Effects of the antidepressant fluoxetine on the subcellular localization of 5-HT\textsubscript{1A} receptors and SERT

Laurent Descarries\textsuperscript{1,2,*} and Mustaph Riad\textsuperscript{1}

\textsuperscript{1}Departments of Pathology and Cell Biology, and \textsuperscript{2}Department of Physiology and Groupe de recherche sur le syst\`eme nerveux central, Faculty of Medicine, Universit\`e de Montr\`eal, Montreal, Quebec, Canada H3C 3J7

Serotonin (5-HT) 5-HT\textsubscript{1A} autoreceptors (5-HT\textsubscript{1A}autoR) and the plasmalemmal 5-HT transporter (SERT) are key elements in the regulation of central 5-HT function and its responsiveness to antidepressant drugs. Previous immuno-electron microscopic studies in rats have demonstrated an internalization of 5-HT\textsubscript{1A}autoR upon acute administration of the selective agonist 8-OH-DPAT or the selective serotonin reuptake inhibitor antidepressant fluoxetine. Interestingly, it was subsequently shown in cats as well as in humans that this internalization is detectable by positron emission tomography (PET) imaging with the 5-HT\textsubscript{1A} radioligand \textsuperscript{[18F]}MPPF. Further immuno-cytocchemical studies also revealed that, after chronic fluoxetine treatment, the 5-HT\textsubscript{1A}autoR, although present in normal density on the plasma membrane of 5-HT cell bodies and dendrites, do not internalize when challenged with 8-OH-DPAT. Resensitization requires several weeks after discontinuation of the chronic fluoxetine treatment. In contrast, the SERT internalizes in both the cell bodies and axon terminals of 5-HT neurons after chronic but not acute fluoxetine treatment. Moreover, the total amount of SERT immunoreactivity is then reduced, suggesting that SERT is not only internalized, but also degraded in the course of the treatment. Ongoing and future investigations prompted by these finding are briefly outlined by way of conclusion.

Keywords: serotonin; selective serotonin reuptake inhibitor; 5-HT\textsubscript{1A} receptors; SERT; internalization; PET imaging

1. INTRODUCTION

The central serotonin (5-hydroxytryptamine, 5-HT) system is implicated in the effects of a vast majority of antidepressant treatments [1], among which the selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine (Prozac), are the most widely used [2]. How the 5-HT system reacts and adapts to chronic exposure to these drugs is an obvious determinant of their therapeutic efficacy. For many years, however, electrophysiological recordings and neurochemical measurements were the only means to investigate in\textit{vivo} changes in 5-HT neuron activity, release and effects resulting from SSRI treatment. The development of approaches for the cellular and subcellular visualization of molecules involved in 5-HT neuron function has opened new perspectives in this regard. This made it possible to examine the subcellular localization of 5-HT\textsubscript{1A} receptors (5-HT\textsubscript{1A}R), the main regulator of 5-HT neuron firing and that of SERT, the plasmalemmal 5-HT transporter, during and after SSRI treatment. The results were of particular interest, because some of the observed changes proved to be detectable by brain imaging in humans.

Here, we first review previous and recent data from our laboratory regarding the internalization of serotonin 5-HT\textsubscript{1A} autoreceptors (5-HT\textsubscript{1A}autoR) after acute but not chronic fluoxetine treatment. Preliminary results are then reported, indicating that the 5-HT transporter, SERT, also internalizes after chronic (but not acute) fluoxetine treatment. By way of conclusion, related questions of biological and clinical relevance are proposed as subject for future investigations.

2. 5-HT\textsubscript{1A} RECEPTORS AND THE MODE OF ACTION OF SELECTIVE SEROTONIN REUPTAKE INHIBITOR ANTIDEPRESSANTS

5-HT\textsubscript{1A}R are one of the fourteen 5-HT receptor subtypes mediating the effects of 5-HT in mammalian brain. Particularly abundant in the midbrain raphe nuclei and the hippocampus, they are also present in cerebral cortex and most components of the limbic system [3,4]. The pharmacological activation of 5-HT\textsubscript{1A}R induces membrane hyperpolarization and reduces neuronal excitability via direct interactions between G proteins and ion channels [5]. In nuclei of origin, such as the dorsal raphe nucleus (DRN), 5-HT\textsubscript{1A}R thus negatively regulate the firing and

* Author for correspondence (laurent.descarries@umontreal.ca).

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hence release of 5-HT neurons as autoreceptors [5], whereas in territories of projection, such as hippocampus (5-HT\textsubscript{1A} heteroreceptors), they mediate an inhibition of non-5-HT neurons.

(a) Essential role of 5-HT\textsubscript{1A} autoreceptors

Numerous neurochemical, electrophysiological and pharmacological studies have characterized the effects of the acute administration of an SSRI on brain 5-HT neurons in rodents, revealing what might be happening at the onset of an SSRI antidepressant treatment. The extracellular concentration of 5-HT is then increased in various brain regions [6–11], which activates 5-HT\textsubscript{1A}autoreceptor in the DRN, and consequently inhibits the firing of 5-HT neurons [12–17] and the release of 5-HT within this nucleus [18] and its territories of projection [18–25] (see earlier studies [26,27] for review). These studies have also shown that a rapid and transient desensitization of 5-HT\textsubscript{1A}autoreceptor (but not of heteroreceptors) ensues, evidenced by reduced responsiveness of 5-HT neurons to a 5-HT\textsubscript{1A} agonist such as 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)tetrailin [19,28–30]). These effects are blocked by the specific 5-HT\textsubscript{1A} autoreceptor antagonist, WAY 100635 [30] which by itself does not alter the firing of 5-HT neurons [31–33].

When the SSRI treatment is chronic (e.g. three weeks in rat), it is not certain that the increases in extracellular 5-HT concentration persist in the nuclei of origin [34–36] or the territories of 5-HT projection [35,37] (but see earlier studies [38–42]). However, during this whole period, the sensitivity of 5-HT neurons to 5-HT\textsubscript{1A} autoreceptor decreases gradually, reflecting a progressive desensitization of 5-HT\textsubscript{1A}autoreceptor [17,43] (see also earlier studies [44,45]). After a decrease in the first week, the electrical activity of DRN 5-HT neurons reverts to its initial levels after three weeks [17]. This temporal course has led to the suggestion that the therapeutic delay in the efficacy of SSRIs (three to four weeks) is due to the time necessary for a persistent deactivation of 5-HT\textsubscript{1A}autoreceptor [44,45], i.e. removal of the ‘brake’ on 5-HT neurons’ activity. 5-HT transmission may then be enhanced even if extracellular 5-HT is no longer increased. Note that after two to three weeks of fluoxetine treatment, neither the $K_{D}$ nor the $B_{\text{max}}$ of 5-HT\textsubscript{1A} binding sites in DRN (or hippocampus) are changed [46–48]. Similar results have been obtained with other SSRIs, such as paroxetine [47].

(b) The internalization of 5-HT\textsubscript{1A} autoreceptors

Our first immuno-electron microscopic studies after immunogold labelling of 5-HT\textsubscript{1A}R in normal and treated rats have shed a new light on these findings [49,50]. Following the development of specific antibodies against 5-HT\textsubscript{1A} receptors in the laboratory of Michel Hamon in Paris [51], it became possible to examine the cellular and subcellular localization of these receptors by light and electron microscopic immunocytochemistry [3,4]. After immunogold pre-embedding labelling, we were able to demonstrate that, under normal conditions, 5-HT\textsubscript{1A}R were mostly located on extrasympatic portions of the somatodendritic plasma membrane of DRN 5-HT neurons, and of non-5-HT neurons in the hippocampus [52]. This immunolabelling approach also allowed us to quantify the respective amount of receptor on the plasma membrane versus the cytoplasm of immunoreactive neurons, by counting the number of silver-intensified immunogold particles in these two cellular compartments.

One hour after the administration of a single dose of either the 5-HT\textsubscript{1A}R agonist 8-OH-DPAT or the prototype SSRI fluoxetine, we could demonstrate an internalization of 5-HT\textsubscript{1A}autoreceptors in rat DRN neurons, evidenced by a 30–40% reduction in the density of 5-HT\textsubscript{1A}R immunogold labelling on the plasma membrane of DRN cell bodies and dendrites, with a concomitant increase in their cytoplasm and preferential labelling of endosomes (figure 1a–c). The total number of receptors was unchanged. This internalization could be observed as soon as 15 min after the injection of either drugs, was not greater after the combined administration of both 8-OH-DPAT and fluoxetine (figure 1d) and it was blocked by prior administration of the 5-HT\textsubscript{1A}R antagonist, WAY 100635 (figure 1e,f), confirming that it was the result of 5-HT\textsubscript{1A}R activation. The fact that it was partial (30–40%) suggests the existence of a functional and a non-functional pool of autoreceptors on the plasma membrane of DRN neurons. In hippocampus, there was no internalization, in keeping with the notion that 5-HT\textsubscript{1A} heteroreceptors do not desensitize [27,48,53], probably due to G-protein interactions different from those in DRN [54] or different type and level of expression of regulatory proteins (e.g. kinases, arrestins). Twenty-four hours after the treatment, the plasmalemmal density of 5-HT\textsubscript{1A} autoreceptor was back to normal, and internalization occurred again upon challenge with 8-OH-DPAT, indicating resensitization.

The subcellular localization of 5-HT\textsubscript{1A}R was then examined after three weeks of treatment with fluoxetine [36]. The 5-HT\textsubscript{1A} autoreceptor are known to be desensitized under these conditions [17,43,47,48], but were unexpectedly found in normal density on the plasma membrane of DRN neurons (figure 2a–c). Moreover, they did not internalize upon 8-OH-DPAT challenge, in keeping with their desensitized state. Together with the results of several $^{[55]}$GTPyS studies [48,55–57], this suggested that repeated internalization and retargeting resulted in a lack of coupling of 5-HT\textsubscript{1A} autoreceptors to their G protein. Thus, acute and chronic SSRI treatment induced two distinct types of 5-HT\textsubscript{1A} autoreceptor desensitization: one rapid and reversible (associated with internalization of the functional pool of membrane-bound receptors), the other being progressive and long-lasting, no longer accompanied by internalization, but which probably resulted from the reiteration of this process throughout the course of the chronic SSRI treatment.

Recently, we also assessed the functional state of 5-HT\textsubscript{1A} autoreceptors by immuno-electron microscopy after 8-OH-DPAT challenge at various time intervals after cessation of a chronic fluoxetine treatment (10 mg kg$^{-1}$ daily for 21 days, by minipump). The density of plasmalemmal and cytoplasmic labelling of DRN dendrites was determined by comparison with saline controls ($n = 5$), in rats administered 8-OH-DPAT (0.5 mg kg$^{-1}$ i.p.) 1 h prior to sacrifice, 24 h, one week
or six weeks after cessation of the chronic treatment (n = 4 in each group). More than 250 5-HT₁₄-autoR immunolabelled dendrites were examined in each group. One day after cessation of the chronic fluoxetine treatment, there was no detectable internalization 1 h after the administration of 8-OH-DPAT (figure 2d). Nor did the 5-HT₁₄-autoR internalize when 8-OH-DPAT was administered one week after cessation of the chronic fluoxetine treatment (figure 2e). After six weeks, however, the internalization was similar to the control (figure 2f). Such a long duration suggested that resensitization required the synthesis of new 5-HT₁₄-autoreceptors (to replace inactivated receptors), or the replenishment of regulatory proteins.

Figure 1. Internalization of 5-HT₁₄-autoR after acute 8-OH-DPAT and/or fluoxetine treatment. (a–f) Immuno-electron microscopic visualization of the subcellular distribution of 5-HT₁₄-autoR in DRN dendrites (d) 1 h after administration of the selective 5-HT₁₄ agonist 8-OH-DPAT or the SSRI fluoxetine. In a saline control (a), note the predilection of the silver-intensified immunogold particles for the plasma membrane as opposed to the cytoplasm of the dendrite. There is a considerable reduction (30–40%) in the density of plasmalemmal labelling and a corresponding increase in the cytoplasmic labelling of dendrites, after the administration of 8-OH-DPAT (0.5 mg kg⁻¹ i.v.; b), fluoxetine (10 mg kg⁻¹ i.p.; c) or both these drugs (d), reflecting 5-HT₁₄-autoR internalization. When the selective 5-HT₁₄ antagonist WAY 100635 (1 mg kg⁻¹ i.p.) is administered 10 min prior to either 8-OH-DPAT (e) or fluoxetine (f), the internalization does not occur. Adapted from earlier studies [50,51] with permission; copyrights © 2001 and 2004, Elsevier. (g,h) β-microprobe measurements of the binding kinetics of the specific 5-HT₁₄ radioligand [¹⁸F]MPPF in the DRN of rats administered fluoxetine (g; white diamonds; control, black diamonds), or fluoxetine preceded by WAY 100635 (h; white diamonds; control, black diamonds). Arrows in each graph indicate the time of saline or drug administration and beginning of data acquisition. Vertical arrows point at the time of [¹⁸F]MPPF injection. Data points are mean ± s.e.m. from five rats in each group. In (g), note the considerable decrease (30–40%) in the amount of radioactivity (Becquerel) detected from the DRN of rats administered fluoxetine. Adapted from [50] with permission; copyright © 2004, Elsevier. Scale bars, 1 μm.

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3. SERT, THE PRIMARY TARGET OF SSRI ANTIDEPRESSANTS

SERT is a member of a family of Na\(^+\)/Cl\(^-\)-dependent transporters in which the transmembrane domains are relatively conserved [58]. In addition to 5-HT neurons, SERT is transiently expressed by thalamo-cortical neurons and other non-5-HT neurons during the postnatal period in the rat [59–61], but in adult human, rat and mouse brain, its mRNA is confined to 5-HT neurons [62–64]. Using immuno-electron microscopy, SERT has been localized to the plasma membrane of 5-HT somata-dendrites, axons and axon terminals outside synaptic contact zones [65,66]; it may thus control not only 5-HT transmission at synapses, but also diffuse 5-HT transmission [65,66] (see also earlier studies [67,68]).

(a) Regulation of SERT during selective serotonin reuptake inhibitor treatment

SERT is selectively blocked by SSRIs, which increase extracellular 5-HT at the onset of a treatment [6–11]. There is strong experimental evidence to suggest that SERT may be regulated [69] and undergoes adaptive changes during SSRI treatment. Changes in the efficacy of SERT have been described in heterologous cell lines under the action of psychostimulants [70], which has been interpreted as the result of a silencing or inactivation of SERT protein already located on the plasma membrane, or else as an endocytosis and recycling of functional protein to the membrane [71–73]. SSRIs may also internalize SERT in heterologous cell lines [74,75] or in 5-HT neurons derived from stem cells [76].

In animal studies, however, radioligand binding and in situ hybridization studies have yielded equivocal results regarding the fate of SERT under chronic treatment with various SSRIs. Increases [77], decreases [78–83] or no changes [84,85] in density of SERT binding sites have been reported in the DRN and various territories of 5-HT innervation, and increases [83–86], decreases [79,83,87–89] or no changes [48,85,90] in SERT mRNA in the DRN.

(b) The internalization of SERT

We have obtained preliminary immuno-electron microscopic evidence in rat indicating that there is internalization of SERT in cell bodies and dendrites of DRN neurons as well as their axon terminals in hippocampus after chronic (three weeks) but not acute

Figure 2. Lack of responsiveness of 5-HT\(_{1A}\)autoR after chronic fluoxetine treatment. (a–c) Subcellular distribution of 5-HT\(_{1A}\)autoR in DRN dendrites (d) of a saline control (a), and rats treated for 1 day (b) or three weeks (c) with fluoxetine (10 mg kg\(^{-1}\) daily, by minipump). In the saline control (a), the predilection of the 5-HT\(_{1A}\)autoR for the plasma membrane of DRN dendrites is again obvious. After one day of fluoxetine administration (b), marked internalization is observed (35% decrease in the density of plasmalemmal labelling), whereas after three weeks of treatment (c), the density of plasmalemmal 5-HT\(_{1A}\)autoR labelling is equivalent to the control. Adapted from earlier studies [49,50]; copyright © 2001 and 2004, Elsevier. (d–f) Responsiveness of 5-HT\(_{1A}\)autoR to 8-OH-DPAT challenge (0.5 mg kg\(^{-1}\) i.p., 1 h prior to kill), at various time intervals after cessation of a chronic fluoxetine treatment (10 mg kg\(^{-1}\) daily for three weeks, by minipump). One day (d) and 1 week (e) after cessation of the chronic treatment, the density of 5-HT\(_{1A}\)autoR on the plasma membrane of DRN dendrites is equivalent to that in control rats or that measured immediately at the end of the treatment. Six weeks after cessation of the treatment, the internalization of 5-HT\(_{1A}\)autoR caused by 8-OH-DPAT is of the same magnitude (30–40%) as in untreated rats (M. Riad 2011, unpublished data). Scale bars, 1 μm.
fluoxetine treatment (figure 3a–c). The subcellular localization of SERT was examined in rats having received a single injection of fluoxetine (10 mg kg\(^{-1}\) i.p.) 1 h prior to killing, after three weeks of treatment with fluoxetine (10 mg kg\(^{-1}\) daily, by minipump), and in saline controls (n = 5 in each group). Almost 300 immunolabelled dendrites in DRN and 200 immunolabelled axon terminals in hippocampus were examined in each group. A quantitative analysis of this data showed no difference in the density of plasmalemmal or cytoplasmic labelling 1 h after fluoxetine, when compared with control. After the chronic treatment, however, the density of plasmalemmal labelling was reduced by 48% in DRN dendrites, and by 71% in hippocampal terminals, with a concomitant increase in the ratio of cytoplasmic/plasmalemmal labelling in both locations. Interestingly, there was also a reduction in the overall density of labelling in both the DRN dendrites and hippocampal terminals, suggesting that SERT was not only internalized, but also degraded, in the course of the chronic SSRI treatment (M. Riad 2011, unpublished data). Scale bars, 1 \(\mu\)m.

It was soon realized that \([18F]\)MPPF binding

Figure 3. Internalization and decrease in 5-HT transporter (SERT) after chronic fluoxetine treatment. (a–c) Immunogold labelling of SERT in DRN dendrites (d) after acute (10 mg kg\(^{-1}\) i.p.) or chronic treatment (10 mg kg\(^{-1}\) daily for three weeks, by minipump) with fluoxetine. One hour after the administration of fluoxetine (b), there were no apparent changes in the subcellular distribution of SERT, either in cell bodies or dendrites of DRN neurons, as illustrated here, nor in axon terminals of the hippocampus (not shown). After chronic treatment (c), however, the localization of the SERT labelling was changed, with a significant reduction in the density of plasmalemmal labelling in both DRN dendrites and hippocampal terminals. There was also a reduction in the overall density of labelling in both DRN dendrites and hippocampal terminals, suggesting that SERT was not only internalized, but also degraded, in the course of the chronic SSRI treatment (M. Riad 2011, unpublished data). Scale bars, 1 \(\mu\)m.

4. FROM CELLULAR AND SUBCELLULAR LOCALIZATION STUDIES IN RAT TO BRAIN IMAGING STUDIES IN HUMANS

It was soon realized that \([18F]\)MPPF binding in vivo might be altered in conditions of 5-HT\(_{1A}\) internalization in the animal brain, offering hopes to detect this phenomenon by PET in the human brain [91].

(a) Radioligand binding studies of 5-HT\(_{1A}\)R in vivo

Indeed, in rats, concordant results were obtained when radiosensitive brain-implanted microprobes (β-microprobes) were used in Luc Zimmer’s laboratory (Lyon, France), to examine the in vivo binding of \([18F]\)MPPF, a selective 5-HT\(_{1A}\)R antagonist amenable to brain imaging in humans, under the same experimental conditions examined by immunoelectron microscopy [50,91]. As measured with one microprobe inserted next to the DRN and another in hippocampus, the amount of radioactivity detected during 1 h after i.v. injection of \([18F]\)MPPF was decreased by 30–40% in the DRN of 8-OH-DPAT-treated rats, without any change in the hippocampus, and this lowering in DRN did not occur after the prior administration of WAY100635. Similar findings were made after acute fluoxetine treatment (figure 1a,b), but, interestingly, when autoradiography of tissue slices was used to measure \([18F]\)MPPF binding in these same conditions of treatment, there were no differences in the regional density of binding sites in either the DRN or the hippocampus of 8-OH-DPAT-treated rats compared to control rats [50]. Of course, the low resolution of ligand binding autoradiography in tissue slices did not allow for distinguishing between radiolabel located on the plasma membrane versus the cytoplasm of neurons. Such a difference between the in vitro and in vivo results indicated that, in vivo, \([18F]\)MPPF did not access internalized 5-HT\(_{1A}\) autoreceptors.

After chronic fluoxetine treatment, there were no differences between the microprobe measurements of \([18F]\)MPPF binding in the DRN or hippocampus of controls versus treated rats, in keeping with the lack of internalization and normal density of plasmalemmal 5-HT\(_{1A}\) receptors in DRN observed in the immuno-electron microscopic experiments [36].
Imaging of 5-HT1A autoR internalization in the cat and the human brain

As the resolution of available microPET instruments was likely to be insufficient to detect a partial decrease in binding potential in such a small anatomical region as the rat DRN [92], a preliminary investigation in animals was carried out in cats using a clinical PET [93]. After demonstrating that the cat DRN could indeed be visualized with [18F]MPPF, [18F]MPPF binding potential was measured in anaesthetized cats given or not a single dose of fluoxetine, or treated chronically with fluoxetine (three weeks). Compared with control, [18F]MPPF binding potential was considerably and visibly decreased (approx. 40%) in the DRN after acute fluoxetine treatment, while it was unchanged in other brain regions. After the chronic fluoxetine treatment, [18F]MPPF binding potential was unchanged in all brain regions, including the DRN, as expected from the immuno-electron microscopic results in rats.

The next step was to try imaging the internalization of 5-HT1A autoR in the human brain. In collaboration with Chawki Benkelfat and co-workers at the McConnell Brain Imaging Center of McGill University, [18F]MPPF PET was therefore performed in normal human volunteers subjected to the double-blind oral administration of a single tablet of fluoxetine (Prozac, 20 mg) or placebo in a random order [94]. The results were clear. In the DRN and nowhere else in the brain, all eight subjects given fluoxetine showed a significant and visible decrease in [18F]MPPF binding potential (44% on average) compared with placebo (figure 4a–c). This was confirmed by a non-parametric (SPM) analysis of the data is shown below. Note that the DRN is the only brain region in which there is a statistically significant change (decrease) in [18F]MPPF binding potential. (Asterisk: spm2, \( k = 25 \) voxels, \( p = 0.001 \) uncorrected, FWHM = 8 mm isotropic.) Adapted from [94] with permission; copyright © 2008, Elsevier.

**Figure 4.** [18F]MPPF PET imaging of 5-HT1A autoR internalization in healthy volunteers administered a single oral dose of fluoxetine. (a) and (b) are co-registered, colour-coded, parametric images of the average [18F]MPPF binding potential in the brain of eight subjects who received placebo (a) or fluoxetine (b), 5 h before the scans. At white arrow in (b), note the selective decrease in density of [18F]MPPF binding confined to a minute region of interest corresponding to the DRN; (c) delineates this small region of interest in an averaged MRI image, in which a mesencephalic area encompassing the DRN is drawn in blue and a 27-voxel cubic region of interest around the most active voxel within this area is drawn in red. (d–f) The non-parametric (SPM) analysis of the data is shown below. Note that the DRN is the only brain region in which there is a statistically significant change (decrease) in [18F]MPPF binding potential. (Asterisk: spm2, \( k = 25 \) voxels, \( p = 0.001 \) uncorrected, FWHM = 8 mm isotropic.) Adapted from [94] with permission; copyright © 2008, Elsevier.

(b) Imaging of 5-HT1A autoR internalization in the cat and the human brain

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5. ONGOING AND FUTURE STUDIES

Some clinical implications of the earlier-mentioned findings have been discussed elsewhere [95]. Here, we will merely list a series of fundamental, yet answerable questions raised by the results thus far obtained on the internalization of 5-HT1A autoreceptors. First: what molecular mechanisms account for this internalization? Are G-protein-coupled receptor kinases (GRKs) and \( \beta \)-arrestins implicated, as in the case of other G-protein-coupled receptors? Second: what accounts for the long-term desensitization (loss of function) of 5-HT1A autoreceptors after chronic SSRI treatment? Third: which elements of the signalling cascade need to be synthesized for these receptors to resensitize after cessation of a chronic fluoxetine treatment?
Identification of these molecules might provide novel targets for antidepressant drugs.

From the physiopathological standpoint, it would also be important to determine the functional state of 5-HT1A autoreceptors in depression. All the earlier-described results have been obtained from normal animals or from the healthy human brain. Further immuno-electron microscopic investigations of 5-HT1A autoreceptors need to be carried out in animal models of depression, and [18F]MPPF PET studies in depressed subjects.

It must also be pointed out that previous radioligand binding and in situ hybridization studies on post-mortem brain of suicide victims [96–103], as well as recent PET studies in unmedicated depressed patients [104,105], suggest that 5-HT1A autoreceptors are present in excessive number in the DRN of depressed subjects (see also earlier studies [106,107]). This could imply that the firing and release of 5-HT neurons is permanently toned down in such patients. Another testable hypothesis is that of an impaired internalization of 5-HT1A autoreceptors in depression, which could also account for low 5-HT tone and/or for limited efficacy of SSRI treatments aimed at long-term desensitization of these receptors and resulting increase in 5-HT transmission. Lastly, another important issue is regards the fate of 5-HT1A autoreceptors after cessation of an SSRI mission. Lastly, another important issue is regards the functional state of 5-HT1A autoreceptors in depression. All the earlier-described models of depression and in human subjects, and could provide crucial information in terms of the mechanisms involved in the permanence of effects versus recurrence of the disease.

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