Serotonin: a regulator of neuronal morphology and circuitry

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Abstract

Serotonin is an important neuromodulator associated with a wide range of physiological effects in the central nervous system. The exact mechanisms for how serotonin influences brain development are not well understood, although studies in invertebrate and vertebrate model organisms are beginning to unravel a regulatory role for serotonin in neuronal morphology and circuit formation. Recent data suggests a developmental window during which altered serotonin levels permanently impact circuitry, however, the temporal constraints and molecular mechanisms responsible are still under investigation. Growing evidence suggests that alterations in early serotonin signaling contribute to a number of neurodevelopmental and neuropsychiatric disorders. Thus, understanding how altered serotonin signaling affects neuronal morphology and plasticity, and ultimately animal physiology and pathophysiology, will be of great significance.

Introduction

In addition to its physiological role, growing evidence suggests the neuromodulator serotonin (5-hydroxytryptamine, 5-HT) also regulates the connectivity of the brain by modulating developmental cellular migration and cytoarchitecture. Data obtained from multiple animal models also support the hypothesis that serotonin autoregulates serotonergic branch morphology. Therefore, the influence of serotonin on neuronal morphology is inherently complex and alterations in serotonergic modulation may have unexpected effects on brain morphology, physiology, and behavior. Serotonin levels during development may be altered by a number of factors, including nutrition [1], stress [2], infection [3], genetic polymorphisms [4], and pharmacological compounds such as selective serotonin reuptake inhibitors (SSRIs) [5] and certain drugs of abuse. Thus, disorders associated with faulty neural connectivity or innervation may be rooted in early circuit errors elicited by primary dysfunction in serotonergic physiology.

Serotonergic innervation is relatively evenly distributed throughout the central nervous system (CNS), indicating that most brain regions receive serotonergic modulation [6,7]. Evidence suggests serotonin is released in an even sprinkler-type fashion termed volume transmission, and functional concentrations of neurotransmitter are maintained several microns from release sites [8]. Serotonin's diverse effects are mediated by a number of receptors distributed throughout the body. To date, at least fourteen different serotonin receptor subtypes have been

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identified in mammals and are grouped into seven families (5-HT$_1$-5-HT$_7$) [112]. All of the serotonin receptors are G-protein coupled receptors except the 5-HT$_3$ ligand-gated ion channel. Figure 1 contains a simplified cartoon of a serotonin release site. Despite the omnipresence of serotonergic innervation, invertebrate and vertebrate models lacking most central serotonergic neurons [7,9,10] or neuronal serotonin synthesis enzymes [11–13] are capable of developing into adulthood with grossly normal brain morphology, although some degree of perinatal mortality is observed. Of the serotonin receptorknockout animals generated thus far, only one, *5-ht2b*, causes embryonic lethality due to defective heart development [14]. However, an important caveat to these studies remains. With the possible exception of the *tph-1 C. elegans* mutant lacking the serotonin biosynthetic enzyme tryptophan hydroxylase (TPH) [12], the serotonin-null animal models generated to date fail to specifically abolish all serotonin detection in the CNS, even when both the central and peripheral serotonin synthesis genes are simultaneously ablated (Table 1). Furthermore, maternally-derived serotonin influences pre-neural embryonic patterning in frog embryos and craniofacial development in mice [15] and may impact early nervous system development in genetic mouse models of central serotonin depletion. The persistence of serotonin detection and survival of animals having reduced serotonin function indicate that redundant mechanisms may ensure adequate serotonin levels during early development and adult animals appear able to adapt to significantly reduced or absent serotonin signaling. The absence of gross brain malformations in these animals suggests that the effects of serotonin on neural morphology are subtle and require analysis on the cellular level in order to be fully appreciated.

Development of serotonergic innervation

Serotonergic differentiation occurs as a result of a transcriptional program driven by early patterning events, and these neurons are generated by embryonic day 12 (E12) in mice (reviewed in Ref. [16]) and within the first gestational month in primates [17]. Serotonergic neurons migrate to and position themselves within the raphe nuclei from the ventricular zone via somal translocation rather than radial-glial guided migration [18]. Subsequent outgrowth and innervation is highly regulated. Adult leech serotonergic interneurons adopt characteristic branching patterns in culture in the absence of external cues [19] and stereotypical serotonergic branching patterns in *Drosophila* larvae form even when initial axonal guidance is disturbed [7]. Typical growth and cell-adhesion mechanisms assist in this initial wiring, as growth associated protein-43 (GAP-43) and protocadherin-a knockout mice exhibit abnormal serotonergic fiber distribution [20,21]. The astrocytic protein S-100β is also a positive regulator of serotonergic outgrowth in culture assays [22] but may not be essential *in vivo*, as S-100β knockout mice exhibit normal serotonergic fiber distribution [23]. Notably, expression of the serotonin transporter (SERT), which is responsible for 5-HT reuptake by the releasing cell, precedes serotonergic neurite outgrowth and synapse formation in mammals and *Drosophila* [24,25], indicating that mechanisms regulating serotonin signaling must be in place before serotonergic terminal arborization occurs. Evidence from multiple animal models suggests that this early regulation may be important for autoregulatory processes guiding serotonergic development.

Evidence for autoregulation of morphology by serotonin

The presence of serotonin receptors on serotonergic neurons (Figure 1) provides an intrinsic feedback mechanism allowing the cell to sense extracellular neurotransmitter levels through autoreceptor activation and downstream signaling cascades, discussed in more detail below (and see Box 1). Evidence suggests that this feedback mediates morphological changes in serotonergic neurons in response to 5-HT and underscores the importance of appropriate neurotransmitter levels during development. Raphe serotonergic neurons of *Tph2* knockout mice [26] and *tph-1* mutant *C. elegans* [12] innervate appropriate target regions in the absence

of serotonin synthesis, echoing the intrinsic patterning observed in the leech and the fruit fly [7,19], although these studies did not include detailed analyses of serotonergic varicosity distribution. Somewhat surprisingly, individual serotonergic varicosities in the fruit fly and frog CNS exhibit clustered fractal-like spatial patterning rather than the expected regular array [27]. Autoregulated fine-tuning of serotonergic terminal patterning may be a conserved function of serotonin signaling acting to develop and maintain the complex distribution of serotonin release sites. Table 2 summarizes evidence from invertebrate and vertebrate model systems indicating autoregulation of serotonergic morphology.

Invertebrate systems

In the snail *Helisoma trivolvis*, embryonic outgrowth of serotonergic neurites was found to be inversely responsive to pharmacological manipulations of serotonin levels [28]. Similarly, serotonergic projections to the larval midgut were found to be over-arborized in *Drosophila Ddc* mutants deficient in serotonin and dopamine synthesis, but neuronal morphologies of other neurotransmitter systems were unaffected [29]. In a model of reversible crush injury of the serotonergic cerebral giant cells (CGC) in the snail *Lymnaea stagnalis*, serotonin synthesis was unchanged following injury while peptide synthesis was downregulated, leading the authors to hypothesize a specific role for serotonin in regeneration of these cells [30]. In primary cell culture, CGC growth cones collapsed following intracellular stimulation of serotonin release. Application of the serotonin receptor antagonist methysergide blocked this effect, indicating that serotonin released by the CGCs activates serotonin autoreceptors to inhibit neurite outgrowth. Thus, serotonin signaling may be necessary for CGC arborization during neuronal regeneration in the snail following injury [30]. In a *Drosophila* larval CNS culture explant preparation, application of exogenous serotonin induced a reversible reduction of serotonin varicosity density, demonstrating plasticity of relatively mature serotonergic neuropil [6]. Therefore, in invertebrate models, it appears that the main function of serotonin feedback during neurite outgrowth is inhibitory, likely acting through receptor-mediated second messenger cascades (Box 1). Importantly, data from *Lymnaea* and *Drosophila* indicate that some level of autoregulation involved in developmental plasticity of serotonergic innervation is retained in the mature CNS [6,30].

Vertebrate systems

The autoregulatory role of serotonin in morphology has proven difficult to assess in mammalian systems *in vivo* due to a lack of cell-labeling tools that are independent of serotonin immunoreactivity or serotonergic marker (eg. SERT) expression levels, both of which may be affected by altered serotonin levels through regulatory feedback mechanisms, however indirect methods have provided some insights into this. In the developing rat brain the serotonin agonist, 5-methoxytryptamine (5-MT), inhibits or promotes serotonergic innervation depending on the dose, as measured by radio-labeled serotonin uptake [31]. 5-MT application to dissociated raphe nuclei culture induces visibly stunted outgrowth of serotonin immunopositive neurons [32]. 5-MT-treated pups also exhibit altered behavior as adults [31], indicating that altered serotonin function during development has lasting effects in the adult. It is important to note, however, that indirect measurements of serotonergic innervation using SERT binding or serotonin uptake assays must be interpreted with caution, as SERT localization and uptake activity is dependent upon a variety of factors, including substrate availability [33].

In 5-HT$_{1B}$ knockout mice, increased binding of a radio-labeled SSRI, [³H]citalopram, in amygdala and hippocampal brain regions correlates with increased axon length and arborization of serotonergic fibers in these areas, suggesting autoregulatory function of terminally located autoreceptors [34]. However, 5-HT$_{1B}$ is also expressed in non-serotonergic neurons and the potential for indirect modulation of serotonergic morphology by other neurotransmitters in the region analyzed cannot be excluded. Similarly, increased serotonergic

sprouting was observed in gerbil amygdala and hippocampal brain regions following isolation rearing and early methamphetamine administration, both manipulations that decrease dopaminergic innervation of these areas [35]. These adaptive changes were speculated to result from dopaminergic disinhibition of serotonin signaling and subsequent promotion of serotonergic sprouting [35]. Data such as this highlights the difficulty involved in isolating factors responsible for altered neural morphology, due to the combination of neurotransmitter cross-talk and autoregulation. Therefore, understanding the impact of serotonin on other neurotransmitter systems is critical.

Evidence for serotonergic modulation of circuit formation

The relatively early differentiation of serotonergic neurons during development suggests serotonergic modulation of other developing neurotransmitter systems. By early postnatal development, adult serotonergic innervation patterns are present in the rat CNS [36]. In rhesus monkeys individual pyramidal cells receive relatively constant serotonergic innervation from 2 weeks to 10 years of age, while dopaminergic inputs are selectively altered over time [37]. Thus, serotonin signaling may be a prominent modulator of development of other neurotransmitter systems and circuitry (Table 3).

Genetic variants of the serotonin transporter in both humans and mice indicate that reduced or absent SERT function alters adult cortical features. Specifically, mice lacking SERT have a significantly thinner cortical layer IV compared to control mice [38]. The serotonin-transporter linked promoter region (*5-HTTLPR*) is a polymorphic region of the human *SERT* promoter sequence that is associated with altered *SERT* transcription [39]. Imaging studies in human subjects indicate that gray matter volumes, assessed by voxel-based morphometric analysis, are affected as a function of the *5-HTTLPR* allele. The most consistent finding is a decrease in gray matter volumes in subjects homozygous for the *S* allele, which is associated with reduced *SERT* transcription [40,41]. Another interesting finding is that *5-HTTLPR* variation modulates basal neural activation [40], implying a broader role for SERT function in brain activity.

One of the most intensely studied examples of serotonergic modulation of morphology is the somatosensory cortex in the rodent. In particular, the sensory cortex, or barrel cortex, receiving input from whisker sensory neurons is morphologically disrupted when serotonin levels are altered during development using genetic or pharmacological methods. This subject has been well reviewed [42] and will not be discussed in great detail here. Importantly, these morphological changes do have functional consequences. *SERT* null mice with altered barrel cortex formation are also impaired in somatosensory cortical responses, as assessed by local glucose utilization during whisker stimulation [43]. Pharmacological depletion of 5-HT during early postnatal development rescues both barrel cortex malformations [44] and the somatosensory cortical response in *SERT* null mice [43], therefore the structural abnormalities and functional consequences are likely a result of elevated extracellular serotonin during a critical developmental window. In order to understand how altered serotonin levels affect cortical features, individual neuronal morphology must be examined.

Recently the role of serotonin in interneuron morphology and migration has been addressed. Pharmacologically decreasing serotonin levels in the embryonic rat using the serotonin synthesis inhibitor parachlorophenylalanine (PCPA) disrupts the maturation of pyramidal neurons of the somatosensory cortex by reducing dendritic arborization [45]. Serotonin depletion also alters migration of interneurons *in vitro* [45]. The cellular mechanisms responsible for serotonergic modulation of interneuron migration and maturation are beginning to be elucidated. Serotonin directs thalamocortical axon pathfinding in slice culture by modulating cellular responses to netrin-1 through 5-HT_{1B} and 5-HT_{1D} receptor activation and the downstream effects on intracellular cAMP levels [46]. A recent study using time-lapse

video directly demonstrates that application of excess serotonin can reduce migration distance of embryonic interneurons in cortical slice cultures [47]. The authors also show altered cortical interneuron positioning in a *SERT* null mouse model of excess serotonin, demonstrating the ability of excess serotonin to alter interneuron migration *in vivo*. 5-HT$_6$ receptor activation is responsible for decreased interneuron migration, again by affecting cAMP production [47]. Clearly the evidence suggests a role for serotonin receptor-activation and signaling cascades in modulating interneuron migration and axon pathfinding directly. In an alternate view, one study proposes that reducing serotonin levels during development reduces expression of cell adhesion molecules in the brain, which in turn impairs migration and induces deficits in synaptogenesis [48]. Both mechanisms may contribute to morphological aberrations associated with altered serotonin signaling during development.

Serotonergic dysfunction in physiology and behavior

Despite the ability to survive to adulthood with grossly normal brain morphology, mice lacking most central serotonergic neurons exhibit defects in development of respiratory circuitry. Ablation of *Lmx1b* [10] and *Pet-1* [49], genes encoding transcription factors necessary for serotonergic differentiation, in central serotonergic neurons of the mouse arrests the development of these cells. These animals exhibit disrupted respiratory rhythms that are normalized by 9–10 days of age, indicating a developmental window where lack of serotonin delays maturation of respiratory circuits due to depressed excitatory drive to the respiratory rhythm generator [9–11]. Application of a 5-HT$_{2A}$ agonist rescues postnatal ventilation defects [10] suggesting that aspects of respiratory circuitry are intact despite the loss of serotonin during development.

Monoamine oxidase-A *(MAO-A)* deficient transgenic mice (*Tg8*) have excess serotonin during development and are similarly unable to generate stable respiratory patterns through 5 days of age [50]. At this time, phrenic motor neurons responsible for rhythmic respiration display altered morphology including expanded dendritic fields, increased spine number, increased varicosity density and increased occurrence of swollen varicosities compared to control animals [50]. These morphological changes can be induced in control animals or rescued in *Tg8* mice by 5-HT$_{2A}$ agonists and antagonists, respectively [50]. Therefore, serotonin levels during development predict temporal aspects of respiratory circuit maturation as well as morphological characteristics of the responsible motor neurons. Rhythmic breathing after this developmental critical window of respiratory circuit maturation does not require serotonin [9,10,50], although *Tph2* null mice exhibit reduced respiration rates [11].

Significant loss of central serotonin also has deleterious effects on reproductive fitness by altering maternal behavior. In *Pet-1*−/− and *Tph2*−/− mice, females can survive to adulthood with normal fertility and milk production but most of their pups fail to survive past postnatal day 5 due to extreme maternal neglect [11,51]. Rescue of pup survival in *Pet1*−/− mice is dependent upon maternal expression levels of the serotonergic transcriptional program [51], however these studies do not indicate whether the requirement for serotonin in maternal behavior circuitry is developmentally or acutely necessary.

A number of studies have suggested that altered serotonin signaling contributes to the development of mood disorders such as depression and anxiety. The human *5-HTTLPR S*-allele that results in reduced *SERT* expression has been associated with the development of depression and anxiety in response to stressful life events [52]. *SERT* knockout mice and *MAO-A* knockout mice contain elevated extracellular serotonin and both exhibit anxious behaviors as adults [53,54]. However, SSRIs and MAO inhibitors are prescribed to alleviate mood disorders, ostensibly by increasing serotonin availability. This apparent paradox may be explained by recent evidence from animal studies suggesting that alterations in the serotonergic system

during development may be responsible for increased susceptibility for depressive and anxious behaviors as an adult.

The 5-HT$_{1A}$ receptor, like 5-HT$_{1B}$, functions as an autoreceptor and heteroreceptor and dysfunction in 5-HT$_{1A}$ signaling is associated with anxiety and depression. Loss of *5-HT$_{1A}$* expression only during early postnatal development results in mice with anxiety-like behaviors similar to *5-HT$_{1A}$* knockout mice, demonstrating a developmental requirement for 5-HT$_{1A}$ activation [55]. Pharmacological blockade of 5-HT$_{1A}$ only during postnatal development also phenocopies *5-HT$_{1A}$* knockout mouse anxiety-like behaviors [56]. Similarly, pharmacological SERT inhibition during early postnatal development in mice causes development of depressive-like behaviors in the adult that mimic behaviors in *SERT* knockout mice [57,58]. Antagonizing the 5-HT$_{1A}$ receptor during early development in *SERT* knockout mice rescues depressive-like behavior in the adult animal [59]. SSRI treatment in adolescent animals does not result in measurable differences in adult anxiety-like behaviors compared to controls [60], therefore the altered emotional behaviors observed in these serotonin knockout animals are likely due to the loss of these proteins during early development. Studies are beginning to associate changes in neuronal morphology with these genetic models. For example, *SERT* knockout mice that exhibit abnormalities in stress-coping and fear extinction assays show altered dendritic morphologies in pyramidal cells of the infralimbic cortex and basolateral amygdala, two brain regions that are associated with responses to emotional stimuli [61].

A genetic animal model of differential *5-HT$_{1A}$*-expression was recently developed using a conditional gene-suppression system [62]. This strategy provides useful information for two reasons: first, gene suppression is targeted to serotonergic neurons so that only 5-HT$_{1A}$ autoreceptors are affected and second, temporal control allows for separation of developmental and acute effects of gene-suppression. The initial study [62] confirmed the previously reported developmental role of 5-HT$_{1A}$ in anxiety-related behaviors [55], but also demonstrated involvement of the 5-HT$_{1A}$ autoreceptor in stress responses, depressive-like behaviors, and SSRI responsiveness in the adult animal. The comprehensive model emerging from these studies is that early serotonin signaling plays a critical and long-lasting role in CNS function and that signaling within serotonergic neurons themselves, via autoreceptor feedback mechanisms, is a key regulatory component.

Evidence for serotonin dysfunction contributing to neurodevelopmental disorders

Early alterations in serotonin-modulated circuit formation may contribute to complex symptoms in disorders that have a developmental component, such as Down's syndrome (DS) and autism. For example, fetal DS brains exhibit a roughly 40% reduction in frontal cortex serotonin levels compared to unaffected brains [63]. This reduction in serotonin levels persists throughout life [64] and SSRIs have been administered to adult DS patients with some positive effects on cognitive function [65], suggesting a role for serotonin dysfunction in DS.

The role of serotonin in autism has been more broadly addressed. Multiple studies report elevated blood serotonin levels in autistic individuals [66], also known as "hyperserotonemia." Central brain serotonin levels may be low 67 and single-photon emission computed tomography (SPECT) in autistic children reveals decreased SERT binding in medial frontal cortex [68], although this may reflect reduced serotonergic innervation or altered SERT expression. Normal developmental changes in serotonin levels are affected in autistic individuals [69] and asymmetries in cortical serotonin synthesis (assessed via indirect measurements of TPH activity) correlate with differences in functions requiring hemispheric specialization, such as language and handedness [70]. At least two serotonin-based models for autism have been proposed in mice. Both result in animals with reduced serotonergic function

either due to developmental downregulation of the serotonergic system or early serotonin toxin administration [71–73]. In both cases, animals exhibit decreased serotonergic terminals in the brain, decreased responsiveness to auditory stimuli, decreased behavioral inhibition, altered social interactions, and cortical defects including metabolic abnormalities that the authors interpret as consistent with autism [72,73]. While it is difficult to model all aspects of a complex human condition like autism in experimental systems, these studies do demonstrate the critical nature of appropriate serotonin levels during brain development and potential consequences of dysfunction.

Serotonergic degenerative morphology

In addition to a role in development, emerging evidence indicates that serotonergic neurons are involved in degeneration. Degenerative 5-HT fibers have been reported in animal models as a result of aging, oxidative stress, neurodegenerative disease and drug administration [74–81]. The morphological aberrations are sometimes accompanied by a reduction in serotonergic innervation density and cell death. Such insult to the serotonergic system in the mature nervous system may contribute to cognitive and psychological defects associated with neurodegenerative diseases and chronic drug abuse, therefore alterations in serotonin signaling due to serotonergic denervation are relevant to the adult organism.

Serotonergic fibers are damaged in the aged animal and in a rat model of oxidative stress, consistent with the hypothesis that increased oxidative stress over time causes degeneration of monoaminergic neurons [74,75]. These morphological characteristics include grossly enlarged varicosities and blunted projections and are also seen in human patients and animal models of neurodegenerative disease [76–78]. In genetic mouse models of Alzheimer's disease, for example, swollen serotonergic fibers are observed in close proximity to amyloid plaques [76, 77]. Depression is one of the earliest symptoms in Alzheimer's disease and is often comorbid with neurodegenerative diseases and dementias, indicating that alterations in serotonergic morphology may contribute to complex disease symptoms.

Neurodegenerative disease states and normal aging are not the only clinical examples of serotonergic dysfunction with potential morphological underpinnings. The amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') is increasingly abused by the adolescent population and is associated with serotonergic toxicity, including reductions in serotonin levels, reduced activity of the serotonin synthetic enzyme Tph and damage to serotonergic fibers in animal models [79]. Notably, serotonergic fibers originating from the dorsal raphe nuclei are preferentially damaged following drug administration while those originating from the median raphe nuclei are spared [80,81]. A recently developed rat model of adolescent recreational MDMA use demonstrates a reduction of SERT immunoreactive fibers in the hippocampus, which may reflect changes in this region contributing to memory deficits [82]. The field of MDMA neurotoxicity in human subjects is controversial, however the well-documented serotonergic changes may be related to negative effects on cognition and mental wellbeing in previous and current ecstasy users [83]. Fenfluramine, one component of the anorectic drug combination, Fen-Phen, has similar mechanistic effects on serotonergic neurons as MDMA, and also induces regionally specific serotonergic degeneration in mammals [84,85]. Enhanced serotonin production in *Drosophila* larvae induces similar degenerative-like swellings along serotonergic branches [86]. As was also observed in mammals, the serotonergic cell bodies of the *Drosophila* larvae were spared, however, unlike the rodent condition, no loss of serotonergic fibers was observed [86]. Substituted amphetamines induce efflux of serotonin from neurons [87], therefore it is unclear whether serotonergic fibers are truly lost following drug administration, as mammalian models to date have utilized serotonergic marker immunoreactivity to assess serotonergic fiber distribution [81,82,84,85]. Further work is necessary to determine whether similar mechanisms contribute to serotonergic

dystrophy in the rodent and fly. Technical advancements with regard to cell-labeling techniques that are independent of serotonergic marker localization will help to clarify effects of MDMA and fenfluramine administration on mammalian serotonergic fiber distribution.

Both MDMA and fenfluramine cause release of serotonin both intracellularly and extracellularly by action at the vesicular transporter (VMAT) and SERT [87,88] and evidence suggests increases in cytoplasmic serotonin are responsible for serotonergic dystrophy in flies synthesizing excess serotonin [86]. There is precedence for receptor independent signaling cascades that are initiated by cytoplasmic serotonin (Box 1) and it will be interesting to ask whether this novel mechanism contributes to degenerative morphology in drug-administered mammals and flies producing excess serotonin. Based on evidence that mature serotonergic neurons are susceptible to autoregulatory processes in invertebrate models [6,30], it is tempting to hypothesize that damaged fibers in cases of serotonin mispartitioning represent novel regulatory pathways gone awry.

Concluding remarks and future directions

While a role for serotonin in brain development has been suggested for some time, the molecular mechanisms responsible for serotonin's effects on physical restructuring of the brain are only beginning to be elucidated and many questions remain (Box 2). A simple circuit with a behavioral readout modulated by serotonin is an ideal platform from which to study the influence of serotonin on neural wiring. For example, the gill-withdrawal reflex in *Aplysia* [89] has been of fundamental importance in achieving an understanding of synaptic plasticity and cellular learning. The fruit fly also offers considerable advantages, as locomotor circuits of the larva are well studied [90,91] and genetic amenability not only provides access to specific gene function but also allows cell-labeling techniques crucial for circuit observation. Electrochemical techniques such as fast scan cyclic voltammetry have been modified to measurereal-time serotonin release in *Drosophila* larval CNS [92,93], meaning that serotonergic input and locomotor output [90] could be quantified simultaneously in addition to monitoring morphology of the serotonergic neurons and the locomotor circuit.

The ability to monitor serotonergic innervation in the vertebrate brain is also improving as genetic tools for these model systems are developed, making it possible to monitor neuronal morphology regardless of endogenous serotonergic marker localization. Engineered transgenes and fluorescent reporters under control of the serotonin-specific *Pet-1* enhancer region are available in mice [94] and zebrafish [95] and this technique has allowed the investigation of developmental serotonergic neuronal migration in slice culture [18] and development of the 5-HT$_{1A}$ conditional knockout that is selectively targeted to serotonergic neurons [62]. In rats, viral vectors have been delivered to central serotonergic neurons *in vivo* and in slice culture, allowing expression of fluorescent reporters and visualization of serotonergic projections [96,97]. Recent identification of discrete transcriptional gene programming in subpopulations of raphe serotonergic neurons [98] may provide new genetic tools, and may reveal differential mechanisms responsible for functional diversity [99] and the disparate susceptibility of these neurons to toxins such as MDMA and fenfluramine [84]. . Thus, there are many potential ways in which serotonin, an incredibly complex neuromodulator, can impact neuronal morphology, development, physiology, behavior and, potentially, neurological diseases

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Box 1. Molecular mechanisms of serotonergic modulation of morphology

The signal transduction pathway responsible for 5-HT induced growth cone collapse in the buccal ganglion neuron (B19) of the freshwater snail, *Helisoma trivolvis*, is largely known (a). Two *Helisoma* 5-HT receptors have been cloned that fall in the 5-HT$_1$ and 5-HT$_7$

families of serotonin receptors based on phylogenetic analysis [104]. 5-HT binding to receptors located on the B19 neuron induces increases in cAMP and subsequent opening of cAMP-gated sodium channels [105]. This depolarization allows voltage-sensitive ion channels to open. The resulting increase in intracellular calcium induces calmodulin-dependent debundling and/or depolymerization of F-actin [106–108]. Similar signal transduction pathways may underlie serotonin-responsive outgrowth and migration of interneurons in mammalian brain slices [46,47].

(b) The gill-withdrawal sensory to motor neuron reflex circuit of the mollusk, *Aplysia*, is a robust model circuit for the study of serotonin-induced synaptic facilitation and plasticity after learning, but what physical changes are induced by 5-HT to promote synapse strengthening presynaptically? The cytoskeletal reorganization responsible for formation of new synapses occurs due to activation of the small Rho- family GTPase Cdc42 by phospholipase C (PLC) and phosphoinositide 3-kinase (PI3 kinase) pathways following 5-HT receptor binding. Cdc42 activation induces filopodia extension and molecular synapse maturation as well as new varicosity formation on sensory neuron axonal processes [89]. It should be noted that a definitive interaction between 5-HT receptor associated G-protein subunits (α,β,γ)and PLC or PI3K in this model has not been identified.

(c) A third mechanism for 5-HT interaction with the cytoskeleton involves receptor-independent cytoplasmic 5-HT signaling. Elevated intracellular 5-HT in conjunction with elevated Ca^{2+} activates transglutaminase (TG) catalysis of a covalent bond between serotonin and small GTPases such as RhoA known as serotonylation. The small GTPases are rendered constitutively active due to the location of 5-HT binding, and go on to influence cytoskeletal dynamics and secretion [109]. In platelets and pancreatic β-cells, serotonylationof small GTPases induces secretion of aggregation factors and insulin, respectively [109,110]. 5-HT activation of Rac1, a member of the Rho family GTPases, has been reported in cultured neurons [111], and elevated serotonin production in fly 5-HT cells induces structural abnormalities that are inhibited by pharmacological inhibition of TG [86]. Therefore, while the structural consequences of serotonylation in neurons have not been directly investigated, it remains a novel potential mechanism contributing to morphological regulation by serotonin.

Box 2. Outstanding questions

- What are the molecular mechanisms responsible for the autoregulation of serotonergic neuronal morphology by serotonin?

- How might alterations in serotonergic branching patterns and the distribution of serotonin release sites impact both serotonergic function and the development and maintenance of local circuitry?

- How do alterations in serotonin signaling differentially influence circuit formation in the central nervous system in early and later development? What are the critical developmental windows for these effects and how do they translate into complex behaviors in the adult?

- Does altered serotonergic morphology significantly impact local circuit modulation? How important are alterations in serotonin signaling to the ontology of various human neurological disorders?

Acknowledgments

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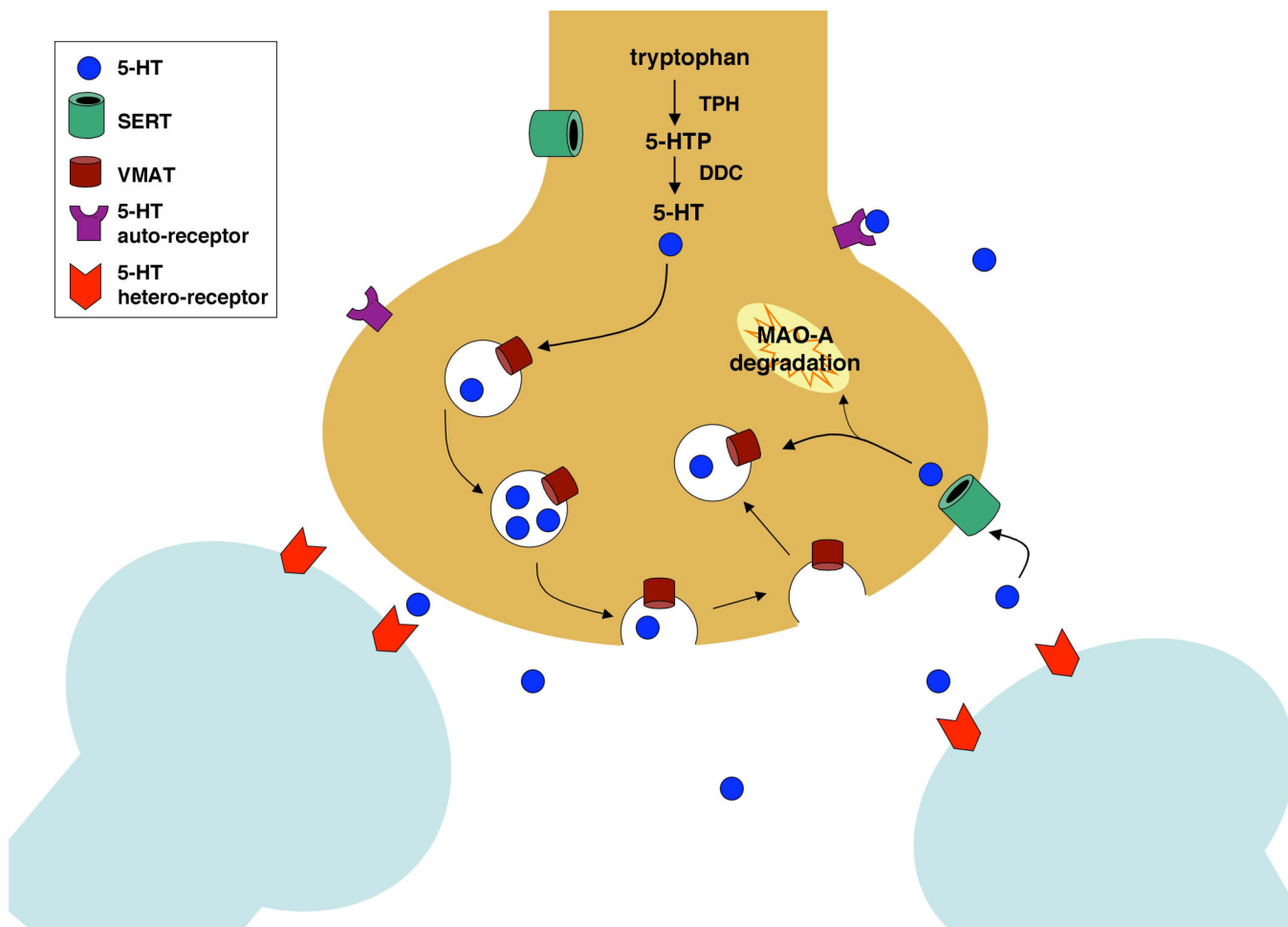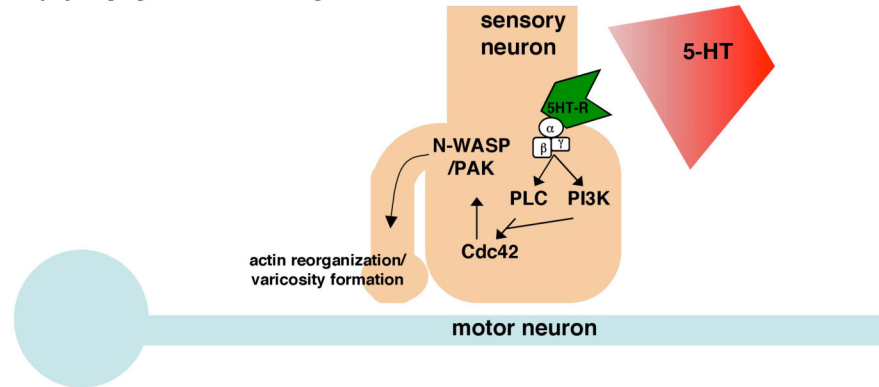
Figure 1.
Molecular machinery of a simplified mammalian serotonin release site. Serotonin is synthesized from the amino acid tryptophan in two enzymatic steps. Hydroxylation of tryptophan by the neuronal form of tryptophan hydroxylase (TPH-2) is rate-limiting. 5-hydroxytryptophan (5-HTP) is converted to 5-hydroxytryptamine (5-HT, serotonin) by dopa-decarboxylase (DDC). After 5-HT synthesis, the vesicular monoamine transporter (VMAT) transports 5-HT into vesicles for storage. Upon vesicle fusion with the plasma membrane, 5-HT is released where it may interact with autoreceptors located on the releasing cell or heteroreceptors, serotonin receptors located on other cell types.. The serotonin transporter (SERT) transports 5-HT back into the releasing cell where it may be repackaged for release by VMAT or degraded by monoamine oxidase A (MAO-A) located on the outer mitochondrial membrane.
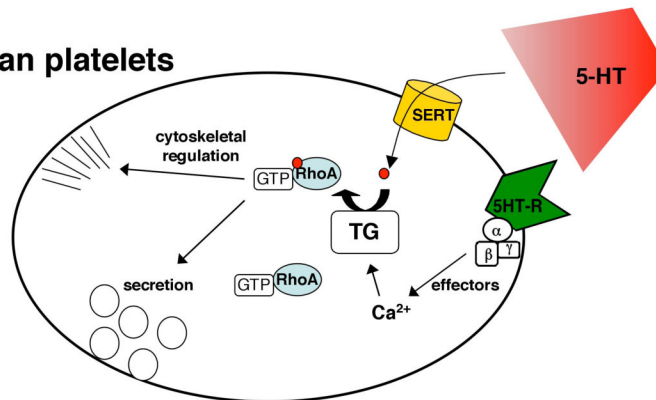
(a) *Helisoma* B19 growth cone
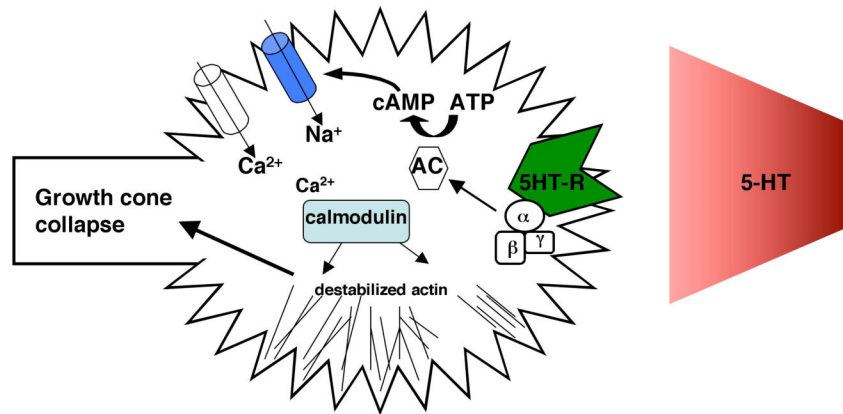
(b) *Aplysia* sensory-to-motor circuit

(c) Mammalian platelets

Figure 2.

Table 1

Genetic ablation of central serotonin in model systems

Animal Model	Genetic manipulation	Effect on serotonergic system	Other effects	Reference
Drosophila	Early *UAS-rpr-hid* induced apoptosis	Loss of all 5-HT cells in VNC but not brain	Slowed development	[7]
Mouse	*Pet-1* −/−	~70% loss of central 5-HT cells	Increased anxiety-like & aggressive behaviors, impaired ventilation & thermoregulation, maternal behavioral defects	[9,49,51]
Mouse	*Lmx1b* conditional knockout (*Lmx1b$^{f/f/p}$*)	Near-complete loss of central 5-HT cells after embryonic day 16.5	Reduced anxiety-like behavior, enhanced fear memory, slowed respiratory circuit development	[10,100]
C. elegans	*tph-1* mutant	5-HT undetectable by immunohistochemistry	Reduced egg-laying and feeding, metabolic defects	[12]
Mouse	*Tph2* −/−	96–99% reduction in 5-HT brain levels	Slowed growth, Maternal Behavioral defects, altered autonomic control	[11]
Mouse	*Tph1/Tph2* double knockout	94–99% reduction in 5-HT brain levels	Conflicting data in tests measuring depressive-like behaviors	[13]
Drosophila	*VMAT* mutant	Loss of 5-HT, DA, OCT storage and vesicle-mediated release	Reduced larval locomotion, sensitive to crowding, altered cocaine responses	[101]
Mouse	*VMAT* knockout	Loss of 5-HT, DA, NE storage and vesicle-mediated release; 5-HT visible in raphe cell bodies	Homozygote lethality, heterozygotes display increased depressive-like behaviors	[102,103]

Abbreviations: 5-HT - 5-hydroxytryptamine (serotonin); VNC - ventral nerve cord; rpr-hid - reaper-hid; Pet-1 - pheochromocytoma 12 ETS factor-1; Lmx1b - LIM homeobox transcription factor 1β; Tph - tryptophan hydroxylase; VMAT - vesicular monoamine transporter; DA - dopamine; OCT - octopamine; NE - norepinephrine

Table 2

Animal Models and Autoregulation of Serotonergic Morphology

Animal model	Cell types/ brain region	Experimental manipulation	Effect on serotonergic morphology	Method of assessment	Reference
Great pond snail (*Lymnaea stagnalis*)	Cerebral giant cells (CGC)	5-HT incubation	Growth cone Collapse	Growth cone observation in culture	[30]
		Stimulated 5-HT release	Growth cone collapse		
Freshwater snail (*Helisoma trivolvis*)	Embryonic neurons C1 (ENC1)	PCPA incubation	Increased neurite outgrowth	5-HT immunoreactivity	[28]
		5-HTP incubation	Decreased Neurite Outgrowth		
Medicinal leech (*Hirudo medicinalis*)	Dorsolateral (DL, 61), ventrolateral (VL, 21)	Primary culture, no agents applied	Normal Patterns in absence of external cues	Dye filled cells in culture	[19]
Fruit fly (*Drosophila melanogaster*)	Midgut innervation	*DDC* mutant	Increased arborization	5-HT immunoreactivity	[29]
Fruit fly (*Drosophila menalogaster*)	Abdominal CNS	5-HT incubation	Decreased 5-HT varicosity density	5-HT immunoreactivity	[6]
Rat (Sprague-Dawley)	Dissociated raphe nuclei cells	5-MT incubation	Stunted outgrowth	5-HT immunoreactivity	[32]
Rat (Sprague Dawley)	Forebrain, brainstem synaptosomes	Maternal 5-MT injections	Decreased 5-HT innervation	[35-HT] uptake	[31]
Mouse (129/SvEvTac)	Hippocampus, amygdale	*5-HT$_{1B}$* knockout	Increased 5-HT innervation	5-HT immunoreactivity	[34]

Abbreviations: 5-HT - 5-hydroxytryptamine (serotonin); PCPA -parachlorophenylalanine; DDC - dopa decarboxylase; 5-MT - 5- methoxytryptamine

Table 3

Serotonergic manipulations affecting neuronal morphology

Molecular target	Method	Effects on neuronal morphology	Reference
TPH	Inhibition (PCPA)	Reduced pyramidal neuron dendritic arborization	[45]
MAO-A	Knockout mouse	Somatosensory cortical barrels absent, retinal axonal segregation lost in thalamus, Altered morphology of phrenic motor neurons	reviewed in [42] [50]
SERT	5-HTTLPR human polymorphism	Decreased gray matter volumes	[40,41]
	Knockout mouse	Decreased cortical layer IV thickness	[38]
		Barrel cortex malformations	[43,44], reviewed in [42]
		Altered cortical interneuron migration	[47]
5-HT$_{1B/1D}$ receptors	Agonist (L694.247) in slice culture	Changed response to netrin-1 of posterior dorsal thalamus axons from attraction to repulsion	[46]
	Over-expression/ siRNA in mouse embryonic brain	Altered thalamocortical axonal trajectories in internal capsule	[46]
5-HT$_{2A}$ receptor	Agonist (DOI)	Altered phrenic motor neuron morphology	[50]
5-HT$_6$ receptor	Agonist(EMD386088)	Reduced interneuron migration	[47]

Abbreviations: 5-HT - 5-hydroxytryptamine (serotonin); PCPA -parachlorophenylalanine; TPH - tryptophan hydroxylase; MAO-A - monoamine oxidase A; SERT- serotonin transporter; 5-HTTLPR - serotonin transporter linked promoter region; DOI -1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane