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Ontogeny and regulation of the serotonin transporter: Providing insights into human disorders

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ABSTRACT

Serotonin (5-hydroxytryptamine, 5-HT) was one of the first neurotransmitters for which a role in development was identified. Pharmacological and gene knockout studies have revealed a critical role for 5-HT in numerous processes, including cell division, neuronal migration, differentiation and synaptogenesis. An excess in brain 5-HT appears to be mechanistically linked to abnormal brain development, which in turn is associated with neurological disorders. Ambient levels of 5-HT are controlled by a vast orchestra of proteins, including a multiplicity of pre- and post-synaptic 5-HT receptors, heteroreceptors, enzymes and transporters. The 5-HT transporter (SERT, 5-HTT) is arguably the most powerful regulator of ambient extracellular 5-HT. SERT is the high-affinity uptake mechanism for 5-HT and exerts tight control over the strength and duration of serotonergic neurotransmission. Perturbation of its expression level or function has been implicated in many diseases, prominent among them are psychiatric disorders. This review synthesizes existing information on the ontogeny of SERT during embryonic and early postnatal development though adolescence, along with factors that influence its expression and function during these critical developmental windows. We integrate this knowledge to emphasize how inappropriate SERT expression or its dysregulation may be linked to the pathophysiology of psychiatric, cardiovascular and gastrointestinal diseases.

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1. Introduction

Serotonin (5-HT) is a neuromodulatory neurotransmitter serving a wide array of physiological and behavioral functions. It has become clear over recent decades that a particularly salient role of 5-HT is as a developmental signal, important for cueing proper wiring of neural circuits (see Gaspar et al., 2003 for review). It is not surprising then, that a growing literature is focused on understanding the consequences of inappropriate ambient levels of 5-HT during development for the adult phenotype. Indeed, abnormal levels of 5-HT result in aberrant morphology and wiring of the nervous system across species, ranging from *Drosophila* (Sykes & Condron, 2005; Daubert et al., 2010), to *Aplysia* (Castellucci et al., 1970; Brunelli et al., 1976) to mammals (see Gaspar

Abbreviations: CpG, Cytosine-phosphate-guanosine; DA, dopamine; DAT, dopamine transporter; DRN, dorsal raphe nucleus; ED, embryonic day; GD, gestational day; GW, gestational week; HPA, hypothalamic–pituitary–adrenal; IBS, irritable bowel syndrome; KO, knockout; MAPK, mitogen activated protein kinase; MDMA, 3,4-methylenedioxymethamphetamine; MCT, monocrotaline; NE, norepinephrine; NET, norepinephrine transporter; OCT, organic cation transporter; PMAT, plasma membrane monoamine transporter; PD, postnatal day; SSRI, selective serotonin reuptake inhibitor; SNP, single nucleotide polymorphisms; VNTR-2, variable number of tandem repeats in the second intron; VMAT2, vesicular monoamine transporter-2; 5-HT, serotonin, 5-hydroxytryptamine; SERT/5-HTT, serotonin transporter; 5-HTTLPR, serotonin transporter linked polymorphic region; +/+, wildtype; +/−, heterozygote; −/−, null, knockout.

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et al., 2003 for review). Ambient levels of 5-HT are controlled by a number of factors including rate of 5-HT synthesis and amount available for release, rate of release, rate of enzymatic breakdown, rate of diffusion through the extracellular milieu and active uptake. The serotonin transporter (SERT) is the high-affinity uptake mechanism for 5-HT. In the mature animal it is located perisynaptically on presynaptic 5-HT nerve terminals as well as on axons and 5-HT cell bodies in the raphe (Rudnick & Clark, 1993; Barker & Blakely, 1995; Blakely et al., 1998). SERTs are also expressed on non-neuronal cells including platelets (Rudnick, 1977; Hranilovic et al., 1996), lymphoblasts (Khan et al., 1996; Faraj et al., 1997), monocytes (Yang et al., 2007), enterochromaffin cells, endothelial cells (Gershon, 1999, 2003; Wheatcroft et al., 2005) and placental syncitiotrophoblasts (Balkovetz et al., 1989). In brain, SERT is arguably the predominant mechanism controlling the strength and duration of serotonergic neurotransmission. As such, factors that perturb SERT expression and/or function during critical periods early in development might be expected to have detrimental consequences in later life. This forms the basis of the present review, which describes what is known about development of SERT in brain under normal conditions, what intrinsic and extrinsic factors can influence SERT expression and function and importantly, how perturbation of SERT early in life can contribute to psychiatric and physiological disease states in later life.

2. Developmental profile of SERT gene and protein expression

To begin, it is necessary to first summarize what is known about "normal" SERT development. Cortical neurogenesis in humans occurs between gestational weeks (GWs) 6 and 16 (Sidman & Rakic, 1973) with neuronal migration leading to distinguishable cortical layers occurring between GW24 and GW26, an event coinciding with synaptogenesis (Huttenlocher & de Courten, 1993). With the advent of SERT specific antibodies (Qian et al., 1995) it became possible to map SERT development in human brain. In fetal brain, SERT antibodies revealed SERT-positive, thick, varicose fibers emerging from the raphe, and reaching the cortical analae at GW8, the subplate at GW10, and arriving at the cortical plate at GW13 (Verney et al., 2002). These findings are consistent with those in non-human primates, where serotonergic axons have been detected in entorhinal cortex in early embryonic life of rhesus macques (Berger et al., 1993) and where the 5-HT terminal network has been shown to mature very rapidly during early postnatal life (Lambe et al., 2000; Verney, 2003).

In addition to SERT-positive fibers arising from the raphe, a second type of SERT-positive fiber was detected that did not resemble classical catecholaminergic fibers (Verney et al., 2002). These non-monoaminergic SERT-positive fibers differ in several respects from those emerging from the raphe, including their anatomic location, the internal capsule, an unusual localization for monoaminergic fibers, and their transient expression between GW12 and GW14. These non-monoaminergic neurons send projections from the anterior and posterior ends of the interior capsule, which lies between the caudate and putamen, and project toward the cerebral and temporal cortex. That these fibers appear at GW12 and disappear by GW14 indicate that 5-HT may play a role in the trophic organization of the somatosensory cortex at this time (Verney et al., 2002).

One of the markers used to define fibers as monoaminergic in these studies was the presence of immunoreactivity for the vesicular monoamine transporter-2 (VMAT2). VMAT2 is responsible for taking up monoamines (including 5-HT, dopamine (DA), and norepinephrine (NE)) from the intracellular fluid and storing them in synaptic vesicles, and so is a useful marker of monoaminergic processes (Henry et al., 1998). Importantly, VMAT2 serves to store 5-HT into synaptic vesicles and protect it from degradation. While SERT and VMAT2 expression do not always go hand in hand in adult brain, for example, in certain neuronal populations within the cerebral cortex (Lebrand et al., 1998), the implications for this discourse during transient phases of early embryonic development remain unclear. Given the important trophic role of 5-HT during early development, and that excess 5-HT during these critical phases can have negative implications for the adult, it is possible that this transient period of SERT expression on non-VMAT2 expressing processes may be important for keeping 5-HT levels in check (for review see Gaspar et al., 2003).

In rodents in situ hybridization (Lebrand et al., 1996; Hansson et al., 1998, 1999), autoradiographic binding (D'Amato et al., 1987; Bennett-Clarke et al., 1997) and immunocytochemical (Lebrand et al., 1998; Zhou et al., 2000) approaches also reveal transient SERT gene and protein expression in brain during early development. Now, with the genetic tractability of mouse models, important insight into the physiological implications for these transient periods of SERT expression in the developing brain are being uncovered. For example, studies using SERT knockout (KO) mice have demonstrated a clear need for SERT in the normal development of thalamic projections (Persico et al., 2001; Salichon et al., 2001).

It is now well established that SERT is expressed much more broadly during development than in adulthood, and is expressed in non-5-HT neurons as well as neural crest derivatives. However, to further interrogate the expression pattern of SERT during development Narboux-Nême et al. (2008) inserted Cre recombinase into the SERT gene ($SERT^{cre}$) of two reporter mouse lines; (1) the ROSA26R mouse, which drives LacZ expression in the cell's cytoplasm regardless of its lineage (Soriano, 1999; Narboux-Nême et al., 2008) and (2) the Tau^{mGFP} mouse which is specific to neurons (Hippenmeyer et al., 2005; Narboux-Nême et al., 2008). This enabled a fate mate for SERT expressing cells to be created. The results, as anticipated, revealed remarkably broad SERT expression in non-5-HT cells during development (Narboux-Nême et al., 2008). SERT is particularly precocious in non-neural cells being robustly expressed in heart and liver at embryonic day (ED) 10.5 (Narboux-Nême et al., 2008). These results from $SERT^{cre}$ mice complement and extend earlier reports using in situ hybridization approaches (Hansson et al., 1998; Lebrand et al., 1998). Moreover, they reveal that brain regions, including CA1–CA2 of hippocampus, dentate gyrus, striatum, piriform cortex and amygdala, previously thought to transiently express SERT (based on in situ hybridization or immunocytochemistry) (Cases et al., 1998; Hansson et al., 1998; Lebrand et al., 1998; Zhou et al., 2000), do not appear to express the SERT gene at any time during development.

What is the fate of SERT after it first appears embryonically? Does SERT protein expression remain static or does its expression wax and wane? As just decribed, SERT protein measured by quantitative autoradiography, is first expressed during prenatal brain development on ED12 in mice, and by ED18 it is found in all of the same brain regions where 5-HT is localized immunohistochemically (Brüning et al., 1997). During the course of embryonic development, in brain regions such as the thalamus and somatosensory cortex, SERT density peaks and subsequently declines by postnatal day (PD) 7 (Brüning & Liangos, 1997). During critical stages of brain development 5-HT levels can influence the expression or function of key mediators of 5-HT neurotransmission, including SERT. In the juvenile brain, SERT binding measured using a SERT selective radioligand in homogenates prepared from rodent frontal cortex, increases steadily from weaning until late adulthood (Moll et al., 2000). However, in other brain regions such as the dorsal raphe and parietal cortex, SERT density measured using quantitative autoradiography peaks and declines prior to PD20, possibly in response to a peak in extracellular 5-HT in those regions during brain development and maturation in rats (Galineau et al., 2004). In agreement with these findings in rats, Sidor et al. (2010) more recently reported that mRNA for SERT in several regions of the dorsal raphe was highest at PD14, the youngest age examined in their study, and declined to relatively stable expression levels across the age range PD17–28 in adolescent mice.

Thus, SERT gene and protein expression is transient in some brain regions, peaks and wanes in other regions and follows a steady incline to adult levels in others. However, as discussed in the following sections,