \( p < 0.05 \); ** \( p < 0.01 \); *** \( p < 0.001 \), significant difference between groups.
4.2. Desensitization of 5-HT1A autoreceptors after chronic fluoxetine treatment

Chronic fluoxetine treatment induced early (starting in the first week of treatment) and long-lasting, reversible increases in dialysate 5-HT in the RN and hippocampus in adult BALB/cJ mice. No gradual increase in extracellular 5-HT levels throughout the duration of the treatment was noticed (Smith et al., 2000). A limited number of studies have previously examined dialysate levels of 5-HT in the raphe nuclei following a SSRI treatment, but a similar increase in 5-HT levels was reported at the end of 14 days with another SSRI (paroxetine, 0.5 mg/kg/day twice a day: Malagie et al., 2000).

Intra-raphe perfusion of WAY 100635 revealed that presynaptic 5-HT1A autoreceptors are activated by the 5-HT released in the raphe. We found a significant desensitization of these 5-HT1A autoreceptors only after 4 weeks of chronic fluoxetine treatment. This effect was revealed after intra-raphe perfusion of WAY 100635. Our finding confirms previous chronic studies indicating that repeated activation of the 5-HT1A autoreceptors during several weeks leads to a functional loss (desensitization - internalization) of these receptors (Blier and de Montigny, 1994; Jolas et al., 1994; Pineyro and Blier, 1999), but extends these studies by detailing the time course of this desensitization. An immediate, but transient, 5-HT1A autoreceptors desensitization after acute SSRI treatments has also been described (Le Poul et al., 2000), due to a temporary internalization observed by a decrease in their binding (Aznavour et al., 2006) and in the density of the labeling at the plasma membrane of raphe dendrites (Riad et al., 2008). We did not check for the presence of such effect, our first session being performed after a week of treatment. Thus, under our conditions, the autoreceptor desensitization is essentially occurring in the late phase of the chronic treatment.

4.3. Desensitization of 5-HT1A autoreceptors after chronic fluoxetine treatment

In conclusion, our results provide further functional evidence that 5-HT1A autoreceptor desensitization–internalization is an important molecular factor involved in the anxiolytic and antidepressant-like activities of fluoxetine in rodents. The desensitization of 5-HT1A autoreceptors should attenuate the negative feedback control exerted by these somatodendritic autoreceptors on 5-HT neuronal activity.

4.4. Anxiolytic-like activity of chronic fluoxetine treatment in BALBc mice

SSRIs administered acutely or sub-chronically are known to have limited beneficial effects or even adverse effects on anxiety and depression (Griebel, 1995; Dulawa et al., 2004). However, chronic SSRIs treatments are effective in depressed or anxious patients (Barr et al., 1997; Gelfin et al., 1998) as well as in highly emotional animal models (Dulawa et al., 2004; Popa et al., 2008). BALBc mice are highly emotional as shown in many paradigms including open-field test (Kim et al., 2002) and show anxiolytic-like effects after chronic fluoxetine (Dulawa et al., 2004). We confirmed such effects in the tail suspension test and novelty suppressed feeding test performed on D28. In addition, we studied the time course of anxiolytic-like activity induced by fluoxetine treatment by monitoring weekly the performance of mice in the open-field test. We found the strongest anxiolysis also on D28, i.e., after 4 weeks of fluoxetine treatment. Moreover, the number of the center entries, a reliable measure of anxiety (for review Prat and Belzung, 2003), was directly correlated with the degree of 5-HT1A autoreceptor desensitization observed after 4 weeks of treatment: the mice with the strongest desensitization of 5-HT1A autoreceptors in the raphe exhibited the weakest anxiety on D28. Therefore our results indicate that the beneficial effects of chronic SSRI treatments are temporally and quantitatively correlated with the desensitization of 5-HT1A autoreceptors.

4.5. 5-HT1A autoreceptors and hippocampus serotonin during chronic treatment

In the hippocampus, we found significantly increased extracellular 5-HT levels only during the first 3 weeks of the fluoxetine treatment, i.e., before the desensitization of the raphe 5-HT1A autoreceptors. This indicates that these autoreceptors are not the main determinant of the basal extracellular 5-HT levels during the chronic fluoxetine treatment. Several groups have previously reported an increase in extracellular 5-HT levels following chronic SSRIs, while others found no change. Chronic fluoxetine administration (14 days (Kreiss and Lucki, 1995)) or other antidepressants (citalopram, paroxetine) (Gundlah et al., 1997; Hajos-Korcso et al., 2000; Wegener et al., 2003) produces an increase in extracellular 5-HT levels in the hippocampus. In contrast, other long-term SSRI administrations (paroxetine, citalopram) failed to induce such an increase (Hjorth and Auerbach, 1994, 1999; Gardier et al., 2003; Keck et al., 2005). In all these studies, the measures of extracellular 5-HT levels were performed only at the end of the chronic treatment. Our longitudinal study demonstrated a waxing and waning of the 5-HT levels in the hippocampus during the treatment. Therefore, a possible cause of discrepancy between these studies could arise from the transient nature of the increase in extracellular 5-HT levels in the hippocampus during the chronic SSRI treatment.

In conclusion, our results provide further functional evidence that 5-HT1A autoreceptor desensitization–internalization is an important molecular factor involved in the anxiolytic and antidepressant-like activities of fluoxetine in rodents. The desensitization of 5-HT1A autoreceptors should attenuate the negative feedback control exerted by these somatodendritic autoreceptors on 5-HT neuronal activity.

![Figure 6](image-url)
However, we also show that basal extracellular 5-HT levels are only transiently increased by the chronic fluoxetine treatment and returned to baseline when 5-HT1A autoreceptors are desensitized. This suggests other concomitant changes in the control of the brain 5-HT system during chronic SSRI treatments and call for further investigations.

Finally, our study suggests that the beneficial anxiolytic/antidepressant-like effects of chronic SSRI treatment indeed depend on 5-HT1A autoreceptor internalization, but do not require a sustained increase in extracellular levels of 5-HT in a territory of 5-HT projection such as the hippocampus. This suggests that the desensitization of 5-HT1A receptors exerts its beneficial effects by means other than the regulation of basal extracellular 5-HT levels in these brain areas. Future studies exploring the direct link between the 5-HT/other systems and the behavioral benefits of SSRIs used combining pharmacology (Millan, 2006) should encourage the development of novel treatments for anxiety and depression with greater efficacy and faster onset.

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