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Excitatory/inhibitory balance of serotonergic axon connectivity in the brain

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ABSTRACT

Serotonin neurons originate from the brainstem raphe nuclei and innervate the entire brain to regulate mood, emotion, sleep, appetite and aggression. Previous electron microscopy (EM) studies have revealed that 5-HT boutons directly contact several neuronal populations via asymmetrical (excitatory) or symmetrical (inhibitory) synapses. Additionally, 5-HT boutons sometimes form "triads" with the pre and postsynaptic components of asymmetrical or symmetrical synapses to modulate their activity. However, the exact proportion and distribution of 5-HT excitatory/inhibitory synapses and triads within the entire brain remains poorly described. Recently, we have published a novel semi-quantitative approach which combines fluorescent confocal microscopy and 3D reconstruction of 5-HT fibers apposed to excitatory and inhibitory neurochemical synapses (triads). Here, we review the similarities and differences in the distribution of 5-HT asymmetrical/symmetrical synapses observed in EM and the distribution of 5-HT excitatory/inhibitory triads quantified in our recent study. We further put into perspective the possible physiological role played by 5-HT triads in the regulation of glutamate and GABA signaling in these various brain regions.

Introduction

Serotonin (5-Hydroxytriptamine, 5-HT) neurons have been extensively involved in the regulation of mood[1], sleep[2], learning[3], memory[4], cognition[5] and impulsivity[6]. As such, the functional connectivity of 5-HT neurons within the brain is complex and remains poorly understood. Pioneer work from electron microscopy (EM) studies have unraveled the heterogeneous connectivity of 5-HT neurons throughout the rodent brain, revealing that 5-HT neurons send axons to almost all brain regions and modulate various populations of neurons by synaptic contacts that are either asymmetrical (excitatory) or symmetrical (inhibitory)[7]. Similarly, some of these electron microscopy studies have also allowed the observation of 5-HT-containing boutons that synapse to the pre or the postsynaptic components of asymmetrical or symmetrical synapses to form "triads". These triads have been observed in the cortex[8], the nucleus accumbens[9], the hippocampus[10], the amygdala[11], the VTA[12] and the striatum[7]. However, the complex and time consuming processing/serial sectioning of samples required for high-quality EM have not allowed the aforementioned studies to quantify these triads in the corresponding brain regions.

Therefore, we have recently developed a semi-quantitative

method combining high-resolution confocal microscopy and 3D reconstruction of 5-HT transporter SERT-positive axons to map the distribution of 5-HT boutons apposed to either excitatory or inhibitory neurochemical synapses in the mouse limbic brain[13]. Briefly, we labeled serotonin transporter (SERT) immunoreactive axons and reconstruct in 3D their distribution within limbic brain regions. We also labelled key pre- (synaptophysin) and postsynaptic components of excitatory (PSD95) or inhibitory (gephyrin) synapses. Using the masking function in IMARIS software, we were able to isolate the synaptophysin punctate labelling inside the SERT-positive fibers (SYNin) to identify 5-HT boutons. In parallel, we used the same masking function to isolate the synaptophysin puncta outside SERT fibers (SYNout) and their engagement into excitatory (SYNout/PSD95 spot pairs) or inhibitory (SYNout/GEPH spot pairs) neurochemical synapses. Finally, we quantified the density of 5-HT boutons forming "triads" with either excitatory or inhibitory neurochemical synapses in various limbic regions of the mouse brain[13].

Importantly, the distribution of excitatory vs inhibitory triads in our study was quite similar to the distribution of direct asymmetrical (excitatory) vs symmetrical (inhibitory) synapses made by 5-HT boutons, as observed in previous electron microscopy studies. This suggests that the excitatory/inhibitory balance of 5-HT axon connectivity is a common structural feature shared by both 5-HTergic direct synapses and synaptic triads in the rodent brain. Here, we review the similarities and differences in the distribution of 5-HT synapses and triads throughout various brain regions where serotonin is known to play an important regulatory role, with an emphasis on the physiological significance previously demonstrated in functional studies.

Cortex

Previous electron microscopy studies in rat motor, visual and somatosensory cortices[8] found that only 20-40% of 5-HT-immunolabelled varicosities (boutons) were involved in synaptic contacts. These contacts were essentially asymmetric on dendritic spines and branches. Similar results were observed in rat frontoparietal cortex using Tryptophan hydroxylase (TPH) immunolabelling[14] or in the prefrontal cortex using SERT immunolabelling[15]. In the prefrontal cortex of monkeys, only 23% of labelled 5-HT boutons were engaged in synaptic contacts, mostly asymmetrical and formed on dendritic shafts[16]. However, in the auditory and sensorimotor cortices of cats and monkeys, respectively, a relatively low synaptic incidence (2-3%) was demonstrated, but again synaptic contacts were all asymmetrical[17,18]. Additionally, in the upper layers of various cortical regions, 5-HT terminals were often apposed to non-5-HT axons engaged in asymmetrical synapses in a triadic formation[8]. This suggests a potential role of cortical 5-HT in the regulation of neurotransmitter release. In line with this, we showed that 5-HT boutons essentially form synaptic triads with excitatory synapses in the upper layer of the mouse prefrontal cortex and were located closer to the presynaptic than the postsynaptic specializations, also suggesting a role of 5-HT in the regulation of excitatory neurotransmitter (glutamate) release[13]. The potential role of 5-HT in the regulation of excitatory transmission has been demonstrated in electrophysiology studies showing a 5-HT-mediated increase in glutamate release and amplitude of glutamatergic EPSCs at the apical dendrites of pyramidal neurons[19]. Blockade of 5-HT receptors (5-HT2A) was also shown to prevent local glutamate release in the medial prefrontal cortex (mPFC) in response to NMDA glutamate receptor antagonist[20].

Hippocampus

In the rat *stratum oriens* layer of CA3, a region known to have the highest density of 5-HT fibers in the hippocampus[21], about 20% of immunolabelled 5-HT boutons were involved in synaptic contacts, with a greater proportion of asymmetrical synapses, only on dendritic shafts. We found that 5-HT boutons form almost twice as much triadic contacts with excitatory than inhibitory neurochemical synapses in the CA3 region of the mouse hippocampus, suggesting that 5-HT signaling is more involved in the regulation of excitatory transmission in this brain region. Similarly, strong evidence has suggested a preferential involvement of 5-HT in the negative regulation of glutamate signaling in other hippocampal regions including CA1 and CA2[22–26]. This was further shown to play an important role in the regulation of long term potentiation (LTP)[27,28]. However, functional studies in the CA3 region have demonstrated that 5-HT both exerts a direct positive modulation of glutamate receptors[29] and depress the $GABA_B$ receptor component of GABA-mediated IPSPs[30–32], suggesting a 5-HT-mediated regulation of inhibitory synapses as well.

Nucleus Accumbens - NAC

In the NAC core and shell, 5-HT labelled boutons were shown to have a relatively high synaptic incidence (39 and 46%, respectively[9]) as compared to other brain regions. The great majority of these 5-HT boutons were in apposition with terminals that often form asymmetric contacts with dendrites, suggesting that 5-HT boutons likely modulate the activity of excitatory axons[9]. We also found a higher proportion of triadic contacts with excitatory synapses (21%) compared to inhibitory synapses (3%) in the NAC core. This result suggests that 5-HT signaling may preferentially modulate the activity of excitatory synapses in this brain region. However, in the NAC shell, we observed an equal distribution of 5-HT triads onto neurochemical excitatory and inhibitory synapses. The NAC shell was

previously shown to be innervated by two functionally different types of 5-HTergic axons that either contain or lack the serotonin transporter SERT[33]. Therefore, our method of labeling 5-HT fibers with an antibody directed against the SERT may have revealed a specific subtype of 5-HTergic axons that is equally engaged in triads with excitatory and inhibitory synapses. Similar differences have been observed in electron microcopy studies where the use of a 5-HT antibody allows for the labelling of 5-HT boutons forming mostly asymmetrical synapses[9], whereas the use of a SERT antibody allowed for the labelling of 5-HT boutons forming both symmetrical and asymmetrical synapses in the rat NAC shell[34]. Interestingly, we found a significantly higher proportion of 5-HT boutons located closer to the presynaptic component of putative inhibitory synapses within the NAC shell, suggesting that 5-HT could have a modulatory effect on GABA release from GABAergic synapses in this region. In addition, functional studies have also revealed a 5-HT-mediated control of glutamate release in rat NAC core and shell slices via activation of presynaptic 5-HT1B receptors[35], however, we did not observe any preferential distribution of 5-HT boutons to the pre- or the postsynaptic specialization of excitatory triads. Whether 5-HT modulates only glutamate release from presynaptic terminals or also postsynaptic activity of glutamatergic synapses in the NAC still needs to be determined.

Ventral Tegmental Area - VTA

Serotonin axons are abundant in the VTA, and it is likely that half of the EM-labelled 5-HT boutons are involved in synaptic contacts, almost exclusively on symmetrical (inhibitory) synapses, as evaluated by quantification extrapolated from a single thin section study in the rat brain[36]. Importantly, we found that the 5-HT triads were exclusively formed with inhibitory neurochemical synapses in our study, which suggests that 5-HT plays a pivotal role in the regulation of inhibitory transmission in the VTA. In line with these findings, limited evidence supports a role of 5-HT in the regulation of glutamate signaling in the VTA, while there is an extensive literature showing a 5-HT-mediated regulation of GABAergic neurotransmission. For instance, in rat VTA slices, 5-HT_{1B} receptor activation was shown to inhibit GABA release[37,38] as well as $GABA_B$-mediated IPSCs[39]. Furthermore, the reduction in $GABA_B$ inhibitory postsynaptic potentials in dopamine neurons of rat VTA slices induced by cocaine was found to be mediated by 5-HT1B receptor[39] which, in turn, facilitates cocaine-induced increases in dopamine levels in the NAC core[40,41]. This suggests that in the VTA, 5-HT signalling is principally involved in the control of inhibitory transmission.

Conclusion

Serotonin axon connectivity is highly heterogeneous along the rostro-caudal axis and, interestingly, the balanced distribution of asymmetrical/symmetrical synapses observed in electron microscopy studies follows a pattern similar to the distribution of 5-HT excitatory/inhibitory triads in our recent study (**Figure 1**). Although asymmetrical and symmetrical synapses have been shown to express PSD95[42] and gephyrin[43] respectively, there is so far no evidence that 5-HT asymmetrical or symmetrical synapses are excitatory or inhibitory, or whether 5-HT postsynaptic specializations express PSD95 or gephyrin. Further studies are therefore needed to identify specific markers of 5-HT synapses and provide a more comprehensive understanding of 5-HT axon connectivity.

**The authors declare that there is no conflict of interest.*

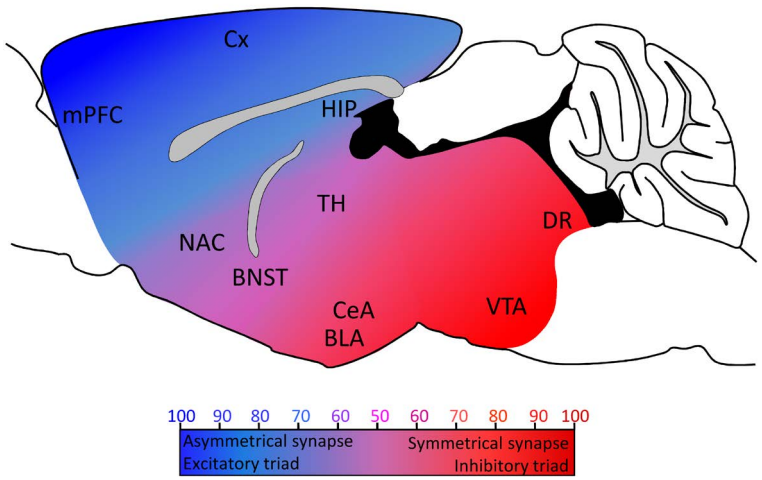

Figure 1: Distribution of 5-HT synaptic contacts and triads in the rodent brain. Cx: cortex; mPFC: medial prefrontal cortex; NAC: nucleus accumbens; BNST: bed nucleus of the stria terminalis; TH: thalamus; CeA: central nucleus of the amygdala; BLA: basolateral nucleus of the amygdala; VTA: ventral tegmental area; DR: dorsal raphe. Color coded from blue to red represents the heterogeneous distribution of 5-HT axons connectivity (blue: asymmetrical synapses/excitatory triads; red: symmetrical synapse/inhibitory triads. *Adapted from* [8–41].

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