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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, Piiaa et al., 2002) and acts as 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 serotoninergic (autoregulation) as also non-serotonergic circuit formation aids to establish CNS organization, supporting as well serotoninergic (autoregulation) as also non-serotonergic circuit formation during pre- and early postnatal periods (Sodhi and Sanders-Bush, 2010; Vitalis et al., 2007). 5-HT signaling is involved in cell division, differentiation, survival, and neuronal migration (Doody et al., 1997; Ladás et al., 1997; Armita, 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 Parnavelas, 2007). 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 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-HT₃ receptors have received the most attention as effectors of the actions of 5-HT during CNS development (Borella et al., 1997; Armita, 2001; Whitaker-Armita, 2001; Gaspar et al., 2003). Paug 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, and the glycine receptor, to the Cys-loop family of ligand-gated ion channels (Barnes and Sharp, 1999; Chameau and van Hooft, 2006; Wålstab et al., 2010; Lummis, 2012). To date, two subunits (5-HT₃A and 5-HT₃B) have been identified in rodents (Martin et al., 1991; Davies et al., 1999), and additional three subunits (5-HT₃C, 5-HT₃D, 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;
Thompson and Lummis, 2007), in such a way that incorporation of 5-HT 3A leads to an increase in single channel conductance and decrease in Ca 2+ permeability (Davies et al., 1999; Noam et al., 2008). Whether the 5-HT 3A subunit is a major determinant of 5-HT 3 receptor function in the CNS is still a subject of debate (van Hoof and Yakel, 2003; Chameau and van Hoof, 2006; Jensen et al., 2008) and appears to, at least in part, depend on species-specific expression patterns. Yet, the putative expression of 5-HT 3A subunits as part of a heteromeric 5-HT 3 receptor complex in the CNS remains of interest, especially in view of the profound effects on Ca 2+ permeability and associated downstream effectors. Most studies of 5-HT 3 receptor expression and function in the CNS in rodents focus on 5-HT 3A receptors and the terms 5-HT 3 and 5-HT 3A are used as equivalent here.

**5-HT 3 RECEPTORS ARE EXPRESSED IN CAUDAL EMINENCE-DERIVED IMMATURE AND MATURE INTERNEURONS DURING CORTICOGENESIS**

In the CNS, the 5-HT 3 receptor is first observed in the subpial ganglionic eminence (GE), the major source of interneurons in the basal telencephalon, at E12.5 (Johnson and Heinemann, 1995; Miquel et al., 1995; T ecott et al., 1995). The rodent GE generates later neocortical GABAergic interneurons which migrate tangentially into the cortical plate. In contrast, neocortical glatumatergic 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 Bossier, 2011).

5-HT 3 receptor-positive interneurons compromise ~30% of the superficial GABAergic interneurons in the somatosensory cortex (Lee et al., 2010). They comprise 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; Férézou et al., 2002; Inta et al., 2008; Lee et al., 2010; Vucurovic et al., 2010). Further expressing several morphological and electrophysiological properties, 5-HT 3 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-HT 3 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-HT 3 receptor-positive cells receives monosynaptic thalamocortical input leading to strong depolarization of these cells (Lee et al., 2010). Therefore, 5-HT 3 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-HT 3 receptors on these interneurons was published recently (Rudy et al., 2011).

The major source of 5-HT 3 receptor-expressing neocortical interneurons is the caudal part of the GE (CGE; Lee et al., 2010; Vucurovic et al., 2010). Based on recent publications, there is no expression of 5-HT 3 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 3 receptor expression was mistakenly described in the MGE in earlier publications (Tecott et al., 1995).

Recently, the generation of enhanced green fluorescent protein (EGFP)-expressing 5-HT 3 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-HT 3 receptor-positive cells during embryonic corticogenesis (Lee et al., 2010; Vucurovic et al., 2010). 5-HT 3 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., 2009; Miyoshi et al., 2007; peak of MGE-derived cortical interneuron genesis at E14.5; Miyoshi et al., 2010). 5-HT 3 receptor-positive neuroblasts thereby migrate at least partly through the neocortical subventricular zone (SVZ) and intermediate zone (IZ; Tanaka and Nakajima, 2012). Further, unlike MGE-derived interneurons, 5-HT 3 receptor-expressing interneurons do occupy preferentially superficial cortical layers I–III (Miyoshi et al., 2007; Lee et al., 2010; Vucurovic et al., 2010). Additionally, they migrate into the neocortical layers in an "outside-in" (Vucurovic et al., 2010) rather than the "inside-out" integration manner of PV- and SST-expressing interneurons. Such "outside-in" neurogenesis was previously described as a feature of CR interneurons (Rymar and Sadikot, 2007). Interestingly, in contrast to PV-interneurons, the birthdate of these CR-expressing interneurons does not match that of neighboring projection neurons in the corresponding layer (Vucurovic et al., 2004; Rymar and Sadikot, 2007). This might be true as well for the 5-HT 3 receptor-positive interneurons. Therefore, 5-HT 3 receptor-expressing CGE-derived neocortical interneurons might form a group of cells with very specific, yet unknown, characteristics and might follow different migration- and integration cues than other major groups of interneurons like PV-positive interneurons (Lee et al., 2010; Miyoshi et al., 2010).

In grafting experiments, Vucurovic et al. (2010) found that CGE-derived cells also populated several limbic structures including the bed nucleus, hippocampus, and amygdala. These were derived earlier from the CGE than the neocortical cells, which is in line with earlier genesis of interneurons in these regions (Vucurovic et al., 2010).

Furthermore, next to the CGE, embryonic 5-HT 3 receptor expression was also observed in cells of the entopeduncular area (AEP) and perirhinal area (POA; 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-HT 3 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-HT3 RECEPTORS ARE EXPRESSED IN POSTNATAL IMMATURE NEURONS

5-HT3 receptors are expressed in migrating neuroblasts in several migratory streams derived from the SVZ in the early postnatal brain (Inta et al., 2008; Vucurovic 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 cortical cortices (Le Magueresse et al., 2011). Additionally, 5-HT3 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-HT3 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-HT3 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-HT3 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-HT3 receptor-positive neuroblasts integrate into the cortex and mature into CR-positive interneurons (Kreuzberg et al., 2010). However, the majority of these cells loses 5-HT3 receptor expression upon maturation (Kreuzberg et al., 2010).

To conclude, 5-HT3 receptor-expressing neuroblasts are present in several locations in the early postnatal and adult brain. Nevertheless, both the regulation of migration and maturation of embryonic CGE- and adult SVZ-derived neuroblasts as well as the functional role of 5-HT3 receptors during these processes are yet unresolved. Only little is known about downstream signaling upon activation of 5-HT3 receptors and subsequent Ca2+ intracellular influx. Investigating a potential function of 5-HT3 receptors in regulating neuroblast migration and maturation therefore would be promising. Some recent studies proposed regulation of cytoskeletal remodeling in neurons by 5-HT3 receptors. For example, 5-HT3 receptor agonists were found to promote neurite elongation of GABAergic cortical interneurons (Vitalis and Parnavelas, 2003). Activation of 5-HT3 receptors further promotes dendrite formation in primary bitemalic neurons in vitro (Persico et al., 2006; note contradictory; Lotito et al., 1999). In growth cones, cohesin spots, and dendrites of hippocampal neurons and in human embryonic kidney (HEK) cells, 5-HT3 receptors were found to form clusters with the light chain (LC1) of microtubule-associated protein 1B (MAP1B) and the tubulin cytoskeleton (Sun et al., 2008) and these clusters lead to the formation of F-actin-rich lamellipodia (Emerit et al., 2002). 5-HT3 receptors follow the tubulin and F-actin networks for receptor routing and precise tuning at the neuronal membrane surface (Gralle et al., 2004; Ilegems et al., 2004). Further, LC1 might regulate the receptor function in these cells (Sun et al., 2008). Therefore, 5-HT3 receptors and the cytoskeleton are highly interacting, which might not only lead to the specific transport of 5-HT3 receptors into synaptic sites and regulation of receptor function, but also 5-HT3 receptors might evoke signaling involved in cytoskeletal remodeling. 5-HT3 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 relay- and CR-, but not VIP-positive neurons (Garcia et al., 2011). Whereas this activity is glutamate-dependent after P3, the source of activity before P3 is yet unclear. Serotonergic input via 5-HT3 receptors might be a candidate source of such perinatal activity.

CONCLUSION I: 5-HT3 RECEPTORS ARE POTENTIAL CENTRAL PART OF MATURATING INTERNEURONS DURING PRE- AND POSTNATAL CORTICAL DEVELOPMENT

5-HT3 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-HT3 RECEPTORS ON CEREBELLAR GRANULE AND CORTICAL CAJAL–RETZIUS CELLS

Next to the pre- and postnatal central expression of 5-HT3 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-HT3 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-HT3 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...
FIGURE 1 | Summary of (A) 5-HT₃ receptor expression on GABAergic interneurons during pre- and postnatal brain development and (B) recently described mechanisms of 5-HT₃ receptor-mediated regulation of maturation of cortical pyramidal cells and cerebellar Purkinje cells in the early postnatal brain.

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The time course of early postnatal morphological maturation of Purkinje cells as indicated by higher dendritic length and complexity in 5-HT₃ receptor knock-out mice and in vitro after treatment with a 5-HT₃ receptor antagonist (Oostland et al., 2013). 5-HT₃ receptor knock-out animals further show delayed climbing-fiber elimination (Oostland et al., 2013). However, morphology and physiology of Purkinje cells in 5-HT₃ receptor knock-out mice appears normal in adult mice, thus indicating a narrow postnatal time window of serotonergic, 5-HT₃ receptor-mediated regulation of cerebellar maturation and connectivity (Oostland et al., 2013). Further research might explore a function of 5-HT₃ 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 pyramidial 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., 2009). The regulation of corticogenesis by Cajal–Retzius cells is at least partly dependent on 5-HT3 receptor signaling (Chameau et al., 2009). Cajal–Retzius cells 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 pyramidial 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 antagonist 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 (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 pathologies such as ASD. 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 distortion of the serotonergic system and ASD pathology (Anderson et al., 1987; Naffah-Mazzucchelli et al., 1993; Chugani, 2002).

In a functional level, the increase in 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-Rijgers et al., 2011). On a functional level, the increase in dendritic complexity of cortical layer II/III pyramidal neurons in 5-HT3 receptor knockout 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 microcircuit, 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 knockout mice display reduced anxiety-like behavior (Kelley et al., 2003; Bhatnagar et al., 2004) and impaired social behavior (Smit-Rijgers 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 are associated with several psychiatric disorders in humans. Single nucleotide polymorphism, especially the C178T 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 (Iidaka et al., 2005), prefrontal and hippocampal gray matter loss, and early life quality-dependent elevated depressed mood (Gaut et al., 2010a,b). These variants are associated with changes in 5-HT3 receptor function and expression (Krzysikowski 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-HT3C, −E), 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 in brain development and ASD. 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 distortion of the serotonergic system and ASD pathology (Anderson et al., 1987; Naffah-Mazzucchelli et al., 1993; Chugani, 2002).

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 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-HT3C, −E), whose function and expression in the CNS have yet to be investigated.

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and increased cortical volume (Bailey et al., 1998; Casanova et al., 2002; Carpenter and Courchesne, 2005). Further, reelin signaling was proposed to be impaired in ASD as a result of reelin deficiency (Smit-Rigter et al., 2012). 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 pathobiology 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 (Craen et al., 2011). In mice, early postnatal exposure to SSRIs leads to increased anxiety-like behavior (Ansorge et al., 2004). In addition, in vitro exposure to fluoxetine leads to life-long abnormalities of cortical cytoarchitecture and increased anxiety-like behavior (Smit-Rigter et al., 2012). These findings could anticipate.

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