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

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The enteric nervous system (ENS) is crucial for essential gastrointestinal physiologic functions such as motility, fluid secretion, and blood flow. The gut is colonized by trillions of bacteria that regulate host production of several signaling molecules including serotonin (5-HT) and other hormones and neurotransmitters. Approximately 90% of 5-HT originates from the intestine, and activation of the 5-HT$_4$ receptor in the ENS has been linked to adult neurogenesis and neuroprotection. Here, we tested the hypothesis that the gut microbiota could induce maturation of the adult ENS through release of 5-HT and activation of 5-HT$_4$ receptors. Colonization of germ-free mice with a microbiota from conventionally raised mice modified the neuroanatomy of the ENS and increased intestinal transit rates, which was associated with neuronal and mucosal 5-HT production and the proliferation of enteric neuronal progenitors in the adult intestine. Pharmacological modulation of the 5-HT$_4$ receptor, as well as depletion of endogenous 5-HT, identified a mechanistic link between the gut microbiota and maturation of the adult ENS through the release of 5-HT and activation of the 5-HT$_4$ receptor. Taken together, these findings show that the microbiota modulates the anatomy of the adult ENS in a 5-HT–dependent fashion with concomitant changes in intestinal transit.

microbiota | enteric nervous system | serotonin | 5-HT4R

The gastrointestinal tract is unique in the body, as it has an intrinsic nervous system, the enteric nervous system (ENS), 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-HT$_4$ receptor (5-HT$_4$R) in the ENS exerts neurogenerative and neuroprotective actions (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-HT$_4$ receptor (5-HT$_4$R). 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-HT$_4$ 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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Fig. 1. The gut microbiota regulates ENS anatomy and function. (A) Intestinal transit in GF mice, GF mice colonized (CONV-D) with microbiota from a CONV-R donor for 3 or 15 d, and CONV-R mice. *$P < 0.05$; **$P < 0.01$ vs. GF; N.S., not significant; one-way ANOVA followed by Dunnett's post hoc test. (B) Representative images of the LMMP of the colon showing pan-neuronal marker HuC/D (green) and neuron-specific beta-III tubulin (Tuj1, red). (C and D) Quantification of HuC/D$^+$ cells (C) and the Tuj1$^+$ area (D). (E and F) Representative images (E) and quantification (F) of the innervation of the colonic crypts of the mice using the peripheral neuronal marker Tuj1. *$P < 0.05$ vs. GF; N.S., not significant; Kruskal–Wallis test followed by Dunn's post hoc test. (Scale bars: 50 μm.)

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-HT$_4$ receptor, we established a link between the gut microbiota and neuronal activation of 5-HT$_4$Rs.

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. 1*A*) (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. 1 *B*–*D*), innervation of the colonic epithelium was reduced in GF animals. These phenotypes were restored 15 d after colonization (Fig. 1 *E* and *F*), mirroring the faster transit (Fig. 1*A*). 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

Fig. 2. Colonization of GF mice with a gut microbiota induces maturation of neuronal precursors in the myenteric plexus of the colon. (A) Immunostaining with HuC/D (red) and the neuronal precursor marker Nestin (green). (B) Colonization reduced the proportion of Nestin$^+$ neurons in the myenteric ganglia ($n > 1,000$ cells counted per group). (Scale bars: 20 μm.)
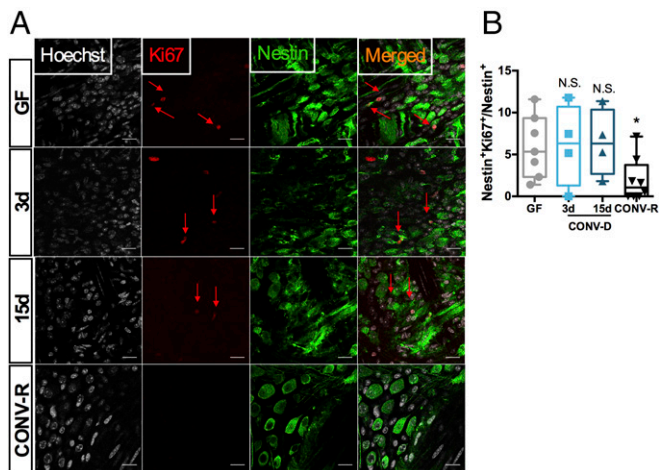
Fig. 3. Colonization of adult GF mice with a gut microbiota results in cycling of neuronal progenitors. (*A*) Representative images of a colonic myenteric ganglion of a mouse stained with the cycling cell marker Ki67 (red arrows), neuronal precursor marker Nestin (green), and with nuclei counterstained 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 colon mucosa. Microbial depletion was confirmed by qPCR and was associated with cecum enlargement (*SI Appendix*, Fig. S3*A* and Table S1). Unlike GF mice, the depletion resulted in reduced innervation of the colonic mucosa and LMMP, 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*A*). Colonization of GF mice induced a global decrease in the proportion of Nestin^{+} neurons ($\chi^2 = 208.7$; df = 3; $P < 10^{-4}$) (Fig. 2*B*), 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 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.

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. S4*A*) (18, 19, 31), likely by inducing de novo 5-HT synthesis by increasing the expression of the rate-limiting enzyme for 5-HT synthesis, *Tph1*, in the mucosa (*SI Appendix*, Fig. S4*B*). Furthermore, depletion of the microbiota with antibiotics reduced circulating 5-HT levels (*SI Appendix*, Fig. S4*C*).

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. S4*D*), 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*
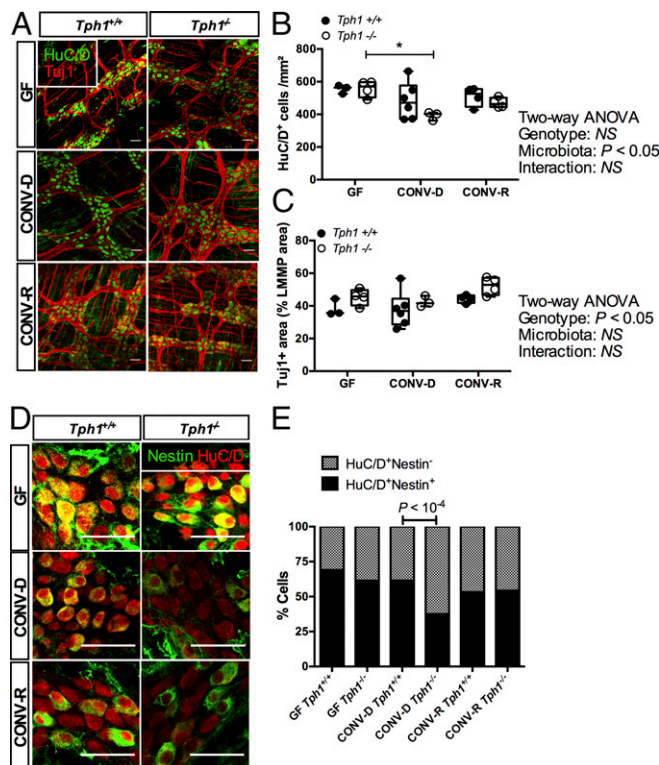

Fig. 4. Mucosal 5-HT is neuroprotective in CONV-D mice. (*A*) Representative images of the colonic LMMP showing pan-neuronal marker HuC/D (green) and neuron-specific β-III tubulin (Tuj1, red). (*B* and *C*) Quantification of HuC/D^{+} cells (*B*) and the Tuj1^{+} area (*C*). *P* values are reported after two-way ANOVA followed by Dunnett's multiple comparisons test. *P < 0.05. (*D*) Immunostaining with HuC/D (red) and the neuronal precursor marker Nestin (green). (*E*) Quantification of results in *D*. Split panels are provided in *SI Appendix*, Fig. S4*E*. *P* values reported are knockout vs. wild type; Fisher's exact test (n > 750 cells were counted per group). (Scale bars: 50 μm.)
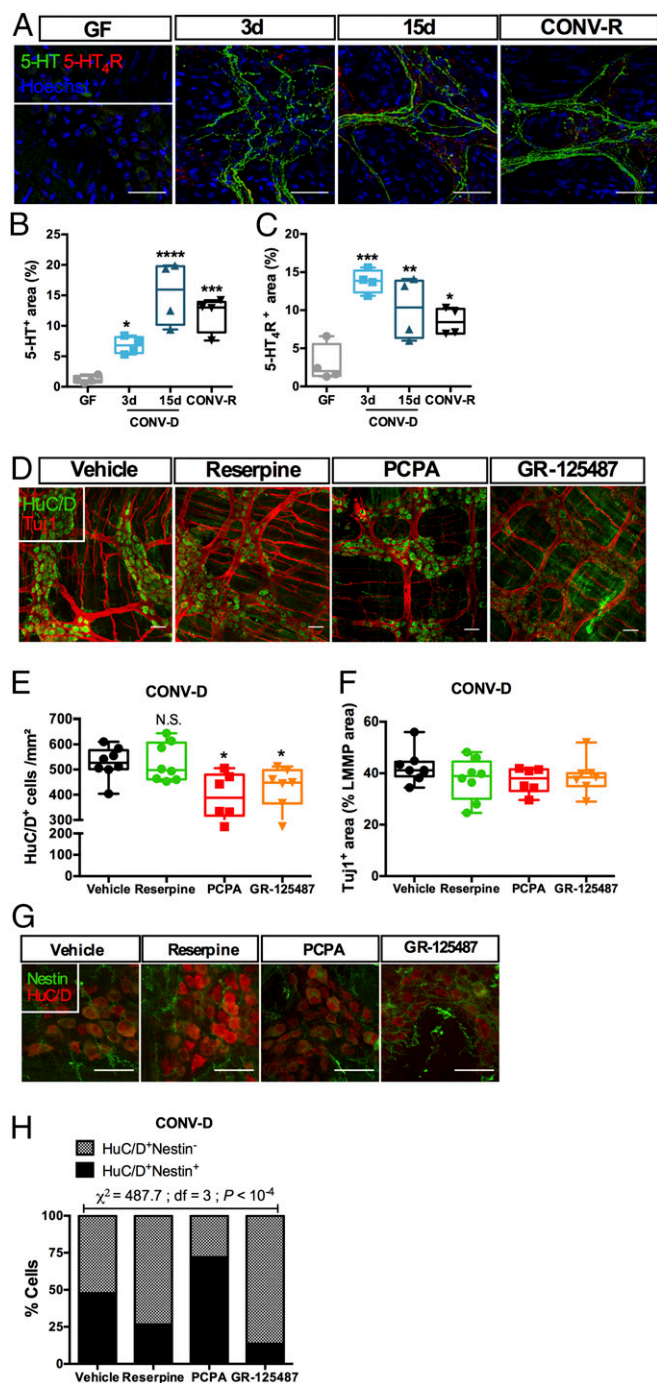
Fig. 5. The gut microbiota regulates neuronal 5-HT release and 5-HT4R activation in the myenteric plexus. (*A*) Representative images of the colonic myenteric plexus showing immunoreactivity to 5-HT (green) and 5-HT$_4$R (red). Localization of 5-HT and 5-HT$_4$R was altered by colonization. (*B* and *C*) Quantification of immunoreactivity to 5-HT (*B*) and 5-HT$_4$R (*C*). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 10^{-4}$ vs. GF; Kruskal–Wallis test followed by Dunn's post hoc test. (*D*) Representative images of the colonic LMMP of CONV-D mice treated with a vehicle solution, the 5-HT$_4$R antagonist GR-125487, the TPH inhibitor PCPA, or the vesicular monoamine transporter blocker reserpine, showing the pan-neuronal marker HuC/D (green) and neuron-specific β-III tubulin (Tuj1, red). (*E* and *F*) Quantification of HuC/D$^+$ cells (*E*) and the Tuj1$^+$ area (*F*). *$P < 0.05$ vs. GF; N.S., not significant; Kruskal–Wallis test followed by Dunn's post hoc test. (*G*) Immunostaining of HuC/D (red) and the neuronal precursor marker Nestin (green). Split panels are shown in *SI Appendix*, Fig. S6. (*H*) Quantification of the proportion of Nestin$^+$ neurons ($n > 500$ cells counted per group). (Scale bars: 50 µm.)
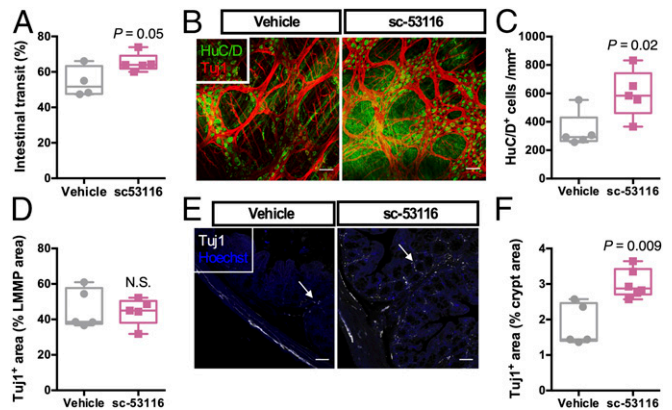
Fig. 6. 5-HT$_4$R regulates ENS anatomy and function in GF mice. (*A*) Intestinal transit in GF mice that were given the 5-HT$_4$R antagonist sc-53116 or a vehicle solution. $P = 0.05$; Student's unpaired *t* test. (*B*) Representative images of the colonic LMMP of the aforementioned mice showing the pan-neuronal marker HuC/D (green) and neuron-specific β-III tubulin (Tuj1, red). (*C* and *D*) Quantification of HuC/D$^+$ 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.)

and *SI Appendix*, Fig. S4*E*). Taken together, our data show that the microbiota-induced release of mucosal 5-HT is neuroprotective for the ENS in the early phases of colonization.

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. 5 *A* 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. S5 *B* 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. S5 *E–H*).

Treatment with the TPH inhibitor PCPA resulted in reduced density of myenteric neurons (Fig. 5 *D* and *E*). Surprisingly, the treatment also led to a significant increase in the proportion of Nestin$^+$ neurons (Fig. 5 *G* 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. S5 *J* and *K*) or CONV-D (Fig. 5 *D–F*) mice, the treatment significantly decreased the proportion of Nestin$^+$ neurons (Fig. 5 *G* 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.

MICROBIOLOGY

Activation of the 5-HT$_4$R in GF Mice Induces Maturation of the Adult ENS. The 5-HT$_4$R is widely expressed in the intestine (33, 34), and mucosal application of 5-HT$_4$R agonists activates mucus discharge by goblet cells and Cl$^-$ secretion by enterocytes, whereas 5-HT$_4$R antagonists block these actions (33). Stimulation of 5-HT$_4$R also induces adult neurogenesis and neuroprotection in the gut (4, 23). Here, we confirmed that 5-HT$_4$R was expressed in the ENS, and specifically in myenteric neurons (*SI Appendix*, Fig. S5*A*), and found that its expression was dependent upon the presence of a gut microbiota (Fig. 5 *A* and *C* and *SI Appendix*, Fig. S5 *B* and *D*). Similar to 5-HT, we observed that 5-HT$_4$R expression was located in the soma of the cells in GF mice but in serotonergic neurites after colonization (Fig. 5*A* and *SI Appendix*, Fig. S5*A*).

We next colonized GF mice and concomitantly treated them for 3 d with chronic injections of the 5-HT$_4$R antagonist GR-125487. Treatment with the antagonist did not affect the density of the neuronal network but resulted in a decrease in the number of myenteric neurons (Fig. 5 *D–F*). The proportion of Nestin$^+$ neurons (Fig. 5 *G* and *H* and *SI Appendix*, Fig. S6) was significantly reduced by the treatment. This suggests that neuronal 5-HT release regulates the stemness of neuronal progenitors in the ENS, while specific activation of 5-HT$_4$R also regulates proliferation.

To test directly whether the effects of colonization on ENS anatomy could be phenocopied in GF mice, we treated GF mice with the 5-HT$_4$R agonist sc-53116 for 4 d. We observed that activation of 5-HT$_4$R in GF mice was associated with faster intestinal transit (Fig. 6*A*), concomitant with an increased number of myenteric neurons (Fig. 6 *B–D*) and increased innervation of the colonic mucosa (Fig. 6 *E* and *F*).

Taken together, our data suggest that, upon colonization, the gut microbiota stimulates neuronal and mucosal 5-HT release and that 5-HT$_4$R activation in GF mice leads to differentiation and maturation of enteric neurons.

Discussion

The ENS contains cells that give rise to neurons in vitro and in vivo (2, 3, 9). In this study, we showed that colonization of adult GF mice induced maturation of the ENS associated with the proliferation of Nestin$^+$ progenitors precursors present in GF mice. While blocking 5-HT$_4$R or depleting endogenous 5-HT prevented the process, GF mice had restored innervation of the gut when treated with 5-HT$_4$R agonists. Here we show that maturation of the adult ENS requires 5-HT$_4$R–specific signaling and that colonization by gut microbes of GF *Tph1*$^{-/-}$ mice resulted in fewer myenteric neurons, suggesting that both pools of enteric 5-HT are crucial for maintenance of the adult ENS.

Our findings confirm that the maturation capacity of the ENS (i.e., growth of neurites and neurogenesis) continues in adult mice in a microbiota-naive gut. Interestingly, GF rodents show an abnormal proportion of calbindin and nitrergic myenteric neurons, suggesting that the microbiota influences the maturation of intestinal neural networks (15, 16, 35). Furthermore, functional activity of the ENS can be regulated by the microbiota, either directly through Toll-like receptors (36) or by inducing the secretion of BMP2 by macrophages (37). A recent study showed that adult enteric neurogenesis is an active process that is balanced with apoptosis (12). Our results complement these findings and suggest that Nestin$^+$ cells can undergo proliferation and differentiation into functional neurons. However, the mechanisms by which the gut bacteria impact the organization of the ENS remained unexplored. For example, in cases of dysbiosis, NO production in the ENS is impaired (38). Thus, it is likely that nitrergic and serotonergic pathways interact with the microbiota to establish a functional ENS.

The gastrointestinal tract is the site where most 5-HT is synthesized, and recently two studies have shown that the gut microbiota plays a key role in promoting 5-HT secretion (18, 19). We confirmed these findings, but our results suggest that neuronal and mucosal 5-HT play complementary roles in the ENS maturation (*SI Appendix*, Fig. S7). Neuronal 5-HT has been identified as a major neuronal growth factor during development (32) while also acting as a promoter of growth and a suppressor of inflammation in the intestinal mucosa (22, 39). We complemented these findings by rederiving *Tph1*$^{-/-}$ mice as germ-free. Similar to the PCPA-treated mice, colonization of these mice resulted in a decreased number of myenteric neurons, showing that mucosal 5-HT is crucial for the integrity of the ENS in the early stages of colonization. However, unlike *Tph1*$^{-/-}$ and reserpine-treated CONV-D mice, PCPA induced an increase in the ratio of Nestin$^+$ cells in the myenteric plexus, an observation that requires future investigation.

The altered organization and properties of enteric neurons in GF mice also influence gastrointestinal function. Treatment with the 5-HT$_4$R agonist prucalopride protects enteric neurons against oxidative stress (23), and activation of 5-HT$_4$R in the epithelium reduces inflammation in mice with colitis (40). Changes in the gut microbiota triggered, for example, by infection, stress, or antibiotic usage have been suggested to cause irritable bowel syndrome (41). Thus, improved understanding of the dialogue between the microbiota and the ENS opens the possibility for new treatments for inflammatory diseases of the gut.

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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