Multiple serotonergic paths to antidepressant efficacy

Pierre-Eric Lutz (pierreeric.lutz@gmail.com)
McGill Group for Suicide Studies, Douglas Mental Health University Institute, McGill University, Montreal, QC, Canada.

Abstract
Depression is a leading cause of disability worldwide. Brain mechanisms underlying the clinical antidepressant efficacy of selective serotonin reuptake inhibitors (SSRI), currently the first-line treatment, remain poorly understood. Recent animal studies have implicated multiple serotonin receptor subtypes in SSRI response, opening new therapeutic perspectives.

Keywords: serotonin receptor, selective serotonin reuptake inhibitor, depression, neurogenesis.

Depression, defined mainly by depressed mood and anhedonia, is a leading cause of disability worldwide. The serendipitous discovery 60 years ago of the antidepressant properties of tricyclics, and later of selective serotonin reuptake inhibitors (SSRI), has prompted scientists to investigate the role of the serotonergic system in antidepressant medication and depression pathophysiology. Here, we summarize recent findings from rodent models indicating that different subtypes of serotonin (5-HT) receptors regulate the activity of 5-HT neurons, and mediate the antidepressant-like efficacy of SSRI.

Serotonergic neurons are mainly located in the midbrain dorsal raphe nucleus (DRN) and send axonal projections throughout the whole brain, where released 5-HT targets different receptor types (Hannon and Hoyer 2008). Serotonergic neurons can be identified by their typical pattern of electrophysiological activity (a slow and regular firing rate, with long-duration positive action potentials). These neurons express the molecular target of SSRI, the 5-HT transporter (SERT), in both their somato-dendritic compartment and axon terminals (Fig. 1A). At both levels, acute SSRI treatment inhibits SERT and increases 5-HT concentration in the extra-cellular space. In the DRN, increased release of 5-HT activates the inhibitory Gi-coupled 5-HT_{1A} receptor (5-HT_{1A}R), which is expressed by 5-HT neurons as an autoreceptor. 5-HT_{1A}R activation in turn decreases the firing rate of 5-HT neurons and their neurotransmitter release, thus opposing the effect of SSRI on axon terminals. Therefore, due to DRN 5-HT_{1A}R negative feedback, acute SSRI treatment only slightly increases the endogenous serotonergic tone in projection regions (Blier and de Montigny 1994).

SSRI typically require 4 to 6 weeks to achieve antidepressant efficacy in the clinic, and neuroadaptations responsible for this delayed onset-of-action are extensively studied. One of the earliest proposed mechanisms was the desensitization of 5-HT_{1A}R in the DRN (Blier and de Montigny 1994). Upon chronic SSRI treatment, 5-HT neurons return to a normal firing rate through 5-HT_{1A}R desensitization, thereby potentiating the serotonergic tone in virtually all DRN projection regions (see Fig. 1B). Accordingly, decreased 5-HT_{1A}R function would be expected to result in decreased autoinhibition and faster SSRI response. However, combining SSRI and a specific 5-HT_{1A}R antagonist (such as WAY100635) has failed to produce fast antidepressant response in animal models.

Additional complexity comes from the fact that the 5-HT_{1A}R is also expressed as a heteroreceptor by forebrain non-serotonergic neurons. During chronic SSRI treatment, the serotonergic tone acting at every post-synaptic 5-HT receptor, including 5-HT_{1A} heteroreceptor, is potentiated. Activation of these heteroreceptors, notably in the hippocampus, has been implicated in SSRI antidepressant efficacy, and may be blocked during combined therapy with a 5HT_{1A}R antagonist (Lucas et al. 2007). Moreover, 5-HT_{1A}...
heteroreceptors indirectly regulate the activity of 5-HT neurons. Activation of 5-HT1A receptors expressed by layer 5 pyramidal neurons in the prefrontal cortex (PFC) was shown to decrease the firing rate of 5-HT neurons, a negative long-feedback loop that mimics activation of DRN autoreceptors (Celada et al. 2001). How this cortical receptor pool adapts during chronic SSRI treatment, and its behavioural relevance for antidepressant efficacy, remains poorly understood.

To manipulate specifically 5-HT1A autoreceptors and determine their role in SSRI behavioural effects, Richardson-Jones et al recently used a conditional genetic approach (Richardson-Jones et al. 2010). They compared adult mice showing normal levels (Auto1A-High) or a 30% genetic deletion (Auto1A-Low) of 5-HT1A autoreceptors, while expression of 5-HT1A heteroreceptors was unchanged. Surprisingly, this modest decreased autoinhibition in Auto1A-Low mice had strong effects on 5-HT neurons and emotional responses, and was sufficient to increase the spontaneous firing activity of 5-HT neurons. Upon acute SSRI exposure, reduced autoinhibition led to increased 5-HT release in the 2 projection regions examined (PFC and hippocampus). Importantly, Auto1A-Low mice showed enhanced behavioural response to SSRI in the novelty suppressed feeding test, a measure of hyponeophagia classically responsive to chronic but not acute treatment. Eight or 26 days of treatment with the SSRI fluoxetine had antidepressant-like effects in Auto1A-Low mice, while Auto1A-High mice showed no response for either treatment duration. These results therefore suggest that the level of 5-HT1AR expression in the DRN partly determines the onset of SSRI antidepressant-like effects.

The 5-HT1B receptor (5-HT1BR) is another Gi-coupled receptor, expressed both as an auto- and heteroreceptor. In contrast to the somato-dendritic 5-HT1A autoreceptor, the 5-HT1B is localized in axon terminals of 5-HT neurons (Fig. 1A), thereby controlling 5-HT release in projection regions. Chronic SSRI treatment was shown to induce desensitization and reduced expression of 5-HT1B autoreceptors, likely contributing to increased serotonergic tone during SSRI exposure. These effects, however, appear less robust than for the 5-HT1AR, and their relevance in the behavioural onset of action or efficacy of SSRI remains poorly characterized in animal models (see (McDevitt and Neumaier 2011) for a review). More recently, it was shown that activity of the 5-HT1B is regulated by the S100α, a protein that anchors the 5-HT1B and other 5-HT receptors at the cell membrane, notably during chronic SSRI exposure (see below and (Svenningsson et al. 2006)).

Another neuroadaptation triggered by chronic, but not acute, SSRI treatment is the generation of new neurons in the dentate gyrus of the hippocampus. Ablation of hippocampal neurogenesis by X-ray irradiation was shown to abolish SSRI antidepressant-like efficacy in the novelty suppressed feeding test (Santarelli et al. 2003). Interestingly, neurogenic and antidepressant-like effects of the SSRI fluoxetine were lost in 5-HT1B constitutive KO mice, indicating that they occur downstream of this receptor’s signaling (Fig. 1B). Available data suggest that both 5-HT1A auto- and heteroreceptors may mediate SSRI neurogenic effects. Decreased 5-HT1A autoinhibition may favor increased SSRI-induced serotonergic activation, promoting faster neurogenesis and enhanced antidepressant response. Genetic polymorphisms affecting the expression (Lemonde et al. 2003), coupling efficiency or transcriptional regulation of the 5-HT1A R, have been suggested and will require further investigation (see (Albert 2012; McDevitt and Neumaier 2011) for detailed reviews).

Alternatively, hippocampal 5-HT1A heteroreceptors may directly control neurogenesis. Genetic deletion of 5-HT1A heteroreceptors has been recently achieved in glutamatergic neurons of the forebrain (Richardson-Jones et al. 2011), and future studies should explore SSRI response in these mice, in which 5-HT1A R are undetectable in the whole hippocampus.

To bypass 5-HT1A and 5-HT1B autoinhibitions, researchers have looked for direct activators of 5-HT neurons, which may be endowed with fast neurogenic and antidepressant properties. Within this line, activation of the excitatory Gq-coupled 5-HT2B receptor (5-
HT₂BR) in the DRN was shown to increase local 5-HT release (Doly et al. 2008). Considering that the 5-HT₂BR is expressed by DRN 5-HT neurons (Diaz et al. 2012), this autoreceptor appears to be responsible for a local positive feedback that opposes 5-HT₁A and 5-HT₁B activities (Fig. 1A). In addition, the 5-HT₂BR is a crucial mediator of SSRI pharmacological and behavioural effects. Microdialysis experiments indicated that enhanced 5-HT release induced in the hippocampus by acute SSRI exposure was strongly reduced by either systemic pharmacological blockade or genetic ablation of the 5-HT₂BR (Diaz et al. 2012). Further, neurogenic and antidepressant-like effects of chronic SSRI treatment were also abolished by pharmacological blockade or genetic ablation of 5-HT₂BR (Diaz et al. 2012). Thus, 5-HT₁A and 5-HT₂BR autoreceptors oppositely modulate the activity of 5-HT neurons, and both receptors are controlling behavioural response to chronic SSRI treatment.

In view of these data, activation of the 5-HT₂BR may now emerge as a therapeutic target in depression, despite concerns about potential cardiac toxicity (Hutcheson et al. 2011). In the forced swim test, a classical rodent screen for antidepressants, acute systemic administration of the 5-HT₂BR agonist BW723C86 decreased despair-like behaviour with similar efficacy compared to acute treatment with the SSRI fluoxetine. In the novelty suppressed feeding test, BW723C86 also had an antidepressant-like effect, similar to chronic fluoxetine. Somewhat surprisingly, a 4-week treatment with BW723C86 was required in the later paradigm, suggesting that activation of serotonergic neurons through the 5-HT₂BR does not achieve fast antidepressant-like effect, and may be hampered by homeostatic processes. Additional studies are required to assess, during the course of acute and prolonged 5-HT₂BR signaling, both 5-HT₂BR sensitivity and adaptations at other 5-HT receptors, which together determine the electrophysiological activity pattern of 5-HT neurons. Alternately, considering that 5-HT₂BR expression is low in the DRN and restricted to a few regions of the brain (Hannon and Hoyer 2008), one could speculate that systemic activation of this receptor may act on a sub-set of 5-HT neurons, possibly targeting specific projection areas with an overall limited impact on mood circuits.

The 5-HT₄R is another excitatory 5-HT receptor type that is coupled to Gs-proteins. This receptor is poorly expressed in the DRN, but is enriched in the striatum, the hippocampus and cortical areas. In rat, continuous systemic administration of a 5-HT₄R agonist (prucalopride or RS67333) rapidly increased the firing of 5-HT neurons, reaching a maximum after 3 days (Lucas et al. 2005). This potentiation persisted after a 3-week treatment, suggesting that the 5-HT₄R does not desensitize. To localize where the 5-HT₄R operates to control 5-HT neurons, over-expression experiments were performed using viral vectors. In the PFC but not in the striatum or the hippocampus, 5-HT₄R over-expression enhanced the firing rate of around 50% of DRN 5-HT neurons, revealing a positive long-feedback loop originating in the cortex (Fig. 1A). The next step was to evaluate the antidepressant potential of 5-HT₄R agonists in comparison with SSRI (Lucas et al. 2007). In the forced swim test, acute systemic administration of 5-HT₄R agonists decreased despair-like behaviour, similar to the SSRI citalopram. In olfactory bulbectomy and chronic mild stress, two models of depression, prolonged depressive-like behaviours were also reversed by the 5-HT₄R agonist RS67333. The latter antidepressant-like effect appeared in 3 days only, much faster than with citalopram (2 weeks). Therefore, 5-HT₄R agonists represent the first putative fast-acting serotonergic antidepressants.

The authors then wondered whether neuronal mechanisms classically implicated in SSRI actions would be recruited faster upon 5-HT₄R activation (Fig. 1B): desensitization of DRN 5-HT₁A autoreceptors, activation of hippocampal 5-HT₁A heteroreceptors and neurogenesis (Lucas et al. 2007). As already mentioned, acute SSRI exposure decreases the firing of 5-HT neurons in a 5-HT₁A, dose-dependent manner. After systemic activation of the 5-HT₄R over 3 days, higher doses of the SSRI citalopram were required to inhibit 5-HT neurons, indicating that 5-HT₁A desensitized more rapidly than previously described for
SSRI (2-3 weeks). In the hippocampus, CA3 pyramidal neurons are classically under tonic inhibition, notably through 5-HT₁₆ heteroreceptors. As expected, systemic activation of the 5-HT₁₆R potentiated the inhibition of CA3 neurons, an effect that was blocked by local infusion of a 5-HT₁₆R antagonist in the hippocampus. Finally, a 3-day treatment with RS67333 was sufficient to increase both cellular proliferation and survival in the hippocampus, which were known to require at least 11 days of SSRI treatment (Santarelli et al. 2003). Thus, sub-acute 5-HT₁₆R signaling recapitulates various effects of chronic SSRI treatment. 5-HT₁₆R agonists primarily activate a PFC-DRN circuitry, in turn enhancing the serotonergic transmission to the hippocampus and correlating with fast antidepressant-like response.

It remains to be determined whether the observed increased hippocampal 5-HT₁₆R signaling and neurogenesis are responsible for the remarkably fast antidepressant-like properties of the 5-HT₁₆R agonist RS67333. It would be interesting to assess RS67333 behavioural effects in the absence of hippocampal neurogenesis, such as previously performed for SSRI (using X-ray irradiation, see (Santarelli et al. 2003)). In addition, activation of 5-HT₁₆R impacts roughly half of DRN 5-HT neurons, and beyond the hippocampus, is likely to modify 5-HT release in other brain regions relevant for mood regulation. Ultimately, activation of 5-HT₁₆R may have fast-acting properties independently of any change in 5-HT neurons firing, and may act directly at striatal or cortical structures to promote antidepressant-like effects.

Schmidt et al recently reported important evidence in support of this provocative hypothesis (Schmidt et al. 2012). The authors used translating ribosome affinity purification (TRAP), a technological breakthrough based on the transgenic tagging of a ribosomal protein in specific cell types. Tagged ribosomes can be immunopurified so as to isolate associated, translating mRNAs. Here, this method was applied to S100α-expressing neurons. This population was chosen because previous reports indicated that S100α (also called p11) acts as an anchoring protein that binds to several 5-HT receptors - including 5-HT₁₆R - and stabilizes their localization at the cell surface (Warner-Schmidt et al. 2009). In a first step, retrograde tracing experiments indicated that cell bodies of S100α-tagged neurons are located in layer 5 of frontal, motor and sensory cortices, and send axonal projections to the dorsal striatum and contra-lateral cortex. Microarray technology and TRAP were then combined to characterize cortico-striatal neurons. These neurons showed a specific transcriptomic signature that was highly distinct from that of cortico-pontine neurons, another pyramidal cell type located in the cortical layer 5. Cortico-striatal neurons also showed a strong sensitivity to chronic SSRI treatment, because 2 weeks of fluoxetine treatment regulated 62 genes in this cell type (but only 4 genes in cortico-pontine neurons).

Most importantly, chronic SSRI treatment induced a striking 16-fold up-regulation of the 5-HT₁₆R in S100α-positive neurons, while the expression of other serotonin receptors was left unaffected.

Further, the authors investigated the role of cortico-striatal neurons in SSRI behavioural response. A genetic conditional strategy was used to delete S100α in the cortex. In these mice, antidepressant-like response to chronic fluoxetine was abolished in the tail suspension test (another measure of despair-like behaviour, close to the forced swim) and the novelty suppressed feeding test, while the up-regulation of the 5-HT₁₆R was significantly blunted. Therefore, these results show that the expression of the anchoring protein S100α in cortico-striatal neurons is necessary for chronic SSRI antidepressant-like effects. They also strongly suggest a new mechanism for the delayed onset-of-action of SSRI, whereby an enhanced serotonergic tone acts on an up-regulated pool of 5-HT₁₆R in S100α-expressing neurons. It is therefore possible that fast-acting properties of the 5-HT₁₆R agonist RS67333 rely on the direct pharmacological activation of this cortico-striatal signaling, mimicking chronic effects of SSRI. Interestingly, the same group previously demonstrated that the
effects of RS67333 in the forced swim and tail suspension tests are lost in S100α constitutive
KO mice (Warner-Schmidt et al. 2009). Future studies should explore models of depression
and chronic SSRI efficacy (novelty suppressed feeding and chronic mild stress for example),
and test whether fast effects of RS67333 are retained after conditional deletion of S100α in
the cortex. Altogether, these findings reveal another brain region, in addition to the
hippocampus, where SSRI act to achieve antidepressant-like response. The molecular
mechanisms mediating up-regulation of a single 5-HT receptor type, in a single cell
population, during chronic SSRI exposure remain unknown. In the future, elucidating these
mechanisms may open new therapeutic avenues.

Adding to the list of adaptations occurring at single 5-HT receptors described in this
review, new molecular mechanisms regulating monoamine receptors have recently
emerged, such as lipid rafts and adaptor proteins (see (Bjork and Svenningsson 2011) for a
review). Adaptor, or anchoring, proteins are capable of recruiting simultaneously multiple
receptor types, and the aforementioned S100α protein represents a promising target.
Chronic SSRI treatment increases the cortical expression of S100α (Schmidt et al. 2012), thus
favoring the localization at the cell membrane of the 5-HT₄R, as already mentioned, as well
as of other 5-HT receptors: 5-HT₁B and 5-HT₁D (Warner-Schmidt et al. 2009). The difficult task
of assessing the role of anchoring proteins, and the specific combinations of 5-HT receptors
they recruit, during SSRI treatment and antidepressant-like response is only beginning
(Egeland et al. 2010).

In summary, the results from animal models reviewed here implicate several
subtypes of 5-HT receptors in SSRI antidepressant-like efficacy. These anatomically dispersed
5-HT receptors together control the firing pattern of 5-HT neurons, in an intricate
modulatory network. Cortical layer 5 pyramidal neurons express 5-HT₁₅A heteroreceptors and
inhibit 5-HT neurons through a negative long-feedback loop. Two populations of cortical
layer 5 neurons expressing 5-HT₄ heteroreceptors either activate 5-HT neurons through a
positive long-feedback loop, or project to the striatum (Fig. 1A), and both likely contribute to
mood control. 5-HT neurons also self-regulate their firing activity through autoreceptors,
with opposed negative (5-HT₁₅A) and positive (5-HT₂B) feedbacks in the DRN, as well as a
negative feedback in axon terminals (5-HT₂B). Finally, while they received less attention,
other 5-HT receptors are recruited by SSRI, such as 5-HT₆ (Svenningsson et al. 2007) and 5-
HT₇ (Mnie-Filali et al. 2011) receptors. SSRI ultimately potentiate the serotonergic
transmission throughout the brain, and simultaneously act at auto- and hetero-
receptors. Notably, available evidence reveals that both hippocampal neurogenesis and cortico-striatal
neurons are necessary for SSRI antidepressant-like effects. It remains unclear whether all
these 5-HT receptors and neuronal substrates must act in concert to achieve antidepressant-
like efficacy. A major goal for future studies will be to determine their relative contributions
as a function of inter-individual sources of variability, in animal models and in humans.

Figure 1. Brain substrates and serotonin (5-HT) receptors implicated in antidepressant-like
properties of selective serotonin reuptake inhibitors (SSRI) in rodent models. (A) SSRI
target the serotonin transporter (SERT, green arrows) in both the dorsal raphe nucleus
(DRN), the cell-body region of 5-HT neurons, and in their axon terminals. Thus, acute SSRI
exposure potentially increases the endogenous 5-HT tone at every receptor type. In
particular, activations of 5-HT₁₅A and 5-HT₂B autoreceptors in the DRN, respectively,
decreases and increases the firing rate of 5-HT neurons, and correspond to local negative
and positive feedback mechanisms (blue and pink circular arrows). 5-HT₁₅A expressed by 5-HT
neurons in their axon terminals also contribute to auto-inhibition. Furthermore, 5-HT₁₅A
and 5-HT₄ heteroreceptors expressed by prefrontal cortex (PFC) layer 5 pyramidal neurons,
respectively, inhibit and activate 5-HT neurons, in negative and positive long-feedback loops.
Upon acute and chronic SSRI treatment (2-3 weeks), a complex cascade of brain adaptations occurs at different brain sites and contributes to antidepressant-like efficacy. Notably, desensitization of 5-HT1A and 5-HT1B autoreceptors is thought to increase the endogenous serotonergic tone, in turn acting on: (i) cortical 5-HT2R, (ii) hippocampal (Hipp) neurogenesis, and (iii) CA3 5-HT1A heteroreceptors. Sub-acute (3 days) activation of 5-HT4 receptors mimics several effects of SSRI, and achieves fast antidepressant responses. Whether CA3 5-HT1AR contributes to increased neurogenesis during chronic SSRI treatment, and whether cortical-striatal 5-HT2R in S100α-positive cells (see text for details) mediates the fast-acting properties of 5-HT4R agonists, remain to be addressed (dashed arrows). Abbreviation: Dorsal Striat, dorsal striatum.

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References
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Lutz. Fig. 1A
Chronic SSRI treatment
(eg. Fluoxetine)

Decreased autoinhibition of
5-HT neurons (5-HT$_{1A}$ & 5-HT$_{1B}$)

Enhanced endogenous 5-HT tone

5-HT$_4$R on cortico-striatal neurons

Increased hippocampal neurogenesis

? 5-HT$_{1A}$R on hippocampal neurons

Sub-acute 5-HT$_4$R agonist treatment
(eg. RS67333)

Antidepressant-like response