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# Serotonin 1A and Serotonin 4 Receptors: Essential Mediators of the Neurogenic and Behavioral Actions of Antidepressants

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## Abstract

Selective serotonin reuptake inhibitors are the mostly widely used treatment for major depressive disorders and also are prescribed for several anxiety disorders. However, similar to most antidepressants, selective serotonin reuptake inhibitors suffer from two major problems: They only show beneficial effects after 2 to 4 weeks and only about 33% of patients show remission to first-line treatment. Thus, there is a considerable need for development of more effective antidepressants. There is a growing body of evidence supporting critical roles of 5-HT<sub>1A</sub> and 5-HT<sub>4</sub> receptor subtypes in mediating successful depression treatments. In addition, appropriate activation of these receptors may be associated with a faster onset of the therapeutic response. This review will examine the known roles of 5-HT<sub>1A</sub> and 5-HT<sub>4</sub> receptors in mediating both the pathophysiology of depression and anxiety and the treatment of these mood disorders. At the end of the review, the role of these receptors in the regulation of adult hippocampal neurogenesis will also be discussed. Ultimately, we propose that novel antidepressant drugs that selectively target these serotonin receptors could be developed to yield improvements over current treatments for major depressive disorders.

## Keywords

major depressive disorders, neurogenesis, 5-HT<sub>1A</sub> receptor, 5-HT<sub>4</sub> receptor, mood disorders, anxiety

## Introduction

Depressive and anxiety disorders are a major burden on society. Major depressive disorder (MDD) affects more than 20 million Americans every year and is currently the second leading cause of disability worldwide (Ferrari and others 2013; Kessler and others 2005). In addition, the World Health Organization predicts that depression will be the leading cause of disease burden globally by 2030 (World Health Organization 2011). MDD also displays high comorbidity with anxiety disorders. A reported 50% to 60% of patients with MDD also have a history of anxiety disorders that usually precede depression (Kaufman and Charney 2000). These findings raise the question of whether mood and anxiety disorders, despite the diagnostic distinctions made clinically, share a common pathophysiology.

Since the discovery and development of these medications, depression has been associated with impairment of serotonergic, noradrenergic, and to a lesser extent dopaminergic neurotransmissions. Most drugs that are currently used to treat MDD, such as selective serotonin

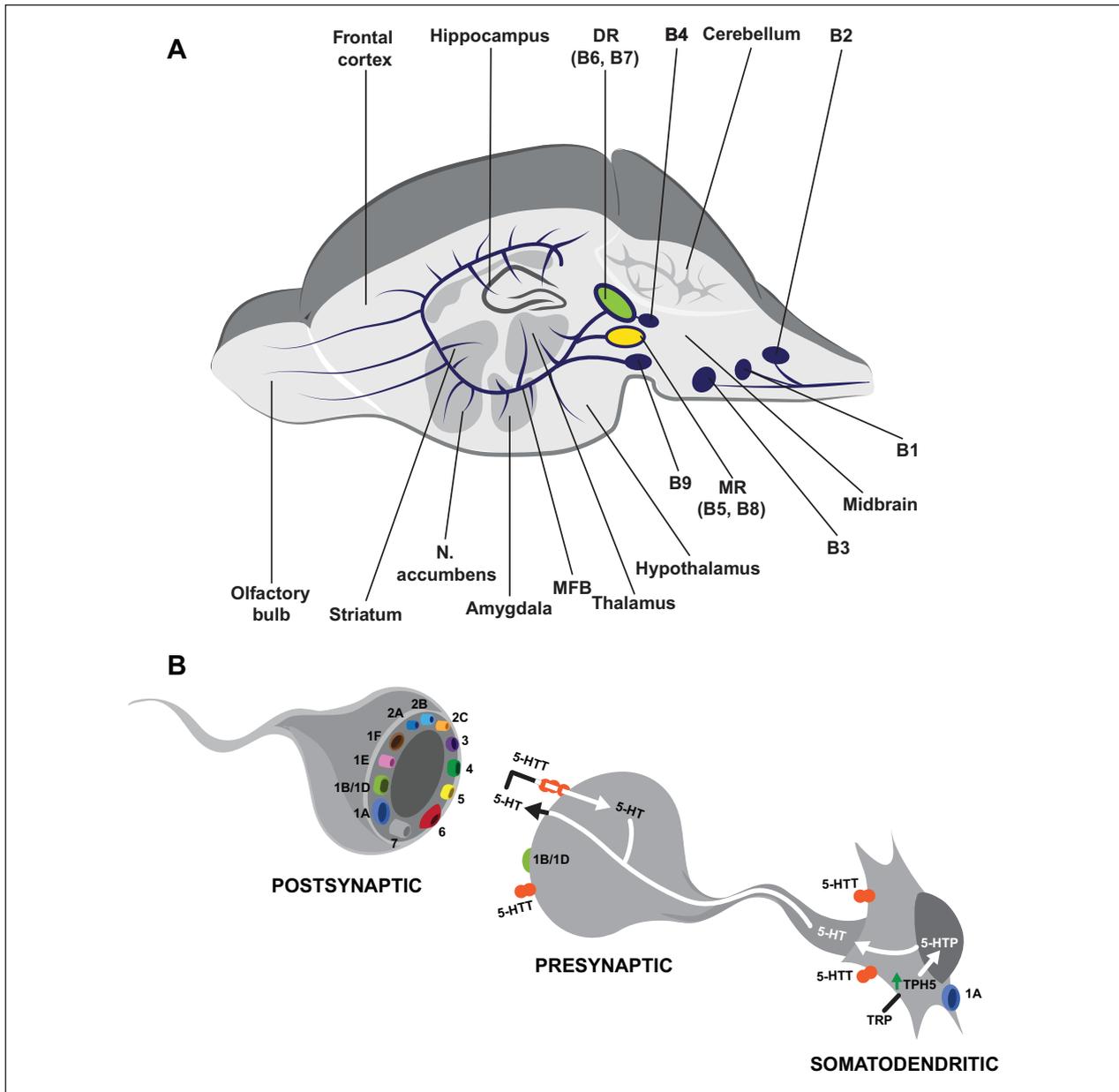
reuptake inhibitors (SSRIs; the most commonly prescribed), activate serotonin neurotransmission and also are effective treatments for generalized anxiety (Burghardt and Bauer 2013; Samuels and others 2011; Schatzberg and Nemeroff 2009). SSRIs act as indirect agonists of serotonin receptors, blocking the serotonin transporter (SERT). After chronic SSRI treatment, serotonin (5-HT) is released throughout the forebrain by axons emanating from cell bodies located in the midbrain raphe (Barnes and Sharp 1999) (Figure 1A). The largely neuromodulatory

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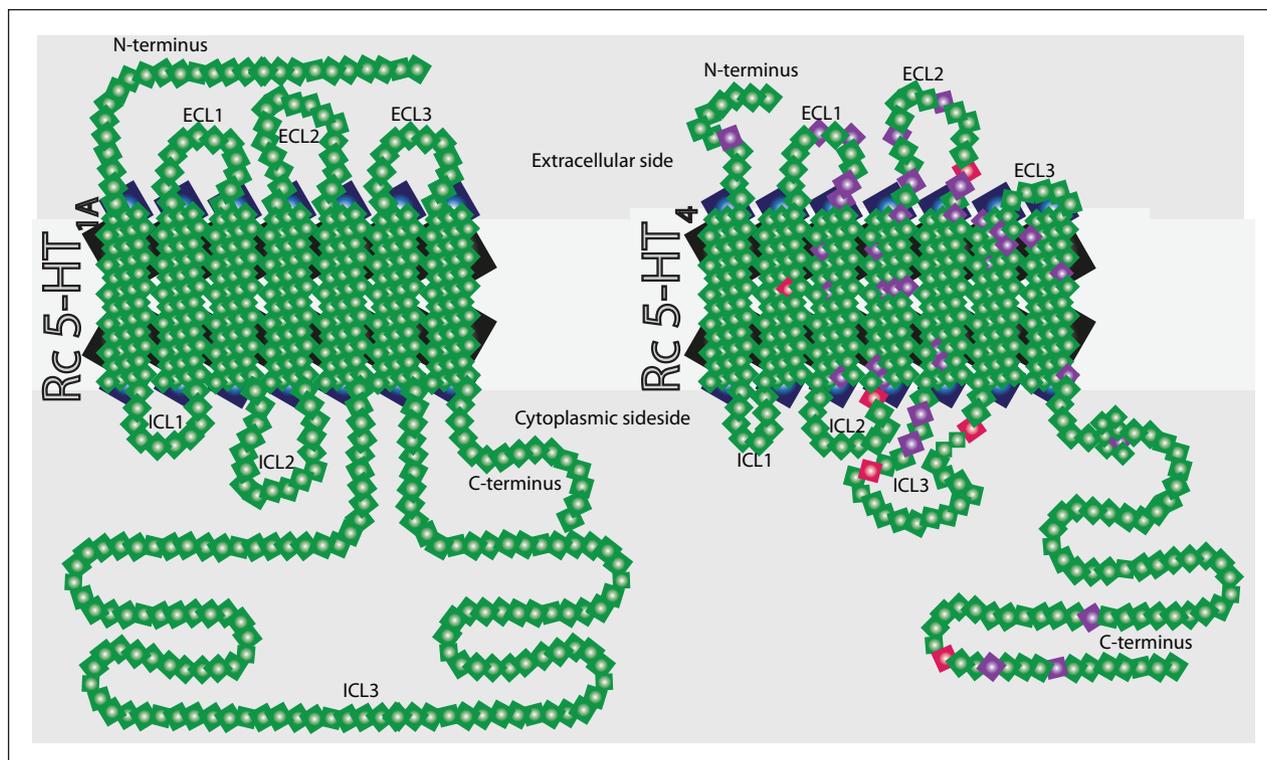
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**Figure 1.** Serotonergic system in rodent brain. (A) The serotonergic system is organized in nine raphe nuclei (from B1 to B9). B1 to B3 nuclei project axons to the spinal cord and the periphery, Dorsal raphe nuclei (DR, B6-B7), Medial Raphe nuclei (MR, B5, B8), and the B9 nucleus project throughout the brain. (B) Steps involved in the synthesis and release of serotonin. Serotonin (5-HT) synthesis depends on the uptake of tryptophan (Trp) into the presynaptic cells localized in the raphe nuclei. The conversion of Trp to 5-hydroxytryptophan (5-HTP) is catalyzed by tryptophan hydroxylase (TPH). 5-HTP is converted to 5-HT by aromatic amino acid decarboxylase (AADC) before being stored in vesicles. The release of 5-HT into the synapse is calcium-dependent. Once released, 5-HT binds to postsynaptic receptors (e.g., 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>1D</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, 5-HT<sub>2C</sub>, 5-HT<sub>3</sub>, 5-HT<sub>4</sub>, 5-HT<sub>5</sub>, 5-HT<sub>6</sub>, 5-HT<sub>7</sub>), pre-synaptic autoreceptors (5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>1D</sub>), or is transported back into the serotonergic cell by the 5-HT transporter (SERT).

effects of serotonin are mediated through 14 distinct receptor subtypes (heteroreceptors) located postsynaptic to serotonergic nerve terminals (Figure 1B). In addition, 5-HT levels are limited by two inhibitory autoreceptors (5-HT<sub>1A</sub> and 5-HT<sub>1B</sub>) expressed in the somatodendritic

compartments (5-HT<sub>1A</sub>) and nerve terminals (5-HT<sub>1B</sub>) of the serotonergic raphe neurons (Barnes and Sharp 1999). However, it is largely unknown which of the 14 receptor subtypes actually mediate the clinical effects of SSRIs. While there is some evidence that 5-HT<sub>2</sub>, 5-HT<sub>3</sub>, 5-HT<sub>6</sub>,



**Figure 2.** Two-dimensional representation of the 5-HT<sub>1A</sub> and the 5-HT<sub>4</sub> receptor sequences. The 5-HT<sub>1A</sub> and the 5-HT<sub>4</sub> receptors are metabotropic receptors that are coupled to G proteins and contain a seven-transmembrane domain structure. The 5-HT<sub>1A</sub> and 5-HT<sub>4</sub> receptors possess three intracellular loops and three extracellular loops. The amino terminus is oriented toward the extracellular space, whereas the carboxyl terminus (C-terminus) is oriented toward the cytoplasm. Primary sequences of the different isoforms are identical throughout the first 358 residues but diverge at their C-terminal end resulting in differential G protein coupling.

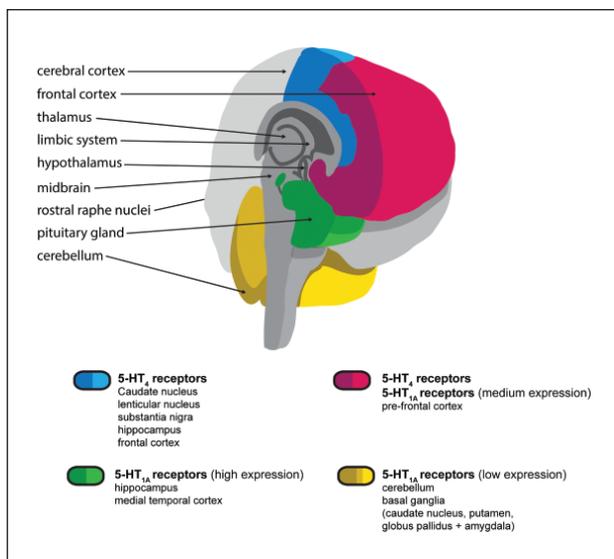
and 5-HT<sub>7</sub> receptor subtypes may play roles in mood disorders and the treatment response (Middlemiss and others 2002), there is a wealth of historical and recent data implicating 5-HT<sub>1A</sub> and 5-HT<sub>4</sub> receptors. This review summarizes the roles that 5-HT<sub>1A</sub> and 5-HT<sub>4</sub> receptors play in mood disorders and the mechanisms underlying their antidepressant action. The impact of these receptors on adult hippocampal neurogenesis, a phenomenon that may be required for some of the clinical response to antidepressants, is also addressed.

### 5-HT<sub>1A</sub> Receptor Expression Pattern and Signal Transduction

With the exception of the 5-HT<sub>3</sub> receptor, which is a ligand-gated ion channel, all serotonin receptors are G-protein coupled receptors containing seven hydrophobic transmembrane helices, three extracellular loops, and three intracellular loops that activate intracellular second messenger cascades to yield either excitatory or inhibitory responses (Hannon and Hoyer 2008) (Figure 2). The first evidence that there were multiple distinct 5-HT

receptor types came in the late 1950s, when Gaddum and colleagues found that the effects of 5-HT in guinea pigs could be blocked in part by morphine and in part by dibenzylamine (Gaddum and Picarelli 1957). By the late 1970s, radioligand binding studies were beginning to hint at the diversity of the 5-HT receptor family (Hannon and Hoyer 2008). Then, in the late 1980s, advances in molecular biology permitted cloning of the 5-HT<sub>1A</sub> receptor (Fargin and others 1988; Kobilka and others 1987).

5-HT<sub>1A</sub> heteroreceptors are expressed in the brain primarily in the septum, hippocampus, amygdala, thalamus, and hypothalamus, and in these areas are mainly located on pyramidal and granule neurons as well as GABAergic interneurons (Albert and others 1996; Garcia-Garcia and others 2014; Tanaka and others 2012) (Figure 3). At these postsynaptic sites, 5-HT<sub>1A</sub> heteroreceptor activation is thought to primarily exert an inhibitory effect on the neuronal activity induced by various neurotransmitters (Hannon and Hoyer 2008; Li and others 1996). By contrast, 5-HT<sub>1A</sub> autoreceptors are located on the soma and dendrites of serotonergic neurons in the dorsal and median raphe nuclei where they exert inhibitory control



**Figure 3.** Localization of the 5-HT<sub>1A</sub> and 5-HT<sub>4</sub> receptor in the human brain. Both 5-HT<sub>1A</sub> and 5-HT<sub>4</sub> are heteroreceptors, but only the 5-HT<sub>1A</sub> is also an autoreceptor localized in the raphe nuclei. 5-HT<sub>1A</sub> and 5-HT<sub>4</sub> heteroreceptors are both expressed in the brain primarily in the hippocampus, the cortex, the globus pallidus, and the caudate nucleus.

over raphe firing rates and 5-HT release through a negative feedback mechanism (Hannon and Hoyer 2008).

5-HT<sub>1A</sub> receptors are coupled to G(i/o) type  $\alpha$  subunits, which act on downstream effectors to induce inhibition of neuronal firing (Albert and others 1996). Specifically, the G(i/o) subunit inhibits adenylyl cyclase, which results in a reduction in cellular levels of cyclic adenosine monophosphate (cAMP), the closing of calcium channels, and a reduction in the intracellular calcium concentration.

Activation of 5-HT<sub>1A</sub> receptors in different brain regions can yield at times opposing intracellular effects. This is because of the fact that different cell types express distinct G $\alpha$  subunits. For example, 5-HT<sub>1A</sub> autoreceptors in the dorsal raphe nuclei (DRN) primarily couple to G $\alpha_3$ , while heteroreceptors in the hippocampus primarily couple to G $\alpha$  (Mannoury la Cour and others 2006). Therefore, the differential effects of 5-HT<sub>1A</sub> receptors are mediated both by distinct anatomical localizations and distinct inhibitory G $\alpha$ /o subunit couplings (Garcia-Garcia and others 2014; Polter and Li 2010).

### 5-HT<sub>4</sub> Receptor Expression Pattern and Signal Transduction

The 5-HT<sub>4</sub> receptor was originally identified by its pharmacology, which was unique among the serotonin receptor subtypes known at the time. In the late 1980s there

was speculation that a novel 5-HT receptor subtype was expressed in collicular and hippocampal neurons that stimulated adenylyl cyclase activity and increased cAMP production. However, this receptor subtype was insensitive to known antagonists of the 5-HT<sub>1</sub>, 5-HT<sub>2</sub>, and 5-HT<sub>3</sub> receptor subtypes (Bockaert and others 1990; Bockaert and others 2004; Dumuis and others 1988). For several years, investigators thought that the 5-HT<sub>3</sub> and 5-HT<sub>4</sub> receptors were closely related because they have similar pharmacological profiles. The first ligands discovered for the 5-HT<sub>4</sub> receptor also had high affinity for the 5-HT<sub>3</sub> receptor (Bockaert and others 2004; Dumuis and others 1988; Eglen and others 1990). The 5-HT<sub>4</sub> receptor was finally recognized as a new serotonergic receptor subtype in 1992 and subsequently several ligands with high affinity and/or selectivity for this receptor subtype were developed. In the late 1990's, the gene encoding the 5-HT<sub>4</sub> receptor, which is exceptionally large and complex (700 kb, 38 exons), was simultaneously cloned in two different species (Bockaert and others 2004; Claeysen and others 1996; Gerald and others 1995). The 5-HT<sub>4</sub> receptor possesses three intracellular loops and three extracellular loops. The amino terminus is in the extracellular space and the carboxyl terminus (C-terminus) is in the cytoplasm (Figure 2).

The large and complex nature of the gene encoding the 5-HT<sub>4</sub> receptor results in several different isoforms, generated through alternative splicing of the gene, with distinct functional properties (Bockaert and others 2004; Claeysen and others 1999; Pindon and others 2002). The sequences of the different isoforms are identical throughout the first 358 residues, but then diverge, which results in differential G protein coupling (Bockaert and others 2004; Claeysen and others 1999). In brain and peripheral tissues, humans have at least six distinct variants of the 5-HT<sub>4</sub> receptor (a, b, c, g, i, and n), whereas mice are currently thought to have only four (a, b, e, and f) (Claeysen and others 1999). In addition to differences in G protein coupling, these distinct splice variants also show differences in the intracellular loops (i3 in particular), and in both phosphorylation and palmitoylation of the C-terminus (Barthet and others 2005). The exact functional roles of these distinct isoforms remain unresolved. However, numerous studies suggest that isoform-specific differences in 5-HT<sub>4</sub> receptors and their distribution impact the overall coupling and regulation of the receptor and, in turn, the potential for 5-HT<sub>4</sub> receptors to be targets for therapeutic intervention (Barthet and others 2005; Bohn and Schmid 2010; Marin and others 2012; Mnie-Filali and Pineyro 2012; Vilaro and others 2005).

The 5-HT<sub>4</sub> receptor plays important roles in the heart, gastrointestinal tract, adrenal gland, and urinary bladder, as well as in the central nervous system (Hegde and Eglen 1996). The development in 1993 of

two specific radioligands for the 5-HT<sub>4</sub> receptor, the antagonists [<sup>3</sup>H]-GR 113808 and [<sup>125</sup>I]-SB 207710, revolutionized the study of this receptor. The usage of these radioligands in biochemical assays and autoradiography experiments permitted accurate determination of the regional distribution of 5-HT<sub>4</sub> receptors in the brain (Grossman and others 1993). The vast majority of 5-HT<sub>4</sub> receptors are expressed in the hypothalamus, hippocampus, nucleus accumbens, the ventral pallidum, amygdala, the basal ganglia, olfactory bulbs, frontal cortex, the septal area, the substantia nigra, and the fundus striatus (Bockaert and others 2004; Eglén and others 1995; Vilaro and others 1996; Vilaro and others 2005; Waeber and others 1993) (Figure 3). More specifically, 5-HT<sub>4</sub> receptors are located in somatodendritic compartments and in axon terminals of striatal spiny efferent neurons containing  $\gamma$ -aminobutyric acid (GABA) (Cai and others 2002; Compan and others 1996; King and others 2008). 5-HT<sub>4</sub> receptors are also expressed in glutamatergic pyramidal neurons in the medial prefrontal cortex and hippocampus (CA1, CA3) and in granule cells of the dentate gyrus (King and others 2008; Roychowdhury and others 1994; Tanaka and others 2012; Vilaro and others 2005). In the cortex, hippocampus, and amygdala, 5-HT<sub>4</sub> receptors are expressed in cholinergic neurons where the binding of selective agonists can stimulate the release of acetylcholine (Waeber and others 1994). Furthermore, recent work demonstrates that 5-HT<sub>4</sub> receptors are also expressed by efferent neurons of the nucleus accumbens that project to the lateral hypothalamus (Jean and others 2012).

Recent work has also used quantitative analyses of mRNA levels and polymerase chain reaction experiments in rat and guinea pig brains to determine the distribution of the 5-HT<sub>4</sub> receptor splice variants. 5-HT<sub>4</sub> receptor isoforms a, b, c, g, and n are highly expressed in the central nervous system (Bockaert and others 2004; Vilaro and others 2002; Vilaro and others 2005). Isoform (a) is highly expressed in the amygdala, hippocampus, nucleus accumbens, and caudate nucleus. Lower levels of expression are found in the small intestine, the atrium, and pituitary gland. Isoform (b) appears to be the most abundant form in the CNS and periphery, and is expressed in the caudate nucleus, putamen, amygdala, pituitary gland, and small intestine. Isoform (g) seems to be highly expressed in the hypothalamus and cortex and isoform (c) is highly expressed in the pituitary gland and small intestine. Lower levels of isoform (c) are found in the caudate nucleus, hippocampus, and putamen. Isoform (d) is not expressed in the CNS, but is found in the small intestine (Bockaert and others 2004; Vilaro and others 2005; Vilaro and others 2002). The 5-HT<sub>4(n)</sub> variant, which lacks the alternatively spliced C-terminal exon, is widely and abundantly expressed in human peripheral tissues and brain regions including areas involved in mood disorders (frontal cortex, hippocampus) (Vilaro and others 2002).

## Roles of 5-HT<sub>1A</sub> and 5-HT<sub>4</sub> Receptors in Mood Disorders and Treatment Response: Evidence from Clinical Studies

In general, across therapeutic areas, there is often an overall paucity of clinical data that link the pharmacodynamic effects of drugs to the underlying disease or to treatment response. However, several recent complementary studies support important roles of the 5-HT<sub>1A</sub> and 5-HT<sub>4</sub> receptors in the treatment of anxiety and/or depression (Lucas 2009; Lucas and others 2007; Mendez-David and others 2014; Pascual-Brazo and others 2012). Electrophysiology, behavioral, and binding studies in different brain regions all suggest that 5-HT<sub>1A</sub> and 5-HT<sub>4</sub> receptors play a role in the pathophysiology of mood disorders and in the treatment response (Licht and others 2009; Lucas 2007; Lucas and others 2009).

For 5-HT<sub>1A</sub> receptors, human genetic and imaging studies demonstrate that differences in receptor levels and regulation are associated with depression, anxiety, and the response to antidepressants (Le Francois and others 2008; Lesch and Gutknecht 2004; Strobel and others 2003). Postmortem analyses of brainstem samples from depressed suicide patients show a significant increase in levels of 5-HT<sub>1A</sub> autoreceptors compared with non-depressed control individuals, especially in the dorsal raphe nuclei (Boldrini and others 2008; Stockmeier and others 1998). Positron emission tomography (PET) studies have yielded some contrasting results when attempting to confirm these data, but this may be due to differences in the characteristics of the populations studied (Descarries and Riad 2012; Drevets and others 2007; Meltzer and others 2004; Parsey and others 2006b; Parsey and others 2010). One of the most recent PET studies, which used a method that made the fewest possible assumptions about nonspecific binding and also used a reference region that did not express 5-HT<sub>1A</sub> receptors, indeed found that 5-HT<sub>1A</sub> receptor binding potential was higher in antidepressant-naïve major depressive disorder subjects than in control subjects (Parsey and others 2010). Importantly, these PET findings indicate that 5-HT<sub>1A</sub> auto- and heteroreceptors are both affected in MDD, but do not decipher whether alterations in binding are causal or a consequence of the disorder.

A C(-1019)G polymorphism in the promoter of the gene encoding the 5-HT<sub>1A</sub> receptor is associated with several mood-related variables, including trait anxiety, depression, and the response to chronic antidepressant treatment (Fakra and others 2009; Le Francois and others 2008). Depressed patients are more likely to be homozygous for the GG genotype at the C(-1019) allele. They are also more likely to have higher 5-HT<sub>1A</sub> binding potential (Lemondé and others 2003; Parsey and others 2006b). Furthermore, higher 5-HT<sub>1A</sub> binding potential and the

GG genotype also predict remission failure to antidepressant treatment (Parsey and others 2006a). Taken together, these clinical findings strongly implicate an essential role for 5-HT<sub>1A</sub> receptors in both the pathophysiology of mood disorders and in the response to antidepressants.

For 5-HT<sub>4</sub> receptors, there are two studies suggesting a role in clinical depression. One study reported that polymorphisms in the splice variant region of the gene encoding the 5-HT<sub>4</sub> receptor are associated with unipolar depression (Ohtsuki and others 2002). The second study revealed alterations in both 5-HT<sub>4</sub> receptor binding and cAMP concentration levels in several brain regions of depressed violent suicide victims (Rosel and others 2004). These results, while more preliminary than the collection of data from the 5-HT<sub>1A</sub> studies, also implicate a role for 5-HT<sub>4</sub> receptors in mood disorders.

### **Roles of 5-HT<sub>1A</sub> Receptors in Mood Disorders and Treatment Response: Evidence from Preclinical Studies**

In addition to the clinical studies, there is a wealth of preclinical data from rodent studies that indicate a role for 5-HT<sub>1A</sub> receptors in mood disorders and treatment response. Initial preclinical studies led to the hypothesis that 5-HT<sub>1A</sub> autoreceptors delay the therapeutic action of SSRIs and other drugs that increase serotonin levels (Blier and others 1998). More specifically, since 5-HT<sub>1A</sub> autoreceptors exert negative feedback inhibition in response to increased serotonin levels, progressive autoreceptor desensitization may underlie the delayed onset of SSRIs (Blier and others 1998; Gardier and others 1996; Richardson-Jones and others 2010). The functional desensitization of 5-HT<sub>1A</sub> autoreceptors is because of decreased levels of G(o) proteins following chronic SSRI treatment (Li and others 1996), and not down-regulation of the receptor (Le Poul and others 1995). In rats treated for up to 21 days with SSRIs (either fluoxetine or paroxetine), *in vitro* recordings show attenuation of the inhibitory effects of 8-OH-DPAT (a 5-HT<sub>1A</sub> receptor agonist) on firing rates of DRN 5-HT neurons that develops over time (Le Poul and others 1995). Therefore, with sustained SSRI treatment the firing rates of DRN 5-HT neurons initially decreases because of 5-HT<sub>1A</sub> autoreceptor-mediated inhibition, but then recovers as the receptors desensitize, and is fully restored by 14 days after the initiation of the chronic SSRI treatment (Blier and others 1990; Czachura and Rasmussen 2000). Chronic treatment with 5-HT<sub>1A</sub> receptor agonists also produces desensitization of somatodendritic 5-HT<sub>1A</sub> autoreceptors as indicated by electrophysiological and *in vivo* microdialysis studies (Blier and de Montigny 1987; Kreiss and Lucki 1997). Interestingly, local administration in the dorsal raphe of the 5-HT<sub>1A/1B</sub> receptor antagonist, WAY-100635, or

(±)-pindolol potentiates the effects of paroxetine on mouse cortical extracellular dialysate 5-HT levels ([5-HT]<sub>ext</sub>), which suggests that the onset of action of antidepressant treatment is mediated by somatodendritic 5-HT<sub>1A</sub> autoreceptors (Guilloux and others 2006). A more recent study suggests that 5-HT<sub>1A</sub> autoreceptor desensitization alone is not sufficient to induce a SSRI response. Rather, serotonergic tone, governed by intrinsic autoreceptor levels prior to the onset of treatment, is critical for establishing responsiveness and the onset of the SSRI response (Richardson-Jones and others 2010).

Several studies have used 5-HT<sub>1A</sub> receptor germline deficient mice to investigate the role of these receptors in anxiety and depression-related behavior. However, these studies are confounded by the fact that they cannot distinguish between the effects of auto- and heteroreceptors. Generally these studies have found a robust anxiety-like phenotype in conflict anxiety paradigms and a decrease in behavioral despair in the forced swim and tail suspension test (Heisler and others 1998; Klemenhagen and others 2006; Mayorga and others 2001; Parks and others 1998; Ramboz and others 1998). In addition, other studies have used mice that are germline deficient for 5-HT<sub>1A</sub> receptors to determine that 5-HT<sub>1A</sub> receptors are required for some (Mayorga and others 2001; Santarelli and others 2003), but not all (Holick and others 2008), behavioral effects of antidepressants. More specifically, constitutive 5-HT<sub>1A</sub> receptor knockout mice do not respond to acute administration of the SSRIs fluoxetine and paroxetine in the tail suspension test (Mayorga and others 2001), or to chronic treatment with fluoxetine in the novelty-suppressed feeding paradigm (Santarelli and others 2003).

More recent studies have used mice engineered to specifically manipulate either auto- or heteroreceptors while preserving the other receptor population. One study used a conditional and inducible transgenic strategy to assess 5-HT<sub>1A</sub> receptor gain-of-function by conferring temporal and spatial control over receptor expression. This study found that postsynaptic 5-HT<sub>1A</sub> receptors expressed during a specific developmental window (from postnatal day 5 to 21) are important for establishing normal anxiety-like behavior in the adult mouse (Gross and others 2002). More specifically, spatially selective overexpression of postsynaptic 5-HT<sub>1A</sub> receptors in the hippocampus and cortex on the knockout mouse background results in mice that perform similarly to wild-type controls in anxiety-related tasks. However, interpretation of these results is slightly confounded by the approach that used ectopic overexpression. More recently, another study developed a genetic system to independently decrease levels of the 5-HT<sub>1A</sub> auto- and heteroreceptor populations (Richardson-Jones and others 2011). In this study, 5-HT<sub>1A</sub> autoreceptors affected anxiety-like behavior, while 5-HT<sub>1A</sub> heteroreceptors affected behavioral despair responses.

Ultimately, these lines of work are in their infancy and future studies are necessary to investigate not only auto- versus heteroreceptor populations but also subpopulations of heteroreceptors and the temporal roles of all of the different populations.

There are also pharmacological data suggesting a role for 5-HT<sub>1A</sub> receptors in mood disorders and the response to antidepressant and anxiolytic treatments (Table 1). 5-HT<sub>1A</sub> receptor agonists induce behavioral effects that are comparable to antidepressant drugs (Bluer 2003; Lucki 1991; Santarelli and others 2003). In addition, buspirone and 8-OH-DPAT are 5-HT<sub>1A</sub> receptor agonists that reduce anxiety (Barrett and Vanover 1993; Griebel 1995; Tunnicliff 1991). Drugs that target 5-HT<sub>1A</sub> receptors, such as pindolol, have led to somewhat disappointing results in clinical trials (McAskill and others 1998). However, a large-scale clinical study found that buspirone was equally effective as other drugs, such as the dopaminergic agent bupropion, when used as an augmentation therapy for depressed patients that did not remit on initial treatment with a SSRI (Warden and others 2007). Ultimately, given the differences between auto- and heteroreceptors regarding their distribution and function in the brain, it is now clear that future treatments targeting 5-HT<sub>1A</sub> receptors will need to specifically target only one of these populations of receptors to improve the antidepressant response.

### **Roles of 5-HT<sub>4</sub> Receptors in Mood Disorders and Treatment Response: Evidence from Preclinical Studies**

The understanding of the roles that 5-HT<sub>4</sub> receptors play in mood disorders also mainly comes from preclinical studies. Animal models of anxiety/depression such as the Flinders sensitive line of rats, olfactory bulbectomy, glucocorticoid receptor heterozygous mice (GR<sup>+/-</sup>), and maternal prenatal stress, show changes in 5-HT<sub>4</sub> receptor density in limbic areas such as the hippocampus and the caudal portion of the caudate-putamen complex (Chen and others 2012; Licht and others 2010; Licht and others 2009; Ridder and others 2005). Similarly, some chronic monoaminergic antidepressant drugs, such as fluoxetine or venlafaxine, but not reboxetine, decrease 5-HT<sub>4</sub> receptor density in rat brain (Vidal and others 2010).

Further studies investigated whether 5-HT<sub>4</sub> receptor ligands can directly exert antidepressant-like effects or modify the effects of monoaminergic antidepressants (Table 2). In naïve rats, acute fluoxetine-induced decreases in immobility in the forced swim test (FST) are not affected by co-administration of the 5-HT<sub>4</sub> receptor antagonist SB 204070A. In addition, this antagonist has no independent effects in the FST (Cryan and Lucki 2000). Conversely, in a model of anxiety/depression

based on chronic elevation of glucocorticoids, a brain penetrant 5-HT<sub>4</sub> receptor antagonist (GR 125487) prevents the effects of the SSRI fluoxetine in Open Field, Tail Suspension Test, Novelty Suppressed Feeding, and the Sucrose Splash test (Mendez-David and others 2014). These results suggest that the antidepressant-like effects of SSRIs are mediated in part through activation of 5-HT<sub>4</sub> receptors. In addition, 5-HT<sub>4</sub> receptor activation with the partial agonist RS 67333 increases the effects of acute SSRI (paroxetine) administration on extracellular 5-HT levels in rat ventral hippocampus (Licht and others, 2010). These increased 5-HT levels are observed both immediately and 3 days after administration (Licht and others 2009; Licht and others 2010). 5-HT<sub>4</sub> receptors are only localized postsynaptic to serotonergic nerve terminals and thus are heteroreceptors. An *in vivo* electrophysiology study demonstrated that 5-HT<sub>4</sub> receptors exert excitatory influence on central 5-HT neuron activity (Lucas and others 2005). These data suggest that frontocortical 5-HT<sub>4</sub> receptors exert positive feedback on serotonergic activity by controlling a population of DRN 5-HT neurons.

In addition, administration of 5-HT<sub>4</sub> receptor agonists induces similar molecular and behavioral changes as SSRI antidepressants in rodents (Bockaert and others 2008; Lucas and others 2007; Pascual-Brazo and others 2012). Lucas and colleagues showed that administration of the 5-HT<sub>4</sub> receptor agonists RS 67333 and prucalopride reduce immobility time in rats exposed to the FST by about 50% compared with controls, whereas citalopram only reduces immobility time by about 23%. Additional behavioral experiments also found that the 5-HT<sub>4</sub> receptor agonist RS 67333 is more effective than citalopram in the Rat Forced Swim test and also increases the locomotor activity induced by olfactory bulbectomy (Lucas and others 2007). Depressed-like behavioral phenotypes observed with olfactory bulbectomy or exposure to chronic mild stress are reversed by 10 to 14 days of RS67333 treatment in rats, suggesting that RS67333 displays a faster antidepressant-like response relative to classical antidepressants (Lucas and others 2007). In addition, short periods of RS 67333 treatment results in antidepressant/anxiolytic-like effects in the sucrose consumption test of anhedonia, in socially defeated mice exposed to the FST, and in the novelty suppressed feeding test in rats (Gomez-Lazaro and others 2012; Pascual-Brazo and others 2012) (Table 2).

In addition, activation of 5-HT<sub>4</sub> receptors mediates several intracellular changes that are associated with the antidepressant drug response. These changes include increases in cAMP levels, protein kinase A activation, phosphorylation of cAMP response element-binding protein (CREB), and transcription of brain-derived neurotrophic factor (BDNF) (Pascual-Brazo and others 2012)

**Table 1.** Effects of 5-HT<sub>1A</sub> Receptor Ligands on Anxiety/Depression-Like Phenotypes.

References	Name	Pharmacological Properties	Doses	Species	Paradigms	Effects	Importance
Lucki (1991)	8-OH-DPAT: 8-hydroxy-2-dipropylaminotetralin Buspirone; gepirone; ipsapirone Pindolol	Agonist Partial agonists Partial agonist/ antagonist Antagonist	Varied	Varied (mostly rats)	FST, LH, OF and Feeding after Restraint	Review assessing outcomes found that most studies showed antidepressant-like effects Review assessing outcomes found that most studies showed no effects when administered alone, but reversed antidepressant-like effects of 8-OH-DPAT	Most studies suggest that the behavioral effects of 5-HT <sub>1A</sub> receptor agonists mimic those of antidepressants
Griebel (1995)	NAN 190: 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine hydrobromide Buspirone	Partial agonist	Varied	Varied	Varied	Review assessing outcomes found that 71% of 210 studies show an anxiolytic-like profile	Most studies suggest that the behavioral effects of 5-HT <sub>1A</sub> receptor agonists are anxiolytic
Santarelli and others (2003)	8-OH-DPAT: 8-hydroxy-2-dipropylaminotetralin 8-OH-DPAT: 8-Hydroxy-2-dipropylaminotetralin	Agonist Agonist	Varied 1 mg/kg/d for 28 days	Mice	NSF	Review assessing outcomes found that 61% of 112 studies show an anxiolytic-like profile Decreased latency to feed	Chronic administration of a 5-HT <sub>1A</sub> receptor agonist mimics the behavioral effects of chronic antidepressant treatment

FST = forced swim test; LH = learned helplessness; NSF = novelty suppressed feeding; OF = open field.

**Table 2.** Effects of 5-HT<sub>4</sub> Receptor Ligands on Anxiety/Depression-Like Phenotypes.

References	Name	Pharmacological Properties	Doses	Species	Paradigms	Effects
Silvestre and others (1996)	SB204070: 8-amino-7-chloro-(N-butyl-4-piperidyl)-methylbenzo-1,4-dioxan-5-carboxylate hydrochloride	Antagonist	0.3-3 mg/kg, s.c., acutely	Rat	EPM	Exhibits an anxiolytic-like profile
Kennett and others (1997)	GR 113808: 1-[2-methylsulphonylamino]ethyl]-4-piperidyl]methyl-1-methyl-1H-indole-3-carboxylate maleate	Antagonist	0.3-3 mg/kg, s.c., acutely			Exhibits an anxiolytic-like profile
Kennett and others (1997)	SB204070: 8-amino-7-chloro-(N-butyl-4-piperidyl)-methylbenzo-1,4-dioxan-5-carboxylate hydrochloride	Antagonist	0.01 and 10 mg/kg p.o. 0.01 and 1 mg/kg s.c., acutely	Rat	Social interaction/ EPM	Increased time spent in social interaction / induced anxiolysis
Costall and Naylor (1997)	SB 207266A: 2H-(1,3)oxazino(3,2-a)indole-10-carboxamide, N-((1-butyl-4-piperidyl)methyl)-3,4-dihydro-, monohydrochloride	Antagonist	0.001 and 0.1 mg/kg s.c. 0.01 and 1 mg/kg s.c., acutely		Social interaction / elevated x-maze	Increased time spent in social interaction / induced anxiolysis
Costall and Naylor (1997)	SDZ205-557: 2-methoxy-4-amino-5-chloro-benzoic acid 2-(diethylamino ester)	Antagonist	0.001-100 µg/kg, i.p., acutely	Mice	Light/dark	No effect by itself, but reduced the disinhibitory effect of diazepam
Schreiber and others (1998)	GR 113808: 1-[2-methylsulphonylamino]ethyl]-4-piperidyl]methyl-1-methyl-1H-indole-3-carboxylate maleate	Antagonist	0.001-10 µg/kg, i.p., acutely			No effect by itself, but reduced the disinhibitory effect of diazepam
Schreiber and others (1998)	SB204070: 8-amino-7-chloro-(N-butyl-4-piperidyl)-methylbenzo-1,4-dioxan-5-carboxylate hydrochloride	Antagonist	0.001-10 µg/kg, i.p., acutely			No effect by itself, but reduced the disinhibitory effect of diazepam
Cryan and Lucki (2000)	GR 125487: [1-[2-[(methylsulfonyl)amino]ethyl]-4-piperidyl]methyl 5-fluoro-2-methoxy-1H-indole-3-carboxylate	Antagonist	3 mg/kg, s.c., acutely	Rat	ultrasonic vocalization	No effect
Cryan and Lucki (2000)	SB204070: 8-amino-7-chloro-(N-butyl-4-piperidyl)-methylbenzo-1,4-dioxan-5-carboxylate hydrochloride	Antagonist	3 mg/kg, s.c., acutely	rat	FST	No effect
Lucas and others (2007)	RS 67333: (1-[4-amino-5-chloro-2-methoxyphenyl]-3-[1-butyl-4-piperidyl]-1-propanone)	Agonist	1.5 mg/kg, i.p. during 3 days	Rat	FST	Antidepressant-like profile: decrease immobility duration and increase climbing duration
Tamburella and others (2009)	SL 65.0155: [5-(8-amino-7-chloro-2,3-dihydro-1,4-benzo-dioxin-5-yl)-3-[1-(2-phenylethyl)-4-piperidyl]-1,3,4-oxadiazol-2-(3H)-one]-monohydrochloride]	Partial agonist	1.5 mg/kg, i.p. during 3 and 14 days	OBX rat CMS rat	Locomotor activity Sucrose consumption	Reversed OBX-induced increase in locomotor activity after 14 days Reversed CMS-induced decrease in sucrose consumption after 14 days
Pascual-Brazo and others (2012)	RS 67333: (1-[4-amino-5-chloro-2-methoxyphenyl]-3-[1-butyl-4-piperidyl]-1-propanone)	Agonist	0.1, 0.5, and 1 mg/kg, i.p. during 1 day	Rat	FST	Antidepressant-like profile: increase swimming and climbing and reduce immobility duration
Pascual-Brazo and others (2012)	RS 67333: (1-[4-amino-5-chloro-2-methoxyphenyl]-3-[1-butyl-4-piperidyl]-1-propanone)	Agonist	1.5 mg/kg, i.p. during 3/7 days	Rat	FST/NSF	Anxiolytic/ Antidepressant-like profile: decrease immobility duration at 3/7 days, decrease latency to feed after 7 days

(continued)

Table 2. (continued)

References	Name	Pharmacological Properties	Doses	Species	Paradigms	Effects
Gomez-Lazaro and others (2012)	RS 67333: (1-[4-amino-5-chloro-2-methoxyphenyl]-3-[1-butyl-4-piperidinyl]-1-propanone)	Agonist	1.5 mg/kg, i.p. during 5 days	Cort-treated rat	Sucrose intake	Antidepressant-like profile: increase sucrose intake at 3/7 days
Mendez-David and others (2014)	RS 67333: (1-[4-amino-5-chloro-2-methoxyphenyl]-3-[1-butyl-4-piperidinyl]-1-propanone)	Agonist	1.5 mg/kg, osmotic minipumps during 7 days	Social stress in rat	FST	Antidepressant-like profile: increase swimming behavior
				Cort-treated mice	OF/EPM/ST/TST/NSF	Anxiolytic/ Antidepressant-like profile: increase time spent in the center and ratio of ambulatory distance in the center divided by total distance in the OF, increase time and entries in EPM, increase grooming duration and decrease immobility duration, the latency to feed in ST and TST, and NSF, respectively
	GR 125487: [1-[2-[(methylsulfonyl)amino]ethyl]-4-piperidinyl]methyl 5-fluoro-2-methoxy-1H-indole-3-carboxylate	Antagonist	1 mg/kg, osmotic minipumps during 7 days	Cort-treated mice	OF/EPM/NSF/ST/TST	No effect <sup>a</sup>

CMS = chronic mild stress; EPM = elevated plus maze; FST = forced swim test; i.p. = intraperitoneally; NSF, novelty suppresses feeding; OBX = olfactory bulbectomy; OF = open field; s.c. = subcutaneously; ST = splash test; TST = tail suspension test.

<sup>a</sup>GR 125487 partially prevents fluoxetine-induced increase in time spent in the center in OF, prevents fluoxetine-induced increase in grooming duration in ST and decrease immobility duration in TST, prevents fluoxetine-induced decrease in latency to feed in NSF.

(Nibuya and others 1995). Therefore, mechanistic data also suggest that direct activation of 5-HT<sub>4</sub> receptors yield antidepressant-like effects (Lucas and others 2007; Pascual-Brazo and others 2012; Tamburella and others 2009). Furthermore, signaling molecules that interact with the 5-HT<sub>4</sub> receptor, such as P11 (S100A10), in brain regions important for anxiety/depression and cognition such as hippocampal pyramidal cells in CA1 and the hippocampal granule cells in the dentate gyrus (Egeland and others 2011; Warner-Schmidt and others 2009) may provide novel targets for fast-acting anxiolytic/antidepressant treatments. Recent results suggest that cortical neurons expressing both P11 and 5-HT<sub>4</sub> receptors regulate the behavioral effects of SSRIs in mice and that chronic treatment with fluoxetine increases 5-HT<sub>4</sub> receptor expression in these neurons (Schmidt and others 2012). In addition, in behavioral tests such as FST and tail suspension test (TST), the antidepressant-like activity of RS67333 was abolished in P11 knockout mice (Warner-Schmidt and others 2009). Taken together, these studies suggest a link between the 5-HT<sub>4</sub> receptor and depression and provide an encouraging pharmacological strategy to obtain a faster treatment response.

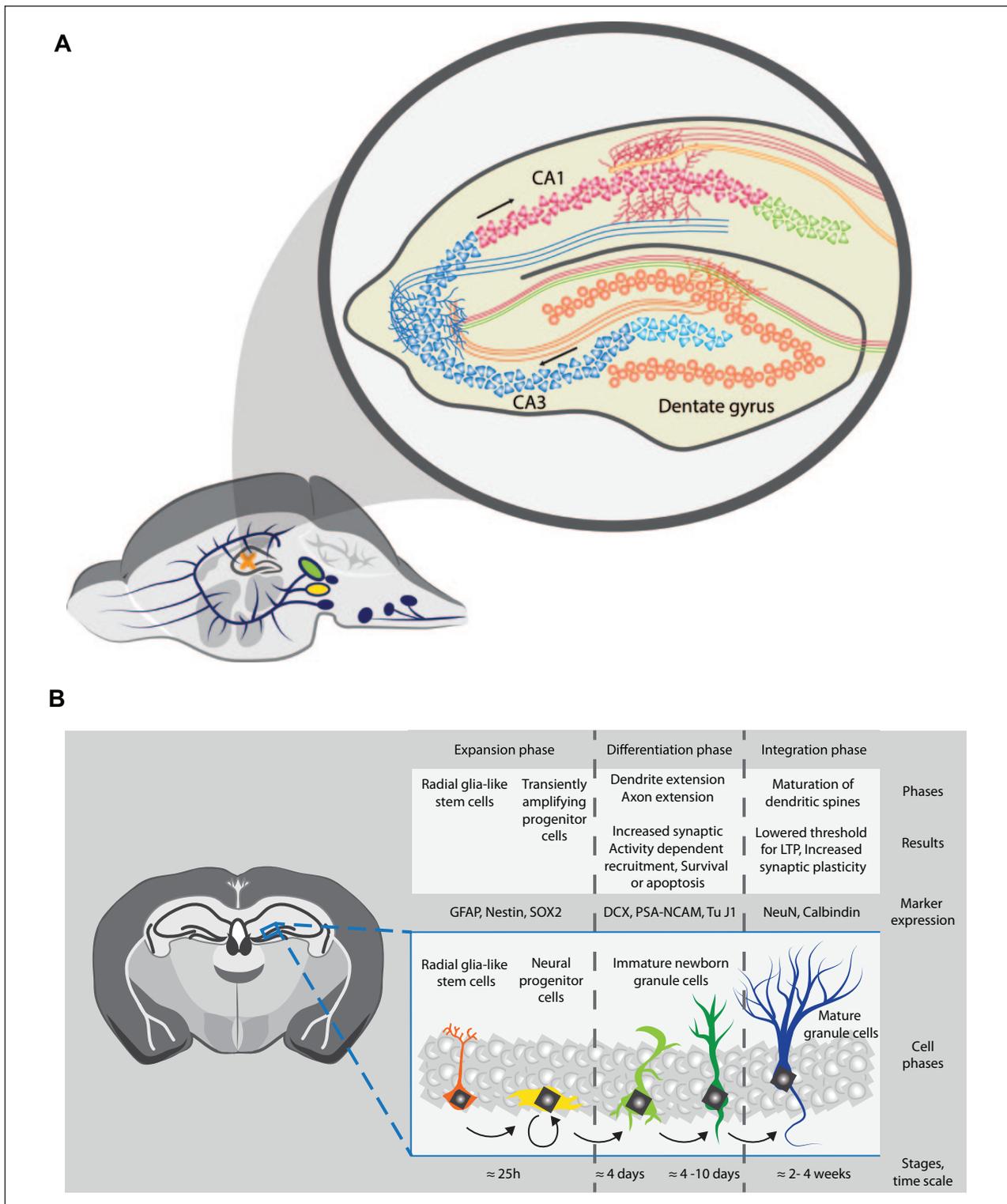
Some historical studies also investigated whether 5-HT<sub>4</sub> receptors mediate the anxiolytic behavioral effects of SSRIs. However, these studies were unable to determine a clear role for 5-HT<sub>4</sub> receptors in anxiety. For instance, in the light/dark choice test, diazepam induces dose-dependent anxiolytic-like effects in mice that are inhibited by 5-HT<sub>4</sub> receptor antagonists (GR 113808, SB 204070, and SDZ 205-557) (Costall and Naylor 1997). These data suggest that activation of 5-HT<sub>4</sub> receptors mediate the anxiolytic effects of diazepam. In addition, while this study did not find any effects of 5-HT<sub>4</sub> receptor antagonists alone on anxiety behavior (Costall and Naylor 1997), others report anxiogenic effects of the 5-HT<sub>4</sub> receptor antagonists SB 204070, GR 113808 (Silvestre and others 1996) and SB 207266A (Kennett and others 1997; Silvestre and others 1996) in the elevated plus maze in rats. In these studies, rats acutely treated with SB 204070 or GR 113808 display an increase in the percentage of total time spent in the open arms, which is indicative of anxiety-like behavior. However, while one study did not detect an effect of the antagonists SB 204070 and GR 113808 on the number of open arm entries when a 10 minute pretest injection interval was used (Silvestre and others 1996), another study reported an increase in the percent of open arm entries after SB 204070 or SB 207266A injections when a one hour pretest injection interval was used (Kennett and others 1997). 5-HT<sub>4</sub> receptor knockout mice do not display an anxious or depressed-like phenotype, but they do show an attenuated response to novelty that may be relevant for mood disorders (Compan and others 2004). In a more recent study,

chronic treatment with GR125487 did not affect the anxiety-like phenotype induced by chronic corticosterone treatment in mice (Mendez-David and others 2014). Interestingly, this study found that, while a 7-day treatment with fluoxetine or RS67333 induced antidepressant-like activity in the TST and FST, only the 5-HT<sub>4</sub> receptor agonist RS67333 displayed an anxiolytic-like activity in the Open Field paradigm and the Elevated Plus Maze. By contrast, a longer duration of treatment (28 days) was required for fluoxetine to exert anxiolytic-like effects in these tests. These data support the idea that 5-HT<sub>4</sub> receptor agonists may treat anxiety and depression disorders with faster efficacy than traditional antidepressants.

Other investigations have found that 5-HT<sub>4</sub> receptor stimulation inhibits the anxiolytic effects of diazepam (an enhancer of GABA response), particularly under conditions of high serotonergic tone (Costall and others 1993). Since GABA<sub>A</sub> receptor-mediated inhibition of synaptic transmission is highly involved in controlling neuronal excitability, and GABA<sub>A</sub> receptors are implicated in the pathogenesis of anxiety disorders (Cai and others 2002; Macdonald and Olsen 1994), these data suggest that 5-HT<sub>4</sub> receptors may also act on GABAergic signaling in PFC neurons. Taken together, these studies demonstrate that 5-HT<sub>4</sub> receptors are important mediators of the antidepressant response. Future work, involving spatially restricted deletions of 5-HT<sub>4</sub> receptors or local administration of pharmacological ligands, is necessary to more precisely determine the cellular and circuit-based mechanisms by which 5-HT<sub>4</sub> receptors influence behavior.

### **Roles of 5-HT<sub>1A</sub> and 5-HT<sub>4</sub> Receptors in Mediating Adult Hippocampal Neurogenesis**

It is well established that new neurons are continuously generated and incorporated into the functional neural network of the mammalian adult brain through a process referred to as adult neurogenesis (Spalding and others 2013). More specifically, neurogenesis occurs in the subventricular zone (SVZ) of the lateral ventricle and in the subgranular zone (SGZ) of the dentate gyrus in most adult mammals (Ming and Song 2005). Adult hippocampal neurogenesis in the SGZ has gained considerable attention over the last decade and a half as a neural substrate potentially underlying the pathophysiology of depression (Figure 4A and B). Most antidepressants, including SSRIs, are potent stimulators of adult hippocampal neurogenesis when administered chronically. Antidepressant treatment increases the proliferation of newborn cells as well as the survival and maturation of the young neurons (Malberg and others 2000; Santarelli and others 2003). The neurogenesis hypothesis originally posited that a decrease in the production of newborn



**Figure 4.** Production of new neurons in the adult dentate gyrus. (A) The hippocampal trisynaptic circuit in mouse brain. Neurons of the entorhinal cortex project to the dentate gyrus, with additional collaterals projecting to the CA3 subfield (perforant pathway). Granule cells in the dentate gyrus project to the CA3 field of the hippocampus via the mossy fiber pathway. The CA3 pyramidal cells project onto themselves and also to the CA1 through Schaffer collaterals. (B) Hippocampal neurogenesis is possible in the subgranular zone (SGZ) of the dentate gyrus of the hippocampus because of the presence of stem cells. These stem cells evolve into neural progenitor cells that can produce multiple cell types in the central nervous system such as neurons, astrocytes, oligodendrocytes, or microglial cells. In rodents, the duration of the mitotic cycle of proliferating precursors is approximately 12 to 24 hours, leading to the production of about 8,000 to 10,000 new neurons per day.

dentate granule cells leads to depression, while enhanced neurogenesis (proliferation, survival, and maturation) is required for treatment of depression (Duman and others 2000; Jacobs and others 2000; Sahay and others 2007; Samuels and Hen 2011). Evidence suggests that this hypothesis is partially correct since adult hippocampal neurogenesis is indeed necessary for some of the behavioral effects of antidepressants (David and others 2009; Santarelli and others 2003; Surget and others 2008; Wang and others 2008). In addition, while no evidence has yet shown that decreasing the production of newborn dentate granule cells leads to depression, a large body of evidence also suggests that mental illness is often marked by diminutions in hippocampal structure and function. For example, patients with depression, posttraumatic stress disorder, schizophrenia, Alzheimer's disease, or stress show decreased hippocampal volume, learning and memory deficits, and mood dysregulation (Nestler and others 2002; Sapolsky 2000). Interestingly, both 5-HT<sub>1A</sub> and 5-HT<sub>4</sub> receptors are implicated in regulating adult hippocampal neurogenesis.

### The Role of 5-HT<sub>1A</sub> Receptors in Mediating Adult Hippocampal Neurogenesis

Several studies, when taken together, suggest that activation of 5-HT<sub>1A</sub> receptors increases adult hippocampal neurogenesis (Table 3). The first evidence that 5-HT<sub>1A</sub> receptors regulated adult hippocampal neurogenesis came from a study assessing the effects of acute administration of antagonists on cell proliferation in the rat dentate gyrus. In this study, three different 5-HT<sub>1A</sub> antagonists (NAN-190, p-MPPI, and WAY-100635) all resulted in an approximately 30% reduction in the number of BrdU-positive cells (Radley and Jacobs 2002), a marker of cell proliferation. A later study then found that the 5-HT<sub>1A</sub> and 5-HT<sub>7</sub> receptor agonist 8-OH-DPAT not only increases cell proliferation in the dentate gyrus but can also reverse decreases in cell proliferation induced by a 5-HT synthesis inhibitor, *para*-chlorophenylalanine (Banar and others 2004). Other 5-HT<sub>1A</sub> receptors partial agonists, buspirone or tandospirone, increases the number of newborn cells and the number of DCX-positive cells in the DG respectively (Grabiec and others 2009; Mori and others 2014). In addition, an *in vitro* study found that 5-HT<sub>1A</sub> receptors regulate self-renewal of precursor cells (Klempin and others 2010).

Another study investigated whether chronic treatment with various antidepressants enhances adult hippocampal neurogenesis in germline 5-HT<sub>1A</sub> receptor knockout mice (Santarelli and others 2003). Interestingly, while the effects of tricyclic antidepressants remain intact, the

effects of the SSRI fluoxetine on both adult hippocampal neurogenesis (newborn cell proliferation) and behavior are abolished in 5-HT<sub>1A</sub> receptor knockout mice. Taken together, these data suggest that 5-HT<sub>1A</sub> receptors are critical mediators of the effects of SSRIs on adult hippocampal neurogenesis and behavior. In addition, this study also showed that the effects of the 5-HT<sub>1A</sub> and 5-HT<sub>7</sub> agonist 8-OH-DPAT are also abolished in 5-HT<sub>1A</sub> receptor knockout mice, confirming the importance of 5-HT<sub>1A</sub> receptors in mediating serotonin-induced enhancements in neurogenesis in the adult DG of the hippocampus.

Mice with decreased 5-HT<sub>1A</sub> autoreceptor levels still show a behavioral and neurogenic response to chronic antidepressants (Richardson-Jones and others 2010), suggesting that 5-HT<sub>1A</sub> heteroreceptors mediate the effects of increased serotonin neurotransmission on neurogenesis and behavior. Future studies are required to determine the anatomical location of the 5-HT<sub>1A</sub> heteroreceptor population that mediates these effects.

### The Role of 5-HT<sub>4</sub> Receptors in Mediating Adult Hippocampal Neurogenesis

5-HT<sub>4</sub> receptor agonists also can induce neurogenesis in the hippocampus as well as in the enteric system in adult rodents (Ishizuka and others 2014; Liu and others 2009; Lucas and others 2007; Pascual-Brazo and others 2012). Interestingly, the beneficial effects of 5-HT<sub>4</sub> receptor agonists seem to appear faster than traditional antidepressants not only on behavior but also on adult hippocampal neurogenesis (Table 4). A recent study performed in naïve, non-stressed rats confirmed that 3 days of treatment with the 5-HT<sub>4</sub> receptor agonist (RS67333) significantly enhanced neurogenesis in the subgranular zone of the dentate gyrus of the hippocampus, an effect that requires at least 2 weeks of treatment with classical antidepressants such as SSRIs (Pascual-Brazo and others 2012). However, no direct evidence currently links the antidepressant-like behavioral effects of 5-HT<sub>4</sub> receptor activation to increased adult hippocampal neurogenesis. A recent study found that the 5-HT<sub>4</sub> receptor agonist RS67333 increases neurogenesis (proliferation and maturation) to a lesser extent than fluoxetine and that the 5-HT<sub>4</sub> antagonist GR125487 partially blocks the neurogenic effects of chronic fluoxetine treatment (Mendez-David and others 2014). Taken together, these results suggest that while 5-HT<sub>4</sub> receptors contribute to the effects of fluoxetine on proliferation and maturation of newborn neurons other 5-HT receptors, such as the 5-HT<sub>1A</sub> receptor, are also important.

Recent work also indicates that 5-HT<sub>4</sub> receptor activation may result in antidepressant-induced dematuration

**Table 3.** Effects of 5-HT<sub>1A</sub> Receptor Ligands on Proliferation and Maturation of Newborn Neurons in the Adult Hippocampus.

References	Name	Pharmacological Properties	Doses	Species	Steps	Effects
Radley and Jacobs (2002)	NAN-190: 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl] piperazine hydrobromide	Antagonists	2.5 mg/kg 2.5 hours before sacrifice	Rats	Proliferation	Decreases the number of newborn cells in the SGZ of DG
	p-MPPI: 4-iodo-N-[2-[4-(methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylbenzamide		10 mg/kg 2.5 hours before sacrifice			
	WAY-100635: (N-(2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl)-N-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride		5 mg/kg 2.5 hours before sacrifice			
Santarelli and others (2003)	8-OH-DPAT: 8-Hydroxy-2-dipropylaminotetralin	Agonist	1 mg/kg/day for 28 days	Mice	Proliferation	Increases the number of newborn cells in the SGZ of DG
Banasr and others (2004)	8-OH-DPAT: 8-hydroxy-2-dipropylaminotetralin	Agonist	1 mg/kg 2.5 hours before sacrifice	Rats	Proliferation	Increases the number of newborn cells in the SGZ of DG
Grabiec and others (2009)	Bupirone	Partial agonist	3 mg/kg 3 hours before sacrifice	Opossums	Proliferation	Increases the number of newborn cells in the SGZ of DG
Klempin and others (2010)	8-OH-DPAT: 8-hydroxy-2-dipropylaminotetralin	Agonist	1 mg/kg 1 day before sacrifice	Mice	Proliferation	Increases the number of newborn cells in the SGZ of DG
	WAY-100635: (N-(2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl)-N-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride	Antagonist	1 mg/kg for 7 days			No effect
			10 mg/kg 1 day before sacrifice			No effect
			10 mg/kg for 7 days			Decreases the number of newborn cells in the SGZ of DG
Mori and others (2014)	Tandospirone	Partial agonist	1 or 10 mg/kg for 10 days	Rats	Maturation	Increases the number of DCX-positive cells in the DG

DCX = doublecortin; DG = dentate gyrus; SGZ = subgranular zone.

of mature dentate granule cells (Kobayashi and others 2010). This study found that upregulation of 5-HT<sub>4</sub> receptor induced cAMP signaling may play an instructive role in the reversal of neuronal maturation induced by chronic antidepressant treatment (Kobayashi and others 2010). However, the exact mechanisms underlying this phenomenon will require further investigation using spatially restricted 5-HT<sub>4</sub> receptor knockout mice.

Analysis of 5-HT<sub>4</sub> receptor-mediated intracellular signaling further suggests that targeting this receptor yields antidepressant-like effects. More specifically, 5-HT<sub>4</sub> receptors are G(s)-coupled G-protein coupled receptors that activate adenylyl cyclase, and thus increase production of cAMP (Dumuis and others 1989; Torres and others 1995). Increased production of cAMP activates protein kinase A, which in turn phosphorylates the transcription factor CREB. Interestingly, chronic antidepressant drug treatment activates the same signal transduction machinery (Nibuya and others 1996). Phosphorylation of CREB is thought to constitute a key step in the facilitation of adult hippocampal neurogenesis as it results in increased BDNF levels (Castren 2014; Duman and others 2001; Malberg and others 2000). Increased BDNF levels can modulate behavior, promote neurite outgrowth and

synaptic plasticity, and enhance survival of new neurons (Duman and Monteggia 2006). Therefore, since activation of 5-HT<sub>4</sub> receptors ultimately increases BDNF expression, it is a reasonable target to achieve antidepressant-like effects. Interestingly, BDNF levels are increased in the rat hippocampus after only 3 days of treatment with the 5-HT<sub>4</sub> receptor agonist RS67333 (Pascual-Brazo and others 2012). Another study found that acute administration of the 5-HT<sub>4</sub> partial receptor agonist SL65.0155 also increases BDNF levels in rats (Tamburella and others 2009). These preclinical studies, when combined with behavioral test results, indicate that 5-HT<sub>4</sub> receptors provide a putative target for faster acting antidepressants.

## Conclusions

Taken together, much evidence indicates that SSRIs mediate some of their effects through both 5-HT<sub>1A</sub> and 5-HT<sub>4</sub> receptors, thus being reasonable targets for future antidepressant drug development. However, 5-HT<sub>1A</sub> receptors in different anatomical locations show distinct brain functions, and therefore it may be necessary to selectively target subpopulations of these receptors to attain the optimal therapeutic outcome. In addition, the

**Table 4.** Effects of 5-HT<sub>4</sub> Receptor Ligands on Proliferation, Maturation, and Survival of Newborn Neurons in the Adult Hippocampus.

References	Name	Pharmacological Properties	Doses	Species	Steps	Effects
Lucas and others (2007)	RS 67333: (1-[4-amino-5-chloro-2-methoxyphenyl]-3-[1-butyl-4-piperidinyl]-1-propanone) versus citalopram (77-300 µg/kg i.v.)	Agonist	1.5 mg/kg, osmotic mini-pumps during 3 days	Rats	Proliferation	Increases the number of newborn cells in the SGZ of DG
Tamburella and others (2009)	SL 65.0155: [5-(8-amino-7-chloro-2,3-dihydro-1,4-benzo-dioxin-5-yl)-3-[1-(2-phenylethyl)-4-piperidinyl]-1,3,4-oxadiazol-2(3H)-one-monohydrochloride]	Partial agonist	0.1, 0.5 and 1 mg/kg, i.p. during 1 day	Rats	Survival	Increases Bcl-2 expression after acute administration
Pascual-Brazo and others (2012)	RS 67333: (1-[4-amino-5-chloro-2-methoxyphenyl]-3-[1-butyl-4-piperidinyl]-1-propanone) versus fluoxetine (10 mg/kg/d)	Agonist	1.5 mg/kg, i.p. during 3/7 days	Rats	Proliferation	Increases the number of newborn cells in the SGZ of DG at 3/7 days and up-regulate some neuroplasticity-related markers as BDNF, CREB, and AKT
Ishizuka and others (2014)	GR 113808: 1-(2-methylsulfonylaminoethyl-4-piperidinyl)methyl-1-methyl-1H-indole-3-carboxylate	Antagonist	1 µM during 30 min and 40 hours later during 30 minutes for 2 days	Mouse induced pluripotent stem cells	Differentiation	Blocks all-trans retinoic acid-induced neural differentiation of mouse iPS cells into NPC
Mendez-David and others (2014)	RS 67333: (1-[4-amino-5-chloro-2-methoxyphenyl]-3-[1-butyl-4-piperidinyl]-1-propanone) versus fluoxetine (18 mg/kg/d)	Agonist	1.5 mg/kg, osmotic mini-pumps during 7 days	Cort-treated mice	Proliferation	Increases by 51% the number of newborn cells in the SGZ of DG
	GR 125487: [1-[2-[(methylsulfonyl)amino]ethyl]-4-piperidinyl]methyl 5-fluoro-2-methoxy-1H-indole-3-carboxylate	Antagonist	1 mg/kg, osmotic mini-pumps during 7 days	Cort-treated mice	Maturation Morphology Proliferation/ maturation/ morphology	Increases by 44% the maturation index Increases the dendritic length and the number of dendritic intersections No effect <sup>a</sup>

Bcl-2 = B-cell lymphoma 2; BDNF = brain-derived neurotrophic factor; CREB = cAMP response element-binding protein; DG = dentate gyrus; i.p. = intraperitoneally; iPS = induced pluripotent stem; i.v. = intravenously; SGZ = subgranular zone.

<sup>a</sup>Partially blocks the effects of chronic fluoxetine-induced increase in proliferation of newborn cells and increase in maturation.

localization of 5-HT<sub>4</sub> receptors may also be a critical consideration for drug targeting since these receptors also play important roles outside the central nervous system. More specifically, 5-HT<sub>4</sub> receptors are also expressed in cardiac and intestinal tissues and administration of 5-HT<sub>4</sub> receptor agonists can lead to arrhythmia (Ferrari and others 2013). Thus, future antidepressants should target either specific anatomical populations of 5-HT<sub>1A</sub> and 5-HT<sub>4</sub> receptors or downstream effectors. To this end, recently developed 5-HT<sub>1A</sub> receptor agonists seem to preferentially target 5-HT<sub>1A</sub> receptor subpopulations (Garcia-Garcia and others 2014). If the appropriate 5-HT<sub>1A</sub> heteroreceptor population can be targeted, then these agonists may be faster acting antidepressants that avoid the delays caused by autoreceptor-mediated feedback inhibition of serotonergic tone observed following chronic administration of SSRIs. In addition, signaling molecules that interact with the 5-HT<sub>4</sub> receptor, such as

P11, may also represent novel targets for faster-acting antidepressant activity (Egeland and others 2011; Warner-Schmidt and others 2009). Perhaps novel multitarget-directed ligands with both 5-HT<sub>1A</sub> and 5-HT<sub>4</sub> agonistic properties could also yield more effective antidepressants.

### Authors' Note

Benjamin Adam Samuels and Indira Mendez-David contributed equally to this work.

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### References

- Albert PR, Lembo P, Storrington JM, Charest A, Saucier C. 1996. The 5-HT<sub>1A</sub> receptor: signaling, desensitization, and gene transcription. *Neuropsychopharmacology* 14(1):19–25.
- Banasr M, Hery M, Printemps R, Daszuta A. 2004. Serotonin-induced increases in adult cell proliferation and neurogenesis are mediated through different and common 5-HT receptor subtypes in the dentate gyrus and the subventricular zone. *Neuropsychopharmacology* 29(3):450–60.
- Barnes NM, Sharp T. 1999. A review of central 5-HT receptors and their function. *Neuropharmacology* 38(8):1083–152.
- Barrett JE, Vanover KE. 1993. 5-HT receptors as targets for the development of novel anxiolytic drugs: models, mechanisms and future directions. *Psychopharmacology (Berl)* 112(1):1–12.
- Barthel G, Gaven F, Framery B, Shinjo K, Nakamura T, Claeysen S, and others. 2005. Uncoupling and endocytosis of 5-hydroxytryptamine 4 receptors. Distinct molecular events with different GRK2 requirements. *J Biol Chem* 280(30):27924–34.
- Blier P. 2003. The pharmacology of putative early-onset antidepressant strategies. *Eur Neuropsychopharmacol* 13(2):57–66.
- Blier P, de Montigny C. 1987. Modification of 5-HT neuron properties by sustained administration of the 5-HT<sub>1A</sub> agonist gepirone: electrophysiological studies in the rat brain. *Synapse* 1(5):470–80.
- Blier P, de Montigny C, Chaput Y. 1990. A role for the serotonin system in the mechanism of action of antidepressant treatments: preclinical evidence. *J Clin Psychiatry* 51(Suppl):14–20.
- Blier P, Pineyro G, el Mansari M, Bergeron R, de Montigny C. 1998. Role of somatodendritic 5-HT autoreceptors in modulating 5-HT neurotransmission. *Ann N Y Acad Sci* 861:204–16.
- Bockaert J, Claeysen S, Compan V, Dumuis A. 2004. 5-HT<sub>4</sub> receptors. *Curr Drug Targets CNS Neurol Disord* 3(1):39–51.
- Bockaert J, Claeysen S, Compan V, Dumuis A. 2008. 5-HT<sub>4</sub> receptors: history, molecular pharmacology and brain functions. *Neuropharmacology* 55(6):922–31.
- Bockaert J, Sebben M, Dumuis A. 1990. Pharmacological characterization of 5-hydroxytryptamine 4 (5-HT<sub>4</sub>) receptors positively coupled to adenylate cyclase in adult guinea pig hippocampal membranes: effect of substituted benzamide derivatives. *Mol Pharmacol* 37(3):408–11.
- Bohn LM, Schmid CL. 2010. Serotonin receptor signaling and regulation via beta-arrestins. *Crit Rev Biochem Mol Biol* 45(6):555–66.
- Boldrini M, Underwood MD, Mann JJ, Arango V. 2008. Serotonin-1A autoreceptor binding in the dorsal raphe nucleus of depressed suicides. *J Psychiatr Res* 42(6):433–42.
- Burghardt NS, Bauer EP. 2013. Acute and chronic effects of selective serotonin reuptake inhibitor treatment on fear conditioning: implications for underlying fear circuits. *Neuroscience* 247:253–72.
- Cai X, Flores-Hernandez J, Feng J, Yan Z. 2002. Activity-dependent bidirectional regulation of GABA(A) receptor channels by the 5-HT<sub>4</sub> receptor-mediated signalling in rat prefrontal cortical pyramidal neurons. *J Physiol* 540(Pt 3):743–59.
- Castren E. 2014. Neurotrophins and psychiatric disorders. *Handb Exp Pharmacol* 220:461–79.
- Chen A, Kelley LD, Janusonis S. 2012. Effects of prenatal stress and monoaminergic perturbations on the expression of serotonin 5-HT<sub>4</sub> and adrenergic  $\beta$ <sub>2</sub> receptors in the embryonic mouse telencephalon. *Brain Res* 1459:27–34.
- Claeysen S, Sebben M, Becamel C, Bockaert J, Dumuis A. 1999. Novel brain-specific 5-HT<sub>4</sub> receptor splice variants show marked constitutive activity: role of the C-terminal intracellular domain. *Mol Pharmacol* 55(5):910–20.
- Claeysen S, Sebben M, Journot L, Bockaert J, Dumuis A. 1996. Cloning, expression and pharmacology of the mouse 5-HT<sub>4L</sub> receptor. *FEBS Lett* 398(1):19–25.
- Compan V, Daszuta A, Salin P, Sebben M, Bockaert J, Dumuis A. 1996. Lesion study of the distribution of serotonin 5-HT<sub>4</sub> receptors in rat basal ganglia and hippocampus. *Eur J Neurosci* 8(12):2591–8.
- Compan V, Zhou M, Grailhe R, Gazzara RA, Martin R, Gingrich J, and others. 2004. Attenuated response to stress and novelty and hypersensitivity to seizures in 5-HT<sub>4</sub> receptor knock-out mice. *J Neurosci* 24(2):412–9.
- Costall B, Naylor RJ. 1997. The influence of 5-HT<sub>2</sub> and 5-HT<sub>4</sub> receptor antagonists to modify drug induced disinhibitory effects in the mouse light/dark test. *Br J Pharmacol* 122(6):1105–18.
- Costall B, Naylor RJ, Tuladhar BR. 1993. 5-HT<sub>4</sub> receptor mediated facilitation of the emptying phase of the peristaltic reflex in the guinea-pig isolated ileum. *Br J Pharmacol* 110(4):1572–8.
- Cryan JF, Lucki I. 2000. 5-HT<sub>4</sub> receptors do not mediate the antidepressant-like behavioral effects of fluoxetine in a modified forced swim test. *Eur J Pharmacol* 409(3):295–9.
- Czachura JF, Rasmussen K. 2000. Effects of acute and chronic administration of fluoxetine on the activity of serotonergic neurons in the dorsal raphe nucleus of the rat. *Naunyn Schmiedeberg Arch Pharmacol* 362(3):266–75.
- David DJ, Samuels BA, Rainer Q, Wang JW, Marsteller D, Mendez I, and others. 2009. Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. *Neuron* 62(4):479–93.

- Descarries L, Riad M. 2012. Effects of the antidepressant fluoxetine on the subcellular localization of 5-HT<sub>1A</sub> receptors and SERT. *Philos Trans R Soc Lond B Biol Sci* 367(1601):2416–25.
- Drevets WC, Thase ME, Moses-Kolko EL, Price J, Frank E, Kupfer DJ, and others. 2007. Serotonin-1A receptor imaging in recurrent depression: replication and literature review. *Nucl Med Biol* 34(7):865–77.
- Duman RS, Malberg J, Nakagawa S. 2001. Regulation of adult neurogenesis by psychotropic drugs and stress. *J Pharmacol Exp Ther* 299(2):401–7.
- Duman RS, Malberg J, Nakagawa S, D'Sa C. 2000. Neuronal plasticity and survival in mood disorders. *Biol Psychiatry* 48(8):732–9.
- Duman RS, Monteggia LM. 2006. A neurotrophic model for stress-related mood disorders. *Biol Psychiatry* 59(12):1116–27.
- Dumuis A, Bouhelal R, Sebben M, Bockaert J. 1988. A 5-HT receptor in the central nervous system, positively coupled with adenylate cyclase, is antagonized by ICS 205 930. *Eur J Pharmacol* 146(1):187–8.
- Dumuis A, Sebben M, Bockaert J. 1989. BRL 24924: a potent agonist at a non-classical 5-HT receptor positively coupled with adenylate cyclase in colliculi neurons. *Eur J Pharmacol* 162(2):381–4.
- Egeland M, Warner-Schmidt J, Greengard P, Svenningsson P. 2011. Co-expression of serotonin 5-HT<sub>1B</sub> and 5-HT<sub>4</sub> receptors in p11 containing cells in cerebral cortex, hippocampus, caudate-putamen and cerebellum. *Neuropharmacology* 61(3):442–50.
- Eglen RM, Cornett CM, Whiting RL. 1990. Interaction of p-F-HHSiD (*p*-fluoro-hexahydrosila-difenidol) at muscarinic receptors in guinea-pig trachea. *Naunyn Schmiedebergs Arch Pharmacol* 342(4):394–9.
- Eglen RM, Wong EH, Dumuis A, Bockaert J. 1995. Central 5-HT<sub>4</sub> receptors. *Trends Pharmacol Sci* 16(11):391–8.
- Fakra E, Hyde LW, Gorka A, Fisher PM, Munoz KE, Kimak M, and others. 2009. Effects of HTR1A C(-1019)G on amygdala reactivity and trait anxiety. *Arch Gen Psychiatry* 66(1):33–40.
- Fargin A, Raymond JR, Lohse MJ, Kobilka BK, Caron MG, Lefkowitz RJ. 1988. The genomic clone G-21 which resembles a  $\beta$ -adrenergic receptor sequence encodes the 5-HT<sub>1A</sub> receptor. *Nature* 335(6188):358–60.
- Ferrari AJ, Charlson FJ, Norman RE, Patten SB, Freedman G, Murray CJ, and others. 2013. Burden of depressive disorders by country, sex, age, and year: findings from the global burden of disease study 2010. *PLoS Med* 10(11):e1001547.
- Gaddum JH, Picarelli ZP. 1957. Two kinds of tryptamine receptor. *Br J Pharmacol* 12(3):323–8.
- Garcia-Garcia AL, Newman-Tancredi A, Leonardo ED. 2014. 5-HT<sub>1A</sub> [corrected] receptors in mood and anxiety: recent insights into autoreceptor versus heteroreceptor function. *Psychopharmacology (Berl)* 231(4):623–36.
- Gardier AM, Malagie I, Trillat AC, Jacquot C, Artigas F. 1996. Role of 5-HT<sub>1A</sub> autoreceptors in the mechanism of action of serotonergic antidepressant drugs: recent findings from in vivo microdialysis studies. *Fundam Clin Pharmacol* 10(1):16–27.
- Gerald C, Adham N, Kao HT, Olsen MA, Laz TM, Schechter LE, and others. 1995. The 5-HT<sub>4</sub> receptor: molecular cloning and pharmacological characterization of two splice variants. *EMBO J* 14(12):2806–15.
- Gomez-Lazaro E, Garmendia L, Beitia G, Perez-Tejada J, Azpiroz A, Arregi A. 2012. Effects of a putative antidepressant with a rapid onset of action in defeated mice with different coping strategies. *Prog Neuropsychopharmacol Biol Psychiatry* 38(2):317–27.
- Grabiec M, Turlejski K, Djavadian RL. 2009. The partial 5-HT<sub>1A</sub> receptor agonist buspirone enhances neurogenesis in the opossum (*Monodelphis domestica*). *Eur Neuropsychopharmacol* 19(6):431–9.
- Griebel G. 1995. 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. *Pharmacol Ther* 65(3):319–95.
- Gross C, Zhuang X, Stark K, Ramboz S, Oosting R, Kirby L, and others. 2002. Serotonin<sub>1A</sub> receptor acts during development to establish normal anxiety-like behaviour in the adult. *Nature* 416(6879):396–400.
- Grossman CJ, Kilpatrick GJ, Bunce KT. 1993. Development of a radioligand binding assay for 5-HT<sub>4</sub> receptors in guinea-pig and rat brain. *Br J Pharmacol* 109(3):618–24.
- Guilloux JP, David DJ, Guiard BP, Chenu F, Reperant C, Toth M, and others. 2006. Blockade of 5-HT<sub>1A</sub> receptors by ( $\pm$ )-pindolol potentiates cortical 5-HT outflow, but not antidepressant-like activity of paroxetine: microdialysis and behavioral approaches in 5-HT<sub>1A</sub> receptor knockout mice. *Neuropsychopharmacology* 31(10):2162–72.
- Hannon J, Hoyer D. 2008. Molecular biology of 5-HT receptors. *Behav Brain Res* 195(1):198–213.
- Hegde SS, Eglen RM. 1996. Peripheral 5-HT<sub>4</sub> receptors. *FASEB J* 10(12):1398–407.
- Heisler LK, Chu HM, Brennan TJ, Danao JA, Bajwa P, Parsons LH, and others. 1998. Elevated anxiety and antidepressant-like responses in serotonin 5-HT<sub>1A</sub> receptor mutant mice. *Proc Natl Acad Sci U S A* 95(25):15049–54.
- Holick KA, Lee DC, Hen R, Dulawa SC. 2008. Behavioral effects of chronic fluoxetine in BALB/cJ mice do not require adult hippocampal neurogenesis or the serotonin 1A receptor. *Neuropsychopharmacology* 33(2):406–17.
- Ishizuka T, Goshima H, Ozawa A, Watanabe Y. 2014. Stimulation of 5-HT<sub>4</sub> receptor enhances differentiation of mouse induced pluripotent stem cells into neural progenitor cells. *Clin Exp Pharmacol Physiol* 41(5):345–50.
- Jacobs BL, van Praag H, Gage FH. 2000. Adult brain neurogenesis and psychiatry: a novel theory of depression. *Mol Psychiatry* 5(3):262–9.
- Jean A, Laurent L, Bockaert J, Charnay Y, Dusticier N, Nieoullon A, and others. 2012. The nucleus accumbens 5-HTR(4)-CART pathway ties anorexia to hyperactivity. *Transl Psychiatry* 2:e203.
- Kaufman J, Charney D. 2000. Comorbidity of mood and anxiety disorders. *Depress Anxiety* 12(Suppl 1):69–76.
- Kennett GA, Bright F, Trail B, Blackburn TP, Sanger GJ. 1997. Anxiolytic-like actions of the selective 5-HT<sub>4</sub> receptor antagonists SB 204070A and SB 207266A in rats. *Neuropharmacology* 36(4–5):707–12.
- Kessler RC, Chiu WT, Demler O, Merikangas KR, Walters EE. 2005. Prevalence, severity, and comorbidity of 12-month

- DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry* 62(6):617–27.
- King MV, Marsden CA, Fone KC. 2008. A role for the 5-HT<sub>1A</sub>, 5-HT<sub>4</sub> and 5-HT<sub>6</sub> receptors in learning and memory. *Trends Pharmacol Sci* 29(9):482–92.
- Klemenhagen KC, Gordon JA, David DJ, Hen R, Gross CT. 2006. Increased fear response to contextual cues in mice lacking the 5-HT<sub>1A</sub> receptor. *Neuropsychopharmacology* 31(1):101–11.
- Klempin F, Babu H, De Pietri Tonelli D, Alarcon E, Fabel K, Kempermann G. 2010. Oppositional effects of serotonin receptors 5-HT<sub>1a</sub>, 2, and 2c in the regulation of adult hippocampal neurogenesis. *Front Mol Neurosci* 3:Article 14.
- Kobayashi K, Ikeda Y, Sakai A, Yamasaki N, Haneda E, Miyakawa T, and others. 2010. Reversal of hippocampal neuronal maturation by serotonergic antidepressants. *Proc Natl Acad Sci U S A* 107(18):8434–9.
- Kobilka BK, Frielle T, Collins S, Yang-Feng T, Kobilka TS, Francke U, and others. 1987. An intronless gene encoding a potential member of the family of receptors coupled to guanine nucleotide regulatory proteins. *Nature* 329(6134):75–9.
- Kreiss DS, Lucki I. 1997. Chronic administration of the 5-HT<sub>1A</sub> receptor agonist 8-OH-DPAT differentially desensitizes 5-HT<sub>1A</sub> autoreceptors of the dorsal and median raphe nuclei. *Synapse* 25(2):107–16.
- Le Francois B, Czesak M, Steubl D, Albert PR. 2008. Transcriptional regulation at a HTR1A polymorphism associated with mental illness. *Neuropharmacology* 55(6):977–85.
- Le Poul E, Laaris N, Doucet E, Laporte AM, Hamon M, Lanfumey L. 1995. Early desensitization of somato-dendritic 5-HT<sub>1A</sub> autoreceptors in rats treated with fluoxetine or paroxetine. *Naunyn Schmiedebergs Arch Pharmacol* 352(2):141–8.
- Lemondé S, Turecki G, Bakish D, Du L, Hrdina PD, Bown CD, and others. 2003. Impaired repression at a 5-hydroxytryptamine 1A receptor gene polymorphism associated with major depression and suicide. *J Neurosci* 23(25):8788–99.
- Lesch KP, Gutknecht L. 2004. Focus on The 5-HT<sub>1A</sub> receptor: emerging role of a gene regulatory variant in psychopathology and pharmacogenetics. *Int J Neuropsychopharmacol* 7(4):381–5.
- Li Q, Muma NA, van de Kar LD. 1996. Chronic fluoxetine induces a gradual desensitization of 5-HT<sub>1A</sub> receptors: reductions in hypothalamic and midbrain Gi and G(o) proteins and in neuroendocrine responses to a 5-HT<sub>1A</sub> agonist. *J Pharmacol Exp Ther* 279(2):1035–42.
- Licht CL, Kirkegaard L, Zueger M, Chourbaji S, Gass P, Aznar S, and others. 2010. Changes in 5-HT<sub>4</sub> receptor and 5-HT transporter binding in olfactory bulbectomized and glucocorticoid receptor heterozygous mice. *Neurochem Int* 56(4):603–10.
- Licht CL, Marcussen AB, Wegener G, Overstreet DH, Aznar S, Knudsen GM. 2009. The brain 5-HT<sub>4</sub> receptor binding is down-regulated in the Flinders Sensitive Line depression model and in response to paroxetine administration. *J Neurochem* 109(5):1363–74.
- Liu MT, Kuan YH, Wang J, Hen R, Gershon MD. 2009. 5-HT<sub>4</sub> receptor-mediated neuroprotection and neurogenesis in the enteric nervous system of adult mice. *J Neurosci* 29(31):9683–99.
- Lucas G. 2009. Serotonin receptors, type 4: a new hope? *Curr Drug Targets* 10(11):1085–95.
- Lucas G, Compan V, Charnay Y, Neve RL, Nestler EJ, Bockaert J, and others. 2005. Frontocortical 5-HT<sub>4</sub> receptors exert positive feedback on serotonergic activity: viral transfections, subacute and chronic treatments with 5-HT<sub>4</sub> agonists. *Biol Psychiatry* 57(8):918–25.
- Lucas G, Rymar VV, Du J, Mnie-Filali O, Bisgaard C, Manta S, and others. 2007. Serotonin(4) (5-HT(4)) receptor agonists are putative antidepressants with a rapid onset of action. *Neuron* 55(5):712–25.
- Lucki I. 1991. Behavioral studies of serotonin receptor agonists as antidepressant drugs. *J Clin Psychiatry* 52(Suppl):24–31.
- Macdonald RL, Olsen RW. 1994. GABAA receptor channels. *Annu Rev Neurosci* 17:569–602.
- Malberg JE, Eisch AJ, Nestler EJ, Duman RS. 2000. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J Neurosci* 20(24):9104–10.
- Mannoury la Cour C, El Mestikawy S, Hanoun N, Hamon M, Lanfumey L. 2006. Regional differences in the coupling of 5-hydroxytryptamine-1A receptors to G proteins in the rat brain. *Mol Pharmacol* 70(3):1013–21.
- Marin P, Becamel C, Dumuis A, Bockaert J. 2012. 5-HT receptor-associated protein networks: new targets for drug discovery in psychiatric disorders? *Curr Drug Targets* 13(1):28–52.
- Mayorga AJ, Dalvi A, Page ME, Zimov-Levinson S, Hen R, Lucki I. 2001. Antidepressant-like behavioral effects in 5-hydroxytryptamine(1A) and 5-hydroxytryptamine(1B) receptor mutant mice. *J Pharmacol Exp Ther* 298(3):1101–7.
- McAskill R, Mir S, Taylor D. 1998. Pindolol augmentation of antidepressant therapy. *Br J Psychiatry* 173:203–8.
- Meltzer CC, Price JC, Mathis CA, Butters MA, Ziolkowski SK, Moses-Kolko E, and others. 2004. Serotonin 1A receptor binding and treatment response in late-life depression. *Neuropsychopharmacology* 29(12):2258–65.
- Mendez-David I, David DJ, Darcey F, Wu MV, Kerdine-Romer S, Gardier AM, and others. 2014. Rapid anxiolytic effects of a 5-HT(4) receptor agonist are mediated by a neurogenesis-independent mechanism. *Neuropsychopharmacology* 39(6):1366–78.
- Middlemiss DN, Price GW, Watson JM. 2002. Serotonergic targets in depression. *Curr Opin Pharmacol* 2(1):18–22.
- Ming GL, Song H. 2005. Adult neurogenesis in the mammalian central nervous system. *Annu Rev Neurosci* 28:223–50.
- Mnie-Filali O, Pineyro G. 2012. Desensitization and internalization mechanisms of the 5-HT<sub>4</sub> receptors. *Wiley Interdisciplin Rev Membrane Transp Signal* 1(6):779–88.
- Mori M, Murata Y, Matsuo A, Tomoyo T, Mine K. 2014. Chronic treatment with the 5-HT<sub>1A</sub> receptor partial agonist tandospirone increases hippocampal neurogenesis. *Neurother* 3(1):67–77.
- Nestler EJ, Gould E, Manji H, Bunce M, Duman RS, Greshenfeld HK, and others. 2002. Preclinical models: status of basic research in depression. *Biol Psychiatry* 52(6):503–28.

- Nibuya M, Morinobu S, Duman RS. 1995. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J Neurosci* 15(11):7539–47.
- Nibuya M, Nestler EJ, Duman RS. 1996. Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. *J Neurosci* 16(7):2365–72.
- Ohtsuki T, Ishiguro H, Detera-Wadleigh SD, Toyota T, Shimizu H, Yamada K, and others. 2002. Association between serotonin 4 receptor gene polymorphisms and bipolar disorder in Japanese case-control samples and the NIMH Genetics Initiative Bipolar Pedigrees. *Mol Psychiatry* 7(9):954–61.
- Parks CL, Robinson PS, Sibille E, Shenk T, Toth M. 1998. Increased anxiety of mice lacking the serotonin<sub>1A</sub> receptor. *Proc Natl Acad Sci U S A* 95(18):10734–9.
- Parsey RV, Ogden RT, Miller JM, Tin A, Hesselgrave N, Goldstein E, and others. 2010. Higher serotonin 1A binding in a second major depression cohort: modeling and reference region considerations. *Biol Psychiatry* 68(2):170–8.
- Parsey RV, Olvet DM, Oquendo MA, Huang YY, Ogden RT, Mann JJ. 2006a. Higher 5-HT<sub>1A</sub> receptor binding potential during a major depressive episode predicts poor treatment response: preliminary data from a naturalistic study. *Neuropsychopharmacology* 31(8):1745–9.
- Parsey RV, Oquendo MA, Ogden RT, Olvet DM, Simpson N, Huang YY, and others. 2006b. Altered serotonin 1A binding in major depression: a [carbonyl-<sup>11</sup>C]WAY100635 positron emission tomography study. *Biol Psychiatry* 59(2):106–13.
- Pascual-Brazo J, Castro E, Diaz A, Valdizan EM, Pilar-Cuellar F, Vidal R, and others. 2012. Modulation of neuroplasticity pathways and antidepressant-like behavioural responses following the short-term (3 and 7 days) administration of the 5-HT<sub>4</sub> receptor agonist RS67333. *Int J Neuropsychopharmacol* 15(5):631–43.
- Pindon A, van Hecke G, van Gompel P, Lesage AS, Leysen JE, Jurzak M. 2002. Differences in signal transduction of two 5-HT<sub>4</sub> receptor splice variants: compound specificity and dual coupling with G $\alpha$ s- and G $\alpha$ i/o-proteins. *Mol Pharmacol* 61(1):85–96.
- Polter AM, Li X. 2010. 5-HT<sub>1A</sub> receptor-regulated signal transduction pathways in brain. *Cell Signal* 22(10):1406–12.
- Radley JJ, Jacobs BL. 2002. 5-HT<sub>1A</sub> receptor antagonist administration decreases cell proliferation in the dentate gyrus. *Brain Res* 955(1-2):264–7.
- Ramboz S, Oosting R, Amara DA, Kung HF, Blier P, Mendelsohn M, and others. 1998. Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. *Proc Natl Acad Sci U S A* 95(24):14476–81.
- Richardson-Jones JW, Craige CP, Guiard BP, Stephen A, Metzger KL, Kung HF, and others. 2010. 5-HT<sub>1A</sub> autoreceptor levels determine vulnerability to stress and response to antidepressants. *Neuron* 65(1):40–52.
- Richardson-Jones JW, Craige CP, Nguyen TH, Kung HF, Gardier AM, Dranovsky A, and others. 2011. Serotonin-1A autoreceptors are necessary and sufficient for the normal formation of circuits underlying innate anxiety. *J Neurosci* 31(16):6008–18.
- Ridder S, Chourbaji S, Hellweg R, Urani A, Zacher C, Schmid W, and others. 2005. Mice with genetically altered glucocorticoid receptor expression show altered sensitivity for stress-induced depressive reactions. *J Neurosci* 25(26):6243–50.
- Rosel P, Arranz B, Urretavizcaya M, Oros M, San L, Navarro MA. 2004. Altered 5-HT<sub>2A</sub> and 5-HT<sub>4</sub> postsynaptic receptors and their intracellular signalling systems IP<sub>3</sub> and cAMP in brains from depressed violent suicide victims. *Neuropsychobiology* 49(4):189–95.
- Roychowdhury S, Haas H, Anderson EG. 1994. 5-HT<sub>1A</sub> and 5-HT<sub>4</sub> receptor colocalization on hippocampal pyramidal cells. *Neuropharmacology* 33(3–4):551–7.
- Sahay A, Drew MR, Hen R. 2007. Dentate gyrus neurogenesis and depression. *Prog Brain Res* 163:697–722.
- Samuels BA, Hen R. 2011. Neurogenesis and affective disorders. *Eur J Neurosci* 33(6):1152–9.
- Samuels BA, Leonardo ED, Gadiant R, Williams A, Zhou J, David DJ, and others. 2011. Modeling treatment-resistant depression. *Neuropharmacology* 61(3):408–13.
- Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, and others. 2003. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* 301(5634):805–9.
- Sapolsky R. 2000. It's not 'all in the genes'. The environment you grow up in is as important as your DNA in determining the person you ultimately become. *Newsweek* 135(15):68.
- Schatzberg AF, Nemeroff CB. 2009. *The American Psychiatric Publishing textbook of psychopharmacology*. Washington, DC: American Psychiatric Publishing.
- Schmidt EF, Warner-Schmidt JL, Otopalik BG, Pickett SB, Greengard P, Heintz N. 2012. Identification of the cortical neurons that mediate antidepressant responses. *Cell* 149(5):1152–63.
- Schreiber R., Melon C., and De Vry J. (1998). The role of 5-HT receptor subtypes in the anxiolytic effects of selective serotonin reuptake inhibitors in the rat ultrasonic vocalization test. *Psychopharmacology (Berl)* 135, 383–391.
- Silvestre JS, Fernandez AG, Palacios JM. 1996. Effects of 5-HT<sub>4</sub> receptor antagonists on rat behaviour in the elevated plus-maze test. *Eur J Pharmacol* 309(3):219–22.
- Spalding KL, Bergmann O, Alkass K, Bernard S, Salehpour M, Huttner HB, and others. 2013. Dynamics of hippocampal neurogenesis in adult humans. *Cell* 153(6):1219–27.
- Stockmeier CA, Shapiro LA, Dilley GE, Kolli TN, Friedman L, Rajkowska G. 1998. Increase in serotonin-1A autoreceptors in the midbrain of suicide victims with major depression-postmortem evidence for decreased serotonin activity. *J Neurosci* 18(18):7394–401.
- Strobel A, Gutknecht L, Rothe C, Reif A, Mossner R, Zeng Y, and others. 2003. Allelic variation in 5-HT<sub>1A</sub> receptor expression is associated with anxiety- and depression-related personality traits. *J Neural Transm* 110(12):1445–53.
- Surget A, Saxe M, Leman S, Iburguen-Vargas Y, Chalon S, Griebel G, and others. 2008. Drug-dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal. *Biol Psychiatry* 64(4):293–301.
- Tamburella A, Micale V, Navarria A, Drago F. 2009. Antidepressant properties of the 5-HT<sub>4</sub> receptor partial

- agonist, SL65.0155: behavioral and neurochemical studies in rats. *Prog Neuropsychopharmacol Biol Psychiatry* 33(7):1205–10.
- Tanaka KF, Samuels BA, Hen R. 2012. Serotonin receptor expression along the dorsal-ventral axis of mouse hippocampus. *Philos Trans R Soc Lond B Biol Sci* 367(1601):2395–401.
- Torres GE, Chaput Y, Andrade R. 1995. Cyclic AMP and protein kinase A mediate 5-hydroxytryptamine type 4 receptor regulation of calcium-activated potassium current in adult hippocampal neurons. *Mol Pharmacol* 47(1):191–7.
- Tunnicliff G. 1991. Molecular basis of buspirone's anxiolytic action. *Pharmacol Toxicol* 69(3):149–56.
- Vidal R, Valdizan EM, Vilaro MT, Pazos A, Castro E. 2010. Reduced signal transduction by 5-HT<sub>4</sub> receptors after long-term venlafaxine treatment in rats. *Br J Pharmacol* 161(3):695–706.
- Vilaro MT, Cortes R, Gerald C, Branchek TA, Palacios JM, Mengod G. 1996. Localization of 5-HT<sub>4</sub> receptor mRNA in rat brain by in situ hybridization histochemistry. *Brain Res Mol Brain Res* 43(1–2):356–60.
- Vilaro MT, Cortes R, Mengod G. 2005. Serotonin 5-HT<sub>4</sub> receptors and their mRNAs in rat and guinea pig brain: distribution and effects of neurotoxic lesions. *J Comp Neurol* 484(4):418–39.
- Vilaro MT, Domenech T, Palacios JM, Mengod G. 2002. Cloning and characterization of a novel human 5-HT<sub>4</sub> receptor variant that lacks the alternatively spliced carboxy terminal exon. RT-PCR distribution in human brain and periphery of multiple 5-HT<sub>4</sub> receptor variants. *Neuropharmacology* 42(1):60–73.
- Waeber C, Sebben M, Grossman C, Javoy-Agid F, Bockaert J, Dumuis A. 1993. [<sup>3</sup>H]-GR113808 labels 5-HT<sub>4</sub> receptors in the human and guinea-pig brain. *Neuroreport* 4(11):1239–42.
- Waeber C, Sebben M, Nicoullon A, Bockaert J, Dumuis A. 1994. Regional distribution and ontogeny of 5-HT<sub>4</sub> binding sites in rodent brain. *Neuropharmacology* 33(3–4):527–41.
- Wang JW, David DJ, Monckton JE, Battaglia F, Hen R. 2008. Chronic fluoxetine stimulates maturation and synaptic plasticity of adult-born hippocampal granule cells. *J Neurosci* 28(6):1374–84.
- Warden D, Rush AJ, Trivedi MH, Fava M, Wisniewski SR. 2007. The STAR\*D Project results: a comprehensive review of findings. *Curr Psychiatry Rep* 9(6):449–59.
- Warner-Schmidt JL, Flajolet M, Maller A, Chen EY, Qi H, Svenningsson P, and others. 2009. Role of p11 in cellular and behavioral effects of 5-HT<sub>4</sub> receptor stimulation. *J Neurosci* 29(6):1937–46.
- World Health Organization. 2011. World health statistics 2011. Geneva, Switzerland: World Health Organization.