

# **Serotonin Signaling and Trophism in the Enteric Nervous System: Roles in the Function, Development, and Maintenance of the “Second Brain”**

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The enteric nervous system is unique in being able to mediate reflexes and integrated neuronal behaviors in the absence of input from the central nervous system (CNS). To do so, the ENS must be informed of conditions prevailing within the lumen of the gut. This involves transepithelial signaling. Serotonin (5-HT) is an important molecule both in transepithelial signaling and neurotransmission. 5-HT is a paracrine messenger utilized by enterochromaffin (EC) cells, which function as sensory transducers. 5-HT activates intrinsic and extrinsic primary afferent neurons to, respectively, initiate peristaltic and secretory reflexes and to transmit nociceptive information to the CNS. As a neurotransmitter, 5-HT is utilized by a system of long descending myenteric interneurons. 5-HT is synthesized through the actions of two different tryptophan hydroxylases, TpH1 and TpH2, which are found, respectively, in EC cells and neurons. Knockout of TpH1 slows but does not stop motility suggesting that 5-HT is not the only paracrine signaling messenger employed by the gut to initiate reflexes. 5-HT is inactivated by the 5-HT reuptake transporter (SERT)-mediated uptake into enterocytes or neurons. The presence of many 5-HT receptor subtypes enables selective drugs to be designed to therapeutically modulate gastrointestinal motility, secretion, and sensation. Current examples include tegaserod, a 5-HT<sub>4</sub> partial agonist, which has been approved for treatment of the irritable bowel syndrome (IBS) with constipation in women and for chronic constipation in men and women. The 5-HT<sub>3</sub> antagonists, granisetron and ondansetron, are useful in combating the nausea associated with cancer chemotherapy and alosetron is employed in the treatment of IBS with diarrhea. Serotonergic signaling abnormalities, especially decreases in mucosal SERT and TpH1 have also been putatively implicated in the pathogenesis of functional bowel diseases. The number of neurons in the enteric nervous system (ENS) falls in later life. In most mammals about 40-60% of neurons are eventually lost. Although the consequences of the age-related decrease in enteric neurons are not completely understood, it may contribute to the high incidence of dysmotility in the aged. 5-HT<sub>4</sub> agonists are effective in the treatments of chronic constipation and irritable bowel syndrome-constipation (IBS-C); moreover, colonic transit slows in aged mice and does so even more in transgenic mice that lack 5-HT<sub>4</sub> receptors (KO). Because the numbers of neurons are also reduced in both plexuses of KO mice, the KO-associated defect in colonic transit could be due to an effect of the knockout on neurotransmission and/or to the loss of neurons. We therefore tested the hypothesis that 5-HT<sub>4</sub> stimulation is neuroprotective or trophic. Numbers of myenteric neurons in the colon were compared between KO and wild-type (WT) littermates at 1, 2.5, 4, 5, and 12 months of age. Neural numbers were not significantly different at 1 month, but at each of the later ages, there were significantly fewer neurons in the KO colon and the neurons of the KO mice were smaller than in their wild-type littermates. Neuronal size and the proportion of myenteric nNOS-immunoreactive neurons were also decreased in KO mice at 12 months, but not at younger ages. To study trophism and/or neuroprotection directly, the 5-HT<sub>4</sub> agonists, tegaserod and RS67506, and the 5-HT<sub>4</sub> antagonist, GR113808, were applied to enteric neurons developing *in vitro* from immunoselected neural crest-derived precursors. Both tegaserod and RS67506 concentration-dependently increased neuronal numbers and length of neurites; these effects were blocked by GR113808, which exerted no effects of its own. Tegaserod and RS67506 decreased apoptosis, assessed by the TUNEL method; these decreases were blocked by GR113808. These observations suggest that 5-HT<sub>4</sub> receptor stimulation is neuroprotective and trophic for enteric neurons. Whether or not 5-HT<sub>4</sub> stimulation can prevent the age-related decline in neuronal numbers remains to be determined. Supported by NIH grant NS12969 and Novartis.