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Regulation of GABAergic Inhibition by Serotonin Signaling in Prefrontal Cortex

Molecular Mechanisms and Functional Implications

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Abstract

Serotonergic neurotransmission in prefrontal cortex (PFC) plays a key role in regulating emotion and cognition under normal and pathological conditios. Increasing evidence suggests that serotonin receptors are involved in the complex regulation of GABAergic inhibitory transmission in PFC. Activation of postsynaptic 5-HT_2 receptors in PFC pyramidal neurons inhibits $GABA_A$-receptor currents via phosphorylation of $GABA_A$ receptor γ2 subunits by RACK1-anchored PKC. In contrast, activation of postsynaptic 5-HT_4 receptors produces an activity-dependent bi-directional regulation of GABA-evoked currents in PFC pyramidal neurons, which is mediated through phosphorylation of $GABA_A$-receptor β subunits by anchored PKA. On the presynaptic side, GABAergic inhibition is regulated by 5-HT through the activation of 5-HT_2, 5-HT_1, and 5-HT_3 receptors on GABAergic intereneurons. These data provide a molecular and cellular mechanism for serotonin to dynamically regulate synaptic transmission and neuronal excitability in the PFC network, which may underlie the actions of many antidepressant and antipsychotic drugs.

Index Entries: Serotonin receptors; prefrontal cortex; $GABA_A$ receptors; inhibitory synaptic transmission; PKC; RACK1; PKA; AKAP; modulation.

Introduction

The serotonergic system plays a key role in regulating emotional processes and cognitive behaviors in the central nervous system (CNS) *(1–3)*. Dysfunction of serotonergic neurotransmission has long been implicated in the pathogenesis of neuropsychiatric disorders including schizophrenia, depression, and anxiety *(4–8)*. The new generation of antipsychotic agents, which constitutes a major advance in the treatment of schizophrenia, has high affin-

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ity for serotonin receptors *(9–12).* Drugs effective in treating depression also act mainly on the serotonergic system *(13,14).*

The serotonergic system has a broad anatomical distribution in the CNS, mediating widespread effects in central neurons. One of the main target structures for the serotonergic system is prefrontal cortex (PFC), a collection of cortical areas in the most anterior portion of the frontal lobes. The anatomical organization of primate PFC can be divided into three major subregions: 1) dorsolateral, 2) orbitofrontal, and 3) medial PFC. In rats, the prelimbic, infralimbic, and ventral anterior cingulate cortex represent the major subdivisions of PFC *(15).* Based on their patterns of neural connectivity, these regions are thought to be related functionally to PFC in the primate *(16–18).* The entire PFC is highly innervated by serotonergic projections from the brain stem raphe nuclei and dopaminergic projections from the ventral tegmental area *(19,20).* The processed and integrated information is transmitted from PFC to distributed neural networks including cortex, thalamus, hypothalamus, brain stem, basal ganglia, and limbic areas. All subregions of PFC are robustly interconnected, suggesting that they participate in concert in central executive functions. The PFC has long been associated with high-level, "executive" processes needed for complicated goal-directed behavior *(21).* Functional studies in primates emphasize that PFC is particularly critical for a form of short-term information storage described as "working memory" *(22).* The firing of some PFC neurons increases throughout the delay period of a delayed-response task when information must be "held" momentarily and used subsequently to guide correct responses *(22).* The excitability and tuning of PFC neurons are subject to modulatory influences by serotonin and many other neurotransmitters *(23).* The damage of PFC in humans results in disturbances in a variety of functions, such as attention, memory, response selection, and planning.

PFC is composed of two major neuronal populations: 1) glutamatergic pyramidal principal neurons and 2) GABAergic interneurons. Local GABAergic neurons form numerous synapses on pyramidal projection neurons *(24),* exerting strong inhibitory control on the excitatory output of PFC. One important function of the GABAergic inhibition in PFC is to control the timing of neuronal activity during cognitive operations and thereby shape the temporal flow of information *(25).* Serotonergic projections target both types of PFC neurons in a synaptic and nonsynaptic manner *(26).* Recent evidence shows that serotonin (5-HT) neurotransmission is predominantly paracrine, raising the possibility that 5-HT can act on receptors that are distant from its release site *(27).* Specific changes of the PFC serotonin system and PFC neuronal activity have been found in patients with neuropsychiatric disorders *(4,5,7,28–31).* It suggests that in PFC, serotonin plays a crucial and unique role in neural computation associated with the execution of complex tasks involved in cognition and emotion.

The pleiotropic functions of serotonin are afforded by the concerted actions of multiple serotonin receptor subtypes. The 5-HT receptors are composed of several families that can be grouped on the basis of conserved structures and signaling mechanisms *(32).* The 5-HT_1 class of receptor (5-$HT_{1A,\ 1B,\ 1D,\ 1E,\ 1F}$) couples to $G_{i/o}$ proteins to inhibit adenylate cyclase. The 5-HT_2 class of receptor (5-$HT_{2A,2B,2C}$) couples to G_q proteins to stimulate the turnover of phospholipids. The 5-HT_4 class of receptor (5-$HT_{4,\ 6,\ 7}$) couples to G_s proteins to stimulate adenylate cyclase. The 5-HT_5 class of receptor (5-$HT_{5A,\ 5B}$) has unknown coupling. In addition, the 5-HT_3 receptor is a ligand-gated channel. Serotonin can have both inhibitory and excitatory functions in neuronal networks through the coupling of different 5-HT receptors to distinct ion channels *(33).* Such a mechanism allows serotonin to simultaneously remodel excitability in a functionally appropriate manner in a wide variety of cell types and neuronal circuits.

Mice lacking different serotonin receptors exhibit diverse phenotypes including epilepsy syndrome *(34),* increased impulsive aggres-

sion *(35)*, elevated anxiety, and antidepressant-like response *(36)*. Given the critical involvement of $GABA_A$ receptor-mediated inhibitory synaptic transmission in epilepsy, anxiety, and depression *(37)*, the phenotypes in 5-HT receptor knockout mice may be correlated to changes in GABAergic transmission of PFC neurons. Indeed, selective alterations in $GABA_A$ receptors, GABA content and GABAergic interneurons have been discovered in PFC of patients with mental disorders *(30,38–40)*. These lines of evidence suggest that $GABA_A$ receptor is one of the cellular substrates of serotonin in PFC, and dysregulation of GABAergic inhibitory transmission by serotonin in PFC is a major contributing factor in neuropsychiatric diseases *(41)*.

The $GABA_A$ receptor contains an intrinsic chloride channel which is thought to be a heteropentameric structure, assembled by combining homologous subunits from five different classes: α (1–6), β (1–4), γ (1–3), δ and ε (1–2) *(37)*. The subunit composition of $GABA_A$ receptors critically affects the functional properties of $GABA_A$ channels. Multiple PKA and PKC phosphorylation sites have been identified in several $GABA_A$-receptor subunits *(42)*. Protein phosphorylation exerts a powerful regulation of GABA-activated currents in recombinant and native $GABA_A$ channels *(43–46)*. Reviewed here are some data showing how serotonin, by activating different receptor-mediated signaling cascades, regulate $GABA_A$-receptor channels and GABAergic inhibitory synaptic transmission in PFC neurons.

Regulation of $GABA_A$-Receptor Channels and GABAergic Synaptic Transmission by 5-HT_2 Signaling

Multiple G-protein-coupled 5-HT receptors have been identified in PFC pyramidal neurons *(47)*, among which 5-HT_{2A} is one of the most prominent subtypes expressed in almost all (approx 90%) of these cells *(48)*. Application of 5-HT_2-class agonists produces a reduction of $GABA_A$ currents in most of PFC pyramidal neurons, and this effect is blocked by 5-HT_2 antagonists, confirming the mediation by 5-HT_2 receptors *(48)*. Postsynaptic 5-HT_{2A} receptors are concentrated in apical dendrites proximal to the soma in PFC pyramidal neurons *(49)*, while $GABA_A$ receptors exhibit a compartmentalized distribution on postsynaptic domains of GABAergic synapses on the soma and proximal dendrites *(50)*. The overlap in the expression pattern between 5-HT_{2A} and $GABA_A$ receptors suggests that they may be co-localized at some synapses in PFC pyramidal neurons. Such spatial organization would enable direct serotonergic modulation of local responses to inhibitory input, which offers a dynamic regulatory mechanism of synaptic integration, hence neuronal output.

Several lines of electrophysiological evidence indicate that the 5-HT_2 modulation of $GABA_A$-receptor currents is through a mechanism involving stimulation of phospholipid hydrolysis and activation of PKC *(48)*. Moreover, in vitro kinase assays show that treatment of PFC slices with a 5-HT_2 receptor agonist significantly increases the kinase activity of PKC towards $GABA_A$-receptor γ2 subunit, but not the β2 subunit over its already high basal level of phosphorylation *(48)*. These results suggest that activation of 5-HT_2 receptors in PFC can enhance the PKC catalytic activity, and potentiate PKC phosphorylation of $GABA_A$ receptors in a subunit-specific manner. Consistent with previous studies on PKC regulation of recombinant $GABA_A$ receptors *(45,51)*, the data have revealed that increased PKC phosphorylation of Ser-327 in γ2 subunit by 5-HT_2 signaling may play a major role in mediating the serotonergic modulation of $GABA_A$ currents in PFC neurons *(48)*.

The broad substrate selectivity of PKC makes it essential to control the specificity of this enzyme. Emerging evidence has suggested that one important mechanism for signaling enzymes to achieve fidelity and efficacy of signal transduction is through association with anchoring proteins that target to specific subcellular localization *(52–54)*. The compartmen-

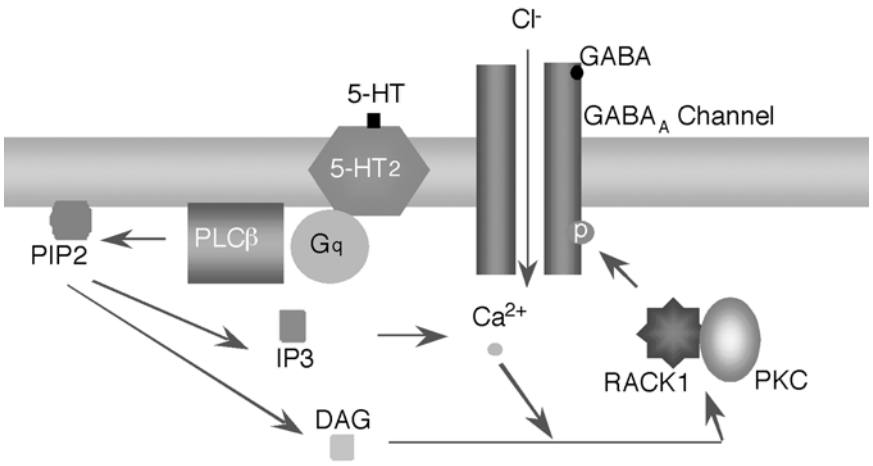

Fig. 1. A diagram illustrating the signal transduction cascade underlying 5-HT_2 regulation of $GABA_A$-receptor channels in PFC pyramidal neurons. Activation of 5-HT_2 receptors stimulates phospholipase C β isoform (PLCβ), leading to the release of ionsitol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) through the hydrolysis of membrane phosphoinositol lipids. Activated PKC is targeted to $GABA_A$ receptors by the association with its anchoring protein RACK1, leading to the phosphorylation of $GABA_AR$ subunits and reduction of $GABA_AR$-mediated currents.

talization and functions of PKC isoforms are also critically dependent on anchoring proteins *(55–57).* RACK1, one member of the RACK family proteins, binds only activated PKC isoforms in a selective manner *(58).* It facilitates the membrane translocation of PKC and PKC phosphorylation of specific substrates *(59–61).* RACK1 is found to strongly associate with neuronal $GABA_A$ receptors in in vitro assays *(62),* suggesting that RACK1 may be responsible for targeting PKC to $GABA_A$-receptor channels and allowing PKC to effectively phosphorylate these receptors. Consistent with this, when the PKC-RACK1 complex is disrupted by a PKC-anchoring inhibitory peptide derived from RACK1, which may lead to the removal of PKC from the proximity of $GABA_A$ receptors, the ability of 5-HT_2 agonists to inhibit $GABA_A$ currents is eliminated *(48).* In contrast, disrupting the binding of PKC to another anchoring protein AKAP79 *(63)* does not affect the 5-HT_2 regulation of $GABA_A$ currents. These results suggest that targeting of activated PKC to $GABA_A$ receptors via RACK1 plays a critical role in the regulation of $GABA_A$ currents by the $5HT_2$/PKC signaling pathway. Thus, activation of 5-HT_2 receptors causes a reduction of $GABA_A$-receptor currents through RACK1-anchored PKC (*see* Fig. 1), which suggests that serotonin signaling may suppress GABAergic inhibition in PFC circuits.

Recordings in frontal cortical slices have shown that application of 5-HT, through activation of 5-HT_{2A} receptors, induces a large, desensitizing enhancement of spontaneous inhibitory postsynaptic currents (sIPSCs), but decreases evoked eIPSCs in pyramidal neurons *(64).* The increase in both frequency and amplitude of sIPSCs and its TTX sensitivity suggest that 5-HT_{2A} receptors can enhance GABAergic inhibitory inputs to PFC pyramidal neurons. One potential reason is that the activation of 5-HT_{2A} receptors on cortical GABAergic interneurons leads to an increase in their excitability *(64),* possibly by inhibiting an inward rectifying potassium conductance *(65,66)* or by activating a cation nonselective current *(67,68),* resulting in the enhanced GABA transmission. On the other hand, the 5-HT reduction of eIPSC amplitude, but not

the paired-pulse depression *(64),* suggests that 5-HT can also decrease the postsynaptic response to GABA in pyramidal neurons, possibly through a 5-HT_2-mediated intracellular signaling cascade *(48).* This 5-HT_2-induced suppression of postsynaptic $GABA_A$-receptors may provide a negative feedback mechanism for 5-HT_2 regulation of GABAergic inhibition in PFC circuits.

Regulation of $GABA_A$-Receptor Channels by 5-HT_4 Signaling

In addition to 5-HT_{2A} receptors, another serotonin receptor subtype that is enriched in PFC pyramidal neurons is 5-HT_4 *(69).* About 60% of these cells express 5-HT_4 receptors *(48).* Emerging evidence contends for a significant role of 5-HT_4 receptors in anxiolysis and cognition *(70).* For example, the anxiolytic effect of diazepam (an enhancer of GABA response) is inhibited by application of 5-HT_4-receptor antagonists, particularly when the serotonergic tone is high *(71).* Activation of 5-HT_4 receptors exerts ameliorative effects on spatial memory tests and reverses cognitive-performance deficits induced by cholinergic hypofunction *(70).* In addition, marked reduction in 5-HT_4-receptor expression has been found in hippocampus and frontal cortex of patients with Alzheimer's disease *(72),* consistent with the potential role of 5-HT_4 receptors in cognition. These findings suggest that 5-HT_4 receptors may play an important role in regulating ion channel activity, therefore affecting synaptic plasticity and neuronal excitability, which is fundamental in learning and memory. Previous studies have found that 5-HT_4-receptor activation exerts an excitatory impact on neurons through regulating different voltage-dependent ion channels *(73–76).* A recent study *(77)* has identified the $GABA_A$-receptor channel as a molecular target of 5-HT_4 receptors in PFC.

Application of 5-HT_4-receptor agonists causes a bi-directional modulation of $GABA_A$ currents in PFC pyramidal neurons, with enhancement in some cells and reduction in others. The dual effects are mediated by PKA, because application of PKA activators mimicks the dual effects of 5-HT_4 agonists on $GABA_A$ currents, while PKA inhibitors block the bi-directional regulation of $GABA_A$ currents by 5-HT_4 agonists *(77).* Due to the broad substrate selectivity of PKA, subcellular targeting through association with anchoring proteins (AKAPs) has been effected as an important mechanism by which PKA achieves specificity in signal transduction *(78–80).* Dialysis of the neurons with a peptide that can specifically disrupt the interaction between AKAPs and PKA *(78)* significantly attenuates the dual effects of 5-HT_4 agonist on $GABA_A$ currents *(77),* suggesting that the 5-HT_4 modulation of $GABA_A$-receptor channels is a highly localized event that requires the fraction of PKA anchored on AKAPs.

Both PKA-induced enhancement and reduction of $GABA_A$ currents have been reported in recombinant systems and native neurons *(43,81).* The dual effects of PKA seem to be attributable to phosphorylation of different $GABA_A$-receptor β subunits, since enhancement occurs when β3 subunit-containing receptors are phosphorylated by PKA on both S408 and S409, whereas reduction occurs when β1 subunit-containing receptors are solely phosphorylated on S409 *(82).* However, single cell RT-PCR profiling results show that both β1 and β3 subunits are co-expressed in PFC pyramidal neurons *(77),* suggesting that reasons other than the differential expression of β subunits may underlie the dual effects of 5-HT_4 agonists on $GABA_A$ currents. A set of experiments suggests that the bi-directional modulation of 5-HT_2 receptors on $GABA_A$ currents is dependent on the basal phosphorylation states of $GABA_A$ receptors *(77).* In the same neuron, manipulations that decrease PKA activation levels switch the effect of 5-HT_4 receptors on $GABA_A$ currents from reduction to enhancement, while manipulations that increase PKA activation levels switch the effect of 5-HT_4 from enhancement to reduction. The molecular

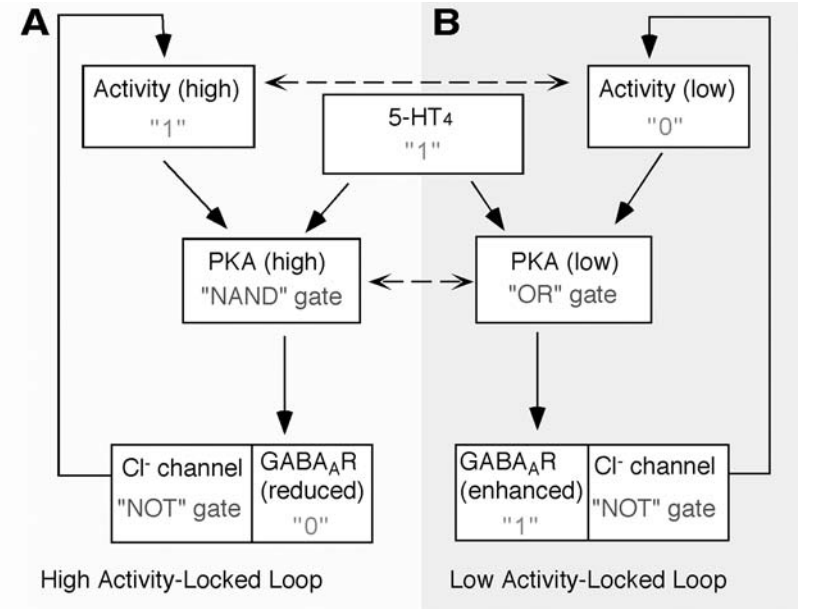

Fig. 2. A neural computational module for the 5-HT_4 actions in PFC pyramidal neurons. For those cells at the "high activity" state, the concomitant activation of PKA to a high level by 5-HT_4 receptors and high neuronal activity leads to a reduction of postsynaptic $GABA_A$-receptor functions ("NAND" gate), and a reduction of GABA-mediated inhibitory synaptic transmission, which results in the neurons being locked at the "high activity" state. However, for those cells at the "low activity" state, the concomitant activation of PKA to a low level by 5-HT_4 receptors and low neuronal activity leads to an enhancement of postsynaptic $GABA_A$-receptor functions ("OR" gate), and an enhancement of GABA-mediated inhibitory synaptic transmission, which results in the neurons being locked at the "low activity" state. "0": logic 0 state; "1": logic 1 state; "NAND" gate: a gate performing the "NOT AND" function, i.e., the output is "0" only when two inputs are both "1";"OR" gate: a gate performing the logic that the output is "1" as long as there is at least one input at "1";"NOT" gate: a gate performing the logic that the output is "1" when the input is "0" and vice versa. (Reproduced with permission from ref. *77*.)

mechanism underlying the changes in the polarity of 5-HT_4 effects remains to be determined. Differential PKA phosphorylation of different β subunits of $GABA_A$ receptors provides one potential mechanism. At low basal activation levels, PKA may preferentially phosphorylate β3 subunits first in response to a 5-HT_4 agonist. This leads to the current enhancement. On the contrary, at high basal activation levels, PKA may cause the phosphorylation of both β1 and β3 subunits in response to the activation of 5-HT_4 receptors. The dominant effect of β1 phosphorylation over β3 phosphorylation leads to the current reduction.

How do different PFC pyramidal neurons have varied basal PKA activation levels under physiological conditions? The role of neuronal activity was further tested *(77)*. Several lines of evidence indicate that the direction of 5-HT_4 regulation is likely to be activity-dependent. First, only reduction of $GABA_A$ currents by a 5-HT_4 agonist is observed in neurons isolated from PFC slices with high neuronal activity. Secondly, the 5-HT_4–mediated enhancement of the amplitude of GABAergic sIPSCs in PFC pyramidal neurons in slices is converted to a reduction after KCI-induced elevation of excitability. Furthermore, it has been found

that the PKA activation level is increased when neuronal activity is enhanced by membrane depolarization in the PFC network. Thus, the direction of 5-HT_4 modulation of $GABA_A$-receptor channels is dependent on the intracellular PKA activation level, which is determined by neuronal activity. Increased neuronal activity can switch the effect of 5-HT_4 receptors on $GABA_A$ channels from enhancement to reduction.

Based on the experimental data, a model which illustrates the potential roles that 5-HT_4 receptors may play in neural computation in PFC pyramidal neurons has been proposed *(77).* The activity-dependent bi-directional regulation of GABAergic signaling by 5-HT_4 receptors could potentially form a neural computational module namely, "Activity-Locked Loop (ALL)" (*see* Fig. 2), which could be simplified to resemble digital circuits controlled by logic gates. This ALL module would allow serotonin, by acting on 5-HT_4 receptors, to use key molecules (e.g. PKA) within a single neuron to perform complex neural computation in maintaining the activity state of a neuron. Non-physiological, activity-uncoupled perturbations of this module at different levels could disrupt the normal reinforcement of this ALL module, leading to altered output that could have dramatic behavioral consequences. This activity-dependent bi-directional modulation of $GABA_A$ receptors by 5-HT_4 receptors provides a novel mechanism for neuromodulators to have either excitatory or inhibitory functions under different physiological conditions *(33,83).* This mechanism ensures that the modulation is flexible, accurate, and dynamic.

Regulation of GABAergic Transmission by 5-HT_1 Receptors

The 5-HT_{1A} receptor is highly concentrated in the limbic system, especially in prefrontal cortex and hippocampus *(84,48),* as well as in the raphe. Postsynaptic 5-HT_{1A} receptors are found only in the dendritic compartment and associated with dendritic spines *(85).* Growing attention is being directed towards developing pharmacological agents that target 5-HT_{1A} receptors for the treatment of schizophrenia, anxiety, depression, and cognition disorders *(86–88).* Increased prefrontal 5-HT_{1A}-receptor density has been found in schizophrenia patients *(28).* 5-HT_{1A}-receptor deficient mice show consistently elevated anxiety alongside antidepressant-like response *(36).* Activation of 5-HT_{1A} receptors has detrimental effects on working memory, and 5-HT_{1A} antagonists ameliorate the cognitive impairment *(89).*

A potential mechanism underlying these actions of 5-HT_{1A} receptors is the change of synaptic transmission and neuronal activity through regulation of ion channels. Previous electrophysiological studies have shown that 5-HT_{1A} receptors can induce a membrane hyperpolarization by activating the inwardly rectifying potassium channels *(90,91).* 5-HT_{1A} receptors are also capable of inhibiting multiple classes of calcium channels *(92,93).* Application of 5-HT_{1A}-receptor agonists has no effect on postsynaptic $GABA_A$-receptor currents in pyramidal neurons of PFC and hippocampus *(48,94).* However, hippocampal slice recordings show that activation of 5-HT_{1A} receptors reduces polysynaptic fast- and slow-inhibitory postsynaptic potentials in CA1 projection neurons *(94).* Presynaptic mechanisms involving the inhibition of GABAergic interneurons have been proposed for the 5-HT_{1A}–mediated reduction of polysynaptic inhibition *(94).* Whether 5-HT_{1A} receptors elicit a similar effect on GABAergic inhibitory synaptic transmission in PFC slices remains to be examined.

Another member of the 5-HT_1 subfamily, 5-HT_{1B}, is expressed in about 60% of PFC pyramidal neurons *(48),* and localized predominantly on axon terminals *(95).* Intracellular recordings show that activation of 5-HT_{1B} receptors in cingulate cortex depresses GABAergic inhibitory postsynaptic potentials evoked by stimulation to the subcortical white matter via a presynaptic mechanism *(66).* These data suggest that activation of presynap-

tic 5-HT_{1B}-receptors reduces the release of GABA at synapses onto PFC pyramidal neurons, and at synapses onto local feed-forward inhibitory interneurons. Similarly, the $GABA_A$-receptor-mediated synaptic input originating from the striatum to substantia nigra pars reticulata neurons is also attenuated by presynaptic 5-HT_{1B} receptors *(96).* The mechanisms underlying 5-HT_{1B}-mediated inhibition of GABAergic transmission are unclear.

Regulation of GABAergic Transmission by 5-HT_3 Receptors

Unlike other G protein-coupled serotonin receptors, 5-HT_3 receptors are ion channels on plasma membrane *(97,98).* An inward current can be induced by 5-HT_3-receptor activation in a variety of neurons *(99,100).* The current is mediated by a cationic influx, activated rapidly upon application of serotonin and desensitized upon prolonged exposure to serotonin. Immunohistochemical studies indicated that 5-HT_3 receptors are expressed only in a subset of GABAergic neurons in neocortex and hippocampus *(101).* Activation of 5-HT_3 receptors in these inhibitory interneurons evokes a large and rapidly desensitizing inward current and induces a transient enhancement of sIPSCs in these brain regions *(64,102).* Though the functional significance of this selective expression of 5-HT_3 receptors is unclear, it is certain that a short pulse of 5-HT can directly excite fast-spiking GABAergic interneurons, leading to a transient increase in GABAergic synaptic activity onto targets innervated by these interneurons. The enhanced inhibition may underlie the 5-HT_3-mediated suppression of the firing rate of PFC neurons *(103).*

Conclusion

The serotonergic system is involved in the regulation of diverse psychophysiological processes including mood, aggression, perception, memory, and anxiety. To mediate this large array of brain functions, a complex set of serotonin receptors has evolved. Alterations of 5-HT-receptor activity have been shown to occur in many psychiatric diseases. A number of effective psychopharmacological agents for these disorders have been developed which either specifically alter brain levels of serotonin or bind to 5-HT-receptor subtypes. To search for new therapeutic agents with higher specificity and efficacy, but lower side effects and toxicity, we need to know more about the physiological functions of serotonin in critical brain regions such as the prefrontal cortex. Genetic and behavior studies have provided important knowledge about the actions of serotonin at the systematic level *(34–36).* The cellular and molecular mechanisms underlying serotonin functions are lacking. By combining electrophysiological, pharmacological, biochemical, and molecular analyses, the data reviewed here demonstrates that by activating different receptors, serotonin plays an important role in regulating GABAergic inhibition in PFC. The information gained from these studies should provide an important basis for understanding physiological functions of serotonergic system in the complex cortical neurocircuits under normal and patahological conditions.

From the postsynaptic loci, 5-HT_2 receptors on PFC pyramidal neurons inhibit $GABA_A$-channel activity *(48),* while 5-HT_4 receptors exert an activity-dependent bi-directional regulation of $GABA_A$ channel activity *(77).* With the convergence of both 5-HT_2/PKC and 5-HT_4/PKA pathways on the same target—postsynaptic $GABA_A$ receptors, serotonin can exert a complex regulation of the GABAergic inhibitory transmission in PFC. For those pyramidal neurons that express 5-HT_2, but not 5-HT_4 receptors, 5-HT may induce a reduction of postsynaptic $GABA_A$-receptor function just through the 5-HT/PKC-mediated cascade. For those pyramidal neurons that express both 5-HT_2 and 5-HT_4 receptors, the 5-HT effect on postsynaptic $GABA_A$ receptors could be complicated. The 5-HT_2/PKC-induced change of

$GABA_A$ receptor γ2 or β2 subunit phosphorylation would compete with the 5-HT_4/PKA-induced change of $GABA_A$ receptor β1 or β3 subunit phosphorylation, and therefore determine the final effect of 5-HT on the channel function. To determine which of the two modifications plays the dominant role may depend on a variety of factors, such as the relative abundance and subcellular localization of 5-HT_2 vs 5-HT_4 receptors, basal levels of phosphorylated $GABA_A$ receptors by constitutively active PKC vs PKA, efficacy in altering $GABA_A$-receptor phosphorylation by the 5-HT receptor-activated PKC/RACk1 vs PKA/AKAP complex, and varied activity states of different PFC pyramidal neurons.

In addition to the postsynaptic modulation of $GABA_A$ receptors by 5-HT in pyramidal neurons, the effect of 5-HT on the excitability of GABAergic interneurons and on the release probability at GABAergic terminals also plays a key role in determining the impact of 5-HT on inhibitory transmission. On one hand, 5-HT could enhance GABAergic transmission by directly exciting GABAergic interneurons via the activation of 5-HT_{2A} or 5-HT_3 receptors *(64).* However, 5-HT could also suppress GABAergic transmission via the activation of presynaptic 5-HT_{1A} or 5-HT_{1B} receptors *(94,66).* To establish which of the two effects plays the dominant role may depend on the expression and properties (e.g., binding affinity and agonist-induced desensitization) of these 5-HT receptors, as well as their coupling to intrinsic ion channels *(64–68)* or release machinery. It has been shown that serotonin at the crayfish neuromuscular junction increases the number of vesicles available for transmitter release, by either an increase in the number of vesicles at each release site, or an activation of previously nonsecreting or silent synapses *(104).* Further studies need to be explored in order to examine whether serotonin in central neurons also regulate synaptic vesicle recycling, and what receptors are mediating this presynaptic effect of serotonin on synaptic transmission.

With so many different receptors acting at both pre- and postsynaptic sites, it is hard to predict what will happen to PFC neuronal activity in response to serotonin. In vivo studies have demonstrated that the predominant effect of 5-HT on cortical pyramidal neurons is an inhibition of spontaneous spiking *(105,106).* The presynaptic increase of GABA transmission mediated by 5-HT_2 and 5-HT_3 receptors *(64),* together with the enhancement of postsynaptic responses to GABA by 5-HT_4 receptors in neurons with "low activity" *(77),* could be one of the underlying mechanisms for the inhibitory actions of 5-HT. However, in vitro, studies suggest that 5-HT induces depolarization and action potential firing in pyramidal neurons *(66,67).* The decrease of postsynaptic responses to GABA by 5-HT_2 receptors *(48)* and by 5-HT_4 receptors in neurons with "high activity" *(77),* as well as the presynaptic suppression of GABA transmission mediated by 5-HT_{1A} and 5-HT_{1B} receptors, could be one of the originating mechanisms for the excitatory actions of 5-HT. Indeed, serotonergic regulation of glutamatergic transmission *(107,108)* could be another important mechanism to regulate PFC neuronal activity. The complex cellular expression and subcellular locations of different 5-HT receptors, their coupling to different intracellular signaling components, and their impact on multiple targets make it too simplistic to catagorize the actions of 5-HT receptors as excitatory vs inhibitory, or cooperative vs antagonistic. However, integration of all the information should help us to understand how serotonin, by acting on various receptor subtypes, modulates the electrophysiological properties of PFC neurons in a coordinated fashion to achieve their precise functions within neuronal networks. Elucidation of signaling components critically engaged in serotonergic actions could provide the potential targets for novel pharmacological agents in the treatment of neuropsychiatric disorders.

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