Gut microbiota requires vagus nerve integrity to promote depression

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Recent studies have shown that gut microbiota (GM) can influence hippocampal plasticity and depression-like behaviors (1,2,3), yet the underlying mechanisms remain elusive. The gastrointestinal branches of the vagus nerve, which constitute a direct bidirectional route of communication between the gut and the brain, are well suited to carry neural messages associated with changes in peripheral states to the brain (4,5). For instance, the ability of some gut bacterial strains to induce anxiety-like behaviors and alter the expression of proteins in the hippocampus depends on vagal afferents (6,7). Similarly, gut vagal sensory signaling affects adult hippocampal plasticity and neurogenesis (8). Impairment in brain plasticity is known to generate depressive states in mice (9,10). Yet, it is not known whether GM abnormalities require a functional, intact vagus nerve to promote depression or if GM borrows other pathways, such as humoral immune responses. Here we used the fecal transplantation paradigm to study whether changes in GM necessitate the integrity of the vagus nerve to impact on adult hippocampal neurogenesis and depressive-like behavior. Alteration of GM was induced by unpredictable chronic mild stress (UCMS), a standard model of depression (11).

We used mice (8 weeks old, C56BL6/j) that sustained 9 weeks of UCMS (UCMS group), and their controls (CT group), collectively called “microbiota donor” mice. Fresh fecal samples were harvested from microbiota donor mice at the end of the 9th week of UCMS and were transferred by oral gavage to “microbiota recipient” mice (8 weeks old, C56BL6/j), which had been treated with broad-spectrum antibiotics (abx) (12) during one week prior the fecal transfer. Abx was discontinued 24h prior microbiota transplantation. Microbiota recipient mice received either the CT (CT-tr group) or the UCMS (UCMS-tr group) fecal suspension at 1 and 4 days following abx discontinuation (Figure 1A,B). In order to assess whether the microbiota transfer can

affect behavior and neurogenesis via the vagus nerve, we distinguished two different subgroups of microbiota recipient mice: animals that had sustained subdiaphragmatic vagotomy (Vx) 4 weeks prior fecal transplantation, and a sham group (Figure 1A,B). Microbiota recipient mice were maintained in isolators for 7 weeks following the fecal transfer.

To assess the impact of fecal transfer on GM composition, we performed 16S rRNA gene V3-V4 region sequencing on the fecal samples. Taxonomic analysis of bacterial families revealed differences between CT and UCMS GM compositions, which were transferred to their respective recipient mice, with the most significant change being observed in Bifidobacteriaceae (Figure 1D). More precisely, both UCMS and UCMS-tr mice had significantly lower levels of Bifidobacteriaceae compared to CT and CT-tr mice respectively ($P=0,0016$ and $P=0,0039$, Tukey post-hoc). This finding supports previous studies showing that Bifidobacteria and prebiotics exert anti-depressant effects in rats (13, 14).

We next sought to determine whether these UCMS-induced GM changes are sufficient to change behavior. We employed a battery of standard behavioral tests to assess anxiety- and depression-like behaviors. Our results showed that UCMS microbiota recipient mice adopted the behavioral phenotype of donor mice, characterized by depressive-like responses, and that transmission of the depressive-like phenotype was abrogated by Vx (Figure 1C,E,F). For instance, in the novelty suppressed feeding (NSF) test (Two-way ANOVA, $F(1, 28)=6.5$; $P=0.02$), Tukey's post-hoc showed that while the transfer of UCMS microbiota significantly increased the latency to eat ($P<0.05$), Vx completely reversed this phenotype ($P=0.005$) (Figure 1C). Moreover, in both the tail suspension and forced swim tests [Two-way ANOVA, $F(1, 28)=10.99$, $P=0.003$; and $F(1, 26)=4.23$; $P=0.05$ respectively], UCMS-tr

mice were more immobile compared to their control counterparts ($P<0.001$; $P=0.02$), and Vx abolished the phenotype ($P=0.02$; $P=0.03$) (Figure 1E,F). Taken together, our observations indicate that UCMS-induced changes in GM lead to depressive states only if the vagus nerve remains functional.

To further investigate whether these depressive states are accompanied by changes in adult hippocampal neurogenesis, we performed immunostaining for Ki67, a marker of proliferating cells, and doublecortin (DCX), a marker of transient proliferating neuronal progenitor cells, in the dentate gyrus (DG) of the hippocampus. The large pool of DCX⁺ cells in the DG, besides being a step in the maturation process of new neurons, is of importance in buffering the negative feedback on the hypothalamic–pituitary-adrenal axis (15). Counting of Ki67⁺ cells in the DG [Two way ANOVA, $F(1, 24)=9.96$; $P=0.005$], revealed that the transfer of UCMS-derived microbiota significantly decreased Ki67⁺ cell number ($P<0.001$), and that this decrease was completely abolished by Vx ($P<0.001$) (Figure 1G,H). Our results on the number of DCX⁺ cells went on the same line. UCMS-tr mice had a significant decrease in DCX⁺ cells ($P=0.001$) that was attenuated by Vx ($P=0.03$) (Figure 1G,I). These data show that vagal nerve activity is essential to mediate the impact of gut dysbiosis on hippocampal adult neurogenesis.

The present study demonstrates that chronic stress induces changes in GM composition, notably diminution of the Bifidobacteriaceae family. When transferred to healthy recipient mice, these changes promote depressive states and decrease adult hippocampal neurogenesis. While the exact underlying cascades of events are not elucidated, we found that the transmitted dysbiosis requires an intact vagus nerve to be effective. Our results are in line with recent studies showing altered cecal and fecal microbiota composition in experimental models of stress (16,17) and in

depressed patients (18). These alterations may contribute to the neuroprogression of stress-related depression by altering physiological and/or metabolism processes that activate vagal afferents, such as microbial peptides, neurotransmitter release and immunity/inflammation (19,20). Our results are in line with recent data showing that the vagus nerve mediates the anxiolytic effects of some probiotic strains in rodents (6,21,22). The two existing studies pertaining to the effect of the vagus nerve on hippocampal plasticity show contradictory results, (23, 24). While the reasons underlying these discrepancies are not clear, they may be due to methodological differences, such as the Vx procedure itself. Relevant electrophysiological data on vagal tone should be implemented in future studies to decipher how GM promote depression. Our data further support the emerging hypothesis that GM can influence brain function and behavior directly through a nervous pathway mediated by vagal afferents.

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123 **Author contributions**

124 ES initiated the project in the P-M.L. laboratory, designed the study, performed the
 125 experiments and wrote the manuscript. S.S. analyzed the 16S data. C.M. contributed
 126 in the subdiaphragmatic vagotomy surgeries. M.B. contributed in some experiments.
 127 P-M.L. supervised the study and edited the manuscript with input from the other
 128 authors.

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Figure 1. Gut microbiota from chronically stressed mice generates depression and decreases hippocampal adult neurogenesis through the vagus nerve.

(A) Experimental design used for assessing the microbiota changes, behavioral responses and hippocampal neurogenesis in mice that were colonized by microbiota from either CT or UCMS mice. Microbiota recipient mice were treated with broad spectrum antibiotics (abx) for one week prior fecal transfer and were kept in isolators in order to maintain their bacterial profile. Mice sustained Vx at 4 weeks prior fecal inoculation. **(B)** Schematic representation of the fecal transplantation paradigm and the different groups used in the study. **(C)** In the NSF test, mice that were inoculated with UCMS-derived microbiota displayed an increased latency to eat the pellet

238 (* $P < 0.05$) that was completely abolished by Vx (** $P < 0.01$). **(D)** Heatmap showing the
 239 mean relative abundances of the different bacterial families in the 16S rRNA
 240 sequencing across all the experimental replicates. **(E, F)** In the tail suspension and
 241 forced swim tests, which are both indicative of despair-like behavior, UCMS-tr mice
 242 displayed increased immobility (*** $P < 0.001$ and * $P < 0.05$), and Vx abolished this
 243 behavior (* $P < 0.05$). **(G)** Representative images of Ki67⁺ and DCX⁺ cells in the
 244 dentate gyrus of CT-tr, UCMS-tr and UCMS-tr-Vx mice. Scale bars: 100 μ m. **(H)**
 245 UCMS-microbiota transfer decreased the number of Ki67⁺ proliferating cells in the
 246 dentate gyrus of recipient mice (*** $P < 0.001$) but not in mice that sustained Vx. **(I)**
 247 Transfer of UCMS microbiota decreased the number of DCX⁺ immature neurons in
 248 the dentate gyrus (*** $P < 0.001$). Vx abolished this decrease (* $P < 0.05$). All data are
 249 represented as mean $\pm$ SEM. Statistical significance was calculated using two-way
 250 ANOVA followed by Tukey post-hoc test. abx: antibiotics, NSF: novelty suppressed
 251 feeding, Vx: vagotomy.