Investigating the role of short-chain fatty acid butyrate on anxiety-like behavior and social recognition in mice

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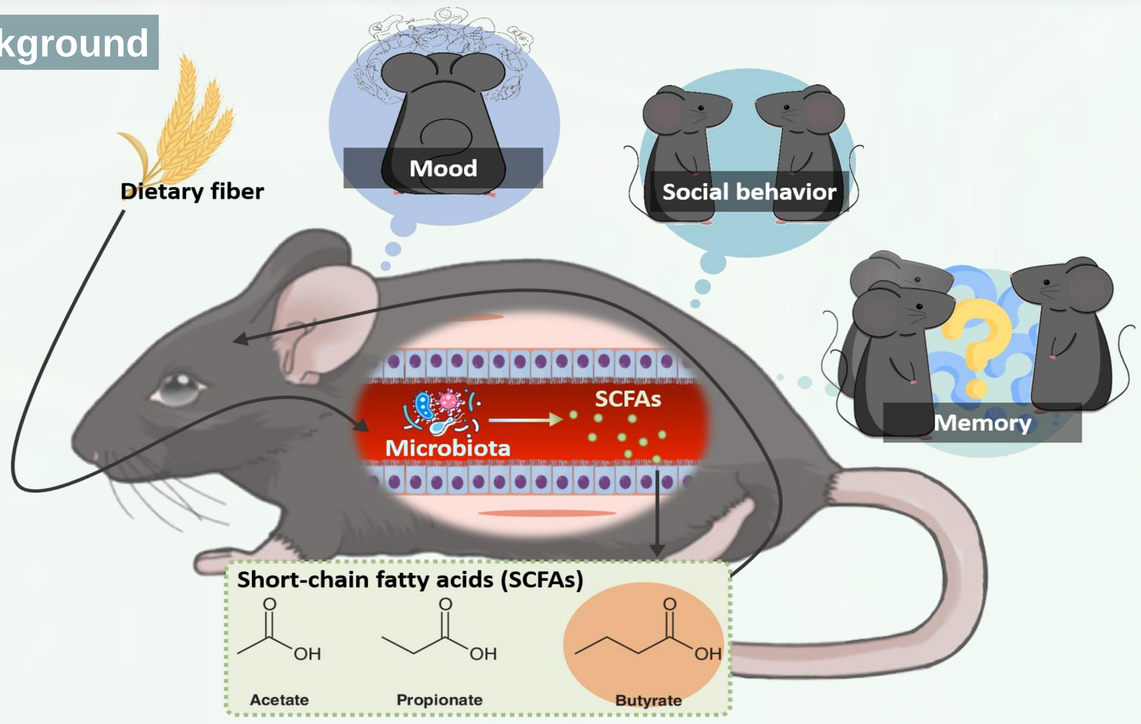
Abstract

The microbiota-gut-brain axis is a concept describing the complicated interactions among gut microbiota, gastrointestinal tract, and central nervous system. Accumulating evidence suggests that signaling from the gut microbes can directly or indirectly impact brain development and functionalities through immune, neural, hormonal, or metabolic pathways. Short-chain fatty acids (SCFA) are metabolites derived from intestinal microbial fermentation of dietary fibers and resistant starch and play a critical role in the host nervous system. Among the various SCFA, previous studies suggest that butyrate exerts beneficial effects on neurodevelopmental disorders and cognitive dysfunction. However, most studies focused on the peripheral effects of butyrate on behaviors. Herein, we examined the effects of central infusion of butyrate on anxiety-like and social recognition in mice. Butyrate was infused into the brain by intracerebroventricular (ICV) injection in mice treated with antibiotics. The data showed that ICV injection of butyrate did not produce any effect on anxiety-like behavior and social recognition in antibiotics-treated mice. However, we found that central infusion of butyrate downregulated the locomotor activity in the open-field (OF) test. In addition, our preliminary data showed that ICV injection of butyrate increased c-Fos+ cells in the paraventricular nucleus of hypothalamus (PVN) and basolateral amygdala (BLA). Altogether, central delivery of butyrate in the acute fashion decreased the locomotion but did not alter mouse anxiety-like and social recognition. We speculate that the lowered locomotion in central butyrate-infused mice might be associated with the neural activity in distinct brain regions.

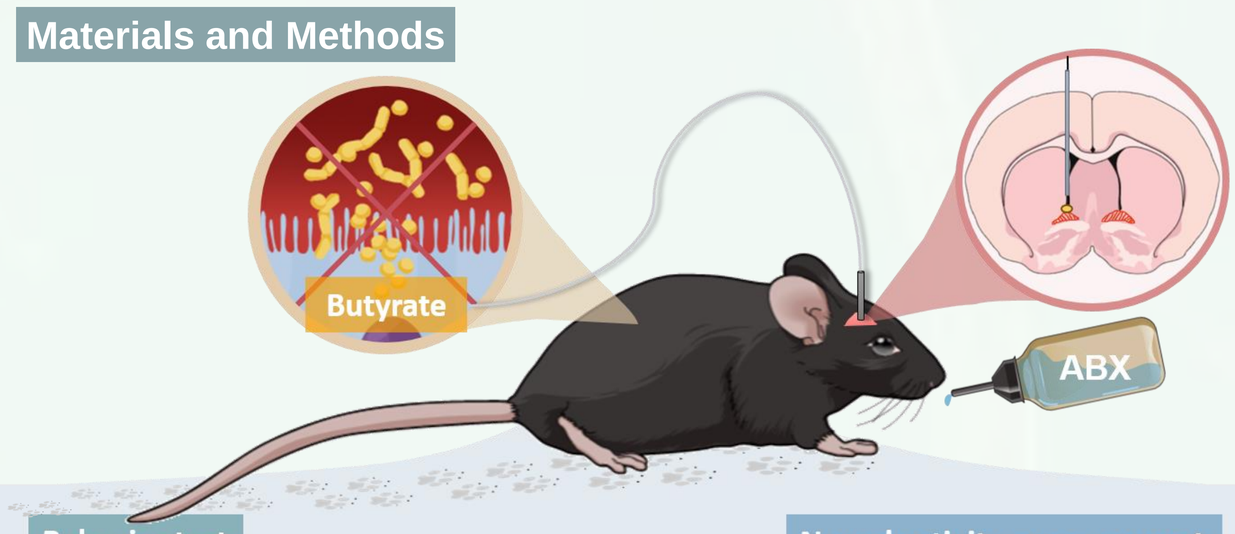
Background



- SCFAs might influence brain function indirectly via peripheral immune, endocrine, and nervous system or directly through the blood-brain barrier.
- In the previous study, sodium butyrate (SB) is a widely known histone deacetylase inhibitor (HDACi) that might attenuate anxiety-like behavior, memory deficits and improve social recognition in animal models.

(Dalile, Van Oudenhove, Vervliet, & Verbeke, 2019)

Materials and Methods



Results

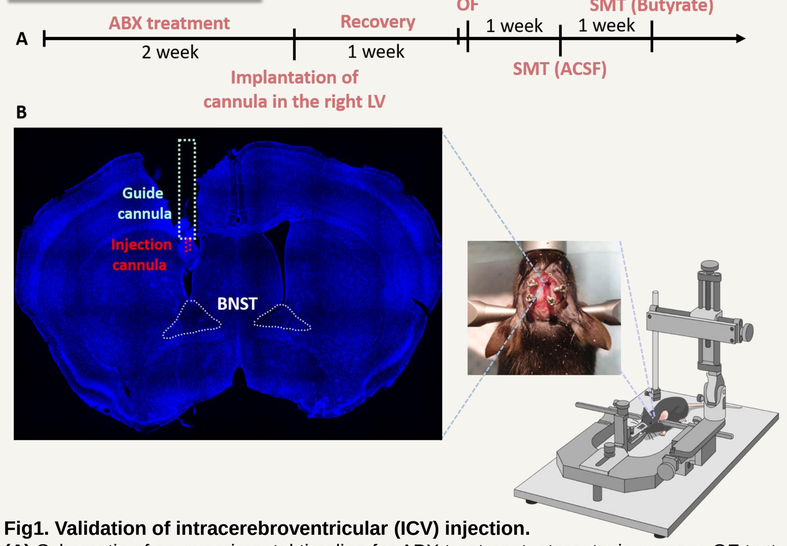


Fig1. Validation of intracerebroventricular (ICV) injection.
(A) Schematic of an experimental timeline for ABX treatment, stereotaxic surgery, OF test, and social memory test (SMT). **(B)** Illustration of the stereotaxic surgery and the guide cannula implantation in the right lateral ventricle. Fluorescent image of DAPI strained brain sections showing the guide cannula track to the lateral ventricle and the site of the BNST. LV: lateral ventricle. ABX: antibiotic cocktail. BNST: bed nucleus of stria terminalis.

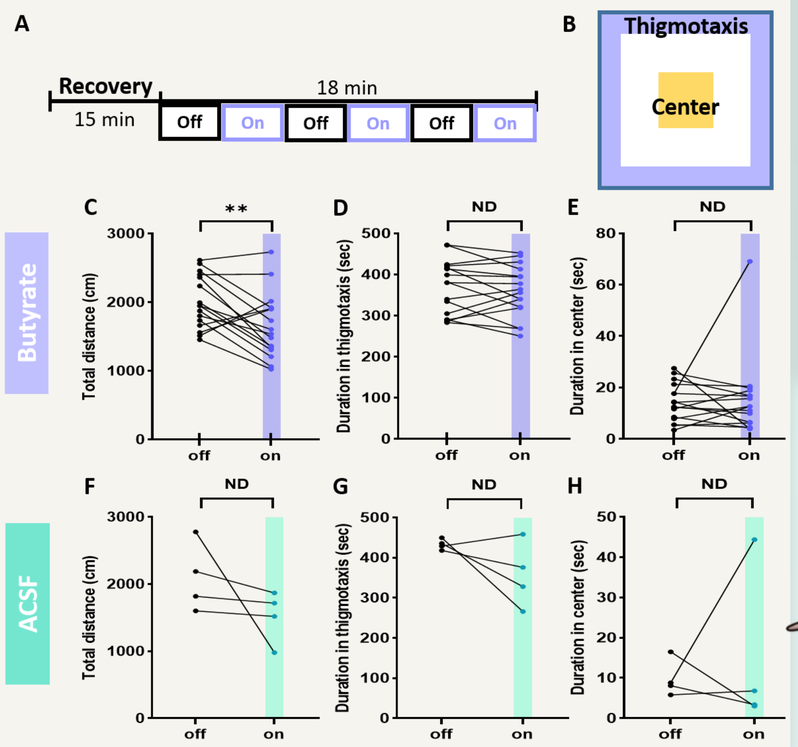


Fig2. ICV injection of butyrate in open-field (OF) test.
(A) Experimental timeline for injection during the OF test. On means the injection pump is turned on. Off means the injection pumps is turned off. Three on-off switches were conducted. Each on and off was lasted for three minutes. On: injection; Off: non-injection. **(B)** Schematic of the OF test. Mice with more anxiety-like behavior spent more time by the wall (thigmotaxis behavior) and less time in the center. Mice with less anxiety-like behavior spent more time in the center zone and less time by the wall. **(C)** Total distance traveled, time spent in **(D)** center zone, **(E)** thigmotaxis zone were analyzed in butyrate-injected mice in OF test. C, $^{**}P$ = 0.0081; D, P = 0.314; E, P = 0.7399. n = 16 per group. **(F)** Total distance mice traveled, time spent in **(G)** center zone, **(H)** thigmotaxis zone, were analyzed in artificial cerebrospinal fluid (ACSF, control)-injected mice in OF test. F, P = 0.2558; G, P = 0.1947; H, P = 0.6977. n=4 per group. Data shown as individual points and analyzed by two-tailed paired t-test. $^{**}P < 0.01$, ND: no difference. ABX: antibiotic cocktail. LV: Lateral ventricle.

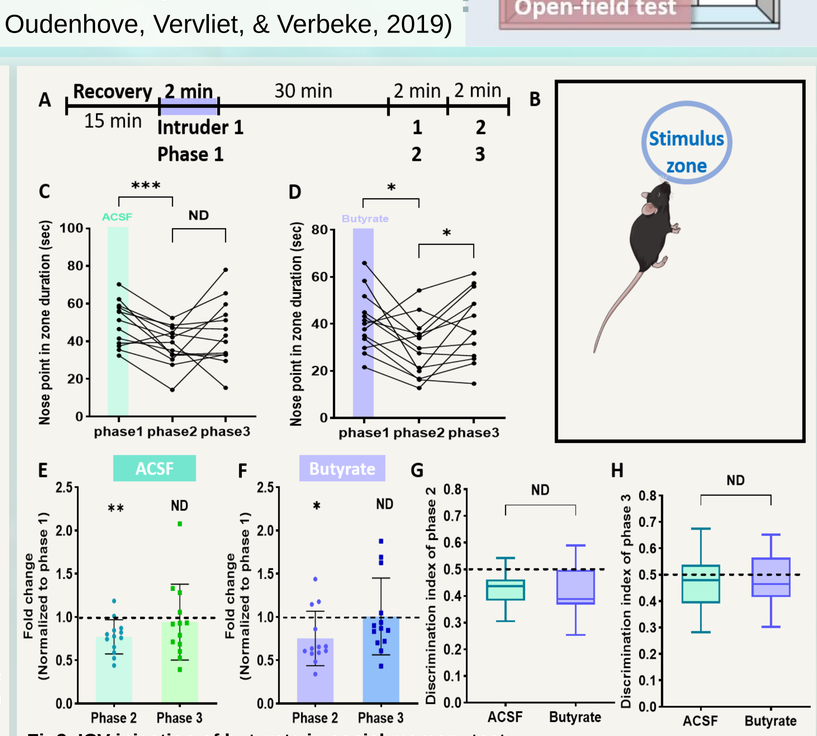


Fig3. ICV injection of butyrate in social memory test.
(A) Experimental timeline for butyrate injection (purple square represents the time of injection) and intruder (female stimulus mouse 1 and 2) placed during the test. Phase 1 and phase 2 placed the same intruder to test the social memory retrieval, and phase 3 placed a novel intruder to test the social novelty. **(B)** Schematic of the social memory and social novelty test. The intruder was placed in the inverted pencil cup, defined as the stimulus zone. Duration of mice nose point toward the stimulus zone in different phase in **(C)** ACSF-injected mice and **(D)** butyrate-injected mice. C, phase 1 vs phase 2 $^{***}P$ = 0.0009, phase 2 vs phase 3 P = 0.146; D, phase 1 vs phase 2 $^{*}P$ = 0.0173, phase 2 vs phase 3 P = 0.0115. Comparison of the time spent on the intruder at phase 2 and the phase 3 (normalized to phase1) in **(E)** the ACSF-injected subjects and **(F)** in the butyrate-injected subjects. E, phase 1 vs phase 2 $^{**}P$ = 0.0013, phase 1 vs phase 3 P = 0.64; F, phase 1 vs phase 2 $^{**}P$ = 0.0151, phase 1 vs phase 3 P = 0.9506. **(G)** Analysis of the ACSF- and butyrate-injected subjects in phase 2 (P2/(P1+P2) and **(H)** phase 3 (P3/(P1+P3)). G, P = 0.6668; H, P = 0.6815. n = 13 per group. Data shown as individual points with mean ± SEM **(E, F)** or box and whisker **(G, H)** and analyzed by two-tailed paired t-test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ND: no difference. ACSF: Artificial cerebrospinal fluid.

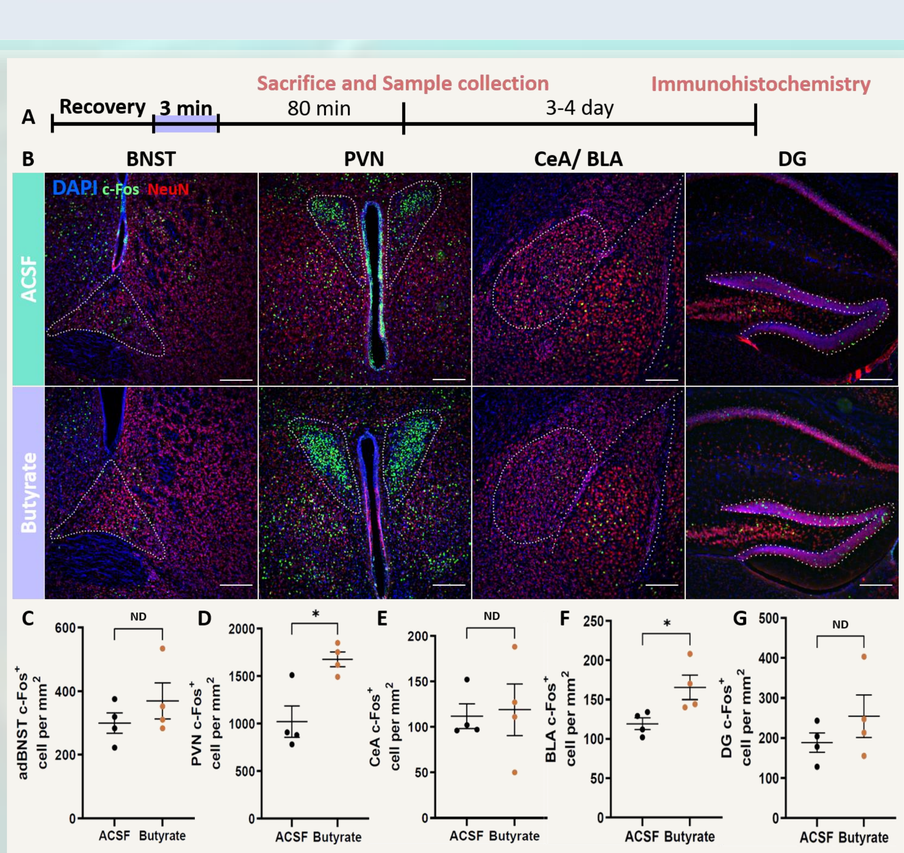
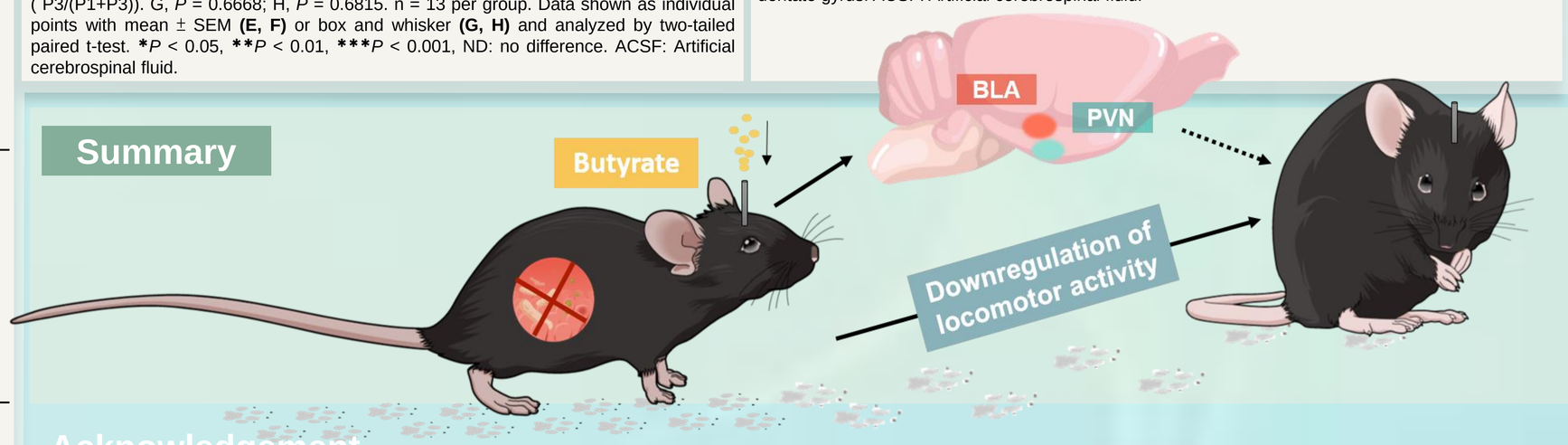


Fig4. Immunohistochemistry staining of c-Fos+ cells in distinct brain regions.
(A) Experimental timeline for butyrate injection (purple square represents the time of injection), sample collection and Immunohistochemistry**. (B)** Representative images of DAPI, neuronal nucleus (NeuN), and c-Fos staining in the ACSF- and butyrate-injected mice. Scale bar: 200 μm. **(C-G)** Quantification of c-Fos+ cells. C, P = 0.3231; D, $^{*}P$ = 0.0115; E, P= 0.8251; F, $^{*}P$= 0.037; G, P = 0.2987. n=4 ACSF-, 4 Butyrate- injected mice. Data shown as individual points with mean ± SEM and analyzed by two-tailed unpaired t-test. $^{*}P < 0.05$, ND: no difference. BNST: bed nucleus of the stria terminalis. PVN: paraventricular nucleus. BLA: basolateral amygdala. CeA: central nucleus of the amygdala. DG: dentate gyrus. ACSF: Artificial cerebrospinal fluid.

Summary



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Reference

Dalile, B., Van Oudenhove, L., Vervliet, B., & Verbeke, K. (2019). The role of short-chain fatty acids in microbiota–gut brain communication. Nature Reviews Gastroenterology & Hepatology, 16(8), 461-478. Retrieved from https://doi.org/10.1038/s41575-019-0157-3. doi:10.1038/s41575-019-0157-3