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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 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 serotonergic (autoregulation) as also non-serotonergic circuit formation and function in the CNS, which could have a major impact on our understanding of neurodevelopmental disorders in which 5-HT plays a role.

Serotonin (5-hydroxytryptamine, 5-HT), next to being an important neurotransmitter, recently gained attention as a key-regulator of pre- and postnatal development in the mammalian central nervous system (CNS). Several receptors for 5-HT are expressed in the developing brain including a ligand-gated ion channel, the 5-HT₃ receptor. Over the past years, evidence has been accumulating that 5-HT₃ receptors are involved in the regulation of neurodevelopment by serotonin. Here, we review the spatial and temporal expression patterns of 5-HT₃ receptors in the pre- and early postnatal rodent brain and its functional implications. First, 5-HT₃ receptors are expressed on GABAergic interneurons in neocortex and limbic structures derived from the caudal ganglionic eminence. Mature inhibitory GABAergic interneurons fine-tune neuronal excitability and thus are crucial for the physiological function of the brain. Second, 5-HT₃ receptors are expressed on specific glutamatergic neurons, Cajal–Retzius cells in the cortex and granule cells in the cerebellum, where they regulate morphology, positioning, and connectivity of the local microcircuitry. Taken together, the 5-HT₃ receptor emerges as a potential key-regulator of network formation and function in the CNS, which could have a major impact on our understanding of neurodevelopmental disorders in which 5-HT plays a role.

Keywords: serotonin, 5-HT₃ receptor, development, interneurons, neoblasts
Thompson and Lumsd, 2007), in such a way that incorporation of 5-HT$_3$A 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$_3$A subunit is a major determinant of 5-HT$_3$ receptor function in the CNS is still a subject of debate (van Hooff and Yäkel, 2003; Chameau and van Hooff, 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$_3$A 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$_3$A receptors and the terms 5-HT$_3$ and 5-HT$_3$A 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; Mädel et al., 1995; Tecott et al., 1995). The rodent GE generates later neocortical GABAergic interneurons which migrate tangentially into the cortical plate. In contrast, neocortical glatumagetic neurons originate in the pallial ventricular zone (VZ) and migrate radially into the cortex (Corbin et al., 2001; Nadara-}


dependent on their birthdate, to cells of the dentate gyrus (DG), amygdala, endopiriform nucleus, and the caudatum.

**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 GEs (LGes). 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-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 García-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 migrate 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 Ca$^{2+}$ 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 GABAAergic cortical interneurons (Vitalis and Parnavelas, 2003). Activation of 5-HT3 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-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 (Giralde et al., 2004; Ilegems et al., 2004). Furthermore, 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 involving 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 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. Serotoninergic input via 5-HT3 receptors might be a candidate source of such perinatal activity.

**Conclusion I: 5-HT3 receptors are a potential central part of maturing interneurons during pre- and postnatal cortical development**

5-HT3 receptors are expressed on embryonic immature CGE-derived GABAAergic 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-nuclear pre-synaptic 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 serotoninergic 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...
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; Lee et al., 2010). Cajal–Retzius cells are transient neurons located in the marginal zones of the neocortex and hippocampus during CNS development (Marin-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., 2012), suggesting that 5-HT3 receptor activity during maturation of neurons is not only important for the wiring of the local microcircuitry, but also consequently for the processing of information within the circuit. As a potential consequence of this disruption of corticogenesis, by 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 C178T polymorphism in the 5′UTR region of the 5-HT3 receptor, was found to be associated with bipolar disorder (Nisier et al., 2001), schizophrenia (Niesler et al., 2001; Thompson et al., 2006), lowered heart avoidance in women (Melke and Westberg, 2003), alcohol and drug dependence (Enoch et al., 2010), lowered activity of amygdala and prefrontal cortex (Idaaka 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-HT3A, the possible expression of heteromeric receptors in the CNS, and three additional receptor genes (5-HT3C−D), 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 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). Like-wise, clinical data from ASD patients points toward a causal relationship of disruption of the serotonergic system and ASD pathology (Anderson et al., 1987; Chugani, 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 (Polleux 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., 2002; Carper and Courchesne, 2005). Further, reelin signaling was proposed to be impaired in ASD (Smit-Rigter et al., 2012). These results suggest that 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 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.

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