Connection re-established: neurotransmission between the medial prefrontal cortex and serotonergic neurons offers perspectives for fast antidepressant action

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Summary points

- **Background**
  - Only a third of depressed patients experience a complete therapeutic improvement with the use of current antidepressant treatments.
  - Even when effective, these molecules display a delayed onset of action. This can be a critical point when considering that a significant proportion of patients are at risk for suicide.
  - The development of new, more effective and more rapid antidepressant treatments constitutes a major issue for current neuropsychopharmacological research.

- **New perspectives of research**
  - Several authors have pointed out a potential role for the molecular factors and/or processes that favor tissular growth and plasticity within the brain, proposing that monoaminergic-based pharmacological approaches might be somewhat outdated.
  - There are, however, a number of recent reports suggesting that the anatomical connections existing between the medial prefrontal cortex (mPFC) and serotonergic (5-HT) neurons located within the dorsal raphé may constitute a promising vector to achieve more rapid and/or more effective antidepressant efficacy. This vector being mobilized by using either pharmacological or surgical tools.

- **Importance of the mPFC/5-HT connection**
  - Deep-brain stimulation of the human Cg25 area produced strong and rapid antidepressant effects in treatment-resistant depressed patients. Animal experiments indicate that the glutamatergic projections connecting the mPFC and 5-HT neurons appear to be crucial for the expression of such effects.
  - Several animal studies also indicate that the stimulation of 5-HT4 receptors may result in antidepressant-like effects within a four- to seven-fold more rapid timeframe than what is observed with standard molecules. This effect appears to involve the mobilization of mPFC/5-HT connections.
  - Similarly, the use of 5-HT7 receptor antagonists led to promising results to achieve a more rapid and/or more effective antidepressant action. Once again, the effect seems to originate from the mPFC, with a possible involvement of the glial system.

- **Conclusion**
  - A more complete characterization of the mPFC/5-HT relationship should open up new perspectives for the treatment of depression, with the possibility to address multiple targets and therefore increase the diversity of approaches.
The search for fast-acting antidepressants has been a major challenge in neuropsychopharmacology for many years. Although the involvement of serotonin (5-HT) in the mechanisms of action of classical antidepressants has been clearly established, the delayed onset of action of these drugs prompted several authors to propose alternative targets in order to achieve a more rapid relief of symptoms in depressed patients. However, recent studies indicate that it may be possible to elaborate fast-acting antidepressant strategies based on 5-HT, provided that such strategies would directly target 5-HT neuron electrical activity. Furthermore, glutamatergic pyramidal neurons projecting from the medial prefrontal cortex to the dorsal raphé appear to play a critical role. This article presents some of the data that support this hypothesis, including results from medial prefrontal cortex deep-brain stimulation studies, as well as those related to treatments with 5-HT4 agonists and 5-HT7 antagonists.

The search for a rapid-acting antidepressant (AD) strategy is still considered as a “quest for the holy grail” in the field of neuropsychopharmacology [1]. Typical ADs, among which selective serotonin reuptake inhibitors (SSRIs) are the most frequently used, display a delayed onset of action, sometimes requiring administration for several weeks before a clinical improvement can be seen. In addition, their therapeutic efficacy is not entirely guaranteed, as up to two-thirds of patients may be partially or fully resilient to such treatments [2]. The delayed action of ADs becomes a critical factor in particular cases of major/severe depression, where the risks of suicide are strongly increased. Several clinical reports indicate that suicide attempts are maximal during the first weeks of treatment, and that this risk is decreased in patients who experience an earlier AD response [3]. Furthermore, the adherence of patients to their medication appears to be more stable if therapeutic efficacy is perceived to occur rapidly [3]. It is now accepted that, despite the variety of their pharmacological profiles and primary targets, a common trait of all the currently used AD molecules resides in their ability to increase central serotonergic (5-HT) transmission [4–6]. In addition, most studies have focused on the dorsal raphé (DR) nucleus, from which the vast majority of brain 5-HT innervation originates [4]. However, SSRIs (e.g., tricyclics, MAO inhibitors or mixed reuptake inhibitors) do not act directly on 5-HT impulse flow, but induce an indirect augmentation of 5-HT extracellular levels via inhibition of inactivation mechanisms (e.g., reuptake or catabolism). Consequently, their facilitatory effect on 5-HT transmission is fully dependent on the actual level of DR 5-HT neuron electrical activity. Numerous animal studies have shown that, during the early stage (a few days) of a SSRI treatment, the 5-HT neuron impulse flow is quasi-suppressed, owing to the existence of inhibitory somatodendritic 5-HT1A autoreceptors [5,7]. It is only after a long-term (>2–3 weeks) period of treatment that these autoreceptors become desensitized, thus allowing the 5-HT neuronal firing rate to recover; it is thought that the delay of action of ADs is precisely related to the period required for this desensitization to occur [5,7].

More recently, new strategies of research have emerged, with the purpose of ‘bypassing’ this 5-HT-related inertia. They were oriented toward assessing the effects of AD beyond the postsynaptic 5-HT receptor level, searching for the cellular/molecular mechanisms that were affected by the treatment. An extensive and seminal work has been conducted in this field for more than a decade, in particular by the groups of Duman and Nestler [8,9]. These studies enabled the isolation of a number of biological parameters that are deeply modified after a sustained AD treatment. Initially, the first changes identified were an activation (phosphorylation) of the transcription factor CREB [10] and enhanced adult neurogenesis [11], both of which are selectively expressed within the hippocampus. More recently, the BDNF (for review see [12]) and VGF [13,14] growth factors were found to play a significant role in the effect of ADs, as well as the MAPK cascade [15] and p11, a Ca2+-binding protein of the EF-hand (helix-loop-helix) type [16,17]. These observations led to the general idea that ADs share the common ability to positively modulate cellular growth and plasticity in mood-related brain areas. As such, they would act in an opposite manner to stressful experiences, which are also known as risk factors for the emergence of depression in humans [18].

Should the emergence of these hypotheses mean that ‘old’ approaches based on the modulation of neurotransmission, such as...
neuropharmacological ones, have to be considered outdated? It has, for instance, been recently proposed that a viral-mediated gene therapy, aimed at increasing brain levels of p11, could constitute a promising new way to achieve AD efficacy [16,19]. Similarly, it is suggested that the development of therapeutic strategies able to directly target the molecular factors mentioned earlier would allow a more rapid onset of action than the currently used ADs [12,20]. Some recent clinical trials have been successful in this context. These studies were notably based on the use of the NMDA antagonist ketamine or consisted of sleep deprivation, both strategies being effective within hours [3]. The idea was to favor a rapid increase of synaptic/brain plasticity, involving, at least partially, an imbalance of the AMPA to NMDA throughput [3]. There is, however, a growing body of evidence that indicates that acting upon the connections existing between different neuronal networks, which includes pharmacological approaches, may also allow fast AD effects. In the present work, we will review the importance of the relationship existing between the medial prefrontal cortex (mPFC) and 5-HT neurons, with a particular focus on the role of 5-HT\textsubscript{1A} and 5-HT\textsubscript{7} receptors (some excellent reviews and articles have already highlighted a role for cortical 5-HT\textsubscript{1A} and 5-HT\textsubscript{7} receptors in this context [21–23]). We will first discuss a series of data showing that a direct, intracerebral electrical stimulation of the mPFC itself may result in spectacular AD efficacy in patients, and displays strong AD-like properties in rodents. The importance of central 5-HT transmission in the underlying mechanisms will also be discussed. We will thereafter address the relevance of studying 5-HT\textsubscript{1A} receptors, their agonists potentially constituting a new and yet ‘monoaminergic’ class of fast-acting ADs. In a similar manner, we will present a body of evidence suggesting that 5-HT\textsubscript{7} receptor antagonists may also be of importance for the purpose of accelerating AD efficacy. For both 5-HT receptor types, as already mentioned, the connection between the mPFC and 5-HT neurons appears to play a pivotal role in the expression of the reported features.

**Deep-brain stimulation of the mPFC**

Within the human brain, several limbic structures have been associated with affective and anxiety disorders. Historically, ablative stereotactic procedures of the anterior cingulate cortex (anterior cingulotomy), of projections from the orbitofrontal (subcaudate tractotomy) [24,25] and/or cingulate cortex to the basal ganglia and medial thalamus (limbic leucotomy) constituted surgical options for treatment-refractory depression (for review see [26–28]). However, these procedures, although partially effective in alleviating symptoms in patients with affective and obsessive–compulsive disorders, have significant and irreversible side effects [27,29]. More recently, functional imaging studies emphasized the involvement of limbic dysfunction in depression, and revealed decreases in metabolism of dorsal limbic and neocortical regions (prefrontal, pre-motor and parietal cortex) and relative increases in ventral paralimbic areas (subgenual cingulate, anterior insula, hypothalamus and caudate) in depressed patients [30,31]. Interestingly, the subgenual cingulate gyrus (SCG), adjacent to the Brodmann area 25, is able to modulate the activity of both the frontal cortex and the limbic system, which are respectively underactive and overactive in depressed patients [32–34]. Moreover, it has been reported that this region, involved in the mediation of depressive symptoms [35,36] or acute sadness [37,38], is also metabolically overactive in treatment-resistant depression [39,40] and is normalized after successful treatments including pharmacological treatment [30,32,41], electroconvulsive therapy [39,40] and transcranial magnetic stimulation [43]. Mayberg et al. conducted a pilot study to examine the efficacy of SCG area deep-brain stimulation (DBS) in treatment-resistant patients [41]. In the initial report, four of six patients demonstrated a significant reduction of depressive symptoms after only 1 week of high-frequency DBS. In addition, the authors observed acute, quasi-instantaneous behavioral changes during the daily short sessions of DBS that occurred within the first postoperative week, before the protocol was established in a continuous mode [44]. Therefore, it may be hypothesized that the efficacy of DBS actually occurs within a very rapid time-frame, similar, or even inferior, to the one observed with ketamine or sleep deprivation (see earlier). The recruitment of 14 more refractory patients confirmed these preliminary results: the benefits observed after 1 week on the Hamilton Depression Rating Scale were still maintained after 6 or 12 months of stimulation, with 35% of patients (seven out of 20) in total remission, and 25% (five out of 20) displaying a partial response [45,46]. Additional trials now
need to be conducted in a larger group of patients with the addition of ‘true’ double-blinded methods of evaluation to fully validate the method. However, it should be mentioned that, although the studies were not blinded as such, they were subject to strict control conditions: during the first postoperative week, patients were stimulated using either a 0.0 V, a subthreshold or the best-set condition of stimulation, being unaware of the setting chosen. In the first two cases, stimulation failed to elicit any behavioral changes. Also, in order to assess whether the long-term benefit in responders could be related to placebo or nonspecific factors, the continuous stimulation of patient one was stopped after 6 months. Following blinded discontinuation of bilateral stimulation (stimulators set at 0.0 V), AD effects were maintained for 2 weeks but a progressive change in behavior, characterized by loss of energy and initiative, impaired concentration and reduced activities were observed during weeks 3 and 4. These symptoms were normalized 48 h after the stimulator was turned back to the previous optimal settings.

Although the neurobiological bases of DBS as a therapeutic AD strategy are not currently determined, several lines of evidence suggest that there are specific neural circuits within the cortico-limbic system that mediate stress responsiveness, mood and emotional regulation. Regarding its mode of action, one of the commonly proposed hypotheses is that high-frequency stimulation reduces neural transmission through inactivation of voltage-dependent ion channels [47–49]. However, currently available animal studies do not support this possibility. Thus, Hamani et al. have modeled the antidepressant effect of SCG DBS by stimulating the rat mPFC [50], a brain area that is likely to be homologous to the human SCG in rodents [51]. They clearly demonstrated that high-frequency stimulation of mPFC (130 Hz) produced an AD-like response in the forced swim test, and improved anxiety and hedonic states in both the novelty suppressed feeding test and sucrose consumption test. Importantly, they also showed the involvement of central 5-HT in DBS. First, using microdialysis, they showed that high-frequency stimulation of the mPFC correlated with a sustained increase of hippocampal 5-HT release. Second, AD-like behaviors induced by mPFC DBS are suppressed by a lesion of the 5-HT system. In agreement with these results, it has also been reported in rodents that a moderate to high (20–60 Hz) frequency stimulation of mPFC glutamatergic neurons increases 5-HT release in the DR, as well as dopamine output in both the ventral tegmental area and the nucleus accumbens [21,52–55]. More recently, we have studied the effects of mPFC DBS (130 Hz) on the firing activity of DR 5-HT neurons by using electrophysiological paradigms. We found that after only 1 h of DBS, the mean firing activity of 5-HT neurons increased by 30% [56].

Surprisingly, Hamani et al. also observed that a neuronal lesion in the mPFC, induced by using ibotenic acid, failed to affect the AD-like effects of DBS [50], which caused the authors to propose a possible involvement of ‘en passage’ fibers rather than local cell bodies [50]. Although it cannot be excluded that other cortical areas may exert an influence on DR 5-HT activity, it remains that anatomical studies report a dense innervation of the DR coming from the mPFC [57,58]. Accordingly, in vivo ‘collision’ electrophysiological experiments, using orthodromic and antidromic stimulations, have shown that a significant proportion of mPFC cell bodies project directly into the DR [21]. It is therefore possible that the persistence of DBS effects after ibotenic lesion could be caused by the recruitment of the few surviving pyramidal neurons. If so, the activity of these survivors would necessitate a strong metabolic supply, and the role of glial cells becomes more important than in normal conditions. Interestingly, Banasr and Duman have characterized the effects of a pharmacological glial reduction in the mPFC of adult rats in behavioral tests known to be affected by stress or AD treatments [59]. Remarkably, they demonstrated that mPFC infusions of an astrocyte-specific toxin, 1-α-aminoadipic acid, induced anhedonia, anxiety and helplessness while a ibotenic acid neurotoxic lesion failed to induce any behavioral impairment [59]. These effects of 1-α-aminoadipic acid, similar to chronic unpredictable stress-induced depressive-like behaviors, support the hypothesis that loss of glia contributes to the core symptoms of depression. Based on these observations, we conducted experiments in order to assess the implication of glia in the AD response to DBS. Our preliminary results showed that 1-α-aminoadipic acid infusion in the mPFC attenuated the facilitating effect of DBS on 5-HT firing activity [56]. We concluded that the glial system plays an important role in the AD effect of DBS, likely by enhancing
gliotransmitters and glutamate uptake in the mPFC. Indeed, Banasr et al. have recently shown that glia-mediated glutamate uptake in the mPFC plays a key role in the behavioral and physiological response to stress and, therefore, in the pathophysiology of depression [60].

While DBS of the mPFC is at the early stage of testing, it seems to be a very promising procedure, owing both to its ability to improve depressive symptoms in refractory patients, and the amazing speed (see earlier) with which it might operate. The characterization of mechanisms mediating these AD effects may provide new perspectives for less invasive and/or pharmacological treatments. Some of these hypotheses are summarized in Figure 1.

### 5-HT4 agonists as a putative new class of fast-acting antidepressants

Since 5-HT4 receptors were first identified in 1988 [61,62], a growing number of studies have been conducted to assess both their

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**Figure 1.** Some hypotheses regarding the mechanisms underlying fast antidepressant responses related to the cortico-raphé neurotransmission. The key factor appears to be the enhanced release of glutamate in the synaptic cleft between pyramidal neuron terminals and DR 5-HT cell bodies. (1) High-frequency stimulation (DBS, 130 Hz) of the mPFC leads to an increased firing rate of glutamatergic pyramidal neurons. Consequently, extracellular levels of glutamate augment in the vicinity of 5-HT neurons within the DR, which receive an important innervation from the mPFC. This, in turn, results in an enhanced stimulation of glutamatergic receptors located on 5-HT cell bodies, particularly those of the ionotropic AMPA/kai type, increasing the electrical activity of DR 5-HT neurons. It seems that glial cells (represented here by the astrocyte) contribute to the effect of DBS by allowing the challenged metabolism of the pyramidal neuron to be sustained. (2) The stimulation of mPFC 5-HT4 receptors by an agonist also increases the firing rate of pyramidal neurons, in a similar manner to that described for (1). Ultimately, this leads to an enhanced activity of DR 5-HT neurons. (3) The blockade of 5-HT4 receptors by an antagonist may also contribute to the excitatory control exerted by mPFC pyramidal cells on DR 5-HT function. Although the precise mechanisms involved remain to be determined, 5-HT4 receptors might play a role in the modulation of the glia–neuron interaction within the mPFC, thus exerting an influence on the ability of pyramidal cells to maintain an increased activity. Alternatively, it is also possible that inhibitory GABA interneurons within the mPFC (and/or the DR) express a certain level of 5-HT4 receptors. As these receptors are usually excitatory, their blockade should induce a disinhibition of pyramidal neurons (and/or 5-HT ones). Whatever the different mechanisms responsible for the antidepressant-like properties of 5-HT4 antagonists, we hypothesize, as in the case of (1 & 2), that they are mediated, at least in part, by an increase of the extracellular levels of glutamate within the DR. (In this model, the role of DR GABA interneurons, which also receive glutamatergic projections from the mPFC is unclear. However, their influence seems to be mostly secondary in the context of a stimulated cortex [21].)

5-HT: Serotonin; DBS: Deep-brain stimulation; DR: Dorsal raphé; Glu: Glutamate; kai: Kainate; mPFC: Medial prefrontal cortex.
physiological features, and the potential therapeutic perspectives related therewith. At the central level, they have been shown to be strongly involved in several functions, including memory processes [63–68] and the regulation of food intake [69,70]. More interestingly, in the context of the present review, a single study conducted in the 1990s reported that preferential 5-HT4 agonists, such as renzapride, seemed to be able to facilitate the release of 5-HT within the hippocampal area [71]. It is noteworthy that these experiments, initially conducted to better understand the mechanisms underlying the effect of some anxiolytic drugs [72], brought the first clue of a putative role played by 5-HT4 receptors in AD therapy.

As explained in detail earlier, the possibility to acutely, directly enhance central 5-HT outflow with a given class of compounds resulted in promising possibilities for the development of new ADs, which could display a more rapid onset of action than SSRIs and other ‘indirect’ agents. First, we decided to assess the ability of selective 5-HT4 pharmacological agents to influence 5-HT neuronal impulse flow, measured using single-cell extracellular recordings. When acutely administered, 5-HT4 agonists, such as cisapride or prucalopride, are able to enhance the firing rate of a subpopulation of responding DR 5-HT neurons (‘responders’), which represent approximately 45% of the whole [73]. We also observed that prucalopride counteracts the inhibitory effect of acute SSRIs on DR responders [74]. The 5-HT4-dependent control was then assessed in a more global manner, by performing successive recording tracks (‘descents’) along the DR to draw a picture of the average 5-HT neuronal firing rate. Both prucalopride and RS 67333, another selective 5-HT4 agonist, enhanced the mean activity of 5-HT neurons when administered 30 min before starting the descents [75]. This effect was fully blocked by an acute injection of the selective 5-HT4 antagonist GR 125487 [75]. More importantly, it was also present at very similar levels after chronic administration of each drug for either 3 or 21 days [75]. Consistent with these findings, the mean firing rate of DR 5-HT neurons was found to be reduced by more than 50% in 5-HT4 knockout mice, with respect to their wild-type littermates [76]. Also, and similarly to the acute conditions, the continuous co-administration of either prucalopride or RS 67333 with the SSRI citalopram during 3 days increased 5-HT neuron activity, with an amplitude very similar to that observed when each 5-HT4 agonist was given alone [74].

The aforementioned positive influence exerted by 5-HT4 receptors on central 5-HT neuron activity was paralleled by the apparition of various biological markers within the brain, occurring typically after chronic AD administration, and postsynaptically reflecting an increased 5-HT transmission. Thus, after 3 days of chronic administration, RS 67333 and/or prucalopride were already able to induce the manifestation of a 5-HT1A-mediated inhibitory tone on hippocampal pyramidal neurons, an increased phosphorylation of CREB and an enhancement of adult mitogenesis within this region [77]. In agreement with our results, the same regimen (using RS 67333) has recently been reported to increase 5-HT release in the ventral hippocampus [78]. The 5-HT4 partial agonist SL 65.0155 has also been shown to facilitate CREB phosphorylation after three subchronic administrations, performed within a 24-h timeframe [79]. By contrast, classical ADs such as SSRIs produce similar postsynaptic effects after only 2 or 3 weeks when given alone [8–11,80–83]. Interestingly, in line with our previous findings, 3 days of combined treatment of the SSRI citalopram with either prucalopride or RS 67333 augmented both the inhibitory tone on hippocampal 5-HT1A receptors, and the phosphorylation of CREB, with a much higher amplitude than what was observed with each 5-HT4 agonist on its own [74]. In addition, a 3-day treatment with RS 67333 proved effective in alleviating the syndromes thought to reflect ‘depression’ in the olfactory bulbectomy and chronic mild stress behavioral tests. In the first one, RS 67333 reduces the hyperactivity of olfactory bulbectomy rats when exposed to a stressful (hyper-illuminated) environment, with the same amplitude as is observed after 14 days of citalopram [77]. A very similar difference in the respective kinetics of RS 67333 and citalopram is observed concerning their ability to reverse the chronic mild stress-induced anhedonia, measured by sucrose consumption [77]. It appears, therefore, that 5-HT4 agonists are able to trigger AD-like effects at least four- to seven-times more rapidly than classical molecules do in numerous experimental models [77]. Clinical trials have yet to be conducted with such compounds; interestingly, both animal [84,85] and human [86] post-mortem...
studies revealed important changes of 5-HT$_4$ receptor expression in (pseudo)depressed subjects. In addition, a recent report has shown that the p11 protein is apparently required for the expression of some behavioral AD-like actions elicited by 5-HT$_4$ receptor stimulation [87]. In addition, both proteins are co-expressed in brain regions relevant for depression, and p11 increases surface expression of the 5-HT$_4$ receptor and facilitates its intracellular signaling [87]. However, anatomical data indicate that both 5-HT$_4$ mRNA and protein are poorly expressed within the rat DR [88,89], with 5-HT$_4$ cell bodies being virtually devoid of these receptors [88]. Therefore, it appears unlikely that such a weak distribution could account for the effects described earlier. In the rodent brain, the most 5-HT$_4$-enriched areas are found in the basal ganglia, especially along the nigrostriatal and mesoaccumbal dopaminergic pathways, and in limbic areas such as the olfactory tubercles and the hippocampus [89–91]. Although the levels were reported to be moderate in the cortical mantle [89,90], a more detailed examination of the available figures revealed that the mPFC (both infralimbic and prelimbic subregions) expressed substantially more 5-HT$_4$ mRNA and protein than other cortical areas [91], a feature also displayed by the human brain [92]. Furthermore, it had already been reported that the electrical stimulation of the mPFC facilitated the activity of 40–45% of DR 5-HT cell bodies [21]. This proportion was strikingly close to what we observed concerning ‘responding’ 5-HT neurons [73]. In addition, an inhibitory, 5-HT$_4$-mediated, ‘long-loop feedback’ originating from the mPFC has been demonstrated, and was thought to concern approximately half of DR 5-HT neurons [93]. Finally, there was evidence suggesting that 5-HT$_4$ and 5-HT$_4$ receptors are mostly co-localized within the same pyramidal neurons in the mPFC [94]. Altogether, these data favored the possibility that the 5-HT$_4$-dependent facilitatory control also originates from the mPFC. To confirm this hypothesis, we used herpes simplex virus particles, transformed to induce an overexpression of 5-HT$_4$ receptors in discrete brain areas. In a particularly striking manner, the microinjection of viral particles into the mPFC induced a marked increase of DR 5-HT neuron firing rate [75]. Conversely, it had no effect when administered in the striatum, the hippocampus or the olfactory bulbs [75], three brain areas being among those which constitutively display the highest 5-HT$_4$ receptor expression (see earlier). Interestingly, recent data indicate that, in contrast to what is seen in the striatum and hippocampus, 5-HT$_4$ receptor binding is not downregulated in the mPFC after 21 days of treatment with the SSRI fluoxetine [95]. This result adds further support to the idea that the mPFC constitutes a major site of origin of the 5-HT$_4$-mediated positive control on 5-HT neuron activity, considering that this control also is not desensitized by long-term (21 days) administration of a 5-HT$_4$ agonist [75]. We propose that the stimulation of 5-HT$_4$ receptors within the mPFC triggers a positive, ‘long-loop feedback’ on DRN 5-HT function (Figure 1). According to this model, the projections connecting the mPFC to the DRN, and using glutamatergic neurotransmission, would constitute the vector of the feedback.

5-HT$_4$ receptor antagonists: a possible role for mPFC glial cells

5-HT$_4$ receptors constitute another 5-HT receptor subtype that has recently received attention as a new target for the development of fast-acting ADs. These receptors are the latest identified members of the 5-HT receptor family and have been found to activate adenylate cyclase [96–100]. Earlier studies have suggested that the therapeutic action of ADs might be mediated, at least in part, by 5-HT$_4$ receptors. Thus, chronic treatment with AD drugs led to a downregulation of these receptors in the hypothalamus [101,102] and to a reduction of the effectiveness of their activation within the rat hippocampus [103]. Conversely, several atypical antipsychotics, such as amisulpride, risperidone, olanzapine or aripiprazole, which possess antagonistic properties for the 5-HT$_4$ receptors [98,104–106], have therapeutic indications as an adjunctive treatment for depression [107–113]. In particular, recent studies have demonstrated that the AD actions of amisulpride and aripiprazole require 5-HT$_4$ receptors, because these agents had no AD-like effect in 5-HT$_4$ receptor knockout mice compared with what was seen in their wild-type littermates [114,115]. More direct evidence of the involvement of 5-HT$_4$ receptors in AD responses was provided by studies showing that pharmacological blockade of 5-HT$_4$ receptors, using the potent and selective 5-HT$_4$ receptor antagonist SB-269970 [116–118], or inactivation of the 5-HT$_4$ receptor gene, produces AD-like effects in behavioral rodent models [119–123]. In addition, similarly to SSRIs, the blockade or
inactivation of the 5-HT7 receptors affect sleep parameters in a pattern opposite to that seen in depressed patients [121,124]. Finally, it has been reported that SB-269970 potentiated both the effects of several ADs, and the rapid eye movement sleep suppression induced by the SSRI citalopram [123,125,126], suggesting that blockade of 5-HT7 receptors may facilitate the actions of AD treatments. In keeping with this hypothesis, we recently reported that 5-HT7 antagonism prevents the suppressant effect of SSRIs on DR 5-HT neuron electrical activity [119].

More interestingly, there is recent evidence suggesting that the AD properties of 5-HT7 receptor antagonists may rise with a faster onset of action than classical compounds. As outlined previously, all ADs have been demonstrated to promote hippocampal neurogenesis and, in turn, such a cellular plasticity seems to be essential for the achievement of an AD response [127–129]. Whereas cell proliferation in the rat hippocampus is enhanced after 2–3 weeks of treatment with classical molecules [11,130], a 1-week treatment with the 5-HT7 receptor antagonist SB-269970 was sufficient to increase this same parameter [119]. Also in support of a faster AD-like profile of 5-HT7 receptor antagonists, we reported that a 1-week treatment with SB-269970 fails to modify the firing activity of DR 5-HT neurons, but desensitizes the inhibitory action of 5-HT1A autoreceptors [119]. By contrast, a similar reduced 5-HT1A receptor responsiveness takes place only after 2–3 weeks of treatment with other ADs [131–133]. In addition, our recent data showing that SB-269970 counteracted the depressive-like behavior in olfactory bulbectomized rats after a 7-day treatment, while fluoxetine remained ineffective within the same timeframe [119], further supports the hypothesis that 5-HT7 receptor antagonists may act with a more rapid onset of action. The precise mechanism underlying the AD-like effects of 5-HT7 receptor antagonism is presently not known. Our electrophysiological data give direct evidence of a negative control exerted by 5-HT7 receptors on 5-HT neuron activity. Thus, the systemic administration of the potent and selective 5-HT7 receptor agonist AS-19 [134] inhibited the firing rate of DR 5-HT neurons in anesthetized rats, an effect prevented by prior systemic injection of SB-26970 [119]. This inhibitory control seems to be indirect. Indeed, biochemical studies performed on midbrain slices suggest that 5-HT7 receptors are not located on 5-HT-containing neurons in the DR [135,136]. Interestingly, microdialysis studies have found that SB-269970 enhanced the release of 5-HT induced by citalopram within the mPFC, suggesting that this region could be implicated in the aforementioned synergic interaction between 5-HT7 receptor antagonists and ADs [125]. In support of this hypothesis, functional 5-HT7 receptors, positively coupled to adenylyl cyclase, have been identified in cultured astrocytes derived from the rat cortex [137,138]. Furthermore, a continuous (3 day) exposure to the ADs mianserin or amitriptyline enhanced 5-HT7 receptor-mediated adenylyl cyclase activation of cortical astrocytes [138]. Considering the recently evidenced importance of mPFC glia on the effects of ADs [59], and the fact that mPFC astrocytes are directly activated by SSRIs ADs [139], 5-HT7 receptor antagonists might, therefore, act via a glia-mediated modulation of the cortico-DR transmission to exert their AD-like influence. Indeed, it has been shown from single cell real-time PCR studies that 5-HT7 receptors are not directly expressed by glutamatergic pyramidal neurons within the mPFC [94]. This latter data caused us to propose the alternative hypothesis that 5-HT7 receptors localized in GABAergic interneurons of the mPFC (and/or of the DR) may also contribute to the aforementioned effects [97]. The possible mechanisms discussed earlier are summarized in Figure 1; whatever the exact cellular localization of the mPFC 5-HT7 receptors involved, we hypothesize that their blockade induces an enhanced release of glutamate in the DR, which in turn increases 5-HT neuron activity.

**Conclusion & future perspective**

The data presented earlier confirm that it is actually possible to obtain faster AD responses than what has been achieved using standard molecules. They also point out that current pharmacological approaches have not reached their maximum efficacy/onset of action, and that the supposedly incompressible long delay of action related to monoaminergic drug treatment is not an immutable dogma. Therefore, the connection existing between the mPFC and DR 5-HT neurons appear to constitute a critical vector to develop new AD strategies. The glutamatergic neurotransmission stemming from mPFC pyramidal neurons can apparently act as a potent motor to boost central 5-HT function, whether it is triggered by a direct electrical stimulation or by a pharmacological one (Figure 1). It seems that the function of this motor
is particularly dependent on the ‘fueling’ properties of the glial system, to the extent that manipulating the mPFC glia results in tangible effects on depression-related behaviors. Several studies have pointed out that the blockade of some types of glutamate receptors, such as NMDA receptors, can also produce very fast AD-like effects. These effects appear to be related to the existence of a complex network within the mPFC itself, involving inhibitory interneurons and/or reciprocal collaterals, which regulate the activity of pyramidal cells [146]. Interestingly, the fast AD-like effects of the NMDA antagonist ketamine are paralleled by a significant increase of synaptogenesis within the mPFC [146]; it is tempting to speculate that this enhanced connectivity may improve the excitability of pyramidal neurons, resulting, again, in an increased release of glutamate in the vicinity of 5-HT cell bodies. Moreover, these very results demonstrate that, at least within the mPFC, morphological changes can be achieved within a very rapid time-frame by using pharmacological tools. They therefore contribute to re-establish a connection between two concepts in the field of ‘fast AD’ research. Should indeed the tools be of viral, molecular, pharmacological, electrical or even magnetic nature, as the increasing use of repetitive transcranial magnetic stimulation in the field of depression already gives a glimpse of, modulating the activity of the descending mPFC-DR pathway will likely constitute a major strategy to develop new fast-acting AD treatments in the near future.

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- of interest
- of considerable interest

12 First full demonstration of an enhanced hippocampal mitogenesis following antidepressant treatment.

Reports on the complex relationship existing between glutamatergic efferents from the medial prefrontal cortex (mPFC), and midbrain serotonin (5-HT) neurons.


First demonstration of a clinical efficacy for deep-brain stimulation (DBS) in depressed patients.


Confirmation of DBS efficacy in a greater number of patients.


Study of DBS effects in rodent brain. Demonstration that 5-HT plays a pivotal role in its behavioral expression.


Medial prefrontal cortex & serotonergic neurons for fast antidepressant action  

**REVIEW**


**A very complete anatomical study, describing the dense innervation projecting from the mPFC toward dorsal raphé 5-HT neurons.**


**Evidence that the glial system within the mPFC plays an important role in mood control and expression.**


**First demonstration that 5-HT<sub>4</sub> agonists may be used as a novel class of antidepressants, with a four- to seven-fold more rapid onset of action than standard molecules.**


83 Tiraboschi E, Tardito D, Kasahara J et al.: Selective phosphorylation of nuclear CREB by fluoxetine is linked to activation of CaM kinase IV and MAP kinase cascades. *Neuropsychopharmacology* 29(10), 1831–1840 (2004).


Medial prefrontal cortex & serotonergic neurons for fast antidepressant action  REVIEW


First full demonstration of an antidepressant potential for 5-HT, receptor antagonists, and more specifically for SB-269970.


151 Evidence that changes in brain plasticity can occur within hours after a single injection of a pharmacological blocker of NMDA receptors.