Gut microbiota regulates maturation of the adult enteric nervous system via enteric serotonin networks

Filipe De Vadder1,2, Estelle Grasset3, Louise Mannerås Holm3, Gérard Karsenty1, Andrew J. Macpherson3, Louise E. Olofsson3, and Fredrik Bäckhed1,4,5

1Wallenberg Laboratory, Department of Molecular and Clinical Medicine, University of Gothenburg, 41345 Gothenburg, Sweden; 2Department of Genetics and Development, Columbia University Medical Center, New York, NY 10032; 3Maurice Müller Laboratories, Departement Klinische Forschung, Universitätsklinik für Viszerale Chirurgie und Medizin Inselspital, University of Bern, 3008 Bern, Switzerland; and 4Novo Nordisk Foundation Center for Basic Metabolic Research, Section for Metabolic Receptology and Enteroendocrinology, Faculty of Health Sciences, University of Copenhagen, DK-2200 Copenhagen, Denmark

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Abstract

The gut microbiota colonizes the gastrointestinal tract after birth and undergoes proliferation, and then colonizes the entire bowel, giving rise to the enteric nervous system (ENS). The ENS is crucial for essential gastrointestinal functions such as motility, fluid secretion, and blood flow. The ENS is composed of the outer myenteric plexus and the inner submucosal plexus. The ENS has several essential physiologic functions, such as regulation of gastrointestinal motility, fluid secretion and absorption, and blood flow (1). In rodents, most enteric neurons are formed during embryogenesis and early postnatal life (2–5). During this process, a small subpopulation of Sox10-expressing neural crest-derived cells colonizes the foregut, subsequently undergoes proliferation, and then colonizes the entire bowel, giving rise to both neurons and glial cells (6–8). Furthermore, enteric neuronal stem cells, which express markers such as Nestin, have been reported in the postnatal murine intestine (9–11). While it was long thought that no enteric neurons were formed after postnatal day 21 in mice, except in cases of inflammation or injury (2–4), a recent study has demonstrated that the ENS undergoes continuous renewal in adult mice, with apoptosis balancing neurogenesis (12). Thus, this report demonstrates that the adult ENS is capable of maturation and plasticity, but the mechanisms driving these processes remain unknown.

The microbiota colonizes the gastrointestinal tract after birth with continuous maturation during the first year of life (13). Concomitantly with colonization of the gastrointestinal tract and maturation of the mucosal immune system, the ENS undergoes extensive development during the early postnatal life (14). Accordingly, germ-free (GF) mice, which lack a gut microbiota, have early postnatal structural and functional abnormalities of the ENS (15). These features can be reversed by colonization with the microbiota from a conventionally raised (CONV-R) donor (16).

About 90% of the body’s serotonin (5-hydroxytryptamine, 5-HT) is produced by enterochromaffin (EC) cells, a type of enteroendocrine cells which are present in the epithelium of the gut (17). Recently, two studies have reported that the gut microbiota is able to induce mucosal 5-HT secretion in the gut (18, 19). The concept that mucosal and neuronal 5-HT are distinct pools is supported by the fact that different forms of the rate-limiting synthetic enzyme tryptophan hydroxylase (TPH) are used by neuronal and nonneuronal cells, with TPH2 used by neurons and TPH1 used by EC cells (20). While mucosal 5-HT is strongly proinflammatory (21, 22), activation of the 5-HT4 receptor (5-HT4R) in the ENS exerts neuroprotective effects (4, 23).

In this study, we tested the hypothesis that the gut microbiota modulates the function and the anatomy of the ENS through release of 5-HT and activation of the 5-HT4 receptor. Colonization of GF mice reduced intestinal transit time and was associated with the release of 5-HT. We studied GF and

Significance

The gut microbiota affects several physiological processes, including gut motility. Here we observed that germ-free mice have an immature enteric nervous system (ENS) that is normalized upon colonization with a normal microbiota. We identified the mechanism of communication between the microbiota and enteric neurons as the initiation of serotonin release and subsequent activation of the 5-HT4 receptor. This demonstrates a strong interaction between the microbiota and the ENS and indicates potential mechanisms linking microbial dysbiosis to gastrointestinal disorders. The ability to modulate the microbiota, e.g., by diet, will open new perspectives of research in neurogastroenterology.

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Conflict of interest statement: F.B. is cofounder of and shareholder in Metabogen AB.

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F.D.V. and E.G. contributed equally to this work.

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colonized *Tph1*-deficient mice and demonstrated that mucosal 5-HT is neuroprotective in the early phases of colonization. Next, we studied the respective roles of mucosal and neuronal 5-HT in the observed phenotype through inhibition of TPH1 and TPH2 with parachlorophenylalanine (PCPA) or depletion of neuronal 5-HT with reserpine. Finally, using pharmacological modulation of the 5-HT₄ receptor, we established a link between the gut microbiota and neuronal activation of 5-HT₄Rs.

**Results**

The Gut Microbiota Regulates Intestinal Transit Accompanied by Neuroanatomic Changes of the ENS. Recent work has shown that the gut microbiota is essential for normal brain development and function in mice (24), but much less is known about the implications of the gut microbiota in the development of the ENS. A recent study established that the microbiota is required for the postnatal development of enteric glial cells of the mucosa (25), but so far mechanistic studies on the role of the gut microbiota in the maturation of ENS neurons, and particularly colonic enteric neurons, are lacking.

We determined a link between the gut microbiota and ENS function and anatomy by colonizing 12-wk-old GF mice with the microbiota from the cecum of age-matched donor mice, generating CONV-D mice. In agreement with previous findings (26), we found that GF mice exhibited significantly slower intestinal transit than CONV-R controls and that intestinal transit was normalized after 15 d, but not 3 d, of colonization, i.e., when the microbial community is fully established after colonization (Fig. 1A) (27, 28). While we did not observe any difference in the number of myenteric neurons or innervation of the longitudinal muscle/myenteric plexus (LMMP) between GF, CONV-D, and CONV-R mice, as revealed by immunohistochemical quantification of the pan-neuronal marker HuC/D and the neurite marker Tuj1 (Fig. 1B–D), innervation of the colonic epithelium was reduced in GF mice. These phenotypes were restored 15 d after colonization (Fig. 1E and F), mirroring the faster transit (Fig. 1A). It is noteworthy that, in accordance with a previous study (25), colonization increased the density of the glial network (as revealed by immunostaining for the glia-specific marker S100β; see SI Appendix, Fig. S1 A and B). However, the number of Sox10⁺ cells (a marker of neuronal and glial progenitors) was not affected by the microbial status of the mice (SI Appendix, Fig. S1 C and D), suggesting that (i) glia are larger after colonization or (ii) a greater percentage of glia express S100β. Sox10⁺ cells have been shown to give rise to neurons and glial cells in vitro and in vivo (2, 3, 25). A previous study using reporter mice found that, after colonization, Sox10⁺ cells undergo proliferation in the myenteric plexus, followed by migration to the mucosa, giving rise to enteric glial cells (25). In line with that finding, we found that about 1% of Sox10⁺ cells expressed Ki67 in the myenteric plexus 15 d after colonization (SI Appendix, Fig. S2).

Next, we investigated whether depletion of the gut microbiota by 3-wk antibiotic treatment in CONV-R mice reduced innervation of...
Interactions Between the Microbiota and Mucosal 5-HT are Neuroprotective. 5-HT has been implicated in neurogenesis as well as in promoting the survival of neurons. We confirmed previous observations that colonization of GF mice partially restored serum levels of 5-HT (SI Appendix, Fig. S4A) (18, 19, 31), likely by inducing de novo 5-HT synthesis and increasing the expression of the rate-limiting enzyme for 5-HT synthesis, Tph1, in the mucosa (SI Appendix, Fig. S4B). Furthermore, depletion of the microbiota with antibiotics reduced circulating 5-HT levels (SI Appendix, Fig. S4C).

To investigate whether mucosal 5-HT production was required to maintain the neuroanatomy of the ENS, we quantified neuronal density in Tph1-knockout (Tph1−/−) mice. After confirming that Tph1 was not expressed in the colon of our knockout mice (SI Appendix, Fig. S4D), we analyzed the anatomy of the ENS in Tph1−/− mice and wild-type littermates. CONV-R Tph1−/− mice do not show any evident alterations in the neuroanatomy of the ENS (32), but no study so far has focused on these mice under GF conditions. In line with previous studies in the ileum of CONV-R mice (32), we did not observe significant changes in the neuroanatomy of the ENS of CONV-R and GF mice (Fig. 4). However, when GF mice were colonized for 3 d with the microbiota of wild-type C57BL/6 CONV-R mice (yielding CONV-D mice), we found that Tph1−/− mice (Fig. 4 A and B) had a decreased number of myenteric neurons (effect of microbiota: 35% of total variation; two-way ANOVA, P = 0.02), highlighting the importance of mucosal 5-HT in maintaining the integrity of the ENS during the early colonization period. Moreover, the proportion of Nestin+ neurons was significantly reduced in CONV-D Tph1−/− mice (Fig. 4 D and E).

Colonization of GF Mice Induces Proliferation of a Preexisting Nestin+ Subpopulation of Neural Precursors. To test the hypothesis that colonization of GF mice with a normal microbiota induces the proliferation of neural precursor cells, we examined the coexpression of Nestin with the cycling marker Ki67 in the myenteric ganglia of the colon. We found that in GF mice as many as 5% of the Nestin+ cells retained the capacity to undergo proliferation and that this state persisted 3 and 15 d after colonization (Fig. 3 A and B). In contrast, less than 1% of Nestin+ cells in CONV-R mice expressed Ki67 (P = 0.03 vs. GF; Kruskal–Wallis test followed by Dunn’s post hoc test). We also observed that some Ki67+ cells had a small rim of Nestin+ cytoplasm (Fig. 3). Since myenteric Nestin+ cells are responsible for adult neurogenesis in the ENS (12), our results suggest that GF mice retain a potential for neurogenesis and maturation of the ENS which can be activated upon colonization. However, since we observed no difference in the density of neurons in GF mice, proliferation is likely compensated for by cell loss. Taken together, our results suggest that neuronal differentiation from Nestin+ cells occurs after exposure to the gut microbiota.

the colon mucosa. Microbial depletion was confirmed by qPCR and was associated with cecum enlargement (SI Appendix, Fig. S3A and Table S1). Unlike GF mice, the depletion resulted in reduced innervation of the colonic mucosa and LMM, which was associated with a decrease in the glial network (SI Appendix, Fig. S3 B–H). This suggests that the presence of a gut microbiota is not necessary for the development of the nervous myenteric network but is crucial for the maintenance of the network.

Nestin is a cytoskeletal protein that is expressed by a variety of neural stem cells (10, 12, 29, 30). Nestin+ cells in the myenteric plexus of adult mice give rise to neurons in vivo (12), and studies of Nestin-GFP transgenic mice suggest that coexpression of Nestin and HuC/D is a signature of neuronal plasticity in the adult ENS (10). Thus, we determined the proportion of Nestin+ neurons in GF, CONV-D, and CONV-R mice (Fig. 2). Colonization of GF mice induced a global decrease in the proportion of Nestin+ neurons (χ² = 208.7; df = 3; P < 10⁻⁵⁰) (Fig. 2B), suggesting that GF mice retain a higher plasticity in the ENS, which is maintained for at least 3 d after colonization but disappears when the microbiota has reached a steady state.

Colonization of adult GF mice with a gut microbiota results in cycling of neuronal progenitors. (A) Representative images of a colonic myenteric ganglion of a mouse stained with the cycling cell marker Ki67 (red arrows), neuronal precursor marker Nestin (green), and with nuclei counterstained with Hoechst (gray). (B) Quantification of double-positive Nestin/Ki67 cells. *P < 0.05 vs. GF; N.S., not significant; Kruskal–Wallis test followed by Dunn’s post hoc test. (Scale bars: 20 μm.)
The gut microbiota regulates neuronal 5-HT release and 5-HT4R.

5-HT, R regulates ENS anatomy and function in GF mice. (A) Intestinal transit in GF mice that were given the 5-HT4R antagonist sc-53116 or a vehicle solution. P = 0.05; Student’s unpaired t test. (B) Representative images of the colonic LMMP of the aforementioned mice showing the pan-neuronal marker HuCD (green) and neuron-specific β-III tubulin (Tuj1, red). (C and D) Quantification of HuCD+ cells (C) and the Tuj1+ area (D). (E) Representative images of the innervation of the colonic crypts of the mice (white arrows) using the peripheral neuronal marker Tuj1. (F) Quantification of the Tuj1+ area. P values were determined by the Mann-Whitney test. N.S., not significant. (Scale bars: 50 μm.)

The Gut Microbiota Induces Neuronal 5-HT Production. It has been shown that the release of 5-HT from enteric neurons influences the development and survival of dopaminergic neurons (32), showing the importance of serotonergic neurons in organizing more mature ENS network components. We performed immunohistochemistry of 5-HT in the LMMP and found that serotonergic neuronal networks were almost absent in GF mice but were gradually restored by colonization with a gut microbiota (Fig. 5A and B). Importantly, the presence of a gut microbiota was crucial to maintain the serotonergic networks, since depletion with antibiotics abolished 5-HT immunoreactivity (SI Appendix, Fig. S5B and C). To distinguish the specific roles of serotonin on the observed phenotype, we chronically treated CONV-D mice for 3 d with PCPA, a selective and irreversible inhibitor of TPH1 and TPH2, or with reserpine, an irreversible blocker of vesicular monoamine transporter (thus depleting neuronal 5-HT). We confirmed that PCPA and reserpine diminished 5-HT immunoreactivity in the LMMP (SI Appendix, Fig. S5E-H).

Treatment with the TPH inhibitor PCPA resulted in reduced density of myenteric neurons (Fig. 5D and E). Surprisingly, the treatment also led to a significant increase in the proportion of Nestin+ neurons (Fig. 5G and H and SI Appendix, Fig. S6). These data suggest that blocking 5-HT production results in (i) loss of myenteric neurons similar to that observed in Tph1−/− CONV-D mice and (ii) inhibition of neuronal differentiation from Nestin+ progenitors. While there was no significant difference in the number of neurons or in Tuj1+ neurite density in reserpine-treated CONV-R (SI Appendix, Fig. S5J and K) or CONV-D (Fig. 5D-F) mice, the treatment significantly decreased the proportion of Nestin+ neurons (Fig. 5G and H and SI Appendix, Fig. S6). It is noteworthy that reserpine is an irreversible blocker of the vesicular monoamine transporter, which also transports dopamine and noradrenaline, so we cannot exclude the possibility that these neurotransmitters contribute to the phenotype. Further studies with GF and CONV-D Tph2-deficient mice might confirm the specificity of the action of neuronal 5-HT on maturation of the ENS after colonization.
Data are presented as box plots showing maximum, minimum, median, and interquartile range. Each dot represents a single mouse. Statistical analysis was performed using GraphPad Prism 7 software.

Materials and Methods
For detailed procedures, see SI Appendix, SI Methods.

Animals. Adult C57BL/6J female mice, aged 12 wk at the beginning of the experiments, were housed in a climate-controlled room (22 ± 2°C) subjected to a 12-h light/dark cycle (lights on, 7:00 AM–7:00 PM), with free access to water and food. Tph1+/− mice were described previously (42). All procedures in mice were approved by the Ethics Committee on Animal Care and Use in Gothenburg, Sweden. Antibiotic treatment is described in SI Appendix, SI Methods.

Statistical Analysis. Data are presented as box plots showing maximum, minimum, median, and interquartile range. Each dot represents a single mouse. Statistical analysis was performed using GraphPad Prism 7 software.

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1. Furness JB (2012) The enteric nervous system and neurogastroenterology. Nat Rev Gastroenterol Hepatol 9:286–294.
2. Joseph NM, et al. (2011) Enteric glia are multipotent in culture but primarily form glia in the adult rodent gut. J Clin Invest 121:3398–3411.
3. Lararajeva C, et al. (2011) Giall cells in the mouse enteric nervous system can undergo neurogenesis in response to injury. J Clin Invest 121:3412–3424.
4. Liu M-T, Kuan Y-H, Wang J, Hen R, Gershon MD (2009) 5-HT4 receptor-mediated neuroprotection and neurogenesis in the enteric nervous system of adult mice. J Neurosci 29:9683–9699.
5. Pham TD, Gershon MD, Rothman TP (1991) Time of origin of neurons in the murine enteric nervous system: Sequence in relation to phenotype. J Comp Neurol 314:789–798.
6. Wang X, Chan AKK, Sham MH, Burns AJ, Chan WY (2011) Analysis of the sacral neural crest cell contribution to the hindgut enteric nervous system in the mouse embryo. Gastroenterology 141:992–1002.e1–6.

7. Lasrado R, et al. (2017) Lineage-dependent spatial and functional organization of the mammalian enteric nervous system. Science 356:722–726.

8. Memic F, et al. (2018) Transcription and signaling regulators in developing neuronal subtypes of mouse and human enteric nervous system. Gastroenterology 154: 624–636.

9. Kruger GM, et al. (2002) Neural crest stem cells persist in the adult gut but undergo changes in self-renewal, neural subtype potential, and factor responsiveness. Neuron 35:657–669.

10. Grundmann D, Markwart F, Scheller A, Kirchhoff F, Schäfer K-H (2016) Phenotype and distribution pattern of nestin-GFP-expressing cells in murine myenteric plexus. Cell Tissue Res 366:573–586.

11. Belkind-Gerson J, et al. (2013) Nestin-expressing cells in the gut give rise to enteric neurons and glial cells. Neurogastroenterol Motil 25:61–69.e7.

12. Kulkarni S, et al. (2017) Adult enteric nervous system in health is maintained by a dynamic balance between neuronal apoptosis and neurogenesis. Proc Natl Acad Sci USA 114:E3709–E3718.

13. Bäckhed F, et al. (2015) Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host Microbe 17:690–703, and erratum (2015) 17:852.

14. Kabouridis PS, Pachnis V (2015) Emerging roles of gut microbiota and the immune system in the development of the enteric nervous system. J Clin Invest 125:956–964.

15. Collins J, Borojevic R, Verdu EF, Huizinga JD, Ratcliffe WA (2014) Intestinal microbiota influence the early postnatal development of the enteric nervous system. Neurogastroenterol Motil 26:98–107.

16. McVey Neufeld KA, Perez-Burgos A, Mao YK, Bienenstock J, Kunze WA (2015) The gut microbiome restores intrinsic and extrinsic nerve function in germ-free mice accompanied by changes in calbindin. Neurogastroenterol Motil 27:627–636.

17. Mave GM, Hoffman JM (2013) Serotonin signalling in the gut-Functions, dysfunctions and therapeutic targets. Nat Rev Gastroenterol Hepatol 10:473–486.

18. Reigstad CS, et al. (2015) Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. FASEB J 29:1395–1403.

19. Yano JM, et al. (2015) Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell 161:264–276.

20. Spohn SN, Mave GM (2017) Non-conventional features of peripheral serotonin signalling-The gut and beyond. Nat Rev Gastroenterol Hepatol 14:412–420.

21. Ghia J-E, et al. (2009) Serotonin has a key role in pathogenesis of experimental colitis. Gastroenterology 137:1649–1660.

22. Margolis KG, et al. (2014) Pharmacological reduction of mucosal but not neuronal serotonin opposes inflammation in mouse intestine. Gut 63:928–937.

23. Bianco F, et al. (2016) Prucalopride exerts neuroprotection in human enteric neurons. Am J Physiol Gastrointest Liver Physiol 310:G768–G775.

24. Diaz Heijtz R, et al. (2011) Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci USA 108:3047–3052.

25. Kabouridis PS, et al. (2015) Microbiota controls the homeostasis of glial cells in the gut lamina propria. Neuron 85:289–295.

26. Wichmann A, et al. (2013) Microbial modulation of energy availability in the colon regulates intestinal transit. Cell Host Microbe 14:582–590.

27. El Aidi S, et al. (2012) Temporal and spatial interplay of microbiota and intestinal mucoza drive establishment of immune homeostasis in conventionalized mice. Mucosal Immunol 5:567–579.

28. Molinari A, et al. (2017) Host-microbiota interaction induces bi-phasic inflammation and glucose intolerance in mice. Mol Metab 6:1371–1380.

29. Bonaguidi MA, et al. (2011) In vivo clonal analysis reveals self-renewing and multipotent adult neural stem cell characteristics. Cell 145:1142–1155.

30. Becker L, Peterson J, Kulkarni S, Parikh PC (2013) Ex vivo neurogenesis within enteric ganglia occurs in a PTEN dependent manner. PLoS One 8:e59452.

31. Wilkoff WR, et al. (2009) Metabolomics analysis reveals large effects of gut microbiota on mammalian blood metabolites. Proc Natl Acad Sci USA 106:3698–3703.

32. Li Z, et al. (2011) Essential roles of enteric neuronal serotonin in gastrointestinal motility and the development/survival of enteric dopaminergic neurons. J Neurosci 31:8998–9009.

33. Hoffman JM, et al. (2012) Activation of colonic mucosal 5-HT4 receptors accelerates propulsive motility and inhibits visceral hypersensitivity. Gastroenterology 142: 844–854.e4.

34. Liu M, Geddis MS, Wen Y, Setlik W, Gershon MD (2005) Expression and function of 5-HT4 receptors in the mouse enteric nervous system. Am J Physiol Gastrointest Liver Physiol 289:G1148–G1163.

35. McVey Neufeld KA, Mao YK, Bienenstock J, Foster JA, Kunze WA (2013) The microbiome is essential for normal gut intrinsic primary afferent neuron excitability in the mouse. Neurogastroenterol Motil 25:183–188.

36. Brun P, et al. (2013) Toll-like receptor 2 regulates intestinal inflammation by controlling integrity of the enteric nervous system. Gastroenterology 145:1323–1333.

37. Muller PA, et al. (2014) Crosstalk between muscularis macrophages and enteric neurons regulates gastrointestinal motility. Cell 158:390–391, and erratum (2014) 158:1210.

38. Grasset E, et al. (2017) A specific gut microbiota dysbiosis of type 2 diabetic mice induces GLP-1 resistance through an enteric NO-dependent and gut-brain axis mechanism. Cell Metab 25:1075–1090.e5.

39. Gross ER, et al. (2012) Neuronal serotonin regulates growth of the intestinal mucosa in mice. Gastroenterology 143:408–417.e2, and erratum (2013) 144:249.

40. Spohn SN, et al. (2016) Protective actions of epithelial 5-hydroxytryptamine 4 receptors in normal and inflamed colon. Gastroenterology 151:933–944.e3.

41. Collins SM (2014) A role for the gut microbiota in IBS.

42. Yadav VK, et al. (2008) Lrp5 controls bone formation by inhibiting serotonin synthesis in the duodenum. Cell 135:825–837.