Roles of Serotonin Signaling in Embryonic and Postnatal Neurogenesis

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Serotonin (5-hydroxytryptamine, 5-HT) is perhaps the most well-studied of the classical neurotransmitters and has been implicated in many behavioral adaptations as well as essential physiological functions. In addition to its role in these processes, 5-HT is also a vital regulator of development and begins acting as early as the end of gastrulation and continues throughout postnatal development. A rich body of research has established that 5-HT is intimately involved in the survival, proliferation, migration, differentiation, and maturation of neurons, and the formation of functional synapses. This review surveys experiments that have demonstrated the role of 5-HT signaling in each of these processes, most of which are mediated by G-protein coupled receptor signaling cascades. However, 5-HT3, the only ligand-gated cation channel member of the 5-HT receptor gene family, has been understudied in the context of development. Evidence presented here suggests a significant role for the 5-HT3 receptor in neurogenesis, but much is still left to be learned about the mechanisms by which it acts. The information gained from the study of 5-HT signaling in neurogenesis has important implications for a more thorough understanding of the development of the central and peripheral nervous systems.

Keywords *Serotonin, serotonin receptors, neurogenesis, development, signaling cascade, molecular biology*

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is an evolutionarily ancient molecule[1] that is critical for the modulation of many physiological and behavioral adaptations. Among these adaptations are appetite, gastrointestinal function, nociception, sexual behavior, mood, learning and memory. The role of 5-HT in these processes has been extensively studied for decades. Outside the realm of classical neurotransmission, 5-HT is considered to be a key regulator of neurogenesis through neurotrophic effects[2,3], and this notion is well supported in the literature. 5-HT expression is highly abundant in the developing central and peripheral nervous systems[4]. Additionally, serotonergic neurons housed in the brainstem are among the earliest born[5,6] and they innervate most regions of the brain[7]. One of the foundational experiments identifying 5-HT in neurodevelopment demonstrated that pharmacological depletion of 5-HT during rat embryogenesis results in impaired neural differentiation in the brain[8]. Since then, advances in molecular biology and pharmacology have permitted study of the modulation of neurogenesis by 5-HT during embryonic and postnatal development, specifically by the activity of 5-HT receptors and the 5-HT transporter, SERT. This review highlights research that attests to the importance of 5-HT signaling throughout neurogenesis in mammalian development and identifies questions in the field that remain unanswered.

Neurogenesis:
The process by which neurons are generated from neural stem and/or precursor cells.

Differentiation:
The developmental process of neural progenitors acquiring the genetic and phenotypic characteristics of their terminal neuronal fates.

Serotonin in the Modulation of Survival, Proliferation and Differentiation

Numerous studies support the hypothesis that 5-HT promotes the proliferation and survival of neuronal progenitors early in embryogenesis. One way 5-HT acts in these processes is by mediating the activity of glycogen synthase kinase-3β (GSK3β). GSK3β

Wnt signaling pathway: Highly conserved cellular signal transduction pathway that plays a crucial role in embryonic and fetal development; known downstream effects include modulation of gene expression, cytoskeletal dynamics, and intracellular calcium levels.

Specification: Also referred to as lineage segregation or divergence, the means by which neural precursors are assigned neuronal identities but have yet to become determined or undergo differentiation.

acts as a negative regulator of neurogenesis by inhibiting β-catenin in the canonical Wnt signaling pathway. Inhibition of GSK3β allows β-catenin to enter the nucleus and activate target genes that promote the survival and proliferation of neuronal progenitors[9]. The effect of 5-HT on GSK3β activity was first observed in the mouse brain—upon administration of d-fenfluramine, a SERT inhibitor, GSK3β becomes phosphorylated and subsequently inhibited in the cerebral cortex and hippocampus[10]. A later experiment showed a similar effect in animals lacking functional tryptophan hydroxylase-2 (Tph2), a key enzyme in neuronal 5-HT synthesis: Tph2 null mice also display phosphorylated, inhibited GSK3β[11]. In both experiments, inhibited GSK3β results in perpetuation of the neural stem cell profile, such that survival and proliferation programs are maintained. Interestingly, the selective serotonin reuptake inhibitor (SSRI) fluoxetine has been proposed to mediate its antidepressant effects by fostering neurogenesis in the hippocampus[12-14]. This hypothesis is supported by the observation that fluoxetine increases phosphorylation and inhibition of GSK3β, thereby promoting survival and proliferation[15,16]. Further experimentation revealed that inhibition of GSK3β occurs by signaling through the serotonin type 1A receptor (5-HT1A). Specifically, 5-HT1A signaling stimulates the kinase activity of Akt[17,18], which is known to phosphorylate and inhibit GSK3β[19-21]. In favor of this mechanism, it was shown that 5-HT1A-specific antagonists block phosphorylation of GSK3β by Akt[10]. Collectively these findings demonstrate that 5-HT1A signaling causes inhibition of GSK3β, stimulating survival and proliferation in neurogenesis.

While there is no evidence substantiating a direct role for 5-HT in specification of neurons, there is support for 5-HT regulating mechanisms of differentiation. Contradictory to its influence on GSK3β inhibition, 5-HT1A signaling mediates effects that oppose the proliferation and survival of neuronal precursors by promoting neuronal differentiation. Autoinhibition of the 5-HT1A receptor moderates serotonergic neuronal differentiation in the developing raphe nucleus, as evidenced by increased serotonergic density in this region in animals lacking 5-HT1A[22]. It has also been noted that the 5-HT1A receptor is expressed early in the mouse fetal brain, and over developmental time its autoinhibitory activity results in decreased 5-HT1A expression as serotonergic neuronal differentiation increases[23]. Corroborating these results, other experiments showed that the absence of SERT leads to excessive stimulation of 5-HT1A receptors and subsequent inhibition of serotonergic neuronal development[24]. Aside from serotonergic neurons, 5-HT signaling also stimulates differentiation of glutamatergic neurons in the embryonic cerebral cortex[25], demonstrating that 5-HT affects the differentiation of multiple neuronal subtypes. In an effort to tease apart the mechanisms underlying these effects, work in cultured cells from the developing brainstem and cortex of fetal mice showed that 5-HT1A activity stimulates the release of S100β from astroglial cells expressing this receptor[26-29]. S100β, when secreted from astroglial cells and taken in by neighboring neuronal progenitors, is anti-apoptotic[30,31] and promotes neuronal differentiation[32]. This factor appears to be involved in regulating the stability of tubulin in the construction of microtubules, thus serving to help stabilize the neuronal cytoskeletal architecture required for differentiation[33]. These collective reports indicate that the 5-HT1A receptor plays opposing roles in neurogenic processes and further study is needed to dissect its mechanisms of action in various settings.

The influence of 5-HT signaling on survival, proliferation and differentiation of neuronal progenitors is not unique to the central nervous system. While 5-HT2B promotes survival in cortical neuronal progenitors[34,35], it also modulates survival and differentiation during peripheral neurogenesis. Depletion of 5-HT2B at the onset of neurulation in mice results in precocious neuronal differentiation

and failure of cranial neural crest cells to migrate to the periphery[36]. In the enteric nervous system (ENS), it was shown by Michael Gershon's group that this receptor is important for initiating differentiation of enteric neurons[37]. Moreover, they have also published studies demonstrating the requirement of 5-HT4 for the generation and survival of neurons in the developing postnatal enteric nervous system[38]. By ablation of Tph2 gene expression, they showed that 5-HT is necessary for the proper differentiation and maintenance of dopaminergic neurons in the gut[39]. These experiments collectively emphasize the significance of 5-HT signaling in the development of several neuronal populations, although the underlying molecular mechanisms have yet to be dissected. How 5-HT functions in the neurodevelopment of other visceral organs outside the ENS is still largely unknown. Clearly, the means by which serotonin signaling impacts the beginning stages of neurogenesis are highly diverse and the downstream effects of several 5-HT receptors vary throughout the course of development.

Serotonin in Neuronal Maturation and Synaptogenesis

An extensive body of literature reports the effects of 5-HT signaling on the maturation of the neuronal phenotype following differentiation and the formation of functional synapses. The involvement of 5-HT in neurite outgrowth and dendritic arborization is especially well-supported by numerous studies. In embryonic development of the mouse raphe nucleus, loss of the 5-HT1A receptor results in an increase in neurite number and length[22]. A comparable autoinhibitory effect of this receptor was noted in cultured fetal rat cortical neurons[40]. However, deprivation of 5-HT during postnatal rat development ultimately leads to deficient dendritic branching on granule cells of the dentate gyrus, which can be rescued with a 5-HT1A receptor agonist[41]. As described earlier in the context of proliferation and differentiation, it appears that 5-HT1A activity has varying effects either by promoting or inhibiting neuritic outgrowth and maturation. Other 5-HT receptors are also influential in neuronal maturation. For example, in fetal mice, enhanced neurite outgrowth is seen when 5-HT1B receptors expressed in thalamic neurons are stimulated[42]. Similarly, detailed studies have revealed that the 5-HT7 receptor activity regulates neuronal architecture in the construction of cortical columns as serotonergic inputs connect to Cajal-Retzius cells[43]. Coupling between stimulated 5-HT7 and the Gα-12 protein activates the RhoA and Cdc42 signaling cascades. When activated by Gα-12, these factors promote and impede, respectively, neurite outgrowth and growth cone motility[20,44-46]. Conversely, 5-HT4 activity, by coupling to the heterotrimeric G13 protein and activating the RhoA signaling cascade, inhibits neurite outgrowth and cell-rounding during neurogenesis in the hippocampus[47,48]. Analogous to the complexity of actions seen in survival and proliferation, the opposing effects of 5-HT in neurite outgrowth and maturation attest to the diversity of the roles this neurotransmitter can play in multiple neurogenic processes.

In addition to its influence on dendritic morphology, 5-HT has also been implicated in moderating axon guidance mechanisms in development. Work from Levitt and colleagues in recent years have clearly demonstrated a unique role for 5-HT signaling in guidance of thalamocortical axons in the developing forebrain of rodents and humans. Using 5-HT1A/1D receptor-specific drugs, they found that activation of the $G_{i/o}$-protein signaling pathway through these receptors, which inhibits adenylyl cyclase and decreases intracellular levels of cAMP[49], causes the axonal attractant netrin-1 to become a repulsive cue for migrating axons[50]. Additionally, segregation of axons in developing thalamic sensory projections relies on appropriate levels of 5-HT signaling in the early postnatal brain—overstimulation of 5-HT1B receptors results in cytoarchitectural aberrations of somatosensory projections to the thalamus and thalamic projections to the barrel field cortex[51]. All the studies reported here emphasize the importance of 5-HT signaling in neuronal maturation processes.

The role of 5-HT signaling in neurogenesis extends from neuronal maturation and axon guidance to the formation of synapses during embryonic and postnatal development. Recalling the early neurogenic effects of 5-HT signaling on s100β expression, 5-HT continues to act through s100β to support synaptogenesis and synaptic plasticity. Transient depletion of 5-HT during rat postnatal brain development leads to a subsequent loss of s100β, which ultimately results in a thinning of synaptic density in the hippocampus of adult rats[52]. The consequence of 5-HT and s100β loss during this time is permanent, such that even after restoration of endogenous 5-HT these synapses cannot be reformed in adulthood. The actions of 5-HT signaling on s100β not only affect synapse formation but also plasticity. When s100β is lost early in mouse development, the remaining hippocampal synapses are less likely to adapt to learning and memory formation compared to control animals[53]. It

is important to recognize that the effects of 5-HT signaling during developmental neurogenesis continue to impact adult brain structure and function.

A Potential Role for the 5-HT3 Receptor in Neurogenesis

Throughout this review of serotonin's modulation of neurogenesis, the focus has been on the members of the 5-HT receptor family that are G-protein coupled receptors. The 5-HT3 receptor is the only serotonin receptor that is a ligand-gated ion channel[54,55], and there are several lines of evidence that implicate an important role for 5-HT3 in central nervous system development. Loss of 5-HT3 activity in cortical development results in decreased levels of reelin. The expression of reelin, an extracellular matrix glycoprotein involved in neurogenesis, has been demonstrated in Cajal-Retzius cells in layer I of the cortex in mice[56]. Reelin expression in this cell population is dependent upon the reception of excitatory input through 5-HT3A receptors[57]. It has also been demonstrated that reelin helps coordinate the migration of sympathetic preganglionic neuronal progenitors to the spinal cord in fetal development[58], which suggests a role for 5-HT signaling in this system. 5-HT3 receptors have also been shown to be involved in the migratory patterns and maturation of GABAergic interneurons in mouse cortical development. Application of a 5-HT3 agonist causes this cell population to have abnormally long neuritic processes with few branches, while a 5-HT3 antagonist results in the formation of numerous, short processes and failure to migrate to the cortical plate[59]. Moreover, fluoxetine has been demonstrated to functionally suppress the activity of 5-HT3 receptors[60], and very recently it was discovered that prenatal exposure to fluoxetine reduces dendritic complexity by nearly 50% in pyramidal neurons of the cortex in mice[61]. This receptor's actions in neurogenesis extend into the construction of neuronal architecture. Elegant experiments have demonstrated an interaction between the intracellular portion of the 5-HT3 receptor and F-actin, implying that 5-HT3 modulates cytoskeletal structure during neuronal migration and maturation[62]. 5-HT3 activity appears to mediate multiple neurogenic processes throughout the course of neurodevelopment—yet, the causal mechanisms underlying the function of 5-HT3 are poorly understood.

It is widely known that regulation of calcium flux in neural progenitors is required for proper nervous system development to occur. Interestingly, presynaptically localized 5-HT3 is permeable to calcium in neurons of the corpus striatum, hippocampus and amygdala[63,64]. 5-HT3 receptors have enhanced calcium permeability in several neuroblastoma cell lines as well[65,66]. Given this information, it is plausible that 5-HT3 exerts its effects on neurogenesis via calcium signaling. Research supporting this hypothesis has only been conducted fairly recently. PC12 cells express 5-HT3, and an increase in intracellular calcium levels was observed in response to treatment with a 5-HT3 agonist[67]. This effect led to Nerve Growth Factor (NGF) upregulation, resulting in neurite outgrowth and differentiation, and is blocked by a 5-HT3 antagonist. Surprisingly, the L-type calcium channel antagonist nifedipine also inhibits the 5-HT-induced increase in intracellular calcium and its stimulation of NGF. The response to nifedipine suggests that 5-HT3 stimulation likely affects the activity of voltage-gated calcium channels, providing another mechanism by which 5-HT3 receptors are responsible for regulating calcium flux in developing neurons. This hypothesis is especially intriguing, as the calcium-dependent promotion of Brain Derived Neurotrophic Factor (BDNF) transcription by the CREB pathway is mediated by L-type calcium channels[68-70]. It was also shown that 5-HT3 receptors are expressed transiently in the glutamatergic granule cells of the developing cerebellum, where they were shown to be critical for promoting plasticity during synaptogenesis of Purkinje cells and parallel fibers[71]. The authors of this work postulate that 5-HT3 may be mediating its effects via control of calcium flux, either through 5-HT3 itself or its activation of L-type calcium channels, but this possibility has not yet been pursued.

In contrast to the intriguing studies conducted in the CNS, the role of 5-HT3 in PNS development is almost entirely unknown. In 1996, Johnson and Heinemann reported expression of 5-HT3 in the neural crest cells of rat embryos aged 15 days post coitus (dpc), including sympathetic and parasympathetic ganglia of the enteric nervous system and the dorsal root ganglia (DRG)[72]. This observation is corroborated by demonstration of 5-HT3 gene expression in the DRG of 14.5 dpc fetal mice[73]. There has been no follow up on these findings, so the function of 5-HT3 throughout neural crest development remains unclear. As mentioned previously, the development of some aspects of the PNS is poorly studied; among these is the innervation of the lower urinary tract (LUT). Proper 5-HT3 function is critical for maintaining the autonomic innervation of the LUT in adult mice[74], but its role in the development of this system has not yet been explored. Based on the importance

of 5-HT signaling in neurogenesis and the preliminary observations outlined here, there is strong support for the hypothesis that signaling through the 5-HT3 receptor influences neural crest development and the innervation of the LUT.

To investigate the function of 5-HT3 in neural crest cells, a wide variety of tools is available. Transgenic reporter mouse lines[75] allow visualization of 5-HT3 expression throughout embryonic and postnatal development. A 5-HT3 knockout line exists[76] and would be valuable to study the effects of loss of this receptor on neural crest survival, differentiation and migration to the LUT. *In vitro* study of mechanisms by which 5-HT3 mediates neuronal specification of neural crest progenitors is possible with numerous drugs affecting 5-HT3 activity[4]. Pharmacological agents may also be used to tease apart the downstream signaling cascades regulating neurogenesis that are stimulated by 5-HT3. Additionally, emerging technology facilitating imaging of calcium flux in live cells permits examination of the functionality of 5-HT3 in neurogenesis[77]. These and other molecular biology techniques will allow researchers to begin to elucidate the role of 5-HT3 signaling in PNS neurogenesis.

Conclusions

A broad body of evidence, including studies described here and others not mentioned, underscores the importance of 5-HT signaling in multiple processes that comprise neurogenesis. 5-HT has been shown to be intimately involved in the survival, proliferation and differentiation of neuronal progenitors. Additionally, 5-HT functions to modulate the migration of differentiating neurons, the augmentation of neurites, and the construction of synapses and cellular architecture. The diversity of the processes regulated by 5-HT is reflected in the myriad signaling mechanisms by which 5-HT acts via its receptors. In fact, the same receptor can even serve oppositional functions throughout the course of development, as is the case with 5-HT1A and 5-HT7. While some of the signaling cascades mediating these processes have been dissected, much work remains to be done in order to discover the ways in which 5-HT signaling is able to take on so many roles throughout the course of neurogenesis. Especially compelling for future study is the 5-HT3 receptor, the only ligand-gated ion channel in the serotonin receptor family. Several publications implicate a significant role of 5-HT3 in neurogenesis—however, no one has yet assembled the pieces of the puzzle to understand precisely the ways by which this receptor affects neurogenic processes. The subject of 5-HT3 in peripheral neurogenesis is still largely untouched. Fortunately, pharmacological and molecular tools currently available make it possible to investigate how 5-HT3 guides neurogenesis in embryonic and postnatal development.

References

1. Turlejski K (1996). Evolutionary ancient roles of serotonin: long-lasting regulation of activity and development. Acta Neurobiol Exp. 56: 619-636.

2. Baker P and Quay W (1969). 5-hydroxytryptamine metabolism in early embryogenesis, and the development of brain and retinal tissues. Brain Res. 12: 273-295.

3. McMahon D (1974). Chemical messengers in development: a hypothesis. Science. 185: 1012-1021.

4. Nichols DE and Nichols CD (2008). Serotonin receptors. Chem Rev. 108 (5): 1614-1641.

5. Olson L and Seiger A (1972). Early prenatal ontogeny of central monoamine neurons in the rat: fluorescence histochemical observations. Z Anat EntwGesch. 137: 301-316.

6. Lauder J and Bloom F (1974). Ontogeny of monoamine neurons in the locus coeruleus, raphe nuclei, and substantia nigra of the rat: I. Cell Differentiation. J Comp Neurol. 155: 469-481.

7. Jacobs B and Azmitia EC (1992). Structure and function of the brain serotonin system. Physiol Rev. 72: 165-229.

8. **Lauder J and Krebs H (1978). Serotonin as a differentiation signal in early neurogenesis. Dev Neurosci. 1: 15-30.**

   This paper was one of the earliest studies that examined the role of serotonin in embryonic neurogenesis. The researchers found that serotonin signaling is necessary for differentiation and maintenance of neurons in the brain.

9. Kim W, Wang X, Wu Y, Doble B, Patel S, Woodgett J and Snider W (2009). GSK-3 is a master regulator of neural progenitor homeostasis. Nat Neurosci. 12 (11): 1390-1399.

10. Li X, Zhu W, Roh M, Friedman A, Rosborough K and Jope R (2004). In vivo regulation of glycogen synthase kinase-3beta (GSK3beta) by serotonergic activity in mouse brain. Neuropsychopharmacology. 29: 1426-1431.

11. Beaulieu J, Zhang X, Rodriguez R, Sotnikova T, Cools M, Wetsel W, Gainetdinov R and Caron M (2008). Role of GSK3 beta in behavioral abnormalities induced by serotonin deficiency. Proc Natl Acad Sci U S A. 105 (4): 1333-1338.

12. Bachis A, Mallei A, Cruz MI, Wellstein A and Mocchetti I (2008). Chronic antidepressant treatments increase basic fibroblast growth factor and fibroblast growth factor-binding protein in neurons. Neuropharmacology. 55 (7): 1114-1120.

13. Czeh B, Muller-Keuker J, Rygula R, Abumaaria N, Hiemke C, Domenici E and Fuchs E (2007). Chronic social stress inhibits cell proliferation in the adult medial prefrontal cortex: hemispheric asymmetry and reversal by fluoxetine treatment. Neuropsychopharmacology. 32: 1490-1503.

14. Encinas JM, Vaahtokari A and Enikolopov G (2006). Fluoxetine targets early progenitor cells in the adult brain. Proc Natl Acad Sci U S A. 103 (21): 8233-8238.

15. McManus E, Sakamoto K, Armit L, Ronaldson L, Shpiro N, Marquez R and Alessi D (2005). Role that phosphorylation of GSK3 plays in insulin and Wnt signalling defined by knockin analysis. EMBO J. 24: 1571-1583.

16. Polter A, Beurel E, Yang S, Garner R, Song L, Miller C, Sweatt J, McMahon L, Bartolucci A, Li X and Jope R (2010). Deficiency in the inhibitory serine-phosphorylation of glycogen synthase kinase-3 increases sensitivity to mood disturbances. Neuropsychopharmacology. 35: 1761-1774.

17. Cowen DS, Sowers R and Manning D (1996). Activation of a mitogen-activated protein kinase (ERK2) by the 5-hydroxytryptamine 1A receptor is sensitive not only to inhibitors of phosphatidylinositol 3-kinase, but to an inhibitor of phosphatidylcholine hydrolysis. J Biol Chem. 271 (22): 297-322.

18. Cowen DS, Johnson-Farley N and Travkina T (2005). 5-HT1A receptors couple to activation of Akt, but not extracellular-regulated kinase (ERK) in cultured hippocampal neurons. J Neurochem. 93: 910-917.

19. Fang X, Yu S, Lu Y, Bast RJ, Woodgett J and Mills G (2000). Phosphorylation and inactivation of glycogen synthase kinase 3 by protein kinase A. Proc Natl Acad Sci U S A. 97 (22): 11960-11965.

20. Li M, Wang X, Meintzer M, Laessig T, Birnbaum M and Heidenreich K (2000). Cyclic AMP promotes neuronal survival by phosphorylation of glycogen synthase kinase 3beta. Mol Cell Biol. 20: 9356-9363.

21. Cross D, Alessi D, Cohen P, Andjelkovich M and Hemmings B (1995). Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature. 378: 785-789.

22. Rumajogee P, Verge D, Hanoun N, Brisorgueil MJ, Hen R, Lesch KP, Hamon M and Miquel MC (2004). Adaption of the serotonergic neuronal phenotype in the absence of 5-HT autoreceptors or the 5-HT transporter: involvement of BDNF and cAMP. Eur J Neurosci. 19 (4): 937-944.

23. Hillion J, Milne-Edwards JB, Catelon J, de Vitry F, Gros F and Hamon M (1993). Prenatal developmental expression of rat brain 5-HT1A receptor gene followed by PCR. Biochem Biophys Res Commun. 191 (3): 991-997.

24. Galter D and Unsicker K (2000). Brain-derived neurotrophic factor and trkB are essential for cAMP-mediated induction of the serotonergic neuronal phenotype. J Neurosci Res. 61 (3): 295-301.

25. Lavdas AA, Blue ME, Lincoln J and Parnavelas JG (1997). Serotonin promotes the differentiation of glutamate neurons in organotypic slice cultures of the developing cerebral cortex. J Neurosci. 17 (20): 7872-7880.

26. Whitaker-Azmitia P and Azmitia EC (1989). Stimulation of astroglial serotonin receptors produces media which regulates development of serotonergic neurons. Brain Res. 497: 80-85.

27. Azmitia EC, K D and Whitaker-Azmitia P (1990). S-100B, but not NGF, EGF or insulin functions as a serotonergic growth factor. Brain Res. 516: 354-360.

28. Whitaker-Azmitia P, Clarke C and Azmitia EC (1993). Localization of 5-HT-1A receptors to astroglial cells in adult rats. Synapse. 14: 201-205.

29. Ramos A, Tagliaferro P, Lopez E, Pecci Saavedra J and Brusco A (2000). Neuroglial interactions in a model of para-chlorophenylalanine-induced serotonin depletion. Brain Res. 883 (1): 1-14.

30. Brewton L, Haddad L and Azmitia EC (2001). Colchicine-induced cytoskeletal collapse and apoptosis in N-18 neuroblastoma cultures is rapidly reversed by applied S-100b. Brain Res. 912 (1): 9-16.

31. Bhattacharyya A, Oppenheim RW, Prevette D, Moore BW, Brackenbury R and Ratner N (1992). S100 is present in developing chicken neurons and schwann cells and promotes motor neuron survival in vivo. J Neurobiol. 23 (4): 451-466.

32. Selinfreund RH, Barger SW, Welsh MJ and van Eldik LJ (1990). Antisense inhibition of glial S100B production results in alterations in cell morphology, cytoskeletal organization, and cell proliferation. J Cell Biol. 111: 2021-2028.

33. Hesketh J and Baudier J (1986). Evidence that S100 proteins regulate microtubule assembly and stability in rat brain extracts. Int J Biochem. 18 (8): 691-695.

34. Stankovski L, Alvarez C, Ouimet T, Vitalis T, El-Hachimi KH, Price D, Deneris ES, Gaspar P and Cases O (2007). Developmental cell death is enhanced in the cerebral cortex of mice lacking the brain vesicular monoamine transporter. J Neurosci. 27 (6): 1315-1324.

35. Persico AM, Baldi A, Dell'Acqua ML, Moessner R, Murphy DL, Lesch KP and Keller F (2003). Reduced programmed cell death in brains of serotonin transporter knockout mice. Neuroreport. 14 (3): 341-344.

36. Choi D, Kellerman O, Richard S, Colas J, Bolanos-Jimenez F, Tournois C, Launay J and Maroteaux L (1998). Mouse 5-HT2B receptor-mediated serotonin trophic functions. Annals of the New York Academy of Sciences. 861: 67-73.

37. Fiorica-Howells E, Maroteaux L and Gershon MD (2000). Serotonin and the 5-HT(2B) receptor in the development of enteric neurons. J Neurosci. 20 (1): 294-305.

38. Liu MT, Kuan YH, Wang J, Hen R and Gershon MD (2009). 5-HT4 receptor-mediated neuroprotection and neurogenesis in the enteric nervous system of adult mice. J Neurosci. 29 (31): 9683-9699.

39. **Li Z, Chalazonitis A, Huang YY, Mann JJ, Margolis KG, Yang QM, Kim DO, Cote F, Mallet J and Gershon MD (2011). Essential roles of enteric neuronal serotonin in gastrointestinal motility and the development/survival of enteric dopaminergic neurons. J Neurosci. 31 (24): 8998-9009.**

This paper provides strong evidence for a significant role of serotonin signaling in neurogenesis during the fetal development of the peripheral nervous system. The researchers were able to use a wide variety of molecular biology techniques to demonstrate the effect of 5-HT signaling on the development of dopaminergic neurons in the gut in vivo.

40. Sikich L, Hickok JM and Todd RD (1990). 5-HT1A receptors control neurite branching during development. Dev Brain Res. 56 (2): 269-274.

41. Yan W, Wilson CC and Haring JH (1997). 5-HT1a receptors

mediate the neurotrophic effect of serotonin on developing dentate granule cells. Dev Brain Res. 98 (2): 185-190.

42. Lotto B, Upton L, Price DJ and Gaspar P (1999). Serotonin receptor activation enhances neurite outgrowth of thalamic neurones in rodents. Neurosci Lett. 269 (2): 87-90.

43. Janusonis S, Gluncic V and Rakic P (2004). Early serotonergic projections to Cajal-Retzius cells: relevance to cortical development. J Neurosci. 24 (7): 1652-1659.

44. Kobe F, Guseva D, Jensen TP, Wirth A, Renner U, Hess D, Muller M, Medrihan L, Zhang W, Zhang M, Braun K, Westerholz S, Herzog A, Radyushkin K, El-Kordi A, Ehrenreich H, Richter DW, Rusakov DA and Ponimaskin E (2012). 5-HT7R/G12 signaling regulates neuronal morphology and function in an age-dependent manner. J Neurosci. 32 (9): 2915-2930.

45. Lee T, Winter C, Marticke SS, Lee A and Luo L (2000). Essential roles of Drosophila RhoA in the regulation of neuroblast proliferation and dendritic but not axonal morphogenesis. Neuron. 25 (2): 307-316.

46. Newey SE, Velamoor V, Govek EE and van Aelst L (2005). Rho GTPases, dendritic structure, and mental retardation. J Neurobiol. 64 (1): 58-74.

47. Ponimaskin E, Profirovic J, Vaiskunaite R, Richter DW and Voyno-Yasenetskaya TA (2002). 5-Hydroxytryptamine 4(a) receptor is coupled to the Galpha subunit of the heterotrimeric G13 protein. J Biol Chem. 277 (23): 20812-20819.

48. Kvachnina E, Liu G, Dityatev A, Renner U, Dumuis A, Richter DW, Dityateva G, Schachner M, Voyno-Yasenetskaya TA and Ponimaskin EG (2005). 5-HT7 receptor is coupled to G alpha subunits of heterotrimeric G12-protein to regulate gene transcription and neuronal morphology. J Neurosci. 25 (34): 7821-7830.

49. Hamblin MW and Metcalf MA (1991). Primary structure and functional characterization of a human 5-Ht1D-type serotonin receptor. Mol Pharmacol. 40 (2): 143-148.

50. Bonnin A, Torii M, Wang L, Rakic P and Levitt P (2007). Serotonin modulates the response of embryonic thalamocortical axons to netrin-1. Nat Neurosci. 10 (5): 588-597.

51. Salichon N, Gaspar P, Upton AL, Picaud S, Hanoun N, Hamon M, De Maeyer E, Murphy DL, Moessner R, Lesch KP, Hen R and Seif I (2001). Excessive activation of serotonin (5-HT) 1B receptors disrupts the formation of sensory maps in monoamine oxidase A and 5-HT transporter knock-out mice. J Neurosci. 21 (3): 884-896.

52. Mazer C, Muneyyrici J, Taheny K, Raio N, Borella A and Whitaker-Azmitia P (1997). Serotonin depletion during synaptogenesis leads to decreased synaptic density and learning deficits in the adult rat: a possible model of neurodevelopmental disorders with cognitive deficits. Brain Res. 760: 68-73.

53. Nishiyama H, Knopfel T, Endo S and Itohara S (2002). Glial protein S100B modulates long-term neuronal synaptic plasticity. Proc Natl Acad Sci U S A. 99 (6): 4037-4042.

54. Derkach V, Surprenant A and North R (1989). 5-HT3 receptors are membrane ion channels. Nature. 339: 706-709.

55. Boess FG, Beroukhim R and Martin IL (1995). Ultrastructure of the 5-Hydroxytryptamine 3 receptor. J Neurochem. 64: 1401-1405.

56. Alcantara S, Ruiz M, D'Archangelo G, Ezan F, de Lecea L, Curran T, Sotelo C and Soriano E (1998). Regional and cellular patterns of reelin mRNA expression in the forebrain of the developing and adult mouse. J Neurosci. 18 (19): 7779-7799.

57. **Chameau P, Inta D, Vitalis T, Monyer H, Wadman WJ and van Hooft JA (2009). The N-terminal region of reelin regulates postnatal dendritic maturation of cortical pyramidal neurons. Proc Natl Acad Sci U S A. 106 (17): 7227-7232.**

The authors of this work report that 5-HT3 activity affects embryonic cortical development by modulation of reelin. It is one of the first demonstrations of serotonin signaling through this receptor having a direct impact on neurogenesis in the mouse brain.

58. Yip YP, Mehta N, Magdaleno S, Curran T and Yip JW (2009). Ectopic expression of reelin alters migration of sympathetic preganglionic neurons in the spinal cord. J Comp Neurol. 515: 260-268.

59. Vitalis T and Parnavelas JG (2003). The role of serotonin in early cortical development. Dev Neurosci. 25 (2): 245-256.

60. Eisensamer B, Rammes G, Gimpl G, Shapa M, Ferrari U, Hapfelmeier G, Bondy B, Parsons C, Gilling K, Zieglgansberger W, Holsboer F and Rupprecht R (2003). Antidepressants are functional antagonists at the serotonin type 3 (5-HT3) receptor. Mol Psychiatry. 8: 994-1007.

61. Smit-Rigter LA, Noorlander CW, von Oerthel L, Chameau P, Smidt MP and van Hooft JA (2012). Prenatal fluoxetine exposure induces life-long serotonin 5-HT(3) receptor-dependent cortical abnormalities and anxiety-like behaviour. Neuropharmacology. 62 (2): 865-870.

62. Emerit MB, Doucet E, Darmon M and Hamon M (2002). Native and cloned 5-HT(3A)(S) receptors are anchored to F-actin in clonal cells and neurons. Mol Cell Neurosci. 20 (1): 110-124.

63. **Nichols RA and Mollard P (1996). Direct observation of serotonin 5-HT3 receptor-induced increases in calcium levels in individual brain nerve terminals. J Neurochem. 67 (2): 581-592.**

This paper demonstrates that 5-HT3 receptors modulate calcium flux not only by its own channel activity, but also by stimulating other voltage-gated calcium channels found on the same synapses. This observation underlies the foundation of the hypothesis that 5-HT3 activity regulates neuronal calcium flux in several ways that are conducive for instigating neurogenic programs during development.

64. Nayak S, Ronde P, Spier A, Lummis SC and Nichols RA (1999). Calcium changes induced by presynaptic 5-hydroxytryptamine-3 serotonin receptors on isolated terminals from various regions of the rat brain. Neurosci. 91 (1): 107-117.

65. Reiser G, Donie F and Binmoller F-J (1989). Serotonin regulates cytosolic Ca2+ activity and membrane potential in a neuronal and in a glial cell line via 5-HT3 and 5-HT2 receptors by different mechanisms. J Cell Sci. 93: 545-555.

66. Yang J (1990). Ion permeation through 5-hydroxytryptamine-gated channels in neuroblastoma N18 cells. J Gen Physiol. 96 (6): 1177-1198.

67. Homma K, Kitamura Y, Ogawa H and Oka K (2006). Serotonin induces the increase in intracellular Ca2+ that enhances neurite outgrowth in PC12 cells via activation of 5-HT3 receptors and voltage-gated calcium channels. J Neurosci Res. 84: 316-325.

68. Shieh PB, Hu S-C, Boob K, Timmusk T and Ghosh A (1998). Identification of a signaling pathway involved in calcium regulation of BDNF expression. Neuron. 20 (4): 727-740.

69. Tao X, Finkbeiner S, Arnold DB, Shaywitz AJ and Greenberg ME (1998). Ca2+ influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. Neuron. 20 (4): 709-726.

70. Chen WG, Chang Q, Lin Y, Meissner A, West AE, Griffith EC, Jaenisch R and Greenberg ME (2003). Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. Science. 302: 885-889.

71. Oostland M, Sellmeijer J and van Hooft JA (2011). Transient expression of function serotonin 5-HT3 receptors by glutamatergic granule cells in the early postnatal mouse cerebellum. J Physiol. 589 (Pt 20): 4837-4846.

72. Johnson D and Heinemann S (1995). Embryonic expression of the 5-HT3 receptor subunit, 5-HT3R-A, in the rat: an in situ hybridization study. Mol Cell Neurosci. 6: 122-138.

73. Diez-Roux G, Banfi S, Sultan M, Geffers L, Anand S, Rozado D, Magen A, Canidio E, Pagani M, Peluso I, Lin-Marq N, Koch M, Bilio M, Cantiello I, Verde R, De Masi C, Bianchi SA, Cicchini J, Perroud E, Mehmeti S, Dagand E, Schrinner S, Nurnberger A, Schmidt K, Metz K, Zwingmann C, Brieske N, Springer C, Hernandez AM, Herzog S, Grabbe F, Sieverding C, Fischer B, Schrader K, Brockmeyer M, Dettmer S, Helbig C, Alunni V, Battaini MA, Mura C, Henrichsen CN, Garcia-Lopez R, Echevarria D, Puelles E, Garcia-Calero E, Kruse S, Uhr M, Kauck C, Feng G, Milyaev N, Ong CK, Kumar L, Lam M, Semple CA, Gyenesei A, Mundlos S, Radelof U, Lehrach H, Sarmientos P, Reymond A, Davidson DR, Dolle P, Antonarakis SE, Yaspo ML, Martinez S, Baldock RA, Eichele G and Ballabio A (2011). A high-resolution anatomical atlas of the transcriptome in the mouse embryo. PLoS Biol. 9 (1): e1000582.

74. Bhattacharya A, Dang H, Zhu QM, Schnegelsberg B, Rozengurt N, Cain G, Prantil R, Vorp DA, Guy N, Julius D, Ford AP, Lester HA and Cockayne DA (2004). Uropathic observations in mice expressing a constitutively active point mutation in the 5-HT3A receptor subunit. J Neurosci. 24 (24): 5537-5548.

75. Gong S, Zheng C, Doughty ML, Losos K, Didkovsky N, Schambra UB, Nowak NJ, Joyner A, Leblanc G, Hatten ME and Heintz N (2003). A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. Nature. 425 (6961): 917-925.

76. Zeitz KP, Guy N, Malmberg AB, Dirajlal S, Martin WJ, Sun L, Bonhaus DW, Stucky CL, Julius D and Basbaum AI (2002). The 5-HT3 subtype of serotonin receptor contributes to nociceptive processing via a novel subset of myelinated and unmyelinated nociceptors. J Neurosci. 22 (3): 1010-1019.

77. Tian L, Akerboom J, Schreiter E and Looger L (2012). Neural activity imaging with genetically encoded calcium indicators. Prog Brain Res. 196: 79-94.

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