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► **To cite this version:**

Luc Zimmer, Laurent Descarries. Internalization of serotonin 5-HT 1A autoreceptors as an imaging biomarker of antidepressant response. Wiley Interdisciplinary Reviews: Membrane Transport and Signaling, 2012, 1 (3), pp.239-245. <10.1002/wmts.11>. <hal-00797786>

HAL Id: hal-00797786

<https://hal.science/hal-00797786v1>

Submitted on 23 Jan 2025

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Internalization of serotonin 5-HT_{1A} autoreceptors as an imaging biomarker of antidepressant response

Luc Zimmer^{1,2*} and Laurent Descarries³

Serotonin (5-hydroxytryptamine, 5-HT) and its various receptors are involved in numerous CNS functions. Among the currently known 5-HT receptors, the 5-HT_{1A} receptor is the best characterized subtype. It is tightly implicated in the pathogenesis of mood disorders, notably in depression, and thus represents an important target for drug therapy. Binding to 5-HT_{1A} receptors can be visualized and quantified by positron emission tomography (PET), facilitating the translation from animal research to man. Using the 5-HT_{1A} radiotracer [¹⁸F]MPPF, recent PET studies in cat and human have provided evidence that internalization of 5-HT_{1A} autoreceptors is amenable to *in vivo* neuroimaging at the very onset of specific serotonin reuptake inhibitor (SSRI) administration. The *in vivo* detection of this phenomenon in human is promising in terms of clinical management, particularly as an early biomarker of responsiveness to SSRI treatment. However, several questions are still pending regarding the correlation between 5-HT_{1A} internalization at the onset of the treatment and the ensuing therapeutic efficacy.

THE SEROTONIN 5-HT_{1A} RECEPTORS

The Serotonin System

The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) is implicated in a broad spectrum of functions and behaviors,¹ as well as in a number of psychiatric disorders, notably pathological anxiety and depression.² This system originates in 5-HT neurons mostly regrouped in raphe nuclei of the brainstem, and particularly in the midbrain dorsal (DRN) and median (MRN) raphe nuclei, whose ascending projections spread to all regions

of the forebrain.³ In rat DRN, the 5-HT cells represent approximately one third of the total neuron population.⁴ In most regions of 5-HT innervation, only 30–40% of 5-HT axon terminals (varicosities) are endowed with junctional complexes of synaptic specialization, allowing for diffuse as well as hard-wired synaptic transmission.⁵ Since the introduction of radioligand binding techniques, followed by the advent of molecular cloning approaches, at least fourteen 5-HT receptor subtypes belonging to seven families have been identified in mammalian CNS.⁶

Brain Distribution and Function of the 5-HT_{1A} Receptors

The 5-HT_{1A} receptors, which were the first to be cloned in human and in rat, are among the best known in terms of molecular structure, distribution in brain, and biological properties. They possess all typical features of G protein-coupled receptors,^{7,8} including seven putative trans-membrane

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domains. Interestingly, these receptors are very well conserved among mammalian species; their sequence in human displays 89% of homology with that in rat.⁹ Activation of 5-HT_{1A} receptors triggers different biochemical pathways. It typically inhibits cAMP accumulation and Ca²⁺ currents, induces the opening of K⁺ channels, regulates phospholipase C activity, and activates a variety of kinases. The signaling pathways that are triggered depend on the G protein subtypes present in neurons.¹⁰

Early autoradiographic studies in rat demonstrated that 5-HT_{1A} receptor binding sites are abundant in various components of the limbic system, such as the cingulate cortex, infralimbic cortex, and hippocampus, as well as in the raphe nuclei.¹¹ In DRN, these receptors have been well characterized electrophysiologically and pharmacologically as autoreceptors (i.e., receptors located on the 5-HT neurons themselves) mediating a negative feedback on 5-HT neuronal firing and thus an inhibition of 5-HT release.¹² In regions of 5-HT innervation, 5-HT_{1A} receptors also mediate an inhibition of neuronal firing, but are located on non-5-HT neurons (e.g., pyramidal and granule cells in hippocampus),¹³ hence their designation as heteroreceptors.

5-HT_{1A} Receptors and Mood Disorders

Because of their capacity to control the firing and release of 5-HT neurons, and to their own distribution in brain, 5-HT_{1A} receptors are involved in the control of numerous physiological processes, including neuroendocrine responses, body temperature, sleep states, neurogenesis, and mood.^{14,15} Considerable data has accumulated over the last three decades to suggest a pivotal role of these receptors in the action of antidepressants and anxiolytics, such as the specific serotonin reuptake inhibitors (SSRIs) and the azapirones.^{16,17} Their proposed role in the modulation of anxiety-related behaviors is strongly supported by recent experimental studies in 5-HT_{1A} receptor knockout mice.^{18–20} Because the entire cerebral cortex and the various components of the limbic system are innervated by the DRN 5-HT neurons, and because 5-HT_{1A} autoreceptors set the tone of the 5-HT system, much emphasis has been put on the mechanisms which regulate their activity. For example, when 5-HT_{1A} autoreceptors are overexpressed in the mid-brain raphe nuclei, the behavioral response of mice to antidepressants appears to be abolished,²¹ a result in keeping with clinical studies suggesting that overexpression of 5-HT_{1A} autoreceptors is associated with increased susceptibility to depressive traits, including suicide risk.²²

INTERNALIZATION OF 5-HT_{1A} AUTORECEPTORS: FROM CELLULAR AND SUBCELLULAR STUDIES IN RATS TO IMAGING STUDIES IN HUMANS

Immunocytochemical and Radiopharmacological Demonstration in Rats

Following the development of a specific antibody against 5-HT_{1A} receptors,²³ light microscopic immunocytochemistry in rat allowed to visualize strong 5-HT_{1A} immunoreactivity in the mesencephalic raphe nuclei, hippocampus, and septum.¹³ After immunogold pre-embedding labeling with the same specific 5-HT_{1A} antibody, the subcellular localization of 5-HT_{1A} receptors was investigated by electron microscopy in both the DRN and the hippocampus.²⁴ This allowed to demonstrate that, in both regions, 5-HT_{1A} receptors were exclusively somatodendritic and predominantly located on the plasma membrane (as opposed to the cytoplasm). Subsequently, a similar approach was used to examine the localization of these receptors after the i.v. administration of a single dose of the selective 5-HT_{1A} receptor agonist 8-OH-DPAT.^{25,26} It was then shown that 1 h after the injection of 8-OH-DPAT, there was a 30–40% decrease in the density of 5-HT_{1A} autoreceptors on the plasma membrane of DRN dendrites, and a corresponding increase in their cytoplasm, accounting for the prior electrophysiological demonstration of a partial and transient desensitization of these autoreceptors under these conditions.²⁷ This internalization was already present and of a similar degree 15 min after the administration of 8-OH-DPAT, but no longer apparent after 24 h.²⁵ It did not occur following the prior administration of the specific 5-HT_{1A} receptor antagonist WAY100635, confirming that it was the result of 5-HT_{1A} receptor activation. The fact that it was partial (30–40%) was compatible with the existence of a ‘functional’ and a ‘reserve’ pool of autoreceptors on the plasma membrane of DRN neurons. In hippocampus, there was no internalization, in keeping with the notion that 5-HT_{1A} heteroreceptors do not desensitize,²⁷ perhaps as a result of the activation of a different signaling cascade than in the DRN.²⁸

A similar internalization of 5-HT_{1A} autoreceptors was observed 1 h after the administration of a single dose of the SSRI fluoxetine (Prozac®),^{29,30} which indirectly activates 5-HT_{1A} receptors by increasing the extracellular concentration of 5-HT in brain. Again, in hippocampus where the 5-HT_{1A} heteroreceptors do not desensitize, internalization did not occur. Interestingly, the combined administration of both

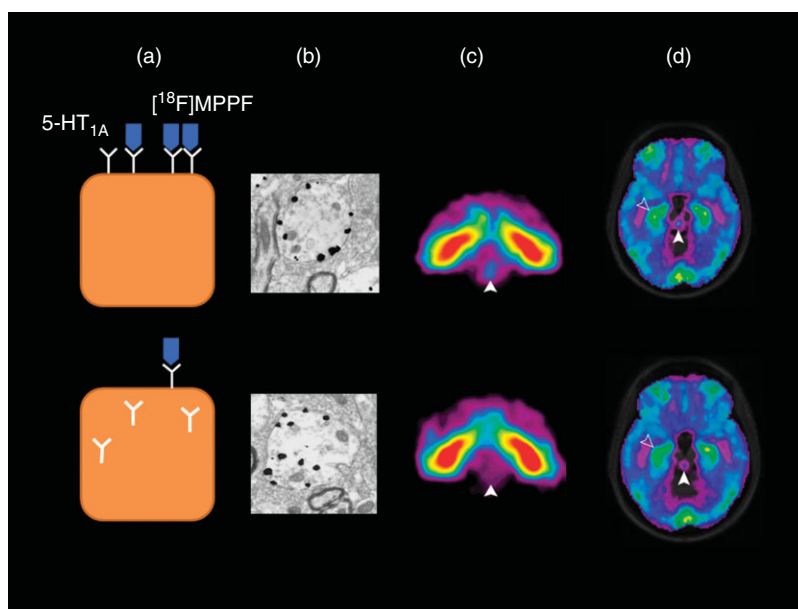


FIGURE 1 | Immunocytochemical and brain imaging of 5-HT_{1A} autoreceptor internalization. (a) Schematic representation of [¹⁸F]MPPF binding to 5-HT_{1A} autoreceptors in 5-HT neurons. Under basal conditions (upper image), 5-HT_{1A} autoreceptors are mostly located on the somatodendritic plasma membrane of 5-HT neurons and available for [¹⁸F]MPPF binding (blue shapes). After indirect activation of 5-HT_{1A} autoreceptors by acute administration of fluoxetine (which increases the extracellular concentration of 5-HT), 5-HT_{1A} receptors are internalized in the cytoplasm (lower image) and no longer available for [¹⁸F]MPPF binding *in vivo*. (b) Electron micrographs illustrating the subcellular localization of 5-HT_{1A} receptors in DRN dendrites, as revealed by immuno-electron microscopy (immunogold labeling). In a control rat (upper image), the immunogold particles are mostly found on the plasma membrane, whereas 1 h after fluoxetine administration (10 mg/kg i.p.), there is a 30–40% reduction in the density of this plasmalemmal labeling and a corresponding increase in the cytoplasmic labeling (lower image). (Reprinted with permission from Ref 26. Copyright 2004 Elsevier) (c) Color-coded PET images of a cat brain showing the summed distribution of [¹⁸F]MPPF, before (upper image) and 1 h after (lower image) the acute administration of fluoxetine (10 mg/kg i.p.). White arrowheads point at the DRN, in which a selective decrease in [¹⁸F]MPPF binding is observed after the treatment. (Reprinted with permission from Ref 32. Copyright 2006 Elsevier) (d) Color-coded PET images of the summed distribution of [¹⁸F]MPPF in the brain of eight subjects who received placebo (upper image) or a unique oral dose of fluoxetine (20 mg), 5 h before the scan (lower image). White arrowheads point at the DRN, in which [¹⁸F]MPPF binding is selectively decreased after fluoxetine. Empty arrowheads point at the hippocampus in which [¹⁸F]MPPF binding is unchanged. (Reprinted with permission from Ref 33. Copyright 2008 Elsevier)

8-OH-DPAT and fluoxetine did not induce a greater amount of internalization than either drug alone, confirming that a fraction only of 5-HT_{1A} autoreceptors present on the plasma membrane of DRN neurons could be activated and internalized at any given time (Figure 1a and b).

Concordant results were obtained when radiosensitive brain-implanted microprobes (β -microprobes) were used to measure the *in vivo* binding of [¹⁸F]MPPF, a selective 5-HT_{1A} radioligand amenable to brain imaging, in the same experimental conditions, i.e., after the acute administration of either 8-OH-DPAT or fluoxetine.^{26,29} When one radiosensitive probe was inserted next to the DRN and another in hippocampus, the amount of radioactivity detected from the DRN during 1 h was decreased by 30–40% in the 8-OH-DPAT-treated rats, without any change in hippocampus. In contrast, when autoradiography was used to measure [¹⁸F]MPPF binding after incubation of tissue slices *in vitro*, there

was no apparent difference in the regional density of binding sites in either the DRN or hippocampus of 8-OH-DPAT-treated rats compared to control rats.²⁵ The lowering of [¹⁸F]MPPF binding *in vivo* (β -microprobes) but not *in vitro* (autoradiography) suggested that internalized receptors were not accessed by [¹⁸F]MPPF administered *in vivo* (Figure 1a). A relatively low lipophilicity or a lesser affinity of [¹⁸F]MPPF for internalized 5-HT_{1A} receptors in a low-sodium environment could also be considered as possible explanations for this reduced binding of [¹⁸F]MPPF to internalized 5-HT_{1A} receptors *in vivo*.

Imaging of 5-HT_{1A} Autoreceptor Internalization in Cat and Human

It was soon realized that PET imaging with [¹⁸F]MPPF might provide a means to detect 5-HT_{1A} receptor internalization in human brain and eventually assess the biological responsiveness to antidepressant

treatment.²⁶ 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,³¹ a preliminary investigation in animal was carried out in cat using a clinical PET.³² After demonstrating that the DRN could indeed be imaged with [¹⁸F]MPPF in this species, [¹⁸F]MPPF binding potential was measured in anesthetized cats given or not a single dose, or treated chronically with fluoxetine for 21 days. Compared to control, [¹⁸F]MPPF binding potential was considerably and visibly decreased (~40%) in the DRN after acute fluoxetine treatment, while it remained unchanged in other brain regions (Figure 1c). Moreover, as expected from concomitant immuno-electron microscopic results in rats, [¹⁸F]MPPF binding potential was not affected in any brain region after the chronic fluoxetine treatment.

The next step was to try imaging the internalization of 5-HT_{1A} autoreceptors in human brain. [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.³³ The results were clear. In the DRN and nowhere else in brain, all eight subjects given fluoxetine showed a significant and visible decrease in [¹⁸F]MPPF binding potential (44% on average) compared to placebo (Figure 1d). This was confirmed by a non parametric analysis, which indicated that the DRN was the only brain region showing a significant difference in [¹⁸F]MPPF binding between fluoxetine and placebo.³³

INTERNALIZATION OF 5-HT_{1A} AUTORECEPTORS: A BIOMARKER OF ANTIDEPRESSANT RESPONSE?

The above study suggesting that PET imaging with [¹⁸F]MPPF might be used to detect a subcellular process like 5-HT_{1A} internalization in living human brain at the very onset of an SSRI treatment raised great hopes in terms of personalized clinical management. Even though it is hard to imagine how this approach might be applicable on a large scale, because of its costs and the small number of PET centers, it could become more accessible in a near future owing to the rapid progresses in SPECT ligands and technology (single photon emission computed tomography in nuclear medicine departments). Yet, many questions remain to be answered before clinical use. It has not yet been shown that SSRIs other than fluoxetine will trigger a comparable internalization of 5-HT_{1A} autoreceptors in animal or human brain. Nor

is it known whether other radioligands of 5-HT_{1A} receptors, such as [¹¹C]WAY100635, which have a lipophilicity and affinity for these receptor different from that of [¹⁸F]MPPF, would similarly reveal the internalization of 5-HT_{1A} autoreceptors. Moreover, the crucial issue of the chain of events through which this process leads to positive responsiveness to an SSRI antidepressant remains to be resolved.

Is 5-HT_{1A} Internalization Correlated with the 5-HT_{1A} Desensitization Occurring During the Antidepressant Treatment?

Although SSRI penetration in brain and blockade of 5-HT reuptake is rapid, chronic (3–4 week) treatment is required to obtain a clinical response. Animal studies have confirmed that a 2–3-week period of treatment is needed to achieve a maximal desensitization of 5-HT_{1A} autoreceptors, and the lack of autoinhibition of 5-HT neuron firing and release that allows for increased 5-HT neurotransmission in key brain regions of 5-HT innervation and the therapeutic efficacy of these drugs.^{16,17} Although PET studies have highlighted changes in 5-HT_{1A} receptors in depressive disorders, with modifications of pre- and postsynaptic 5-HT_{1A} receptor binding potential in depressed patients,³⁴ the underlying mechanisms remain speculative and several methodological limitations have to be considered. A major one is that the sole study having demonstrated 5-HT_{1A} internalization after a single oral dose of SSRI was not carried out in depressed patients.³³ Obviously, such a study will need to be repeated in unmedicated depressed patients to know whether 5-HT_{1A} autoreceptors are similarly responsive in the depressed state.

It must be pointed out that all studies having thus far demonstrated 5-HT_{1A} autoreceptor internalization upon treatment with fluoxetine or the direct 5-HT_{1A} receptor agonist 8-OH-DPAT have been carried out in ‘normal’ animal or human subjects. Besides PET studies in depressed patients, it will be important to pursue immuno-electron microscopic studies in animal models of depression, such as bilateral olfactory bulbectomy and unpredictable chronic stress in rat, to determine the state of 5-HT_{1A} autoreceptors in the depressed-like condition, the occurrence of 5-HT_{1A} autoreceptor internalization upon acute SSRI treatment, and substantiate the link between acute receptor internalization and antidepressant efficacy in these animal models.

Another limitation concerns the relatively short duration of the period during which 5-HT_{1A} autoreceptors internalize in response to agonist or SSRI treatment. After 3 weeks of fluoxetine treatment

in rat or cat, internalization is no longer observed upon treatment with agonist (8-OH-DPAT challenge), even though the receptors are present in normal density on the plasma membrane of DRN neurons.^{30,32} We have postulated that during chronic treatment, the repeated activation and internalization of the pool of functional 5-HT_{1A} autoreceptors leads to the recycling of desensitized receptors to the plasma membrane of DRN 5-HT neurons.³⁰ Acute and chronic SSRI treatment therefore seem to induce two distinct types of 5-HT_{1A} autoreceptor desensitization: one rapid and reversible, associated with internalization of the functional pool of membrane bound receptors, the other progressive and long lasting, no longer accompanied by internalization, but which probably results from the reiteration of this process throughout the course of the chronic SSRI treatment.³⁰

Can 5-HT_{1A} Internalization Imaging Discriminate Antidepressant Responders and Non-Responders?

Another challenge is to demonstrate that PET imaging provides a biological index of eventual therapeutic efficacy at the very onset of the treatment. It is known that clinical response to SSRI antidepressants is variable, and that less than half of depressed patients respond favorably to their first drug treatment.³⁵ The others are considered as 'non-responders' (or 'partial responders'), and their medication needs to be changed, often after long months of repeated failures. Because there is still no consensus to guide psychiatrists in selecting the best antidepressant therapy, or predicting its possible efficacy, a PET biomarker that would discriminate rapidly the responders from the non-responders would have a significant impact. Moreover, elucidating the nature of the cellular and molecular mechanisms underlying treatment resistance remains an important need. 5-HT_{1A} autoreceptors could be a relevant target in this regard, as their expression and density might be abnormally high in patients suffering from major depressive disorder.³⁶⁻³⁸

However, before using PET to determine whether subjects are likely to respond to SSRI medication or should be candidate to alternative treatments, there is a need for further experimental data demonstrating the link between the functional state of the receptor and the antidepressant response. Thus far, it has been assumed that 5-HT_{1A} autoreceptor desensitization suffices to determine the behavioral

response to antidepressant treatment.^{16,17} However, a recent study has shown that transgenic mice with a 30% higher level of 5-HT_{1A} autoreceptors in the DRN do not respond behaviorally to a chronic fluoxetine treatment even if their 5-HT_{1A} autoreceptors are then desensitized.²¹ Conversely, mice that differed only by lower autoreceptor levels before chronic treatment displayed a robust behavioral response to fluoxetine.²¹

It is nevertheless tempting to speculate that the internalization process that was observed in healthy subjects upon the initial administration of SSRI antidepressant might be deficient in depressed patients, particularly in those which do not respond to SSRI antidepressants. It would also be interesting to determine if subjects expressing 5-HT_{1A} polymorphisms which are known to be associated with deficient antidepressant response, and notably the C-1019G SNP,³⁹ exhibit deficient 5-HT_{1A} receptor internalization. A dysfunction in internalization could prevent the rapid desensitization which counterbalances sudden rises in extracellular 5-HT, and could therefore explain the lack of therapeutic effect of the antidepressant. Decreased or increased internalization of 5-HT_{1A} autoreceptors is a testable hypothesis warranting further PET investigations in patients with major depression. Because several teams (but not all) reported an abnormally high density of 5-HT_{1A} autoreceptors in the DRN of depressed patients,³⁶⁻³⁸ a decrease in [¹⁸F]MPPF binding associated with 5-HT_{1A} autoreceptor internalization might be even greater and more easily detectable in depressed patients than in healthy subjects.

CONCLUSIONS

PET imaging holds the potential to facilitate the translation of knowledge from laboratory animal to man in the search for a better understanding of the mode of action of antidepressant drugs and the pathophysiology of depression. The studies reviewed above have provided convincing evidence that internalization of 5-HT_{1A} autoreceptors may be amenable to *in vivo* neuroimaging. However, it remains to be demonstrated that imaging of this subcellular process in depressed patients will allow for distinguishing between responders from non-responders to treatment, and help to select the best medication in individual cases. Although challenging, these possibilities need to be validated both in animal models of depression and in depressed patients.

ACKNOWLEDGMENTS

The authors thank Mustapha Riad for his critical reading of the manuscript. This research was supported by the French program CNRS-CEA 'Imagerie du Petit Animal' to L. Z., and grant NRF 3544 from the Canadian Institutes of Health Research and infrastructure support (GRSNC) from the Fonds de la Recherche en Santé du Québec to L. D.

REFERENCES

1. Meneses A. 5-HT system and cognition. *Neurosci Biobehav Rev* 1999, 23:1111–1125.
2. Jone BJ, Blackburn TP. The medical benefit of 5-HT research. *Pharmacol Biochem Behav* 2002, 71: 555–568.
3. Hornung JP. The human raphe nuclei and the serotonergic system. *J Chem Neuroanat* 2003, 26:331–343.
4. Descarries L, Watkins KC, Garcia S, Beaudet A. The serotonin neurons in nucleus raphe dorsalis of adult rat: a light and electron microscope radioautographic study. *J Comp Neurol* 1982, 207:239–254.
5. Descarries L, Riad M, Parent M. Ultrastructure of the serotonin innervation in the mammalian central nervous system. In: Muller CP, Jacobs B, eds. *Handbook of the Behavioural Neurobiology of Serotonin*. Amsterdam: Elsevier; 2009, 65–101.
6. IUPAHR Database. Available at: <http://www.iuphar-db.org/index.jsp> (August 2010 version). (Accessed April 3, 2011).
7. Raymond JR, Mukhin YV, Gettys TW, Garnovskaya MN. The recombinant 5-HT_{1A} receptor: G protein coupling and signalling pathways. *Br J Pharmacol* 1999, 127:1751–1764.
8. Harrington MA, Oksenberg D, Peroutka SJ. 5-Hydroxytryptamine_{1A} receptors are linked to a Gi-adenylate cyclase complex in rat hippocampus. *Eur J Pharmacol* 1988, 154:95–98.
9. Albert PR, Zhou QY, Van Tol HH, Bunzow JR, Civelli O. Cloning, functional expression, and mRNA tissue distribution of the rat 5-hydroxytryptamine_{1A} receptor gene. *J Biol Chem* 1990, 265:5825–5832.
10. Raymond JR, Mukhin YV, Gelasco A, Turner J, Collinsworth G, Gettys TW, Grewal JS, Garnovskaya MN. Multiplicity of mechanisms of serotonin receptor signal transduction. *Pharmacol Ther* 2001, 92:179–212.
11. Marcinkiewicz M, Vergé D, Gozlan H, Pichat L, Hamon M. Autoradiographic evidence for the heterogeneity of 5-HT₁ sites in the rat brain. *Brain Res* 1984, 291:159–163.
12. Sprouse JS, Aghajanian GK. Electrophysiological responses of serotonergic dorsal raphe neurons to 5-HT_{1A} and 5-HT_{1B} agonists. *Synapse* 1987, 1:3–9.
13. Kia HK, Miquel MC, Brisorgueil MJ, Daval G, El Mestikawy S, Riad M, Hamon M, Vergé D. Immunocytochemical localization of 5-HT_{1A} receptors in the rat central nervous system. *J Comp Neurol* 1996, 365:289–305.
14. Lanfumey L, Hamon M. 5-HT₁ receptors. *Curr Drug Targets CNS Neurol Disord* 2004, 3:1–10.
15. Borg J. Molecular imaging of the 5-HT_{1A} receptor in relation to human cognition. *Behav Brain Res* 2008, 195:103–111.
16. Blier P, de Montigny C. Current advances and trends in the treatment of depression. *Trends Pharmacol Sci* 1994, 15:220–226.
17. Piñeyro G, Blier P. Autoregulation of serotonin neurons: role in antidepressant drug action. *Pharmacol Reviews* 1999, 51:533–591.
18. Heisler LK, Chu HM, Brennan TJ, Danao JA, Bajwa P, Parsons LH, Tecott LH. Elevated anxiety and antidepressant-like responses in serotonin 5-HT_{1A} receptor mutant mice. *Proc Natl Acad Sci U S A* 1998, 95:15049–15054.
19. Parks CL, Robinson PS, Sibille E, Shenk T, Toth M. Increased anxiety of mice lacking the serotonin_{1A} receptor. *Proc Natl Acad Sci U S A* 1998, 95:10734–10739.
20. Ramboz S, Oosting R, Amara DA, Kung HF, Blier P, Mendelsohn M, Mann JJ, Brunner D, Hen R. Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. *Proc Natl Acad Sci U S A* 1998, 95:14476–14481.
21. Richardson-Jones JW, Craige CP, Guiard BP, Stephen A, Metzger KL, Kung HF, Gardier AM, Dranovsky A, David DJ, Beck SG, et al. 5-HT_{1A} autoreceptor levels determine vulnerability to stress and response to antidepressants. *Neuron* 2010, 65:40–52.
22. Le François B, Czesak M, Steubl D, Albert PR. Transcriptional regulation at a HTR_{1A} polymorphism associated with mental illness. *Neuropharmacology* 2008, 55:977–985.
23. El Mestikawy S, Riad M, Laporte AM, Vergé D, Daval G, Gozlan H, Hamon M. Production of specific anti-rat 5-HT_{1A} receptor antibodies in rabbits injected with a synthetic peptide. *Neurosci Lett* 1990, 118:189–192.
24. Riad M, Garcia S, Watkins KC, Jodoin N, Doucet E, Langlois X, El Mestikawy S, Hamon M, Descarries L. Somatodendritic localization of 5-HT_{1A} and preterminal axonal localization of 5-HT_{1B} serotonin receptors in adult rat brain. *J Comp Neurol* 2000, 417:181–194.
25. Riad M, Watkins KC, Doucet E, Hamon M, Descarries L. Agonist-induced internalization of serotonin-1A receptors in the dorsal raphe nucleus (autoreceptors) but

- not hippocampus (heteroreceptors). *J Neurosci* 2001, 21:8378–8386.
26. Zimmer L, Riad M, Rbah L, Belkacem-Kahlouli A, Le Bars D, Renaud B, Descarries L. Toward brain imaging of serotonin 5-HT_{1A} autoreceptor internalization. *NeuroImage* 2004, 22:1421–1426.
 27. Beer M, Kennett GA, Curzon G. A single dose of 8-OH-DPAT reduces raphe binding of [³H]8-OH-DPAT and increases the effect of raphe stimulation on 5-HT metabolism. *Eur J Pharmacol* 1990, 178:179–187.
 28. La Cour CM, El Mestikawy S, Hanoun N, Hamon M, Lanfumey L. Regional differences in the coupling of 5 hydroxytryptamine-1A receptors to G proteins in the rat brain. *Mol Pharmacol* 2006, 70:1013–1021.
 29. Riad M, Zimmer L, Rbah L, Watkins KC, Hamon M, Descarries L. Acute treatment with the antidepressant fluoxetine internalizes 5-HT_{1A} autoreceptors and reduces the in vivo binding of the PET radioligand [¹⁸F]MPPF in the nucleus raphe dorsalis of rat. *J Neurosci* 2004, 24:5420–5426.
 30. Riad M, Rbah L, Verdurand M, Aznavour N, Zimmer L, Descarries L. Unchanged density of 5-HT_{1A} autoreceptors on the plasma membrane of nucleus raphe dorsalis neurons in rats chronically treated with fluoxetine. *Neuroscience* 2008, 151:692–700.
 31. Moulin-Sallanon M, Charnay Y, Ginovart N, Perret P, Lanfumey L, Hamon M, Hen R, Fagret D, Ibanez V, Millet P. Acute and chronic effects of citalopram on 5-HT_{1A} receptor-labeling by [¹⁸F]MPPF and coupling to receptors-G proteins. *Synapse* 2009, 63:106–116.
 32. Aznavour N, Rbah L, Riad M, Reilhac A, Costes N, Descarries L, Zimmer L. A PET imaging study of 5-HT_{1A} receptors in cat brain after acute and chronic fluoxetine treatment. *NeuroImage* 2006, 33:834–842.
 33. Sibon I, Benkelfat C, Gravel P, Aznavour N, Costes N, Mzengeza S, Booiij L, Baker G, Soucy J-P, Zimmer L, et al. Decreased [¹⁸F]MPPF binding potential in the dorsal raphe nucleus after a single oral dose of fluoxetine: a PET study in healthy volunteers. *Biol Psychiatry* 2008, 63:1135–1140.
 34. Savitz J, Lucki I, Drevets WC. 5-HT_{1A} receptor function in major depressive disorder. *Prog Neurobiol* 2009, 88:17–31.
 35. Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, Warden D, Niederehe G, Thase ME, Lavori PW, Lebowitz BD, et al. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *Am J Psychiatry* 2006, 163:1905–1917.
 36. Stockmeier CA, Shapiro LA, Dilley GE, Kolli TN, Friedman L, Rajkowska G. Increase in serotonin-1A autoreceptors in the midbrain of suicide victims with major depression—Postmortem evidence for decreased serotonin activity. *J Neurosci* 1998, 18:7394–7401.
 37. Parsey RV, Oquendo MA, Ogden RT, Olvet DM, Simpson N, Huang YY, Van Heertum RL, Arango V, Mann JJ. Altered serotonin 1A binding in major depression: a [carbonyl-C-11]WAY100635 positron emission tomography study. *Biol Psychiatry* 2006, 59:106–113.
 38. Parsey RV, Ogden RT, Miller JM, Tin A, Hesselgrave N, Goldstein E, Mikhno A, Milak M, Zanderigo F, Sullivan GM, et al. Higher serotonin 1A binding in a second major depression cohort: Modeling and reference region considerations. *Biol Psychiatry* 2010, 68:170–178.
 39. Kishi T, Tsunoka T, Ikeda M, Kawashima K, Okochi T, Kitajima T, Kinoshita Y, Okumura T, Yamanouchi Y, Inada T, et al. Serotonin 1A receptor gene and major depressive disorder: an association study and meta-analysis. *J Hum Genet* 2009, 54:629–633.