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# Enhanced serotonin signaling stimulates ordered intestinal mucosal growth

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ABSTRACT

*Background:* Significant quantities of serotonin (5-HT) are found in the intestine, and studies have demonstrated that 5-HT can stimulate enterocyte cell division, suggesting regulatory roles in mucosal homeostasis and intestinal adaptation. We hypothesized that excess enteric 5-HT signaling enhances mucosal growth without changing intestinal villous cellular makeup.

*Methods:* Mice lacking the serotonin reuptake transporter (SERT) and wild-type littermates (WTLM) were euthanized and their ileum analyzed. Villus height (VH), crypt depth (CD), and enterocyte height (EH) were measured. Enterocyte cell division was measured using Ki-67 immunofluorescence to calculate crypt proliferation index (CPI). Cellular distribution along villi was investigated by immunofluorescent staining for enterocytes, enteroendocrine cells, and goblet cells. Group measurements were compared using *t*-test and chi-squared test.

*Results:* SERT knock-out (SERTKO) mice had significantly taller villi, deeper crypts, and taller enterocytes compared with WTLM ($P < 0.0001$). Similarly, enterocyte proliferation was greater in SERTKO compared with WTLM ($P < 0.01$). For SERTKO, mean values were: VH, 255.6 μm; CD, 66.7 μm; EH, 21.2 μm; and CPI, 52.8%. For WTLM, corresponding values were: VH, 207.8 μm; CD, 56.1 μm; EH, 19.5 μm; and CPI, 31.9%. The cellular composition along villi was not significantly different between genotypes ($P > 0.05$).

*Conclusions:* Enhancing 5-HT signaling in mice increases VH, CD, EH, and crypt cell proliferation in the intestinal mucosa. 5-HT-associated growth did not alter the cellular composition of the villi. Serotonin may represent an important physiologic regulator of intestinal growth and adaptation and holds promise as a target for therapies aimed at enhancing intestinal recovery after injury or mucosal surface area loss.





## Introduction

Short bowel syndrome (SBS), a clinical manifestation of intestinal failure, results from reduction in intestinal mucosal surface area below what is needed for adequate digestion and absorption of nutrients from ingested food.[1] In the setting of SBS, intestinal adaptation represents a well-orchestrated growth in various areas of the intestine in response to tissue loss; this adaptive response allows for recovery from significant loss of intestine. The complex mechanisms responsible

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for regulating intestinal adaptation after intestinal resection are not fully understood but may be intimately related to the enteric nervous system (ENS), likely involving multiple mediators.[2]

The ENS consists of a complex network of neurons, enteric glia, and other cells that extends the entire length of the gastrointestinal (GI) tract.[3] Anatomically, the ENS is composed of two distinct plexuses: the myenteric and the submucosal plexus. The cells that make up these plexuses have extensive processes allowing communication between the mucosa, muscular layers, and different cells within the ENS. The ENS functions independently from the central nervous system, giving it the moniker of "the second brain"; it is responsible for controlling or modulating nearly all aspects of GI activity, including motility, secretion, absorption, and immunity.[3-5] The ENS employs a wide variety of chemical and peptide neurotransmitters to exert these effects. ENS-associated peptides have been shown to promote adaptation after intestinal resection; however, the ENS nonpeptide neurotransmitter serotonin (5-HT) and its role in regulating recovery from injury and growth are not well studied.

5-HT is a versatile signaling molecule that acts as a paracrine messenger, hormone, and neurotransmitter in different locations.[6] Although 5-HT is most recognized as an important central nervous system neurotransmitter, the GI tract actually contains more than 95% of the body's 5-HT stores; this seems to suggest that 5-HT plays an important regulatory role in the intestine. Previous work has shown that neural processes extend deeply into the tip of the intestinal villus, suggesting the presence of sensory and/or effector functions of the ENS at the mucosal level.[7] Cell-surface receptors for 5-HT have been localized to the intestinal epithelium, and 5-HT has been shown to induce proliferation of a variety of intestinal epithelial cell types both *in vivo* and *in vitro*.[8-14] These facts suggest an active role for 5-HT in mucosal processes.

Previous work demonstrated that neuronal 5-HT modulates intestinal mucosal homeostasis.[7] This study demonstrated enhancement of villus height (VH), crypt depth (CD), and enterocyte proliferation when excess serotonin was available. This early work was limited by the fact that comparisons were not made between knock-out mice and their wild-type littermates (WTLM), and it did not go on to evaluate how the cellular composition of the villus changed as villus growth was stimulated by 5-HT.

It was therefore hypothesized that enhanced serotonin signaling stimulates intestinal mucosal growth without a change in the cellular composition of villi. This work set out to confirm previous findings in a more controlled experimental model using knock-out mice and their WTLM, and to obtain additional data regarding the makeup of villi that are stimulated to grow.

## Material and methods

### Animals

In the GI tract, 5-HT is secreted by either enteroendocrine cells or enteric neurons. In normal physiology, inactivation of the effect of 5-HT requires the serotonin reuptake transporter (SERT); without SERT, 5-HT remains at its site of action, and therefore, its effects are enhanced. SERT knock-out (SERTKO) animals lack the SERT protein and have potentiated 5-HT signaling. Two SERT ± heterozygous mice, one male and one female, bred on a C57BL/6 background at Jackson Labs (Farmington, CT) were transferred to Yale University. This pair and all subsequent mice were housed under pathogen-free conditions on a 12-h light/dark cycle with food and water ad libitum. With the goal of creating wild-type (WT) and SERTKO (SERT −/−) littermates, the pair of heterozygous mice were bred to supply a familial lineage to provide the experimental WT and SERTKO littermates. All male mice used in experiments came from the same original parental lineage bred at Yale University. 8-wk-old adult mice were used for all experiments. Mice were humanely euthanized with $CO_2$ asphyxiation, and their ilea were analyzed. Yale University's Institutional Animal Care and Use Committee approved all experimental protocols involving the use of animals.

### Intestinal harvesting

All experimental mice underwent the same process of intestinal harvest regardless of the genotype. Each mouse was first euthanized with $CO_2$ asphyxiation. After confirmation of death, the mouse carcass was placed in a supine position, and the abdominal cavity was opened, and the small intestine eviscerated. The small intestine was then sharply excised from the ileocecal junction to the proximal jejunum along the mesenteric border of the intestinal tube. After removal, the bowel was then irrigated with phosphate-buffered saline (PBS) slowly using a blunt syringe to remove fecal content. This was performed with the intestine submerged in PBS to avoid dehydration and degradation.

The irrigated tube was then placed along a moistened cutting surface with a metric ruler, positioning the ileocecal junction at 0 and the jejunum at the other end. The tube was then separated sharply in 2-cm sections starting at the ileocecal junction. The first 2 cm of terminal ileum at the ileocecal junction were discarded. The next 4 cm were used for unrelated experiments. The next 2-cm section was used for tubular pathology permanent sectioning, and the following 2-cm section was used for linear pathology permanent sectioning. These pieces were then placed on tack-free silicone dissection plates filled with PBS and were pinned down for fixation. The first 2-cm cylindrical tube section was left intact with a single pin. The second 2-cm section was opened sharply along the mesenteric border to expose the inner mucosal layer. The rectangular specimen was then pinned flat at the corners before fixation. PBS was removed and replaced with 10% formalin. The sections remained in formalin for at least 12 h before being sectioned and mounted on slides for staining.

### Anatomic measurement of mucosal parameters

Paraffin sections underwent standard hematoxylin and eosin staining. These slides were examined at 400× using bright field microscopy. The investigator was blinded to the genotype of the specimen. Measurements were made by photographing magnified slides and then using Aperio ImageScope software to measure VH, CD, and enterocyte height (EH; Leica