Can we increase speed and efficacy of antidepressant treatments?

Part I: General aspects and monoamine-based strategies

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Running title. Antidepressant improvement: Monoaminergic strategies

Abstract. Major depressive disorder (MDD) is a severe psychiatric syndrome with high prevalence and socioeconomic impact. Current antidepressant treatments are based on the blockade of serotonin (5-hydroxytryptamine, 5-HT) and/or noradrenaline transporters. These drugs show slow onset of clinical action and limited efficacy, partly due to the activation of physiological negative feed-back mechanisms operating through autoreceptors (5-HT_{1A}, 5-HT_{1B}, α_2 -adrenoceptors) and postsynaptic receptors (e.g., 5-HT₃). As a result, clinically-relevant doses of reuptake inhibitors increase extracellular (active) 5-HT concentrations in the midbrain raphe nuclei but not in forebrain, as indicated by rodent microdialysis studies and by PET-scan studies in primate/human brain. The prevention of these self-inhibitory mechanisms by antagonists of the above receptors augments preclinical and clinical antidepressant effects. Hence, the mixed β -adrenoceptor/5-HT_{1A} antagonist pindolol accelerated, and in some cases enhanced, the clinical action of selective serotonin reuptake inhibitors (SSRI). This strategy has been incorporated into two new multi-target antidepressant drugs, vilazodone and vortioxetine, which combine 5-HT reuptake inhibition and partial agonism at 5-HT_{1A} receptors. Vortioxetine shows also high affinity for other 5-HT receptors, including excitatory 5-HT₃ receptors located in cortical and hippocampal GABA interneurons. 5-HT₃ receptor blockade by vortioxetine enhances pyramidal neuron activity in prefrontal cortex as well as cortical and hippocampal 5-HT release. It is still too soon to know whether these new antidepressants will represent a real advance over existing drugs in the real world. However, their development opened the way to future antidepressant drugs based on the prevention of local and distal self-inhibitory mechanisms attenuating monoamine activity.

Keywords: 5-hydroxytryptamine (serotonin) receptors; antidepressant drugs; autoreceptors; multi-target agents;

1. Socioeconomic impact of Major Depressive Disorder

Major Depressive Disorder (MDD) is a severe psychiatric syndrome with very high socioeconomic impact worldwide (Murray et al., 2012; Whiteford et al., 2013). A study from the European Brain Council indicated that brain disorders cost almost 800 billion € per year to European countries (Gustavsson et al., 2011). More than one quarter of this high burden is attributable to psychiatric disorders, including mood and anxiety disorders, which account for 113 billion €, and psychotic disorders, which account for 94 billion €. A recent study by the Global Burden of Disease Study Group indicates that MDD is one of the leading causes of illness-induced disability worldwide. It is the first leading cause of years lived with disability in 56 countries, the second one in another 56 countries and the third one in 34 countries (Global Burden of Disease Study 2013 Collaborators, 2015).

The large impact of MDD is attributable to three main factors. On the one hand, MDD is a highly prevalent disorder in the general population (Kessler et al., 2005, 2007; Phillips et al., 2009; Baxter et al., 2013). On the other hand, depressive episodes have a long duration and appear during active periods of adult life (Ferrari et al., 2013), which results in very large labor costs. Finally, standard MDD treatments are far from optimal, which leaves a high percentage of patients with incomplete responses and poor quality of life, thus increasing suicide risk.

2. Slow and limited action of monoamine-based antidepressant drugs

The limited efficacy of antidepressant drugs is a very important contributor to the large -and possibly increasing- impact of MDD. The treatment of MDD is mainly based on SSRI (Selective Serotonin -5-HT- Reuptake Inhibitors) and SNRI (Serotonin and Noradrenaline -NA- Reuptake Inhibitors), which increase serotonergic and noradrenergic neurotransmission in order to relieve depressive symptoms. A few antidepressant drugs antagonize postsynaptic monoamine receptors with little or no inhibition of the serotonin transporter (SERT), such as agomelatine, mirtazapine, trazodone, nefazodone, etc. SSRI and SNRI are pharmacological refinements of first-generation antidepressant drugs -tricyclic antidepressants (TCA) such as imipramine or clomipramine- which were discovered by serendipity 6 decades ago when searching for antipsychotic drugs with a

chemical structure similar to that of chlorpromazine (the first antipsychotic drug, also discovered by serendipity when searching for sedative agents to potentiate general anesthetics; reviewed in Brown and Rosdolsky, 2015; Ban, 2007). The current use of TCA and monoamine oxidase inhibitors (MAOI) is minimal, due to their severe side effects. The development of SSRI -and subsequently, of SNRI- enabled to “clean” antidepressant treatments, removing additional pharmacological activities responsible for the severe side effects of TCAs (blockade of α_1 -adrenoceptors, histamine H1receptors, muscarinic receptors, etc.). Moreover, MAOI can cause severe hypertensive episodes when associated with tyramine-containing foods. However, despite that the lack of severe side effects and increased treatment compliance, newer drugs did not surpass the efficacy of some TCA, such as clomipramine (Danish University Antidepressant Group 1986, 1990).

Clinical trials in research settings with selected patient populations typically yield response and remission rates of ~60% and ~40%, respectively, with standard antidepressant drugs (Tollefson et al., 1994; Stahl, 2000; Thase et al., 2001). However, data from naturalistic studies, such as the STAR*D (Sequenced Treatment Alternatives to Relieve Depression) revealed that 80% of MDD patients have recurrent or chronic depression, and that response and remission rates are substantially lower than in research settings (e.g., 47% and 28%, respectively, after a 8-wk treatment with the SSRI citalopram; Trivedi et al., 2006a). Augmentation strategies with drugs not targeting SERT in patients not responding to SSRI yielded similar remission rates (Rush et al., 2006a; Trivedi et al., 2006b). The STAR*D study also showed that the overall remission rate increased to 67% after four sequenced treatments with different antidepressant drugs during 1 yr (Rush et al., 2006b). These figures from the real world indicate that nearly one third of treated depressed patients do not respond adequately to standard treatments.

An important element of complexity when assessing treatment response is the existence of generic polymorphisms. Hence, polymorphisms of the promoter region of the SERT gene, targeted by SSRI and SNRI, are involved in the clinical response. Homozygotes for the long variant (l/l) and heterozygotes (l/s) showed a better response to SSRI than homozygotes for the short variant (s/s) (Smeraldi et al., 1998; Zanardi et al., 2000; Serretti et al., 2007). Interestingly, the organic cation transporter 3 (OCT3), a high-capacity, corticosterone-sensitive transporter mediating the bidirectional transport of monoamines, may take the role of SERT when this is down-regulated or absent (Baganz et al., 2008). Likewise, an increased expression or function of 5-HT_{1A} autoreceptors -which limit antidepressant effects, see below - is associated to MDD, suicide

and poor response to antidepressant drugs (Stockmeier et al., 1998; Lemonde et al., 2003; Neff et al., 2009; Albert et al., 2014). Other genes, not directly related to the mechanism of action of SSRI/SNRI play also important roles, such as polymorphisms of FKBP5, a glucocorticoid receptor-related gene, involved in antidepressant response and recurrence of depressive episodes (Binder et al., 2004).

A second limitation of current antidepressant treatments is their slowness of action, likely related to their limited efficacy. Controlled trials with SSRI or SNR show that active drugs separate from placebo after two or more weeks of daily treatment, and maximal differences between placebo and active arms are typically observed after 6-8 weeks of treatment (Tollefson et al., 1994; Stahl, 2000; Thase et al., 2001).

In terms of antidepressant drug design, a key question is whether the slowness of clinical action is a temporal requirement of brain networks to switch from a depressive to a euthymic state or is simply a limitation of existing drugs. The first view is supported by a large number of clinical and preclinical observations. Indeed, all marketed antidepressant drugs show a delayed clinical action, irrespectively of their mechanism of action (TCA, MAOI-, SSRI, SNRI, monoamine receptor antagonists, etc.). The delay in the clinical action of these drugs is attributable to the many pre- and postsynaptic adaptive mechanisms evoked in brain, including changes in receptor expression or sensitivity, increased expression of tropic factors and neurogenesis, changes in signaling pathways, increases in synaptic plasticity and neuronal complexity, etc., which lead in the end to the remodeling of brain circuits involved in the therapeutic effect. All these changes occur after repeated exposure of laboratory animals to antidepressant drugs (Blier and de Montigny, 1994; Santarelli et al., 2003; Berton and Nestler, 2006; Sahay and Hen, 2007; Samuels et al., 2015). According to these observations, speed and perhaps clinical efficacy of antidepressant drugs would always be limited by these neurobiological adaptive mechanisms, inherent to the antidepressant action.

In contrast to the above findings, a number of clinical and experimental observations support the opposite view, i.e., that fast antidepressant responses can be achieved. We summarize some of these observations, as follows: 1) some non-standard antidepressant strategies, like sleep deprivation or electroconvulsive therapy (ECT) can evoke rapid antidepressant efficacy in depressed patients resistant to standard treatments (Kellner et al., 2006); 2) addition of some drugs, like pindolol or atypical antipsychotics, can accelerate and/or

enhance the antidepressant action of SSRI by preventing pre- or postsynaptic adaptive mechanisms of monoamine neurotransmission (Artigas et al., 1994; Pérez et al., 1997; Shelton et al., 2001; Berman et al., 2007); 3) the reduction of SERT expression with RNAi strategies evokes much faster antidepressant-like effects in laboratory animals than its pharmacological blockade with SSRI (Ferrés-Coy et al., 2016; see also part II of this review); 4) tryptophan depletion studies showed that the impairment of 5-HT synthesis produced a rapid relapse of the clinical state of depressed patients responding to SSRI, with a subsequent recovery of the euthymic state within hours (Delgado et al., 1990; Smith et al., 1997); 5) deep brain stimulation of midbrain areas, such as the substantia nigra reticulata, evoked a depressive-like state in Parkinsonian patients, which immediately disappeared after the cessation of the stimulation current (Bejjani et al., 1999; Blomstedt et al., 2008; Tommassi et al., 2008); 6) on the contrary, deep brain stimulation of the subgenual cingulate gyrus (Brodmann's area 25; Cg25) evoked a very rapid antidepressant response in MDD patients refractory to any other treatment strategy (Mayberg et al., 2005; Kennedy et al., 2011; Puigemont et al., 2012; Lozano and Lipsman, 2013); 7) a rapid (in hours) and persistent (up to 1 wk) antidepressant effect has also been observed in treatment-resistant unipolar and bipolar depressed patients after the intravenous infusion of single doses of the non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist ketamine (Berman et al., 2000; Zarate et al., 2006). Interestingly, unlike monoamine-based drugs, some fast-acting antidepressants, such as ketamine, are able to evoke rapid changes in neuroplasticity mechanisms (Duman et al., 2016; see also part II for an extended review on the antidepressant action of ketamine), and 8) similarly to ketamine, the intravenous infusion of the cholinergic muscarinic antagonist scopolamine also evoked rapid antidepressant responses in unipolar and bipolar depressed patients (Furey and Drevets, 2006; Drevets et al., 2013).

Overall, these observations indicate that the human brain is able to rapidly switch between depressive and euthymic states (and vice-versa), provided that adequate pharmacological targets are used. The challenge for the scientific community in Neuropsychopharmacology, both in academia and industry, is to develop new strategies overcoming the limitations of existing treatments. Here we will review 3 different approaches namely *i)* the development of multi-target monoamine-based drugs, *ii)* the use of glutamatergic agents, and *iii)* the development of RNAi strategies for the treatment of CNS disorders, and particularly MDD. The latter two approaches are covered in part II of the present review.

3. Improving serotonin-based antidepressants: Rationale for the development of multi-target drugs

Despite the generalised use of serotonin-enhancing drugs, the precise neurobiological mechanisms involved in the therapeutic action of these drugs are not fully understood. In a separate publication, one of us reviewed the role of the different 5-HT receptors in antidepressant action (Artigas, 2013). We can summarize the existing evidence as follows: presynaptic 5-HT_{1A} and 5-HT_{1B} autoreceptors play a major detrimental role in antidepressant treatments, since their activation by the excess of the active 5-HT fraction produced by SERT blockade reduces presynaptic serotonergic function. Conversely, stimulation of postsynaptic 5-HT_{1A} receptors in cortico-limbic networks appears beneficial for antidepressant action. The 5-HT₂ receptor family may also be involved, since 5-HT_{2A/2C} receptor blockade by antipsychotic drugs improves the antidepressant action of SSRIs (Shelton et al., 2001; Berman et al., 2007) and the antidepressant drug agomelatine is a mixed 5-HT_{2C} receptor antagonist and melatonin receptor agonist (Millan et al., 2003). Further, activation of 5-HT_{2B} receptors appears to be required for SSRI actions in rodents (Diaz et al., 2012). Also, as stated above, 5-HT₃ receptor blockade augments the 5-HT increase evoked by SERT inhibition. On the other hand, 5-HT₄ receptor activation may have antidepressant effects on its own and blockade of 5-HT₆ and 5-HT₇ receptors augments the antidepressant effects produced by SERT inhibition. Hence, it is unlikely that a single 5-HT receptor can account for the widespread actions of SSRI/SNRI on depressive symptoms, and therefore, agonists/antagonists of a single receptor are unlikely to overcome the limitations of current treatments, despite some preclinical observations being highly suggestive of clinical antidepressant efficacy (e.g., Lucas, 2009).

3.1. Presynaptic attenuation of serotonergic function by single clinical antidepressant doses

Preclinical research indicated that self-inhibitory mechanisms evoked by autoreceptor activation in serotonergic neurons attenuated the increase in serotonergic function induced by SERT blockade and MAO inhibition. This was supported by independent electrophysiological and neurochemical data more than two decades ago. On the one hand, antidepressant drugs blocking SERT or

inhibiting MAO suppressed the activity of dorsal raphe serotonergic neurons through the activation of 5-HT autoreceptors (5-HT_{1A} subtype), thus reducing serotonergic activity (Blier et al., 1987; Blier and de Montigny 1994). In parallel, the development of the microdialysis techniques allowed to examine the effects of antidepressant drugs on the extracellular (active) 5-HT concentration in multiple brain areas of behaving animals. These studies revealed that antidepressant drugs of different families, such as TCAs, MAO inhibitors and SSRI preferentially enhanced the extracellular 5-HT concentration in the midbrain raphe nuclei, containing the cell bodies of 5-HT neurons, as compared to forebrain areas rich in nerve terminals, such as the prefrontal cortex (PFC) or hippocampus (Adell and Artigas, 1991; Bel and Artigas, 1992; Celada and Artigas, 1993; Hervás and Artigas, 1998). Interestingly, low SSRI doses, comparable to those used clinically only enhanced extracellular 5-HT in the midbrain raphe nuclei (Hervás and Artigas, 1998). Interestingly, repeated -but not single- treatment with low SSRI doses produced a marked increase in extracellular 5-HT (Bel and Artigas, 1993), an effect likely accounted for by the desensitization of 5-HT_{1A} autoreceptors produced by repeated antidepressant treatment (Blier and de Montigny 1994; Hervás et al., 2001).

Two main factors account for this preferential effect of antidepressant drugs in the midbrain raphe. On the one hand, this is a neurochemically active area, which contains a high density of 5-HT axonal varicosities in addition to 5-HT cell bodies, and the highest densities of SERT and MAO in rodent and human brain (Fuxe et al., 1983; Cortés et al., 1988; Saura et al., 1992; 1996). On the other hand, 5-HT neurons express a high density of somatodendritic 5-HT_{1A} autoreceptors (Pazos and Palacios, 1985; Pompeiano et al., 1992; Kia et al., 1996), coupled to inward rectifying GIRK channels, whose activation by 5-HT and exogenous agonists hyperpolarizes 5-HT neurons and reduces their discharge (Blier et al., 1987; Innis et al., 1988; Montalbano et al., 2015). PET-scan studies also indicate that the dorsal and median human raphe nuclei contain the highest density of SERT in human brain, in association with a very high density of 5-HT_{1A} receptors (Spies et al., 2015).

The physiological role of 5-HT_{1A} autoreceptors is to maintain the slow pacemaker activity of 5-HT neurons in front of excitatory inputs. Hence, the activation of excitatory afferents from the medial prefrontal cortex (mPFC) to the raphe nuclei increases the local release of 5-HT, which then inhibits neuronal activity through 5-HT_{1A} receptor activation via recurrent axons or cross-talk between different 5-HT neurons (Celada et al., 2001).

This regional coincidence of the highest brain density of SERT and MAO with a very abundant expression of 5-HT_{1A} receptors results in a paradoxical effect of antidepressants, which reduce serotonergic activity and 5-HT release in forebrain due to their action on these midbrain targets (Adell and Artigas, 1991; Rutter et al., 1995; Romero and Artigas, 1997), an effect opposed to the desired one, i.e., the increase in forebrain serotonergic function via SERT blockade. The involvement of 5-HT_{1A} autoreceptors in this self-inhibitory effect is supported by its reversal by the selective 5-HT_{1A} receptor antagonist WAY-100635 which potentiated the elevation of extracellular 5-HT produced by SSRI, the TCA clomipramine (but not desipramine) and the MAO inhibitor phenelzine (Romero et al., 1996; Romero and Artigas, 1997; Hervás and Artigas, 1998). These observations supported the hypothesis that 5-HT_{1A} autoreceptor antagonists could accelerate, and perhaps augment, the therapeutic action of SSRI (Artigas, 1993). Further, terminal 5-HT_{1B} autoreceptors add a second element of self-attenuation of 5-HT release (Artigas et al., 2001; Fig. 1). In agreement with this hypothesis the combination of SSRI and 5-HT_{1A} and/or 5-HT_{1B} receptor antagonists elevated extracellular 5-HT in microdialysis studies more than SSRI alone (Gartside et al., 1995; Artigas et al., 1996; Hjorth and Auerbach, 1996; Romero et al., 1996; Gobert and Millan, 1997; Romero and Artigas, 1997). Likewise, SSRI induced a greater elevation of extracellular 5-HT in mutant mice lacking 5-HT_{1A} or 5-HT_{1B} receptors (Knobelman et al., 2001; Bortolozzi et al., 2004).

Whether these changes also occur in human brain has been an enigma until recently. On the one hand, the similarities between rodent and human brain in terms of the elements involved -very high density of SERT and MAO in the midbrain raphe area, plus expression of somatodendritic 5-HT_{1A} autoreceptors by 5-HT neurons- suggested that a reduction of 5-HT release could also occur in human brain after single treatment with drugs blocking SERT or MAO. On the other hand, the different techniques and variables used to study antidepressant effects in rodent brain (mainly microdialysis, examining changes in extracellular 5-HT) and human brain (PET scan imaging, assessing radioligand displacement), -together with the higher complexity of human brain- are a limitation to compare studies in both species.

Unlike in the dopamine field (e.g., Laruelle et al., 1996), the assessment of drug effects on 5-HT release in human brain using PET scan imaging has been hampered by the lack of suitable ligands displaceable by endogenous 5-HT (Paterson et al., 2010). Recently, [¹¹C]AZ10419369, an antagonist ligand for 5-HT_{1B} receptors was developed, which allows the assessment of drug-induced changes on the free 5-HT brain concentration. Hence, administration of the 5-HT releaser

fenfluramine reduced the binding potential of this ligand in some brain regions of nonhuman primates (Finnema et al., 2012). Subsequent studies revealed that the i.v. infusion of escitalopram (2 mg/kg) to non-human primates produced a similar result, with a decrease of the binding potential of [^{11}C]AZ10419369 in all brain areas examined, as a result of the displacement of the radioactive ligand from 5-HT_{1B} sites by the increased extracellular 5-HT concentration produced by SERT blockade (Nord et al., 2013; Fig. 2A). However, the administration of a single clinical dose (20 mg p.o., equivalent to 0.25-0.3 mg/kg) to human volunteers evoked an opposite effect in the midbrain raphe nuclei and in forebrain areas, in agreement with the regionally-selective elevation of extracellular 5-HT by low SSRI doses in rat midbrain (Hervás and Artigas, 1998). Hence, escitalopram reduced the binding potential of [^{11}C]AZ10419369 in the midbrain raphe nuclei but increased it in forebrain, indicating a reduction of the availability 5-HT to compete with the radioligand, likely reflecting a reduction of 5-HT release (Fig. 2B). These observations are fully consistent with the above rodent data and suggest that 1) except in the midbrain raphe, single clinical doses of SSRI *reduce* 5-HT function in human brain, and 2) the negative feed-back exerted at autoreceptor level can be overcome by the administration of higher antidepressant doses, as observed in non-human primates (2 mg/kg i.v. vs 0.25-0.30 mg/kg p.o. in human volunteers). Therefore, more rapid and effective antidepressant treatments could be achieved using higher antidepressant doses, an uncommon clinical practice due to the increased incidence of side effects. In agreement with these views, intravenous administration of antidepressant doses higher than the classical oral doses resulted in more rapid and/or effective responses. Hence, the intravenous infusion of relatively high doses of clomipramine remitted treatment resistant patients in less than two weeks (from a HAM-D score of 22.6 ± 2.4 to 6.8 ± 1.5 in 11 days) (Pollock et al., 1985). Likewise intravenous citalopram was superior to oral citalopram in MDD patients (Guelfi et al., 2000).

As mentioned above, the partial prevention of 5-HT_{1A} autoreceptor-mediated inhibitory mechanisms by the non-selective agent pindolol (with mixed partial agonist actions at β -adrenoceptors and at 5-HT_{1A} receptors) accelerated, and in some studies, enhanced the efficacy of SSRI (Artigas et al., 1994; Blier and Bergeron, 1995; Pérez et al., 1997; see meta-analyses in Ballesteros and Callado, 2004; Whale et al., 2010; Portella et al., 2011). Pindolol shows a preferential activity for presynaptic vs. postsynaptic 5-HT_{1A} receptors, as assessed by a variety of methods (Romero et al., 1996; Martinez et al., 2001; Serrats et al., 2004). This regional selectivity allows for the activation of postsynaptic 5-HT_{1A} receptors, involved in antidepressant actions

(Haddjeri et al., 1998; Samuels et al., 2015). In contrast to pindolol, DU 125530, a potent and selective 5-HT_{1A} receptor antagonist not discriminating between pre- and postsynaptic 5-HT_{1A} receptors, did not accelerate nor enhance the antidepressant action of the SSRI fluoxetine (Scorza et al., 2012). The latter study was conducted using the same trial protocol and in the same setting than previous pindolol studies (Pérez et al., 1997).

3.2. Development of multi-target antidepressants

Given the difficulty of combining SERT blockade and 5-HT_{1A} receptor antagonist properties in a single molecule, together with the need to stimulate postsynaptic 5-HT_{1A} receptors, pharmaceutical companies have developed agents blocking SERT and acting as partial agonists at 5-HT_{1A} receptors. To date, two new antidepressant drugs have been developed based on the above strategy, vilazodone (Merck) and vortioxetine (Lundbeck). Figure 3 shows a schematic representation of the connectivity between mPFC and brainstem monoamine nuclei, with the main sites of action of vilazodone and vortioxetine.

Preclinical microdialysis studies revealed that vilazodone increased extracellular 5-HT in rat forebrain more than the SSRI fluoxetine after a single administration and exhibited antidepressant-like responses in the forced swim test at low doses. The failure to evoke the same behavioral effect at a high dose may be attributable to the blockade of postsynaptic 5-HT_{1A} receptors and the subsequent cancellation of the presynaptic enhancement of 5-HT function (Page et al., 2002). Despite these preclinical observations, the few controlled studies conducted so far do not support a faster and more effective therapeutic action than SSRI (Sahli et al., 2016).

The other antidepressant drug developed on the basis of combining SERT blockade and partial agonist activity at 5-HT_{1A} receptors is vortioxetine. However, vortioxetine shows also high affinity for other 5-HT receptors, including 5-HT₃, 5-HT_{1B}, 5-HT_{1D} and 5-HT₇ (Sanchez et al., 2015). In particular, vortioxetine shows very high affinity for 5-HT₃ receptors, expressed by a subpopulation of GABA interneurons in PFC and the hippocampal formation (Morales and Bloom, 1997; Puig et al., 2004). In the PFC, GABA interneurons expressing 5-HT₃ receptors are located in upper cortical layers (Puig et al., 2004; Lee et al., 2010) and the physiological activation of 5-HT₃ receptors by endogenous 5-HT markedly enhances interneuron activity (Puig et al., 2004). This

effect results in the activation of GABA_A receptors in pyramidal neurons and the subsequent attenuation of the excitation/inhibition balance in cortical pyramidal neurons.

Conversely, blockade of 5-HT₃ receptors by vortioxetine reduces the GABAergic tone on GABA_A and GABA_B receptors, which then translates into a disinhibition of the activity of midbrain-projecting pyramidal neurons in the mPFC (Riga et al., 2016) (Fig. 4). The presence of a high population of pyramidal neurons projecting to the brainstem monoamine nuclei in mPFC (Gabbott et al., 2005), and the tight control exerted by the mPFC on the activity of monoamine neurons (Jodo et al., 1998; Sara and Hervé-Minvielle, 1995; Hajós et al., 1998; Tong et al., 1996; Celada et al., 2001; Warden et al., 2012) suggests that 5-HT₃ blockade in mPFC may distally affect the activity of serotonergic and noradrenergic neurons involved in antidepressant responses. This prefrontal-brainstem connectivity may account for the rapid recovery of serotonergic activity after repeated vortioxetine treatment (Bétry et al., 2013). In addition, 5-HT₃ receptor blockade potentiates the increase in extracellular 5-HT produced by SERT blockade, an effect likely involving the removal of a GABA_B tone on 5-HT axon terminals (Riga et al., 2016). These effects may account for the greater enhancement of forebrain monoamine release produced by vortioxetine, as compared with SSRI (Mork et al., 2012).

In clinical trials, vortioxetine shows an efficacy similar to high doses of the SNRI venlafaxine, with significant separation from placebo at 2 weeks of treatment (Alvarez et al., 2012; see metanalysis in Thase et al., 2016). Vortioxetine also shows pro-cognitive effects in animal models and MDD patients (Wallace et al., 2014; Mahableshwarkar et al., 2015; see Sanchez et al., 2015 for review). Given the relevance of the dorsolateral PFC –and possibly sensory areas– in short-term (working) memory (Fuster, 2001; Curtis and D'Esposito, 2003; Sreenivasan et al., 2014) it is possible that pro-cognitive effects of vortioxetine can be mediated by the enhancement of neuronal function in this cortical area.

Given the short time spent since the marketing of these two antidepressant drugs, it is still soon to know whether these multi-target antidepressants will represent a real advance over existing drugs in terms of speed of action, overall efficacy, relapse prevention and reduction of the suicide risk. However, their development has opened the way to future monoaminergic drugs based on the same rationale, i.e., the prevention of a large number of local and distal self-inhibitory mechanisms attenuating the activity of monoamine neurons, which delay and limit the benefits of enhancing monoamine activity to treat mood disorders. One should not forget that

5-HT and NA systems are essentially of tonic nature, with a slow and regular discharge rate that ceases during REM sleep (REM-off neurons). These systems show a strong homeostasis and react to excitatory phasic inputs with a release of the neurotransmitter and the subsequent activation of somatodendritic autoreceptors. Antidepressant drugs aim to increase monoamine function, which then breaks this homeostasis and activates physiological self-limiting responses through auto- and heteroreceptors. These inhibitory responses need to be prevented or attenuated to obtain the full benefits of reuptake blockade.

Funding and Disclosure

Support from the following grants is acknowledged: SAF2015-68346-P and Retos-Colaboración Subprograma RTC-2014-2812-1, MINECO co-financed by European Regional Development Fund - ERDF), UE (Ministry of Economy and Competitiveness and European Regional Development Fund), PI12/00156 and PI13/01390 (Instituto de Salud Carlos III, co-financed by ERDF), and Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red de Salud Mental, CIBERSAM. Support from the Generalitat de Catalunya (2014 SGR798) is also acknowledged. The sponsors had no further role in the design of the review, in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication. All authors revised critically the manuscript and they gave final approval. We thank María Jaramillo for secretarial assistance.

F.A. has received consulting honoraria from Lundbeck and he and P.C. are PI and Co-PI respectively of a grant from Lundbeck. He is also member of the scientific advisory board of Neurolinx. F.A. and A.B. are co-inventors of a patent on conjugated RNAi molecules.

References

- Adell, A., Artigas, F., 1991. Differential effects of clomipramine given locally or systemically on extracellular 5-hydroxytryptamine in raphe nuclei and frontal cortex. An in vivo brain microdialysis study. *Naunyn Schmiedeberg's Arch. Pharmacol.* 343, 237-244.
- Albert, P.R., Vahid-Ansari, F., Luckhart, C., 2014. Serotonin-prefrontal cortical circuitry in anxiety and depression phenotypes: pivotal role of pre- and post-synaptic 5-HT_{1A} receptor expression. *Front. Behav. Neurosci.* 8, 199.
- Alvarez, E., Perez, V., Dragheim, M., Loft, H., Artigas, F., 2012. A double-blind, randomized, placebo-controlled, active reference study of Lu AA21004 in patients with major depressive disorder. *Int. J. Neuropsychopharmacol.* 15, 589-600.
- Artigas, F., 1993. 5-HT and antidepressants: new views from microdialysis studies. *Trends Pharmacol. Sci.* 14, 262.
- Artigas, F., 2013. Serotonin receptors involved in antidepressant effects. *Pharmacol Ther* 137, 119-131.
- Artigas, F., 2015. Developments in the field of antidepressants, where do we go now? *Eur. Neuropsychopharmacol.* 25, 657-670.
- Artigas, F., Celada, P., Laruelle, M., Adell, A., 2001. How does pindolol improve antidepressant action? *Trends Pharmacological Sciences* 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-HT_{1A} antagonists. *Trends. Neurosci.* 19, 378-383.
- Baganz, N.L., Horton, R.E., Calderon, A.S., Owens, W.A., Munn, J.L., Watts, L.T., Koldzic-Zivanovic, N., Jeske, N.A., Koek, W., Toney, G.M., Daws, L.C... 2008. Organic cation transporter 3: Keeping the brake on extracellular serotonin in serotonin-transporter-deficient mice. *Proc Natl Acad Sci U S A.* 105, 18976-18978
- Ballesteros, J., Callado, L.F., 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.
- Ban, T.A., 2007. Fifty years chlorpromazine: a historical perspective. *Neuropsychiat. Dis. Treat.* 3, 495-500
- Baxter, A.J., Patton, G., Scott, K.M., Degenhardt, L., Whiteford, H.A., 2013. Global epidemiology of mental disorders: what are we missing? *PLoS One* 8, e65514.
- Bejjani, B.P., Damier, P., Arnulf, I., Thivard, L., Bonnet, A.M., Dormont, D., Cornu, P., Pidoux, B., Samson, Y., Agid, Y., 1999. Transient acute depression induced by high-frequency deep-brain stimulation. *N. Engl. J. Med.* 340, 1476-1480.
- Bel, N., Artigas, F., 1992. Fluvoxamine preferentially increases extracellular 5-hydroxytryptamine in the raphe nuclei: an in vivo microdialysis study. *Eur. J. Pharmacol.* 229, 101-103.

Bel, N., Artigas, F., 1993. Chronic treatment with fluvoxamine increases extracellular serotonin in frontal cortex but not in raphe nuclei. *Synapse* 15, 243-245.

Berman, R.M., Cappiello, A., Anand, A., Oren, D.A., Heninger, G.R., Charney, D.S., Krystal, J.H., 2000. Antidepressant effects of ketamine in depressed patients. *Biol. Psychiatry* 47, 351-354.

Berman, R.M., Marcus, R.N., Swanink, R., McQuade, R.D., Carson, W.H., Corey-Lisle, P.K., Khan, A., 2007. The efficacy and safety of aripiprazole as adjunctive therapy in major depressive disorder: a multicenter, randomized, double-blind, placebo-controlled study. *J. Clin. Psychiatry* 68, 843-853.

Berton, O., Nestler, E.J., 2006. New approaches to antidepressant drug discovery: beyond monoamines. *Nat. Rev. Neurosci.* 7, 137-151.

Bétry, C., Pehrson, A.L., Etiévant, A., Ebert, B., Sánchez, C., Haddjeri, N., 2013. The rapid recovery of 5-HT cell firing induced by the antidepressant vortioxetine involves 5-HT(3) receptor antagonism. *Int. J. Neuropsychopharmacol.* 16, 1115-1127.

Binder, E.B., Salyakina, D., Lichtner, P., Wochnik, G.M., Ising, M., Putz, B., Papiol, S., Seaman, S., Lucae, S., Kohli, M.A., Nickel, T., Kunzel, H.E., Fuchs, B., Majer, M., Pfennig, A., Kern, N., Brunner, J., Modell, S., Baghai, T., Deiml, T., Zill, P., Bondy, B., Rupprecht, R., Messer, T., Kohnlein, O., Dabitz, H., Bruckl, T., Müller, N., Pfister, H., Lieb, R., Mueller, J.C., Lohmusaar, E., Strom, T.M., Bettecken, T., Meitinger, T., Uhr, M., Rein, T., Holsboer, F., Müller-Myhsok, B., 2004. Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. *Nat. Genet.* 36, 1319-1325.

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(6 Suppl), 24S-35S.

Blomstedt, P., Hariz, M.I., Lees, A., Silberstein, P., Limousin, P., Yelnik, J., Agid, Y., 2008. Acute severe depression induced by intraoperative stimulation of the substantia nigra: a case report. *Parkinsonism Relat. Disord.* 14, 253-256.

Bortolozzi, A., Amargós-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.

Brown, W.A., Rosdolsky, M., 2015. The clinical discovery of imipramine. *Am. J. Psychiatry* 172, 426-429.

Celada, P., Artigas, F., 1993. Monoamine oxidase inhibitors increase preferentially extracellular 5-hydroxytryptamine in the midbrain raphe nuclei. A brain microdialysis study in the awake rat. *Naunyn Schmiedeberg's Arch. Pharmacol.* 347, 583-590.

- Celada, P., Puig, M.V., Casanovas, J.M., Guillazo, G., Artigas, F., 2001. Control of dorsal raphe serotonergic neurons by the medial prefrontal cortex: Involvement of serotonin-1A, GABA(A), and glutamate receptors. *J. Neurosci.* 21, 9917-9929.
- Cortés, R., Soriano, E., Pazos, A., Probst, A., Palacios, J.M., 1988. Autoradiography of antidepressant binding sites in the human brain: localization using [3H]imipramine and [3H]paroxetine. *Neuroscience* 27, 473-496.
- Curtis, C.E., D'Esposito, M., 2003. Persistent activity in the prefrontal cortex during working memory. *Trends Cogn. Sci.* 7, 415-423.
- Danish University Antidepressant Group, 1986. Citalopram: clinical effect profile in comparison with clomipramine. A controlled multicenter study. *Psychopharmacology (Berl)* 90, 131-138.
- Danish University Antidepressant Group, 1990. Paroxetine: a selective serotonin reuptake inhibitor showing better tolerance, but weaker antidepressant effect than clomipramine in a controlled multicenter study. *J. Affect. Disord.* 18, 289-299.
- Delgado, P.L., Charney, D.S., Price, L.H., Aghajanian, G.K., Landis, H., Heninger, G.R., 1990. Serotonin function and the mechanism of antidepressant action. Reversal of antidepressant-induced remission by rapid depletion of plasma tryptophan. *Arch. Gen. Psychiatry* 47, 411-418.
- Diaz, S.L., Doly, S., Narboux-Nême, N., Fernández, S., Mazot, P., Banas, S.M., Boutourlinsky, K., Moutkine, I., Belmer, A., Roumier, A., Maroteaux, L. 2012. 5-HT(2B) receptors are required for serotonin-selective antidepressant actions. *Mol Psychiatry* 17, 154-163
- Drevets, W.C., Zarate, C.A. Jr., Furey, M.L., 2013. Antidepressant effects of the muscarinic cholinergic receptor antagonist scopolamine: a review. *Biol. Psychiatry* 73, 1156-1163.
- Duman, R.s., Aghajanian, G.K., Sanacora, G., Krystal, J.H. (2016) Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. *Nat Med* 22:238-249.
- Ferrari, A.J., Charlson, F.J., Norman, R.E., Patten, S.B., Freedman, G., Murray, C.J., Vos, T., Whiteford, H.A., 2013. Burden of depressive disorders by country, sex, age, and year: findings from the global burden of disease study 2010. *PLoS Med.* 10, e1001547.
- Ferrés-Coy, A., Galofré, M., Pilar-Cuellar, F., Vidal, R., Paz, V., Ruiz-Bronchal, E., Campa, L., Pazos, Á., Caso, J.R., Leza, J.C., Alvarado, G., Montefeltro, A., Valdizán, E.M., Artigas, F., Bortolozzi, A., 2016. Therapeutic antidepressant potential of a conjugated siRNA silencing the serotonin transporter after intranasal administration. *Mol. Psychiatry* 21, 328-338.
- Finnema, S.J., Varrone, A., Hwang, T.J., Halldin, C., Farde, L., 2012. Confirmation of fenfluramine effect on 5-HT(1B) receptor binding of [(11)C]AZ10419369 using an equilibrium approach. *J. Cereb. Blood Flow Metab.* 32, 685-695.
- Furey, M.L., Drevets, W.C., 2006. Antidepressant efficacy of the antimuscarinic drug scopolamine: a randomized, placebo-controlled clinical trial. *Arch. Gen. Psychiatry* 63, 1121-1129.
- Fuster, J.M., 2001. The prefrontal cortex--an update: time is of the essence. *Neuron* 30, 319-333.

- Fuxe, K., Calza, L., Benfenati, F., Zini, I., Agnati, L.F., 1983. Quantitative autoradiographic localization of [3H]imipramine binding sites in the brain of the rat: relationship to ascending 5-hydroxytryptamine neuron systems. *Proc. Natl. Acad. Sci. U.S.A.* 80, 3836-3840.
- Gabbott, P.L., Warner, T.A., Jays, P.R., Salway, P., Busby, S.J., 2005. Prefrontal cortex in the rat: projections to subcortical autonomic, motor, and limbic centers. *J. Comp. Neurol.* 492, 145-177.
- Gartside, S.E., Umbers, V., Hajós, M., Sharp, T., 1995. Interaction between a selective 5-HT_{1A} receptor antagonist and an SSRI in vivo: effects on 5-HT cell firing and extracellular 5-HT. *Br. J. Pharmacol.* 115, 1064-1070.
- Global Burden of Disease Study 2013 Collaborators, 2015. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 386, 743-800.
- Gobert, A., Millan, M.J., 1997. Potentiation of the fluoxetine-induced increase in dialysate levels of serotonin (5-HT) in the frontal cortex of freely moving rats by combined blockade of 5-HT_{1A} and 5-HT_{1B} receptors with WAY 100,635 and GR 127,935. *J. Neurochem.* 68, 1159-1163.
- Guelfi, J.D., Strub, N., Loft, H., 2000. Efficacy of intravenous citalopram compared with oral citalopram for severe depression. Safety and efficacy data from a double-blind, double-dummy trial. *J. Affect. Disord.* 58, 201-209.
- Gustavsson, A., Svensson, M., Jacobi, F., Allgulander, C., Alonso, J., Beghi, E., Dodel, R., Ekman, M., Faravelli, C., Fratiglioni, L., Gannon, B., Jones, D.H., Jenum, P., Jordanova, A., Jonsson, L., Karampampa, K., Knapp, M., Kobelt, G., Kurth, T., Lieb, R., Linde, M., Ljungcrantz, C., Maercker, A., Melin, B., Moscarelli, M., Musayev, A., Norwood, F., Preisig, M., Pugliatti, M., Rehm, J., Salvador-Carulla, L., Schlehofer, B., Simon, R., Steinhausen, H.C., Stovner, L.J., Vallat, J.M., den Bergh, P.V., van Os, J., Vos, P., Xu, W., Wittchen, H.U., Jonsson, B., Olesen, J., 2011. Cost of disorders of the brain in Europe 2010. *Eur. Neuropsychopharmacol.* 21, 718-779.
- Haddjeri, N., Blier, P., de Montigny, C., 1998. Long-term antidepressant treatments result in a tonic activation of forebrain 5-HT_{1A} receptors. *J. Neurosci.* 18, 10150-10156.
- Hajos, M., Richards, C.D., Szekely, A.D., Sharp, T., 1998. An electrophysiological and neuroanatomical study of the medial prefrontal cortical projection to the midbrain raphe nuclei in the rat. *Neuroscience* 87, 95-108.
- Hervás, I., Artigas, F., 1998. Effect of fluoxetine on extracellular 5-hydroxytryptamine in rat brain. Role of 5-HT autoreceptors. *Eur. J. Pharmacol.* 358, 9-18.
- Hjorth, S., Auerbach, S.B., 1996. 5-HT_{1A} autoreceptors and the mode of action of selective serotonin reuptake inhibitors (SSRI). *Behav. Brain Res.* 73, 281-283.
- Hervás, I., Vilaró, M.T., Romero, L., Scorza, M.C., Mengod, G., Artigas, F., 2001. Desensitization of 5-HT(1A) autoreceptors by a low chronic fluoxetine dose effect of the concurrent administration of WAY-100635. *Neuropsychopharmacology.* 24, 11-20.
- Innis, R.B., Nestler, E.J., Aghajanian, G.K., 1988. Evidence for G protein mediation of serotonin- and GABA-induced hyperpolarization of rat dorsal raphe neurons. *Brain Res.* 459, 27-36.

Jodo, E., Chiang, C., Aston-Jones, G., 1998. Potent excitatory influence of prefrontal cortex activity on noradrenergic locus coeruleus neurons. *Neuroscience* 83, 63-79.

Kellner, C.H., Knapp, R.G., Petrides, G., Rummans, T.A., Husain, M.M., Rasmussen, K., Mueller, M., Bernstein, H.J., O'Connor, K., Smith, G., Biggs, M., Bailine, S.H., Malur, C., Yim, E., McClintock, S., Sampson, S., Fink, M., 2006. Continuation electroconvulsive therapy vs pharmacotherapy for relapse prevention in major depression: a multisite study from the Consortium for Research in Electroconvulsive Therapy (CORE). *Arch. Gen. Psychiatry* 63, 1337-1344.

Kennedy, S.H., Giacobbe, P., Rizvi, S.J., Placenza, F.M., Nishikawa, Y., Mayberg, H.S., Lozano, A.M., 2011. Deep brain stimulation for treatment-resistant depression: follow-up after 3 to 6 years. *Am. J. Psychiatry* 168, 502-510.

Kessler, R.C., Angermeyer, M., Anthony, J.C., De Graaf, R., Demyttenaere, K., Gasquet, I., De Girolamo, G., Gluzman, S., Gureje, O., Haro, J.M., Kawakami, N., Karam, A., Levinson, D., Medina Mora, M.E., Oakley Browne, M.A., Posada-Villa, J., Stein, D.J., Adley Tsang, C.H., Aguilar-Gaxiola, S., Alonso, J., Lee, S., Heeringa, S., Pennell, B.E., Berglund, P., Gruber, M.J., Petukhova, M., Chatterji, S., Ustün, T.B., 2007. Lifetime prevalence and age-of-onset distributions of mental disorders in the World Health Organization's World Mental Health Survey Initiative. *World Psychiatry* 6, 168-176.

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.

Kia, H.K., Miquel, M.C., Brisorgueil, M.J., Daval, G., Riad, M., El Mestikawy, S., Hamon, M., Vergé, D., 1996. Immunocytochemical localization of serotonin_{1A} receptors in the rat central nervous system. *J. Comp. Neurol.* 365, 289-305.

Knobelman, D.A., Hen, R., Lucki, I., 2001. 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.

Laruelle, M., Abi-Dargham, A., van Dyck, C.H., Gil, R., D'Souza, C.D., Erdos, J., McCance, E., Rosenblatt, W., Fingado, C., Zoghbi, S.S., Baldwin, R.M., Seibyl, J.P., Krystal, J.H., Charney, D.S., Innis, R.B., 1996. Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. *Proc. Natl. Acad. Sci. U.S.A.* 93, 9235-9240.

Lee, S., Hjerling-Leffler, J., Zagha, E., Fishell, G., Rudy, B., 2010. The largest group of superficial neocortical GABAergic interneurons expresses ionotropic serotonin receptors. *J. Neurosci.* 30, 16796-16808.

Lemondé, S., Turecki, G., Bakish, D., Du, L., Hrdina, P.D., Bown, C.D., Sequeira, A., Kushwaha, N., Morris, S.J., Basak, A., Ou, X.M., Albert, P.R., 2003. Impaired repression at a 5-hydroxytryptamine 1A receptor gene polymorphism associated with major depression and suicide. *J. Neurosci.* 23, 8788-8799.

Lozano, A.M., Lipsman, N., 2013. Probing and regulating dysfunctional circuits using deep brain stimulation. *Neuron* 77, 406-424.

Lucas G. 2009. Serotonin receptors, type 4: a new hope? *Curr Drug Targets.* 10, 1085-1095.

- Mahableshwarkar, A.R., Zajecka, J., Jacobson, W., Chen, Y., Keefe, R.S., 2015. A randomized, placebo-controlled, active-reference, double-blind, flexible-dose study of the efficacy of vortioxetine on cognitive function in major depressive disorder. *Neuropsychopharmacology* 40, 2025-2037.
- Martinez, D., Hwang, D., Mawlawi, O., Slifstein, M., Kent, J., Simpson, N., Parsey, R.V., Hashimoto, T., Huang, Y., Shinn, A., Van Heertum, R., Abi-Dargham, A., Caltabiano, S., Malizia, A., Cowley, H., Mann, J.J., Laruelle, M., 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.
- Mayberg, H.S., Lozano, A.M., Voon, V., McNeely, H.E., Seminowicz, D., Hamani, C., Schwalb, J.M., Kennedy, S.H., 2005. Deep brain stimulation for treatment-resistant depression. *Neuron* 45, 651-660.
- Millan, M.J., Gobert, A., Lejeune, F., Dekeyne, A., Newman-Tancredi, A., Pasteau, V., Rivet, J.M., Cussac, D., 2003. The novel melatonin agonist agomelatine (S20098) is an antagonist at 5-hydroxytryptamine_{2C} receptors, blockade of which enhances the activity of frontocortical dopaminergic and adrenergic pathways. *J Pharmacol Exp Ther.* 306, 954-964.
- Montalbano, A., Corradetti, R., Mlinar, B., 2015. Pharmacological Characterization of 5-HT_{1A} Autoreceptor-Coupled GIRK Channels in Rat Dorsal Raphe 5-HT Neurons. *PLoS One* 10, e0140369.
- Morales, M., Bloom, F.E., 1997. The 5-HT₃ receptor is present in different subpopulations of GABAergic neurons in the rat telencephalon. *J. Neurosci.* 17, 3157-3167.
- 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.
- Murray, C.J., Vos, T., Lozano, R., Naghavi, M., Flaxman, A.D., Michaud, C., et al. 2012. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 380, 2197-2223.
- Neff, C.D., Abkevich, V., Packer, J.C., Chen, Y., Potter, J., Riley, R., Davenport, C., DeGrado Warren, J., Jammulapati, S., Bhatena, A., Choi, W.S., Kroeger, P.E., Metzger, R.E., Gutin, A., Skolnick, M.H., Shattuck, D., Katz, D.A., 2009. Evidence for HTR1A and LHPP as interacting genetic risk factors in major depression. *Mol. Psychiatry* 14, 621-630.
- Nord, M., Finnema, S.J., Halldin, C., Farde, L., 2013. Effect of a single dose of escitalopram on serotonin concentration in the non-human and human primate brain. *Int. J. Neuropsychopharmacol.* 16, 1577-1586.
- Page, M.E., Cryan, J.F., Sullivan, A., Dalvi, A., Saucy, B., Manning, D.R., Lucki, I., 2002. Behavioral and neurochemical effects of 5-(4-[4-(5-Cyano-3-indolyl)-butyl]-butyl)-1-piperazinyl)-benzofuran-2-carboxamide (EMD 68843): a combined selective inhibitor of serotonin reuptake and 5-hydroxytryptamine(1A) receptor partial agonist. *J. Pharmacol. Exp. Ther.* 302, 1220-1227.
- Paterson, L.M., Tyacke, R.J., Nutt, D.J., Knudsen, G.M., 2010. Measuring endogenous 5-HT release by emission tomography: promises and pitfalls. *J. Cereb. Blood Flow Metab.* 30, 1682-1706.

Pazos, A., Palacios, J.M., 1985. Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. *Brain Res.* 346, 205-230.

Pérez, V., Gilaberte, I., Faries, D., Alvarez, E., Artigas, F., 1997. Randomised, double-blind, placebo-controlled trial of pindolol in combination with fluoxetine antidepressant treatment. *Lancet* 349, 1594-1597.

Phillips, M.R., Zhang, J., Shi, Q., Song, Z., Ding, Z., Pang, S., Li, X., Zhang, Y., Wang, Z., 2009. Prevalence, treatment, and associated disability of mental disorders in four provinces in China during 2001-05: an epidemiological survey. *Lancet* 373, 2041-2053.

Pollock, B.G., Perel, J.M., Shostak, M., 1985. Rapid achievement of antidepressant effect with intravenous chlorimipramine. *N. Engl. J. Med.* 312, 1130.

Pompeiano, M., Palacios, J.M., Mengod, G., 1992. Distribution and cellular localization of mRNA coding for 5-HT_{1A} receptor in the rat brain: correlation with receptor binding. *J. Neurosci.* 12, 440-453.

Portella, M.J., Diego-Adelino, J., Ballesteros, J., Puigdemont, D., Oller, S., Santos, B., Alvarez, E., Artigas, F., Perez, V., 2011. Can we really accelerate and enhance the selective serotonin reuptake inhibitor antidepressant effect? A randomized clinical trial and a meta-analysis of pindolol in nonresistant depression. *J. Clin. Psychiatry* 72, 962-969.

Puig, M.V., Santana, N., Celada, P., Mengod, G., Artigas, F., 2004. In vivo excitation of GABA interneurons in the medial prefrontal cortex through 5-HT₃ receptors. *Cereb. Cortex* 14, 1365-1375.

Puigdemont, D., Perez-Egea, R., Portella, M.J., Molet, J., Diego-Adelino, J., Gironell, A., Radua, J., Gomez-Anson, B., Rodriguez, R., Serra, M., de Quintana, C., Artigas, F., Alvarez, E., Perez, V., 2012. Deep brain stimulation of the subcallosal cingulate gyrus: further evidence in treatment-resistant major depression. *Int. J. Neuropsychopharmacol.* 15, 121-133.

Riga, M.S., Sánchez, C., Celada, P., Artigas, F., 2016. Involvement of 5-HT₃ receptors in the action of vortioxetine in rat brain: Focus on glutamatergic and GABAergic neurotransmission. *Neuropharmacology* 108, 73-81.

Romero, L., Hervás, I., Artigas, F., 1996 The 5-HT_{1A} antagonist WAY-100635 selectively potentiates the presynaptic effects of serotonergic antidepressants in rat brain. *Neurosci Lett.* 219, 123-126.

Romero L, Artigas F., 1997. Preferential potentiation of the effects of serotonin uptake inhibitors by 5-HT_{1A} receptor antagonists in the dorsal raphe pathway: role of somatodendritic autoreceptors. *J. Neurochem.* 68, 2593-2603.

Romero, L., Bel, N., Artigas, F., De Montigny, C., Blier, P., 1996. Effect of pindolol on the function of pre- and postsynaptic 5-HT_{1A} receptors: in vivo microdialysis and electrophysiological studies in the rat brain. *Neuropsychopharmacology* 15, 349-360.

Rush, A.J., Trivedi, M.H., Wisniewski, S.R., Stewart, J.W., Nierenberg, A.A., Thase, M.E., Ritz, L., Biggs, M.M., Warden, D., Luther, J.F., Shores-Wilson, K., Niederehe, G., Fava, M., STAR*D Study Team, 2006a. Bupropion-SR, sertraline, or venlafaxine-XR after failure of SSRIs for depression. *N. Engl. J. Med.* 354, 1231-1242.

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., 2006b. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *Am. J. Psychiatry* 163, 1905-1917.
- Rutter, J.J., Gundlach, C., Auerbach, S.B., 1995. Systemic uptake inhibition decreases serotonin release via somatodendritic autoreceptor activation. *Synapse* 20, 225-233.
- Sahay, A., Hen, R., 2007. Adult hippocampal neurogenesis in depression. *Nat. Neurosci.* 10, 1110-1115.
- Sahli, Z.T., Banerjee, P., Tarazi, F.I., 2016. The preclinical and clinical effects of vilazodone for the treatment of major depressive disorder. *Expert Opin. Drug Discov.* 11, 515-523.
- Samuels, B.A., Anacker, C., Hu, A., Levinstein, M.R., Pickenhagen, A., Tsetsenis, T., Madroñal, N., Donaldson, Z.R., Drew, L.J., Dranovsky, A., Gross, C.T., Tanaka, K.F., Hen, R., 2015. 5-HT_{1A} receptors on mature dentate gyrus granule cells are critical for the antidepressant response. *Nat. Neurosci.* 18, 1606-1616.
- Sanchez, C., Asin, K.E., Artigas, F., 2015. Vortioxetine, a novel antidepressant with multimodal activity: review of preclinical and clinical data. *Pharmacol. Ther.* 145, 43-57.
- 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.
- Sara, S.J., Herve-Minvielle, A., 1995. Inhibitory influence of frontal cortex on locus coeruleus neurons. *Proc. Natl. Acad. Sci. U.S.A* 92, 6032-6036.
- Saura, J., Bleuel, Z., Ulrich, J., Mendelowitsch, A., Chen, K., Shih, J.C., Malherbe, P., Da Prada, M., Richards, J.G., 1996. Molecular neuroanatomy of human monoamine oxidases A and B revealed by quantitative enzyme radioautography and in situ hybridization histochemistry. *Neuroscience* 70, 755-774.
- Saura, J., Kettler, R., Da Prada, M., Richards, J.G., 1992. Quantitative enzyme radioautography with 3H-Ro 41-1049 and 3H-Ro 19-6327 in vitro: localization and abundance of MAO-A and MAO-B in rat CNS, peripheral organs, and human brain. *J. Neurosci.* 12, 1977-1999.
- Scorza, M.C., Llado-Pelfort, L., Oller, S., Cortés, R., Puigdemont, D., Portella, M.J., Pérez-Egea, R., Alvarez, E., Celada, P., Pérez, V., Artigas, F., 2012. Preclinical and clinical characterization of the selective 5-HT_{1A} receptor antagonist DU- 125530 for antidepressant treatment. *Br. J. Pharmacol.* 167, 1021-1034.
- Serrats, J., Artigas, F., Mengod, G., Cortes, R., 2004. An autoradiographic study of the influence of pindolol upon [35S]GTPgammaS binding in rat, guinea pig and human brain. *Int. J. Neuropsychopharmacol.* 7, 27-34.
- Serretti, A., Kato, M., De Ronchi, D., Kinoshita, T., 2007. Meta-analysis of serotonin transporter gene promoter polymorphism (5-HTTLPR) association with selective serotonin reuptake inhibitor efficacy in depressed patients. *Mol. Psychiatry* 12, 247-257.
- Shelton, R.C., Tollefson, G.D., Tohen, M., Stahl, S., Gannon, K.S., Jacobs, T.G., Buras, W.R., Bymaster, F.P., Zhang, W., Spencer, K.A., Feldman, P.D., Meltzer, H.Y., 2001. A novel augmentation strategy for treating resistant major depression. *Am. J. Psychiatry* 158, 131-134.

- Smeraldi, E., Zanardi, R., Benedetti, F., Di Bella, D., Perez, J., Catalano, M., 1998. Polymorphism within the promoter of the serotonin transporter gene and antidepressant efficacy of fluvoxamine. *Mol. Psychiatry* 3, 508-511.
- Smith, K.A., Fairburn, C.G., Cowen, P.J., 1997. Relapse of depression after rapid depletion of tryptophan. *Lancet* 349, 915-919.
- Spies, M., Knudsen, G.M., Lanzenberger, R., Kasper, S. 2015. The serotonin transporter in psychiatric disorders: insights from PET imaging. *Lancet Psychiatry* 2, 743-755
- Sreenivasan, K.K., Curtis, C.E., D'Esposito, M., 2014. Revisiting the role of persistent neural activity during working memory. *Trends Cogn. Sci.* 18, 82-9.
- Stahl, S.M., 2000. Placebo-controlled comparison of the selective serotonin reuptake inhibitors citalopram and sertraline. *Biol. Psychiatry* 48, 894-901.
- Stockmeier, C.A., Shapiro, L.A., Dilley, G.E., Kolli, T.N., Friedman, L., Rajkowska, G., 1998. Increase in serotonin- 1A autoreceptors in the midbrain of suicide victims with major depression-postmortem evidence for decreased serotonin activity. *J. Neurosci.* 18, 7394-7401.
- Thase, M.E., Entsuah, A.R., Rudolph, R.L., 2001. Remission rates during treatment with venlafaxine or selective serotonin reuptake inhibitors. *Br. J. Psychiatry* 178, 234-241.
- Thase, M.E., Mahabeshwarkar, A.R., Dragheim, M., Loft, H., Vieta, E., 2016. A meta-analysis of randomized, placebo-controlled trials of vortioxetine for the treatment of major depressive disorder in adults. *Eur. Neuropsychopharmacol.* 2016 Mar 25. pii: S0924-977X(16)30005-0. doi: 10.1016/j.euroneuro.2016.03.007. (Epub ahead of print).
- Tollefson, G.D., Holman, S.L., 1994. How long to onset of antidepressant action: a meta-analysis of patients treated with fluoxetine or placebo. *Int. Clin. Psychopharmacol.* 9, 245-250.
- Tommasi, G., Lanotte, M., Albert, U., Zibetti, M., Castelli, L., Maina, G., Lopiano, L., 2008. Transient acute depressive state induced by subthalamic region stimulation. *J Neurol Sci.* 273:135-138
- Tong, Z.Y., Overton, P.G., Clark, D., 1996. Stimulation of the prefrontal cortex in the rat induces patterns of activity in midbrain dopaminergic neurons which resemble natural burst events. *Synapse* 22, 195-208.
- Trivedi, M.H., Rush, A.J., Wisniewski, S.R., Nierenberg, A.A., Warden, D., Ritz, L., Norquist, G., Howland, R.H., Lebowitz, B., McGrath, P.J., Shores-Wilson, K., Biggs, M.M., Balasubramani, G.K., Fava, M., STAR*D Study Team, 2006a. Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. *Am. J. Psychiatry* 163, 28-40.
- Trivedi, M.H., Fava, M., Wisniewski, S.R., Thase, M.E., Quitkin, F., Warden, D., Ritz, L., Nierenberg, A.A., Lebowitz, B.D., Biggs, M.M., Luther, J.F., Shores-Wilson, K., Rush, A.J., STAR*D Study Team, 2006b. Medication augmentation after the failure of SSRIs for depression. *N. Engl. J. Med.* 354, 1243-1252.
- Wallace, A., Pehrson, A.L., Sanchez, C., Morilak, D.A., 2014. Vortioxetine restores reversal learning impaired by 5-HT depletion or chronic intermittent cold stress in rats. *Int. J. Neuropsychopharmacol.* 17, 1695-1706.

Warden, M.R., Selimbeyoglu, A., Mirzabekov, J.J., Lo, M., Thompson, K.R., Kim, S.Y., Adhikari, A., Tye, K.M., Frank, L.M., Deisseroth, K., 2012. A prefrontal cortex-brainstem neuronal projection that controls response to behavioural challenge. *Nature* 492, 428-432.

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.* 24, 513-520.

Whiteford, H.A., Degenhardt, L., Rehm, J., Baxter, A.J., Ferrari, A.J., Erskine, H.E., Charlson, F.J., Norman, R.E., Flaxman, A.D., Johns, N., Burstein, R., Murray, C.J., Vos, T., 2013. Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. *Lancet* 382, 1575-1586.

Zanardi, R., Benedetti, F., Di Bella, D., Catalano, M., Smeraldi, E., 2000. Efficacy of paroxetine in depression is influenced by a functional polymorphism within the promoter of the serotonin transporter gene. *J. Clin. Psychopharmacol.* 20, 105-107.

Zarate, C.A. Jr., Singh, J.B., Carlson, P.J., Brutsche, N.E., Ameli, R., Luckenbaugh, D.A., Charney, D.S., Manji, H.K., 2006. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch. Gen. Psychiatry* 63, 856-864.

Figure legends

Figure 1. Schematic representation of the net effects of antidepressant drugs inhibiting SERT (TCA, SSRI, SNRI) on the active (extracellular) 5-HT concentration. SERT blockade in forebrain nerve terminals has a positive influence, which is partly attenuated by the reduction of 5-HT release by axon terminals produced by the activation of somatodendritic 5-HT_{1A} autoreceptors in the midbrain raphe after SERT blockade in this area. The activation of terminal 5-HT_{1B} autoreceptors also contributes to reduce 5-HT release in a local manner. Further, the activation of postsynaptic 5-HT receptors may influence 5-HT release, either locally, via modulation of local microcircuits involving GABA interneurons, or distally, in postsynaptic structures projecting to the midbrain raphe nuclei, such as the prefrontal cortex. Hence, activation of 5-HT_{1A} receptors in pyramidal neurons and of 5-HT₃ receptors in GABA interneurons exerts a distal negative feedback on serotonergic activity, whereas the activation of 5-HT_{2A} or 5-HT₄ receptors in pyramidal neurons has positive effects on 5-HT neuronal activity and 5-HT release. 5-HT₃ receptors in GABA interneurons may also exert a local control on 5-HT through the activation of GABA_B receptors. The overall balance between positive and negative effects depends on the antidepressant dose. Hence, SSRI doses >10-fold greater than clinical p.o. doses increase forebrain active 5-HT concentration yet clinical doses do not produce such increase or even reduce 5-HT release below basal levels. Modified from Artigas et al. (2001).

Figure 2. Bar graphs showing the displacement of the 5-HT_{1B} receptor ligand [¹¹C]AZ10419369 by the SSRI escitalopram in positron emission tomography (PET) scan studies conducted in non-human primates and healthy volunteers. **A)** After a first scan (PET1), primate subjects were infused i.v. with 2 mg/kg escitalopram and healthy volunteers received 20 mg escitalopram p.o..A second scan was performed after escitalopram administration (PET2), which revealed a reduction of the binding potential in all brain areas of primate brain, as expected from the displacement of [¹¹C]AZ10419369 by the increased extracellular 5-HT concentration produced by SERT blockade. **B)** However, a clinical escitalopram dose (20 mg p.o., equivalent to 0.25-0.3 mg/kg) evoked an opposite effect in the midbrain raphe nuclei and in forebrain areas, with a reduction of the binding potential in the raphe nuclei (yet not statistically significant) and an increase of the binding potential in projection areas of 5-HT neurons, most likely resulting from a decreased release of 5-HT induced by the excess 5-HT in the raphe nuclei and the subsequent activation of 5-HT_{1A} receptors. *Abbreviations used:* **A)** vB, cerebellum; DLPFC, dorsolateral prefrontal cortex; CN,

caudate nucleus; Put, putamen; Thal, thalamus; GP, globus pallidus; MB, midbrain; HC, hippocampus; RN, raphe nuclei. **B)** CB, cerebellum; FC, frontal cortex; TC, temporal cortex; CN, caudate nucleus ; Put, putamen ; Thal, thalamus ; RN, raphe nuclei. Data from 7 primate subjects and 9 healthy volunteers. * $p < 0.05$; ** $p < 0.01$. Reproduced with permission from Nord et al., (2013).

Figure 3. Schematic representation of the reciprocal connectivity between the medial prefrontal cortex (mPFC) and the dorsal and median raphe nuclei of the midbrain (DR and MnR, respectively), showing also the main targets for vilazodone and vortioxetine. Axons from pyramidal neurons in the mPFC innervate the DR and MnR and make excitatory synapses mediated by AMPA and NMDA receptors on 5-HT neurons and GABAergic interneurons. In anesthetized rats, the physiological stimulation of the mPFC results mainly in inhibitory responses of 5-HT neurons due to 1) the excitatory effect on raphe GABAergic interneurons, and 2) self-inhibitory responses in 5-HT neurons mediated by 5-HT_{1A} autoreceptors, following excitatory inputs that release 5-HT (Celada et al., 2001). In addition to blocking SERT, vilazodone and vortioxetine partially prevent 5-HT_{1A} autoreceptor activation by 5-HT due to its partial agonist character, thus allowing a greater elevation of extracellular 5-HT in forebrain areas after SERT blockade. This action may also contribute to desensitize 5-HT_{1A} autoreceptors. In addition, vortioxetine shows high affinity for other 5-HT receptors (5-HT_{1B}, 5-HT_{1D}, 5-HT₃ and 5-HT₇) with highest affinity for 5-HT₃ receptors.

These receptors are expressed by a subpopulation of GABA interneurons located in cortical layers I-III. Blockade of 5-HT₃ receptors reduces the GABA tone on GABA_A and GABA_B receptors, which leads, respectively, to *i*) an increased activity of pyramidal neurons projecting to the brainstem monoamine nuclei (DR/MnR, ventral tegmental area –VTA- and locus coeruleus –LC-), which in turn project back to the PFC (not shown for VTA and LC in the present figure), and *ii*) increased 5-HT release following the fall of the tone on GABA_B receptors, likely located on 5-HT nerve terminals. In turn, serotonergic neurons from the DR/MnR modulate the activity of mPFC pyramidal neurons via inhibitory 5-HT_{1A} receptors and excitatory 5-HT_{2A} receptors located on pyramidal and GABAergic neurons. Adapted from Artigas, 2015 and Riga et al., 2016.

Figure 4. Enhancement of the discharge of layer V pyramidal neurons in rat medial prefrontal cortex (mPFC) by vortioxetine (VOR) administration. Pyramidal neurons were identified by antidromic stimulation from midbrain monoamine nuclei (dorsal raphe and/or ventral tegmental area) and recorded extracellularly with glass electrodes (Riga et al., 2016). **A)** Representative example of a pyramidal neuron whose discharge rate was increased by the intravenous administration of vortioxetine (0.1-1.6 mg/kg). **B)** Bar graph showing the average effect of

vortioxetine in all neurons examined (n= 13). Of these, 10 were excited by vortioxetine and 3 were unaffected. Maximal effect was observed at 0.4 mg/kg i.v. The involvement of 5-HT₃ receptors is supported by the prevention of VOR effects by the previous administration of the 5-HT₃ receptor agonist SR57227A and by the similar effect produced by the 5-HT₃ receptor antagonist ondansetron and by escitalopram + ondansetron combinations (Riga et al., 2016). **C and D)** In contrast to vortioxetine, the intravenous administration of escitalopram at the same cumulative doses (0.1-1.6 mg/kg i.v.) did not alter the discharge of layer V mPFC pyramidal neurons (n=9). *p<0.05 vs. basal and saline conditions. Redrawn from data in Riga et al., 2016.