Neuropsychopharmacology (2006) 31, 2162–2172

www.neuropsychopharmacology.org

Blockade of 5-HT_{1A} Receptors by (±)-Pindolol Potentiates Cortical 5-HT Outflow, but not Antidepressant-Like Activity of Paroxetine: Microdialysis and Behavioral Approaches in 5-HT_{1A} Receptor Knockout Mice

Jean-Philippe Guilloux[1], Denis JP David[1], Bruno P Guiard[1], Franck Chenu[2], Christelle Repérant[1], Miklos Toth[3], Michel Bourin[2] and Alain M Gardier*[,1]

[1]Laboratoire de Neuropharmacologie EA 3544, Faculté de Pharmacie, Université Paris-Sud, Châtenay-Malabry Cedex, France; [2]Laboratoire Neurobiologie de l'anxiété et de la dépression EA3256, Faculté de Médecine, Université de Nantes, Nantes, France; [3]Department of Pharmacology, Weill Medical College of Cornell University, New York, NY, USA

Selective serotonin reuptake inhibitors like paroxetine (Prx) often requires 4–6 weeks to achieve clinical benefits in depressed patients. Pindolol shortens this delay and it has been suggested that this effect is mediated by somatodendritic 5-hydroxytryptamine (5-HT) 1A autoreceptors. However clinical data on the beneficial effects of pindolol are conflicting. To study the effects of (±)-pindolol–paroxetine administration, we used genetical and pharmacological approaches in 5-HT_{1A} knockout mice (5-HT_{1A}−/−). Two assays, *in vivo* intracerebral microdialysis in awake mice and the forced swimming test (FST), were used to assess the antidepressant-like effects of this drug combination. Basal levels of extracellular serotonin, 5-HT ($[5\text{-HT}]_{ext}$) in the frontal cortex (FCX) and the dorsal raphe nucleus (DRN) did not differ between the two strains of mice, suggesting a lack of tonic control of 5-HT_{1A} autoreceptors on nerve terminal 5-HT release. Prx (1 and 4 mg/kg) dose-dependently increased cortical $[5\text{-HT}]_{ext}$ in both genotypes, but the effects were greater in mutants. The selective 5-HT_{1A} receptor antagonist, WAY-100635 (0.5 mg/kg), or (±)-pindolol (5 and 10 mg/kg) potentiated the effects of Prx (4 mg/kg) on cortical $[5\text{-HT}]_{ext}$ in 5-HT_{1A} +/+, but not in 5-HT_{1A}−/− mice. Similar responses were obtained following local intra-raphe perfusion by reverse microdialysis of either WAY-100635 or (±)-pindolol (100 μM each). In the FST, Prx administration dose-dependently decreased the immobility time in both strains of mice, but the response was much greater in 5HT_{1A}−/− mice. In contrast, (±)-pindolol blocked Prx-induced decreases in the immobility time while WAY-100635 had no effect in both genotypes. These findings using 5-HT_{1A}−/− mice confirm that (±)-pindolol behaves as an antagonist of 5-HT_{1A} autoreceptor in mice, but its blockade of paroxetine-induced antidepressant-like effects in the FST may be due to its binding to other neurotransmitter receptors.
Neuropsychopharmacology (2006) **31,** 2162–2172. doi:10.1038/sj.npp.1301019; published online 25 January 2006

Keywords: microdialysis; 5-HT_{1A} receptor antagonist; (±)-pindolol; selective serotonin reuptake inhibitor; forced swimming test; dorsal raphe nucleus

INTRODUCTION

The clinical benefits of selective serotonin (5-HT) reuptake inhibitors (SSRI), like paroxetine are only evident after 4–6 weeks of treatment (Blier and de Montigny, 1994). One possible explanation for the long delay of action could be a negative feedback control exerted by 5-HT_{1A} autoreceptors on nerve terminal 5-HT release (Artigas *et al*, 1996). Although this initially blunts the effects of SSRI, 5-HT_{1A} autoreceptors are gradually desensitized during chronic SSRI administration allowing the development of the antidepressant effect (Blier *et al*, 1987; Invernizzi *et al*, 1996). This notion led clinicians to propose a pharmacological strategy to accelerate the antidepressant response by blocking the action of presynaptic 5-HT_{1A} receptors during SSRI administration. (±)-Pindolol is a β_{1-2} adrenergic receptor antagonist with a putative antagonistic action on 5-HT_{1A} receptors (Newman-Tancredi *et al*, 1998; Castro *et al*, 2000), with a greater occupation at somatodendritic 5-HT_{1A} receptors than at post synaptic receptors (Martinez *et al*, 2000, 2001). Several open clinical studies have shown a

*Correspondence: Dr AM Gardier, Laboratoire de Neuropharmacologie EA 3544, Tour D1, 2ème étage, Faculté de Pharmacie, Université Paris-Sud, 5 rue J-B. Clément, 92296 Châtenay-Malabry Cedex, France, Tel: +33 1 46 83 54 16, Fax: +33 1 46 83 53 55,
E-mail: alain.gardier@cep.u-psud.fr
Received 28 January 2005; revised 22 September 2005; accepted 28 November 2005
Online publication: 29 November 2005 at http://www.acnp.org/citations/Npp112905050068/default.pdf

faster onset of antidepressant effects of SSRIs when combined with (±)-pindolol in depressed patients (Artigas *et al*, 1994; Blier and Bergeron, 1995). Nevertheless, the recent meta-analysis of Ballesteros and Callado (2004), pooling nine randomized controlled trials, has come to a different conclusion, as that the efficacy of pindolol + SSRI in depression is restricted to approximately the first 2 weeks of treatment, period needed for the desensitization of 5-HT$_{1A}$ receptors.

Preclinical studies in rats showed contradictory results. Except the study of Romero *et al* (1996), electrophysiological experiments failed to demonstrate the ability of (±)-pindolol to block the inhibitory effects of SSRI on the firing of 5-HT neurons in the dorsal raphe nucleus (DRN) (Fornal *et al*, 1999; Sprouse *et al*, 2000), whereas microdialysis experiments almost always show a potentiation of SSRI-induced 5-HT release in various brain areas (Hjorth and Auerbach, 1994; Hjorth, 1996; Dawson and Nguyen, 2000).

Owing to the conflicting clinical and preclinical results, we decided to address the question of the mechanism of action of pindolol and to study the effects of an optimal combination of (±)-pindolol and paroxetine by using genetically modified, 5-HT$_{1A}$−/− mice (5-HT$_{1A}$−/−, for a review, see Toth, 2003) to study the effects of an optimal combination of (±)-pindolol and paroxetine, which seem to be the more efficient (Plenge and Mellerup, 2003). We also studied the selectivity of (±)-pindolol by using reverse microdialysis to perfuse this compound directly in the DRN. To assess the neurochemical actions of (±)-pindolol at presynaptic 5-HT$_{1A}$ receptors, we performed a series of *in vivo* microdialysis experiments in both the DRN and frontal cortex (FCX). We also tested the effect of (±)-pindolol in combination with paroxetine in a behavioural model, the forced swimming test (FST), a useful model that predicts the antidepressant-like activity of new compounds with a good reliability and predictive validity for the screening of antidepressant drugs (Petit-Demouliere *et al*, 2005).

MATERIALS AND METHODS

Animals

Male C57Bl/6 wild-type (5-HT$_{1A}$+/+) and homozygous 5-HT$_{1A}$ receptor knockout mice (5-HT$_{1A}$−/−) (Parks *et al*, 1998), 6–8 weeks old, weighing 24–32 g, were used in all experiments. Mice maintained at Weill Medical College of Cornell University were transferred to our laboratory in order to grow a stable colony in the animal facility of the Faculté de Pharmacie, University of Paris XI, France. Experimental animals were housed in the animal care facility in groups of 4–6 and kept under standard conditions. Procedures were conducted in conformity with the institutional guidelines that are in compliance with national and policy (Council directive # 87–848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale, permissions # 92–196 to AM Gardier).

Chemicals and Drugs

Racemic 8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT) and *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide (WAY-100635) were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). The SSRI, paroxetine hydrochloride was a gift from GlaxoSmithKline Laboratory (Harlow, UK), (±)-pindolol base from Novartis laboratory (Rueil-Malmaison, France), and citalopram hydrobromide from Lundbeck laboratory (Copenhagen, Denmark). All chemical compounds except (±)-pindolol were dissolved in distilled water. (±)-Pindolol was dissolved in Tween 20% for systemic administration. Paroxetine was administered by intraperitoneally (i.p.) and the 5-HT$_{1A}$ receptor agonists and antagonists were administered subcuteanously (s.c.) or perfused locally. Control animals were injected using the appropriate vehicle and the same administration route. For their local perfusion into the DRN or the FCX, WAY-100635 (100 μM) and citalopram (1 μM) were dissolved in artificial cerebrospinal fluid (aCSF) and perfused at a flow rate of 0.5 and 1.5 μl/min, respectively; (±)-pindolol (100 μM) was first dissolved in perchloric acid (1%) and then diluted in aCSF and perfused at 0.5 μl/min.

Microdialysis Procedure

Concentric dialysis probes were made of cuprophan fibers and constructed as previously described (Malagie *et al*, 2001; Guiard *et al*, 2004). All probes present an active length of 2 and 5 mm for the FCX and DRN, respectively (×0.30 mm outer diameter). Animals were anaesthetized with chloral hydrate (400 mg/kg, i.p.) and were implanted with a probe, cemented in place in the FCX and/or in the DRN, based on coordinates taken from the mouse brain atlas (Franklin and Paxinos, 1997) (coordinates: from bregma (in mm) FCX, $A = +1.6$, $L = +1.3$, $V = -1.6$; DRN, $A = -4.5$, $L = 0$, $V = -4.0$ (*A*, anterior; *L*, lateral; and *V*, ventral)). Animals were allowed to recover from the surgery overnight. The next day, ≈20 h after surgery, the probes were continuously perfused with an artificial cerebrospinal fluid (composition in mM: NaCl 147, KCl 3.5, $CaCl_2$ 1.26, $MgCl_2$ 1.2, NaH_2PO_4 1.0, $NaHCO_3$ 25.0, pH 7.4±0.2) at a flow rate of 1.5 μl/min in the FCX and 0.5 μl/min in the DRN using a CMA/100 pump (Carnegie Medicin, Stockholm, Sweden). Dialysate samples were collected every 15 min in the FCX and every 30 min in the DRN in tubes and were analyzed for 5-HT by a high-performance liquid chromatography apparatus (XL-ODS, 4.6 × 7.0 mm, particle size 3 m; Beckman) coupled to an amperometric detector (1049A, Hewlett-Packard, Les Ulis, France) (Malagie *et al*, 2001). Usually four fractions were collected to obtain basal values (means±SEM) before drug administration. The limit of sensitivity for 5-HT was ≈0.5 fmol/sample (signal-to-noise ratio = 2). At the end of the experiments, localisation of microdialysis probes was verified histologically (Figure 1).

Histological Verification of Microdialysis Probes' Implantation

The placement of microdialysis probes was verified using adaptation of the technique described previously (Bert *et al*, 2004). After the microdialysis study, mice were killed by cervical dislocation, brains were removed

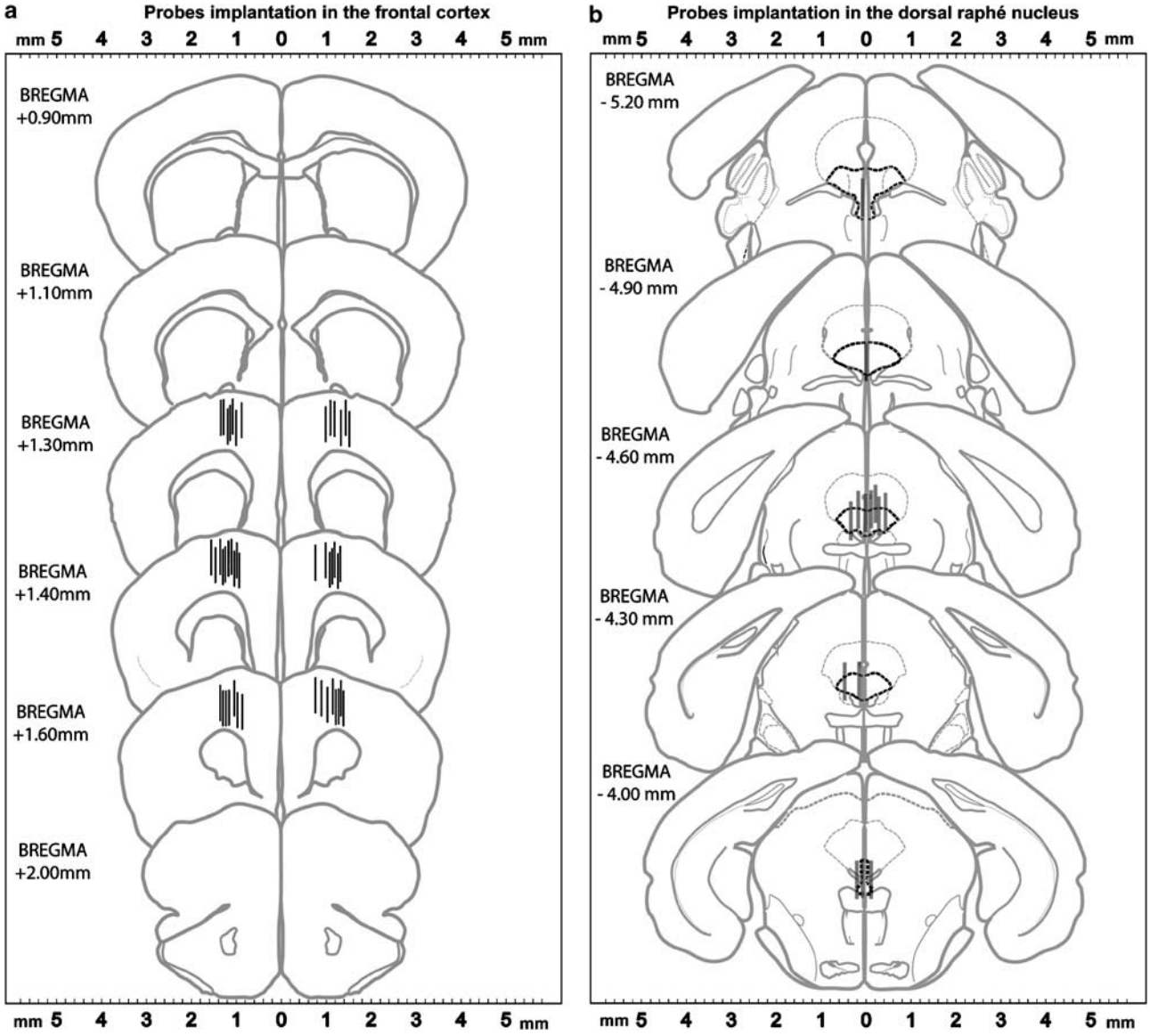

Figure 1 Histological verification of microdialysis probes' implantation. Coronal sections drawings of a C57BL/6 mouse brain showing the location of the concentric microdialysis probes in the frontal cortex (a) and dorsal raphe nucleus (b) according to Hof *et al* 'Mouse brains' atlas (2000). (a) The black line indicates the brain zone of dialysis of the membrane. The probes were implanted with the following stereotaxic coordinates (coordinates for bregma in mm) in the FCX: AP + 1.6, *L* ± 1.3, *V* −1.6. (b) The dotted line indicates the location of the dorsal raphe nucles (DRN). The black continuous line indicates the zone of dialysis of the membrane. The probes were implanted with the following stereotaxic coordinates in the DRN: AP −4.5, *L* 0, *V* −4.

and stored at +4°C in Formalin 2%. To determine the exact probe implantation in the FCX or DRN, brains were placed in a Kryomat apparatus and kept at −25°C. Brain regions were identified by using the 'Mouse brains' atlas (Hof *et al*, 2000) and coronal frozen sections of brain were sliced serially at 50 μm intervals. Slices were prepared from AP +2 to +1 mm and AP −4 to −5 mm for the FCX and DRN, respectively. Each slice was photographed using a digital camera (C-4000 Zoom, Olympus) and the appropriate placement of the probe estimated in comparison to the corresponding slices obtained by the 'Mouse brains' atlas software (Hof *et al*, 2000). Only mice with probes confined to either the FCX or the DRN were used for subsequent data analysis and a cartography of probes implantation in the DRN was performed (Figure 1).

The Mouse FST

The FST was applied as described by Porsolt *et al* (1977): mice were dropped individually into glass cylinders (height: 25 cm, diameter: 10 cm) containing 10 cm water height, maintained at 23–25°C. Animals were tested for a total of 6 min. Two mice were tested simultaneously and the time of immobility was recorded during the last 4 min of the 6-min testing period, after 2 min of habituation. The test was performed by the same well-trained experimenter, who was unaware of the treatment administered.

Data Analysis and Statistics

Statistical analysis was performed using the computer software StatView 5.0. (Abacus Concepts, Inc, Berkely, CA,

Table 1 Basal 5-HT Levels in the Frontal Cortex (FCX) and in the Dorsal Raphe Nucleus (DRN) in 5-HT$_{1A}$+/+ and 5-HT$_{1A}$−/− Mice

	5-HT$_{1A}$ +/+ mice	5-HT$_{1A}$−/− mice
FCX (fmol/20 µl)	4.30 (±0.19; $n=122$)	4.35 (±0.15; $n=132$)
DRN (fmol/10 µl)	23.70 (±4.00; $n=46$)	23.95 (±3.12; $n=25$)

No Statistical differences for the 5-HT basal levels were observed between both genotype in the FCX or the DRN. Mean ± SEM, n = number of determinations.

USA). Dialysate 5-HT levels were calculated as the amount of 5-HT outflow collected during the post-treatment period from the FCX or the DRN, and expressed as a percentage of basal values. Statistical analyses were realized on the area under the curve (AUC; mean ± SEM) values for the amount of 5-HT outflow collected during the post-treatment period. To compare different AUC values in each group of treated animals, statistical analysis was performed using a two-way ANOVA with drug treatment and genotype as main factors, followed by Fisher Protected Least Significance Difference *post hoc* test when appropriate. Significant level was set at $p<0.05$.

In the FST, data collected were expressed as a mean of immobility time (in seconds ± SEM). A two-way ANOVA analysis with treatment and genotype as main factors followed by Fisher Protected Least Significance Difference *post hoc* test was performed to compare immobility time values.

RESULTS

Basal Extracellular Levels of 5-HT in the FCX and Dorsal Raphe in 5-HT$_{1A}$ +/+ and 5-HT$_{1A}$−/− Mice

Table 1 shows the mean ± SEM of basal [5-HT]$_{ext}$ levels in the FCX (in fmol/20 µl) and DRN (in fmol/10 µl) of the various groups of mice studied. Basal extracellular 5-HT levels were not different between 5-HT$_{1A}$ +/+ and 5-HT$_{1A}$−/− mice either in the FCX ($F(1,252)=0.47$, $p=0.82$) or in the DRN ($F(1,69)=0.01$, $p=0.97$).

Effects of Systemic Administration of 8-OH-DPAT on Cortical Dialysate 5-HT in 5-HT$_{1A}$ +/+ and 5-HT$_{1A}$−/− Mice

Two-way ANOVA (treatment × genotype) of 5-HT outflow, measured as AUC calculated during a 60 min post-treatment period revealed a significant main effect of genotype factor ($F(1,34)=5.06$; $p<0.05$), treatment ($F(1,34)=5.83$; $p<0.05$), and treatment × genotype interaction ($F(1,34)=12.45$; $p<0.01$) (Figure 2). Specifically, the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT (0.5 mg/kg, s.c.) decreased cortical [5-HT]$_{ext}$ in 5-HT$_{1A}$ +/+ mice by about 38% ($p<0.01$), but caused no change in 5-HT$_{1A}$−/− mice (Figure 2c) ($p=0.34$).

Effects of Local Intra-Raphe Infusion of (±)-Pindolol Alone on Cortical and DRN [5-HT]$_{ext}$ in the Presence of Citalopram (1 µM) in 5-HT$_{1A}$ +/+ and 5-HT$_{1A}$−/− Mice

Two-way ANOVA (treatment × genotype) of AUC values, however, revealed no significant effect of treatment factor ($F(1,18)=1.76$; $p=0.20$) and genotype factor ($F(1,18)=0.34$; $p=0.57$) indicating that administration of (±)-pindolol (100 µM) in the DRN had no effect either on local (ie DRN) or on cortical dialysate 5-HT levels in both

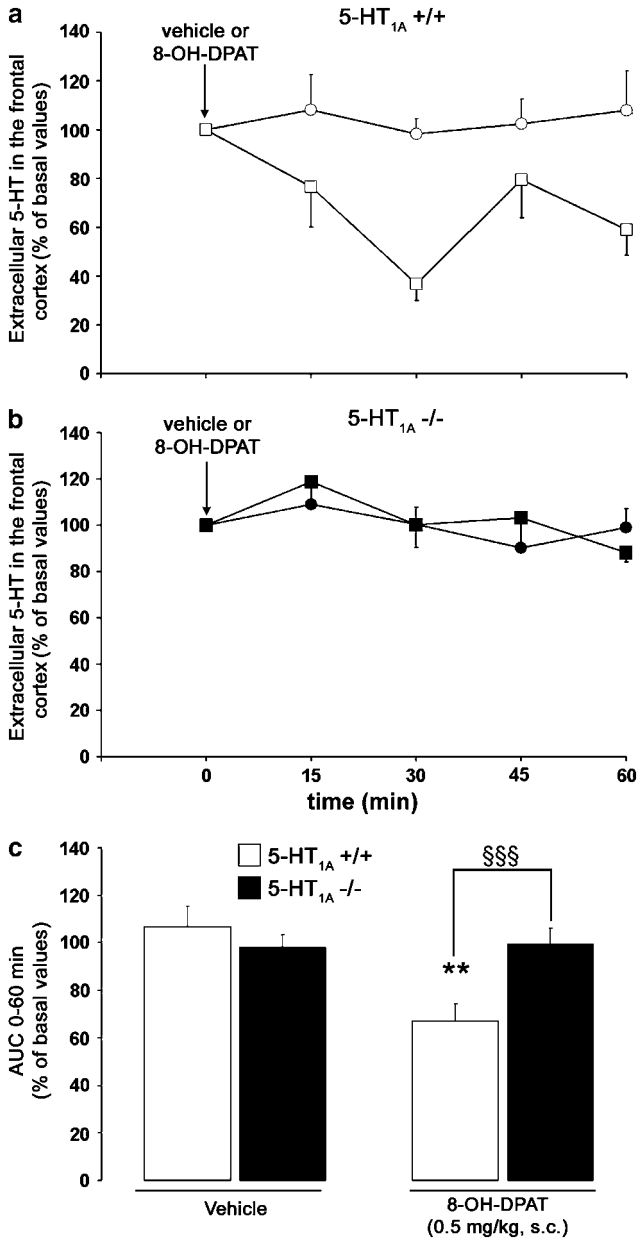

Figure 2 Effects of systemic administration of 8-OH-DPAT on cortical [5-HT]$_{ext}$ in 5-HT$_{1A}$ +/+ and 5-HT$_{1A}$−/− mice. Results are expressed as means ± SEM of cortical [5-HT]$_{ext}$ (percentages of basal values). (a) 5-HT$_{1A}$ +/+ and (b) 5-HT$_{1A}$−/− mice received (arrow) either the vehicle (○, ●) or 8-OH-DPAT (0.5 mg/kg; s.c.) (□, ■). Citalopram 1 µM was perfused by reverse microdialysis in the FCX for the all duration of the experiment. (c) Data are also expressed as area under the curve (AUC; mean ± SEM). AUC values are calculated for the amount of 5-HT outflow measured in the FCX during the 0–60 min post-treatment period with the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT or the vehicle, and expressed as percentages of baseline ($n=7$–13 mice per group) in 5-HT$_{1A}$ +/+ (empty bars) and 5-HT$_{1A}$−/− (full bars) mice. $^{**}p<0.01$ significantly different from the vehicle-treated group; $^{§§§}p<0.001$ and significantly different from 5-HT$_{1A}$ +/+ mice (two-way ANOVA followed by a PLSD *post hoc* *t*-test).

5-HT$_{1A}$+/+ and 5-HT$_{1A}$−/− mice (see Supplementary information, Figure 8).

Effect of Systemic Administration of Paroxetine on Cortical Dialysate 5-HT in 5-HT$_{1A}$+/+ and 5-HT$_{1A}$−/− Mice

Two-way ANOVA (treatment × genotype) on AUC values revealed significant main effect of genotype ($F(1,39) = 14.42$; $p < 0.001$), treatment ($F(2,39) = 11.26$; $p < 0.001$), and treatment × genotype interaction ($F(2,39) = 5.63$; $p < 0.01$) (Figure 3). While both a low (1 mg/kg) and a high (4 mg/kg) paroxetine dose increased dialysate 5-HT levels in 5-HT$_{1A}$−/− mice ($p < 0.05$ and $p < 0.001$ respectively), only the higher paroxetine dose (4 mg/kg) increased $[5\text{-HT}]_{ext}$ in 5-HT$_{1A}$+/+ mice (Figure 3c) ($p < 0.05$).

Antidepressant-Like Effects of Paroxetine on the Immobility Time in the Mouse FST in 5-HT$_{1A}$+/+ and −/− Mice

Next, FST was carried out during the peak of paroxetine effect (30 min after the drug, see gray area in Figure 3) and immobility time was measured in the last 4 min of the 6 min test in both genotypes. Two-way ANOVA (treatment × genotype) on the immobility time revealed significant main effect of genotype ($F(1,88) = 57.17$; $p < 0.001$), treatment ($F(4,88) = 13.69$; $p < 0.001$), and an interaction between these two factors ($F(4,88) = 6.69$; $p < 0.001$). As Figure 4 shows, paroxetine (1–4 mg/kg) had a more robust effect on the behavior of 5-HT$_{1A}$−/− mice ($p < 0.05$, $p < 0.001$, $p < 0.01$ for 1, 2, and 4 mg/kg, respectively).

These data indicate that 5-HT$_{1A}$−/− mice have a dramatically increased sensitivity to the antidepressant-like effects of paroxetine.

Dose–Response Study of the Systemic Administration of (±)-Pindolol on Paroxetine (4 mg/kg)-Induced Increases in Cortical Dialysate 5-HT in 5-HT$_{1A}$+/+ and 5-HT$_{1A}$−/− Mice

Two-way ANOVA (treatment × genotype) on AUC values revealed significant main effect of treatment ($F(5,122) = 5.54$; $p < 0.001$) and genotype ($F(1,122) = 4.04$; $p < 0.05$) (Figure 5). Specifically, (±)-pindolol at 5 and 10 mg/kg s.c. (but not at 1 mg/kg), administered 1 h after paroxetine, potentiated the elevation in $[5\text{-HT}]_{ext}$ when compared to the paroxetine/vehicle group in 5-HT$_{1A}$+/+ mice ($p < 0.01$ for 5 and 10 mg/kg of pindolol). Similarly, the 5-HT$_{1A}$ receptor antagonist WAY-100635 potentiated the effects of paroxetine ($p < 0.001$). In contrast, neither (±)-pindolol nor WAY-100635 potentiated the effect of paroxetine in 5-HT$_{1A}$−/− mice ($p = 0.65$ and $p = 0.76$, respectively). In both genotypes, neither (±)-pindolol nor WAY-100635 have an effect on extracellular 5-HT levels when administered alone (data not shown). These data indicated that the presence of 5-HT$_{1A}$ receptor is essential for the potentiation of paroxetine's effect by either pindolol or WAY-100635 (for the time course study, see Figure 9 in Supplementary information).

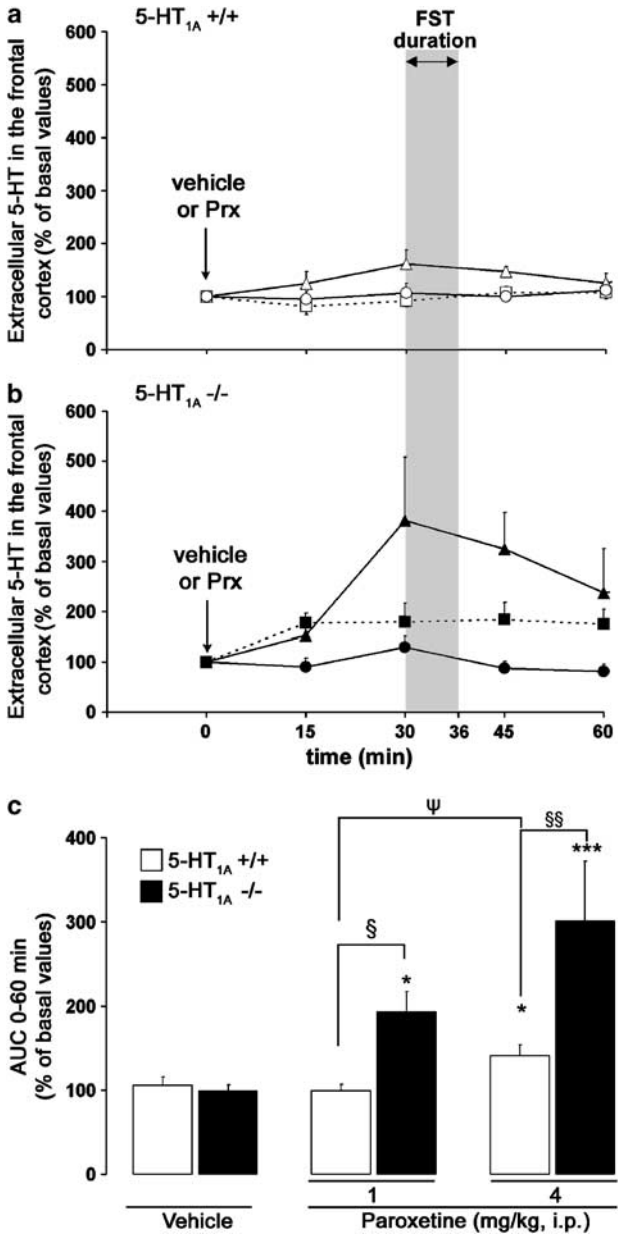

Figure 3 Effect of systemic administration of paroxetine on cortical $[5\text{-HT}]_{ext}$ in 5-HT$_{1A}$+/+ and 5-HT$_{1A}$−/− mice. Results are expressed as means ± SEM of cortical $[5\text{-HT}]_{ext}$ (percentages of basal values). (a) 5-HT$_{1A}$+/+ and (b) 5-HT$_{1A}$−/− mice received (arrow) either the vehicle (○,●), paroxetine (1 mg/kg; s.c.) (□,■), or paroxetine (4 mg/kg; s.c.) (△,▲). (c) Data are also expressed as area under the curve (AUC; mean ± SEM). AUC values are calculated for the amount of 5-HT outflow measured during the 0–60 min post-treatment period with paroxetine or the vehicle and expressed as percentages of baseline ($n = 6$–10 mice per group) in 5-HT$_{1A}$+/+ (empty bars) and 5-HT$_{1A}$−/− (full bars) mice. $^{*}p < 0.05$; $^{***}p < 0.001$ significantly different from the appropriate vehicle-treated group; $^{\S}p < 0.05$; $^{\S\S}p < 0.01$ significantly different from 5-HT$_{1A}$+/+ mice; $^{\Psi}p < 0.05$ significantly different from the paroxetine 1 mg/kg-treated group (two-way ANOVA followed by a PLSD *post hoc t*-test). FST and microdialysis have been performed separately: the gray area indicates the duration time of the FST (ie 6 min). It suggests that microdialysis and behavioral experiments (in the present Figure 3 and in Figure 4, respectively) were carried out by using the same protocol and that FST has been realized at the maximum effect of paroxetine.

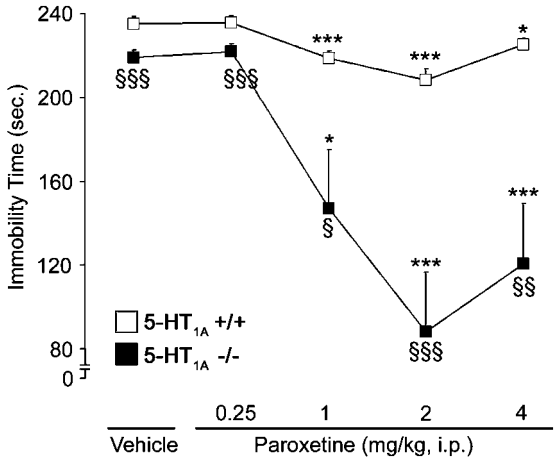

Figure 4 Antidepressant-like effects of paroxetine on the immobility time in the mouse forced swimming test (FST) in 5-HT$_{1A}$+/+ and 5-HT$_{1A}$−/− mice. Results are expressed as means ± SEM of the immobility time (in seconds). 5-HT$_{1A}$+/+ (□) and 5-HT$_{1A}$−/− mice (■) received either the vehicle or paroxetine (0.25, 1, 2, 4 mg/kg, i.p.). Statistical analysis were carried out using a two-way ANOVA followed by Fisher PLSD: *$p<0.05$; ***$p<0.001$ significantly different from the appropriate control group; $^{\S}p<0.05$; $^{\S\S}p<0.01$; $^{\S\S\S}p<0.001$ and significantly different from 5-HT$_{1A}$+/+ mice. Statistical analysis was carried out using a two-way ANOVA followed by Fisher PLSD *post hoc t*-test.

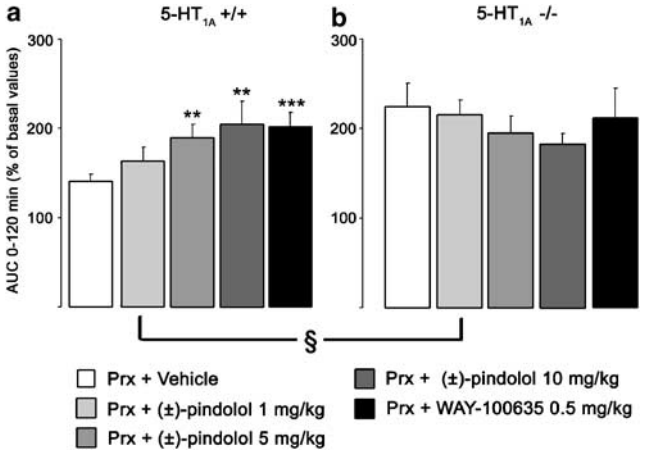

Figure 5 Dose–response study of the systemic administration of (±)-pindolol or WAY-100635 combined with paroxetine (4 mg/kg) on cortical [5-HT]$_{ext}$ in 5-HT$_{1A}$+/+ and 5-HT$_{1A}$−/− mice. Data are area under the curve (AUC; mean ± SEM) values calculated for the amount of 5-HT outflow measured during the 0–120 min post-treatment period with paroxetine or paroxetine coadministered with either pindolol (1, 5 or 10 mg/kg, s.c.) or WAY-100635 and expressed as percentages of basal values ($n=8$–15 mice per group) in 5-HT$_{1A}$+/+ (a) and 5-HT$_{1A}$−/− (b) mice. **$p<0.01$; ***$p<0.001$ significantly different from the paroxetine-treated group; $^{\S}p<0.05$ significantly different from the 5-HT$_{1A}$+/+ mice (two-way ANOVA followed by a PLSD *post hoc t*-test).

Effects of Local Intra-Raphe Infusion of (±)-Pindolol or WAY-100635 Following Systemic Paroxetine Administration on Dialysate 5-HT in the FCX and DRN in 5-HT$_{1A}$+/+ and 5-HT$_{1A}$−/− Mice

FCX. Next, effect of pindolol was studied following local administration to DRN. Two-way ANOVA analysis on AUC values revealed significant main effects of treatment factor ($F(5,61)=8.06$, $p<0.001$), but not genotype factor ($F(1,61)=0.85$; $p=0.36$) (Figure 6a–b).

In 5-HT$_{1A}$+/+ mice, a 1 h intra-raphe perfusion of (±)-pindolol given 1 h after a systemic administration of paroxetine (Figure 6a) potentiated the effects of the SSRI on cortical [5-HT]$_{ext}$ when compared to the paroxetine/aCSF group (AUC values: 146 ± 9 *vs* 258 ± 41% ($p<0.01$) for paroxetine/aCSF *vs* paroxetine/aCSF + (±)-pindolol, respectively).

Similarly, intra-raphe perfusion of WAY-100635 (100 μM) (Figure 6a) potentiated the effects of paroxetine (AUC values: 146 ± 9 *vs* 227 ± 36% ($p<0.05$) for paroxetine/aCSF *vs* paroxetine/aCSF + WAY-100635, respectively).

In 5-HT$_{1A}$−/− mice, neither intra-raphe perfusion of (±)-pindolol nor WAY-100635 potentiated the effects of paroxetine (Figure 6b). AUC values were 275 ± 48% for paroxetine/aCSF and 222 ± 46% ($p=0.31$), 253 ± 14% ($p=0.67$), for paroxetine/aCSF + (±)-pindolol and paroxetine/aCSF + WAY-100635, respectively (for the time course study, see Figure 10 in Supplementary information).

DRN. Two-way ANOVA analysis on AUC values revealed significant main effects of treatment factor ($F(5,70)=11.68$; $p<0.001$) and genotype factor ($F(1,70)=6.86$; $p<0.05$)) (Figure 6c–d).

As we observed in the FCX, the effects of paroxetine (4 mg/kg) were higher in 5-HT$_{1A}$−/− mice than in 5-HT$_{1A}$+/+ mice.

In 5-HT$_{1A}$+/+ mice, intra-raphe perfusion of (±)-pindolol given 1 h after a systemic administration of paroxetine (Figure 6c) potentiated the effects of the SSRI on dialysate 5-HT in the DRN, when compared to the paroxetine/aCSF group (AUC values were 284 ± 38% for the paroxetine/aCSF + (±)-pindolol group ($p<0.05$)).

Local intra-raphe perfusion of WAY-100635 (100 μM) (Figure 6d and f) also potentiated the effects of paroxetine (AUC values were 273 ± 60% for paroxetine/aCSF + WAY-100635 group ($p<0.05$)).

In 5-HT$_{1A}$−/− mice, neither intra-raphe perfusion of (±)-pindolol nor WAY-100635 potentiated the effects of paroxetine (Figure 6d). AUC values were 352 ± 78% for paroxetine/aCSF, 312 ± 39 and 365 ± 117%, for paroxetine/aCSF + (±)-pindolol and paroxetine/aCSF + WAY-100635, respectively ($p=0.70$ and $p=0.17$ for paroxetine/aCSF + (±)-pindolol and paroxetine/aCSF + WAY-100635, respectively) (for the time course study, see Figure 10 in Supplementary information).

Antidepressant-Like Effects of the Co-Administration of Paroxetine and (±)-Pindolol or WAY-100635 in 5-HT$_{1A}$+/+ and 5-HT$_{1A}$−/− Mice in the FST

Two-way ANOVA analysis on immobility time values revealed significant main effect of treatment ($F(5,99)=9.76$; $p<0.001$) and genotype factors ($F(1,99)=4.51$; $p<0.05$) (Figure 7). Neither (±)-pindolol ($p=0.52$ and $p=0.75$ in 5-HT$_{1A}$+/+ and 5-HT$_{1A}$−/− mice, respectively) nor WAY-100635 ($p=0.55$ and $p=0.44$ in 5-HT$_{1A}$+/+ and 5-HT$_{1A}$−/− mice, respectively) alone had an effect on the immobility time when compared to the appropriate vehicle-treated group (see Figure 11 in Supplementary information). Pindolol, but not WAY100635 ($p=0.21$ and $p=0.35$

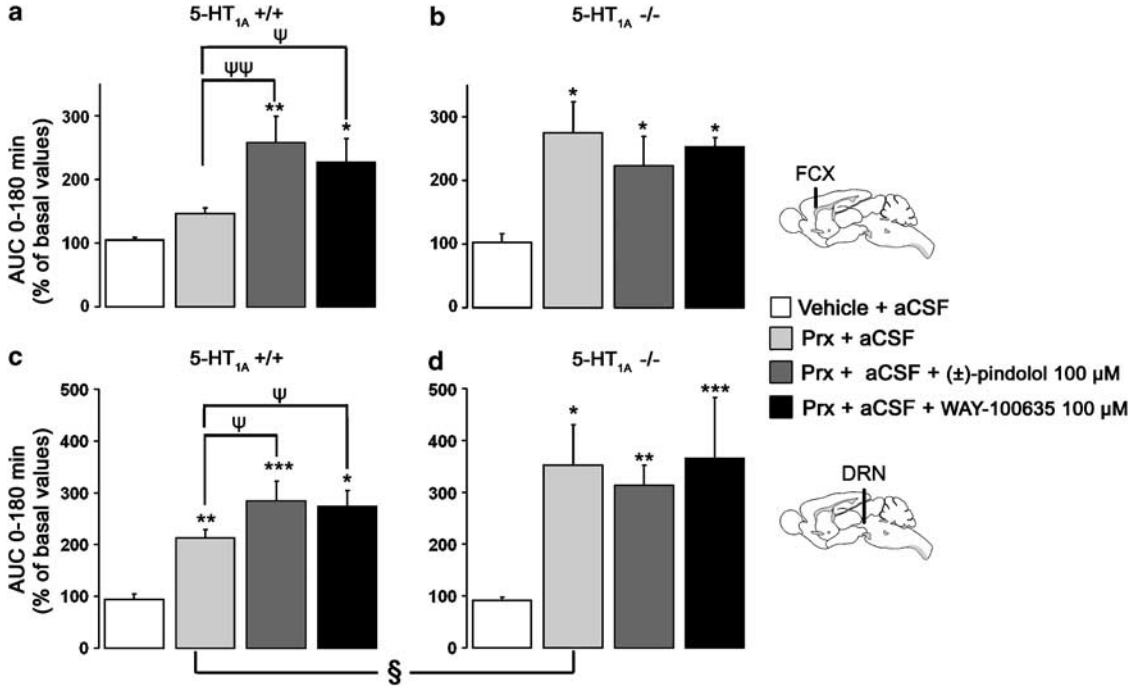

Figure 6 Effects of local intra-raphe infusion of either (±)-pindolol or WAY-100635 on $[5\text{-HT}]_{ext}$ levels in the FCX and DRN following systemic paroxetine (4 mg/kg) administration in 5-HT$_{1A}$+/+ and 5-HT$_{1A}$−/− mice. In the FCX, (a) in 5-HT$_{1A}$+/+, (b) in 5-HT$_{1A}$−/−: data are area under the curve (AUC; mean ± SEM) values calculated for the amount of 5-HT outflow measured during the 0–180 min post-treatment period in the FCX of 5-HT$_{1A}$+/+ and 5-HT$_{1A}$−/− mice, and expressed as percentages of baseline ($n = 4$–9 mice per group). In the DRN, (c) in 5-HT$_{1A}$+/+, (d) in 5-HT$_{1A}$−/− mice: data are area under the curve (AUC; mean ± SEM) values calculated for the amount of 5-HT outflow measured during the 0–180 min post-treatment period in the DRN of 5-HT$_{1A}$+/+ and 5-HT$_{1A}$−/− mice, and expressed as percentages of baseline ($n = 4$–9 mice per group). $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ significantly different from the vehicle-treated group; $^{\S}p < 0.05$ and significantly different from 5-HT$_{1A}$+/+ mice; $^{\Psi}p < 0.05$; $^{\Psi\Psi}p < 0.01$ significantly different from the paroxetine 4 mg/kg/aCSF-treated group (two-way ANOVA followed by a PLSD *post hoc t*-test).

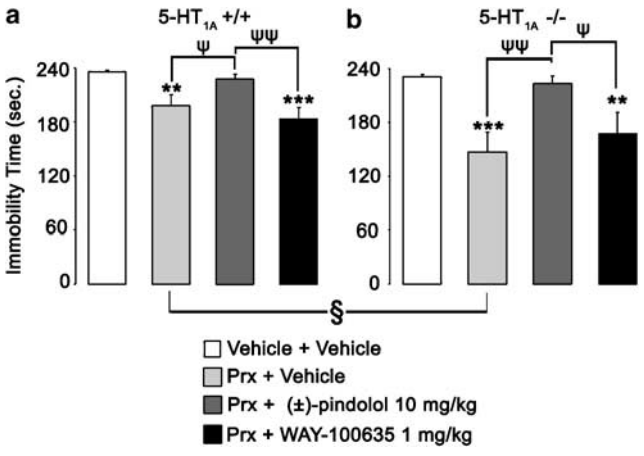

Figure 7 Antidepressant-like effects of the coadministration of paroxetine and (±)-pindolol or WAY-100635 on 5-HT$_{1A}$+/+ and 5-HT$_{1A}$−/− mice' behaviour in the FST. Results are means ± SEM of the immobility time (in seconds). 5-HT$_{1A}$+/+ and 5-HT$_{1A}$−/− received either the vehicle, paroxetine (1 mg/kg), paroxetine and (±)-pindolol (10 mg/kg), or paroxetine and WAY-100635 (1 mg/kg). Statistical analyses were carried out using a two-way ANOVA followed by Fisher PLSD: $^{**}p < 0.01$; $^{***}p < 0.001$ significantly different from the 5-HT$_{1A}$+/+ control, vehicle-treated group; $^{\S}p < 0.05$ and significantly different from 5-HT$_{1A}$+/+ mice; $^{\Psi}p < 0.05$; $^{\Psi\Psi}p < 0.01$ significantly different from paroxetine and (±)-pindolol.

when compared to the appropriate paroxetine/vehicle group in 5-HT$_{1A}$+/+ and 5-HT$_{1A}$−/− mice, respectively), blocked the antidepressant-like effects of paroxetine in both genotypes ($p < 0.05$ and $p < 0.01$ when compared to the appropriate paroxetine/vehicle group in 5-HT$_{1A}$+/+ and 5-HT$_{1A}$−/− mice, respectively).

DISCUSSION

In the present study, after the validation of our genetic mice model (5-HT$_{1A}$−/−) using the intracerebral *in vivo* microdialysis and the mouse FST, we investigated, using these two different approaches (neurochemical and behavioral ones), the mechanism of action of (±)-pindolol alone or in combination with paroxetine.

Neurochemical Validation of the Genetic Model, 5-HT$_{1A}$−/− Mice

Baseline dialysate 5-HT levels in the FCX and DRN. Basal fronto-cortical $[5\text{-HT}]_{ext}$ in 5-HT$_{1A}$−/− mice were similar to those found in 5-HT$_{1A}$+/+ mice. These results confirm that dialysate 5-HT levels at serotonergic nerve terminals are not enhanced in the mutant mice, as most studies have revealed (see Knobelman's study (2001b) but on a background strain different from ours). These results differ from those of Parsons *et al* (2001) who also used mice on C57BL/6 genetic background, but generated elsewhere (Heisler *et al*, 1998): they found that basal dialysate 5-HT levels were higher in mutants than in controls, both in the FCX and hippocampus. Age of these mice may account for this discrepancy since Parsons *et al* (2001) used 8- to 10-month-old male mice. Our results provide additional evidence that

somatodendritic 5-HT$_{1A}$ autoreceptors located in the DRN do not exert a tonic inhibitory control on 5-HT release at serotonergic nerve terminals. Also, in agreement with a recent study performed in a C57BL/6 genetic background, in the DRN, we also found no differences between basal [5-HT]$_{ext}$ in 5-HT$_{1A}$+/+ and 5-HT$_{1A}$−/− mice (Bortolozzi *et al*, 2004). Furthermore, the role of 5-HT$_{1A}$ receptors on the noradrenergic system is also important: the blockade of 5-HT$_{1A}$ autoreceptors is known to decrease the noradrenaline release (Haddjeri *et al*, 1997, 2004).

Effects of a 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT. Since the 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT decreased cortical [5-HT]$_{ext}$ in 5-HT$_{1A}$+/+ mice, presumably by acting on presynaptic 5-HT$_{1A}$ autoreceptors located in the DRN and because it had no effect in 5-HT$_{1A}$−/− mice, we validated our experimental knockout model. Similar results have been obtained in the striatum and ventral hippocampus either in 5-HT$_{1A}$−/− mice or in rats (Adell *et al*, 1993; Knobelman *et al*, 2001a).

Dose-Dependent Neurochemical Effects and Antidepressant-Like Activity of Paroxetine in 5-HT$_{1A}$−/− Mice

Differences between 5-HT$_{1A}$+/+ and 5-HT$_{1A}$−/− mice were observed in the effects of a single paroxetine administration (1–4 mg/kg) on [5-HT]$_{ext}$. This SSRI slightly increased cortical [5-HT]$_{ext}$ in wild-type mice, as most studies have reported in this brain region (Malagie *et al*, 2001; Parsons *et al*, 2001; David *et al*, 2003a), as well as in the striatum and hippocampus (Knobelman *et al*, 2001a). For each paroxetine dose tested, its effects were almost three-fold higher in 5-HT$_{1A}$−/− mice than in controls: these potentiated effects were unlikely due to differences between wild-type controls and 5-HT$_{1A}$−/− mice in the serotonin transporter density in various brain regions (Ase *et al*, 2001). This larger neurochemical effect of SSRI measured in mutant *vs* wild-type mice, in forebrain regions was likely due to the lack of presynaptic 5-HT$_{1A}$ autoreceptors and has been described elsewhere (Parsons *et al*, 2001; Knobelman *et al*, 2001b). However, the absence of postsynaptic 5-HT$_{1A}$ receptors in constitutive knockout mice generated by homologous recombination cannot be ruled out as contributing to this effect. Indeed, this latter receptor is involved in a long postsynaptic inhibitory feedback loop to raphe nuclei and the brain stem, thus regulating activities of cell bodies of serotonergic neurons (Casanovas *et al*, 1999). In other way, this loop has been described using microdialysis in the medial pre-FCX and our work has been performed in a more posterior part of the brain.

Our behavioral data obtained in the mouse FST confirm other reports showing the dose-dependent efficacy of SSRIs in reducing the immobility time in 5-HT$_{1A}$+/+ mice (David *et al*, 2003b). As in other studies using Porsolt's Test or the Tail Suspension Test (Steru *et al*, 1985), we found a difference in baseline activity in this model between wild-type 5-HT$_{1A}$+/+ and mutant 5-HT$_{1A}$−/− mice (Heisler *et al*, 1998; Parks *et al*, 1998; Ramboz *et al*, 1998; Mayorga *et al*, 2001). Overall, these results suggest that the genetic inactivation of 5-HT$_{1A}$ receptors abolished the inhibitory feedback control exerted by somatodendritic 5-HT$_{1A}$ autoreceptors, thus enhancing the response of mutant mice to stressful conditions: indirect activation of presynaptic 5-HT$_{1A}$ receptors by endogenous 5-HT may limit the antidepressant-like effects of the SSRI in the FST in wild-type mice. However, pharmacological studies performed in wild-type mice (O'Neill and Conway, 2001) and rats (Wieland and Lucki, 1990) described an antidepressant-like activity of full and partial 5-HT$_{1A}$ receptor agonists, such as 8-OH-DPAT and buspirone, respectively. It has been suggested that postsynaptic 5-HT$_{1A}$ receptors could mediate the behavioral effect of these agonists (Detke *et al*, 1995).

Neurochemical Effects and Antidepressant-Like Activity of (±)Pindolol Associated with Paroxetine in 5-HT$_{1A}$−/− Mice

Our microdialysis results suggest that, *in vivo*, in awake mice, (±)-pindolol acts as an antagonist at 5-HT$_{1A}$ receptors. Indeed, (±)-pindolol displayed similar effects as those obtained with the antagonist of reference WAY-100635, that is, potentiating the effects of paroxetine on extracellular cortical 5-HT levels. Preclinical studies have reported conflicting results regarding the ability of (±)-pindolol to antagonize SSRI-induced inhibition of cell firing of 5-HT neurons or 5-HT release at nerve terminals. Numerous electrophysiological studies performed either *in vivo* in anesthetized animals or *in vitro* on brain slices suggested that (±)-pindolol behaves as a 5-HT$_{1A}$ receptor antagonist by blocking the SSRI-induced inhibition of 5-HT cell firing (Romero *et al*, 1996; Corradetti *et al*, 1998; Rasmussen *et al*, 2004), while others showed that it has either no effects or an agonistic effect on 5-HT$_{1A}$ receptors (Fornal *et al*, 1999; Sprouse *et al*, 2000). Results of intracerebral *in vivo* microdialysis studies brought up less discrepancies: almost all studies demonstrated that pindolol can potentiate the effects of SSRI on dialysate 5-HT levels in brain regions enriched in serotonergic nerve terminals (Dreshfield *et al*, 1996; Hjorth, 1996; Romero *et al*, 1996; Gobert and Millan, 1999; Dawson and Nguyen, 2000; Cremers *et al*, 2001; Miguez *et al*, 2002).

The lack of (±)-pindolol effects in 5-HT$_{1A}$−/− mice suggests that changes in cortical dialysate 5-HT are unlikely due to its antagonistic action on other neurotransmitter receptor subtypes: it binds to 5-HT$_{1B}$ receptors with a third and 1000-fold lower affinity than to 5-HT$_{1A}$ receptors in rats and human, respectively (Castro *et al*, 2000; Artigas *et al*, 2001). However, it seems that, in brain regions innervated by the median raphe (hippocampus) rather than by the dorsal raphe (FCX) higher effects of 5-HT$_{1B}$ receptor antagonist on 5-HT release occurred (Malagie *et al*, 2001; Hughes and Dawson, 2004). Furthermore, other studies have shown that selective β_1 or β_2-adrenoceptor antagonists do not modulate dialysate 5-HT levels in the FCX or ventral hippocampus in rats (Hjorth *et al*, 1996; Gobert and Millan, 1999).

As we cannot exclude that (±)-pindolol binds to other neurotransmitter receptors and/or in other brain regions following its systemic administration, we studied whether its main site of action was confined to somatodendritic 5-HT$_{1A}$ autoreceptors located in the DRN. Thus, we performed local intra-raphe perfusion of (±)-pindolol (or WAY-100635) by reverse microdialysis: we found responses

in the FCX and DRN similar to those measured following systemic administration of these compounds. It is likely that 5-HT$_{1A}$ autoreceptors were completely blocked following either systemic or intra-raphe (±)-pindolol administration. This assertion is based (1) on a pharmacokinetic studies performed in guinea-pigs (Hasegawa *et al*, 1989; Cremers *et al*, 2001), and (2) on the hypothesis that (±)-pindolol displays an affinity for 5-HT$_{1A}$ receptors in mice comparable to that measured in rats or guinea-pigs (Ki in a 6–34 nM range).

We used, here for the first time, a dual microdialysis approach by implanting two probes in the brain of awake, freely moving mice. Thus, the neurochemical responses depicted a neurochemical circuitry between the DRN and FCX. Our results show that intra-raphe perfusion of either (±)-pindolol or WAY-100635 had no effects on cortical and DRN dialysate 5-HT levels, when given alone, but potentiated paroxetine's effects on extracellular 5-HT levels in the two brain regions studied. In rats, similar results were found in the ventral hippocampus following the local perfusion of (±)-pindolol in the median raphe nucleus (Miguez *et al*, 2002).

In the FST, the effect of the inactivation of the 5-HT$_{1A}$ receptor in combination with paroxetine differed depending on the experimental strategy used. By using a genetic strategy, we found that paroxetine-induced decreases in the immobility time was dramatically potentiated in 5-HT$_{1A}$−/− mice compared to wild-type controls. Conversely, by using a pharmacological strategy, we found that (±)-pindolol, but not WAY-100635, blocked paroxetine-induced effects in both genotypes in the FST. Conflicting results were reported in numerous studies using this behavioral test or the Tail Suspension Test that found either no potentiation or a blockade of SSRI effects by 5-HT$_{1A}$ receptor antagonists pindolol in rats or wild-type mice (Moser and Sanger, 1999; Tatarczynska *et al*, 2002).

Receptor Subtypes Involved in the Antidepressant-Like Activity of Pindolol

Non-5-HT$_{1A}$ receptor, 5-HT$_{1B}$ receptors, or $\beta_{1,2}$-adrenoceptors? In the present study, the effects of pindolol in the behavioral test were different when combined with paroxetine, from those of WAY-100635 in both genotypes. It is likely that pindolol bound to non-5-HT$_{1A}$ receptors to induce these effects in the FST because (1) WAY-100635 had no effect in wild-type mice and (2) pindolol did block the effects of paroxetine in 5-HT$_{1A}$−/− mice. Such non-5-HT$_{1A}$ receptor subtypes could be either 5-HT$_{1B}$ receptors, or $\beta_{1,2}$-adrenoceptors. Indeed, pindolol has an affinity for 5-H$_{1B}$ receptors in rats (Titeler *et al*, 1987; Hoyer, 1988; Tsuchihashi *et al*, 1990; Langlois *et al*, 1993), or humans (Gobert and Millan, 1999). Furthermore, the guinea-pig has often served as an animal model to assess 5-HT$_{1B/1D}$ receptor function. Thus, (−)-pindolol displayed a significant binding affinity to 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (Zgombick *et al*, 1997; Moret and Briley, 1997). (−)-Pindolol potentiated the effect of milnacipran, a dual noradrenaline (NA) and serotonin reuptake inhibitor, on the extracellular levels of NA and 5-HT in the hypothalamus of guinea-pig (Moret and Briley, 1997). Tatarczynska *et al* (2004) found that 5-HT$_{1B}$ receptor agonists combined with an SSRI can reduce the immobility time in the mice FST. Results from our laboratory have demonstrated that the 5-HT$_{1B}$ receptor antagonist GR127935 (as pindolol here) blocks the anti-immobility effects of paroxetine in this paradigm in wild-type mice, but not in 5-HT$_{1B}$−/− mice, suggesting that activation of 5-HT$_{1B}$ receptors mediate, at least in part, the antidepressant-like activity of this SSRI (Gardier *et al*, 2001). Another possibility could be an antagonistic action of pindolol on $\beta_{1,2}$-adrenoceptors. However, Detke et al (1995) found no blockade of the anti-immobility effects of 8-OHDPAT by either betaxolol or ICI 118551, antagonists at β_1 and β_2 adrenoceptor subtypes, respectively.

The results of the present study in wild-type mice are also in line with others, who found no potentiation of the effects of paroxetine by WAY-100635 (Cryan *et al*, 1998), but a blockade of this effect by pindolol in another model (Cryan *et al*, 1999). Only one pharmacological study found a potentiation of the antidepressant-like effects of an SSRI (fluoxetine) with (±)-pindolol in mice, but the high dosage of pindolol administered (32 mg/kg) is questionable (Redrobe *et al*, 1996). The dosage of both the antidepressant and the 5-HT$_{1A}$ receptor antagonist may be critical for the potentiation of the antidepressant-like effect of SSRIs in the FST.

Pre- vs *postsynaptic 5-HT$_{1A}$ receptors?* In wild-type mice, two different approaches (neurochemical and behavioral ones) studying the effects of coadministration of pindolol with paroxetine gave opposite responses: a potentiation or blockade in microdialysis experiments and the FST, respectively. It suggests that two different receptors having a distinct cellular distribution have been mobilized in these responses. Since intracerebral microdialysis is known to be a presynaptic test, changes in cortical extracellular 5-HT levels likely involved the effects of (±)-pindolol on presynaptic 5-HT$_{1A}$ autoreceptors. From this assertion, we can conclude that responses in the FST likely involved postsynaptic receptors in wild-type mice.

In conclusion, the results of microdialysis experiments suggest that activation of presynaptic 5-HT$_{1A}$ autoreceptors limits the effects of SSRI on cortical dialysate 5-HT because the paroxetine response in the FCX was potentiated by pindolol in 5-HT$_{1A}$+/+, but not in 5-HT$_{1A}$−/− mice. The results of behavioral experiments suggest that (1) activation of 5-HT$_{1A}$ receptors limits the antidepressant-like activity of paroxetine and (2) the effects of pindolol in the FST are not mediated by activation of 5-HT$_{1A}$ receptors because pindolol gave similar responses in both genotypes (it blocked the antidepressant-like activity of paroxetine). Thus, a non-5-HT$_{1A}$ postsynaptic receptor, which remains to be identify, seems to be involved in the antidepressant-like activity of the combined treatment paroxetine + (±)-pindolol in this paradigm in mice.

ACKNOWLEDGEMENTS

We thank the participation of Isabelle Seif for the genotyping of animals and for her scientific support. This work has been supported by the technical assistance of the Animal Care Facility of the 'Institut Fédératif de Recherche—IFR75' of the Paris XI University.

REFERENCES

Adell A, Carceller A, Artigas F (1993). In vivo brain dialysis study of the somatodendritic release of serotonin in the Raphe nuclei of the rat: effects of 8-hydroxy-2-(di-n-propylamino)tetralin. *J Neurochem* **60**: 1673–1681.

Artigas F, Celada P, Laruelle M, Adell A (2001). How does pindolol improve antidepressant action? *Trends Pharmacol Sci* **22**: 224–228.

Artigas F, Perez V, Alvarez E (1994). Pindolol induces a rapid improvement of depressed patients treated with serotonin reuptake inhibitors. *Arch Gen Psychiatry* **51**: 248–251.

Artigas F, Romero L, de Montigny C, Blier P (1996). Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT1A antagonists. *Trends Neurosci* **19**: 378–383.

Ase AR, Reader TA, Hen R, Riad M, Descarries L (2001). Regional changes in density of serotonin transporter in the brain of 5-HT1A and 5-HT1B knockout mice, and of serotonin innervation in the 5-HT1B knockout. *J Neurochem* **78**: 619–630.

Ballesteros J, Callado LF (2004). Effectiveness of pindolol plus serotonin uptake inhibitors in depression: a meta-analysis of early and late outcomes from randomised controlled trials. *J Affect Disord* **79**: 137–147.

Bert L, Favale D, Jego G, Greve P, Guilloux J-P, Guiard BP et al (2004). Rapid and precise method to locate microdialysis probe implantation in the rodent brain. *J Neurosci Methods* **140**: 53–57.

Blier P, Bergeron R (1995). Effectiveness of pindolol with selected antidepressant drugs in the treatment of major depression. *J Clin Psychopharmacol* **15**: 217–222.

Blier P, de Montigny C (1994). Current advances and trends in the treatment of depression. *Trends Pharmacol Sci* **15**: 220–226.

Blier P, de Montigny C, Chaput Y (1987). Modifications of the serotonin system by antidepressant treatments: implications for the therapeutic response in major depression. *J Clin Psychopharmacol* **7**: 24S–35S.

Bortolozzi A, Amargos-Bosch M, Toth M, Artigas F, Adell A (2004). In vivo efflux of serotonin in the dorsal raphe nucleus of 5-HT1A receptor knockout mice. *J Neurochem* **88**: 1373–1379.

Casanovas JM, Hervas I, Artigas F (1999). Postsynaptic 5-HT1A receptors control 5-HT release in the rat medial prefrontal cortex. *NeuroReport* **10**: 1441–1445.

Castro ME, Harrison PJ, Pazos A, Sharp T (2000). Affinity of (+/−)-pindolol, (−)-penbutolol, and (−)-tertatolol for pre- and postsynaptic serotonin 5-HT(1A) receptors in human and rat brain. *J Neurochem* **75**: 755–762.

Corradetti R, Laaris N, Hanoun N, Laporte AM, Le Poul E, Hamon M et al (1998). Antagonist properties of (−)-pindolol and WAY 100635 at somatodendritic and postsynaptic 5-HT1A receptors in the rat brain. *Br J Pharmacol* **123**: 449–462.

Cremers TI, Wiersma LJ, Bosker FJ, den Boer JA, Westerink BH, Wikstrom HV (2001). Is the beneficial antidepressant effect of coadministration of pindolol really due to somatodendritic autoreceptor antagonism? *Biol Psychiatry* **50**: 13–21.

Cryan JF, McGrath C, Leonard BE, Norman TR (1998). Combining pindolol and paroxetine in an animal model of chronic antidepressant action—can early onset of action be detected? *Eur J Pharmacol* **352**: 23–28.

Cryan JF, McGrath C, Leonard BE, Norman TR (1999). Onset of the effects of the 5-HT1A antagonist, WAY-100635, alone, and in combination with paroxetine, on olfactory bulbectomy and 8-OH-DPAT-induced changes in the rat. *Pharmacol Biochem Behav* **63**: 333–338.

David DJ, Bourin M, Jego G, Przybylski C, Jolliet P, Gardier AM (2003a). Effects of acute treatment with paroxetine, citalopram and venlafaxine in vivo on noradrenaline and serotonin outflow: a microdialysis study in Swiss mice. *Br J Pharmacol* **140**: 1128–1136.

David DJ, Renard CE, Jolliet P, Hascoet M, Bourin M (2003b). Antidepressant-like effects in various mice strains in the forced swimming test. *Psychopharmacology (Berl)* **166**: 373–382.

Dawson LA, Nguyen HQ (2000). The role of 5-HT(1A) and 5-HT(1B/1D) receptors on the modulation of acute fluoxetine-induced changes in extracellular 5-HT: the mechanism of action of (+/−)pindolol. *Neuropharmacology* **39**: 1044–1052.

Detke MJ, Wieland S, Lucki I (1995). Blockade of the antidepressant-like effects of 8-OH-DPAT, buspirone and desipramine in the rat forced swim test by 5HT1A receptor antagonists. *Psychopharmacology (Berl)* **119**: 47–54.

Dreshfield LJ, Wong DT, Perry KW, Engleman EA (1996). Enhancement of fluoxetine-dependent increase of extracellular serotonin (5-HT) levels by (−)-pindolol, an antagonist at 5-HT1A receptors. *Neurochem Res* **21**: 557–562.

Fornal CA, Martin FJ, Metzler CW, Jacobs BL (1999). Pindolol, a putative 5-hydroxytryptamine(1A) antagonist, does not reverse the inhibition of serotonergic neuronal activity induced by fluoxetine in awake cats: comparison to WAY-100635. *J Pharmacol Exp Ther* **291**: 220–228.

Franklin KB, Paxinos G (1997). *The Mouse Brain in Stereotaxic Coordinates*. Academic Press Inc.: San Diego, CA.

Gardier AM, Trillat AC, Malagie I, David D, Hascoet M, Colombel MC et al (2001). 5-HT1B serotonin receptors and antidepressant effects of selective serotonin reuptake inhibitors. *C R Acad Sci III* **324**: 433–441.

Gobert A, Millan MJ (1999). Modulation of dialysate levels of dopamine, noradrenaline, and serotonin (5-HT) in the frontal cortex of freely-moving rats by (−)-pindolol alone and in association with 5-HT reuptake inhibitors: comparative roles of beta-adrenergic, 5-HT1A, and 5-HT1B receptors. *Neuropsychopharmacology* **21**: 268–284.

Guiard BP, Przybylski C, Guilloux JP, Seif I, Froger N, De Felipe C et al (2004). Blockade of substance P (neurokinin 1) receptors enhances extracellular serotonin when combined with a selective serotonin reuptake inhibitor: an in vivo microdialysis study in mice. *J Neurochem* **89**: 54–63.

Haddjeri N, de Montigny C, Blier P (1997). Modulation of the firing activity of noradrenergic neurones in the rat locus coeruleus by the 5-hydroxtryptamine system. *Br J Pharmacol* **120**: 865–875.

Haddjeri N, Lavoie N, Blier P (2004). Electrophysiological evidence for the tonic activation of 5-HT(1A) autoreceptors in the rat dorsal raphe nucleus. *Neuropsychopharmacology* **29**: 1800–1806.

Hasegawa R, Murai-Kushiya M, Komuro T, Kimura T (1989). Stereoselective determination of plasma pindolol in endotoxin-pretreated rats by high-performance liquid chromatography. *J Chromatogr* **494**: 381–388.

Heisler LK, Chu HM, Brennan TJ, Danao JA, Bajwa P, Parsons LH et al (1998). Elevated anxiety and antidepressant-like responses in serotonin 5-HT1A receptor mutant mice. *Proc Natl Acad Sci USA* **95**: 15049–15054.

Hjorth S (1996). Pindolol, but not buspirone, potentiates the citalopram-induced rise in extracellular 5-hydroxytryptamine. *Eur J Pharmacol* **303**: 183–186.

Hjorth S, Auerbach SB (1994). Further evidence for the importance of 5-HT1A autoreceptors in the action of selective serotonin reuptake inhibitors. *Eur J Pharmacol* **260**: 251–255.

Hjorth S, Bengtsson HJ, Milano S (1996). Raphe 5-HT1A autoreceptors, but not postsynaptic 5-HT1A receptors or beta-adrenoceptors, restrain the citalopram-induced increase in extracellular 5-hydroxytryptamine in vivo. *Eur J Pharmacol* **316**: 43–47.

Hof PR, Young WG, Bloom FE, Belichenko PV, Celio MR (2000). *Comparative Cytoarchitectonic Atlas of the C57BL/6 and 129/Sv Mouse Brains with CD ROM*. Elsevier: San Diego, CA.

Hoyer D (1988). Functional correlates of serotonin 5-HT1 recognition sites. *J Recept Res* **8**: 59–81.

Hughes ZA, Dawson LA (2004). Differential autoreceptor control of extracellular 5-HT in guinea pig and rat: species and regional differences. *Psychopharmacology (Berl)* **172**: 87–93.

Invernizzi R, Bramante M, Samanin R (1996). Role of 5-HT1A receptors in the effects of acute chronic fluoxetine on extracellular serotonin in the frontal cortex. *Pharmacol Biochem Behav* **54**: 143–147.

Knobelman DA, Hen R, Blendy JA, Lucki I (2001a). Regional patterns of compensation following genetic deletion of either 5-hydroxytryptamine(1A) or 5-hydroxytryptamine(1B) receptor in the mouse. *J Pharmacol Exp Ther* **298**: 1092–1100.

Knobelman DA, Hen R, Lucki I (2001b). Genetic regulation of extracellular serotonin by 5-hydroxytryptamine(1A) and 5-hydroxytryptamine(1B) autoreceptors in different brain regions of the mouse. *J Pharmacol Exp Ther* **298**: 1083–1091.

Langlois M, Bremont B, Rousselle D, Gaudy F (1993). Structural analysis by the comparative molecular field analysis method of the affinity of beta-adrenoreceptor blocking agents for 5-HT1A and 5-HT1B receptors. *Eur J Pharmacol* **244**: 77–87.

Malagie I, Trillat AC, Bourin M, Jacquot C, Hen R, Gardier AM (2001). 5-HT1B autoreceptors limit the effects of selective serotonin re-uptake inhibitors in mouse hippocampus and frontal cortex. *J Neurochem* **76**: 865–871.

Martinez D, Broft A, Laruelle M (2000). Pindolol augmentation of antidepressant treatment: recent contributions from brain imaging studies. *Biol Psychiatry* **48**: 844–853.

Martinez D, Hwang D, Mawlawi O, Slifstein M, Kent J, Simpson N et al (2001). Differential occupancy of somatodendritic and postsynaptic 5HT(1A) receptors by pindolol: a dose-occupancy study with [11C]WAY 100635 and positron emission tomography in humans. *Neuropsychopharmacology* **24**: 209–229.

Mayorga AJ, Dalvi A, Page ME, Zimov-Levinson S, Hen R, Lucki I (2001). Antidepressant-like behavioral effects in 5-hydroxytryptamine(1A) and 5-hydroxytryptamine(1B) receptor mutant mice. *J Pharmacol Exp Ther* **298**: 1101–1107.

Miguez JM, Paz-Valinas L, Miguez I, Aldegunde M (2002). Somatodendritic action of pindolol to attenuate the paroxetine-induced decrease in serotonin release from the rat ventral hippocampus: a microdialysis study. *Naunyn Schmiedebergs Arch Pharmacol* **365**: 378–387.

Moret C, Briley M (1997). Effects of milnacipran and pindolol on extracellular noradrenaline and serotonin levels in guinea pig hypothalamus. *J Neurochem* **69**: 815–822.

Moser PC, Sanger DJ (1999). 5-HT1A receptor antagonists neither potentiate nor inhibit the effects of fluoxetine and befloxatone in the forced swim test in rats. *Eur J Pharmacol* **372**: 127–134.

Newman-Tancredi A, Chaput C, Gavaudan S, Verriele L, Millan MJ (1998). Agonist and antagonist actions of (−)pindolol at recombinant, human serotonin1A (5-HT1A) receptors. *Neuropsychopharmacology* **18**: 395–398.

O'Neill MF, Conway MW (2001). Role of 5-HT(1A) and 5-HT(1B) receptors in the mediation of behavior in the forced swim test in mice. *Neuropsychopharmacology* **24**: 391–398.

Parks CL, Robinson PS, Sibille E, Shenk T, Toth M (1998). Increased anxiety of mice lacking the serotonin1A receptor. *Proc Natl Acad Sci USA* **95**: 10734–10739.

Parsons LH, Kerr TM, Tecott LH (2001). 5-HT(1A) receptor mutant mice exhibit enhanced tonic, stress-induced and fluoxetine-induced serotonergic neurotransmission. *J Neurochem* **77**: 607–617.

Petit-Demouliere B, Chenu F, Bourin M (2005). Forced swimming test in mice: a review of antidepressant activity. *Psychopharmacology (Berl)* **177**: 245–255.

Plenge P, Mellerup ET (2003). Pindolol and the acceleration of the antidepressant response. *J Affect Disord* **75**: 285–289.

Porsolt RD, Bertin A, Jalfre M (1977). Behavioral despair in mice: a primary screening test for antidepressants. *Arch Int Pharmacodyn Ther* **229**: 327–336.

Ramboz S, Oosting R, Amara DA, Kung HF, Blier P, Mendelsohn M et al (1998). Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. *Proc Natl Acad Sci USA* **95**: 14476–14481.

Rasmussen K, McCreary AC, Shanks EA (2004). Attenuation of the effects of fluoxetine on serotonergic neuronal activity by pindolol in rats. *Neurosci Lett* **355**: 1–4.

Redrobe JP, MacSweeney CP, Bourin M (1996). The role of 5-HT1A and 5-HT1B receptors in antidepressant drug actions in the mouse forced swimming test. *Eur J Pharmacol* **318**: 213–220.

Romero L, Bel N, Artigas F, de Montigny C, Blier P (1996). Effect of pindolol on the function of pre- and postsynaptic 5-HT1A receptors: in vivo microdialysis and electrophysiological studies in the rat brain. *Neuropsychopharmacology* **15**: 349–360.

Sprouse J, Braselton J, Reynolds L (2000). 5-HT(1A) agonist potential of pindolol: electrophysiologic studies in the dorsal raphe nucleus and hippocampus. *Biol Psychiatry* **47**: 1050–1055.

Steru L, Chermat R, Thierry B, Simon P (1985). The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology (Berl)* **85**: 367–370.

Tatarczynska E, Klodzinska A, Chojnacka-Wojcik E (2002). Effects of combined administration of 5-HT1A and/or 5-HT1B receptor antagonists and paroxetine or fluoxetine in the forced swimming test in rats. *Pol J Pharmacol* **54**: 615–623.

Tatarczynska E, Klodzinska A, Stachowicz K, Chojnacka-Wojcik E (2004). Effects of a selective 5-HT1B receptor agonist and antagonists in animal models of anxiety and depression. *Behav Pharmacol* **15**: 523–534.

Titeler M, Lyon RA, Davis KH, Glennon RA (1987). Selectivity of serotonergic drugs for multiple brain serotonin receptors. Role of [3H]-4-bromo-2,5-dimethoxyphenylisopropylamine ([3H] DOB), a 5-HT2 agonist radioligand. *Biochem Pharmacol* **36**: 3265–3271.

Toth M (2003). 5-HT1A receptor knockout mouse as a genetic model of anxiety. *Eur J Pharmacol* **463**: 177–184.

Tsuchihashi H, Nakashima Y, Kinami J, Nagatomo T (1990). Characteristics of 125I-iodocyanopindolol binding to beta-adrenergic and serotonin-1B receptors of rat brain: selectivity of beta-adrenergic agents. *Jpn J Pharmacol* **52**: 195–200.

Wieland S, Lucki I (1990). Antidepressant-like activity of 5-HT1A agonists measured with the forced swim test. *Psychopharmacology (Berl)* **101**: 497–504.

Zgombick JM, Bard JA, Kucharewicz SA, Urquhart DA, Weinshank RL, Branchek TA (1997). Molecular cloning and pharmacological characterization of guinea pig 5-HT1B and 5-HT1D receptors. *Neuropharmacology* **36**: 513–524.

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