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The serotonin 5-HT₃ receptor: a novel neurodevelopmental target

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INTRODUCTION

In addition to its role as a classical neurotransmitter, it is now well established that serotonin (5-hydroxytryptamine, 5-HT) plays a pivotal role in the development of the mammalian central nervous system (CNS). 5-HT is one of the first neurotransmitters to appear during development (E13 in the rat; Lauder, 1990; and E11 in the mouse; Piaar et al., 2002) and acts a neurotrophic factor in early embryonic CNS development and thus even before synapse formation of cortical neurons is completed. Therefore, it aids to establish CNS organization, supporting as well serotonergic (autoregulation) as also non-serotonergic circuit formation during pre- and early postnatal periods (Sodhi and Sanders-Bush, 2004; Vitalis et al., 2007). 5-HT signaling is involved in cell division, differentiation, survival, and neuronal migration (Dooley et al., 1997; Lerdas et al., 1997; Armitia, 2001; Vitalis et al., 2007). It further regulates dendrite formation (Vitalis et al., 2007) and synaptogenesis of cortical neurons (Chubakov et al., 1986; Matsukawa et al., 2003) and is released from sprouting axons even before initial synapse formation (Vitalis and Parnavas, 2003). Genetic or pharmacological disruption of 5-HT signaling leads to disruption of circuit formation as well as alteration of cell morphology, for example in the somatosensory cortex (Gaspar et al., 2003) and interneuronal circuits (Vitalis et al., 2007). Further, disruption of the 5-HT system during early development by stress or drug exposure is associated with altered cognitive ability, neurodevelopmental disorders such as autism spectrum disorders (ASD) and increased incidence of psychopathologies such as schizophrenia (Whitaker-Azmitia, 2001).

The myriad of functions of 5-HT in developmental processes corresponds to the expression of a vast amount of receptors, each with its spatial and temporal expression patterns. Seven receptor families for 5-HT have been identified, including the G protein-coupled receptors 5-HT₁, 5-HT₂, and 5-HT₄, and the only ligand-gated ion channel 5-HT₃. Thus far, 5-HT₁ and 5-HT₂ receptors have received the most attention as effectors of the actions of 5-HT during CNS development (Borella et al., 1997; Armitia, 2001; Whitaker-Azmitia, 2001; Gaspar et al., 2003; Puig et al., 2004; Bonnin et al., 2006). However, recent evidence suggests that the 5-HT₃ receptor is involved in several mechanisms which determine the formation of neuronal circuits from embryonic stages onward. In this review, we summarize these recent findings which suggest that 5-HT₃ receptors emerge as a novel target during the development of the CNS.

EXPRESSION OF 5-HT₃ RECEPTORS DURING DEVELOPMENT

The 5-HT₃ receptor belongs, together with the nicotinergic acetylcholine, the GABA_A, and the glycine receptor, to the Cys-loop family of ligand-gated ion channels (Barnes and Sharp, 1999; Chameau and van Hooft, 2006; Walstab et al., 2010; Lummis, 2012). To date, two subunits (5-HT₃A and 5-HT₃B) have been identified in rodents (Maricq et al., 1991; Davies et al., 1999), and additional three subunits (5-HT₃C–5-HT₃E) have been identified in humans (Niesler et al., 2007). Functional 5-HT₃ receptors can be built from the same (only 5-HT₃A) or different subunits (5-HT₃A and 5-HT₃B receptor subunits). The receptor composition is crucial for its function (Chameau and van Hooft, 2006;
In the CNS, the 5-HT3 receptor is first observed in the subpallial ganglionic eminence (GE), the major source of interneurons in the basal telencephalon, at E12.5 (Johnson and Heinemann, 1995; Miquel et al., 1995; Tector et al., 1995). The rostral GE generates later neocortical GABAergic interneurons which migrate tangentially into the cortical plate. In contrast, neocortical glatamatergic neurons originate in the pallial ventricular zone (VZ) and migrate radially into the cortex (Corbin et al., 2001; Nadarajah and Parnavelas, 2002). Different areas of the GE give rise to various subpopulations of GABAergic interneurons which can be subclassified by their morphology and neuropeptide expression (Flames and Marin, 2005; Rudy et al., 2011; Vitalis and Rossier, 2011).

5-HT3 receptor-positive interneurons compromise ~30% of the superficial GABAergic interneurons in the somatosensory cortex (Lee et al., 2010). They express cholecystokinin (CCK), vasoactive intestinal peptide (VIP), and/or neuropeptide Y (NPY), and, at smaller fractions, calretinin (CR) and/or reelin, but not parvalbumin (PV) or somatostatin (SST; Morales and Bloom, 1997; Ferenczi et al., 2002; Inta et al., 2008; Lee et al., 2010; Vucurovic et al., 2010). Further expressing several morphological and electrophysiological properties, 5-HT3 receptor-positive interneurons form a rather heterogeneous group of cells, whose potential common properties remain to be fully characterized (for a recent review, see Rudy et al., 2011). 5-HT3 receptor-expressing neocortical interneurons are not only excited by 5-HT but also acetylcholine via nicotinic receptors (Lee et al., 2010). At least a subset of 5-HT3 receptor-positive cells receives monosynaptic thalamocortical input leading to strong depolarization of these cells (Lee et al., 2010). Therefore, 5-HT3 receptor-expressing cells might be part of potential feedforward inhibitory thalamocortical networks whose sensitivity is potentially regulated by serotonergic and/or cholinergic input (Lee et al., 2010; Rudy et al., 2011). Further discussion of potential functional significance of 5-HT3 receptors on these interneurons was published recently (Rudy et al., 2011).

The major source of 5-HT3 receptor-expressing neocortical interneurons is the caudal part of the GE (Lee et al., 2010; Vucurovic et al., 2010). Based on recent publications, there is no expression of 5-HT1 receptor in the medial GE (MGE; Lee et al., 2010; Vucurovic et al., 2010), which is the area PV- and SST-expressing cortical interneurons are derived exclusively from (Miyoshi et al., 2007). Note that embryonic 5-HT receptor expression was mistakenly described in the MGE in earlier publications (Tector et al., 1995).

Recently, the generation of enhanced green fluorescent protein (EGFP)-expressing 5-HT3A receptor reporter mice by Inta et al. (2008) and the GENSAT (Gene Expression Nervous System Atlas) project allowed for detailed analysis and fate mapping of 5-HT3 receptor-positive cells during embryonic corticogenesis (Lee et al., 2010; Vucurovic et al., 2010). 5-HT3 receptor-positive superficial neocortical interneurons were found to be generated in the CGE around E13.5–14.5 (Vucurovic et al., 2010). Similar, Miyoshi et al. (2010) described the genesis of cortical interneurons in the CGE to begin at E12.5 and peak at E16.5. Therefore, CGE-derived interneurons are some of the latest cells to integrate into neocortical layers, which by this time point are already populated by other interneurons including MGE-derived interneurons (Butt et al., 2005; Miyoshi et al., 2007), while CGE-derived cortical interneurons are generated earlier from the CGE (Gelman et al., 2009, 2011) and MGE (Lee et al., 2010; Vucurovic et al., 2010). The further development of these cells has not been characterized yet. Cells from the POA might contribute to interneurons in the neocortex (Gelman et al., 2009, 2011) and thus it was proposed that the POA might also give rise to 5-HT3 receptor-positive interneurons of the neocortex (Rudy et al., 2011). However, Vucurovic et al. (2010) found no evidence of POA cells migrating into neocortical regions but the cells rather contributed,
dependent on their birthdate, to cells of the dentate gyrus (DG), amygdala, endopiriform nucleus, and the claustrum.

**5-HT	extsubscript{3} RECEPTORS ARE EXPRESSED IN POSTNATAL IMMATURE NEURONS**

5-HT	extsubscript{3} receptors are expressed in migrating neuroblasts in several migratory streams derived from the SVZ in the early postnatal brain (Inta et al., 2008; Vuciurovic et al., 2010). The SVZ, and therefore these neuroblasts, are not derived from the CGE but from the lateral GE (LGE). Migratory streams in the early postnatal rodent brain are part of the ongoing neurogenesis and migration of neurons after birth. These migratory streams include the rostral migratory stream (RMS) populating mainly the olfactory bulb (OB), the dorsal migratory pathway (DMP) above the hippocampus directed toward the occipital cortex, the ventral migratory pathway (VMP) heading toward the striatum and nucleus accumbens, and the external migratory pathway (EMP) aiming toward latero-dorsal brain regions (Inta et al., 2008). Neuroblasts of the RMS do not only migrate into and mature within the OB but also integrate into the cortex (Le Magueresse et al., 2011). Next to cortical interneurons derived from embryonic interneuron genesis, these neuroblasts mature into a novel, recently described subclass of CR-positive interneurons with unique firing patterns (“small axonless neurons”) which are uniquely generated in the early postnatal period and mainly integrate into deeper layers of olfactory and orbital cortices (Le Magueresse et al., 2011). Additionally, 5-HT	extsubscript{3} receptor-positive postnatal SVZ-derived neuroblasts, so-called immature white matter interstitial cells, were recently described to populate the corpus callosum (von Engelhardt et al., 2011).

Of the several postnatal migratory streams harboring 5-HT	extsubscript{3} receptor-positive neuroblasts, only the RMS persists into adulthood as an area of secondary neurogenesis (Alvarez-Buylla and Garcia-Verdugo, 2002; Abrous et al., 2005) containing 5-HT	extsubscript{3} receptor-positive neuroblasts (Inta et al., 2008; Chen et al., 2012). Similar to early postnatal RMS neuroblasts, they migrate and integrate into the OB, where they mature to CR- and VIP-positive but calbindin-(CB) negative interneurons. Interestingly, and in contrast to cortical interneurons derived from the CGE, about one-third and one-tenth of the 5-HT	extsubscript{3} receptor-expressing interneurons in the OB are PV- and SST-positive, respectively (Chen et al., 2012). Adult SVZ neurogenesis is of particular clinical interest because SVZ-derived neuroblasts can migrate into the cortex upon traumatic events or in neurodegenerative diseases to replace cortical neurons. Indeed, upon stroke in adult mice 5-HT	extsubscript{3} receptor-positive neuroblasts migrate and mature therefore would be promising. Some recent studies proposed regulation of cytoskeletal remodeling in neurons by 5-HT	extsubscript{3} receptors. For example, 5-HT	extsubscript{3} receptor agonists were found to promote neurite elongation of GABAergic cortical interneurons (Vitalis and Parnavelas, 2003). Activation of 5-HT	extsubscript{3} receptors further promotes dendrite formation in primary thalamic neurons in vitro (Persico et al., 2006; note contradictory: Lotto et al., 1999). In growth cones, cohesion spots, and dendrites of hippocampal neurons and in human embryonic kidney (HEK) cells, 5-HT	extsubscript{3} receptors were found to form clusters with the light chain (LC1) of microtubule-associated protein 1B (MAP1B) and the tubulin cytoskeleton (Su et al., 2008) and these clusters lead to the formation of F-actin-rich lamellipodia (Emerit et al., 2002). 5-HT	extsubscript{3} receptors follow the tubulin and F-actin networks for receptor routing and precise tuning at the neuronal membrane surface (Giraldo et al., 2004; Legems et al., 2004). Further, LC1 might regulate the receptor function in these cells (Sun et al., 2008). Therefore, 5-HT	extsubscript{3} receptors and the cytoskeleton are highly interacting, which might not only lead to the specific transport of 5-HT	extsubscript{3} receptors into synaptic sites and regulation of receptor function, but also 5-HT	extsubscript{3} receptors might evoke signaling involving in cytoskeletal remodeling. 5-HT	extsubscript{3} receptor activity in immature and mature interneurons might be crucial for their activity as well as development.

Interestingly, it was recently reported that electrophysiological activity is essential for the postnatal correct migration and axonal and dendritic integration of CGE-derived reelin- and CR-, but not VIP-positive neurons (García et al., 2011). Whereas this activity is glutamate-dependent after P3, the source of activity before P3 is yet unclear. Serotonergic input via 5-HT	extsubscript{3} receptors might be a candidate source of such perinatal activity.

**CONCLUSION I: 5-HT	extsubscript{3} RECEPTORS ARE A POTENTIAL CENTRAL PART OF MATURATING INTERNEURONS DURING PRE- AND POSTNATAL CORTICAL DEVELOPMENT**

5-HT	extsubscript{3} receptors are expressed on embryonic immature CGE-derived GABAergic interneurons as well as neuroblasts in early postnatal migratory streams and the adult SVZ. Therefore, they might be involved in (fine)regulation of neuronal excitability and thus migration, maturation, and network formation of inhibitory networks from early embryonic to adult stages (Figure 1).

**EXPRESSION OF 5-HT	extsubscript{3} RECEPTORS ON CEREBELLAR GRANULE AND CORTICAL CAJAL–RETZIUS CELLS**

Next to the pre- and postnatal central expression of 5-HT	extsubscript{3} receptors on mature and immature interneurons, recent evidence showed also expression on two specific types of glutamatergic cells: cerebellar granule cells and cortical Cajal–Retzius cells. First, ubiquitous post-/extra- and presynaptic expression of 5-HT	extsubscript{3} receptors was recently observed in glutamatergic granule cells of the cerebellum within the first three postnatal weeks in rodents (Oostland et al., 2011, 2013). 5-HT	extsubscript{3} receptors are important for the serotonergic regulation of short-term synaptic plasticity at parallel fiber–Purkinje cell synapses during the early postnatal sensitive period and regulate the maturation state of these synapses (Oostland et al., 2011). They further regulate the ionic influx.
FIGURE 1 | Summary of (A) 5-HT\textsubscript{3} receptor expression on GABAergic interneurons during pre- and postnatal brain development and (B) recently described mechanisms of 5-HT\textsubscript{3} receptor-mediated regulation of maturation of cortical pyramidal cells and cerebellar Purkinje cells in the early postnatal brain.

time course of early postnatal morphological maturation of Purkinje cells as indicated by higher dendritic length and complexity in 5-HT\textsubscript{3} receptor knock-out mice and in vitro after treatment with a 5-HT\textsubscript{3} receptor antagonist (Oostland et al., 2013). 5-HT\textsubscript{3} receptor knock-out animals further show delayed climbing-fiber elimination (Oostland et al., 2013). However, morphology and physiology of Purkinje cells in 5-HT\textsubscript{3} receptor knock-out mice appears normal in adult mice, thus indicating a narrow postnatal time window of serotonergic, 5-HT\textsubscript{3} receptor-mediated regulation of cerebellar maturation and connectivity (Oostland et al., 2013). Further research might explore a function of 5-HT\textsubscript{3} receptors in the development of early life motor coordination and learning.
Second, glutamatergic Cajal–Retzius cells were recently described to express 5-HT3 receptors upon birth (Chameau et al., 2009). Cajal–Retzius cells are transient neurons located in the marginal zones of the neocortex and hippocampus during CNS development (Marín-Padilla, 1998). In the cortex, they are strategically located in layer I, the area where the apical dendrites of pyramidal neurons terminate and secrete the extracellular matrix glycoprotein reelin. Reelin plays a major role as guidance factor for cell migration, cell positioning, and neuronal process outgrowth (Frotscher, 1997). Cajal–Retzius cells in mice are innervated by serotonergic fibers as early as E16. Disruption of the serotonergic system during embryonic development results in lower levels of reelin and a disturbed corticogenesis with disrupted formation of cortical columns (Janusonis et al., 2004). The regulation of corticogenesis by Cajal–Retzius cells is at least partly dependent on 5-HT3 receptor signaling (Chameau et al., 2009). Chameau et al. (2009) not only reported expression of 5-HT3 receptors specifically on Cajal–Retzius cells (but not on pyramidal neurons), but further established a novel role of 5-HT3 receptors, Cajal–Retzius cells, and reelin in the postnatal maturation of cortical pyramidal neurons. Cajal–Retzius cells limit the apical dendritic outgrowth of cortical layer II/III pyramidal cells and thus complexity of cytoarchitecture and network formation. Blocking 5-HT3 receptor activity with an antagonists or reelin signaling with an anti-reelin antibody leads to hypercomplexity of the apical dendrites of layer II/III pyramidal neurons in the somatosensory cortex. A similar phenotype is also present in 5-HT3 receptor knock-out mice and can be rescued by application of recombinant reelin (Chameau et al., 2009). However, it remains to be investigated if, and how, the release of reelin from Cajal–Retzius cells is directly regulated by 5-HT3 receptor activity. Similar findings of possibly indirect regulation of migration and regulation of cytoarchitecture in cortical pyramidal neurons were shown in vitro in mixed GABA- and non-GABAergic cortical neuron cultures, where 5-HT3 receptor activation inhibited axonal and dendritic outgrowth and dendritic branching only in non-GABAergic cells (Hayashi et al., 2010).

The increased dendritic complexity of cortical layer II/III pyramidal neurons in 5-HT3 receptor knock-out mice has been associated with altered cortical spatial organization and connectivity with larger dendritic bundles in layer III tangential sections, whereas spine density was not affected (Smit-Rigter et al., 2011). On a functional level, the increase in dendritic complexity of cortical layer II/III pyramidal neurons in 5-HT3 receptor knock-out mice results in a different firing pattern of these cells (van der Velden et al., 2012), suggesting that 5-HT3 receptor activity during maturation of neurons is not only important for the wiring of the local microcircuity, but also consequently for the processing of information within the circuit. As a potential consequence of this disturbed cortical wiring and function, 5-HT3 receptor knock-out mice display reduced anxiety-like behavior (Kelley et al., 2003; Bhatnagar et al., 2004) and impaired social behavior (Smit-Rigter et al., 2010), although a direct link between the cortical abnormalities and the behavioral phenotypes remains to be established.

CONCLUSION II: 5-HT3 RECEPTORS REGULATE MATURATION AND DENDRITE COMPLEXITY OF NON-INTERNEURON CELLS

5-HT3 receptors regulate the wiring of the local microcircuit in the cortex and the cerebellum by yet unknown direct or indirect mechanisms via Cajal–Retzius cells and granule cells, respectively. Therefore, 5-HT3 receptors may be crucially involved in the formation of higher-level neuronal structures (Figure 1).

PUTATIVE IMPLICATIONS FOR NEURODEVELOPMENTAL DISORDERS

5-HT3 receptors are associated with several psychiatric disorders in humans. Single nucleotide polymorphism, especially the C175T polymorphism in the 5′UTR region of the 5-HT3 receptor, were found to be associated with bipolar disorder (Niesler et al., 2001), schizophrenia (Niesler et al., 2001; Thompson et al., 2006), lowered harm avoidance in women (Melke and Westberg, 2003), alcohol and drug dependence (Enoch et al., 2010), lowered activity of amygdala and prefrontal cortex (Ideaka et al., 2005), prefrontal and hippocampal gray matter loss, and early life quality-dependent elevated depressed mood (Gaud et al., 2010a,b). These variants are associated with changes in 5-HT3 receptor function and expression (Krzypkowski et al., 2007). However, it has to be noted that 5-HT3 receptor genetics is fundamentally different between humans and rodents. 5-HT3 receptor expression in humans is much more complicated including additional splice variants of 5-HT3α, the possible expression of heteromeric receptors in the CNS, and three additional receptor genes (5-HT3β−γ), whose function and expression in the CNS have yet to be investigated.

The data presented in this review highlights the 5-HT3 receptor as a crucial regulator of brain development. This also makes it interesting as novel candidate to be involved brain development and related abnormalities. Indeed, several studies present evidence that ASD might be caused by disruptions of the serotonergic system during brain development. Common ASD animal models are based on alterations of prenatal 5-HT levels (Whitaker-Azmitia, 2005; Boylan et al., 2007; Hohmann et al., 2007). Likewise, clinical data from ASD patients points toward a causal relationship of dysfunction of the serotonergic system and ASD pathology (Anderson et al., 1987; Naffah-Mazzocarotti et al., 1993; Chugani, 2002).

Investigating a potential role of 5-HT3 receptors in the development of ASD, it is apparent that 5-HT3 receptor knock-out mice display some features similar to ASD symptoms including impaired social behavior (Smit-Rigter et al., 2010) and a reduction in basal anxiety-related behavior (Kelley et al., 2003; Bhatnagar et al., 2004; Smit-Rigter et al., 2010). Further, in line with the potential role of the 5-HT3 receptor outlined earlier in this review, these animals display some alterations in neocortical development as hypercomplexity of apical dendrites of cortical layer II/III pyramidal neurons (Chameau et al., 2009) and increased apical dendrite bundling (Smit-Rigter et al., 2011). Disruptions of neocortical development, especially in the balance between excitatory and inhibitory circuits, might at least partially underlie autism neurobiology (Pollex and Lauder, 2004; Levitt, 2005). For example, in parallel with 5-HT3 receptor knock-out animals, ASD patients display a cortical column pathology with changes in cortical minicolumn size, number and cellular distribution,
and increased cortical volume (Bailey et al., 1998; Casanova et al., 2005). Further, reelin signaling was proposed to be impaired in ASD (Luo et al., 2005). Indeed, 5-HT3 gene polymorphisms were recently found to be associated with ASD (Anderson et al., 2009; Rehnström et al., 2009). However, there is yet no evidence of a role of 5-HT3 receptors in the pathophysiology of ASD.

Finally, recent literature draws attention to the potential risk of disturbing serotonergic circuits during fetal brain development via exposure of fetuses to selective serotonin reuptake inhibitors (SSRIs). The use of SSRIs by pregnant women, especially during the first trimester, may increase the risk of ASD in the offspring (Croen et al., 2011). In mice, early postnatal exposure to SSRIs leads to increased anxiety-like behavior (Ansorge et al., 2004). In addition, in utero exposure to fluoxetine leads to life-long abnormalities of cortical cytoarchitecture and increased anxiety-like behavior (Smit-Rijgers et al., 2012). These effects are seen in 5-HT3 receptor knock-out mice suggesting that the adverse effect of fluoxetine-exposure during brain development might be 5-HT3 receptor-dependent (Smit-Rijgers et al., 2012). We conclude that, although current data is still limited, 5-HT3 receptors are important for proper brain development. The 5-HT3 receptor knock-out mouse has proven to be a valuable tool to elucidate some of the roles of 5-HT3 receptors in neuronal development. However, the availability of more advanced tools to knock-out or down-regulate 5-HT3 receptors in a more spatially and temporally controlled manner is eagerly anticipated.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.