Investigation of influence of the gut microbial composition associated with colitis on mice behavior

Maryana V. Morozova[1,2,*], Mariya A. Borisova[3,*], Olga A. Snytnikova[4], Kseniya M. Achasova[1,2], Ekaterina A. Litvinova[1,5], Yuri P. Tsentalovich[4], Elena N. Kozhevnikova[1,2,6,#]

[1] Scientific Research Institute of Neurosciences and Medicine, [2] Institute of Molecular and Cellular Biology SB RAS, [3] The Federal Research Center Institute of Cytology and Genetics SB RAS, [4] International Tomography Center SB RAS, [5] Novosibirsk State Technical University, Center of Technological Excellence, [6] Novosibirsk State Agrarian University, Novosibirsk, Russia

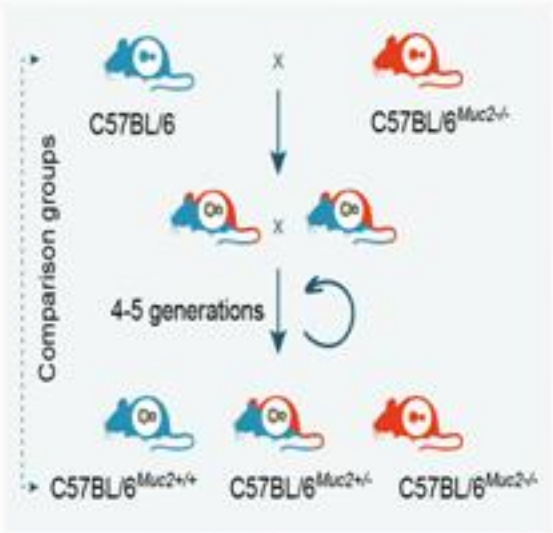
*e-mail: morozova.maryana@mail.ru

Introduction

Inflammatory bowel diseases (IBD) are chronic and relapsing inflammatory disorders of the gastrointestinal tract with complex etiology and no strategies for complete cure. IBD are often complicated by mental disorders like anxiety and depression, indicating substantial shifts in the gut-brain axis. However, the mechanisms connecting IBD to mental diseases are still under debate. Here we use *Muc2* mutant mouse model of chronic colitis to uncouple the effects of the intestinal microbiota on host behavior from chronic inflammation in the gut.

Methods

Littermate method



Drug treatments:

- Strychnine 0.75 mg/kg (i.p.) 15 minutes before the behavioral test.
- L-701,324 10 mg/kg (i.p.) 45 minutes before the behavioral test

Behavioral tests:

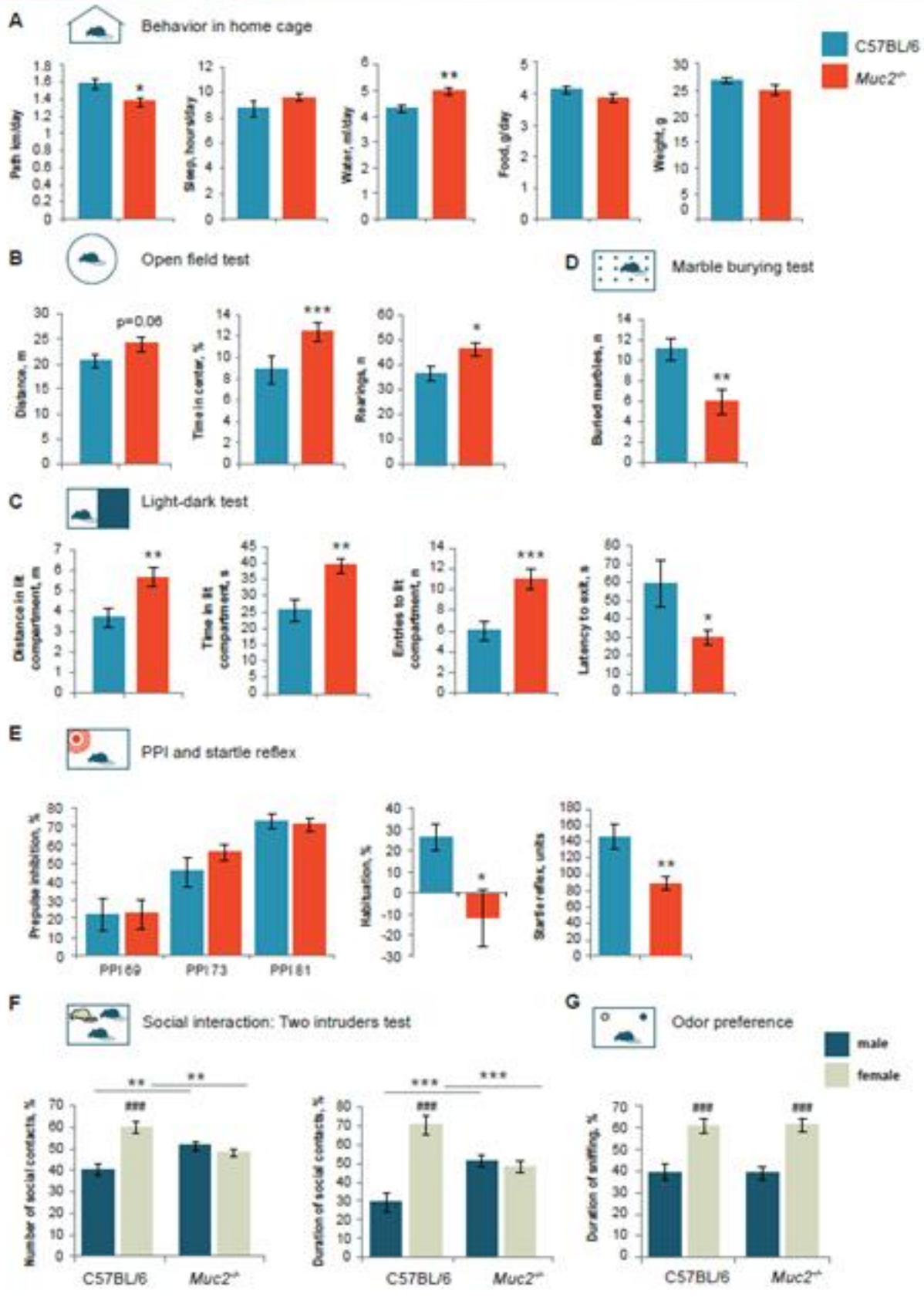
- Home-cage behavior
- Open field test
- Light-dark test
- Elevated plus maze
- Marble burying
- Tail suspension test
- Forced swim test
- Rotarod test
- T-maze spontaneous alternation test
- Barnes maze
- Sucrose preference test
- Active Avoidance test
- Startle reflex, prepulse inhibition (PPI), habituation
- Social preference test
- Odor preference test
- Resident-intruder tests

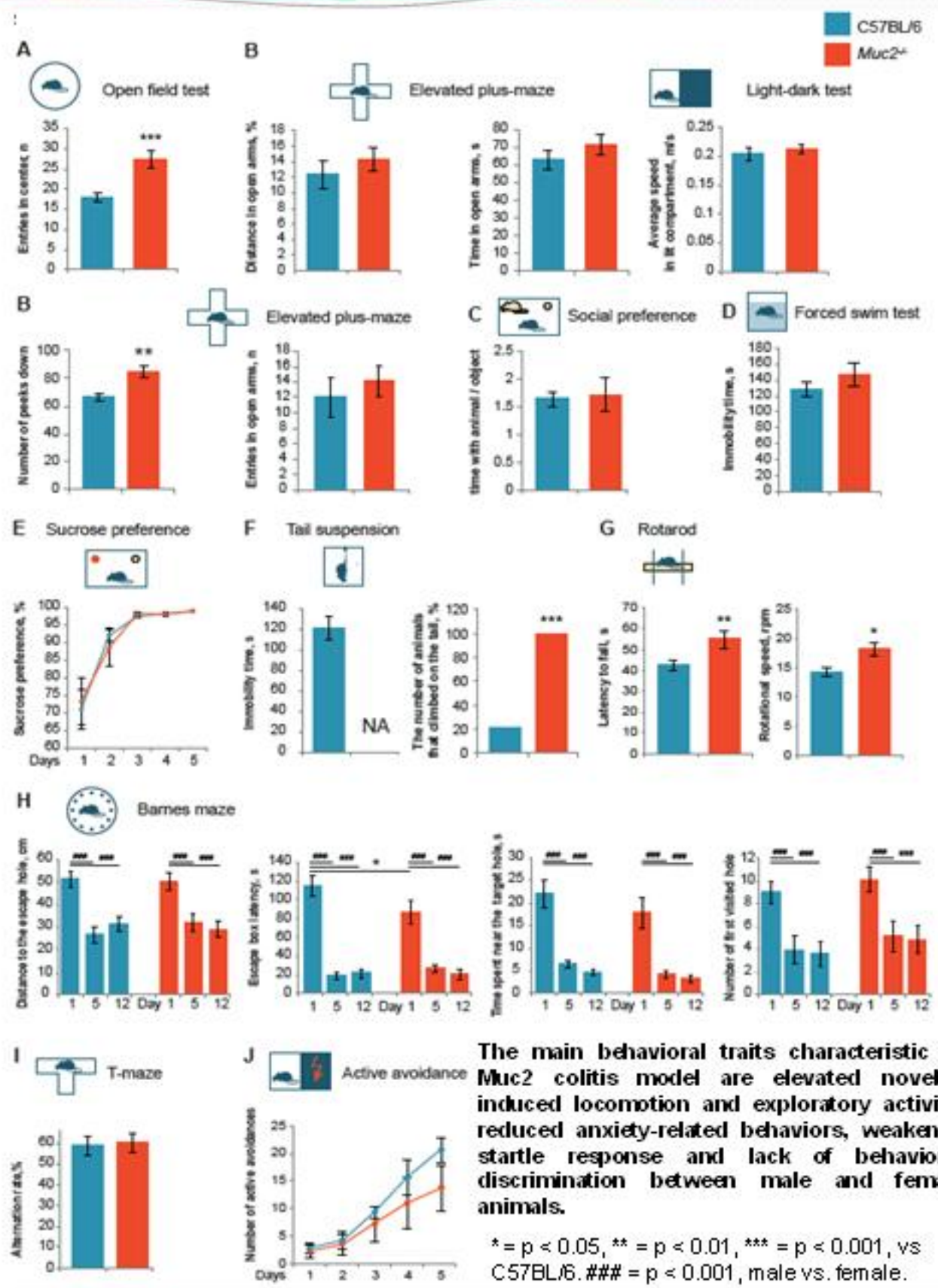
Nuclear magnetic resonance (NMR) spectroscopy

Metagenomic analysis

Real-time PCR

Results. Behavioral traits of *Muc2*$^{-/-}$ animals

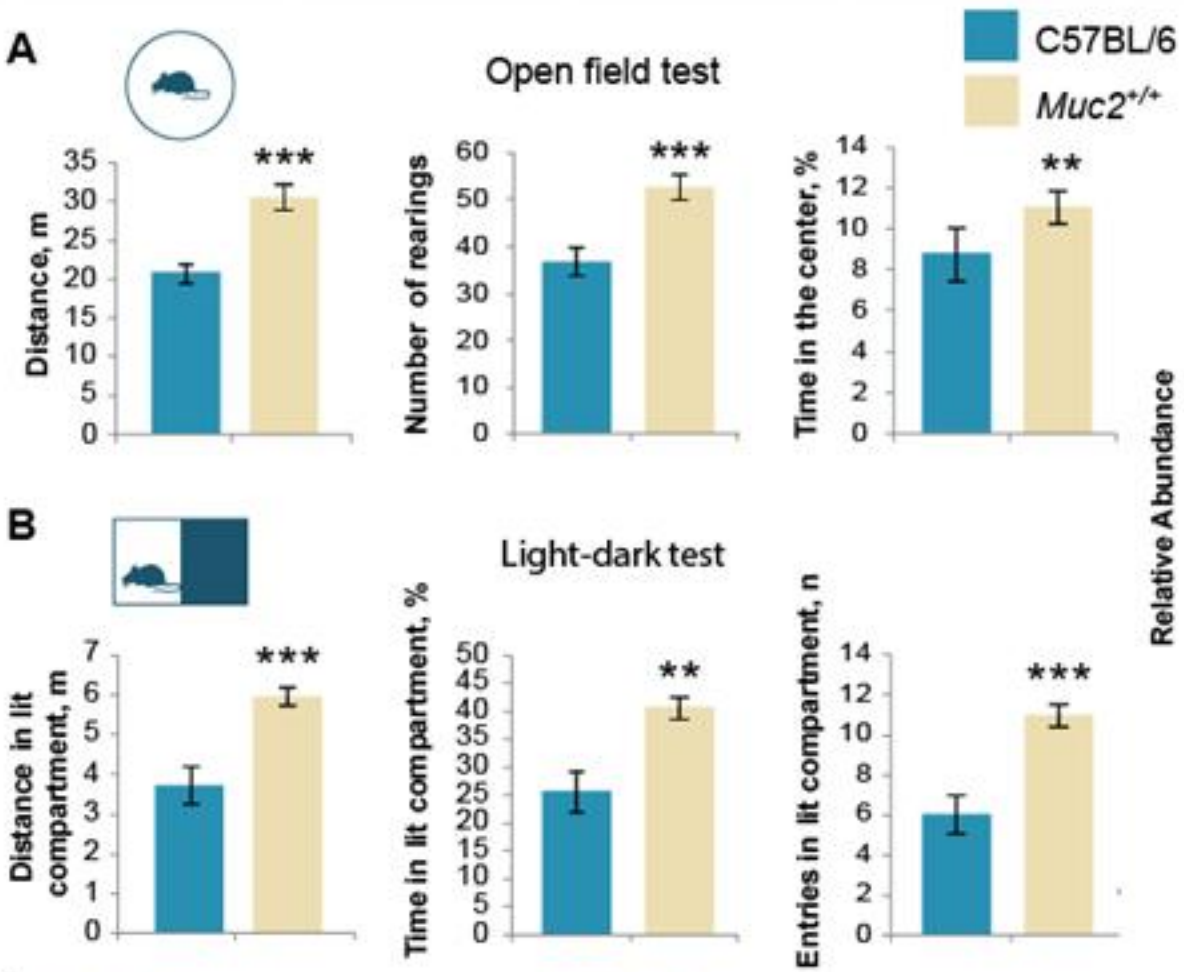


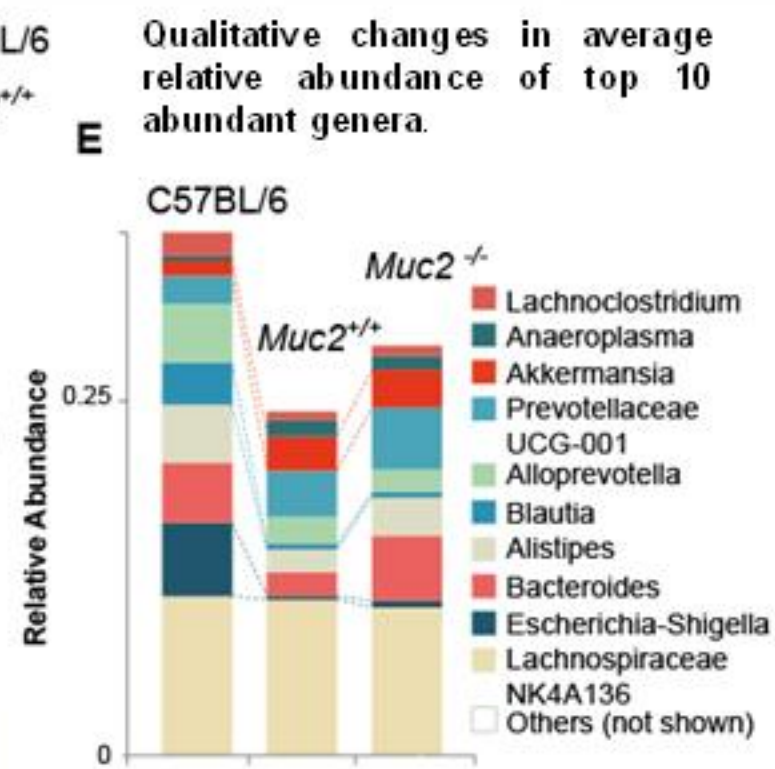


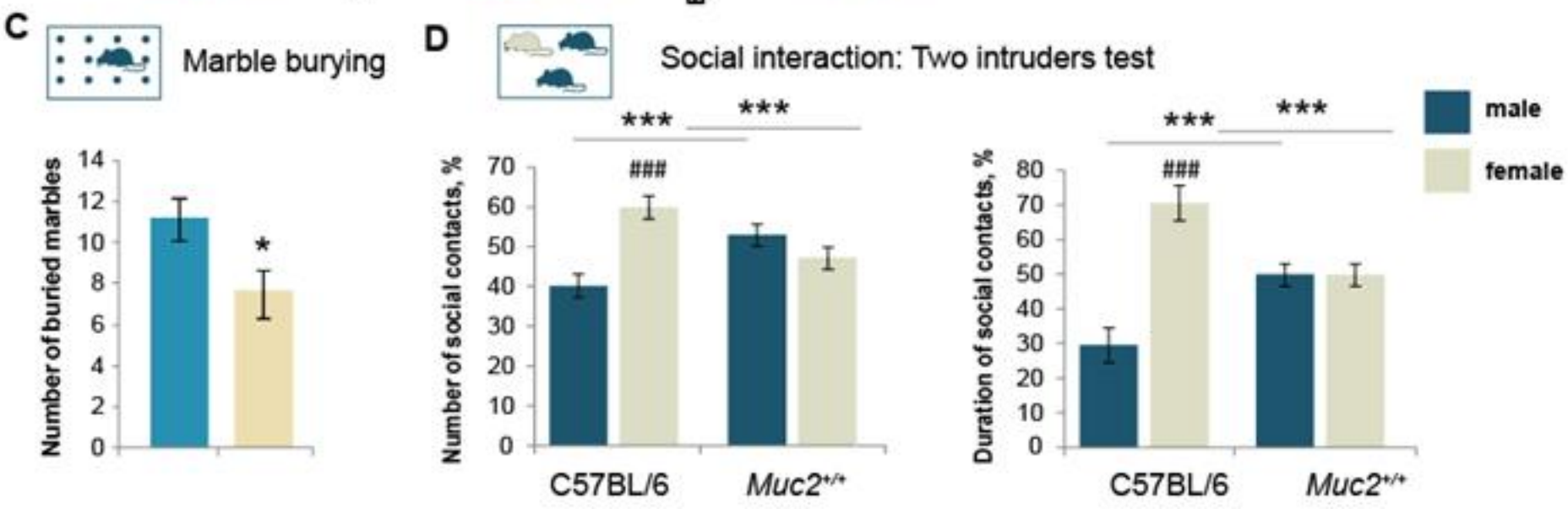
The main behavioral traits characteristic to Muc2 colitis model are elevated novelty-induced locomotion and exploratory activity, reduced anxiety-related behaviors, weakened startle response and lack of behavioral discrimination between male and female animals.

* = p < 0.05, ** = p < 0.01, *** = p < 0.001, vs C57BL/6. ### = p < 0.001, male vs. female.

Results. Behavioral traits are associated with microbiota of *Muc2*$^{-/-}$ mice.



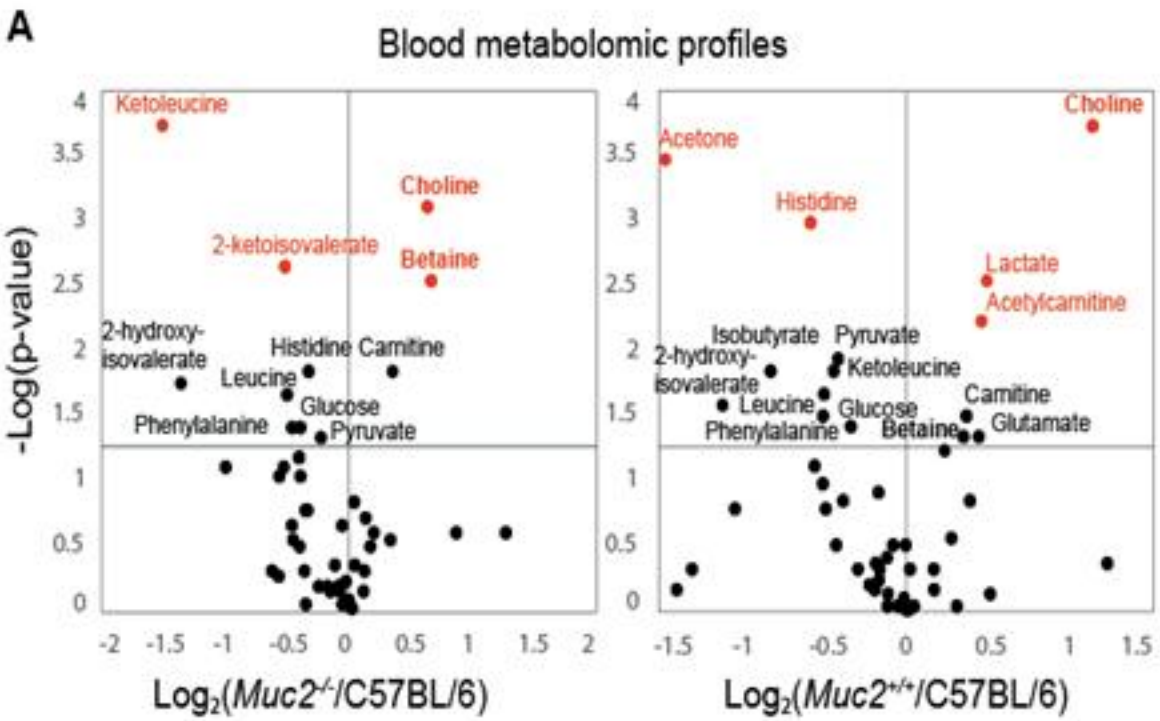


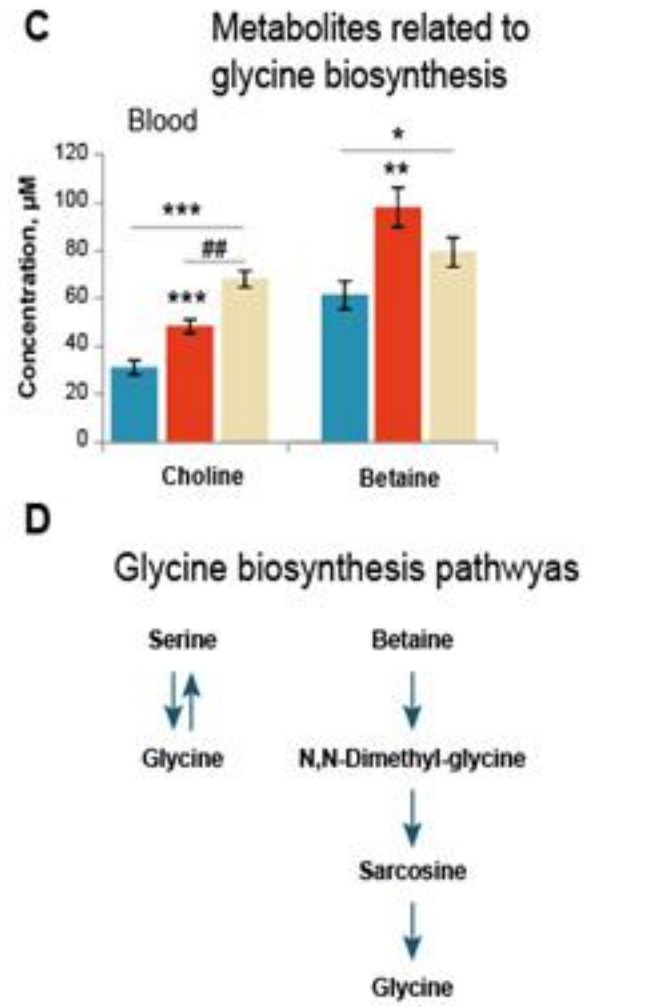


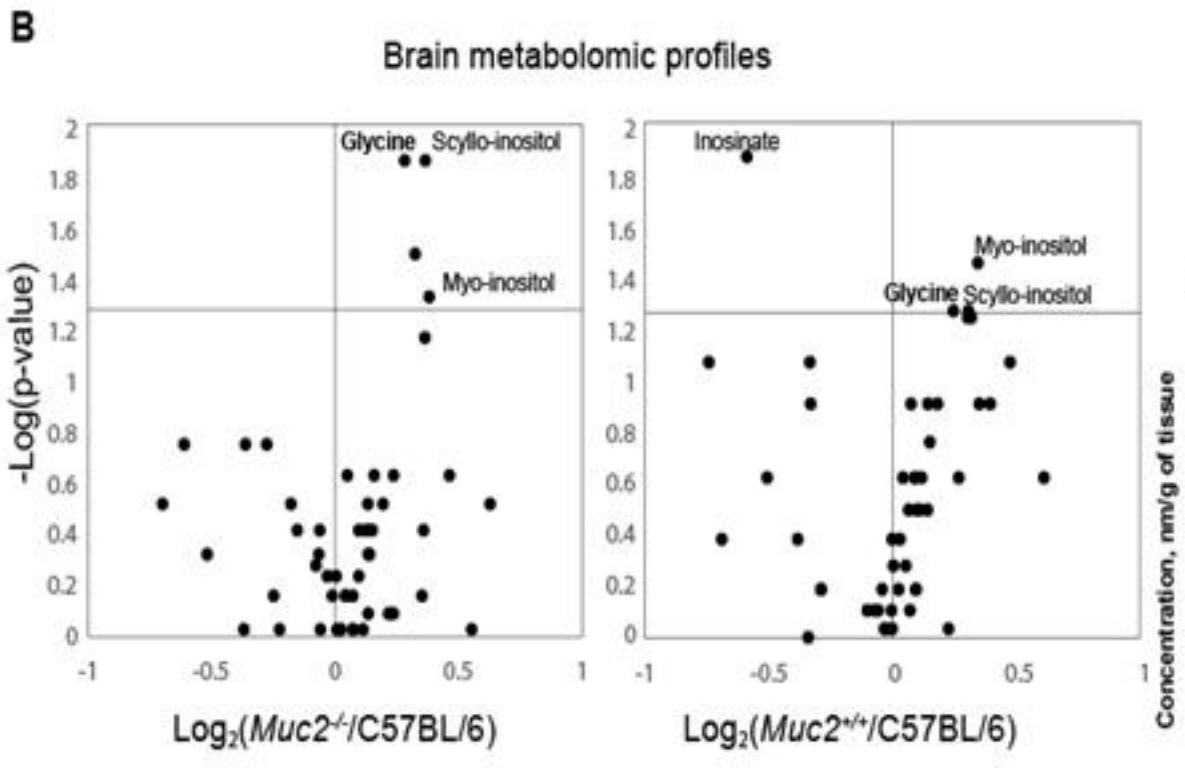
These data demonstrate that the behavioral traits in Muc2$^{-/-}$ animals, at least in part, are attributed to the microbiota composition rather than intestinal inflammation itself.

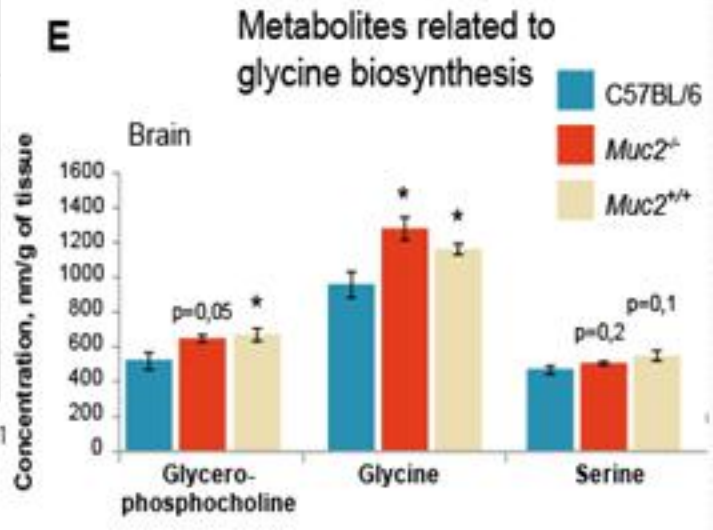
* = p < 0.05, ** = p < 0.01, *** = p < 0.001, vs. C57BL/6. ### = p < 0.001, male vs. female.

Results. Metabolomic profiling of blood and brain of *Muc2*$^{-/-}$ and *Muc2*$^{+/+}$ mice.







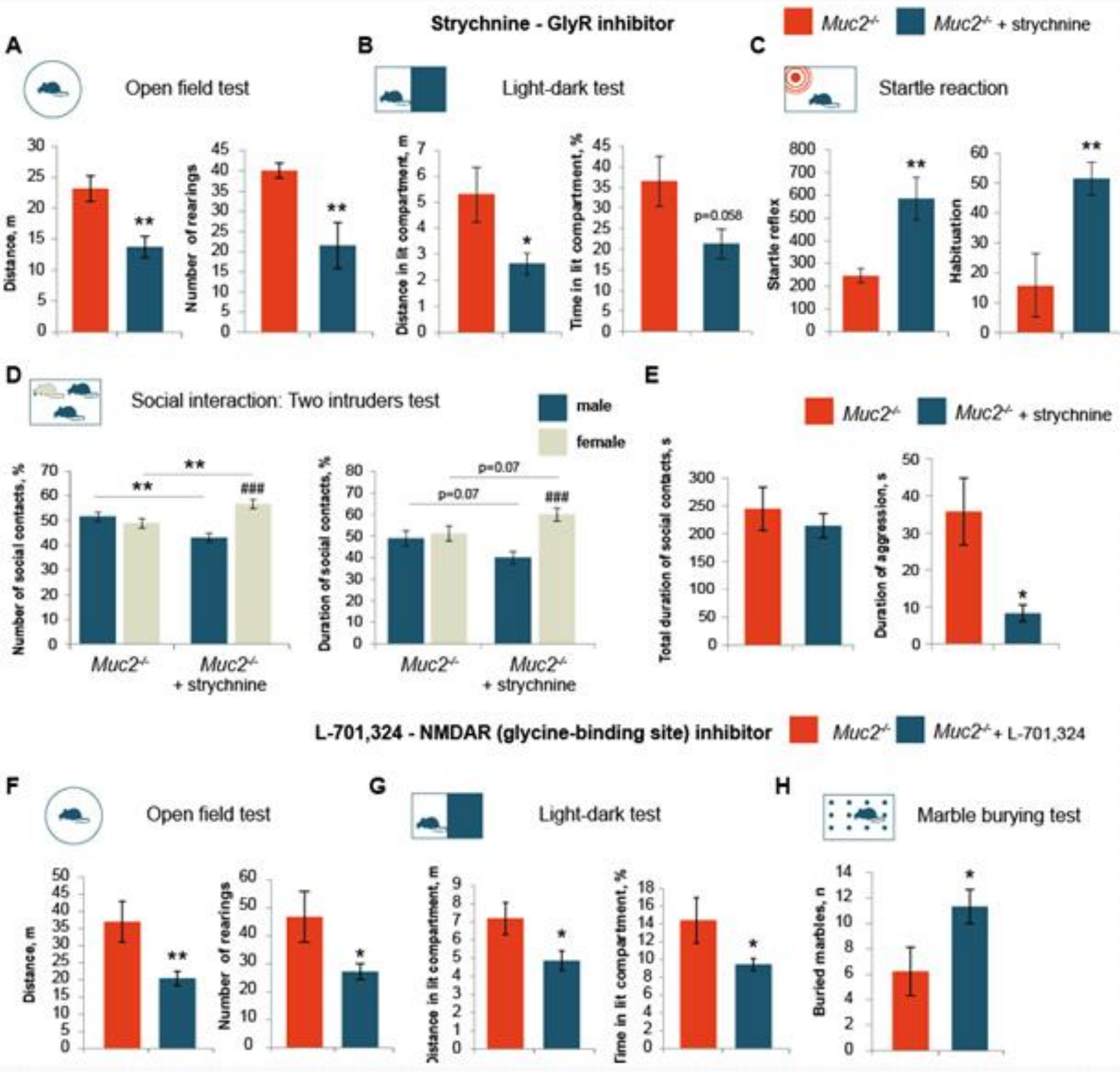


We suggested that glycine might be a potential central mediator of the behavioral phenotype observed in Muc2 colitis model.

Blood and brain metabolic profiles suggest that microbiota-dependent changes in choline metabolism might be involved in regulation of central glycine neurotransmission.

*= $p < 0.05$, **= $p < 0.01$ *vs.* C57BL/6. ##= $p < 0.01$ *vs.* *Muc2*$^{+/+}$.

Results. Glycine neurotransmission mediates behavioral abnormalities in *Muc2* model of colitis.



Both inhibitors ameliorated all behavioral effects related to novelty-induced hyperactivity, anxiety, sensorimotor gating, and social behavior. Therefore, glycine appears to be the major microbiota gut-brain axis regulator in the mucus deficiency model of colitis.

** = p < 0.05, *** = p < 0.001 vs. C57BL/6. ### = p < 0.001, male vs. female.*

Conclusions

- **Muc2 knockout mouse model demonstrates high clinical and histological score of colitis.**
- **Muc2 do not have anxiety - or depression-related behavior, as patients with IBD.**
- **We suggest that microbiota defines the mutant behavioral profile.**
- **We hypothesize that the behavioral change in Muc2 mice is associated with increased levels of *A. muciniphila* in the gut.**
- **Brain metabolomic profiles, revealed glycine as a potential neurotransmitter responsible for the observed behavior.**
- **Blood metabolomic profiling revealed elevation of choline and betaine in both $Muc2^{-/-}$ and $Muc2^{+/+}$ mice. Choline and betaine are precursors of glycine biosynthesis in the glycine-betaine pathway**
- **Free choline either comes from the diet or is released by phospholipase D from phosphatidylcholine, known as a main choline depot.**
- **A possible mechanism might involve a shift in the intestinal phospholipid metabolism induced by *Akkermansia* via Toll-like receptor 2.**

Link to the article

https://www.biorxiv.org/content/10.1101/2022.03.07.483210v1

Acknowledgmen

This study was supported by the Russian Science Foundation (RSF) Grant #20-74-10022.