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Pharmacological Blockade of 5-HT_7 Receptors as a Putative Fast Acting Antidepressant Strategy

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Current antidepressants still display unsatisfactory efficacy and a delayed onset of therapeutic action. Here we show that the pharmacological blockade of serotonin 7 (5-HT_7) receptors produced a faster antidepressant-like response than the commonly prescribed antidepressant fluoxetine. In the rat, the selective 5-HT_7 receptor antagonist SB-269970 counteracted the anxiogenic-like effect of fluoxetine in the open field and exerted an antidepressant-like effect in the forced swim test. *In vivo*, 5-HT_7 receptors negatively regulate the firing activity of dorsal raphe 5-HT neurons and become desensitized after long-term administration of fluoxetine. In contrast with fluoxetine, a 1-week treatment with SB-269970 did not alter 5-HT firing activity but desensitized cell body 5-HT autoreceptors, enhanced the hippocampal cell proliferation, and counteracted the depressive-like behavior in olfactory bulbectomized rats. Finally, unlike fluoxetine, early-life administration of SB-269970, did not induce anxious/depressive-like behaviors in adulthood. Together, these findings indicate that the 5-HT_7 receptor antagonists may represent a new class of antidepressants with faster therapeutic action.
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INTRODUCTION

There are extensive data demonstrating the involvement of the brain serotonin (5-HT) system in both the pathogenesis of depression and the action of antidepressant (AD) drugs. However, optimal therapeutic effectiveness is not achieved with current ADs, because most agents present major drawbacks in clinical use, such as a delayed onset of their action and a high percentage of non-responders. Selective 5-HT reuptake inhibitors (SSRIs) exert their actions by enhancing synaptic availability of 5-HT. However, a series of neuroadaptive changes must first occur for this to happen, and these changes are thought to underlie the prolonged therapeutic latency of these drugs (Blier and de Montigny, 1999; Duman *et al*, 2001; Nestler *et al*, 2002). Examples of the above mentioned changes include a progressive desensitization of raphe 5-HT_{1A} autoreceptors and a stimulation of the cell proliferation in the dentate gyrus (DG) of the hippocampus following long-term treatment with SSRIs, with a time course consistent with their delayed AD effects (Blier and de Montigny, 1999; Hensler, 2002; Santarelli *et al*, 2003; Castrén, 2004; Lanfumey and Hamon, 2004; Faure *et al*, 2006a).

Recently, attention has been given to 5-HT_7 receptors because of their potential role in mood disorders including depression (for reviews, see Hedlund, 2009 and Mnie-Filali *et al*, 2009). The 5-HT_7 receptor is the most recently identified member of the 5-HT receptor family and has been cloned from several species including humans (Hedlund and Sutcliffe, 2004). The 5-HT_7 receptors have been shown to be involved in various functions modulated by the 5-HT system such as circadian rhythms and thermoregulation (Thomas *et al*, 2003; Hedlund *et al*, 2004; Faure *et al*, 2006b). Moreover, 5-HT_7 receptors in the hippocampal formation also seem to be involved in interactions between 5-HT system and the hypothalamic-pituitary-adrenal axis (Yau *et al*, 2001; Laplante *et al*, 2002). In addition, the localization of 5-HT_7 receptors in corticolimbic areas related to affective processes and their involvement in functions impaired in depressed patients suggest an important role of these receptors in mood disorders (Ruat *et al*, 1993; To *et al*, 1995; Mnie-Filali *et al*, 2007a, 2009). More direct evidence, such as downregulation of 5-HT_7 receptors in hypothalamus after chronic AD treatments (Sleight *et al*, 1995; Mullins *et al*, 1999)

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and antidepressant-like behaviors produced by 5-HT$_7$ receptor knock-out mice (Hedlund *et al*, 2005; Guscott *et al*, 2005) strengthen this hypothesis.

In this study, the AD potential of the selective 5-HT$_7$ receptor antagonist SB-269970 was first assessed using the forced swim test (FST) in rats. Then, we investigated whether only a 1-week treatment with SB-269970 is able to elicit the same functional and cellular changes as those induced by classical ADs, including desensitization of dorsal raphe nucleus (DRN) 5-HT$_{1A}$ autoreceptors, enhanced inhibitory tone mediated by postsynaptic 5-HT$_{1A}$ receptors in the hippocampus, and the stimulation of hippocampal cell proliferation. The effects of SB-269970 were compared with those induced by 1-week treatment with the widely used SSRI fluoxetine. The action of SB-269970 and fluoxetine was then assessed in the olfactory bulbectomy (OBX) paradigm, considered as a 'chronic' behavioral model of depression in which classical AD treatments require the administration for 2–3 weeks before any AD-like effects can be observed (Song and Leonard, 2005). Finally, the potential of SB-269970 to produce, like fluoxetine, anxious- and/or depressive-like effects following early life exposure was examined.

MATERIALS AND METHODS

Animals

Experiments were carried out in male Sprague-Dawley rats weighing 250–300 g (Harlan (Gannat, France) or Charles River (Saint-Constant, QC, Canada)). Animals were kept under standard laboratory conditions (12-h light:12-h dark cycle) with free access to food and water. They were habituated at least 1 week to the laboratory facility before each experiment. Experiments were in accordance to the European Communities Council Directives 86/609, OJ L 358,1, 12 December, 1987, for the care and use of laboratory animals. Also, the electrophysiological experiments performed in Canada were approved by the local Animal Care Committee and were in accordance with the guidelines set by the Canadian Council for Animal Care.

Drugs

SB-269970 ((*R*)-3-(2-(2-(4-methyl-piperidin-1-yl)ethyl)-pyrrolidine-1-sulphonyl)-phenol), AS19 ((2*S*)-(+)-5-(1,3,5-trimethylpyrazol-4-yl)-2-(dimethylamino)tetralin), and paroxetine were obtained from TOCRIS (Illkirch, France). L-5-HTP (L-5-Hydroxytryptophan), PCPA parachlorophenylalanine, WAY-100635 (N-(2-(4(2-methoxyphenyl)-1-piperazinyl) ethyl) -N-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride), 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)tetralin), and BrdU (5-Bromo-3′deoxyuridine) were purchased from Sigma Aldrich (St-Quentin Fallavier, France). Fluoxetine was obtained from LKT Laboratories (St Paul, MN, USA).

Open-Field Test

Open field was performed in a white wooden chamber ($100 \times 100 \times 40$ cm). Testing took place under bright ambient light conditions (400 lux) to increase the anxiety component of the center area of the open field. Rats were intraperitoneally injected with the vehicle (water) (vehicle and SB-269970 groups) or fluoxetine (10 mg/kg) (fluoxetine and fluoxetine + SB-269970 groups) 1 h before the test and then with the vehicle (vehicle and fluoxetine groups) or SB-269970 (2 mg/kg) (SB-269970 and fluoxetine + SB-269970 groups) 30 min later. Each rat was then placed in a corner of the open field and allowed to explore freely for 5 min. Exploratory activity was monitored by a video camera fixed above the arena and relayed to a computer system. Anxiety-like behavior was assessed as the number of visits to the center area of the open field (defined as the central of 60×60 cm portion) over a 5 min period.

FST

The FST was used as previously described (Porsolt *et al*, 1977) to evaluate potential AD effects. Briefly, rats experienced a pre-test session followed 24 h later by a test session. For both the pre-test and the test sessions, conducted under low illumination (12 lux), the rats were placed in a plastic cylindrical tank (50 cm high by 20 cm in diameter) filled with water at $23 \pm 2^{\circ}$C, with a depth of 40 cm, for which the hind limbs could not reach the tank floor. In all experiments, the pre-test was carried out for 15 min and the test for 6 min in the same tank. Vehicle (water) or SB-269970 (0.5 mg/kg, i.p.) was administered between these two sessions (23, 5, and 1 h before the test sessions). Following either pre-test or test sessions, rats were dried with a towel and kept warm for 30 min before returning to their home cage. A camera coupled with a computer recorded the behavior of the animal online during the FST and the immobility duration was then assessed by image analysis through a specialized digital interface (Videotrack, ViewPoint, Lyon, France) as previously described by Haddjeri *et al*. (2004). Software from ViewPoint permitted us to analyze data offline avoiding observer subjectivity.

Locomotor Activity

These experiments were performed in order to ensure that behaviors in the FST were not secondary to a nonspecific alteration in locomotor activity produced by the treatments. In order to simulate the treatment procedure used in the FST, rats were injected with vehicle (water) or SB-269970 (0.5 mg/kg, i.p.) three times within 24 hours: 23 h, 5 h, and 1 h before the test. They were then placed in activity cages in an actimeter (Imetronic, France) equipped with two infrared beams (one at the front and one at the back) positioned 4 cm above the floor. Locomotor activity was estimated by the determination of successive beam breaks at the front and at the back, and vice versa (crossovers) during a 10-min session.

Head-Twitch Response (HTR)

The global design of these experiments was chosen on the basis of previous work of Sánchez and Kreilgaard (2004). Thirty minutes after drugs or vehicle (saline or polyethylene glycol 5% for AS19) injection, the rats were given L-5-HTP (87.3 mg/kg, s.c.). Each animal was then placed in an observation cage and the cumulative number of head twitches (which consists of a characteristic, rapid and rotational

flick of the head, ears, and neck) was counted during 10 min. Assessments were performed by observers blind to the drug condition of rats.

Extracellular Unitary Recordings of DRN 5-HT Neurons

Extracellular unitary recordings of DRN 5-HT neurons were performed in chloral hydrate anesthetized rats (400 mg/kg, i.p.), with single-barrelled glass micropipettes positioned 1 mm anterior to lambda on the midline (Haddjeri and Blier, 1995). Presumed DRN 5-HT neurons were identified using the criteria of Aghajanian and Vandermaelen (1982), ie a slow (0.5–2.5 Hz) and regular firing rate and long-duration (0.8–1.2 ms) positive action potentials. For drug administration, a baseline firing rate was established over 1–2 min and the 5-HT$_7$ agonist AS19 (dissolved in polyethylene glycol 5%) at a dose of 2.5–10 mg/kg was intravenously injected in 1–2-min intervals until cell firing was completely suppressed with or without prior injection (2–3 min) of SB-269970 (0.1 mg/kg, i.v. dissolved in saline) as well as in rats pre-treated for 15 days with fluoxetine (10 mg/kg per day, i.p. dissolved in saline). In each rat, only one neuron was studied.

For the chronic studies, in order to determine the possible changes of the spontaneous firing activity of dorsal raphe 5-HT neurons, rats were injected daily for 1 week with fluoxetine (10 mg/kg, i.p. dissolved in saline) or SB-269970 (2 mg/kg, i.p. dissolved in saline). Four to five electrode descents were carried out through this nucleus about 1 h after the last i.p. injection. Finally, the responsiveness of 5-HT neurons to enhanced 5-HT levels was evaluated by using the SSRI paroxetine (0.5 mg/kg, i.v.) in control and treated rats for 1 week with SB-269970 (2 mg/kg, i.p.). The percentage of inhibition of cell firing (% of inhibition) was calculated by comparison of the mean baseline firing obtained from a 1–2 min interval before the initial drug application and the mean cell firing rate obtained from a 1–2 min interval following each drug injection.

Recordings from Dorsal Hippocampus CA3 Pyramidal Neurons

Rats received a daily injection of vehicle (saline), fluoxetine (10 mg/kg, i.p.), or SB-269970 (2 mg/kg, i.p.) for 1 week. Recording and microiontophoresis were performed with five-barreled glass micropipettes broken back to 8–12 μM under microscopic control. The central barrel was filled with a 2 M NaCl solution and used for extracellular unitary recordings. Pyramidal neurons were identified by their large amplitude (0.5–1.2 mV) and long-duration (0.8–1.2 ms) simple spikes alternating with complex spike discharges (Kandel and Spencer, 1961). The side barrels contained the following solutions: 5-HT creatinine sulfate (2 mM in 200 mM NaCl, pH 4), quisqualate (1.5 mM in 200 mM NaCl, pH 8), and 2 M NaCl used for automatic current balancing. The rats were mounted in a stereotaxic apparatus and the microelectrodes were lowered at 4.2 mm lateral and 4.2 mm anterior to lambda into the CA3 region of the dorsal hippocampus. As most hippocampus pyramidal neurons are not spontaneously active under chloral hydrate anesthesia, a leak or a small ejection current of quisqualate (+2 to −8 nA) was used to activate them within their physiological firing range (10–15 Hz; Ranck, 1975). A 10 or 20 nA ejection current of 5-HT was used, each ejection period lasting 50 s. In order to assess the degree of activation of the postsynaptic 5-HT$_{1A}$ receptors exerting an inhibitory influence on the firing activity of CA3 pyramidal neurons, WAY 100 635 was administered intravenously to disinhibit the hippocampal neurons resulting in an increase of their firing activity. The saline injection corresponds to the 0 mg/kg dose. WAY 100 635 was administered in incremental doses of 25 μg/kg at time intervals of 2 min. To avoid residual drug effects, only one cell was studied in each rat. The change of firing activity was assessed by calculating the mean firing rate of neurons from about 1–2 min before and after (until a 'plateau') the i.v. administration of the drugs and the percent of change was calculated.

Cell Proliferation in the Subgranular and the Subventricular Zones

As previously described (Mnie-Filali *et al*, 2007b), rat hippocampal cell proliferation was evaluated after seven daily injections with saline, fluoxetine (10 mg/kg, i.p.), or SB-269970 (2 mg/kg, i.p.). A group of rats treated with SB-269970 received a daily administration of PCPA (400 mg/kg, i.p.) during the last 3 pretreatment days. On the sixth day, all the groups of rats received four injections of BrdU (50 mg/kg, i.p.) separated by 2 h intervals. Twenty-four hours after the last BrdU injection, animals were deeply anesthetized and transcardially perfused with NaCl 0.9% for 5 min followed by 4% cold formaldehyde in phosphate buffer (PB: 0.1 M, pH 7.4) for 17 min. The brains were then removed, post-fixed overnight in formaldehyde and stored at 4°C in 30% sucrose. The 30 μm coronal sections were cut through the olfactory bulb or the hippocampus, (respectively from +2.28 to −0.48 mm and from −1.8 to −5.8 mm, posterior to bregma, according to Paxinos and Watson, 1998) collected as a series of 12 serial sections and stored in phosphate buffer saline (PBS) containing 0.1% of sodium azide at 4°C. These free-floating sections were used in the determination of BrdU labeling. DNA denaturation was conducted by incubation for 2 h in 50% formamide/2 × SSC (0.3 M NaCl, 0.003 M sodium citrate) at 65°C followed by two rinses in 2 × SCC buffer. Sections were incubated for 30 min in HCl (2N) and then 10 min in borate buffer (0.1 M, pH 8). All incubations were followed by three rinses of 10 min in PBST (PBS + 0.1% Triton X-100) at room temperature. Sections were processed for 3 min on pepsin (0.45 U/ml in 0.1N HCl) and transferred for 15 min onto 3% H_2O_2 to eliminate endogenous peroxidase activity. They were subsequently incubated overnight with anti-BrdU antibody (ABCys, Paris, France) and diluted 1/200 in PBST-1% bovine serum albumin at room temperature. Sections were then washed by three rinses in PBST and incubated in a biotinylated secondary rabbit antirat IgG (1/250, Vector Laboratories, Burlingame, CA) for 90 min followed by amplification with an avidin horseradish peroxidase diluted 1/200 in PBST (Vector Laboratories). Visualization of bound peroxidase was achieved by a reaction in a solution of 50 mM Tris-HCl (pH 7.6) containing 0.003% H_2O_2, 0.8% $NiCl_2$, and 0.02% diaminobenzidine. Every twelfth section throughout the hippocampus was processed for BrdU immunohistochemistry. All BrdU-labeled

cells in the granule cell layer were counted in each section by an experimenter blinded to the identity of the sections and data were collected with the help of mapping software (Mercator Pro, Explora Nova, La Rochelle, France), coupled to a Zeiss microscope (Axioskop 20, Zeiss, Lena, Germany). A cell was counted as being in the subgranular zone (SGZ) of the DG if it was located in a 40 μm wide band immediately adjacent to the surface of the DG granule cell layer. The total number of BrdU-labeled cells per section was determined and multiplied by 12 to obtain the total estimated number of cells per DG. Ten sections were examined per animal ($n = 7$–8 rats per group). We used the protocol of Kuhn *et al.* (1996) to assess cell proliferation in the subventricular zone (SVZ). BdrU-labeled cells were counted in the walls of the lateral ventricles on four sections of SVZ per brain taken between 9.4 and 8.8 mm from bregma and the total number of BrdU-labeled cells was counted and expressed per SVZ and per section.

OBX and Assessment of Hyperlocomotion

Bilateral OBX was performed in rats anaesthetized with chloral hydrate (400 mg/kg i.p.) and mounted in a stereotaxic frame. The head was shaved and a midline sagittal incision was made. Bilateral burr holes (2 mm diameter) were drilled at 7 mm anterior to bregma and ± 2.5 mm lateral from midline and olfactory bulbs were removed by suction with a vacuum pump attached to a Pasteur pipette. The cavities were then packed with hemostatic sponges (Bloxang, Bausch and Lomb-Laboratoires Chauvin, France) and the wound was closed with sterile suture. Sham-operated rats were treated similarly, with the exception that olfactory bulbs were not removed. After surgery, animals were injected 5 ml i.p. with 5% glucose. The animals were allowed to recover for 14 days following surgery during which they were handled daily to eliminate any aggressiveness that may have otherwise arisen (Song and Leonard, 2005).

After recovery, rats were daily injected (between 08:30 and 10:30) for 7 days with SB-269970 (2 mg/kg per day, i.p.), 7 or 21 days with fluoxetine (10 mg/kg per day, i.p.), or with the vehicle (water). A 5-min open-field test was conducted 24 h after the last drug injection (ie, 22 and 36 days after bulbectomy, respectively). The open-field chamber consisted of a square wooden box ($85 \times 85 \times 70\ cm^3$). As conditions of very high brightness are crucial to observe the hyperactivity in OBX-rats (Mar *et al*, 2000; Song and Leonard, 2005), the inner faces of the walls were covered with aluminium foil and illumination was provided by a 75 W bulb positioned 90 cm directly over the center of the open field (1700–2000 lux). Each rat was placed into the center of the open field and exploratory activity was monitored by a video camera fixed above the arena and relayed to a computer system. To estimate locomotor activity, the open field was virtually divided into 25 squares of 17×17 cm and the number of crossed squares was calculated.

Neonatal Exposure to 5-HT Agents

Female Sprague-Dawley rats with litters (standardized to 10 male pups) of postnatal day 1 were purchased. From their arrival, litters were randomly assigned to receive either fluoxetine (10 mg/kg), SB-269970 (2 mg/kg), or AS19 (5 mg/kg). Corresponding to their group, rat pups were given i.p. injections from days P8 to P21 of either drug or an equivalent volume of saline twice daily. As previously described, behavioral testing in the FST and the open field was conducted at 2 months of age (Hansen *et al*, 1997; Ansorge *et al*, 2004).

Statistical Analysis

Electrophysiological data were expressed as percentage of baseline values ± SEM. Behavioral results were presented as mean ± SEM. In all experiments, statistical differences between the groups were analyzed with Student's *t* test or with one-way ANOVA or two-way ANOVA repeated measures followed by the *post-hoc* PLSD Fisher's test. Differences were considered to be statistically significant when p values were less than 0.05.

RESULTS

Effect of 5-HT$_7$ Receptor Antagonist in the Open-Field Test and the FST

In a first set of experiments, the illuminated open-field test was used to assess the effect of 5-HT$_7$ receptor blockade on the anxiogenic-like effect of fluoxetine. Analysis of data illustrated in Figure 1 revealed a significant effect of treatment ($F_{3,28} = 4.87$, $p < 0.01$, one-way ANOVA). When compared with vehicle-treated animals, acute administration of fluoxetine induced a 80% decrease in the number of visits to the center of the open-field apparatus ($p < 0.01$, PLSD Fisher's test), whereas no significant change was observed after acute administration with SB-269970. Interestingly, administration of SB-269970 after fluoxetine counteracted the decrease of the number of visits to the center induced by fluoxetine ($p < 0.05$, PLSD Fisher's test). These results demonstrated that fluoxetine, acutely administered, induced an anxiogenic-like effect that was prevented by the 5-HT$_7$ receptor antagonist SB-269970.

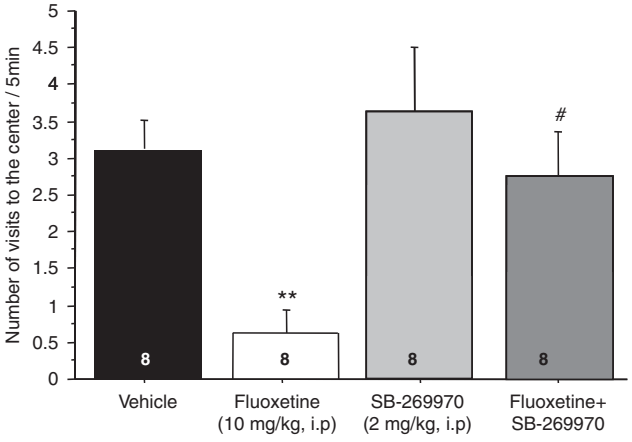

Figure 1 Effect of the selective 5-HT$_7$ antagonist SB-269970 (2 mg/kg, i.p.) and of the SSRI fluoxetine (10 mg/kg, i.p.) on the number of visits to the center of an illuminated open-field. Results are expressed as mean ± SEM. The different compounds were administered 30 or 60 min before the test session. $^{**}p < 0.01$ *vs* vehicle, $^{\#}p < 0.05$ *vs* fluoxetine; PLSD Fisher's test. The number at the bottom of the columns indicates the number of rats tested.

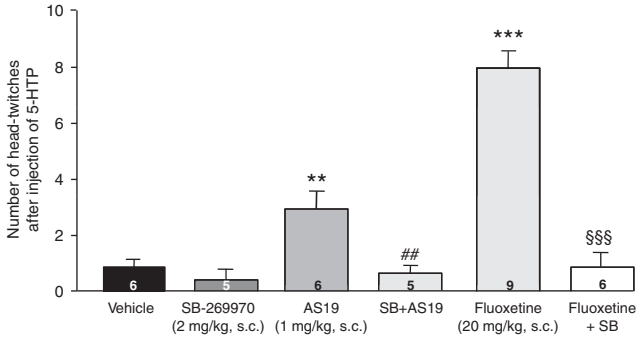

Figure 2 Number of head twitches elicited in 10 min by SB-269970 (2 mg/kg, s.c.) alone or combined with AS19 (1 mg/kg, s.c.) or fluoxetine (20 mg/kg, s.c.) after injection of L-5-HTP (87.3 mg/kg, s.c.). Results are presented as mean ± SEM. $^{**}p<0.01$, $^{***}p<0.001$ *vs* vehicle; $^{\#\#}p<0.01$ *vs* AS19; $^{\S\S\S}p<0.001$ *vs* fluoxetine; PLSD Fisher's test. The number at the bottom of the columns indicates the number of rats tested.

In a second set of experiments, the AD potential of SB-269970 was assessed using the FST. As previously shown (Wesołowska and Kowalska, 2008), a sub-acute treatment with the 5-HT$_7$ receptor antagonist SB-269970 (0.5 mg/kg, i.p.) significantly reduced immobility time compared with controls, mean immobility duration in vehicle and SB-269970-treated rats: 73 ± 8 s ($n=19$) and 48 ± 6 s ($n=15$), respectively ($t_{32}=2.34$, $p<0.05$; Student's *t* test). SB-269970 (0.5 mg/kg, i.p.) did not, however, alter locomotion (mean locomotor activity in vehicle and SB-269970-treated rats: 49 ± 7 crossovers/10 min ($n=6$) and 52 ± 7 crossovers/10 min ($n=6$), respectively ($t_{10}=-0.22$, $p>0.05$; Student's *t* test)). Hence, these results suggest that SB-269970 produced an antidepressant-like profile that was not attributable to an increase in locomotor activity.

Effect of 5-HT$_7$ Receptor Manipulation on Head-Twitches Responses Induced by L-5-HTP

Analysis of 5-HTP-induced HTR showed a significant effect of treatment ($F_{5,31}=39.61$, $p<0.0001$, one-way ANOVA; Figure 2). The number of HTR following an injection of L-5-HTP (87.3 mg/kg, s.c.) was significantly enhanced by 3.5 fold in AS19-injected rats (1 mg/kg, s.c., $p<0.01$, PLSD Fisher's test). SB-269970 (2 mg/kg, s.c.) by itself had no significant effect on the number of 5-HTP-induced HTR, but abolished the enhancing action of AS19 ($p<0.01$, PLSD Fisher's test). As reported in previous studies, the SSRI fluoxetine (20 mg/kg, s.c.) potentiated the head-twitch behavior evoked by 5-HTP (>10 fold; $p<0.001$, PLSD Fisher's test). Interestingly, SB-269970 completely blocked the enhancement of HTR following fluoxetine administration ($p<0.001$, PLSD Fisher's test). These results therefore indicate that 5-HT$_7$ receptor blockade did not facilitate the motoric component of the 5-HT syndrome but prevented the potentiating effect of fluoxetine.

Modulation of Dorsal Raphe Nucleus 5-HT Neuronal Firing Activity by 5-HT$_7$ Receptors

The effect of cumulative (2.5–10 mg/kg) intravenous injections of the 5-HT$_7$ receptor agonist AS19 in controls and rats pre-injected with the selective 5-HT$_7$ receptor antagonist SB-269970 (0.1 mg/kg, i.v.) and with the SSRI fluoxetine (10 mg/kg per day, i.p, 15 days) are illustrated in Figure 3. These data showed significant effects of treatment ($F_{2,12}=11.65$; $p<0.01$), AS19 doses ($F_{2,24)}=5.66$; $p<0.01$), and a treatment x AS19 dose interaction ($F_{(4,24)}=10.34$; $p<0.0001$; two-way ANOVA with repeated measures). *Post-hoc* PLSD Fisher's test revealed that dose-response curves of both SB-269970 and fluoxetine-treated rats were significantly different from that of the control rats ($p<0.05$; Figure 3c). Typical examples showing the reduction of the spontaneous firing activity of 5-HT induced by cumulative doses of AS19 in the vehicle-treated rats and the prevention of this suppressant effect in rats previously injected with SB-269970 are shown in Figure 3a and b. These results indicate that 5-HT$_7$ receptors negatively modulated DRN 5-HT neurons activity and that long-term treatment with fluoxetine desensitized these receptors.

A 1-Week Regimen of the 5-HT$_7$ Receptor Antagonist SB-269970 did not Alter DRN 5-HT Neuronal Firing Activity but Enhanced the Tonic Activation of Postsynaptic 5-HT$_{1A}$ Receptors in the Hippocampus

It has been demonstrated that chronic administration of SSRIs, such as fluoxetine or citalopram, induces a robust decrease of 5-HT neuronal activity that is followed by a full recovery, with a delay that corresponds to the onset of their therapeutic effects. Analysis of DRN 5-HT spontaneous firing showed a significant effect of treatment ($F_{2,119}=16.81$, $p<0.0001$, one-way ANOVA). As expected, a 1-week regimen of the SSRI fluoxetine (10 mg/kg per day, i.p.) significantly decreased by 63% the spontaneous firing activity of 5-HT neurons ($p<0.001$, PLSD Fisher's test, Figure 4a). This neuronal activity was not, however, modified by a 1-week regimen of SB-269970 (2 mg/kg per day) in comparison with controls (Figure 4a). It is assumed that the recovery of the firing rate of 5-HT neurons after chronic SSRIs is due to 5-HT$_{1A}$ receptor desensitization (Chaput *et al*, 1986; Le Poul *et al*, 1995; Czachura and Rasmussen, 2000). In order to assess the sensitivity of presynaptic 5-HT$_{1A}$ autoreceptors after a 1-week regimen of SB-269970, the capacity of the SSRI paroxetine (0.5 mg/kg, i.v.) to inhibit DRN 5-HT neurons firing activity was examined in vehicle- and SB-269970-treated rats. This probe was deemed optimal to assess the responsiveness of 5-HT$_{1A}$ autoreceptors because it is devoid of 5-HT$_7$ affinity and its inhibitory effect is not dependent on 5-HT$_{1A}$ receptors on a cortical-raphe feedback loop involved in inhibiting 5-HT neuron firing (Blier and de Montigny, 1987). Paroxetine (0.5 mg/kg, i.v.) completely suppressed the firing activity of DRN 5-HT neurons in control rats, whereas it reduced this parameter by only 29% in rats pre-injected with SB-269970 ($n=5$ rats per group, Student's *t*-test; $t_8=3.2$; $p<0.05$). These results suggest that a 1-week regimen of SB-269970 produced an early desensitization of 5-HT$_{1A}$ and/or 5-HT$_7$ receptors. In addition, it has been previously reported that clinically effective ADs induced an augmentation of the tonic stimulation of inhibitory postsynaptic 5-HT$_{1A}$ receptors (Haddjeri *et al*, 1998a, b). As most CA3 hippocampal pyramidal neurons are not spontaneously active under chloral hydrate anesthesia, an ejection current of quisqualate (0 to −2 nA) is necessary to activate them (Haddjeri *et al*, 1998a, b, 2000; El Mansari *et al*, 2005; Lucas *et al*, 2007). The

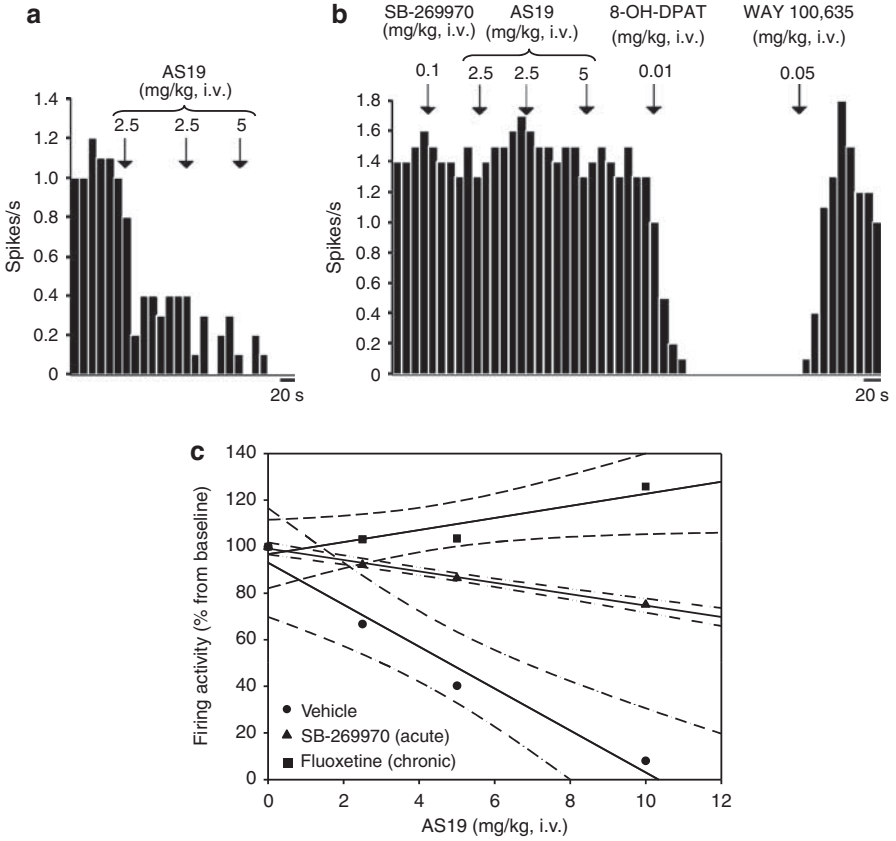

Figure 3 Examples of integrated firing histograms of dorsal raphe 5-HT neurons showing the effect of AS19 (2.5–10 mg/kg, i.v.) alone in control rats (a) or pre-injected with SB-269970 (b). Dose-response curves (solid lines) illustrating the effect of cumulative intravenous doses of AS19 on dorsal raphe nucleus (DRN) 5-HT neuron average of firing rate, expressed as percentage of basal activity, in controls ($n=5$), in rats pre-treated with SB-269970 (0.1 mg/kg, i.v., $n=5$) and in rats chronically treated with fluoxetine (10 mg/kg per day, i.p., 15 days, $n=4$). Dashed lines depict the 95% confidence interval of the regression (c).

effect of WAY 100 635 was then assessed in control and drug-treated rats (Figure 4b). WAY 100 635 slightly increased the quisqualate-activated firing activity of CA3 pyramidal neurons within a dose range of 50–100 μg/kg (i.v.) in controls but more potently in drug-treated rats. A two-way ANOVA with repeated measures revealed a significant effect of treatment ($F_{(2,14)}=4.34$; $p<0.05$), WAY 100 635 doses ($F_{(3,42)}=13.06$; $p<0.0001$), and treatment x WAY 100 635 dose interaction ($F_{(6,42)}=3.33$; $p<0.01$). *Post-hoc* PLSD Fisher's tests revealed that dose-response curves for both SB-269970 and fluoxetine treated rats were significantly different from that of the control rats ($p<0.05$, Figure 4b). Hence, a 1-week treatment with SB-269970 and fluoxetine induced a facilitation of 5-HT neurotransmission, as correlated by an enhanced 5-HT$_{1A}$-mediated inhibitory tone on CA3 pyramidal neurons.

A 1-Week Regimen of SB-269970, but not with Fluoxetine, Promoted Rat Hippocampal Cell Proliferation

It is now widely accepted that, despite the variety of their pharmacological profiles, a common feature of ADs is their ability to enhance hippocampal cell proliferation. Hence, it has been suggested that assessing an increase of this parameter might be a useful paradigm to screen compounds with AD activity. Thus, the ability of SB-269970 to enhance such cell proliferation was investigated. The examination of hippocampal sections showed that BrdU-positive nuclei in both control and drug-treated rats were restricted in the subgranular cell layer (SGZ) of the DG at the border between the granule cell layer and hilus, and appeared in irregular shapes as clusters of 2–3 cells (Figure 5a). Analysis of data obtained in the SGZ of control and treated rats revealed a significant effect of treatment ($F_{2,19}=3.79$; $p<0.05$, one-way ANOVA). Interestingly, as illustrated in Figure 5b, a 1-week regimen of SB-269970 (2 mg/kg per day, i.p.) significantly enhanced the number of BrdU-positive cells in the SGZ of the DG by 41% when compared with controls ($p<0.05$, PLSD Fisher's test), whereas no significant effect was observed after a 7-day regimen of the SSRI fluoxetine (10 mg/kg per day, i.p.). Moreover, this enhancement was specific to hippocampal cell proliferation as neither SB-269970 nor fluoxetine affected the number of newborn cells in the SVZ of the olfactory bulb (Figure 5c; $F_{2,17}=0.53$; $p>0.05$, one-way ANOVA). Altogether, these results showed that the 5-HT$_7$ receptor antagonist promoted cell proliferation selectively in the SGZ of the DG of the hippocampus, more promptly than with SSRIs. In order to determine that the effect of SB-269970 on cell proliferation was mediated through the 5-HT system, the inhibitor of 5-HT synthesis, PCPA, was administered to control and

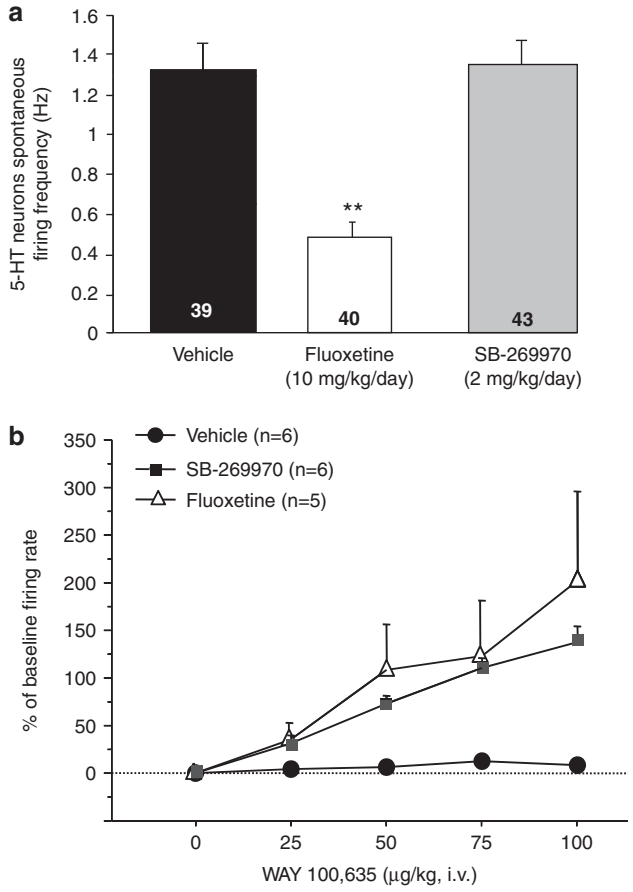

Figure 4 (a) Histograms representing the spontaneous firing rate of dorsal raphe 5-HT neurons (expressed in Hz) in rats administered the vehicle, fluoxetine (10 mg/kg per day, i.p.) or SB-269970 (2 mg/kg per day, i.p.) for 7 days. The number at the bottom of each column indicates the number of neurons recorded. $^{**}p<0.01$ *vs* vehicle. (b) Effect of cumulative intravenous doses of the selective 5-HT$_{1A}$ antagonist WAY 100 635 on the average (mean ± SEM) of firing activity of hippocampal pyramidal neurons of the CA3 sub-field, in rats administered i.p. SB-269970 (2 mg/kg per day, $n=6$), fluoxetine (10 mg/kg per day, 7 days, $n=5$) or the vehicle ($n=7$).

SB-269970 pre-treated rats. As illustrated in Figure 5d, a statistical difference was found when groups were compared using one-way ANOVA ($F_{2,18}=4.41$; $p<0.05$). Data obtained for the SGZ of rats exposed to PCPA alone (100 mg/kg per day for 4 days, i.p.) showed that the number of newborn cells in the SGZ was decreased by 54% compared with controls ($p<0.05$, PLSD Fisher's test). Interestingly, in rats receiving regimen of SB-269970 for 1 week, the enhancement of cell proliferation in the SGZ previously obtained was no longer present after PCPA, as evidenced by the significant 42% decrease compared with controls ($p<0.05$, PLSD Fisher's test). These results suggest that a functional 5-HT system is necessary for SB-269970 to exert its effect on hippocampal cell proliferation.

Effects of Treatment with SB-269970 or Fluoxetine in OBX Rats

It had previously been reported that OBX rats exhibit locomotor hyperactivity that is suppressed after long-term antidepressant treatment. The effect of SB-269970 was thus investigated on this behavioral parameter. Analysis of data illustrated in Figure 6a revealed a significant effect of treatment ($F_{2,15}=10.55$; $p<0.01$, one-way ANOVA). As expected, OBX rats that received the vehicle for 7 days displayed a strong increase (+135%) of locomotor activity when placed in the open field in comparison with the sham-operated animals ($p<0.001$, PLSD Fisher's test). Interestingly, a 1-week treatment with SB-269970 (2 mg/kg per day, i.p.) significantly reduced this hyperlocomotion ($p<0.05$, PLSD Fisher's test). In contrast, the OBX-fluoxetine-treated rats (7 days, 10 mg/kg per day, i.p.) still exhibited a strong hyperactivity compared with the sham-operated animals (+121%; $p<0.05$; PLSD Fisher's test performed after a global one-way ANOVA ($F_{2,15}=5.84$; $p<0.05$), Figure 6b). Only a 3-week treatment with fluoxetine was able to reverse the OBX-induced hyperactivity ($p<0.001$; PLSD Fisher's test performed after a global one-way ANOVA ($F_{2,15}=11.25$; $p<0.01$), Figure 6c)). This suggests that SB-269970 had a faster antidepressant-like action than fluoxetine.

Neonatal Exposure to SB-269970 or AS19 had no Detectable Effects in Adulthood

In neonatal rodents, sustained administration of 5-HT ADs (including SSRIs and tricyclic antidepressants) during the early life period from postnatal 8 (PN8) to PN21 results in a pattern of maladaptive behaviors that are evident and persistent in adulthood (Mirmiran *et al*, 1981; Maudhuit *et al*, 1995; Hansen *et al*, 1997; Ansorge *et al*, 2004). These behavioral changes include alterations in locomotor activity and increased immobility in the FST (Mirmiran *et al*, 1981; Hilakivi and Hilakivi, 1987; Alexandre *et al*, 2006; Hansen *et al*, 1997). The effect of postnatal exposure to fluoxetine (10 mg/kg per day for 14 days, i.p.), SB-269970 (2 mg/kg per day for 14 days, i.p.), or with AS19 (5 mg/kg per day for 14 days, i.p.) was examined in adulthood (Figure 7). A significant effect of treatment ($F_{3,40}=3.01$; $p<0.05$, one-way ANOVA) was obtained. In comparison with control animals, rats neonatally exposed to the SSRI fluoxetine exhibited a 58% decrease in the number of visits to the center of the open-field apparatus ($p<0.05$, PLSD Fisher's test). No significant difference was observed after neonatal exposure to SB-269970 or AS19 (Figure 7a). These results showed an increase in anxiety-like behavior by postnatal exposure to the SSRI fluoxetine, but not with the 5-HT$_7$ receptor agonist or the antagonist. In the FST, a significant effect of treatment was observed ($F_{3,34}=5.35$; $p<0.01$, one-way ANOVA). The immobility duration was significantly enhanced by 32% in fluoxetine-exposed rats ($p<0.05$, PLSD Fisher's test) during the neonatal period, but not in AS19- or SB-269970-exposed animals (Figure 7b). Taken together, these results showed that neonatal exposure to the SSRI fluoxetine, but not to AS19 or SB-269970, produced anxiety- and depression-like behaviors in early adulthood of the rats.

DISCUSSION

The present results show that behavioral, electrophysiological and neuro-anatomical changes that usually correlate with the onset of beneficial action of long-term treatment

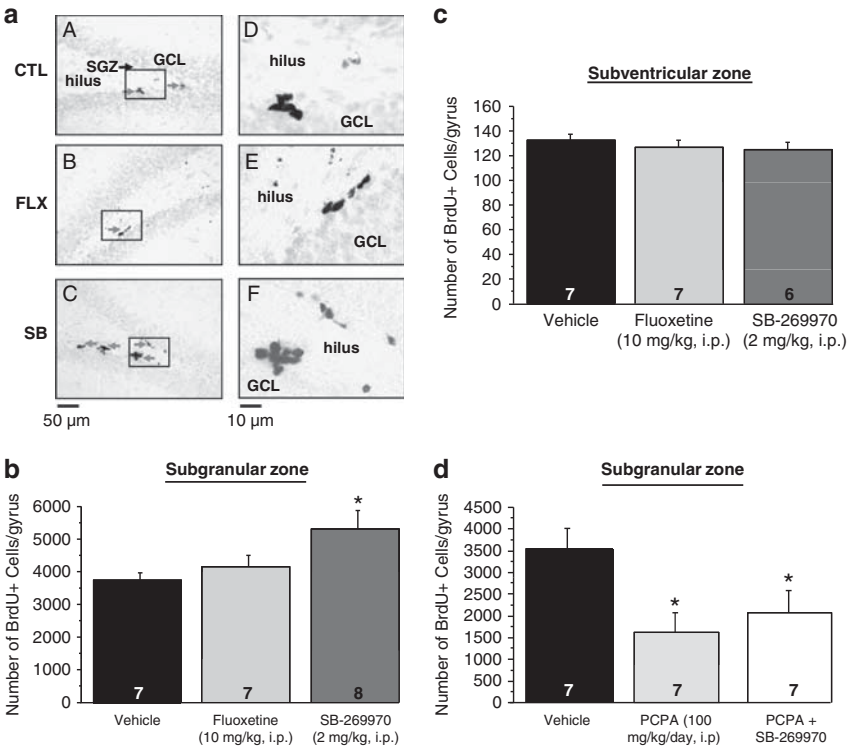

Figure 5 Effect of SB-269970 (2 mg/kg per day, 7 days) and fluoxetine (10 mg/kg per day, 7 days) on the number of BrdU-positive cells in the sub-ventricular zone (SVZ) and the sub-granular zone (SGZ) of the hippocampus. (a) Photomicrographs (magnification: × 10 and × 50 for the *small rectangles*) representative of the vehicle (CTL), fluoxetine (FLX), and SB-269970 (SB) groups. Please note the clusters of positive cells found in the presence of SB-269970 (*small rectangles*). (b, c, d) show the summary (mean ± SEM) of the effects of SB-269970 and fluoxetine in the sub-ventricular zone, the SGZ of the hippocampus and the SGZ of 5-HT depleted rats with PCPA, respectively. The quantification of BrdU-labeled cells was performed 24 h after the last injection of BrdU. GCL, granule cell layer. *$p < 0.05$ *vs* vehicle, PLSD Fisher's test.

with SSRIs are already observed after only 7 days of treatment with the 5-HT$_7$ receptor antagonist SB-269970.

The first results showed that SB-269970 reduced immobility in the FST without modifying locomotor activity. These data confirm previous results showing an antidepressant-like action of SB-269970 in the same test, with doses ranging from 1.25 to 2.5 mg/kg (Wesołowska and Kowalska, 2008). This group reported that an intrahippocampal injection of SB-269970 also reduces immobility time, suggesting that the hippocampus is one of the brain structures involved in the beneficial action of 5-HT$_7$ antagonism (Wesolowska *et al*, 2006). It has also been reported that both pharmacological inhibition and genetic inactivation of 5-HT$_7$ receptors induce antidepressant-like behavior in the mouse tail suspension test (Hedlund *et al*, 2005; Bonaventure *et al*, 2007; Sarkisyan *et al*, 2010), as well as an antianxiety effect in the Vogel drinking test in rats, the elevated plus maze in rats, and in the four-plate test in mice (Wesolowska *et al*, 2006). Interestingly, a dose of 2 mg/kg of SB-269970 had no detectable effect in the present study by itself, suggesting a lack of modification of 5-HT release as previously shown (Bonaventure *et al*, 2007), it counteracted the anxiogenic-like effect of acute administration of fluoxetine in the illuminated open field (Figure 1). Previous reports have shown that the SSRIs fluoxetine and citalopram display anxiogenic behaviors via the activation of 5-HT$_{2C}$ receptors as their effects were prevented by a 5-HT$_{2C}$ antagonist (Burghardt *et al*, 2007; Dekeyne *et al*, 2000; Greenwood *et al*, 2008). Hence, it can be suggested that an enhanced activation of 5-HT$_{2C}$, and also of 5-HT$_7$, receptors might be a mechanism for the anxiogenic effects of SSRIs observed initially during treatment, based on the present results. These findings are of particular interest as several atypical antipsychotics, such as amisulpride and aripiprazole, which are potent 5-HT$_7$ antagonist, are used in the treatment of mood disorders (Smeraldi, 1998; Montgomery, 2002; Na *et al*, 2008: Berman *et al*, 2009). Additionally, Abbas *et al*. (2009) demonstrated that, in contrast to their wild-type littermates, 5-HT$_7$ receptor knock-out mice did not respond to amisulpride in the FST and the tail suspension test. This indicates that 5-HT$_7$ receptor antagonism may underly, at least in part, the antidepressant-like actions of antipsychotics such as amisulpride and aripiprazole (Abbas *et al*, 2009; Sarkisyan *et al*, 2010). Taken together, these data suggest that 5-HT$_7$ receptor antagonists are of potential interest for the treatment of both depression and anxiety.

Another acute action of 5-HT$_7$ receptor manipulation was tested in the 5-HTP-induced HTR paradigm. The increase of monoamine contents in brain, especially 5-HT, is known to cause several abnormal behaviors in animals, such as HTR and head-weaving (Kim *et al*, 1998). It is also reported that HTR is induced by 5-HT$_{2A}$ receptor stimulation in the prefrontal cortex (Willins and Meltzer, 1997; González-Maeso *et al*, 2003; Nakagawasai *et al*, 2004; Moya *et al*, 2007). It was documented for the first time that 5-HT$_7$ receptor stimulation is also able to produce HTR (Figure 2). This effect was, however, smaller in amplitude than that

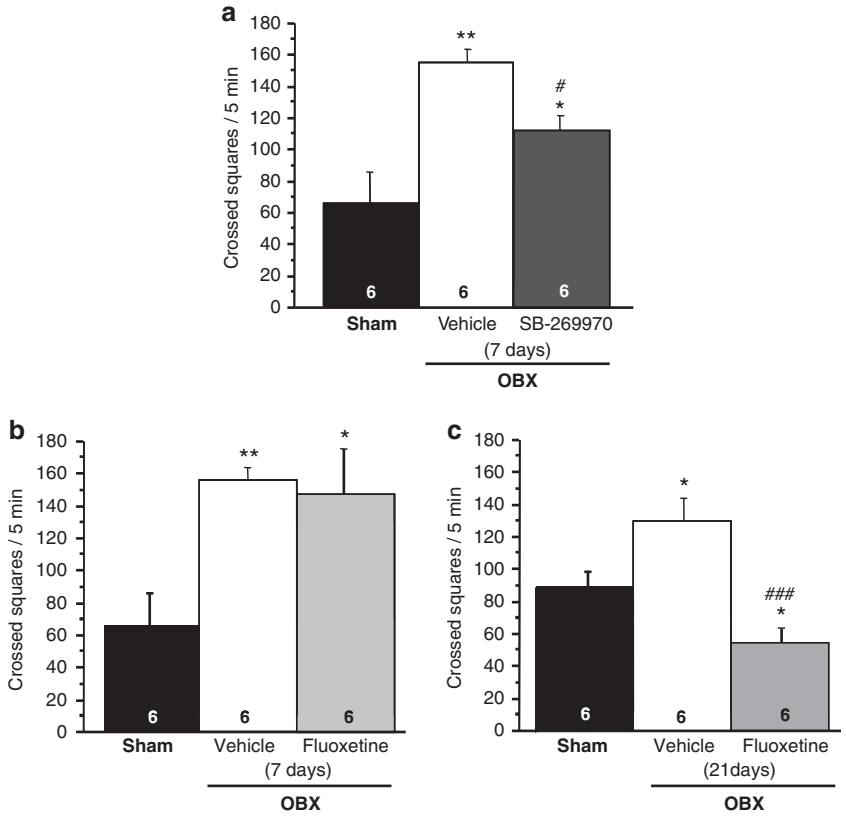

Figure 6 Effect of SB-269970 (2 mg/kg per day i.p., 7 days) (a) and fluoxetine (10 mg/kg per day i.p.) 7 (b) or 21 days (c) on the locomotor activity of olfactory bulbectomized (OBX) rats. The OBX surgery was performed (see Materials and Methods section for more details), and rats were allowed to recover for a period of 14 days. SB-269970 and fluoxetine were then administered and locomotion was measured in an open-field apparatus after 7 and 21 days of drug treatment. Results represent the mean (±SEM) values of six animals per group. $^{*}p<0.05$, $^{**}p<0.01$ vs sham, $^{\#}p<0.05$, $^{\#\#\#}p<0.001$ *vs* OBX-Vehicle; PLSD Fisher's test.

produced by fluoxetine, suggesting that the increase of cerebral 5-HT level induced by fluoxetine and 5-HTP activates a larger number of 5-HT receptors to induce HTR. Similarly, several studies have previously shown that ADs including SSRIs (citalopram, escitalopram, and fluoxetine) and monoamine oxidase inhibitors (tranylcypromine, pargyline) potentiate the 5-HTP-induced behavioral syndrome (Ortmann *et al*, 1980; Shimomura *et al*, 1981; Hyttel *et al*, 1992; Stórustovu *et al*, 2004; Sánchez and Kreilgaard, 2004). Notably, the 5-HT$_7$ receptor antagonist SB-269970 did not modify the 5-HTP-induced HTR but prevented both the AS19- and fluoxetine-stimulating effects. Interestingly, various drugs with 5-HT$_7$ antagonistic activity, including atypical antipsychotics olanzapine and risperidone, antagonize HTR induced by 5-HTP or tryptamine (Kitaichi *et al*, 1994; Fu *et al*, 2000; Van Oekelen *et al*, 2002).

The present study also showed that raphe-hippocampus 5-HT transmission can be markedly modified by 5-HT$_7$ receptor blockade. Indeed, acute activation of 5-HT$_7$ receptors by AS19 suppressed the firing activity of all 5-HT neurons recorded in DRN and SB-269970 prevented this effect (Figure 3), suggesting that 5-HT firing activity is under negative 5-HT$_7$ receptor control. This uniform responsiveness of 5-HT neurons stands in contrast with the observation that 5-HT$_4$ receptor agonists, which have been proposed to be fast acting ADs, activate only a subpopulation of 5-HT neurons (Lucas *et al*, 2007). In addition, the present data showed that the 5-HT$_7$ receptor antagonists SB-269970 and DR-4365 prevented the inhibition of DRN 5-HT firing activity caused by the SSRIs paroxetine and fluvoxamine, respectively (see Supplementary Figure S1 in the Supplementary Material). Such a result fits well with the potentiating action of SB-269970 on 5-HT release, the antidepressant-like behavior, and REM sleep suppression induced by the SSRI citalopram (Bonaventure *et al*, 2007). The control of DRN neuronal activity by 5-HT$_7$ receptors seems to be indirect and it has been suggested that 5-HT$_7$ receptors are not been localized directly on 5-HT neurons but rather on GABAergic and/or glutamatergic neurons (Harsing *et al*, 2004; Monti *et al*, 2008). On the other hand, in rats chronically treated with the SSRI fluoxetine, the selective 5-HT$_7$ agonist AS-19 no longer suppressed 5-HT firing activity, suggesting a desensitization of 5-HT$_7$ receptors. As previously shown (Czachura and Rasmussen, 2000), a 7-day treatment with fluoxetine decreases DRN 5-HT firing activity, in agreement with previous studies using different SSRIs (Chaput *et al*, 1986; de Montigny *et al*, 1990). This decrease is attributable to an increased level of extracellular 5-HT (Bel and Artigas, 1992; Yoshioka *et al*, 1995; Bonaventure *et al*, 2007) that leads to a greater activation of 5-HT$_{1A}$ autoreceptors (Blier *et al*, 1984; Chaput *et al*, 1986; Le Poul *et al*, 1995; Haddjeri *et al*, 1998a). A full recovery is usually reported after about 2 or 3 weeks treatment with classical SSRIs (Chaput *et al*, 1986; de Montigny *et al*, 1990; Czachura and Rasmussen, 2000), a time course consistent with their delayed therapeutic

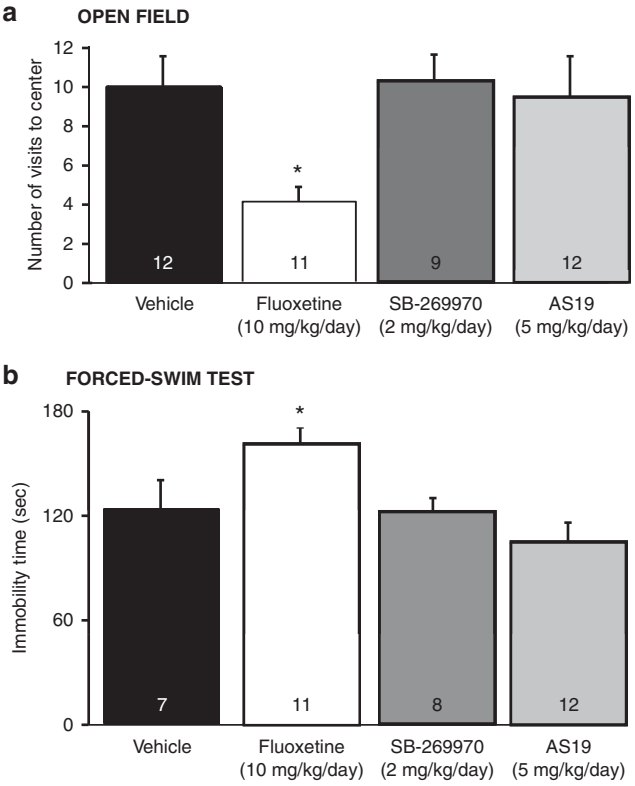

Figure 7 Effect of early life repeated administrations of fluoxetine, SB-269970, and AS19 on the anxiety- and depressive-like behaviors in adulthood. (a) Compared with vehicle-treated animals, rats neonatally treated with the SSRI fluoxetine (10 mg/kg per day for 14 days, i.p.) displayed lower number of visits to the center in the open-field apparatus whereas no difference was observed after a neonatal treatment with SB-269970 (2 mg/kg per day for 14 days, i.p.), or with AS19 (5 mg/kg per day for 14 days, i.p.). (b) In the FST, the immobility duration was significantly enhanced in fluoxetine-exposed rats during the neonatal period, but not in rats administered with SB-269970 or AS19. *$p<0.05$ *vs* vehicle, PLSD Fisher's test.

effect (Blier and de Montigny, 1999; Lanfumey and Hamon, 2004). The recovery of the firing rate is believed to be due to a desensitization of the somatodendritic 5-HT$_{1A}$ autoreceptors of 5-HT neurons in DRN (Blier and de Montigny, 1994). Importantly, 5-HT firing activity was not modified after 7 days of SB-269970 administration, but acute injection of paroxetine failed to induce a complete suppression of the 5-HT firing activity, indicating a desensitization of these 5-HT$_{1A}$ autoreceptors. Hence, a reduced 5-HT$_{1A}$ and/or 5-HT$_7$ receptor responsiveness in the DRN after 5-HT$_7$ receptor blockade takes place in a faster manner than most ADs. This desensitization may be secondary to an early increase of the extracellular 5-HT levels after only a 7-day treatment with SB-269970. This would be consistent with an enhancement of 5-HT transmission in projection areas such as the dorsal hippocampus after 1 week of SB 269970 administration. In fact, this enhancement was shown by the manifestation of an enhanced inhibitory tone on post-synaptic 5-HT$_{1A}$ receptors, as the selective antagonist WAY 100 635 was able to increase CA3 pyramidal neuron activity (Figure 4). Such an effect was reported using classical ADs after 2 to 3 weeks of treatment (Haddjeri *et al*, 1998b; Besson *et al*, 2000; El Mansari *et al*, 2005, Lucas *et al*, 2007). However, it was observed that fluoxetine was also effective after a 1-week treatment. Hence, a sub-acute treatment with this SSRI may be sufficient to induce a disinhibition of CA3 pyramidal neurons in dorsal hippocampus, but not a recovery of 5-HT neuronal activity in DRN. Then, both desensitization of 5-HT$_{1A}$ autoreceptors and increased tonus on hippocampal postsynaptic 5-HT receptors may be necessary to induce detectable AD effects.

The current findings on 5-HT$_{1A}$ neurotransmission are in line with the fact that a 1-week treatment with SB-269970, but not fluoxetine, promoted specifically the proliferation rate in the DG of hippocampus (Figure 5). This result brings additional support to the idea that 5-HT$_7$ receptor antagonists display a fast antidepressant-like profile. In fact, such progenic effect was previously observed after only long-term (2 to 3 weeks) treatment with various ADs including fluoxetine (Malberg *et al*, 2000; Santarelli *et al*, 2003; Malberg and Duman, 2003; Mnie-Filali *et al*, 2007b). To our knowledge, only non-selective 5-HT$_7$ receptors antagonists (such as atypical neuroleptics risperidone or clozapine) were used to assess the effects on hippocampal cell proliferation but the results were not conclusive (Halim *et al*, 2004; Kodama *et al*, 2004; Schmitt *et al*, 2004). Also, Sarkisyan and Hedlund (2009) reported that the rate of cell proliferation in the DG was identical in 5-HT$_7$ receptor wild-type and knock-out mice even if such mice present antidepressant-like phenotypes (Guscott *et al*, 2005; Hedlund *et al*, 2005). As it has been recently proposed (Bessa *et al*, 2009), neuronal plasticity (dendritic remodeling and synaptic contacts) in the hippocampus, rather than neurogenesis itself, could be responsible for the latter behavioral phenotypes of 5-HT$_7$ mutant mice. Interestingly, the stimulation of hippocampal cell proliferation induced by SB-269970 was prevented by PCPA exposure, in line with previous studies showing a robust decline in hippocampal proliferation rate following PCPA exposure (Banasr *et al*, 2001; Jha *et al*, 2006). Hence, the present results suggest that the stimulating effect of 5-HT$_7$ receptor antagonism on cell proliferation in hippocampus necessitates an intact 5-HT system, and is not mediated by a direct action of SB-269970.

As previously discussed, SB-269970 produced an AD effect in the FST. Despite the fact that FST is a useful, high-throughput, and reliable test, classical ADs such as fluoxetine produce a response after only 2 days of treatment, which is inconsistent with their delayed therapeutic action. It is therefore difficult to determine if SB-269970 has a more rapid onset of action in comparison with these ADs based on this test. To address this issue, we used OBX rats, which require long-term (2–3 weeks) treatment to observe an AD-like response (McGrath and Norman, 1998; Song and Leonard, 2005). An exaggeration of locomotor activity in OBX animals was observed compared with sham rats when placed in a high illuminated open-field (Figure 6). This is consistent with previous reports and is believed to be due to a deficit of habituation to a new stressful environment (Cairncross *et al*. 1979; Van Riezen and Leonard, 1990; McNish and Davis, 1997; Song and Leonard, 2005; Romeas *et al*, 2009). Importantly, although fluoxetine was ineffective after a 7-day treatment (as expected for an SSRI; Lucas *et al*, 2007; Breuer *et al*, 2007), SB-269970 reversed the OBX-induced hyperactivity. This is the first report of a rapid effect of 5-HT$_7$ receptor antagonist in a 'chronic' behavioral

model of depression. The faster effect of SB-269970 in the OBX paradigm is an important indication of the 5-HT$_7$ receptor antagonists as putative fast-acting ADs.

Finally, the profile of 5-HT$_7$ receptor antagonists was tested on neonatal AD exposure paradigm. Neonatal administration of ADs (such as clomipramine, citalopram, or fluoxetine) to rodents during the early life period (from PN8 to PN21) was shown to produce a constellation of maladaptive behaviors, possibly related to anxiety and depression, that persist in adulthood (Vogel *et al*, 1988; Andersen *et al*, 2002; Ansorge *et al*, 2004; Maciag *et al*, 2006; Popa *et al*, 2008). In this study, postnatal exposure to the SSRI fluoxetine significantly decreased visits to an open-field center in adulthood while increasing total immobility time in the FST, in agreement with previous reports (Ansorge *et al*, 2004; Karpova *et al*, 2009; Popa *et al*, 2010). 5-HT$_{1A}$ receptors have been proposed to have a crucial role in the mediation of such a deleterious action of SERT blockade (Alexandre *et al*, 2006; Popa *et al*, 2008). The use of preferential 5-HT receptor agonists in this paradigm also indicated the involvement of other 5-HT receptor subtypes. Indeed, early life stimulation of 5-HT$_{1B}$, 5-HT$_{2A}$, 5-HT$_{2C}$, and 5-HT$_4$ receptors induced a significant decrease in the number of visits in the open-field center (see Supplementary Figure S2 in the Supplementary Material). In contrast, such behavioral disturbances were not observed after a neonatal administration of AS19 or SB-269970.

Overall, the multiple experimental approaches used herein provided important support for the hypothesis that 5-HT$_7$ receptor antagonists may act as AD agents with a rapid onset of action. Thus far, only one 5-HT$_7$ receptor antagonist has been assessed in a clinical trial for moderate to severe depression (JNJ-18038683; Johnson and Johnson Pharmaceutical Research and Development). It is hoped that the present results will further stimulate the development of selective 5-HT$_7$ receptor antagonists as a novel and potentially improved class of ADs.

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DISCLOSURE

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)