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Review Article

Serotonergic modulation of Neural activities in the entorhinal cortex

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Abstract: The entorhinal cortex (EC) is considered as the gate to control the flow of information into and out of the hippocampus. The EC is important for numerous physiological functions such as emotional control, learning and memory and pathological disorders including Alzheimer's disease, schizophrenia and temporal lobe epilepsy. Serotonin is a classical neurotransmitter which may modify these physiological functions and pathology of neurological diseases. The EC receives profuse serotonergic innervations from the raphe nuclei in the brainstem and expresses high density of serotonergic receptors including 5-HT_{1A}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{2A}, 5-HT_3 and 5-HT_6. The prominent innervation by serotonergic neurons and the dense expression of serotonergic receptors in the EC suggest that serotonin is a major modulator in this brain region. Serotonin exerts inhibitory effects in the EC. Serotonin hyperpolarizes entorhinal neurons and inhibits the excitatory synaptic transmission via activation of 5-HT_{1A} receptors but facilitates GABA release via activation of 5-HT_{2A} receptors. Both 5-HT_{1A} and 5-HT_{2A} receptors are required for serotonin-induced inhibition of epileptiform activity although 5-HT_3 receptors may be involved in serotonin-mediated inhibition of acetylcholine release in the EC. Furthermore, the functions of serotonin in the EC may be implicated in Parkinson's disease, Alzheimer's disease and depression. Thus, understanding the roles of serotonergic modulation in the EC is of major clinical importance. Here, I review recent findings concerning the effects of serotonin on neural circuitry activity in the EC.

Keywords: Glutamate, GABA, synaptic transmission, epilepsy, neurotransmitter, G-protein coupled receptor

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is a classical neurotransmitter distributed in both the periphery and the central nervous system. Serotonin in the brain has extensive physiological functions including modulation of sleep, mood, emotion, learning and memory. Serotonergic signaling is altered in many neurological disorders such as Alzheimer's disease, Parkinson's disease, schizophrenia and depression. The cerebral cortices including the EC receive prominent serotonergic innervations from the raphe nuclei which are clustered along the midline of the brainstem. The EC mediates the majority of the connections between the hippocampus and other cortical areas [1, 2]. Sensory inputs converge onto the superficial layers (layers II–III) of the EC [3] which give rise to dense projections to the hippocampus; the axons of the stellate neurons in layer II of the EC form the perforant path that innervates the dentate gyrus and CA3 [4] whereas those of the pyramidal neurons in layer III form the temporoammonic pathway that synapses onto the distal dendrites of pyramidal neurons in CA1 and the subiculum [2, 4, 5]. Reciprocally, neurons in the deep layers of the EC (layers V–VI) relay a large portion of hippocampal output projections back to the superficial layers of the EC [6-9] and to other cortical areas [1]. The EC is part of a network that aids in the consolidation and recall of memories [10-13]. Neuronal pathology and atrophy of the EC are commonly observed in Alzheimer's disease [14, 15] and schizophrenia [16-19]. Furthermore, the EC is closely related to the induction and maintenance of temporal lobe epilepsy [20, 21].

In addition to being innervated by serotonergic fibers, the EC also expresses high density of serotonergic receptors. In the following sec-

tions, I will review the expression of different subtypes of serotonergic receptors and experimental evidence concerning the physiological functions and pathological roles of serotonin in the EC.

Serotonergic innervation and distribution of serotonergic receptors in the EC

Serotonin in the central nervous system is released majorly by raphe nuclei in the brainstem. With the technique of combined retrograde fluorescent tracing and immunohistochemistry, the cells that innervate the EC were found to be situated in the caudal half of the dorsal raphe nucleus, the medial part of the median raphe and throughout the rostrocaudal extension of the nucleus reticularis tegmenti pontis [22, 23]. The distribution of 5-HT was detected in both the medial and lateral EC with antibodies against 5-HT in combination with fluorescence histochemistry [23, 24]. Thin, varicose, branching fibers were found to be distributed in a relatively even, diffuse pattern throughout all layers with the highest innervation in layer I (molecular layer) of the EC. A dense network of 5-HT terminals was also observed in layer III. The EC contains the largest amount of 5-HT among all the monoamines [25].

Serotonin interacts with serotonergic receptors. According to their pharmacological, structural, and transductional characteristics, 5-HT receptors are classified into seven subfamilies, 5-HT_1 to 5-HT_7, which comprise 14 receptor subtypes associated with unique genes [26]. All the 5-HT receptors belong to the G protein-coupled receptor superfamily except 5-HT_3 which is a ligand-gated ion channel [26]. The former 5-HT_{1C} was renamed as 5-HT_{2C}, based on its transductional properties and molecular structure.

The EC expresses serotonergic receptors including 5-HT_{1A}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{2A}, 5-HT_3 and 5-HT_6. Experiments that detect the expression of serotonergic receptors usually include autoradiography using radiolabeled ligands for the receptors, measuring the mRNA for the receptors using in situ hybridization and immunostaining of the receptors with specific antibodies. High density of binding sites for 5-[^{3}H] hydroxytryptamine which may label all the 5-HT receptors was found in layers I and II and layers IV through VI of the EC and moderate to low density of binding was observed in layer III of the EC [27].

Individual 5-HT receptor subtypes have been identified in the EC. The EC expresses high density of 5-HT_1 receptors. High densities of binding sites for the selective 5-HT_{1A} agonist tandospirone [28] and 5-HT_{1A} antagonists 8-OH-DPAT [29] or WAY 100635 [30-32] have been detected in each layer of the EC. High level of 5-HT_{1A} mRNA are found in the EC [33] which is one of the brain regions expressing the highest level of mRNA for 5-HT_{1A} receptors [34, 35]. The highest density of immunostaining for 5-HT_{1A} receptors using 5-HT_{1A} receptor antibody is also found in the limbic areas including the EC [36]. In addition to 5-HT_{1A}, the EC also expresses 5-HT_{1D} [37, 38] and 5-HT_{1E} [37] receptors.

For 5-HT_2 receptors, very high density of ketanserin (selective 5-HT_{2A} antagonist) binding sites is found in layers III and V of the EC [39]. Layer I and layer II of the EC are also labeled by spiperone, an antagonist for both 5-HT_{2A} and D_2-like receptors [40]. mRNAs for 5-HT_{2A} [35] and 5-HT_{2C} [41] are found in the EC. The EC also expresses 5-HT_{2A} proteins [42].

For 5-HT_3 receptors, the homogenates of rat EC contain high-affinity binding sites for 5-HT_3 receptor antagonists, GR65630 [43], zacopride [44, 45] and LY278584 [46]. Autoradiography has also detected high binding sites for GR65630 [47] and (S)-zacopride [48, 49]. Whereas the potent 5-HT_3 receptor antagonist (S)-zacopride only labels 5-HT_3 receptor binding sites, the (R)-enantiomer, (R)-zacopride, labels these receptors and another class of high-affinity binding sites, named the R sites, in membranes from the rat cerebral cortex and NG 108-15 clonal cells [50]. Further study suggests that the R sites and 5-HT_3 receptors are different molecular species [51]. Therefore data obtained by (R)-zacopride should include 5-HT_3 receptors and another unidentified molecular entity. In situ hybridization demonstrates that high density of 5-HT_3 receptor mRNA exists in the EC [52]. Furthermore, 5-HT_6-like immunoreactive material is abundant in the EC [53].

Physiological functions of 5-HT in the EC

Activation of 5-HT_3 receptors inhibits acetylcholine (ACh) release in the EC

5-HT_3 receptors are ligand-gated cation channels. Whereas the binding sites for 5-HT_3 ligands and 5-HT_3 receptor mRNA have been detected in the EC, a direct functional identifi-

cation of 5-HT_3 in the entorhinal neurons has not been determined. Nonetheless, activation of 5-HT_3 receptor has been shown to reduce ACh release in the EC [54]. Furthermore, applications of 5-HT_3 receptor antagonists, ondansetron and granisetron, concentration-dependently increase both spontaneous and K^+-evoked ACh release in the entorhinal slices whereas application of 5-HT_3 receptor agonists exerts no effects on ACh release but fully blocks the ondansetron-induced enhancement in both spontaneous and K^+-evoked ACh release [55]. These results suggest that activation of 5-HT_3 receptors tonically inhibits ACh release in the EC. However, a late study demonstrates that no significant inhibition or increase in K^+-evoked ACh release is observed with either 5-HT_3 receptor agonists or antagonists [56] casting doubts on the effects of 5-HT_3 receptor activation on ACh release in the EC.

The mechanisms underlying 5-HT_3 receptor-mediated inhibition of ACh release are unclear. The release of ACh in the EC is Ca^{2+}-dependent and tetrodotoxin-sensitive. Application of $GABA_A$ receptor antagonists bicuculline and flumazenil by themselves remarkably potentiates ACh release in the EC [55]. The $GABA_A$ receptor antagonists potentiates ondansetron-induced increases in ACh release in a tetrodotoxin-sensitive manner but does not modify the facilitatory effects of MDL 72222 and granisetron, other two 5-HT_3 receptor antagonists [57]. Application of the $GABA_A$ antagonists in a superfusion medium deficient in Cl^- also potentiates ACh efflux. ACh release is also increased by the nonspecific K^+-channel blockers TEA and Ba^{2+} but bicuculline does not modify the effects of TEA and Ba^{2+}. These results support the functional interaction of ondansetron with GABAergic interneurons in the rat EC and GABA-independent mechanisms may be involved in the regulation of cortical cholinergic function by other 5-HT_3 receptor antagonists.

There are further controversies as to the subtypes of 5-HT receptors and the mechanisms involved in the inhibitory effects of serotonin on ACh release in the EC. Serotonin inhibits ACh release induced by depolarization evoked electrically or by high K^+ (20 mM) via activation of 5-HT_{1B} receptors located on cholinergic terminals [58]. However, this inhibition requires the functional elimination of the substance P-containing GABAergic interneurons which express 5-HT_{2A} receptors as shown by in situ hybridization. Activation of these somadendritically located 5-HT_{2A} receptors facilitates the release of substance P which in turn, stimulates ACh release through NK1 receptors present on cholinergic terminals [58].

Modulation of membrane conductance of EC neurons

Serotonin has been shown to induce membrane hyperpolarization of layer II stellate neurons and pyramidal neurons in layer II and III resulting in inhibition of action potential firing frequency (**Figure 1**) [59-63]. Serotonin-induced hyperpolarization is accompanied with the generation of an outward current and a reduction of input resistance suggesting that serotonin opens a conductance to produce membrane hyperpolarization. It is generally agreed that serotonin activates a background K^+ channel to hyperpolarize entorhinal neurons. The involved K^+ channels belong to the family of the two-pore domain K^+ channels [63]. However, the contribution of other ionic channels cannot be completely excluded because serotonin has been shown to evoke a biphasic response consisting of a moderately short latency and large amplitude hyperpolarization followed by a slowly developing, long lasting, and small amplitude depolarization in layer II projection neurons [59]. The 5-HT-induced depolarization is accompanied with an inward current which is sensitive to ZD7288, a blocker for the hyperpolarization-activated channels (H-channel) suggesting multiple ionic mechanisms underlying the effects of serotonin in the EC.

Consistent with the abundant expression of 5-HT_{1A} receptors in the EC, 5-HT-induced hyperpolarization is mediated via activation of 5-HT_{1A} receptors [59-63] whereas 5-HT-mediated late depolarization in layer II projection neurons is independent of 5-HT_{1A} receptors [59].

Modulation of excitatory synaptic transmission in the EC

Glutamate is the major excitatory neurotransmitter in the EC. An initial *in vivo* experiment suggests that 5-HT may facilitate synaptic transmission in the EC. Intraperitoneal injections of 5-HT precursor, 5-hydroxytryptophan, and the 5-HT_{1A} receptor agonist, 8-OH-DPAT, into the urethane-anesthetized rats facilitate

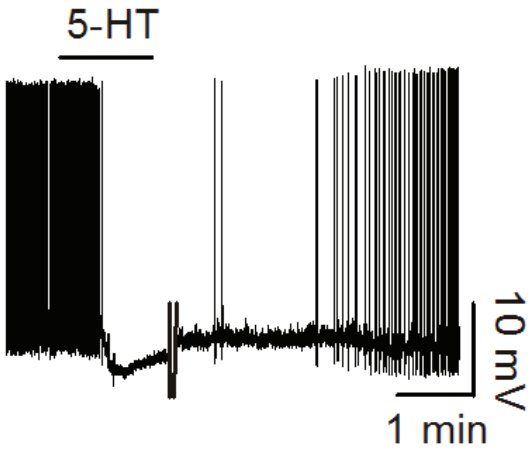

Figure 1. 5-HT depresses the firing frequency of action potentials recorded from a stellate neuron in layer II of the EC (unpublished data).

synaptic transmission between the EC and the dentate gyrus *in vivo* [64]. However, *in vitro* experiments from entorhinal slices demonstrate that 5-HT inhibits synaptic transmission in the EC. Iontophoretic application of 5-HT reduces the depolarization evoked by exogenous application of glutamate but has no apparent action on neuronal responses to iontophoretically ejected GABA in the pyramidal neurons of layers II/III in entorhinal slices [65]. The 5-HT-mediated attenuation of glutamate response persists in the medium containing $CdCl_2$ to block synaptic transmission. Serotonin has no effects on the release of endogenous glutamate measured by a fluorometric enzyme assay. This study suggests that the depressant effect of 5-HT on the response evoked by exogenous application of glutamate is not mediated by modulation of presynaptic glutamate release but due to an effect on glutamate receptors. However, further studies demonstrate that 5-HT decreases presynaptic glutamate release (see below).

The actions of serotonin on excitatory synaptic transmission in the EC were further probed by recording synaptic responses in the entorhinal slices. Serotonin reduces stimulus-evoked EPSPs/EPSCs recorded by whole-cell patch-clamp and intracellular recordings from layers II and III principal neurons [61] and field potentials recoded in the superficial layers [66] of the EC. The depressant effects of 5-HT are presynaptic based on the following lines of evidence. 1) both NMDA and AMPA receptor-mediated responses are reduced to similar extents by 5-HT [60-62]; 2) 5-HT-induced depression is similar in whole-cell patch-clamp versus intracellular recordings, does not require intracellular GTP, and is not visible in glutamate applications to excised patches; 3) 5-HT reduces the frequency with no effects on the amplitude of miniature EPSCs recorded in the presence of tetrodotoxin and bicuculline; 4) 5-HT-mediated depression of field potentials is associated with a significant increase in paired-pulse facilitation [66]. Therefore, 5-HT suppresses excitatory synaptic transmission by reducing presynaptic glutamate release not by inhibiting the functions of postsynaptic glutamate receptors.

The 5-HT_{1A} receptors are also identified to be responsible for 5-HT-induced depression of excitatory synaptic transmission in the EC [61, 66, 67]. The inhibitory effects of 5-HT on EPSPs are mimicked by 5-HT_{1A} receptor agonists but reduced by 5-HT_{1A} receptor antagonists. However, the ionic and signaling mechanisms underlying 5-HT-induced depression of excitatory synaptic transmission in the EC still need to be elucidated.

Modulation of GABAergic transmission in the EC

The principal neurons in the EC receive GABAergic innervations. The effects of 5-HT on GABAergic transmission in the EC have also been explored in the EC. Initial study demonstrate that 5-HT has no apparent action on neuronal responses to iontophoretically ejected GABA in the EC suggesting that 5-HT has no effects on postsynaptic $GABA_A$ receptors [65]. In the entorhinal slices, 5-HT increases both the frequency and amplitude of spontaneous IPSCs recorded from the principal neurons with no effects on the frequency and amplitude of miniature IPSCs recorded in the presence of tetrodotoxin [68]. However, 5-HT reduces the amplitude of IPSCs evoked by extracellular field stimulation and in synaptically connected interneuron and pyramidal neuron pairs. Another study also demonstrates that 5-HT inhibits evoked IPSPs in the EC [67]. Because 5-HT does not modulate the miniature IPSCs which suggests that 5-HT has no effects on postsynaptic $GABA_A$ receptors, the effects of 5-HT on GABAergic transmission should be presynaptic in origin. Because the spontaneous, miniature and evoked IPSCs represent different status of

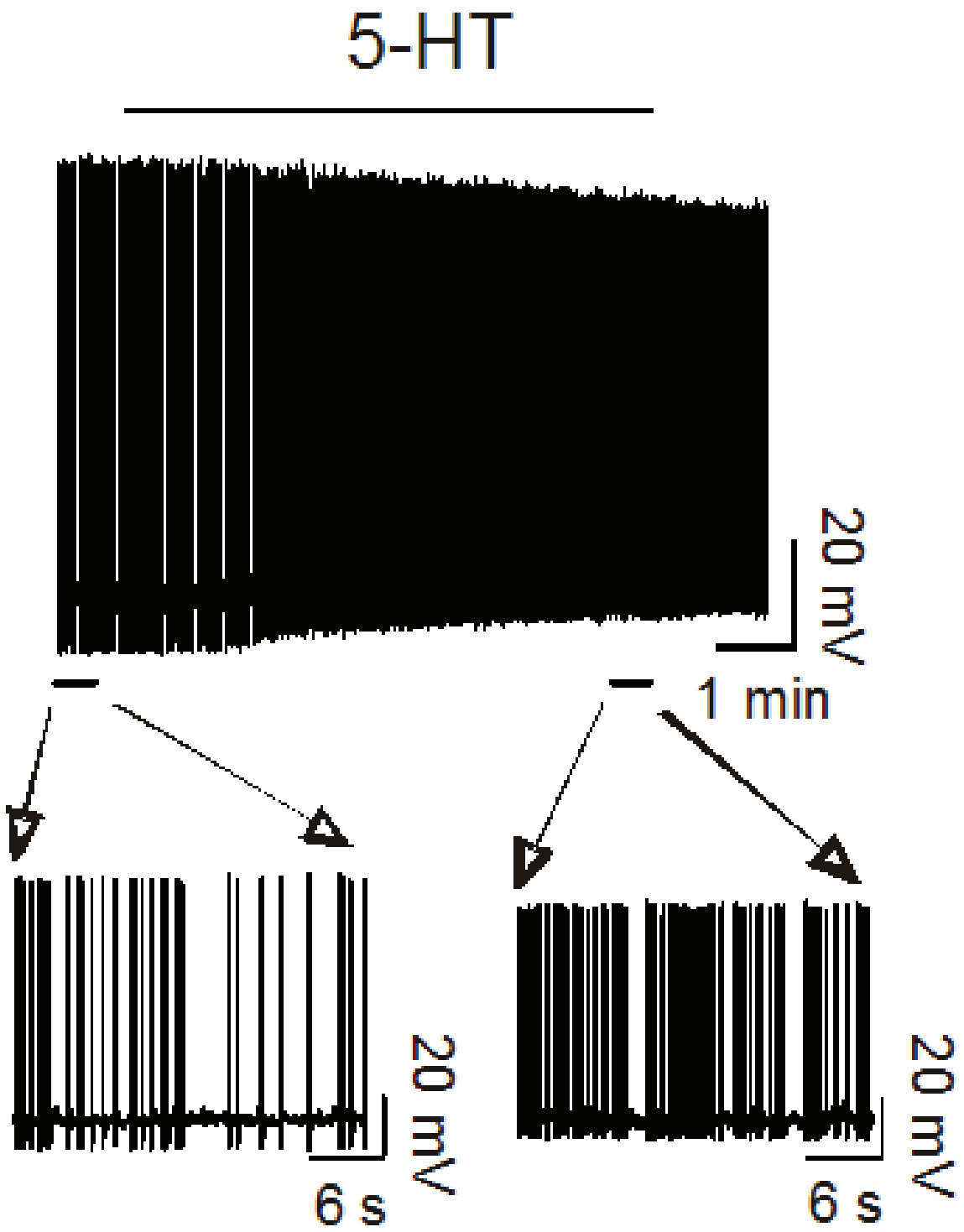

Figure 2. 5-HT enhances the firing frequency of action potentials recorded from an interneuron in the EC (unpublished data).

inhibitory synaptic circuitry, these results suggest that 5-HT exerts diverse functions according to the distinct physiological conditions of neural circuit. 5-HT generates membrane depolarization and increases action potential firing frequency but reduces the amplitude of action potentials recorded directly from presynaptic GABAergic interneurons (**Figure 2**) suggesting

that 5-HT increases GABA release whereas the depressant effects of 5-HT on evoked IPSCs could be explained by 5-HT-induced reduction in action potential amplitude [68]. The depolarizing effect of 5-HT is mediated by inhibition of TASK-3 K^+ channels in interneurons and requires the functions of 5-HT_{2A} receptors and $G\alpha_{q/11}$ proteins but is independent of phospholipase C activity [68]. Consistent with the electrophysiological data, double immunofluorescence confocal microscopy demonstrates the colocalization of 5-HT_{2A} receptors with GABA in the EC [42].

Pathologic roles of 5-HT in the EC

The EC is an indispensable structure participating in the induction and maintenance of temporal lobe epilepsy [20, 21]. The actions of 5-HT on epilepsy have been studied mainly in entorhinal slices. Application of 5-HT to entorhinal slices attenuated the length of epileptiform bursts induced by bath application of the $GABA_A$ receptor inhibitor bicuculline [65]. Lowering the concentration of Mg^{2+} in the extracellular solution in entorhinal slices generates epileptiform activity characterized by an initial expression of seizure-like events followed by late recurrent discharges and bath application of 5-HT blocks the epileptiform activity induced by low Mg^{2+} [68, 69]. Furthermore, application of the 5-HT-releasing agent fenfluramine reversibly blocks epileptiform activity induced by omission of the extracellular Mg^{2+} [70]. 5-HT-induced depression of epileptiform activity is related to 5-HT_{1A} [68, 70] and 5-HT_{2A} [68] receptors. The antiepileptic effects of 5-HT likely represent its inhibitory effects on entorhinal neuronal excitability [59-63] and excitatory synaptic transmission [60-62, 66] and its facilitatory effects on GABA release [68].

The EC is also a predilection site for the pathological alterations underlying Parkinson's disease [71, 72]. The functions of 5-HT are also implicated in Parkinson's disease. A reduction of serotonin level was observed in Parkinson's disease [73] and serotonin fibers in the EC are dystrophic in the brains of individuals with Parkinson's disease [74]. Furthermore, the maximal density of the binding of the selective 5-HT_3 antagonist GR 65630 was reduced in the entorhinal homogenates on the side lesioned with 6-hydroxydopamine [75] suggesting a role of 5-HT in the neuropathology of Parkinson's disease.

Pathological alterations of Alzheimer's disease first occur in the EC [14, 15]. Electrochemical oxidation of 5-HT produces 4,5-diketotryptamine (4,5-DKT) and administration of 4,5-DKT into the lateral ventricles of rats results in cell death and terminal degeneration in the EC [76, 77]. Furthermore, the density of 5-HT_2 receptors was reduced to 45% in postmortem patients with Alzheimer's disease [78]. A significant reduction in serotonin transporter sites was also observed in the EC in Alzheimer's disease [79].

The EC is an important limbic structure involved in emotional control and it is well-known that serotonin plays an important role in emotional control. The level of serotonin is reduced in the EC of demented patients with depression [80] suggesting that the modulatory effects of 5-HT in the EC contribute to depression as well.

Future directions

The EC is a brain region receiving the most abundant serotonergic innervations from the raphe nuclei. The EC also expresses numerous subtypes of serotonergic receptors including 5-HT_{1A}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{2A}, 5-HT_3 and 5-HT_6. Whereas the functions of 5-HT_{1A} (inhibition of neuronal excitability and excitatory synaptic transmission), 5-HT_{2A} (facilitation of GABA release), 5-HT_3 (inhibition of ACh release) receptors are beginning to be revealed, the functions of other 5-HT receptor subtypes have not been determined. Moreover, the EC is an indispensable structure involved in learning and memory and undergoes synaptic plasticity. However, the potential roles of 5-HT in the modulation of long-term potentiation (LTP), long-term depression (LTD), learning and memory in the EC have not been determined. Whereas there is convincing evidence demonstrating an antiepileptic action of 5-HT in *in vitro* slice models of epilepsy, the *in vivo* roles of 5-HT in the EC in antagonizing epilepsy have not been determined. This area is worthy of further investigation because the temporal lobe epilepsy is usually resistant to most antiepileptic drugs and agonists for 5-HT_{1A} and 5-HT_{2A} could be novel antiepileptic drugs. Furthermore, more direct evidence is required to define the roles of 5-HT in other neurological diseases including

Parkinson's disease, Alzheimer's disease and depression.

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