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Regional Distribution of Serotonergic Receptors: A Systems Neuroscience Perspective on the Downstream Effects of the Multimodal-Acting Antidepressant Vortioxetine on Excitatory and Inhibitory Neurotransmission

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REVIEW ARTICLE

Regional distribution of serotonergic receptors: a systems neuroscience perspective on the downstream effects of the multimodal-acting antidepressant vortioxetine on excitatory and inhibitory neurotransmission

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Previous work from this laboratory hypothesized that the multimodal antidepressant vortioxetine enhances cognitive function through a complex mechanism, using serotonergic (5-hydroxytryptamine, 5-HT) receptor actions to modulate gamma-butyric acid (GABA) and glutamate neurotransmission in key brain regions like the prefrontal cortex (PFC) and hippocampus. However, serotonergic receptors have circumscribed expression patterns, and therefore vortioxetine's effects on GABA and glutamate neurotransmission will probably be regionally selective. In this article, we attempt to develop a conceptual framework in which the effects of 5-HT, selective serotonin reuptake inhibitors (SSRIs), and vortioxetine on GABA and glutamate neurotransmission can be understood in the PFC and striatum–2 regions with roles in cognition and substantially different 5-HT receptor expression patterns. Thus, we review the anatomy of the neuronal microcircuitry in the PFC and striatum, anatomical data on 5-HT receptor expression within these microcircuits, and electrophysiological evidence on the effects of 5-HT on the behavior of each cell type. This analysis suggests that 5-HT and SSRIs will have markedly different effects within the PFC, where they will induce mixed effects on GABA and glutamate neurotransmission, compared to the striatum, where they will enhance GABAergic interneuron activity and drive down the activity of medium spiny neurons. Vortioxetine is expected to reduce GABAergic interneuron activity in the PFC and concomitantly increase cortical pyramidal neuron firing. However in the striatum, vortioxetine is expected to increase activity at GABAergic interneurons and have mixed excitatory and inhibitory effects in medium spiny neurons. Thus the conceptual framework developed here suggests that vortioxetine will have regionally selective effects on GABA and glutamate neurotransmission.

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Key words: 5-HT receptor, GABA, glutamate, Lu AA21004, vortioxetine.

Clinical Implications

- Serotonin neurotransmission can modulate the behavior of GABA and glutamate cells.
- Serotonin's effects on GABA and glutamate neurotransmission depend on regional and cellular receptor expression patterns, as well as the structure of local neuronal circuits.
- 5-HT, SSRI antidepressants, and the multimodal antidepressant vortioxetine have markedly different effects on excitatory and inhibitory neurotransmission in the prefrontal cortex and striatum.

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Introduction

Cognitive dysfunction is a common but poorly understood aspect of major depressive disorder (MDD). MDD patients commonly experience impairments in a broad

range of cognitive domains including attention, executive function, working memory, and psychomotor processing speed.[1] Although there are a number of relatively effective treatments for the mood symptoms associated with MDD, empirical data suggest that many patients continue to experience high levels of functional disability despite improvements in depression symptoms.[1] Furthermore, neurocognitive impairment predicts functional outcomes in MDD patients.[2] Therefore, developing treatments capable of normalizing cognitive function in MDD patients is an important goal for restoring normal functional ability.

Vortioxetine is an antidepressant that recently has been approved for treatment of MDD. In 3 double-blind, randomized, placebo-controlled clinical studies, vortioxetine has shown positive effects on predefined cognition outcome measures in MDD patients with cognitive dysfunction, 2 of which included the active reference duloxetine.[3–5] Evidence from a large number of preclinical studies in rodents also supports a procognitive profile of vortioxetine and provides a mechanistic hypothesis for its pharmacological actions. Vortioxetine restored cognitive function across a range of rodent models of memory and cognitive flexibility applying cognitive disruptors such as time, serotonin (5-hydroxytryptamine; 5-HT) depletion, age, ovariectomy, N-methyl-D-aspartate (NMDA) receptor antagonism, and muscarinic cholinergic receptor antagonism.[6–12] Furthermore, in a quantitative electroencephalographic analysis in rats, vortioxetine dose-dependently increased cortical oscillatory power, suggesting that vortioxetine modulates cortical networks recruited during cognitive behaviors.[13]

Vortioxetine is thought to exert its pharmacological effects through a multimodal mechanism of action[14] that involves modulation of 5-HT receptors and inhibition of the serotonin transporter (SERT). *In vitro* studies in cellular assays show that vortioxetine is a 5-HT_3, 5-HT_7, and 5-HT_{1D} receptor antagonist; a 5-HT_{1B} receptor partial agonist; a 5-HT_{1A} receptor agonist; and a 5-HT reuptake inhibitor.[15] However, although the pharmacological effects of this drug are relatively well understood, the precise mechanism underlying vortioxetine's cognitive enhancing properties has yet to be fully elucidated. Although vortioxetine may indirectly modulate several neurotransmitter systems, previous work from this laboratory has advanced the hypothesis that vortioxetine enhances cognitive function by modulating gamma-aminobutyric acid (GABA) neurotransmission through its direct pharmacological actions on serotonergic receptors.[16] These changes in GABA neurotransmission induce downstream increases in glutamate neurotransmission and neuroplasticity in key brain areas, such as the hippocampus and prefrontal cortex. In support of this hypothesis, electrophysiology studies have shown that vortioxetine through 5-HT_3 receptor antagonism stimulated glutamatergic pyramidal neurons in rat hippocampus slices and in the medial prefrontal cortex of anesthetized rats.[17,18] The fact that 5-HT_3 receptors in the rat brain are almost exclusively expressed on GABA interneurons that exert inhibitory control over pyramidal neurons further supports the hypothesis.[19] Work is currently ongoing to assess to what extent other portions of vortioxetine's pharmacological profile play a role in its effects on GABA and glutamate neurotransmission.

An interesting corrollary to the idea that vortioxetine modulates GABA and glutamate neurotransmission through its pharmacological actions on 5-HT receptors is the idea that these effects may be regionally specific. Although serotonergic receptors are broadly expressed throughout the mammalian brain, the presence of individual receptor subtypes is regionally circumscribed. For example, simple visual inspection of autoradiographic maps of vortioxetine's receptor targets demonstrates that although each of vortioxetine's major pharmacological targets is expressed within the medial prefrontal cortex (mPFC; Figure 1), several of those receptor targets (notably 5-HT_{1A} and 5-HT_3 receptors) are not present or are present at very low levels within the striatal caudate and putamen subregions (CPu; Figure 2). Thus, the likelihood that vortioxetine will affect striatal neurotransmission in the same manner as it affects frontal cortex neurotransmission is small.

The goal of this article is to derive specific hypotheses about how vortioxetine will affect neural activity, with a particular focus on principle output cells, within the rodent mPFC and striatum. These brain regions were chosen because they play a role in aspects of cognitive function and have markedly different profiles of serotonergic receptor expression. Toward this end, we will discuss the functional organization of the microcircuits comprising the prefrontal cortex (PFC) and striatum, including a discussion of important neuronal subtypes and their relationships to one another. We will discuss the regional distribution of serotonergic receptor targets and the cell types expressing each target before evaluating the way that 5-HT modulates the function of the various cellular players within the circuit in question. Finally we will make predictions on how an SSRI or the multimodal antidepressant vortioxetine might modulate the function of the circuit before evaluating the electrophysiological data on these effects, where available.

The Prefrontal Cortex

Functional roles and circuit organization

The neocortex is a large structure that serves as an association region, integrating and performing

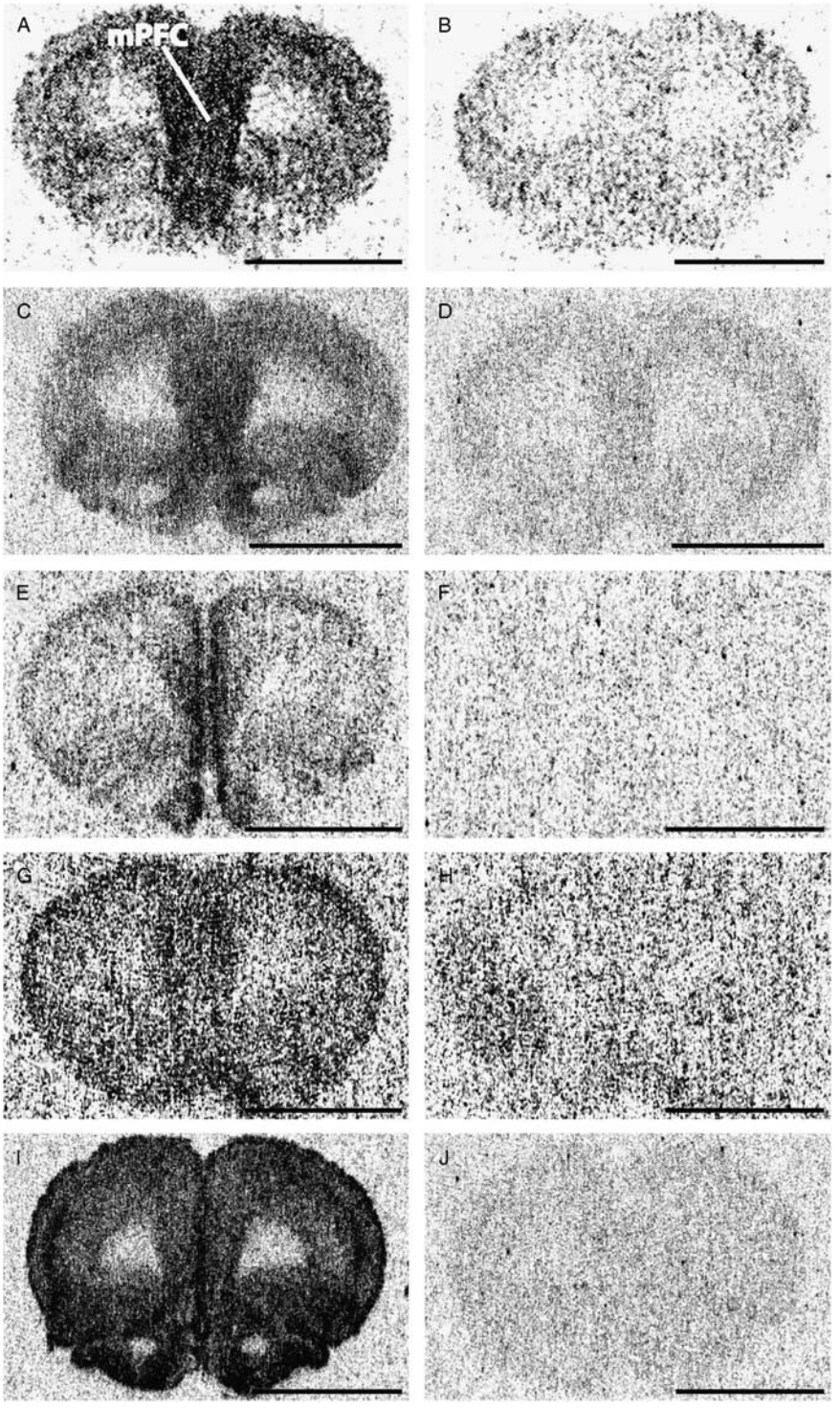

FIGURE 1. Expression pattern of serotonergic targets of the multimodal antidepressant vortioxetine within the rat prefrontal cortex. Autoradiographic images representing total (left panels) and nonspecific binding (right panels) for each of vortioxetine's separate serotonergic targets in coronal rat brain sections at approximately 2.7 mm anterior from Bregma (according to Paxinos and Watson[113]). 5-HT_{1A} receptors were mapped using 3nM [^{3}H]8OHDPAT alone (A) or in combination with 1 μM of the 5-HT_{1A} receptor selective antagonist WAY100635 to determine nonspecific binding (B). 5-$HT_{1B/1D}$ receptors were mapped using 1nM [^{3}H] GR125743 alone (C) or in combination with 1 μM of the 5-HT_{1B} receptor preferring antagonist SB216641 to determine nonspecific binding (D). 5-HT_3 receptors were mapped using 3nM [^{3}H] LY278584 alone (E) or in combination with 1 μM ondansetron to determine nonspecific binding (F). 5-HT_7 receptors were mapped using 4.5nM [^{3}H]SB269970 alone (G) or in combination with 1 μM of unlabeled SB269970 to determine nonspecific binding (H). Finally, SERT was mapped using 4.5 nM [^{3}H] escitalopram alone (I) or in combination with 1 μM paroxetine to determine nonspecific binding (J). Scale bars represent 5 mm. Abbreviations: mPFC – medial prefrontal cortex.

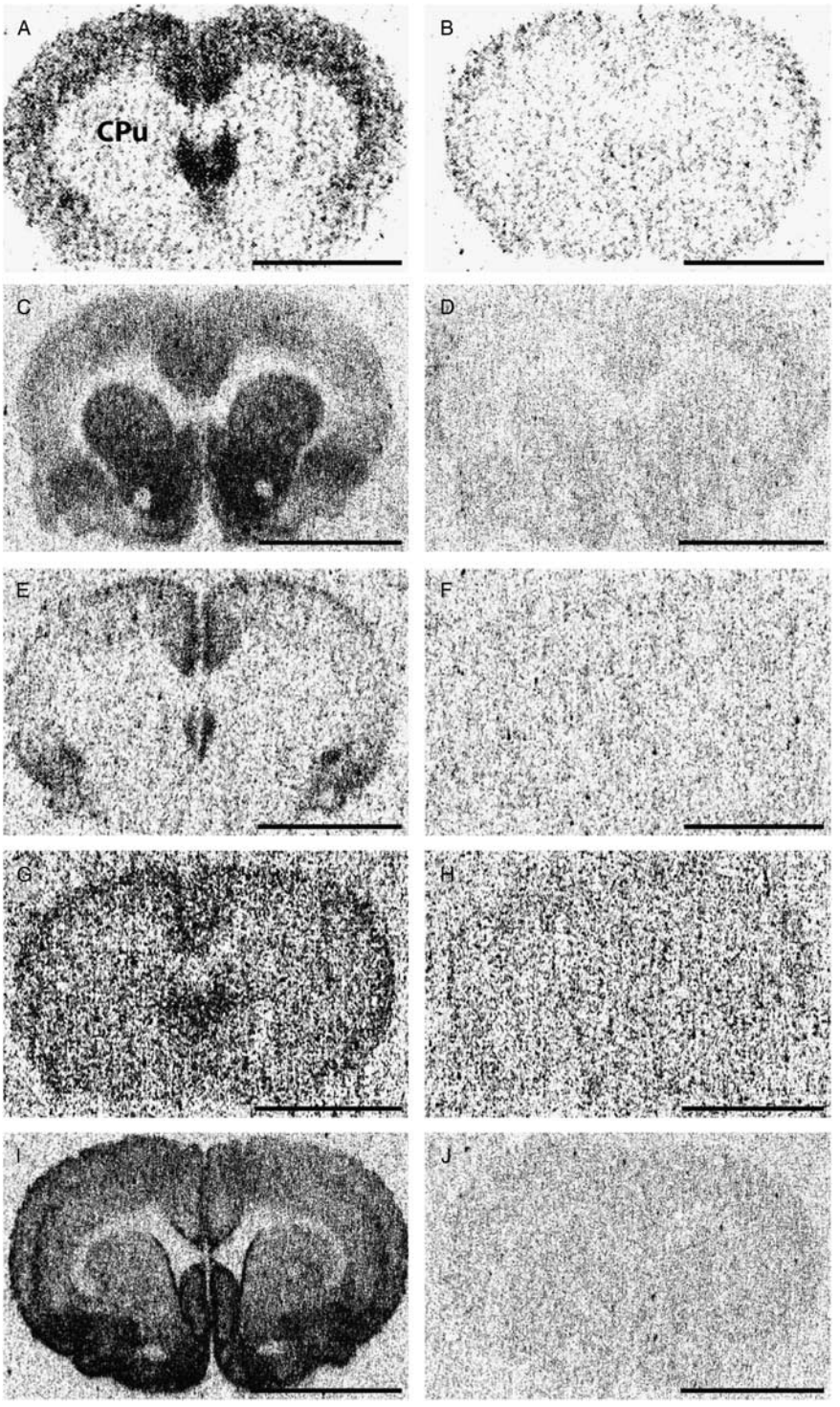

FIGURE 2. Expression pattern of serotonergic targets of the multimodal antidepressant vortioxetine within the rat striatum. Autoradiographic images representing total (left panels) and nonspecific binding (right panels) for each of vortioxetine's separate serotonergic targets in coronal rat brain sections at approximately 1.6 mm anterior from Bregma (according to Paxinos and Watson[113]). 5-HT_{1A} receptors were mapped using 3nM [^{3}H]8OHDPAT alone (A) or in combination with 1 μM of the 5-HT_{1A} receptor selective antagonist WAY100635 to determine nonspecific binding (B). 5-$HT_{1B/1D}$ receptors were mapped using 1nM [^{3}H] GR125743 alone (C) or in combination with 1 μM of the 5-HT_{1B} receptor preferring antagonist SB216641 to determine nonspecific binding (D). 5-HT_3 receptors were mapped using 3nM [^{3}H] LY278584 alone (E) or in combination with 1 μM ondansetron to determine nonspecific binding (F). 5-HT_7 receptors were mapped using 4.5nM [^{3}H]SB269970 alone (G) or in combination with 1 μM of unlabeled SB269970 to determine nonspecific binding (H). Finally, SERT was mapped using 4.5 nM [^{3}H] escitalopram alone (I) or in combination with 1 μM paroxetine to determine nonspecific binding (J). Scale bars represent 5 mm. Abbreviations: CPu – caudate/putamen.

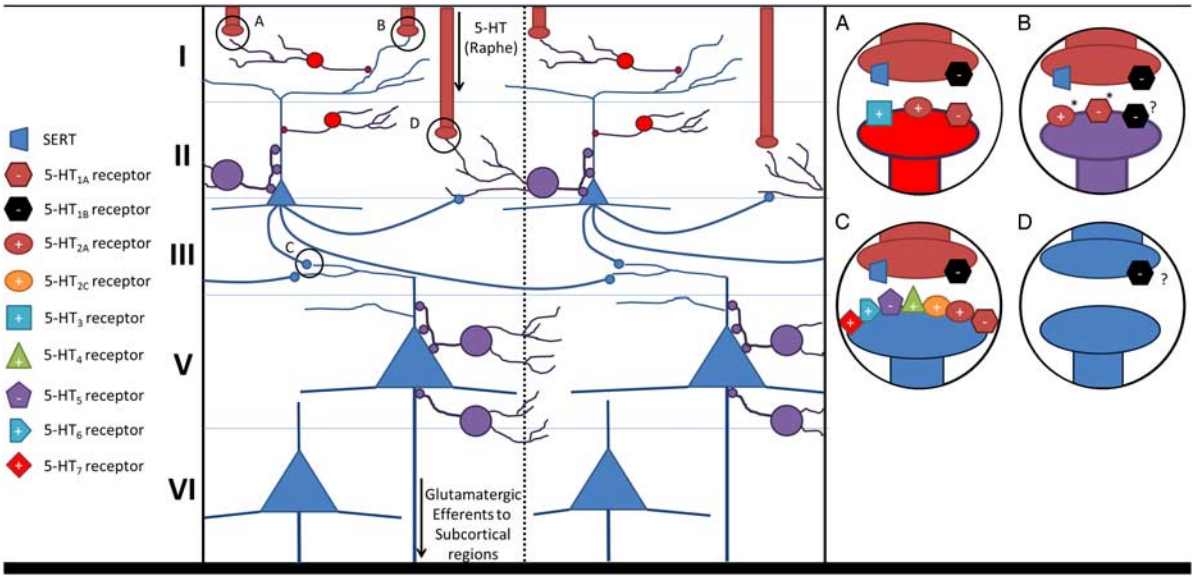

FIGURE 3. Serotonergic influence on a theoretical prefrontal cortex microcircuit. The influence of serotonin on the behavior of cortical pyramidal neurons is mediated by several different factors. Non-parvalbumin-immunoreactive interneurons (depicted in red) in the shallow cortical layers receive serotonergic inputs that have mixed excitatory and inhibitory effects mediated via 5-HT_{1A}, 5-HT_{2A}, and 5-HT_3 receptors (A) and have an inhibitory influence on pyramidal cell dendritic membrane polarization. Additionally, serotonin's effects on parvalbumin-immunoreactive interneurons (depicted in purple) are also probably a mix of excitatory and inhibitory actions that are mediated by 5-HT_{1A}, 5-HT_{2A}, and perhaps 5-HT_{1B} receptors (B). These cells exert a powerful inhibitory effect on pyramidal neuron activity via hyperpolarizing actions at the pyramidal neuron soma or axon hillock. Pyramidal neurons themselves (depicted in blue) also receive a mix of excitatory and inhibitory serotonergic signals, which are mediated by 5-HT_{1A}, 5-$HT_{2A/2C}$, 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7 receptors (C). Finally, glutamate release from pyramidal neuron terminals in the prefrontal cortex may be inhibited by 5-HT_{1B} receptor activation (D).

computations on information from numerous other brain regions. The PFC represents a specialized subset of the neocortex that mediates a variety of cognitive functions, including learning,[20] working memory,[21] attention,[22] and behavioral flexibility.[23]

The PFC is a complex structure where neurons are incorporated into a modular design featuring columns of neurons as a basic unit of information processing. Each column is organized into well-defined layers that feature distinct populations of cells or cellular features, which are depicted in Figure 3. As discussed in a recent review by Puig and Gulledege,[24] layer I is primarily a neuropil layer, featuring afferent terminals from a variety of sources and the cell bodies of regular spiking GABAergic interneurons and glia. Layers II and III contain the cell bodies of small pyramidal neurons and GABAergic interneurons characterized by either regular- or fast-spiking behavior. Layers V and VI contain the cell bodies of large pyramidal neurons as well as GABAergic interneurons primarily characterized by fast-spiking behavior. While an in-depth discussion of each cell type is outside the scope of this review, we will briefly discuss the role of these different cell types and their relationships to one another in the following section.

Important cortical cell types

Parvalbumin-immunoreactive (PV-IR) fast-spiking interneurons

One major category of cortical inhibitory interneuron can be identified by the expression of the calcium binding protein parvalbumin (PV). There are several subclasses of cortical inhibitory interneuron that express PV, which at least include a subset of basket cells and chandelier cells (for an excellent review, see Kubota[25]). PV-IR interneurons primarily have fast-spiking behavior, and thus for the purposes of this article, the terms "PV-IR interneuron" and "fast-spiking interneuron" will be used interchangeably. Importantly, each PV-IR interneuron subtype is thought to have a selective subcellular axonal target. PV-IR basket cells, which comprise approximately 40–50% of cortical non-pyramidal neurons, arborize at the soma and dendrites of pyramidal neurons, with each basket cell innervating between 200 and 1000 pyramidal cells.[25] PV-IR chandelier cells are a relatively small subset of PV-IR interneurons and synapse with pyramidal neurons almost exclusively at the axon hillock.[25] Given the placement of these axonal arborizations, cortical PV-IR interneurons can be expected to have a strong inhibitory effect on the output of pyramidal neurons. Interestingly, PV-IR interneuron subclasses

such as fast-spiking basket cells are thought to be connected to one another via electrical gap junctions,[25] which may allow these cell types to easily form neural assemblies that could multiply the inhibitory signal they represent within the frontal cortex network.

Non-PV-IR interneurons

In addition to PV-IR interneurons, there are a number of other GABAergic interneuron classes in the frontal cortex. These subtypes can be identified by various neurochemical markers, including calbindin, calretinin, cholecystokinin, vasoactive intestinal peptide, somatostatin, and neuropeptide Y. Each interneuron subtype has a distinctive morphology, cellular target, and role in modulating the activity of frontal cortical networks.[25] Furthermore, the electrical activity of these cells can have a regular-spiking, late-spiking, or bursting character. However, information on the placement of serotonergic targets on these cortical interneuron subtypes has generally not reached a high level of detail. Therefore, we have chosen to consider these various classes together as one group for the purposes of this article. The most important group of non-PV-IR interneurons to consider from the perspective of understanding serotonergic neurotransmission's effects on PFC activity is a set of interneurons in the shallow cortical layers (ie, layers I–III) that inhibits cortical pyramidal neuron activity.

Pyramidal neurons

Pyramidal neurons are excitatory glutamatergic cells that are characterized by a triangular-shaped soma, apical dendrites emanating from the soma's vertex, and basal dendrites as well as a single branching axon originating from the soma's base. Within the cortex, pyramidal neurons are thought to fill multiple functional roles. The small pyramidal neurons prevalent in layers II and III integrate subcortical and cortico-cortical afferent inputs and send axonal projections horizontally to layers II and III of neighboring cortical columns.[26,27] Additionally, layer II/III pyramidal neurons project axons vertically to layer V pyramidal neurons.[26,27] This combination of local projections drives lateral inhibitory effects on layer II/III pyramidal neurons from neighboring cortical columns, presumably by activating inhibitory interneurons, while also activating layer V pyramidal neurons both within the originating column and in neighboring columns.[26] Thus, activation of a given layer II/III pyramidal neuron may suppress inputs from neighboring columns while at the same time magnifying the output of its own column.

The pyramidal neurons of the deep cortical layers (ie, layers V and VI) have a large soma in comparison to layer II/III pyramidal neurons, and layer V pyramidal neurons are responsible for sending excitatory glutamatergic projections to subcortical regions, such as the striatum. Thus, the pyramidal neuron is considered the principle cortical projection neuron.

In order to understand how 5-HT modulates PFC microcircuit activity, it is necessary to understand which cells within the network express each receptor and their effect on neural activity. Therefore, the following section will review information on 5-HT receptor expression within the PFC.

Serotonergic receptor expression in the prefrontal cortex

5-HT$_{1A}$ receptors

5-HT$_{1A}$ receptors are inhibitory G-protein coupled receptors. Autoradiographic and immunohistochemical studies have demonstrated strong 5-HT$_{1A}$ receptor expression in some portions of the hippocampal formation (see the detailed review by Dale *et al*[28] elsewhere in this issue) and the brain stem raphe nuclei. 5-HT$_{1A}$ receptor expression within the PFC is moderate in strength (Figure 1, panels A and B), and has been demonstrated in all cortical layers with the exception of layer I.[29]

Aznar *et al*[29] investigated the cellular subtypes that express the 5-HT$_{1A}$ receptor in the rodent cortex, and found that the majority of pyramidal neurons, PV-IR interneurons, and some non-PV-IR interneurons express 5-HT$_{1A}$ receptors (approximately 85% or more in each case). However, it should be noted that other research groups have suggested lower estimates in PFC subregions, with approximately 60% of pyramidal neurons in the prelimbic, and 40% of those in the infralimbic subregion, expressing 5-HT$_{1A}$ receptor messenger ribonucleic acid (mRNA).[30] Approximately 20% of GABAergic interneurons in these PFC subregions express 5-HT$_{1A}$ receptors.[30]

On a subcellular level, 5-HT$_{1A}$ receptors are thought to be somatodendritically expressed, and in frontal cortex pyramidal neurons there is evidence supporting a placement on the axon hillock.[31]

Based on this pattern of expression, 5-HT$_{1A}$ receptors can be expected to have complex effects on the behavior of the frontal cortex. Activation of 5-HT$_{1A}$ receptors expressed on PV-IR interneurons would inhibit these cells, and thereby allow pyramidal neuron firing to increase via disinhibition. However, 5-HT$_{1A}$ receptor expression on pyramidal neurons themselves would reduce their activation state. Therefore, 5-HT$_{1A}$ receptor activation should have mixed effects on the behavior of the PFC microcircuit. Moreover, electrophysiological evidence suggesting that selective activation of 5-HT$_{1A}$ receptors leads to an increase in firing followed by a reduction for most pyramidal neurons[32] is consistent with this conceptual framework.

5-HT$_{1B}$ receptors

Within the frontal cortex, 5-HT$_{1B}$ receptors are expressed at low to moderate levels[33–35] (Figure 1, panels C and D) and may exist with a combination of presynaptic and postsynaptic expression patterns. The observation that 5-HT$_{1B}$ receptor stimulation reduces extracellular 5-HT concentrations elicited by SSRIs in the frontal cortex suggests the presence of 5-HT$_{1B}$ receptors on serotonergic terminals in this region.[36] Additionally, Tanaka and North[37] have suggested that 5-HT$_{1B}$ receptors are present as postsynaptic terminal heteroreceptors on cortical glutamate terminals, as 5-HT$_{1B}$ receptors modulate cortical glutamate release. However, this should be viewed with caution, since there is no histological confirmation. Egeland *et al*[38] demonstrated 5-HT$_{1B}$ receptor immunoreactivity within a subpopulation of PV-IR interneurons of the cingulate gyrus, and this included some weak immunoreactivity in the soma of these cells. It is not known whether this expression pattern reflects somatodendritic insertion of 5-HT$_{1B}$ receptors on the neuronal membrane, as has been observed in the hippocampal formation,[39,40] or simply 5-HT$_{1B}$ receptors that are early in the process of being trafficked to axon terminals.

Thus, activation of 5-HT$_{1B}$ receptors may negatively modulate the release of 5-HT and glutamate within the frontal cortex via their expression as terminal autoreceptors or heteroreceptors. Additionally, if 5-HT$_{1B}$ receptors have a somatodendritic expression in cortical PV-IR interneurons, then activation of these receptors may also serve to reduce the activity of these neurons. However, we have not been able to identify any electrophysiological studies confirming the presence of a 5-HT$_{1B}$ mediated current in cortical PV-IR interneurons.

Given that Bruinvels *et al*[33] found that the closely related 5-HT$_{1D}$ receptor represents a negligible amount of binding in the frontal cortex, we will consider it to be absent in the PFC and will discuss it in more detail below, in the section dealing with the striatum.

5-HT$_{2A/2C}$ receptors

5-HT$_2$ receptors are stimulatory G-protein coupled receptors that exist primarily as postsynaptic heteroreceptors within the CNS. 5-HT$_{2A}$ receptors are strongly expressed in the cortex. Within the frontal cortex, 5-HT$_{2A}$ receptor mRNA has been observed in pyramidal neurons and interneurons, with substantial variation in the proportion of these cellular populations expressing this receptor. In the prelimbic PFC, approximately one-half of pyramidal neurons and one-third of interneurons express 5-HT$_{2A}$ receptor mRNA, while in the infralimbic region only about 12% of pyramidal neurons and 22% of interneurons expressed 5-HT$_{2A}$ receptors.[30] Moreover, the presence of 5-HT$_{2A}$ receptor proteins has been confirmed in each of these cell populations.[41] Furthermore, the presence of 5-HT$_{2A}$ receptors on GABAergic interneurons has been extended, showing these receptors are present in cortical PV-IR and non-PV-IR interneurons.[42,43]

The structurally similar 5-HT$_{2C}$ receptor is present at moderate levels in the frontal cortex,[44] but has not been found in fast spiking interneurons.[42]

Thus, within the frontal cortex, activation of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors is expected to have excitatory direct effects on pyramidal neurons, while 5-HT$_{2A}$ receptors should also excite GABAergic interneurons. However, given the tight interconnection between pyramidal and GABAergic interneurons in the frontal cortical microcircuitry, the effects of 5-HT$_{2A/2C}$ receptor stimulation are expected to be mixed. Moreover, the available electrophysiological evidence is consistent with this interpretation. In vivo recordings of the PFC after administration of the 5-HT$_{2A/2C}$ receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) found that regular spiking cells had a mix of excitatory and inhibitory responses,[45] although the largest group of DOI-responsive neurons exhibited an excitatory response.

5-HT$_3$ receptors

5-HT$_3$ receptors are unique among the known 5-HT receptor subtypes in that they are the only stimulatory ligand-gated ion channel. On a regional level, 5-HT$_3$ receptors are expressed at their strongest levels within the CNS in the nucleus of the solitary tract, and their level of expression in forebrain regions tends to be low to moderate by comparison.[46] Within the frontal cortex, cells expressing 5-HT$_3$ receptor mRNA are segregated primarily into shallow cortical layers (ie, layers I–III), although some 5-HT$_3$ receptor mRNA-positive cells have been observed in deeper layers.[47] 5-HT$_3$ receptor protein expression in the frontal cortex tends to be present throughout the cortical layers, though again it is stronger in the shallow layers[46,48] (see Figure 1, panels E and F). On the level of cellular expression, 5-HT$_3$ receptors are nearly exclusively expressed within GABAergic interneurons, more specifically in those expressing cholecystokinin, calretinin, vasoactive intestinal peptide, or neuropeptide Y, but not in those expressing somatostatin or parvalbumin.[24,49]

Thus, 5-HT$_3$ receptors represent a mechanism of 5-HT-mediated fast excitatory drive that is selective to non-PV-IR interneurons segregated into layers I–III. By extension, it can be expected that 5-HT$_3$ receptor-mediated 5-HT neurotransmission will have an overall inhibitory effect on the output of cortical pyramidal cells, especially in the PFC, where there are somewhat more 5-HT$_3$ receptors than in other cortical subregions. Electrophysiological data are consistent with this

interpretation. Puig *et al*[47] demonstrated that raphe stimulation increased the activity of a subset of GABAergic interneuron that could be blocked by selective 5-HT_3 receptor antagonists, while Ashby *et al*[50] found that 5-HT_3 receptor agonists reduced the activity of putative pyramidal neurons in the PFC. By extension, 5-HT_3 receptor antagonists can be expected to increase pyramidal neuron output via disinhibition.

5-HT_4 receptors

5-HT_4 receptors are stimulatory G-protein coupled receptors for which there is relatively little accumulated knowledge. Autoradiographic evidence suggests that 5-HT_4 receptors are expressed at very low levels in the frontal cortex.[51] Furthermore, there is little and conflicting experimental data addressing their cellular expression in the frontal cortex.[38,52]

5-HT_5 receptors

5-HT_5 receptors are inhibitory G-protein coupled receptors that are broadly present within the rodent brain. Immunohistochemical evidence shows low to moderate 5-HT_5 receptor expression levels within several cortical regions, highest within layers II and IV, where expression tended to be in the soma,[53] but the degree to which this information will translate to the PFC is questionable, since this region lacks a well-defined layer IV. Anatomical data on the cortical neuronal subtypes expressing 5-HT_5 receptors is largely absent; however, Goodfellow *et al*[54] observed a 5-HT_5 receptor-mediated inhibitory current in cortical layer V pyramidal neurons, suggesting that they are at least present in cortical principle cells. Based on these data alone, activating 5-HT_5 receptors should be expected to reduce cortical output. However, given the relative lack of anatomical and electrophysiological data on this receptor subtype, 5-HT_5 receptors may turn out to have more complex actions on the PFC network.

5-HT_6 receptors

5-HT_6 receptors are stimulatory G-protein coupled receptors that are present at low to moderate levels within the rodent frontal cortex, with stronger areas of expression in rostral compared to caudal cortical regions.[55] Similar results were found in human PFC, where 5-HT_6 receptor expression was present at relatively low levels.[56] The strongest 5-HT_6 receptor expression in the human cortex was observed in layer I, where 5-HT_6 receptors were primarily associated with glia. However, 5-HT_6 receptor expression was associated with pyramidal neurons in deeper cortical layers,[56] and expression in non-pyramidal neuronal types has not yet been reported. These data may suggest that 5-HT_6 receptor activation will activate pyramidal neuron firing; however, we are not aware of any electrophysiology data that have investigated the consequences of 5-HT_6 receptor stimulation in the PFC.

5-HT_7 receptors

5-HT_7 receptors are stimulatory G-protein coupled receptors that have varying levels of expression within the cortex, with strong expression in the shallow cortical layers and more moderate levels in deeper layers[57] (Figure 1, panels G and H). More specifically, 5-HT_7 receptor expression has been observed in layer V pyramidal neurons, and in cells with relatively small oval-shaped soma that were not characterized further. Electrophysiological evidence indicates the presence of 5-HT_7 receptors on hippocampal pyramidal neurons[58] and GABAergic interneurons[59]; thus it is possible that they will also be present in cortical interneurons, but this remains to be experimentally confirmed. A study by Tokarski *et al*[60] showed that acute intraperitoneal (i.p.) administration of the selective 5-HT_7 receptor antagonist SB269970 at 1.25 mg/kg had no effect on the firing of frontal cortex pyramidal neurons, while repeated administration reduced firing. But Lundbeck internal data suggest that a 1 mg/kg dose of this drug via the subcutaneous (s.c.) route, which is in many respects similar to the i.p. route, results in negligible occupancy at 5-HT_7 receptors. In fact, in our hands, a 30 mg/kg dose of SB269970 only engendered approximately 30% of 5-HT_7 receptors 1 hour after administration, owing to difficulty penetrating the blood–brain barrier. Given these receptor occupancy data, it remains unclear how 5-HT_7 receptor modulation will alter the activity of cells within the PFC.

Summary of serotonergic receptor mechanisms in frontal cortex neuronal subtypes

This section summarizes the 5-HT receptor subtypes that have been demonstrated on each of the important neuron types within the PFC microcircuit.

PV-IR interneurons

In cortical PV-IR fast-spiking interneurons, there is evidence supporting the expression of inhibitory 5-HT_{1A} as well as excitatory 5-HT_{2A} receptors, although 5-HT_{1A} and 5-HT_{2A} receptors appear to be largely expressed by separate populations of PV-IR interneurons.[42] There is also some evidence suggesting that 5-HT_{1B} receptors are expressed on cortical PV-IR interneurons, but the subcellular localization of 5-HT_{1B} receptors on cortical PV-IR cells is not clear at this time. Based on this evidence, cortical PV-IR cells will likely respond to 5-HT with either an excitatory or inhibitory response on the single-cell level, while on the population scale there will

TABLE 1. 5-HT receptor subtypes in the rat prefrontal cortex and striatum: expression patterns, affinities for 5-HT, and effects on neural activity.

			Expression		Cell Ttypes		
Receptor	Direct effect on neural activation	Affinity for 5-HT K_i or K_D (nM)	PFC	Striatum	PFC	Striatum	Ref.
5-HT_{1A}	I	0.2–0.79	++	+/–	Pyr PV-IR IN Non-PV-IR IN	–	28,29,92
5-HT_{1B}	I	4.0–32	++	++/+++	Pyr IN (PV-IR)	PV-IR IN ChAT-IR IN	32–34,37
5-HT_{1D}	I	2.5–6.3	–	+	?	?	32
5-HT_{2A}	S	1.3	+++	++	Pyr PV-IR IN	MSN	29,40,41
5-HT_{2C}	S	2.5–160	++	++	Pyr	MSN PV-IR IN ChAT-IR IN	41,43,44,97–100
5-HT_3	S	130–320	++	+/–	Non-PV-IR IN	?	19,46,48
5-HT_4	S	1.6–4.0	+	++/+++	Pyr?	MSN	51,101
5-HT_5	I	130–200	++	+	Pyr (L5)	MSN	53,54
5-HT_6	S	13	+/++	+++	Pyr	MSN ChAT-IR IN	55,56,97
5-HT_7	S	1.0–7.9	++/+++	+/++	Pyr	ChAT-IR IN	57,100

Affinities for 5-HT were calculated based on pK_i/pK_D data from the IUPHAR database (www.iuphar-db.org). –: absent; +: low; ++: moderate; +++: strong; ?: unknown; ChAT-IR: choline acetyltransferase immunoreactive; I: inhibitory; IN: interneuron; MSN: medium spiny neuron; PFC: prefrontal cortex; PV-IR: parvalbumin immunoreactive; Pyr: pyramidal neuron; S: stimulatory.

likely be a mixed excitatory and inhibitory response. Whether this population level mix of excitatory and inhibitory responses skews to one side or the other may depend on a number of issues, including (1) how commonly 5-HT_{1A} and 5-HT_{2A} receptors are expressed within frontal cortical PV-IR cells, (2) the affinity of 5-HT for each of these targets (Table 1), and (3) the concentration of 5-HT applied to the neural system.

Puig *et al*[42] have suggested that about half of PV-IR interneurons in layers II/III express 5-HT_{1A} receptor mRNA, while about 20% express 5-HT_{2A} receptor mRNA. However, in layer V, approximately one-third of PV-IR neurons express 5-HT_{1A} receptors, while a separate third of the population express 5-HT_{2A} receptors. Thus, these data may suggest that PV-IR interneurons in shallow cortical layers are more likely to be inhibited by 5-HT than those in deep cortical layers, where there is more likelihood for an even split in excitatory and inhibitory responses within this cell population. However, given that 5-HT's affinity for the 5-HT_{1A} receptor is anywhere from 1.6- to 6.5-fold higher than for the 5-HT_{2A} receptor (Table 1), it is possible that the effect of 5-HT on cortical fast-spiking interneuron behavior will skew more heavily toward inhibition at lower levels of 5-HT concentrations, with excitation becoming more prominent as 5-HT concentrations increase.

Non PV-IR interneurons

In non-PV-IR cortical GABAergic interneurons, there is evidence to support 5-HT_{1A} receptor expression on calbindin-IR interneurons, and 5-HT_3 receptors have been found on CR-IR and CCK-IR interneurons located in shallow cortical layers. Additionally, electrophysiological evidence has suggested that 5-HT_{2A} receptors are also present in cortical non-PV-IR interneurons, although the specific subtype remains unclear at this time. When considering the overall effects of 5-HT in this subpopulation of cells, inhibitory 5-HT_{1A} receptors may again tend to predominate in low concentration ranges of 5-HT, given its higher affinity for the endogenous ligand by comparison to 5-HT_{2A} and especially 5-HT_3 receptors (Table 1). However, it is important to note that it is essentially unknown at this time whether these receptors are expressed in serotonergic synapses. Although SERT expression in the frontal cortex is relatively strong (see Figure 1, panels I and J), it is currently believed that only about one-quarter of serotonergic terminals in this brain region have synaptic specializations.[61] However, the serotonergic terminals that do form synapses are thought to be primarily on GABAergic interneurons residing in the shallow cortical layers,[62] which may include 5-HT_3 receptor-expressing interneurons. If true, this pattern may make it easier to achieve sufficiently high 5-HT concentrations to stimulate 5-HT_3 receptor activity than for those serotonergic receptor targets that are primarily modulated by extracellular serotonin concentrations. Additionally, 5-HT_3 receptors are a fast-desensitizing serotonergic target (for example, see Dale *et al*[17]); thus at high concentrations, it may be the case that a larger proportion of this receptor population is non-functional

than at lower concentrations. Taken together, it is reasonable to suggest that serotonergic neurotransmission is more likely to activate non-PV-IR interneurons in the shallow cortical regions.

Pyramidal neurons

In cortical pyramidal neurons, there is evidence for the expression of a variety of serotonergic receptors. Inhibitory 5-HT_{1A} and 5-HT_5 receptors have been found in somatodendritic subcellular regions of pyramidal neurons, 5-HT_{1B} receptors may be expressed in the terminals of cortical pyramidal neurons. Additionally, several excitatory serotonergic receptors are present on these cells, including 5-HT_{2A}, 5-HT_{2C}, 5-HT_4, 5-HT_6, and 5-HT_7 receptors.

Given the wide range of excitatory and inhibitory serotonergic receptors that have been demonstrated in cortical pyramidal neurons, along with the differences in affinity these receptors have for 5-HT, it is not possible to accurately determine 5-HT's overall effects on cortical pyramidal neurons. However, within the native frontal cortex microcircuit, pyramidal neurons are under powerful inhibitory control from a variety of inhibitory interneurons, and thus some of the effects 5-HT will have on pyramidal neurons will depend on its effects on these inhibitory interneurons.

Electrophysiological effects of 5-HT in the frontal cortex

In vivo electrophysiology

Studies that have investigated the effects of 5-HT on cortical fast-spiking (presumably PV-IR) interneuron behavior in vivo using anesthetized animals have generally found that 5-HT has inhibitory effects. For example, Puig *et al*[42] found that stimulating the midbrain raphe nuclei at low levels and recording in vivo from the medial PFC induced mixed effects in fast-spiking interneurons, with the majority of these cells exhibiting inhibition (61%), and small populations exhibiting excitation (approximately 10%) or excitation followed by inhibition (approximately 6%). Moreover, the inhibitory actions of raphe nucleus stimulation were antagonized by administration of the selective 5-HT_{1A} receptor antagonist WAY100635, whereas the few excitations that were observed were antagonized by the 5-HT_2 receptor antagonist ritanserin.

Superficially, these data appear to be at odds with data from the same lab demonstrating that separate but equal proportions of layer V PV-IR interneurons within the cortex express inhibitory 5-HT_{1A} receptors and excitatory 5-HT_{2A} receptors[42]. A corollary to these data would seem to be that approximately equal proportions of layer V fast-spiking interneurons would exhibit an inhibitory or excitatory response to physiological 5-HT levels. However, during periods of light anesthesia that more closely resembled an "awake" cortical network, Puig *et al*.[42] also found a higher proportion of cells exhibiting an excitatory response to raphe stimulation (approximately 31% compared to 10%). These data fit with an interpretation that *in vivo* electrophysiology in anesthetized rats is biased towards 5-HT_{1A} receptor mediated inhibition.

Although inhibition of PV-IR interneurons would be expected to have a disinhibitory effect on pyramidal neurons, it seems that pyramidal neurons also have an overwhelmingly inhibitory response to raphe nucleus stimulation in anesthetized in vivo electrophysiological experiments. Puig *et al*[63] demonstrated that low-level stimulation of the raphe nucleus inhibited activity in the majority of recorded putative pyramidal neurons (66%), while 13% exhibited an excitatory response and another 20% had mixed effects characterized by inhibition followed by excitation. 5-HT_{1A} receptor antagonism attenuated the inhibitory responses (but did not entirely block them), while 5-HT_{2A} receptor antagonism blocked the excitatory responses. Similar results were found by several other research groups after raphe nucleus stimulation in anesthetized in vivo electrophysiological preparations.[64–66]

While the results of these in vivo anesthetized electrophysiology experiments consistently suggest that 5-HT has an overwhelmingly inhibitory effect on not only pyramidal neurons but also PV-IR interneurons, there are some important limitations to consider when evaluating these data. The presence of anesthesia may cause a number of deviations from normal cortical network states, for example a reduction in tonic or phasic 5-HT concentrations resulting from reduced raphe nucleus activity. This may bias the effects of 5-HT stimulation toward receptors with high affinity for 5-HT, such as the inhibitory 5-HT_{1A} receptor, which is present on pyramidal neurons, as well as multiple subtypes of non-pyramidal neurons in this region. It may be the case that in an awake and behaving animal, stimulation of the raphe nucleus would be more likely to induce excitatory responses mediated by lower-affinity receptors, such as the 5-HT_{2A} receptor (Table 1).

In addition to this distal effect of anesthesia, local cortical networks may also be abnormally regulated by these unusually high $GABA_A$ receptor mediated currents. This may have the effect of partially divorcing the activity of pyramidal neurons and some types of interneurons from the activity of the inhibitory cells governing their behavior, therefore abrogating the downstream effects of serotonergic modulation. This, along with the apparent bias toward 5-HT_{1A} receptor mechanisms, may help to explain why pyramidal neuron firing is overwhelmingly reduced by raphe stimulation or SSRI treatments within anesthetized cortical networks in the face of reduced fast-spiking interneuron firing.

In vitro electrophysiology

In stark contrast to the in vivo electrophysiological techniques in anesthetized rodents, research groups investigating the effects of 5-HT on fast-spiking interneuron behavior in brain slices have generally found that 5-HT has an activating effect on fast-spiking interneurons. For example, Zhong and Yan[67] found that 5-HT (20-40 μM) led to an increased firing rate of fast spiking interneurons, with relatively little effect of 5-HT at lower concentrations (1–2 μM). The idea that 5-HT activates fast-spiking interneuron behavior in slices is further supported by observations that 5-HT depolarized fast-spiking interneurons[43] and increased spontaneous inhibitory postsynaptic currents (sIPSCs) recorded from pyramidal neurons.[43,68,69]

Additionally, in the concentration ranges that drove increases in fast-spiking interneuron activity, Zhong and Yan[67] observed concomitant reductions in the firing rate of cortical pyramidal neurons. But the inhibitory effects of 5-HT on pyramidal neuron firing were not limited to downstream actions on GABAergic interneurons. Araneda and Andrade[70] found that 5-HT hyperpolarized layer V cortical pyramidal neuronal membranes, which could be blocked by 5-HT_{1A} receptor antagonists but not a 5-HT_2 receptor antagonist.

As expected by the fact that both inhibitory 5-HT_{1A} and stimulatory 5-HT_{2A} receptors are expressed in cortical pyramidal neurons, 5-HT's effects on pyramidal neuron firing in slice electrophysiological preparations are not universally inhibitory. Several groups have found that 5-HT application increases spontaneous excitatory postsynaptic currents[69,71] (sEPSCs), induces membrane depolarizations,[70] and modulates after hyperpolarization currents in a manner that supports increased activity.[70,72]

Taken together, in contrast to in vivo recordings in anesthetized animals, these data from brain slices suggest that 5-HT has a primarily excitatory effect on cortical fast-spiking interneurons, as well as mixed excitatory and inhibitory effects on pyramidal neuron activation. Slice electrophysiology has a number of advantages, including a stronger ability to identify the cell type from which each recording is taken and the absence of anesthesia. However, there are also limitations to consider. First, although it can be argued that local neuronal circuits are largely intact, the process of sectioning tissue does attenuate some aspects of the neural network, most obviously from distal regions such as the raphe nucleus. Additionally, it should be noted that slice electrophysiological studies are often conducted in brain slices collected from adolescent rodents, and thus the degree to which the results translate to the adult cortical network are difficult to know. But perhaps the most important limitation of slice electrophysiology in the context of this article is that it is difficult to ascertain what the effects of physiological 5-HT concentrations would be. It is more or less not possible to determine whether the 5-HT concentrations used to elicit a primarily excitatory response via bath application are physiologically relevant, and in fact the concentrations commonly used to elicit these responses are in a very high range. Although it is common for slice electrophysiologists to use relatively high concentrations of a given ligand to ensure that it penetrates to the recorded cells (typically in the range of 100–1000 times Ki), concentrations of 5-HT of 20–40 μM seem disproportionately high from the perspective that the Ki for 5-HT at 5-HT_{2A} receptors is1.3 nM (Table 1).

Thus, it seems likely that in vivo investigations of 5-HT's effects in anesthetized PFC networks primarily represent the actions of this neurotransmitter at low concentrations, while those from the slice represent 5-HT's actions at high concentrations. In order to further advance our understanding of how 5-HT and serotonergic antidepressants modulate prefrontal cortical networks, it would be advantageous to conduct investigations in awake and behaving rodents.

Hypothesized effects of 5-HT, SSRIs, and vortioxetine on the overall frontal cortex circuit

5-HT and SSRIs

Based on the receptor profiles that are present in each of the major cell types we have considered in the frontal cortex microcircuitry, as well as electrophysiological evidence of 5-HT's effects, the emerging theme seems to be that 5-HT has largely inhibitory actions at low concentrations dominated by 5-HT_{1A} receptor mediated actions at some PV-IR and non-PV-IR interneurons. Under normal circumstances, this would be expected to disinhibit pyramidal neuron activity, although pyramidal neurons themselves would also experience direct inhibitory effects of 5-HT_{1A} receptor activation. At higher levels of 5-HT tone, excitatory actions would start coming into play at inhibitory interneurons, primarily mediated by 5-HT_{2A} receptors at PV-IR fast-spiking interneurons and by 5-HT_{2A} and 5-HT_3 receptors in non-PV-IR interneurons. These actions on cortical interneurons would be expected to drive a GABA-mediated inhibitory influence on pyramidal neuron firing, particularly given the powerful inhibitory action PV-IR interneurons have on pyramidal neuron behavior. However, pyramidal neurons would also experience some direct excitatory actions of 5-HT mediated through a variety of serotonergic receptor targets. Therefore, at multiple levels of organization, 5-HT has opposite directed effects that overall do not appear to clearly influence the output of frontal cortical output cells toward excitation or inhibition. On a conceptual level,

5-HT may therefore play a role in fine-tuning the output of frontal cortical networks, acting in a sense as a buffer that will tend to normalize the activity of pyramidal output neurons. Given this concept and the idea that SSRI antidepressants increase synaptic and extrasynaptic serotonin concentrations without directly modulating serotonergic receptors, we hypothesize that, under acute conditions, these treatments will have mixed effects that will not clearly bias frontal cortex output in either direction. Moreover, this idea is supported by electrophysiological studies that have demonstrated that acute and short-term repeated SSRI administration have little effect on PFC firing in anesthetized rats.[73,74] However, long-term SSRI administration seems to have an inhibitory effect on PFC firing,[73,74] which may imply that desensitization of some serotonergic receptor systems are important for the long-term effects of SSRI administration on frontal cortical network behavior. It is not clear based on available anatomical and electrophysiological data precisely which serotonergic receptor mechanisms might be involved in these effects of chronic administration.

Vortioxetine

In contrast, vortioxetine should have markedly different effects on the output of frontal cortex networks that will be driven by its direct pharmacological actions on several serotonergic receptors. Vortioxetine's potent antagonism of 5-HT_3 receptors should remove a source of 5-HT-mediated fast excitatory drive on a selective population of non-PV-IR cortical interneurons localized in shallow cortical layers. Based on the model presented here, this action should disinhibit cortical pyramidal neurons, although it is unclear whether this effect would be segregated purely in layer II/III pyramidal neurons or if it would generalize to layer V pyramidal output cells. 5-HT_3 receptor antagonism can also result in increased extracellular 5-HT concentrations when dosed along with an SSRI,[75] which may lead to more stimulation of serotonergic receptors that are not directly targeted by vortioxetine. Additionally, 5-HT_{1A} receptor agonism should induce a fast inhibitory effect mediated by inwardly rectifying potassium channels in both PV-IR and non-PV-IR interneurons, which may also disinhibit pyramidal neuron activity that would be balanced by inhibitory actions on the pyramidal neurons themselves. Additionally, vortioxetine's partial agonist action at 5-HT_{1B} receptors, which acts to attenuate 5-HT_{1B} receptor actions in vivo,[76] may increase serotonergic tone in the PFC by reducing inhibitory feedback at the site of the serotonergic terminal, which may again lead to greater stimulation of serotonergic targets not directly targeted by vortioxetine. Furthermore, this partial agonist effect at 5-HT_{1B} receptors may also attenuate 5-HT_{1B} receptor mediated inhibition of cortical glutamatergic terminals, leading to increased glutamate release from layer II/III pyramidal neurons at their local cellular targets. Finally, it is unclear at this time what effect vortioxetine's 5-HT_7 receptor antagonist properties will have on PFC activity. But we expect that the net effect of vortioxetine in the PFC will be to reduce aspects of GABAergic neurotransmission and indirectly increase the activity of glutamatergic pyramidal cells.

In support of these ideas, recent in vivo electrophysiology experiments by Riga *et al*[18] in anesthetized rats demonstrated that acute administration of the SSRI escitalopram had no effect on the overall firing rate of putative frontal cortex pyramidal neurons, although a relatively equal minority of cells exhibited excitatory or inhibitory responses. However, in stark contrast, acute i.v. administration of vortioxetine led to an overwhelmingly excitatory response in cortical pyramidal neurons. This research group also found that an acute combination of escitalopram and the selective 5-HT_3 receptor antagonist ondansetron led to significant increases in pyramidal neuron firing, at least implicating a combination of 5-HT reuptake inhibition and 5-HT_3 receptor antagonism in vortioxetine's excitatory effects.

In summary, SSRIs appear to have mixed effects on the function of the PFC circuit that do not clearly modulate activity either toward excitation or inhibition under acute conditions, while acute vortioxetine leads to an increase in PFC pyramidal neuron activity that is driven by a reduction in GABAergic inhibitory processes. After long-term administration, SSRI antidepressants lead to an overall reduction of PFC pyramidal neuron firing that is likely driven by the desensitization of some serotonergic receptors. At this time, empirical data on the long-term effects of vortioxetine on PFC pyramidal neuron activity are not available; however, we hypothesize that vortioxetine will still have an overall excitatory effect on these cells after chronic administration.

The Striatum

Functional role and organization

The striatum, which is a term we will use to refer specifically to the caudate and putamen, is a subcortical structure that receives afferent inputs from a number of different brain regions including association and sensorimotor cortices as well as the thalamus, and is considered the primary input structure for the basal ganglia. Thus, the striatum can be viewed as an assimilation zone for information related to central nervous system functions as broad as sensorimotor integration and motor function, motivation and reinforcement, and some aspects of cognitive function such as working memory and executive function.[77,78]

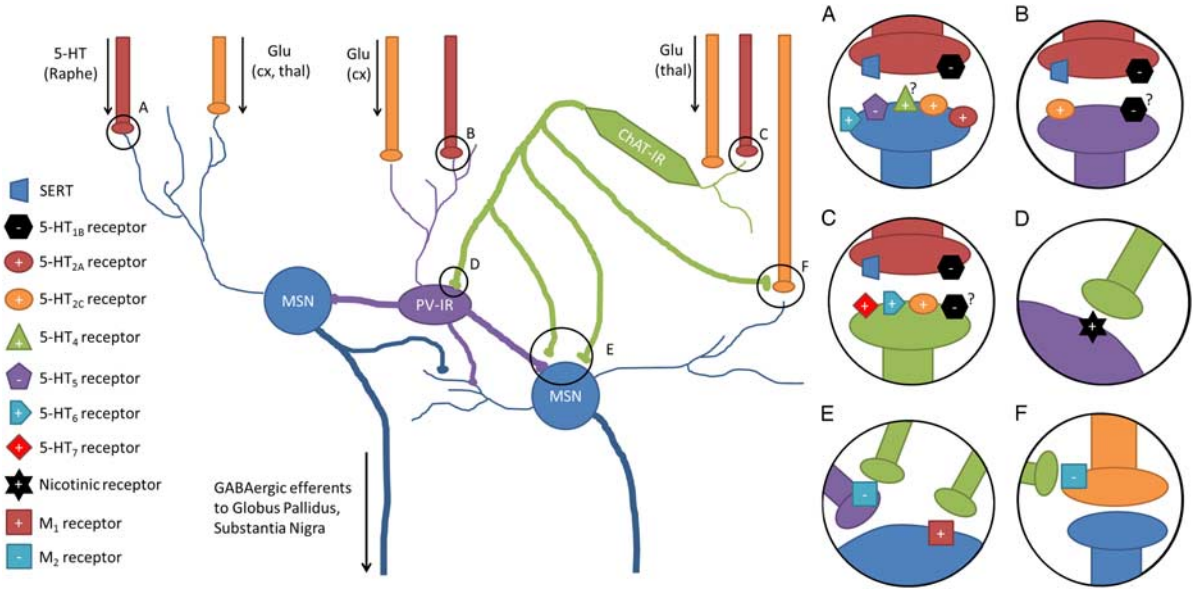

FIGURE 4. Serotonergic influence on a theoretical striatal microcircuit. The effects of serotonin on the behavior of striatal medium spiny neurons (MSNs) are mediated by a number of different factors. Serotonergic afferents from the raphe have a mostly excitatory direct influence on MSNs that are mediated through 5-$HT_{2A/2C}$, 5-HT_4, and 5-HT_6 receptors, although 5-HT_5 receptors have an inhibitory influence on these cells. 5-HT_{1B} receptors are prominently expressed in the striatum, but are thought to exist primarily as presynaptic autoreceptors in this region (A). Similarly, serotonin has an excitatory direct effect on parvalbumin-immuoreactive (PV-IR) interneurons, which are at least mediated by 5-HT_{2C} receptors (B). The excitatory effect of 5-HT observed in choline-acetyltransferase immunoreactive interneurons ChAT-IR cells is mediated by a combination of 5-HT_{2C}, 5-HT_6, and 5-HT_7 receptors (C). MSN behavior is also modulated by the behavior of PV-IR and ChAT-IR cells. PV-IR cells have a powerful inhibitory effect on MSN firing that is mediated by GABAergic neurotransmission localized at the MSN soma or dendrites. ChAT-IR cells have a relatively complex mix of excitatory and inhibitory effects on the behavior of PV-IR cells and MSNs. Cholinergic transmission from ChAT-IR cells has a short-term activating effect on PV-IR cells that are mediated by nicotinic acetylcholinergic receptors (D), but can reduce GABA release from PV-IR cells via presynaptic M_2 receptors (E). ChAT-IR cells can also excite MSNs via M_1 receptors (E), and can reduce release from glutamatergic afferent terminals via M_2 receptors (F).

From a structural perspective, the striatum has a number of important differences compared to the PFC. First, the laminar structure observed in the PFC is absent in the striatum. Additionally, while the PFC has a mix of inhibitory interneurons and excitatory cells that are native to the region, the vast majority of striatal neurons are GABAergic and thus inhibitory in nature. In fact, there are at least four separate types of GABAergic cells in the striatum. These include the medium spiny neuron (MSN), which is this region's principle projection cell, as well as at least three chemically differentiated types of striatal GABAergic interneuron.[79] In addition, a fifth type of striatal interneuron is cholinergic, rather than GABAergic.[79] The section below will focus on describing medium spiny neurons, PV-IR fast-spiking interneurons, and cholinergic interneurons and their relationships to one another. These relationships, as well as the influence of 5-HT on their behavior, are depicted in Figure 4.

Important striatal cell types

PV-IR fast-spiking interneurons

One of the most important classes of striatal GABAergic interneuron is identified immunohistochemically by the expression of the calcium binding protein parvalbumin (PV). As in the frontal cortex, PV-IR interneurons are characterized electrophysiologically by their fast firing rate and short-duration action potential. Therefore we will refer to these cell types interchangeably as striatal PV-IR interneurons or as striatal fast-spiking interneurons.

PV-IR interneurons are believed to make up between 3% and 5% of striatal neurons (for an excellent review, see Kawaguchi *et al*[79]). These interneurons are thought to receive a strong excitatory input from the neocortex, as well as an inhibitory input from other striatal PV-IR cells.[79,80] In addition to these inputs, PV-IR cells are reported to form electrical networks with one another via gap junctions,[79] which may allow for the formation of neural assemblies with other striatal fast-spiking interneurons.

The axons of striatal PV-IR cells have a basket-like arborization pattern,[79] with synapses directed at both the dendritic tree and the soma of MSNs.[80] Studies on the synaptic connections between this class of interneurons and MSNs have suggested that each MSN receives connections from multiple PV-IR cells.[80] Thus, given the ideas that multiple PV-IR interneurons form inhibitory synapses on the soma of each MSN cell, and further that these cells may easily form inhibitory neural ensembles, it

can be expected that PV-IR interneurons will have a powerful inhibitory effect on the output of striatal MSNs. Moreover, this idea is consistent with electrophysiological studies that have suggested that the majority of inhibitory processes in the striatum are mediated by PV-IR cells.[81]

Although there are at least 2 other types of GABAergic interneurons that have been chemically or morphologically identified in the striatum,[79] there is relatively little accumulated knowledge on the calretinin-IR and nitric oxide/neuropeptide Y/somatostatin-IR interneurons in the striatum, particularly in terms of their relationship to serotonergic neurotransmission. Therefore, this review will not consider these cell types further.

Cholinergic interneurons

Striatal non-GABAergic interneurons are differentiated from other striatal interneurons by a number of morphological, histochemical, and electrical properties, including a large soma with broadly branching dendritic and axonal arbors; the expression of the acetylcholine-producing enzyme choline acetyltransferase (ChAT); and a tonic, irregular firing pattern with low activation thresholds.[79] These cells receive excitatory inputs from the cortex and thalamus, although the thalamic inputs are thought to predominate in this cellular population.[79,82] Throughout this article, we will refer to this cellular population as ChAT-IR interneurons.

ChAT-IR interneurons modulate the behavior of other striatal neuronal populations in a complex manner, having a mix of excitatory and inhibitory effects on both MSN and PV-IR cell populations. These influences have been reviewed in detail in Goldberg and Reynolds,[82] but for the purpose of understanding how serotonergic neurotransmission affects neuronal activity in the striatum, we will briefly discuss these effects here (summarized in Figure 4). ChAT-IR neurons have a fast excitatory influence on PV-IR cells that is mediated by nicotinic acetycholinergic receptors expressed on the soma.[83] However, this excitatory effect is balanced by a slower, inhibitory influence on GABA release that is mediated by muscarinic M_2 receptors expressed on striatal GABA terminals.[82] Additionally, Goldberg and Reynolds[82] suggest that M_2 receptors can have a similar effect on the release of glutamate from cortical or thalamic afferent terminals and that striatal ChAT-IR neurons can have a direct stimulatory effect on MSN firing that is mediated via muscarinic M_1 receptors.

Medium spiny neurons

Whereas principle output cells in the cortex are excitatory glutamatergic pyramidal neurons, principle cells in the striatum are GABAergic in nature, and thus efferents originating from the striatum have an inhibitory effect on their projection regions. These striatal principle cells are MSNs, which are thought to represent between 80% and 98% of the neurons present in the neostriatum (for a review, see Tepper *et al*[84]). From a morphological perspective, MSNs are characterized by their medium size and spiny dendritic arbors.[85] MSNs can be further subdivided into at least 2 separate but equally represented populations based on neuropeptide and neurotransmitter receptor expression: those expressing enkephalin and dopamine (DA) D_2 receptors, and those expressing dynorphin, substance P, and DA D_1 receptors.[86–89] MSNs receive excitatory glutamatergic afferents from the neocortex and thalamus,[80] as well as serotonergic afferents from the midbrain raphe nuclei. However, the MSN subpopulations noted above preferentially send efferent projections to separate portions of the basal ganglia. MSNs expressing enkephalin and DA D_2 receptors project preferentially to the globus pallidus, while those expressing dynorphin, substance P, and DA D_1 receptors send efferents to the substantia nigra.[87,88]

In addition to MSN axon efferents sent to basal ganglia targets, MSN neurons also send axon collaterals to other MSN cells in the striatum that synapse in the dendritic arbor.[79] This phenomenon, in combination with the inhibitory nature of these cells, originally led to a hypothesis that striatal MSNs form a competitive lateral inhibitory network, where activation of one MSN leads to inhibition of surrounding MSNs. However, more recently this theory has been modified in light of electrophysiological evidence demonstrating that the inhibitory effect of a given MSN on the firing of surrounding MSNs is very weak, owing probably to the dendritic insertion of MSN-MSN axon collaterals.[80,81] A modified theory suggests that lateral inhibition may still be possible in MSN networks, based on the fact that each MSN sends axon collaterals to about 1 in 6 of its surrounding MSNs and receives inputs from approximately 450 other local MSNs. In this theory, MSNs that are not connected to one another via axon collaterals but receive similar excitatory inputs may form neural assemblies that can inhibit surrounding MSNs in the aggregate (reviewed in Wilson[80]). Thus, it is possible that MSNs have a relatively weak lateral inhibitory influence on other MSNs in the striatum, but this is currently considered to represent a small minority of the total phasic inhibitory drive on striatal MSN activity.[80]

In the following section we will review evidence on the regional and cellular pattern of 5-HT receptor expression for each of the above-mentioned striatal cell types.

Serotonergic receptor expression within the striatum

5-HT$_1$ receptors

5-HT_{1A} receptor binding in the caudate and putamen it is extremely low[29,90] (Figure 2, panels A and B).

Thus, although there may be some very weak expression of 5-HT_{1A} receptors within the striatum of the rodent brain,[91] we will consider it to be essentially absent in this region.

In contrast, studies of 5-HT_{1B} receptor expression in the striatum showed moderate to strong expression levels of mRNA[92] as well as protein[33,35] (Figure 2, panels C and D), which may indicate that 5-HT_{1B} receptors are postsynaptically expressed in the striatum or are expressed on interneurons. A mouse study by Ghavami *et al*,[91] using a conditional 5-HT_{1B} receptor knockout approach, demonstrated that 5-HT_{1B} receptors made by striatal MSNs are primarily sent to the basal ganglia projection regions. These data might suggest that striatal 5-HT_{1B} receptors are localized primarily on terminals originating from the midbrain raphe.[92,93] However, dual-labeling immunohistochemistry has provided evidence demonstrating that 5-HT_{1B} receptors are also present on striatal PV-IR and ChAT-IR interneurons.[38] It is not known whether there is a somatodendritic expression of 5-HT_{1B} receptors in these cell types, and it is possible that 5-HT_{1B} receptors act as terminal heteroreceptors in striatal PV-IR and ChAT-IR interneurons.

5-HT_{1D} receptors are closely related to 5-HT_{1B} receptors in the brain and often have a similar, although much more rarified, expression pattern. However, Bruinvels *et al*[33] demonstrated that, in the striatum, 5-HT_{1D} receptor specific binding constituted about 11% of the total binding of a radioligand that is nonselective for 5-$HT_{1B/1D}$ receptors. However, given evidence that 5-HT_{1D} receptors form a heterodimer with 5-HT_{1B} receptors when they are co-expressed,[94] it can be questioned whether these receptors should be considered separately.

Based on these data, striatal 5-HT_{1B} receptors may act primarily to modulate extracellular concentrations of 5-HT by acting as an inhibitory autoreceptor at serotonergic terminals. Moreover, this idea is supported by data from microdialysis experiments.[36]

If 5-HT_{1B} receptors are present in the somatodendritic region of PV-IR and ChAT-IR interneurons, then stimulation of these receptors should drive down the activation state of these interneurons. Alternatively, 5-HT_{1B} receptors expressed as terminal heteroreceptors in these cells may act to reduce release of GABA or acetylcholine, respectively, without markedly modulating cellular excitability at the soma. Which of these subcellular expression patterns is present in striatal interneurons is essentially unknown at this time.

5-$HT_{2A/2C}$ receptors

5-HT_{2A} receptors are only moderately expressed in the striatum,[41] although it should be noted that at least one research group has noted a gradient in 5-HT_{2A} receptor mRNA expression, with more dense expression in the caudal portions of the striatum.[95] Cornea-Hebert *et al*[41] have confirmed 5-HT_{2A} receptor immunoreactivity in the majority of striatal MSNs. Furthermore, Ward and Dorsa[95] found that 5-HT_{2A} receptor mRNA is present in MSNs that project to both the globus pallidus and substantia nigra. Cornea-Hebert *et al*[41] also observed some 5-HT_{2A} receptor immunoreactivity in a population of neurons characterized by a large soma. These cells are presumably cholinergic interneurons based on the large soma.

5-HT_{2C} receptors are present in the striatum at moderate levels[44] and are expressed in striatal MSN cells,[95,96] and once again do not appear to discriminate between MSN cells projecting to the globus pallidus vs the substantia nigra.[95] Although we have not been able to identify any systematic immunohistochemical studies that have examined whether 5-HT_{2C} receptors are present in striatal interneuron populations, electrophysiological experiments would suggest 5-HT_{2C} receptor expression on striatal fast spiking interneurons[97] and ChAT-IR cells.[98]

Within the frontal cortex, 5-HT_{2A} receptors have mixed effects on the behavior of the microcircuitry by activating pyramidal neurons and GABAergic interneurons. Within the striatum, a similar picture of the effects of 5-$HT_{2A/2C}$ receptor stimulation emerges, with activation of these receptors likely to stimulate the striatal MSN projection cells as well as PV-IR and ChAT-IR interneurons.

5-HT_3 receptors

5-HT_3 receptor expression in the striatum is extremely low[46] (Figure 2, panels E and F). There are reports of a few strongly 5-HT_3 receptor-IR cells present within the striatum[48]; however, the identity of these cells is not known.

5-HT_4 receptors

5-HT_4 receptor expression is moderate to high in the striatum with an increasing rostro-caudal gradient.[51,99] Given that the regional profile of 5-HT_4 receptor mRNA is very similar to that seen using autoradiography within the striatum,[51,99] it can be hypothesized that 5-HT_4 receptors primarily have a somatodendritic expression pattern. Egeland *et al*[38] found that striatal PV-IR and ChAT-IR interneurons do not express 5-HT_4 receptors. Given the prominence of 5-HT_4 receptors in the striatum, it is therefore likely that MSN cells express 5-HT_4 receptors; however, this awaits a definitive confirmation.

5-HT_5 receptors

Striatal 5-HT_5 receptor immunoreactivity is weak, and there is a paucity of evidence on the cell types expressing 5-HT_5 receptors. The little that is available suggests that

these receptors are expressed by MSNs but not cholinergic cells.[53] Thus, 5-HT_5 receptor activation might have an inhibitory effect on striatal MSN activity, and in fact this is one of the few serotonergic inhibitory receptors that have been found in the striatum. It remains to be seen whether 5-HT_5 receptors are present in any of the GABAergic interneurons of the striatum.

5-HT_6 receptors

5-HT_6 receptors are densely expressed in the striatum, where they are primarily associated with a postsynaptic, dendritic expression pattern.[55] 5-HT_6 receptors have been observed in striatal MSN cells[95,100] in a manner that does not differentiate between MSNs projecting to the globus pallidus or the substantia nigra,[95] continuing with the apparent theme that serotonergic receptors do not discriminate between these alternate projections to the basal ganglia. In human striatal tissue, Marazziti *et al*[101] found that 5-HT_6 receptors were present in putatively PV-IR cells. However, in rodent tissue, another group found no evidence of 5-HT_6 receptor mRNA in PV-IR cells.[100] Electrophysiological data have suggested the presence of 5-HT_6 receptors in striatal cholinergic (ChAT-IR) interneurons.[98] Thus, stimulation of striatal 5-HT_6 receptors can be expected to activate striatal MSN and ChAT-IR interneurons, whereas it is unclear that these receptors are present in striatal PV-IR interneurons.

5-HT_7 receptors

Within the striatum, Neumaier *et al*[57] reported moderate levels of 5-HT_7 receptor-like immunoreactivity, and 5-HT_7 receptor-selective autoradiographic binding is present at relatively low levels in the rodent (see Figure 2, panels G and H) and human striatum.[102] We have not been able to identify any anatomical studies that characterize the striatal cellular subtypes expressing 5-HT_7 receptors. However, Bonsi *et al*[98] found that cholinergic interneurons expressed 5-HT_7 receptor mRNA, and further that the activating effect of 5-HT on striatal cholinergic cells could be attenuated by application of the 5-HT_7 receptor selective antagonist SB269970. Thus, 5-HT_7 receptors are likely to be expressed at the least in striatal ChAT-IR interneurons, where they have a stimulatory effect.

Serotonergic modulation of striatal neuronal subtypes and overall striatal output

PV-IR fast-spiking interneurons

In striatal PV-IR fast-spiking interneurons, there is evidence supporting the presence of excitatory 5-HT_{2C} receptors as well as inhibitory 5-HT_{1B} receptors. It is not known whether the 5-HT_{1B} receptor immunoreactivity is associated with a somatodendritic expression, as has been observed in the hippocampus,[39,40] or if it is expressed primarily as a terminal heteroreceptor. Additionally, it is unknown whether 5-HT_{1B} and 5-HT_{2C} receptors constitute the entire complement of serotonergic receptors expressed by striatal PV-IR interneurons. Furthermore, it is somewhat difficult to gauge the relative level of affinity at these targets, as the range of observed affinities for 5-HT at these 2 receptors overlaps (Table 1). Based on these uncertainties, it is unclear how physiological levels of 5-HT will alter the behavior of striatal PV-IR interneurons, although one might expect to find mixed effects of 5-HT stimulation. However, it should be noted that electrophysiological investigations into the effects of 5-HT stimulation on PV-IR interneuron activity have not found any 5-HT_{1B} receptor mediated inhibitory currents in striatal PV-IR interneurons (see discussion below), which may either suggest this receptor is expressed as a terminal heteroreceptor, or may call its presence in these cells into question altogether. Taken together, these data may suggest that striatal PV-IR interneurons will be primarily excited by 5-HT, via a 5-HT_{2C} receptor mediated mechanism.

Cholinergic interneurons

There is evidence supporting the presence of excitatory 5-HT_{2C}, 5-HT_6, 5-HT_7, and inhibitory 5-HT_{1B} receptors in striatal ChAT-IR interneurons. This cellular profile may suggest that 5-HT will bias striatal ChAT-IR interneurons toward excitation.

Medium spiny neurons

In striatal MSNs, the available evidence primarily supports the presence of excitatory 5-HT receptors, including 5-HT_{2A}, 5-HT_{2C}, 5-HT_4, and 5-HT_6 receptors, although inhibitory 5-HT_5 receptors are also present. Given that 5-HT_5 receptors are expressed in a relatively weak manner in the striatum, and have a relatively low affinity for 5-HT by comparison to at least 5-HT_{2A}, 5-HT_4, and 5-HT_6 receptors (Table 1), it is likely that 5-HT will have primarily excitatory direct effects on MSN firing behavior.

Electrophysiological effects of 5-HT in the striatum

In vivo electrophysiology

Although the available evidence suggests that primarily excitatory serotonergic receptor targets are expressed on the major cell types in the striatal circuitry, studies of the effects of 5-HT on neuronal firing have overwhelmingly shown an inhibitory effect. For example, Rebec and Curtis[103] found in paralyzed (not anesthetized) rodents that 5-HT application by microinjection at a 10 μM concentration suppressed firing at neurons close to the

microinjection cannula. Similar results were found in other studies that investigated this issue,[104–106] although in some neurons a brief excitation was followed by inhibition.[104] It should be noted that in at least one case, more units were excited than inhibited.[107] The identities of the recorded units in these in vivo experiments were not further characterized, but, given the ascendant proportion of cells that MSNs represent within the striatum, it is most likely that the recorded neurons are primarily MSNs. Interestingly, although excitatory 5-HT_{2A} receptors are present on MSNs, and further that 5-HT_{2C} receptors are present on all 3 major striatal neuronal types discussed here, 5-$HT_{2A/2C}$ receptor agonists mimicked the overwhelmingly inhibitory effects of 5-HT application. We were not able to identify any in vivo electrophysiology studies that examined the effects of 5-HT on identified fast-spiking interneurons or cholinergic interneurons within the striatum.

In vitro electrophysiology

In the one in vitro study we identified that attempted to examine the response of MSNs to 5-HT in acutely dissociated cells, the authors found that the majority of cells were excited by the bath application of 5-HT at 10–60 μM concentrations. This result stands in stark contrast to the behavior of putative MSNs recorded in vivo using anesthetized and paralyzed rats, but is in line with the primarily excitatory complement of 5-HT receptors that has been found on striatal MSNs. Given that the in vivo experiments reported above used similarly high 5-HT concentrations, the most probable explanation for this difference lies in the fact that the recorded MSNs were acutely dissociated from the striatal network, which divorces the subject cell from the inhibitory cells that would normally govern its behavior.

Blomeley and Bracci[97] used a slice electrophysiology preparation to demonstrate that high concentrations of 5-HT (30 μM) strongly increased the excitability of striatal fast-spiking interneurons. These authors further demonstrated that this excitatory response was blocked by a 5-HT_{2C} receptor antagonist. Moreover, this research group did not report the presence of any 5-HT mediated inhibitory currents in these cells that could be attributed to 5-HT_{1B} receptors, which would seem to be at odds with the somatic 5-HT_{1B} receptor expression observed in striatal PV-IR interneurons by Egeland *et al*.[38]

In vitro electrophysiological evidence from at least 2 research labs also supports the idea that high concentrations of 5-HT have an excitatory effect on striatal cholinergic interneurons. Blomeley and Bracci[108] found that 30 μM 5-HT excited striatal cholinergic cells and further that the 5-HT_2 receptor antagonist ketanserin antagonized this effect. Similarly, Bonsi *et al*[98] found that 50 μM 5-HT excited striatal cholinergic interneurons, that the 5-$HT_{2A/2C}$ receptor agonist DOI mimicked this effect, and finally that a 5-HT_{2C} receptor antagonist reversed this excitation. This group also showed that the excitatory effect of 5-HT could be reduced by 5-HT_6 and 5-HT_7 receptor selective antagonists.

Hypothesized effects of 5-HT, SSRIs, and vortioxetine on the overall striatal circuit

5-HT and SSRIs

The electrophysiological data on the effects of 5-HT on striatal PV-IR and ChAT-IR firing suggest that 5-HT has an excitatory effect on these cell types. Additionally, the profile of 5-HT receptor expression on striatal MSN cells also suggests that 5-HT should have primarily excitatory direct effects. This is supported by data showing that 5-HT activates MSN cell firing when these cells are dissociated from the striatal network, but it is firmly at odds with the electrophysiological effects of 5-HT on putative MSN cells recorded in vivo.

The most plausible explanation for this apparently paradoxical effect of 5-HT on striatal MSN activity is that the concomitant activation of striatal PV-IR interneurons has a downstream inhibitory action on MSNs that overwhelms 5-HT's direct excitatory effects on these cells. This reasoning is based on the fact that striatal PV-IR interneurons have inhibitory axo-somatic insertions on numerous MSN cells, which will tend to powerfully and negatively modulate MSN activity. Furthermore, each MSN cell receives inhibitory inputs from multiple PV-IR interneurons. This phenomenon, along with the presence of electrical gap junctions between striatal PV-IR cells, will tend to multiply the inhibitory influence of PV-IR interneurons on MSN behavior. It is possible that some of 5-HT's effects on MSN firing are also mediated via striatal ChAT-IR interneurons. However, ChAT-IR interneuron activation is expected to have a mixture of excitatory and inhibitory effects that seem unlikely to clearly bias MSN output in either direction. Therefore, the strong inhibitory effect of 5-HT on MSN firing is most likely mediated primarily through PV-IR interneuron activation.

Based on this line of reasoning, we hypothesize that SSRIs will have primarily inhibitory local effects on striatal MSN firing under acute conditions. Given that 5-HT_{2C} receptors are the only excitatory serotonergic targets identified on striatal PV-IR interneurons, the degree to which this local inhibitory effect of SSRIs is maintained after chronic administration might be dictated by the degree to which 5-HT_{2C} receptors desensitize or downregulate in response to long-term stimulation. Thus, it is possible that the local inhibitory effect of SSRIs on MSN firing will be decreased after

chronic administration. Additionally, given that SSRIs tend to reduce cortical pyramidal neuron firing after chronic administration, striatal PV-IR cells may also receive an attenuated excitatory drive from the cortex after chronic SSRI treatment. Thus, it seems plausible to hypothesize that long-term SSRI treatment will attenuate the reduction in MSN firing. However, we were unable to identify in vivo or in vitro electrophysiological studies of the effects of acute or long-term SSRI administration on striatal neuron firing.

Vortioxetine

Although vortioxetine has markedly different effects by comparison to SSRIs within the frontal cortex, this may not be the case within the striatum. 5-HT_3 and 5-HT_{1A} receptors are largely absent in the striatum, and thus cannot be expected to modulate the effects of this network locally. 5-HT_7 receptors, where vortioxetine acts as an antagonist, have been confirmed in striatal ChAT-IR interneurons, and thus vortioxetine may remove some of the excitatory influence of 5-HT on this cell population. But it is seems unlikely that altered activation of striatal ChAT-IR interneurons will clearly bias MSN output in either direction. 5-HT_{1B} receptors, where vortioxetine functions as a partial agonist in vivo,[76] are thought to primarily exist in the striatum as terminal autoreceptors. Thus vortioxetine's primary 5-HT_{1B} receptor mediated effect in this region will probably be an enhancement of extracellular 5-HT by comparison to an SSRI. However, the question of whether vortioxetine drives greater extracellular 5-HT concentrations in the striatum than an SSRI is currently untested. Additionally, if 5-HT_{1B} receptors are expressed in a meaningful way on striatal PV-IR interneurons, then vortioxetine's 5-HT_{1B} receptor partial agonism may have the effect of attenuating the only inhibitory influence 5-HT would be expected to have on PV-IR interneurons, thereby increasing their excitability and further driving down the activity of MSNs.

Therefore, when considering local effects of vortioxetine within the striatum only, the emerging theme is that vortioxetine will increase striatal PV-IR interneuron activation and reduce the output of striatal MSN projection neurons downstream. Moreover, these inhibitory effects on striatal MSN behavior will be qualitatively similar to those expected from SSRIs. However, the question of how vortioxetine may modulate striatal neuron activity is complicated by the fact that striatal MSN and PV-IR interneurons receive excitatory inputs from the cortex,[80] where vortioxetine increases activity in projection neurons.[18] Thus, vortioxetine may have both local and non-local effects that will tend to activate striatal PV-IR interneurons, while in MSN cells, vortioxetine's local GABA-mediated inhibitory effects and nonlocal excitatory effects may be at odds with one another. These contradictory actions may suggest that vortioxetine will produce a mix of excitatory and inhibitory effects on MSN activity, but we hypothesize that these effects will skew toward inhibition of MSN firing.

Conclusions

The recurrent theme underlying this article is that 5-HT modulates GABA and glutamate neurotransmission differently from brain region to brain region, based simply on the circumscribed expression pattern exhibited by each serotonergic target. Using a more complex analysis of receptor expression and electrophysiological data, we have generated several hypotheses about how 5-HT in general and vortioxetine in particular will modulate the behavior of GABAergic and glutamatergic cells in the PFC and striatum.

Within the PFC, we have hypothesized that acute SSRI administration will have mixed excitatory and inhibitory effects on GABAergic interneurons and principle pyramidal neuron firing that will not clearly bias the output of the overall network strongly in either direction. However, chronic SSRI administration may lead to a reduction in glutamatergic pyramidal neuron firing. Within the striatum, SSRIs will acutely reduce the activity of MSNs by enhancing the activity of GABAergic fast-spiking interneurons, and this effect may be attenuated by chronic administration. Recent computational modeling studies have suggested that increased activity in fast-spiking interneurons and the resulting reduction in MSN activity may contribute to reduced impulsive behavior.[109] Although, to our knowledge, this idea has not yet been empirically evaluated, it is consistent with preclinical studies showing that SSRIs reduce some aspects of impulsivity.[110,111]

By contrast, vortioxetine is hypothesized to reduce PFC GABAergic inhibitory tone through a variety of serotonergic mechanisms, including 5-HT_3 receptor antagonism, leading to an increase in the activity of excitatory glutamatergic pyramidal projection neurons. This notion is supported by electrophysiological recordings showing that vortioxetine has strong excitatory effects on putative pyramidal neurons.[18] This increase in cortical glutamate neurotransmission is thought to be responsible in part for vortioxetine's beneficial effects on some aspects of cognitive function.[16] Locally within the striatum, vortioxetine is hypothesized to have qualitatively similar effects as SSRIs, ie, enhancing the activity of striatal GABAergic PV-IR interneurons, leading to an overall reduction in the activation of striatal MSNs. But vortioxetine's effects on striatal MSN function may also be due in part to effects driven by the enhancement of frontal cortex pyramidal neurons, which are thought to

make excitatory connections with striatal PV-IR and MSN cells. Thus, the combination of local and nonlocal effects of vortioxetine may ultimately lead to mixed excitatory and inhibitory effects on striatal MSN output, although in our view, vortioxetine is most likely to bias MSN function toward inhibition. This hypothesis clearly needs to be confirmed by empirical data.

Future research should evaluate these hypotheses in further detail by examining the effects of vortioxetine on cortical and striatal signaling pathways. Additionally, if technically feasible, then future studies should investigate vortioxetine's effects on GABA and glutamate neurotransmission (eg, pyramidal neuron firing) in awake animals during the conduct of a cognitive task. Ideally, it will be important to include translational demonstrations of these effects of vortioxetine in humans. However the technical feasibility of such a demonstration is uncertain, given current limitations in non-invasively measuring GABA and glutamate neurotransmission.

Limitations

There are a number of important limitations to consider when evaluating this body of work. First and foremost, there is a relative paucity of cell-type specific 5-HT receptor expression or electrophysiological data available for the striatum, which implies uncertainty around hypotheses generated for the effects of 5-HT, SSRIs, and vortioxetine. Additionally, we do not currently have electrophysiological data on vortioxetine's effects in the striatum with which to check the validity of these hypotheses. Furthermore, the available electrophysiological data on how 5-HT modulates striatal neurotransmission are generally characterized by the use of high concentrations, which may not be physiologically relevant. It is important to note that the hypotheses of the effects of 5-HT, SSRIs, and vortioxetine are primarily developed using anatomical and electrophysiological data from rodents. The biology that drives serotonergic modulation of GABA and glutamate neurotransmission may not translate across species into humans. Additionally, vortioxetine's affinities for some of its receptor targets, most notably 5-HT_{1A} and 5-HT_{7} receptors, are approximately 10-fold weaker at the rodent versions than they are at their human counterparts.[113] Therefore, the degree to which these concepts of serotonergic modulation of GABA and glutamate neurotransmission will translate into humans is unknown at this time.

Disclosures

Alan L. Pehrson, Theepica Jeyarajah, and Connie Sanchez are employees of Lundbeck Research USA, Inc.

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