Neuropharmacology 73 (2013) 147–159

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

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

Antidepressant and anxiolytic potential of the multimodal antidepressant vortioxetine (Lu AA21004) assessed by behavioural and neurogenesis outcomes in mice☆

Jean-Philippe Guilloux [a], Indira Mendez-David [a], Alan Pehrson [b], Bruno P. Guiard [a], Christelle Repérant [a], Sophie Orvoën [a], Alain M. Gardier [a], René Hen [d,e], Bjarke Ebert [c], Silke Miller [b,1], Connie Sanchez [b], Denis J. David [a,*]

[a] Univ Paris-Sud, EA3544, Faculté de Pharmacie, Châtenay-Malabry F-92296, France
[b] Lundbeck Research USA, 215 College Rd, Paramus, NJ 07652, USA
[c] H. Lundbeck A/S, Medical Affairs Mood & Anxiety, Ottiliavej 9, DK-2500 Valby, Denmark
[d] Department of Neuroscience, Columbia University, New York, NY 10032, USA
[e] Department of Psychiatry, Columbia University, New York, NY 10032, USA

ARTICLE INFO

Article history:
Received 26 December 2012
Received in revised form
6 May 2013
Accepted 7 May 2013

Keywords:
Multimodal antidepressant
Anxiolytic
Antidepressant
Behaviour
Mice
Neurogenesis

ABSTRACT

Vortioxetine (Lu AA21004) is an investigational novel antidepressant with multimodal activity that functions as a 5-HT_3, 5-HT_7 and 5-HT_{1D} receptor antagonist, 5-HT_{1B} receptor partial agonist, 5-HT_{1A} receptor agonist and inhibitor of the 5-HT transporter *in vitro*. Here we explore its anxiolytic and antidepressant potential in adult mice. Vortioxetine was assessed in BalB/cJ@RJ mice using the open-field and forced-swim tests (acute: p.o. 1 h, repeated: daily p.o. 21 days), and in 129S6/SvEvTac mice using the novelty suppressed feeding paradigm (acute: p.o. 1 h, sustained: daily p.o. 14 or 21 days). Fluoxetine and diazepam were controls. Acute and repeated dosing of vortioxetine produced more pronounced anxiolytic- and antidepressant-like activities than fluoxetine. Vortioxetine significantly increased cell proliferation and cell survival and stimulated maturation of immature granule cells in the subgranular zone of the dentate gyrus of the hippocampus after 21 days of treatment. After 14 days, a high dose of vortioxetine increased dendritic length and the number of dendrite intersections, suggesting that vortioxetine accelerates the maturation of immature neurons. Vortioxetine displays an antidepressant and anxiolytic profile following repeated administration associated with increased neurogenesis at several stages. Vortioxetine effects were observed at low levels of 5-HT transporter occupancy, suggesting an alternative mechanism of action to 5-HT reuptake inhibition.

1. Introduction

Depression is a major psychiatric disease, with a ≈17% lifetime prevalence (Kessler et al., 2005). Rates of response to initial pharmacotherapy can vary from 30 to 60% depending on the studies while remission rates in the first step of the STAR*D study was ≈37% (Guilloux et al., 2012; Rush et al., 2006). Side effects with Selective Serotonin Reuptake Inhibitors (SSRIs) are commonly reported during chronic treatment, notably insomnia, somnolence, dizziness, akathisia, and long-term sexual dysfunction (e.g., decreased libido, delayed ejaculation) (Hamon and Bourgoin, 2006). The modest efficacy of conventional antidepressants such as the selective serotonin (5-HT) reuptake inhibitors (SSRIs) calls for novel approaches to treat depression and anxiety disorders. Combinatorial pharmacological therapies, such as additional blockade of aminergic receptors in addition to monoamine transporter inhibition, have earlier been proposed to shorten the time to antidepressant effect and/or to increase efficacy in clinical studies (Artigas et al., 2006; Kennedy et al., 2011).

The 5-HT_{1A} and the 5-HT_{1B} receptors were the first serotonergic receptors targeted to treat anxiety and depression due to their

* Corresponding author. Tel.: +33 1 46 83 59 68; fax: +33 1 46 83 53 55.
E-mail addresses: jean-philippe.guilloux@u-psud.fr (J.-P. Guilloux), indira.david@u-psud.fr (I. Mendez-David), APEH@Lundbeck.com (A. Pehrson), bruno.guiard@u-psud.fr (B.P. Guiard), christelle.reperant@u-psud.fr (C. Repérant), sophie.orvoen@gmail.com (S. Orvoën), alain.gardier@u-psud.fr (A.M. Gardier), rh95@columbia.edu (R. Hen), BJEB@Lundbeck.com (B. Ebert), silkem@amgen.com (S. Miller), cs@Lundbeck.com (C. Sanchez), denis.david@u-psud.fr (D.J. David).
[1] Present address: Amgen Inc., One Amgen Center Dr., MS 29-2-B, Thousand Oaks, CA 91320, USA.

0028-3908/$ – see front matter © 2013 The Authors. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neuropharm.2013.05.014

localization at the pre- and post-synaptic levels. Both receptors modulate serotonergic neurotransmission (Gingrich and Hen, 2001; Guilloux et al., 2011). For instance, pindolol, a beta adrenoceptor blocker with 5-HT_{1A} receptor partial agonism, has shown some efficacy in augmentation strategies with SSRIs; however, the low doses used in clinical studies, its antagonistic action on 5-HT_{1B} heteroreceptors combined with its effects on post-synaptic 5-HT_{1A} receptors limits its efficacy (Guilloux et al., 2006; Whale et al., 2010; Martiny et al., 2012).

A link between the activity of antidepressant drugs and 5-HT_3 receptor function has been suggested since 5-HT_3 receptor antagonists administered alone exert antidepressant- and anxiolytic-like effects in preclinical settings (Costall and Naylor, 2004). Moreover, pretreatment with the 5-HT_3 receptor antagonist ondansetron potentiates the effects of antidepressant drugs in preclinical models (Redrobe and Bourin, 1997; Ramamoorthy et al., 2008). However, the preclinical observations were not confirmed in the few clinical studies that have been conducted. Thus, there is currently a weak support of 5-HT_3 antagonism alone or in combination with SSRI in the treatment of depression. Furthermore, whereas selective 5-HT_3 receptor antagonists are used routinely to attenuate nausea associated with chemotherapy, irradiation or cisplatin treatment, there are only few studies conducted showing that 5-HT_3 receptor antagonism reduces nausea in patients being treated with SSRIs (Bailey et al., 1995).

Early indications of an involvement of 5-HT_7 receptors in mood disorders came from a study showing down-regulation of 5-HT_7 receptor expression after chronic treatment with various antidepressants (Mullins et al., 1999; for a review, see Mnie-Filali et al., 2011). Recent studies further support a role for 5-HT_7 receptors in treating depression. Hence, SB-269970, a 5-HT_7 receptor antagonist, decreased immobility in both the tail suspension and forced swim tests (Guscott et al., 2005; Hedlund et al., 2005; Faure et al., 2006; Wesolowska et al., 2006a, 2006b) and enhanced the antidepressant-like effect of citalopram (Bonaventure et al., 2007; Sarkisyan et al., 2010). In agreement with these pharmacological data, 5-HT_7 receptor knockout mice showed reduced immobility in both the forced swim and the tail suspension tests (Hedlund et al., 2005). Thus, it appears that the efficacy of SSRIs may be enhanced by blocking feedback systems and modulating relevant receptors.

Vortioxetine (Lu AA21004; 1-[2-(2,4-dimethyl-phenylsulfanyl)-phenyl]-piperazine) is a novel investigational antidepressant with multimodal activity. Vortioxetine acts as an inhibitor at the 5-HT transporter (SERT, Ki = 1.6 nM) in recombinant cells expressing human receptors or SERT and as a 5-HT_3, 5-HT_7 and 5-HT_{1D} receptor antagonist (Ki = 3.7, 19 and 54 nM, respectively), a partial agonist at the 5-HT_{1B} receptor (Ki = 33 nM), an agonist at the 5-HT_{1A} receptor (Ki = 15 nM) (Bang-Andersen et al., 2011; Mork et al., 2012; Westrich et al., 2012). In rats the binding affinities are Ki = 1.1, 200, 3.7, 16 and 230 nM, for 5-HT_3, 5-HT_7, 5-HT_{1D}, 5-HT_{1B}, and 5-HT_{1A} receptors, respectively and Ki = 8.6 nM for the SERT (Mork et al., 2012; Westrich et al., 2012). *In vivo*, vortioxetine increases the extracellular levels of 5-HT, noradrenaline (NA), and dopamine (DA) in rat prefrontal cortex and hippocampus (Mork et al., 2012).

Although preclinical findings indicate that, acutely, vortioxetine produces an antidepressant and anxiolytic profile (Mork et al., 2012), the behavioural consequences of chronic administration have not been described. To investigate the effects of chronic vortioxetine treatment, as well as to confirm its anxiolytic- and antidepressant-like activities, we assessed its behavioural effects after acute (1 h) or repeated (14 or 21 days) dosing using the open field (OF) paradigm, the novelty suppressed feeding (NSF) paradigm and the mouse forced swim test (FST). OF and FST studies were conducted in Balb/cJ mice that have been shown to display a high basal anxiety- and depression-like behaviour (Belzung and Griebel, 2001). We confirmed the behavioural effects of 14 or 21 days of treatment with vortioxetine in 129S6/SvEvTac mice in the NSF paradigm. As stimulation of hippocampal neurogenesis has been suggested to underlie the delayed onset of therapeutic efficacy of SSRIs and tricyclic antidepressants (Duman et al., 1999; Malberg et al., 2000; Santarelli et al., 2003), we investigated the effects of vortioxetine dosed for 14 or 21 days on cell proliferation and maturation/survival in the dentate gyrus in 129S6/SvEvTac mice.

2. Methods

2.1. Animals

One hundred and eighty BALB/cJ@Rj male mice, 7–8 weeks old (25–30 g, Centre d'élevage Janvier, Le Genest-St-Isle, France) were used for the acute and repeated dosing experiments in the OF and FST. Eighty 129S6/SvEvTac male mice, 7–8 weeks old (25–30 g, Taconic Farms, Denmark) were used for the acute and repeated dosing NSF and the cell proliferation and survival/maturation study and Sholl analysis.

Mice were maintained under standard conditions (12/12 h light/dark cycle, lights on at 6AM, 22 ± 1 °C, food and water *ad libitum*, 5 mice/cage). The protocols involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (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 # 92-256B to DJD).

2.2. Drugs and treatment

2.2.1. Acute studies

Three doses of vortioxetine (2.5, 5 and 10 mg/kg, free base dissolved in 10% β-cyclodextrin, oral gavage, p.o.,) were used in the OF test, the NSF test and the FST. The effects of vortioxetine were compared to the vehicle control group (10% β-cyclodextrin) and also to a fluoxetine- (18 mg/kg p.o., (David et al., 2007)) and a diazepam-treated group (1.5 mg/kg, s.c. (David et al., 2007)). All doses were corrected for the weight of the salt. All treatments were administered 1 h before testing.

2.2.2. Chronic studies

Two doses of vortioxetine (5 and 20 mg/kg/day, free base dissolved in 10% β-cyclodextrin, oral gavage, p.o.) were tested in mice after 14 days of administration in the NSF and 21 days of administration in the OF test, the NSF test and the FST. The mice were tested 24 h after the last dose. The effects of vortioxetine were compared to a vehicle control group (10% β-cyclodextrin) and also to a fluoxetine-treated group (18 mg/kg/day p.o.).

2.3. Ex vivo SERT and 5-HT_3 receptor occupancy assays

Brains from mice treated with vehicle, fluoxetine, or vortioxetine (1 h after acute administration or 24 h after the 14th or 21st injection) were flash frozen, sectioned coronally using a cryostat, and then mounted on slides and frozen until use. Slices were 20 μm thick, and began at approximately +1.2 mm anterior from bregma for SERT receptor occupancy or −2.7 mm posterior from bregma for 5-HT_3 receptor occupancy determination (Franklin and Paxinos, 2008). Slides were stored for at least 24 h at −20 °C before use in autoradiography experiments.

2.3.1. Assessment of SERT occupancy

Slides were incubated at room temperature for 60 min in buffer (50 mM Tris–HCl, 150 mM NaCl, 5 mM KCl, pH = 7.4) containing 4.5 nM [^{3}H]-escitalopram. Nonspecific binding was determined using 1 μM escitalopram. Slides were washed briefly in cold buffer, dried, and exposed in a Beta imager for 16 h. The region of interest (ROI) for the SERT assay included the lateral and medial septum, the nucleus accumbens and the olfactory tubercle. An example image of the ROI for the SERT assay can be found in Supplementary Fig. 2A.

2.3.2. Assessment of 5-HT3 receptor occupancy

Slides were preincubated for 5 min in a buffer consisting of 50 mM Tris and 150 mM NaCl. Slides were dried under a stream of air for 30–45 min. Subsequently, slides were incubated at room temperature for 60 min in buffer (50 mM Tris–HCl, 150 mM NaCl, 5 mM KCl, pH = 7.4) containing 1 nM [^{3}H]LY278584 (Perkin-Elmer, USA). Nonspecific binding was determined using 1 μM ondansetron. Slides were washed briefly in cold buffer, dried, and exposed in a Beta imager for 24 h. The ROI for the 5-HT_3 receptor occupancy assay consisted of the hippocampus. An example image for the 5-HT_3 receptor occupancy assay can be found in Supplementary Fig. 2B.

2.4. Behavioural analysis

2.4.1. The open-field paradigm

Motor activity was quantified in Plexiglas OF boxes 43 × 43 cm (MED associates, Georgia, VT, USA) during a 10 min session (Popa et al., 2010). Two sets of 16 pulse-modulated infrared photobeams were placed on opposite walls 2.5 cm apart to record x–y ambulatory movements. Activity chambers were computer interfaced for data sampling at 100 ms resolution. The centre was defined as a 32 × 32 cm central arena. Dependent measures were: total time spent in the centre, the numbers of entries into the centre and distance travelled in the centre divided by total distance travelled. Overall motor activity was quantified as the total distance travelled (cm).

2.4.2. The novelty-suppressed feeding test

The NSF paradigm is a conflict test that elicits competing motivations: it does not only measure anxiety components of behaviour, but also measures the animal's motivation to eat the pellet. This implies that the NSF differs from anxiety-related tests such as the open field or the elevated plus maze paradigms. Briefly, animals were food-deprived for 24 h prior to the test. Testing was performed in a 50 × 50 × 20 cm box covered with bedding and illuminated by a 70 W lamp. The NSF test was carried out during a 5 min period as described by Santarelli et al. (2003). At the time of testing, a single pellet of food (regular chow) was placed on a white paper platform positioned in the centre of the box. Mice were tested individually after placing them in the corner of the box for 10 min. The latency to eating was timed. Immediately afterwards, the animal was transferred to its home cage and the amount of food consumed during the subsequent 5 min was measured, serving as a control for change in appetite as a possible confounding factor (home cage food consumption), because antidepressants are known to affect appetite.

2.4.3. The mouse forced swim test

The FST procedure was modified to enhance the sensitivity to detect the putative antidepressant-like activity of drugs (Rainer et al., 2012; Porsolt et al., 1977). Briefly, mice were placed into clear plastic buckets, 20 cm in diameter and 23 cm deep, filled up to two-thirds with water at ≈24 °C. Automated scoring was done using the automated X'PERT FST (Bioseb, Vitrolles, France). Dependent variables were mobility, swimming and climbing duration.

2.5. Immunohistochemistry

2.5.1. 5-Bromo-2-deoxyuridine (BrdU) labelling

2.5.1.1. Proliferation study. Mice were administered BrdU (150 mg/kg i.p.) 2 h before sacrifice and processed as described in David et al. (2009). BrdU-positive ($BrdU^+$) cells were counted using a BX51 microscope (Olympus, Germany).

2.5.1.2. Survival study. Mice were administered BrdU (150 mg/kg, i.p. b.i.d. for 3 days) 4 weeks before sacrifice. We then proceeded as described by Xia et al. (2012). $BrdU^+$ cells were counted under the microscope.

2.5.2. Doublecortin (DCX) labelling for maturation index study

The immunohistochemistry protocol was adapted from David et al. (2009). DCX-positive (DCX^+) cells were subcategorized according to their dendritic morphology: DCX^+ cells and DCX^+ cells with tertiary (or higher order) dendrites. The maturation index was defined as the ratio of DCX^+ cells possessing tertiary dendrites to the total number of DCX^+ cells.

2.6. Sholl analysis

For Sholl analysis, DCX^+ cells with tertiary, relatively untruncated dendritic branches were traced for each 35 μm hippocampal slice using Neurolucida software (MicroBrightField, Williston, VT) on an Olympus BX51 microscope equipped with a motorized stage device and ×100 immersion oil objective. DCX immunohistochemistry was done to maximize the labelling of dendrites. Sholl analysis for dendritic complexity was performed using the accompanying software (NeuroExplorer; MicroBrightField, version 10), calculating dendritic complexity including dendritic length and number of intersections (branch points).

2.7. Statistical analysis

For all experiments except the NSF, a one-way ANOVA was performed and results were expressed as mean ± SEM values. When main effects were significant, treatment comparisons were analysed using either PLSD (behaviour) or Tukey's post-hoc test. Unpaired *t*-test analysis was also used for planned comparisons. In the NSF test, a Kaplan–Meier survival analysis was applied due to the non-normal distribution of data, as described by Samuels and Hen (2012). Animals that did not eat during the 10-min test period were statistically censored. The Mantel–Cox log-rank test was used to evaluate differences between experimental groups. Complete description of statistics can be found in the Supplementals (Supplementary Table 1). Linear correlation analyses of latency values in the NSF vs. number DCX+ cells with tertiary dendrites were performed using the Pearson correlation coefficient "*R*".

For *ex vivo* autoradiography experiments, surface radioactivity (expressed as cpm/mm^2) was measured using Beta vision+ software version 2.0 (Biospace Lab, Paris, France). Radioactivity was quantified from a ROI defined *a priori* on the basis of receptor mapping experiments performed by this laboratory. This region remained consistent across each slice of tissue within a given assay. Specific binding was determined by subtracting nonspecific binding from total binding. Specific binding for each brain was normalized to the average specific-bound radioactivity from vehicle-treated individuals and expressed as a percentage of vehicle binding. These percentages were subtracted from 100% to obtain percent receptor occupancy.

Differences were considered significant when $p \leq 0.05$. All analyses were conducted using Statview 5.0 for IBM-compatible computers.

3. Results

3.1. SERT and 5-HT_3 receptor occupancy after acute and chronic treatment with vortioxetine in BALB/cJ and 129Sv mice

Acute dosing of vortioxetine (5 mg/kg and 10 mg/kg, p.o., 1 h) resulted in 60–70% occupancy of the SERT for both strains at the time of behavioural testing (Table 1). This was significantly lower than the approximately 90% SERT occupancy with fluoxetine ($p < 0.01$ and $p < 0.05$, compared to vortioxetine 5 and 10 mg/kg, respectively). Conversely, the same doses of vortioxetine resulted in >90% occupancy of the 5-HT_3 receptors, while no detectable occupancy was observed after fluoxetine administration (Table 2).

After chronic (daily for 14 or 21 days) administration of vortioxetine (5 or 20 mg/kg), practically no occupancy of the SERT was measurable at the time of behavioural and neurogenic assessment, i.e., 24 h after the last dose in both strains, whereas fluoxetine occupied >90% of the SERT at that time point (Table 1). Chronic dosing of vortioxetine, except in the high-dose BALB/cJ group, resulted in low non-significant occupancy of 5-HT_3 receptors (Table 2).

3.2. Vortioxetine produces acute antidepressant and anxiolytic activity in BALB/cJ and 129Sv mice

In the OF test in BALB/cJ mice (Fig. 1A–D), vortioxetine 2.5 and 5 mg/kg induced an anxiolytic-like effect similar to that of diazepam (1 mg/kg), characterized by an increase in the time spent in the centre (Fig. 1A: $F_{5,62} = 2.7$, $p < 0.05$; $p < 0.05$ for vortioxetine 2.5 mg/kg, $p = 0.053$ for vortioxetine 5 mg/kg; $p < 0.01$ for diazepam). Vortioxetine and diazepam also increased the number of entries (Fig. 1B $p < 0.05$ for vortioxetine 2.5 mg/kg or diazepam, unpaired *t*-test planned comparison) and the distance travelled in the centre (Fig. 1C $p < 0.01$ for diazepam; $p < 0.05$ for vortioxetine 2.5 and 5 mg/kg, unpaired *t*-test planned comparison). Vortioxetine at a higher dose (10 mg/kg) as well as fluoxetine had no significant effect on any of the measured parameters. Distance travelled in the periphery (Fig. 1D), an index of locomotion in a non-stressful environment, was unchanged for all treatments. Similarly, no effect of treatment was observed on overall locomotor activity (Supplementary Fig. 3).

In the FST in BALB/cJ mice (Fig. 1E–G), vortioxetine (5 mg/kg) significantly increased the mobility duration (Fig. 1E $p < 0.01$, unpaired *t*-test planned comparison), and significantly increased swimming (Fig. 1F $p < 0.01$, unpaired *t*-test planned comparison) and climbing behaviour (Fig. 1G $p < 0.05$, unpaired *t*-test planned comparison). Comparatively, fluoxetine also increased mobility (Fig. 1E $p < 0.01$, unpaired *t*-test planned comparison), apparently through an increase of swimming duration only (Fig. 1F $p < 0.05$, unpaired *t*-test planned comparison).

The 5- but not the 10-mg/kg dose of vortioxetine had an antidepressant/anxiolytic-like effect in the NSF test in 129Sv mice similar to that of diazepam (Fig. 2B: $F_{5,54} = 7.3$, $p < 0.0001$; $p < 0.01$ for diazepam or vortioxetine at 5 mg/kg). In contrast, fluoxetine

Table 1
Serotonin transporter occupancy in 129S6/SvEvTac and BALB/cJ mice after acute and chronic treatment. Serotonin transporter occupancy of vortioxetine and fluoxetine after acute (p.o., 1 h) or chronic (daily p.o. administration for 14 and 21 days and measurement 24 h after the last dose) in 129/SvEvTac and Balb/cJ mice. Data ($n = 3$–4 animals/group) are expressed in percentage of occupancy (mean ± SEM) and specific binding values in cpm/mm^2 (mean ± SEM).

Strain	Treatment		Duration					
			Acute		14 days		21 days	
			Occupancy (%)	Specific bound (cpm/mm^2)	Occupancy (%)	Specific bound (cpm/mm^2)	Occupancy (%)	Specific bound (cpm/mm^2)
129S6/SvEvTac	Vehicle		0 ± 4.0	11 ± 0.4	0 ± 5.9	9.7 ± 0.6	0 ± 5.4	10 ± 0.6
	Fluoxetine		91 ± 1.7***	1 ± 0.2***	ND	ND	98 ± 0.3***	0.13 ± 0.03***
	Vortioxetine	5 mg/kg/d	70 ± 2.9$^{***,\#\#}$	3.2 ± 0.3$^{***,\#\#}$	14 ± 10	8.4 ± 1	5.9 ± 2.2$^{\#\#\#}$	9.6 ± 0.2$^{\#\#\#}$
		20 mg/kg/d	ND	ND	−1.1 ± 0.6	9.8 ± 0.1	−0.4 ± 2.9$^{\#\#\#}$	10 ± 0.3$^{\#\#\#}$
BALB/cJ	Vehicle		0 ± 7.9	7.1 ± 0.6	ND	ND	0 ± 3.1	7.7 ± 0.2
	Fluoxetine		93 ± 1.6***	0.5 ± 0.1***	ND	ND	92 ± 0.4***	0.6 ± 0.03***
	Vortioxetine	5 mg/kg/d	63 ± 0.9$^{***,\#\#}$	2.6 ± 0.1$^{***,\#\#}$	ND	ND	0.6 ± 7.0$^{\#\#\#}$	7.6 ± 0.5$^{\#\#\#}$
		10 mg/kg/d	69 ± 3.4$^{***,\#}$	2.17 ± 0.2$^{***,\#}$	ND	ND	ND	ND
		20 mg/kg/d	ND	ND	ND	ND	−5.9 ± 4.1$^{\#\#\#}$	8.1 ± 0.3$^{\#\#\#}$

$^{***}p < 0.0001$, $^{**}p < 0.01$ and $^{*}p < 0.05$ when compared to the vehicle group, within strain and duration of treatment.
$^{\#\#\#}p < 0.0001$, $^{\#\#}p < 0.01$ and $^{\#}p < 0.05$ when compared to the fluoxetine group, within strain and duration of treatment.
ND: not determined.
Detailed statistical results are shown in Supplementary Table 1.

(18 mg/kg) induced no significant effect on latency to eat the pellet and tended to increase latency ($p = 0.057$). The results of the one-way ANOVA were confirmed by a Kaplan–Meier survival analysis followed by a Mantel–Cox log-rank test (Fig. 2A, $p < 0.01$ for diazepam and vortioxetine 5 mg/kg). Food consumption in the home cage was monitored after the behavioural test and displayed no differences between groups ($F_{5,54} = 1.2$, $p = 0.34$, Supplementary Fig. 4).

3.3. Chronic vortioxetine produced sustained antidepressant and anxiolytic activity in BALB/cJ and 129Sv mice

In BALB/cJ mice, chronic (21 days) administration of vortioxetine, 5 mg/kg per day and test 24 h after the last dose, resulted in an anxiolytic-like activity in the OF test characterized by increased time spent in the centre (Fig. 3A: $F_{3,48} = 3.1$, $p < 0.05$; $p < 0.01$ for vortioxetine 5 mg/kg), and increased number of entries (Fig. 3B $p < 0.05$ for vortioxetine 5 mg/kg, unpaired t-test planned comparison). Neither vortioxetine 20 mg/kg per day and test 24 h after the last dose or fluoxetine administration had a significant effect on the number of entries, the time spent in the centre or distance travelled. Distance travelled in the centre or in the periphery was unchanged in all treatment groups (Fig. 3C–D). Similarly, no effect of treatment was observed on overall locomotor activity (Supplementary Fig. 5).

In the FST in BALB/cJ mice (Fig. 3E–G), chronic (21 days) vortioxetine, 5 mg/kg per day, but not 20 mg/kg per day, significantly increased the mobility duration (Fig. 3E: $F_{3,42} = 4.6$, $p < 0.01$; $p < 0.01$ for vortioxetine 5 mg/kg). As in the acute study, the increase was due to a combination of increased duration of swimming (Fig. 3F: $F_{3,42} = 4.0$, $p < 0.05$; $p < 0.01$ for vortioxetine 5 mg/kg) and climbing behaviour (Fig. 3G $p < 0.05$ for vortioxetine 5 mg/kg, unpaired t-test, planned comparison). Fluoxetine had no significant effect on any of the parameters measured in this test ($p = 0.69$, $p = 0.49$ and $p = 0.89$, for mobility, swimming and climbing, respectively).

Vortioxetine, 20 mg/kg per day and test 24 h after last dose, administered for 14 but not 21 days, induced a significant reduction in the latency to feed in the NSF test in 129Sv mice (14 days: Fig. 4A: $p < 0.01$ Kaplan–Meier survival analysis followed by a Mantel–Cox log-rank test, confirmed at the trend level ($p = 0.067$) by one-way ANOVA in Fig. 4B. 21 days: $p = 0.37$, Fig. 4D). At a lower dose (5 mg/kg per day) and test 24 h after the last dose, vortioxetine reduced the latency to feed after 21 days of

Table 2
5-HT$_3$ receptor occupancy in 129S6/SvEvTac and BALB/cJ mice after acute and chronic treatment. 5-HT$_3$ receptor occupancy of vortioxetine and fluoxetine after acute (p.o., 1 h) or chronic (daily p.o. administration for 14 and 21 days and measurement 24 h after the last dose) in 129/SvEvTac and Balb/cJ ice. Data ($n = 3$–4 animals/group) are expressed in percentage of occupancy (mean ± SEM) and specific binding values in cpm/mm^2 (mean ± SEM).

Strain	Treatment		Duration					
			Acute		14 days		21 days	
			Occupancy (%)	Specific bound (cpm/mm^2)	Occupancy (%)	Specific bound (cpm/mm^2)	Occupancy (%)	Specific bound (cpm/mm^2)
129S6/SvEvTac	Vehicle		0 ± 4.2	0.09 ± 0.004	0 ± 6.8	0.1 ± 0.01	0 ± 11	0.11 ± 0.01
	Fluoxetine		−6.3 ± 9.4	0.1 ± 0.01	ND	ND	8 ± 9.3	0.097 ± 0.01
	Vortioxetine	5 mg/kg/d	94 ± 0.4$^{***,\#\#\#}$	0.005 ± 0.0004$^{***,\#\#\#}$	3 ± 13	0.1 ± 0.01	13 ± 10	0.092 ± 0.01
		20 mg/kg/d	ND	ND	8.2 ± 8.4$^{\#}$	0.96 ± 0.01$^{\#}$	19 ± 9.8	0.085 ± 0.01
BALB/cJ	Vehicle		0 ± 7.6	0.22 ± 0.02	ND	ND	0 ± 2.8	0.19 ± 0.01
	Fluoxetine		3.4 ± 9.2	0.21 ± 0.02	ND	ND	12 ± 3.2	0.16 ± 0.01
	Vortioxetine	5 mg/kg/d	92 ± 1.5$^{***,\#\#\#}$	0.016 ± 0.003$^{***,\#\#\#}$	ND	ND	−7 ± 5.3	0.20 ± 0.01
		10 mg/kg/d	97 ± 1.0$^{***,\#\#\#}$	0.005 ± 0.002$^{***,\#\#\#}$	ND	ND	ND	ND
		20 mg/kg/d	ND	ND	ND	ND	42 ± 15*	0.11 ± 0.03*

$^{***}p < 0.0001$, $^{**}p < 0.01$ and $^{*}p < 0.05$ when compared to the vehicle group, within strain and duration of treatment.
$^{\#\#\#}p < 0.0001$, $^{\#\#}p < 0.01$ and $^{\#}p < 0.05$ when compared to the fluoxetine group, within strain and duration of treatment.
ND: not determined.
Detailed statistical results are shown in Supplementary Table 1.

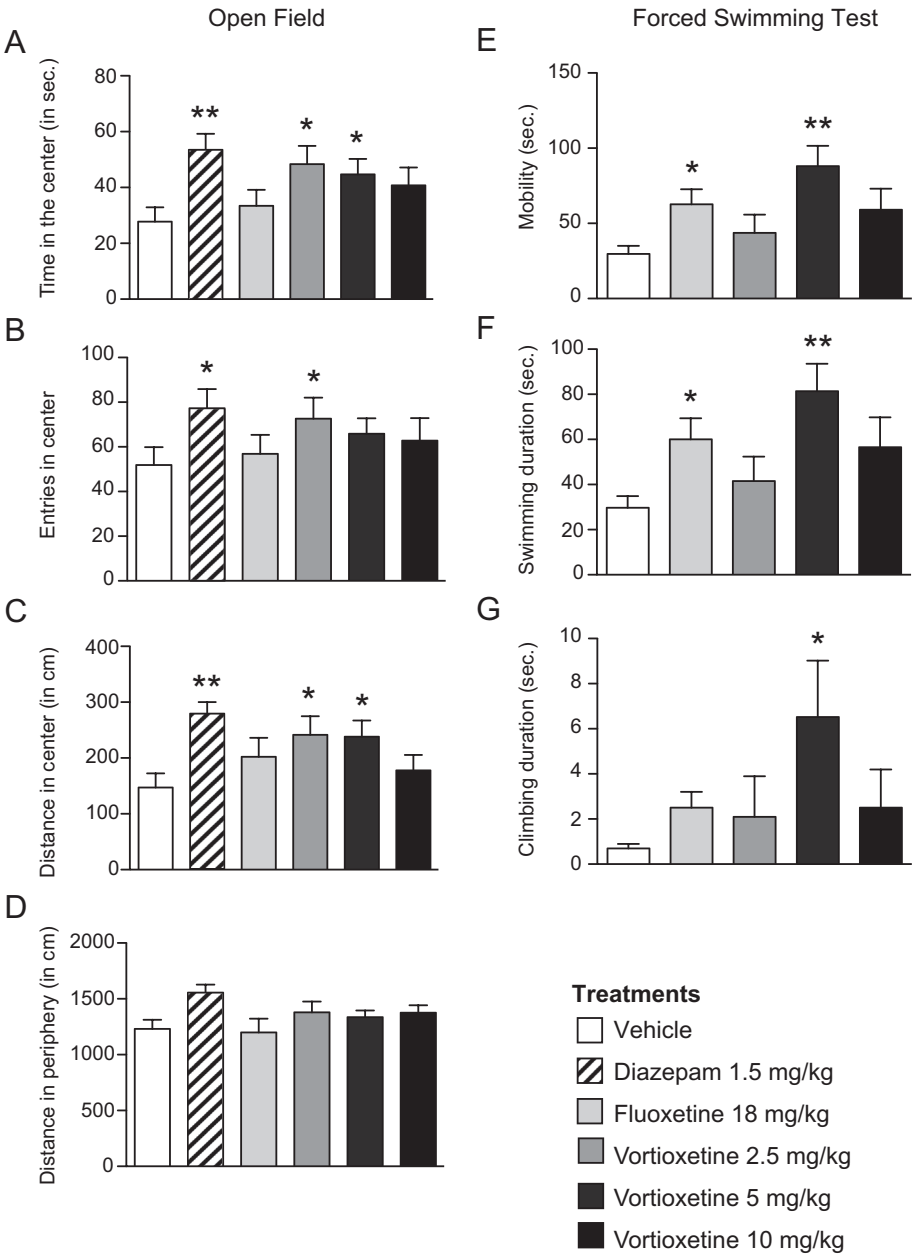

Fig. 1. Effects of acute vortioxetine treatment in the open field paradigm and the forced swim test in BALB/cJ@Rj mice. Behavioural effects of vortioxetine were studied after 1-h administration at doses of 2.5, 5 and 10 mg/kg, compared to those of vehicle, diazepam (1.5 mg/kg) and/or fluoxetine (10 mg/kg) in the open-field paradigm and the mouse forced swim test. Data are expressed as mean ± SEM. Anxiety was expressed as total of the time spent in the centre (**A**) and the number of entries in the centre (**B**) for the entire session. Locomotor activity was reported as total ambulatory distance travelled in the centre (**C**) and in the periphery (**D**) for the entire session. In the FST, mobility (**E**), swimming (**F**) and climbing (**G**) times were recorded. $^{*}p < 0.05$; $^{**}p < 0.01$ for drugs compared to the vehicle-treated group ($n = 10–12$ animals/group).

treatment (Fig. 4C: $p < 0.01$ using a Kaplan–Meier survival analysis followed by a Mantel–Cox log-rank test, confirmed by one-way ANOVA in Fig. 4D: $p < 0.05$). Fluoxetine, 18 mg/kg per day and test 24 h after the last dose, administered for 14 or 21 days failed to reduce the latency to eat significantly ($p = 0.22$ and $p = 0.90$ respectively). This lack of effect after 21 days of treatment has been previously observed (David et al., 2009), while longer duration of treatment induced reduction in latency in the NSF (Santarelli et al., 2003; Wang et al., 2008). Food consumption in the home cage was monitored after the behavioural tests and no effect of vortioxetine on food consumption was observed after both 14 and 21 days of treatment (Supplementary Fig. 6A–B and Supplementary Table 1).

3.4. Effect of chronic (21 days) vortioxetine on the various steps of adult hippocampal neurogenesis in 129Sv mice

Vortioxetine, 5 mg/kg per day and assessment 24 h after the last dose, significantly increased the number of $BrdU^{+}$ cells in the dentate gyrus of the hippocampus (Fig. 5A $p < 0.05$ for vortioxetine 5 mg/kg per day, unpaired *t*-test planned comparison; Supplementary Fig. 7). Furthermore, the same dosing regimen also increased the survival of newborn neurons (Fig. 5B $p < 0.05$ for vortioxetine 5 mg/kg, unpaired *t*-test planned comparison; Supplementary Fig. 8), an effect also seen with fluoxetine (18 mg/kg per day and assessment 24 h after the last dose) ($p < 0.05$, following PLSD *post-hoc* analysis). While the number of

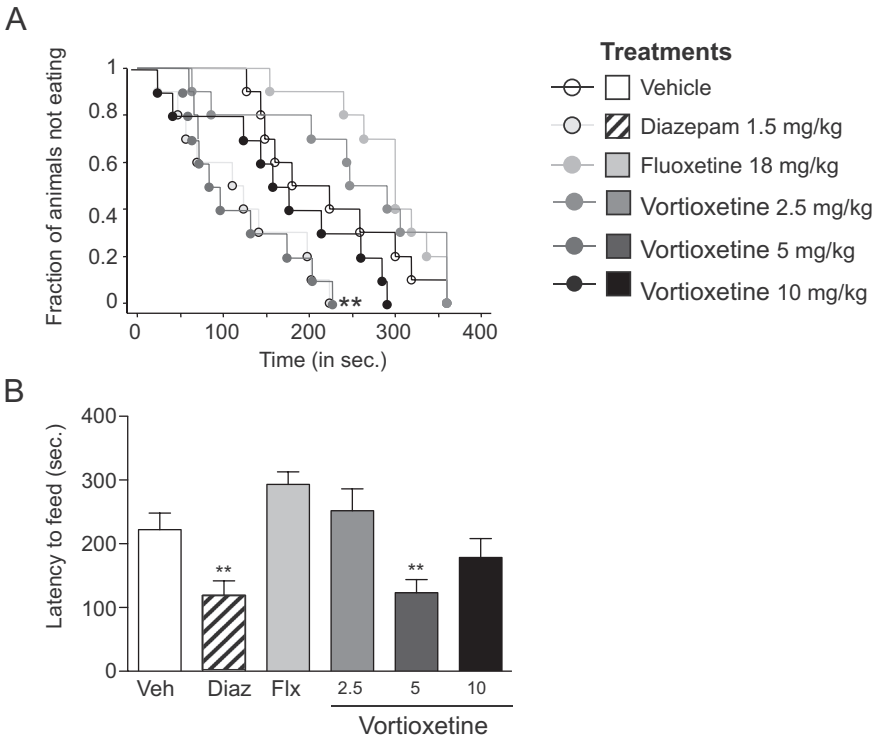

Fig. 2. Effects of acute vortioxetine treatment in the novelty suppressed feeding paradigm in 129/SvEvTac mice. The effects of vortioxetine were studied 1 h after administration at doses of 2.5, 5 and 10 mg/kg, compared to vehicle, fluoxetine (18 mg/kg) and diazepam (1.5 mg/kg). Data are expressed as cumulative survival with percentage of animals that have not eaten for over 10 min (**A**) and mean ± SEM of latency time to feed (**B**). $^{*}p < 0.05$; $^{**}p < 0.01$ for vortioxetine compared to the vehicle-treated group ($n = 15–20$ animals/group).

doublecortin-positive cells remained unchanged after all treatments (Fig. 5C, G), chronic vortioxetine (5 and 20 mg/kg per day) or fluoxetine increased the number of DCX^+ cells with tertiary dendrites (Fig. 5D: $F_{3,19} = 3.3$, $p < 0.05$ for fluoxetine and vortioxetine 5 and 20 mg/kg per day) and the maturation index (Fig. 5E: $F_{3,19} = 5.5$, $p < 0.05$ for fluoxetine and vortioxetine 5 mg/kg per day; $p < 0.0001$ for vortioxetine 20 mg/kg per day). The behavioural measurements in the NSF correlated with maturation and branching of neurons in the adult hippocampus across all drug-treated animals ($R = -0.44$, $p < 0.05$).

In line with the significant effect of vortioxetine 20 mg/kg per day in the NSF test after 14 days of treatment (Fig. 4A), this dosing regimen also had an effect on some stages of neurogenesis. Compared to 21 days of treatment, the number of doublecortin-positive cells was unchanged (Fig. 6A, $p > 0.05$ for all treatment; Supplementary Fig. 9), but the number of DCX^+ cells with tertiary dendrites was significantly increased (Fig. 6B: $p < 0.05$ for vortioxetine 20 mg/kg per day, unpaired *t*-test, planned comparison), as was the maturation index (Fig. 6C: $p < 0.05$ for vortioxetine 20 mg/kg per day, unpaired *t*-test planned comparison). After 21 days, behavioural measurements in the NSF were correlated with maturation and branching of neurons in the adult hippocampus across all drug-treated animals ($R = -0.70$, $p < 0.01$).

To further examine the effects of treatment on the dendritic morphology of newborn cells, we performed Sholl analyses on DCX^+ cells with tertiary dendrites (Fig. 6E). In the groups treated chronically with vortioxetine or fluoxetine, DCX^+ cells displayed increased dendritic length (Fig. 6E: $F_{3,12} = 19.1$, $p < 0.001$, $p < 0.0001$ for vortioxetine 20 mg/kg per day and $p < 0.05$ for fluoxetine) and increased number of intersections (Fig. 6F: $F_{3,12} = 17.3$, $p < 0.001$, $p < 0.0001$ for vortioxetine 20 mg/kg per day and $p < 0.01$ for fluoxetine).

4. Discussion

In this study performed in two different strains of mice, and using various behavioural test associated with emotion-related disorders and/or antidepressant treatment response, we found converging evidence for anxiolytic and antidepressant effects of vortioxetine, a novel antidepressant with multimodal activity, in preclinical models.

4.1. Behavioural effects of vortioxetine

4.1.1. Acute antidepressant/anxiolytic activity

An acute dose of vortioxetine corresponding to 60–70% occupancy of the SERT and >90% 5-HT_3 receptor occupancy decreased spontaneous (OF) and novelty-related (NSF) anxiety in naïve BALB/cJ and 129Sv mice, respectively, to the same extent as the benzodiazepine, diazepam. In line with previous observations, an acute dose of the SSRI, fluoxetine, was inactive at a dose corresponding to >90% SERT occupancy (Holmes and Rodgers, 2003). These observations support the hypothesis that the acute anxiolytic activity of vortioxetine is mediated by a mechanism that does not only rely on occupancy of the SERT. This is in line with the finding that vortioxetine has antidepressant effects in patients at doses where only approximately 50% of the SERT are occupied as shown in PET imaging studies (Areberg et al., 2012).

In both animal models, the anxiolytic effects of vortioxetine were not statistically significant at the highest dose (10 mg/kg) tested. This biphasic dose–response relation was not observed in previously reported acute studies in rat and mouse models of anxiety and depression, i.e., rat social interaction (significant effect at 2, 4 and 8 mg/kg, p.o., corresponding to up to 50% SERT occupancy), rat conditioned fear (significant effect at 3.9 and

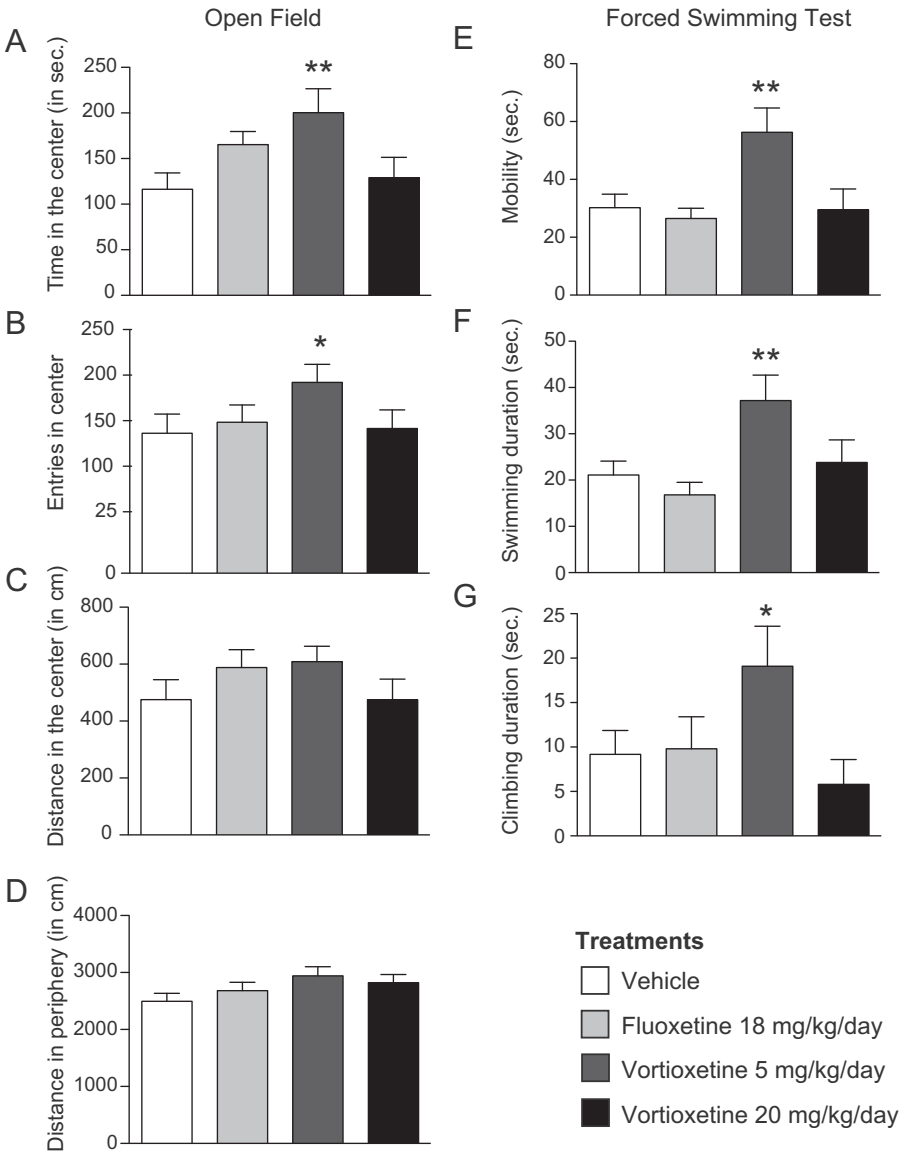

Fig. 3. Effects of chronic vortioxetine treatment in the open field paradigm and the forced swim test in BALB/cJ@Rj mice. Behavioural effects of vortioxetine were studied after 21 days of administration at doses of 5 and 20 mg/kg/day, compared to those of vehicle and fluoxetine (18 mg/kg/day) in the open-field paradigm or the mouse forced swim test. Data are expressed as mean ± SEM. Anxiety was expressed as total of the time spent in the centre (**A**) and the number of entries in the centre (**B**) for the entire session. Locomotor activity was reported as total ambulatory distance travelled in the centre (**C**) and in the periphery (**D**) for the entire session. In the FST, mobility (**E**), swimming (**F**) and climbing (**G**) times were recorded. $^{*}p < 0.05$; $^{**}p < 0.01$ for drugs compared to the vehicle-treated group ($n = 10$–12 animals/group).

7.9 mg/kg, s.c., corresponding to approximately 50 and 90% SERT occupancy) (Mork et al., 2012) and in an unpublished marble burying study in CD-1 mice (significant effects at 3.9 and 7.9 mg/kg, s.c.) (Sanchez, unpublished data). The underlying mechanism of the consistent biphasic dose response observed in BALB/cJ and 129Sv mice in the different models is not readily explained. Indeed, the mechanism of action of vortioxetine is complex, involving modulation of 5-HT receptors and SERT activity, resulting in modulation of several neurotransmitter systems (Mork et al., 2012; Pehrson et al., 2012). Interestingly, 5-HT_7 receptor antagonists have shown a biphasic-response in several anxiety paradigms (Wesolowska et al., 2006a, 2006b), while 5-HT_3 receptor blockade and 5-HT_{1A} receptor activation may be involved in dose-dependent anxiolytic effects (Zhang et al., 2001; Barrett and Gleeson, 1991). Moreover, significant strain differences have been described with respect to stress sensitivity, neurotransmitter tone and responses to antidepressants; e.g., BALB/cJ and 129Sv mice were responsive to fluoxetine, whereas CD-1 and NIH-Swiss mice were not (Lucki et al., 2001). Thus, complex and oppositely directed interactions between neurotransmitter systems and receptor mechanisms may be involved in the net effects of vortioxetine.

In the FST, a paradigm designed for screening of potential antidepressants (Rainer et al., 2012; Petit-Demouliere et al., 2005) an acute dose (5 mg/kg, p.o.) of vortioxetine corresponding to 60–70% SERT occupancy and >90% 5-HT_3 receptor occupancy showed an antidepressant-like activity in BALB/cJ mice. However, while similar SERT and 5-HT_3 occupancy was observed after vortioxetine at both doses tested, only the lowest dose (5 mg/kg) induced a statistically significant antidepressant effect in the FST. Fluoxetine, as well as the highest dose of vortioxetine increased the mobility only at the trend level ($p < 0.07$ and $p < 0.09$ respectively). An antidepressant-like effect was also observed in NIH-Swiss mice (significant effect at 15.9 mg/kg, s.c.)

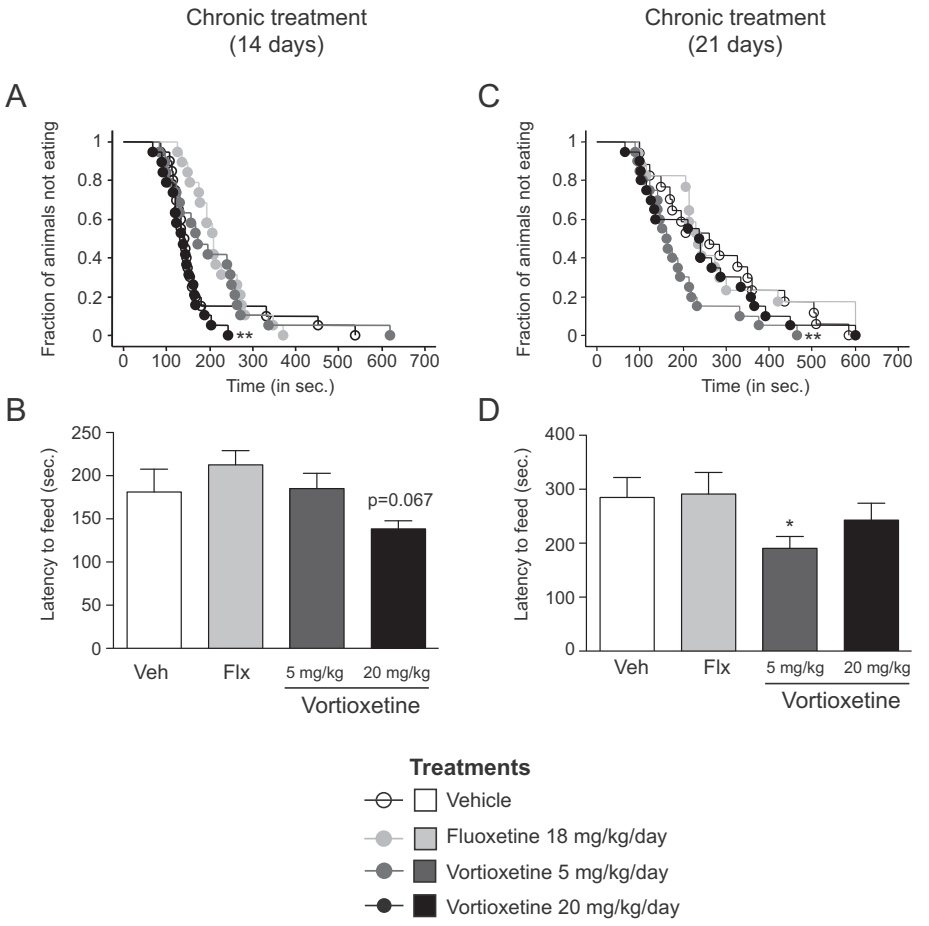

Fig. 4. Effects of chronic vortioxetine treatment in the novelty suppressed feeding paradigm in 129/SvEvTac mice. The effects of vortioxetine were studied after 14 (**A**–**B**) or 21 (**C**–**D**) days of administration at doses of 5 and 20 mg/kg/day, compared to those of vehicle and fluoxetine (18 mg/kg). Data are expressed as cumulative survival with percentage of animals that have not eaten over 10 min (**A & C**) and mean ± SEM of latency time to feed (**B & D**) $^{*}p < 0.05$; $^{**}p < 0.01$ for vortioxetine compared to the vehicle-treated group ($n = 15$–20 animals/group).

(Sanchez, unpublished data). Here, vortioxetine increases both climbing and swimming behaviour, effects related to increases in noradrenergic and serotonergic neurotransmissions, respectively, whereas fluoxetine (>90% SERT occupancy) only affected swimming behaviour. These results corroborate microdialysis experiments showing increases of both neurotransmitters after vortioxetine administration in rats (Mork et al., 2012). In that study, immobility was significantly reduced by acute treatment with 7.8 mg/kg vortioxetine, corresponding to ≈90% SERT occupancy. However, Mork and colleagues observed no specific effects of vortioxetine on swimming and climbing parameters in the Flinders Sensitive Line (FSL) rat model that was used in their study. This discrepancy with our results may be due to the model selected. Indeed, this line, selectively bred for high sensitivity to cholinergic agonism, also display changes in neurotransmitter systems, specifically a lower density of 5-HT_{1A} receptors and a higher density of 5-HT_{1B} receptors in several brain regions (Nishi et al., 2009; Wegener et al., 2011). While the low affinity of vortioxetine for rodent 5-HT_{1A} receptors is unlikely to explain the discrepancy between the findings of the present study in mice and the findings in FSL rats, its partial agonistic action at 5-HT_{1B} receptors may limit the increase in mobility in the FST, i.e., in a model of elevated 5-HT levels, a 5-HT_{1B} receptor partial agonistic effect may block effects on mobility, as observed in other studies (Gardier et al., 2001; Guilloux et al., 2006, 2011).

4.1.2. Chronic antidepressant/anxiolytic activity

Repeated daily dosing of vortioxetine (5 mg/kg, p.o.) for 14 days or 21 days in 129Sv and 21 days in BALB/cJ mice resulted in antidepressant/anxiolytic-like effects. Indeed, anxiolytic and/or antidepressant-like effect was observed in the in the OF paradigm, the NSF test and the FST, whereas the same dosing regimen with fluoxetine (18 mg/kg, p.o.) was inactive. As in the acute studies, a biphasic dose response was observed, as vortioxetine 20 mg/kg per day was not active.

The behavioural testing took place 24 h after the last dose, at which time fluoxetine had >90% SERT occupancy, while vortioxetine had comparatively low occupancies at SERT and 5-HT_3 receptors. Vortioxetine's low occupancies 24 h after the final chronic dose can be explained by its half-life. Indeed, experiments performed in rats showed that the half-life of fluoxetine and norfluoxetine (a potent SERT inhibitor (Owens et al., 1997) and fluoxetine's active metabolite) are about 5 and 15 h, respectively (Caccia et al., 1990). However, vortioxetine has a half-life around 3.2 h (Mork et al., 2012) and does not have an active metabolite on its different targets (Areberg et al., 2012).

The lack of antidepressant/anxiolytic effect of fluoxetine after 21 days dosing is compatible with previous experiences in BALB/cJ and 129SVeV strains, where 28 days of fluoxetine treatment are needed to achieve an effect (Wang et al., 2008; Dulawa et al., 2004). Furthermore, in contrast to benzodiazepines, the delayed onset of

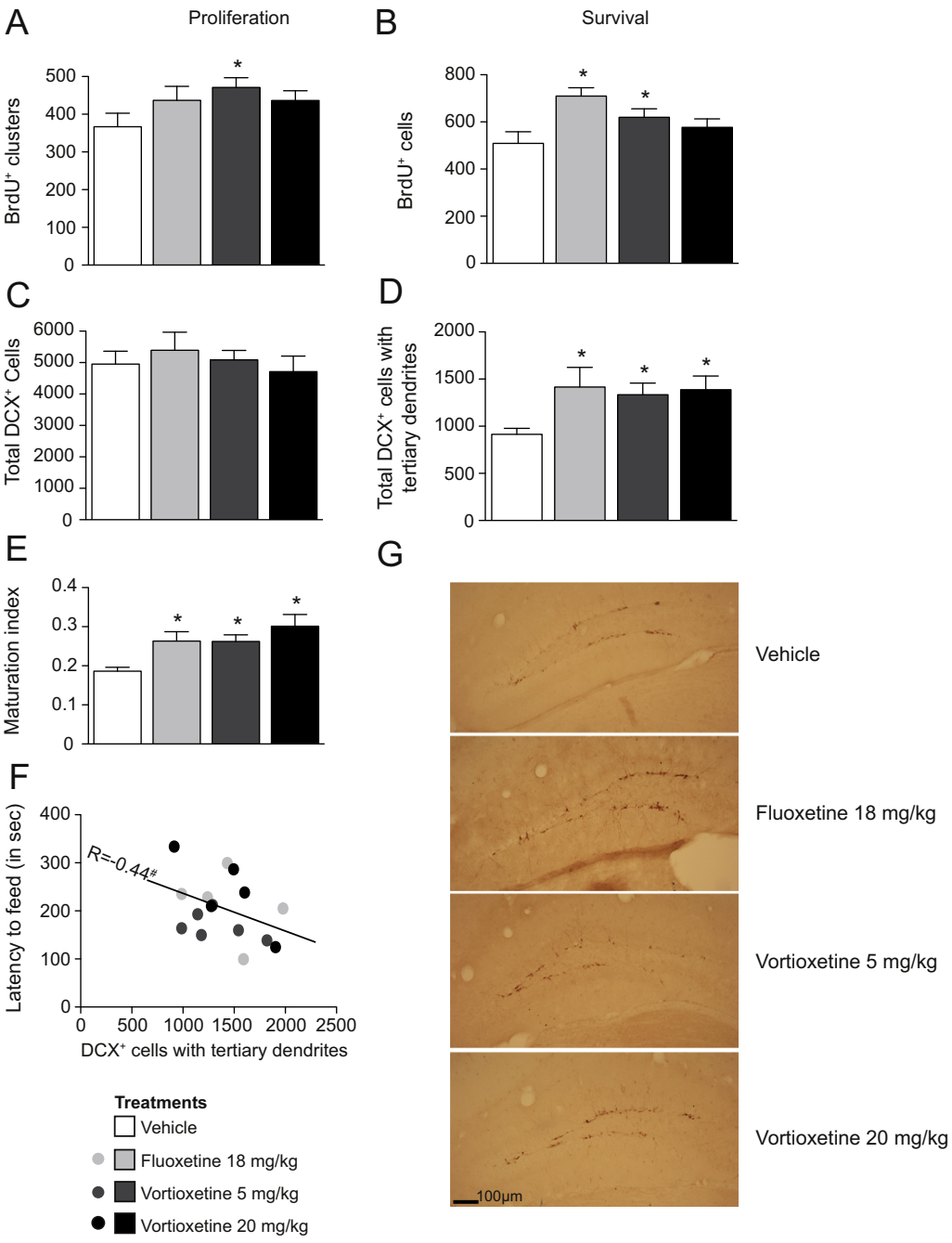

Fig. 5. Neurogenic effects of chronic vortioxetine administration (21 days) in 129/SvEvTac mice. The effects of 21 days of treatment with vortioxetine (5 and 20 mg/kg/day) on cell proliferation (**A**), cell survival (**B**) or cell maturation (**C**–**G**) were compared to those of vehicle and fluoxetine. Maturation was characterized by the total number of DCX^+ cells (**C**), the number of DCX^+ cells with tertiary dendrites (**D**) and the maturation index of newborn granule cells (**E**). Latency to feed in the NSF correlated with the number of DCX^+ cells with tertiary dendrites under pharmacological stimulation (**F**). Representative illustrations of doublecortin staining following chronic administration (21 days) of either fluoxetine (18 mg/kg/d) or vortioxetine (5 or 20 mg/kg/d) (**G**). Data are expressed as mean ± SEM. $^*p < 0.05$, for effects of fluoxetine or vortioxetine compared to vehicle ($n = 4$–10 animals per group). $\#p < 0.05$ for R Pearson value of correlation of latency values with number of DCX^+ cells with tertiary dendrites ($n = 16$).

anxiolytic effect of SSRIs in tests performed in mouse is consistent with the delayed onset in their clinical anxiolytic efficacy (Bespalov et al., 2010).

In conclusion, the findings indicate that the antidepressant activity of vortioxetine is mediated by a mechanism different from that of an SSRI. Overall, these findings may indicate that vortioxetine exerts its sustained antidepressant/anxiolytic activity by a mechanism that is not related to a constant SERT inhibition over time. Furthermore, vortioxetine is equally potent in two mouse strains with different 5-HT tone (Zhang et al., 2004), an effect that has not been observed with SSRIs (Kulikov et al., 2011; Cervo et al., 2005). Here, the lack of strain-dependent effects would support the hypothesis that mechanisms that are independent of 5-HT synthesis or 5-HT tissue content may be involved.

4.2. Neurogenic effects of vortioxetine

Antidepressant action has been putatively associated with changes in adult hippocampal neurogenesis (Airan et al., 2007; David et al., 2007, 2009; Santarelli et al., 2003; Wang et al., 2008).

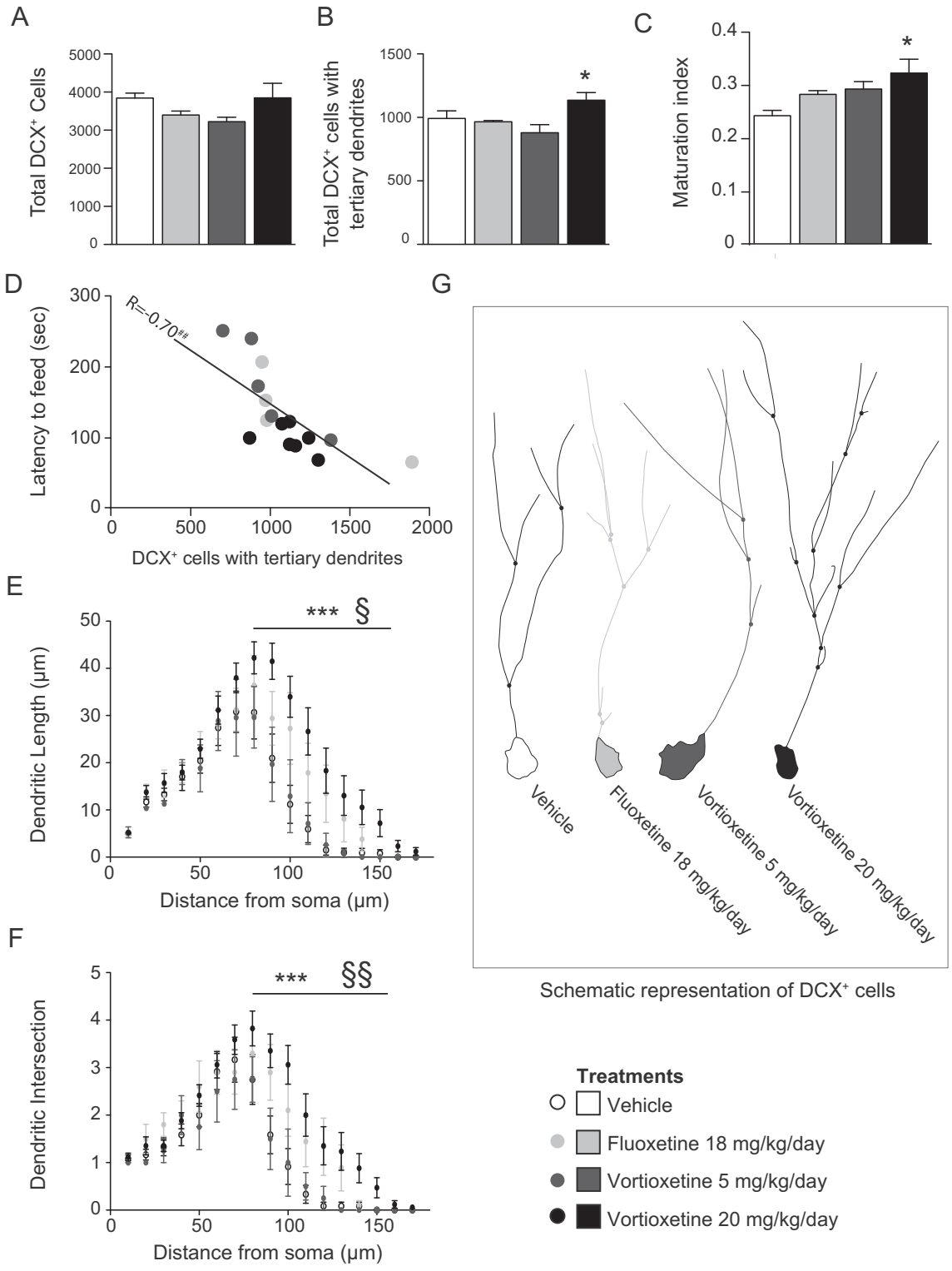

Fig. 6. Neurogenic effects of chronic vortioxetine administration (14 days) in 129/SvEvTac mice. The effects of 14-day treatment with vortioxetine (5 and 20 mg/kg/day) on cell maturation (**A**–**C**) and dendritic complexity (**E**–**G**) were compared to those of vehicle and fluoxetine. Maturation was characterized by the total number of DCX$^+$ cells (**A**), the number of DCX$^+$ cells with tertiary dendrites (**B**) and the maturation index of newborn granule cells (**C**) ($n = 4$–10 animals per group). Latency to feed in the NSF was correlated with number of DCX$^+$ cells with tertiary dendrites under pharmacological stimulation (**D**) ($p < 0.01$, $n = 16$). The effects of vortioxetine treatment on dendritic length (**E**) and the number of intersection (**F**) were measured ($n = 4$–5 mice/group, 4–8 cells/mouse) using a Sholl analysis of DCX$^+$ neurons (**G**). $^*p < 0.05$, $^{***}p < 0.0001$ for effects of vortioxetine compared to the vehicle-treated group ($n = 4$–10 animals per group). §$p < 0.05$, §§$p < 0.01$ for effects of fluoxetine compared to the vehicle-treated group ($n = 4$–10 animals per group). ##$p < 0.01$ for R Pearson value of correlation of latency values with number of DCX$^+$ cells with tertiary dendrites.

However, not all antidepressant effects are related to neurogenesis (David et al., 2009) and increased neurogenesis by itself in naïve animals does not induce antidepressant effects (Sahay et al., 2011). Thus, neurogenesis-related effects of antidepressants may be restricted to specific behaviours or symptoms that remain to be fully detailed.

Among all stages implicated in neurogenesis, maturation of young neurons is a crucial step for the functional integration of young neurons into neural circuits. Studies in rodents have shown that treatment with classical SSRIs such as fluoxetine and also novel antidepressants such as agomelatine can promote maturation of post-mitotic neurons (Rainer et al., 2012; Dagyte et al., 2010; Soumier et al., 2009; Wang et al., 2008). Here, we report that chronic vortioxetine (5 mg/kg, p.o. per day for 21 days) not only significantly increased cell proliferation within the hippocampus, but also increased the survival rate of $BrdU^+$ cells and the number of DCX^+ cells with tertiary dendrites. Fluoxetine (18 mg/kg per day p.o.) produced a similar effect in spite of its overall lack of effect in the NSF test. Similarly, in spite of a lack of an overall effect of vortioxetine at 20 mg/kg per day for 14 days in the NSF test, there was a significant increase in the number of DCX^+ cells with tertiary dendrites, dendritic length and the number of dendritic intersections at this early time point. Interestingly, irrespective of treatment and treatment duration (14 or 21 days), the latency to feed vs. the number of DCX^+ cells with tertiary dendrites were correlated. To our knowledge, this is the first time such a correlation has been observed.

It would be of interest to define which targets of vortioxetine contribute most to its neurogenic effect. There is a large body of evidence, including the present study, indicating that chronic SERT blockade is responsible for an increase in several steps of neurogenesis. Pharmacological stimulation of 5-HT_{1A} receptors increases cell proliferation, while its blockade induces opposite effects (Banasr et al., 2004; Klempin et al., 2010; Radley and Jacobs, 2002; Santarelli et al., 2003). 5-HT_{1B} receptor blockade has no effect on cell proliferation within the hippocampus, but pharmacological dissection of auto- vs. hetero-receptors in rats suggests that activation of the latter may contribute to cell proliferation (Banasr et al., 2004). Furthermore, activation by 5-HT_{1A} receptor agonists in rodents increased cell survival in a time-dependent manner (Klempin et al., 2010; Soumier et al., 2010), while its blockade had opposite effects (Klempin et al., 2010), reinforcing the present results.

Unfortunately, no study has yet observed the effects of chronic pharmacological blockade or stimulation of 5-HT_{1B} receptors on cell survival in the hippocampus in adult rodents.

Overall, while its precise mechanism of action remains to be detailed, vortioxetine produces a robust neurogenic effect that is compatible with its anxiolytic/antidepressant activity.

4.3. Comments and limitations

Here, we studied the effects of vortioxetine in selected strains of mice depending on the readout measured. Adult male 129S6/SvEvTac mice were used for the NSF study because of their sensitivity to chronic antidepressants in this behavioural model (Santarelli et al., 2003), and because the rate of hippocampal neurogenesis allows for study antidepressant-induced neurogenesis. Male BALB/cJ mice were used for the FST studies because of their high sensitivity to chronic antidepressant treatment in these models (Belzung and Griebel, 2001; Dulawa et al., 2004), and because 129S6/SvEVTac mice do not behave adequately in this paradigm.

Furthermore, neurogenesis results may differ depending on the strain used. Indeed, 2 strains may utilize different cellular and molecular machinery to mediate the neurogenic and behavioural effects of chronic antidepressant treatment, and thus pro-neurogenic effects of vortioxetine observed here in 129S6/SvEV-Tac mice may differ in BALB/cJ mice. However, recent reports show that female mice of the BALB/c and a 129 substrain (129SvJ) are quite similar on measures of basal adult neurogenesis including cell proliferation, survival, and neuronal differentiation, although the 129SvJ mice show slightly less survival 4 weeks after BrdU injection (Kempermann et al., 1997).

Interestingly, the 5 mg/kg dose of vortioxetine had a significant impact on animals' behaviour after 21 days exposure, but not after 14 days, while this was the opposite for the highest dose. One possible interpretation of these findings is that development of tolerance occurred at the highest dose administered. However, this needs to be confirmed in other strains of mice, or in appropriate animal models of major depressive disorders.

Indeed, behavioural and neurogenic effects of vortioxetine need to be confirmed in other model. For instance, a putative effect of vortioxetine after stress (unpredictable chronic mild stress [UCMS], social defeat) or neuroendocrine-induced alterations of animal behaviour should be assessed, since these models have shown good predictive validity. Furthermore, studies in genetic models lacking one or more of the targets involved in vortioxetine's mechanism of action would help to dissect the contributions of the individual targets to the overall effects.

5. Conclusion

Vortioxetine displays a sustained antidepressant and anxiolytic profile associated with increased neurogenesis at several stages. The effect of vortioxetine was not associated with SERT occupancy, suggesting that its mechanism of action is different from that of an SSRI.

Conflict of interests

This work received financial support from Lundbeck and the Takeda Pharmaceutical Company, Ltd.

Denis David currently receives investigator-initiated research support from Lundbeck and served as a consultant in the areas of target identification and validation and new compound development to Lundbeck and Servier in 2011–12.

Bruno Guiard currently receives investigator-initiated research support from Neurosearch and served as a consultant in the areas of target identification and validation and new compound development to Lundbeck and Servier in 2011–12.

Acknowledgements

This work was supported by the French Ministry of Higher Education and Research and H Lundbeck. We thank the animal care facility of the "Institut Fédératif de Recherche-IFR141" of the Paris Sud University for their technical assistance.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.neuropharm.2013.05.014.

References

Airan, R.D., Meltzer, L.A., Roy, M., Gong, Y., Chen, H., Deisseroth, K., 2007. High-speed imaging reveals neurophysiological links to behavior in an animal model of depression. Science 317, 819–823.

Areberg, J., Luntang-Jensen, M., Sogaard, B., Nilausen, D.O., 2012. Occupancy of the serotonin transporter after administration of Lu AA21004 and its relation to

plasma concentration in healthy subjects. Basic Clin. Pharmacol. Toxicol. 110, 401–404.
Artigas, F., Adell, A., Celada, P., 2006. Pindolol augmentation of antidepressant response. Curr. Drug Targets 7, 139–147.
Bailey, J.E., Potokar, J., Coupland, N., Nutt, D.J., 1995. The 5-HT3 antagonist ondansetron reduces gastrointestinal side effects induced by a specific serotonin reuptake inhibitor in man. J. Psychopharmacol. (Oxf.) 9, 137–141.
Banasr, M., Hery, M., Printemps, R., Daszuta, A., 2004. Serotonin-induced increases in adult cell proliferation and neurogenesis are mediated through different and common 5-HT receptor subtypes in the dentate gyrus and the subventricular zone. Neuropsychopharmacology 29, 450–460.
Bang-Andersen, B., Ruhland, T., Jorgensen, M., Smith, G., Frederiksen, K., Jensen, K.G., Zhong, H., Nielsen, S.M., Hogg, S., Mork, A., Stensbol, T.B., 2011. Discovery of 1-[2-(2,4-dimethylphenylsulfanyl)phenyl]piperazine (Lu AA21004): a novel multimodal compound for the treatment of major depressive disorder. J. Med. Chem. 54, 3206–3221.
Barrett, J.E., Gleeson, S., 1991. Anxiolytic effects of 5-HT1A agonists, 5-HT3 antagonists and benzodiazepines: conflict and drug discrimination studies. In: Rogers, R.J., Cooper, S.J. (Eds.), 5-HT1A Agonists, 5-HT3 Antagonists and Benzodiazepines: Their Comparative Behavioural Pharmacology. Wiley & Sons Ltd, Chichester, pp. 59–105.
Belzung, C., Griebel, G., 2001. Measuring normal and pathological anxiety-like behaviour in mice: a review. Behav. Brain Res. 125, 141–149.
Bespalov, A.Y., van Gaalen, M.M., Gross, G., 2010. Antidepressant treatment in anxiety disorders. Curr. Top. Behav. Neurosci. 2, 361–390.
Bonaventure, P., Kelly, L., Aluisio, L., Shelton, J., Lord, B., Galici, R., Miller, K., Atack, J., Lovenberg, T.W., Dugovic, C., 2007. Selective blockade of 5-hydroxytryptamine (5-HT)7 receptors enhances 5-HT transmission, antidepressant-like behavior, and rapid eye movement sleep suppression induced by citalopram in rodents. J. Pharmacol. Exp. Ther. 321, 690–698.
Caccia, S., Cappi, M., Fracasso, C., Garattini, S., 1990. Influence of dose and route of administration on the kinetics of fluoxetine and its metabolite norfluoxetine in the rat. Psychopharmacology (Berl.) 100, 509–514.
Cervo, L., Canetta, A., Calcagno, E., Burbassi, S., Sacchetti, G., Caccia, S., Fracasso, C., Albani, D., Forloni, G., Invernizzi, R.W., 2005. Genotype-dependent activity of tryptophan hydroxylase-2 determines the response to citalopram in a mouse model of depression. J. Neurosci. 25, 8165–8172.
Costall, B., Naylor, R.J., 2004. 5-HT3 receptors. Current drug targets. CNS Neurol. Disord. 3, 27–37.
Dagyte, G., Trentani, A., Postema, F., Luiten, P.G., Den Boer, J.A., Gabriel, C., Mocaer, E., Meerlo, P., Van der Zee, E.A., 2010. The novel antidepressant agomelatine normalizes hippocampal neuronal activity and promotes neurogenesis in chronically stressed rats. CNS Neurosci. Ther. 16, 195–207.
David, D.J., Klemenhagen, K.C., Holick, K.A., Saxe, M.D., Mendez, I., Santarelli, L., Craig, D.A., Zhong, H., Swanson, C.J., Hegde, L.G., Ping, X.I., Dong, D., Marzabadi, M.R., Gerald, C.P., Hen, R., 2007. Efficacy of the MCHR1 antagonist N-[3-(1-{[4-(3,4-difluorophenoxy)phenyl]methyl}(4-piperidyl))-4-methylphenyl]-2-m ethylpropanamide (SNAP 94847) in mouse models of anxiety and depression following acute and chronic administration is independent of hippocampal neurogenesis. J. Pharmacol. Exp. Ther. 321, 237–248.
David, D.J., Samuels, B.A., Rainer, Q., Wang, J.W., Marsteller, D., Mendez, I., Drew, M., Craig, D.A., Guiard, B.P., Guilloux, J.P., Artymyshyn, R.P., Gardier, A.M., Gerald, C., Antonijevic, I.A., Leonardo, E.D., Hen, R., 2009. Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. Neuron 62, 479–493.
Dulawa, S.C., Holick, K.A., Gundersen, B., Hen, R., 2004. Effects of chronic fluoxetine in animal models of anxiety and depression. Neuropsychopharmacology 29, 1321–1330.
Duman, R.S., Malberg, J., Thome, J., 1999. Neural plasticity to stress and antidepressant treatment. Biol. Psychiatry 46, 1181–1191.
Faure, C., Mnie-Filali, O., Scarna, H., Debonnel, G., Haddjeri, N., 2006. Effects of the 5-HT7 receptor antagonist SB-269970 on rat hormonal and temperature responses to the 5-HT1A/7 receptor agonist 8-OH-DPAT. Neurosci. Lett. 404, 122–126.
Franklin, K.B.J., Paxinos, G., 2008. The Mouse Brain in Stereotaxic Coordinates. Academic Press, Amsterdam; Boston.
Gardier, A.M., Trillat, A.C., Malagie, I., David, D., Hascoet, M., Colombel, M.C., Jolliet, P., Jacquot, C., Hen, R., Bourin, M., 2001. [5-HT1B serotonin receptors and antidepressant effects of selective serotonin reuptake inhibitors]. C. R. Acad. Sci. III 324, 433–441.
Gingrich, J.A., Hen, R., 2001. Dissecting the role of the serotonin system in neuropsychiatric disorders using knockout mice. Psychopharmacology (Berl.) 155, 1–10.
Guilloux, J.P., David, D.J., Guiard, B.P., Chenu, F., Reperant, C., Toth, M., Bourin, M., Gardier, A.M., 2006. Blockade of 5-HT1A receptors by (+/−)-pindolol potentiates cortical 5-HT outflow, but not antidepressant-like activity of paroxetine: microdialysis and behavioral approaches in 5-HT1A receptor knockout mice. Neuropsychopharmacology 31, 2162–2172.
Guilloux, J.P., David, D.J., Xia, L., Nguyen, H.T., Rainer, Q., Guiard, B.P., Reperant, C., Deltheil, T., Toth, M., Hen, R., Gardier, A.M., 2011. Characterization of 5-HT(1A/1B)−/− mice: an animal model sensitive to anxiolytic treatments. Neuropharmacology 61, 478–488.
Guilloux, J.P., David, D.J., Samuels, B.A., David, I., Gardier, A.M., Guiard, B.P., 2012. Non-response to Initial Antidepressant Therapy. Psychology. InTech, Rijeka, Croatia.
Guscott, M., Bristow, L.J., Hadingham, K., Rosahl, T.W., Beer, M.S., Stanton, J.A., Bromidge, F., Owens, A.P., Huscroft, I., Myers, J., Rupniak, N.M., Patel, S., Whiting, P.J., Hutson, P.H., Fone, K.C., Biello, S.M., Kulagowski, J.J., McAllister, G., 2005. Genetic knockout and pharmacological blockade studies of the 5-HT7 receptor suggest therapeutic potential in depression. Neuropharmacology 48, 492–502.
Hamon, M., Bourgoin, S., 2006. Pharmacological profile of antidepressants: a likely basis for their efficacy and side effects? Eur. Neuropsychopharmacol. 16, S625–S632.
Hedlund, P.B., Huitron-Resendiz, S., Henriksen, S.J., Sutcliffe, J.G., 2005. 5-HT7 receptor inhibition and inactivation induce antidepressantlike behavior and sleep pattern. Biol. Psychiatry 58, 831–837.
Holmes, A., Rodgers, R.J., 2003. Prior exposure to the elevated plus-maze sensitizes mice to the acute behavioral effects of fluoxetine and phenelzine. Eur. J. Pharmacol. 459, 221–230.
Kennedy, S.H., Young, A.H., Blier, P., 2011. Strategies to achieve clinical effectiveness: refining existing therapies and pursuing emerging targets. J. Affect. Disord. 132 (Suppl. 1), S21–S28.
Kempermann, G., Kuhn, H.G., Gage, F.H., 1997. Genetic influence on neurogenesis in the dentate gyrus of adult mice. Proc. Natl. Acad. Sci. U. S. A. 94, 10409–10414.
Kessler, R.C., Berglund, P., Demler, O., Jin, R., Merikangas, K.R., Walters, E.E., 2005. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch. Gen. Psychiatry 62, 593–602.
Klempin, F., Babu, H., De Pietri Tonelli, D., Alarcon, E., Fabel, K., Kempermann, G., 2010. Oppositional effects of serotonin receptors 5-HT1a, 2, and 2c in the regulation of adult hippocampal neurogenesis. Front. Mol. Neurosci. 3.
Kulikov, A.V., Tikhonova, M.A., Osipova, D.V., Kulikov, V.A., Popova, N.K., 2011. Association between tryptophan hydroxylase-2 genotype and the antidepressant effect of citalopram and paroxetine on immobility time in the forced swim test in mice. Pharmacol. Biochem. Behav. 99, 683–687.
Lucki, I., Dalvi, A., Mayorga, A.J., 2001. Sensitivity to the effects of pharmacologically selective antidepressants in different strains of mice. Psychopharmacology (Berl.) 155, 315–322.
Malberg, J.E., Eisch, A.J., Nestler, E.J., Duman, R.S., 2000. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J. Neurosci. 20, 9104–9110.
Martiny, K., Lunde, M., Bech, P., Plenge, P., 2012. A short-term double-blind randomized controlled pilot trial with active or placebo pindolol in patients treated with venlafaxine for major depression. Nord. J. Psychiatry 66, 147–154.
Mnie-Filali, O., Faure, C., Lambas-Senas, L., El Mansari, M., Belblidia, H., Gondard, E., Etievant, A., Scarna, H., Didier, A., Berod, A., Blier, P., Haddjeri, N., 2011. Pharmacological blockade of 5-HT7 receptors as a putative fast acting antidepressant strategy. Neuropsychopharmacology 36, 1275–1288.
Mork, A., Pehrson, A., Brennum, L.T., Nielsen, S.M., Zhong, H., Lassen, A.B., Miller, S., Westrich, L., Boyle, N.J., Sanchez, C., Fischer, C.W., Liebenberg, N., Wegener, G., Bundgaard, C., Hogg, S., Bang-Andersen, B., Stensbol, T.B., 2012. Pharmacological effects of Lu AA21004: a novel multimodal compound for the treatment of major depressive disorder. J. Pharmacol. Exp. Ther. 340, 666–675.
Mullins, U.L., Gianutsos, G., Eison, A.S., 1999. Effects of antidepressants on 5-HT7 receptor regulation in the rat hypothalamus. Neuropsychopharmacology 21, 352–367.
Nishi, K., Kanemaru, K., Diksic, M., 2009. A genetic rat model of depression, Flinders sensitive line, has a lower density of 5-HT(1A) receptors, but a higher density of 5-HT(1B) receptors, compared to control rats. Neurochem. Int. 54, 299–307.
Owens, M.J., Morgan, W.N., Plott, S.J., Nemeroff, C.B., 1997. Neurotransmitter receptor and transporter binding profile of antidepressants and their metabolites. J. Pharmacol. Exp. Ther. 283, 1305–1322.
Pehrson, A.L., Cremers, T., Betry, C., van der Hart, M.G., Jorgensen, L., Madsen, M., Haddjeri, N., Ebert, B., Sanchez, C., 2012. Lu AA21004, a novel multimodal antidepressant, produces regionally selective increases of multiple neurotransmitters-A rat microdialysis and electrophysiology study. Eur. Neuropsychopharmacol. 23 (2), 133–145.
Petit-Demouliere, B., Chenu, F., Bourin, M., 2005. Forced swimming test in mice: a review of antidepressant activity. Psychopharmacology (Berl.) 177, 245–255.
Popa, D., Cerdan, J., Reperant, C., Guiard, B.P., Guilloux, J.P., David, D.J., Gardier, A.M., 2010. A longitudinal study of 5-HT outflow during chronic fluoxetine treatment using a new technique of chronic microdialysis in a highly emotional mouse strain. Eur. J. Pharmacol. 628, 83–90.
Porsolt, R.D., Bertin, A., Jalfre, M., 1977. Behavioral despair in mice: a primary screening test for antidepressants. Arch. Int. Pharmacodyn. Ther. 229, 327–336.
Radley, J.J., Jacobs, B.L., 2002. 5-HT1A receptor antagonist administration decreases cell proliferation in the dentate gyrus. Brain Res. 955, 264–267.
Rainer, Q., Xia, L., Guilloux, J.P., Gabriel, C., Mocaer, E., Hen, R., Enhamre, E., Gardier, A.M., David, D.J., 2012. Beneficial behavioural and neurogenic effects of agomelatine in a model of depression/anxiety. Int. J. Neuropsychopharmacol. 15, 321–335.
Ramamoorthy, R., Radhakrishnan, M., Borah, M., 2008. Antidepressant-like effects of serotonin type-3 antagonist, ondansetron: an investigation in behaviour-based rodent models. Behav. Pharmacol. 19, 29–40.
Redrobe, J.P., Bourin, M., 1997. Partial role of 5-HT2 and 5-HT3 receptors in the activity of antidepressants in the mouse forced swimming test. Eur. J. Pharmacol. 325, 129–135.
Rush, A.J., Trivedi, M.H., Wisniewski, S.R., Nierenberg, A.A., Stewart, J.W., Warden, D., Niederehe, G., Thase, M.E., Lavori, P.W., Lebowitz, B.D., McGrath, P.J., Rosenbaum, J.F., Sackeim, H.A., Kupfer, D.J., Luther, J., Fava, M., 2006. Acute and

longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. Am. J. Psychiatry 163, 1905–1917.
Sahay, A., Scobie, K.N., Hill, A.S., O'Carroll, C.M., Kheirbek, M.A., Burghardt, N.S., Fenton, A.A., Dranovsky, A., Hen, R., 2011. Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. Nature 472, 466–470.
Samuels, B.A., Hen, R., 2012. Novelty-suppressed feeding in the mouse. In: Gould, T.D. (Ed.), Mood and Anxiety Related Phenotypes in Mice. Characterization Using Behavioral Tests. Springer, pp. 107–121.
Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O., Belzung, C., Hen, R., 2003. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301, 805–809.
Sarkisyan, G., Roberts, A.J., Hedlund, P.B., 2010. The 5-HT(7) receptor as a mediator and modulator of antidepressant-like behavior. Behav. Brain Res. 209, 99–108.
Soumier, A., Banasr, M., Goff, L.K., Daszuta, A., 2010. Region- and phase-dependent effects of 5-HT(1A) and 5-HT(2C) receptor activation on adult neurogenesis. Eur. Neuropsychopharmacol. 20, 336–345.
Soumier, A., Banasr, M., Lortet, S., Masmejean, F., Bernard, N., Kerkerian-Le-Goff, L., Gabriel, C., Millan, M.J., Mocaer, E., Daszuta, A., 2009. Mechanisms contributing to the phase-dependent regulation of neurogenesis by the novel antidepressant, agomelatine, in the adult rat hippocampus. Neuropsychopharmacology 34, 2390–2403.
Wang, J.W., David, D.J., Monckton, J.E., Battaglia, F., Hen, R., 2008. Chronic fluoxetine stimulates maturation and synaptic plasticity of adult-born hippocampal granule cells. J. Neurosci. 28, 1374–1384.
Wegener, G., Finger, B.C., Elfving, B., Keller, K., Liebenberg, N., Fischer, C.W., Singewald, N., Slattery, D.A., Neumann, I.D., Mathe, A.A., 2011. Neuropeptide S alters anxiety, but not depression-like behaviour in Flinders Sensitive Line rats: a genetic animal model of depression. Int. J. Neuropsychopharmacol., 1–13.
Wesolowska, A., Nikiforuk, A., Stachowicz, K., 2006a. Potential anxiolytic and antidepressant effects of the selective 5-HT7 receptor antagonist SB 269970 after intrahippocampal administration to rats. Eur. J. Pharmacol. 553, 185–190.
Wesolowska, A., Nikiforuk, A., Stachowicz, K., Tatarczynska, E., 2006b. Effect of the selective 5-HT7 receptor antagonist SB 269970 in animal models of anxiety and depression. Neuropharmacology 51, 578–586.
Westrich, L., Pehrson, A., Zhong, H., Nielsen, S.M., Frederiksen, K., Stensbøl, T.B., Boyle, N., Hentzer, M., Sanchez, C., 2012. In vitro and in vivo effects of the multimodal antidepressant vortioxetine (Lu AA21004) at human and rat targets. Int. J. Psychiatry Clin. Pract. 16 (Suppl. 1), 47.
Whale, R., Terao, T., Cowen, P., Freemantle, N., Geddes, J., 2010. Pindolol augmentation of serotonin reuptake inhibitors for the treatment of depressive disorder: a systematic review. J. Psychopharmacol. (Oxf.) 24, 513–520.
Xia, L., Delomenie, C., David, I., Rainer, Q., Marouard, M., Delacroix, H., David, D.J., Gardier, A.M., Guilloux, J.P., 2012. Ventral hippocampal molecular pathways and impaired neurogenesis associated with 5-HT(1)A and 5-HT(1)B receptors disruption in mice. Neurosci. Lett. 521, 20–25.
Zhang, X., Beaulieu, J.M., Sotnikova, T.D., Gainetdinov, R.R., Caron, M.G., 2004. Tryptophan hydroxylase-2 controls brain serotonin synthesis. Science 305, 217.
Zhang, Z.J., Schmidt, D.E., de Paulis, T., Trivedi, B.L., Onaivi, E.S., Ebert, M.H., Hewlett, W.A., 2001. Anxiolytic-like effects of DAIZAC, a selective high-affinity 5-HT(3) receptor antagonist, in the mouse elevated plus-maze. Pharmacol. Biochem. Behav. 69, 571–578.