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Post-stress changes in the gut microbiota in rat strains with different excitability of the nervous system

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Motivation and aim: The great diversity of bacteria that inhabit the mammalian gut constitutes a complex and dynamic ecosystem [1] that plays an important role in the homeostasis of the host organism. Changes in the microbial community are the influence of various genetic, environmental, and dietary factors. The aim of our study was to show the effect of stress on the gut microbial community of two different strains of rats, with low and high nervous system excitability.

Methods and Algorithms: The experiment was performed using 24 male rats (5-month-old) from two strains which differed in their nervous system excitability; the high excitability HT strain (12 rats) and the low excitability LT strain (12 rats). Animals were divided randomly into two groups. The control and experimental groups consisted of 6 rats each. The same standard environmental conditions were used for all animals. The Hecht protocol for prolonged emotional and painful stress was used on experimental groups of rats [2] as follows: every day for 15 days, the animals were exposed to 6 unsupported (10 seconds each) and 6 current-reinforced (2.5 mA, 2 ms) light signals. Samples of feces were collected before the stress and on the 7th and 24th days after.

Total DNA from fecal samples was isolated using the QIAamp DNA Stool Mini kit (51504, QIAGEN). The V4 region of the bacterial 16S rRNA gene was amplified with dual-indexing bacterial 16S RNA primers F515 (5′-GTGBCAGCMGCCGCGGTAA-3’) and R806 (5’-GGACTACHVGGGTWTCTAAT-3’). Amplification was performed with the HS SYBR qPCR mix reaction mixture (Eurogen, Moscow, Russia) on a CFX96 real-time PCR system (BioRad, Hercules, CA, USA).

The quality of the PCR products was checked by agarose gel electrophoresis. Purification of amplicons was performed with the QIAquick Gel Extraction Kit (250).

The purified libraries were pooled in an equimolar concentration and further paired-end sequencing was performed using an Illumina Miseq instrument (Illumina Inc., San Diego, CA, USA).

The raw reads were checked with FastQC, then filtered with fastp and demultiplexed with the deML package. Further analysis was performed using the Quantitative Insights Into Microbial Ecology 2 (Qiime2-2020.14) software package. The alpha diversity of the operational taxonomic units (OTU) was calculated using the q2-diversity Qiime2 plugin. The taxonomy of the reads was annotated to the Greengenes database using a naive Bayes classifier via the q2-feature-classifier plugin.

Distribution of data was evaluated by Shapiro-Wilk test using the GraphPad Prism software (v.6, GraphPad Software Inc., La Jolla, CA). Non-parametric tests (Mann–Whitney U tests, Fisher's exact test, Wilcoxon) were also performed with the GraphPad Prism software.

Results: In the intact animals of high excitable strain (LT), we detected a significant reduction of the Chao1 and Shannon indexes for the α-diversity of the bacterial

composition compared with the low excitable strain (HT). Our analysis revealed 11 Phyla among all samples. The most diverse taxonomic levels were represented by *Firmicutes* and *Bacteroidetes,* which were found in every sample. Less represented Phylums were *Actinobacteria, Proteobacteria, Euryarchaeota, Tenericutes, Spirochaetes, Cyanobacteria, Deferribacteres, Chlamydiae, Saccharibacteria* (TM7). The most distributed Orders in our samples were *Lactobacillales, Bacteroidales, Clostridiales*. We identified 59 genera, which the most represented were *Lactobacillus, Prevotella, Allobaculum, Enterococcus, Blautia, Collinsella, Faecalibacterium, Dorea, Bifidobacterium.*

We have found the following post-stress changes in the composition of the microbial community. A progressive decrease of *Lactobacillus* Genus in both strains from the 7th until the 24th day after the stress. *Enterococcus* was not detected in the LT strain but appeared in the HT strain after 24 days in response to stress. Genus of *Blautia, Collinsella* and *Methanobrevibacter* were overrepresented in the LT strain but were reduced in the HT strain. In the HT group, the Genera *Faecalibacterium* and *Prevotella* had low relative frequencies before the stress stimulus but increased significantly on days 7 and 24 after stress.

Conclusion: Our present data on dynamic changes in microbial diversity after stress provide support for further studies and may be correlated to the effects of probiotic therapy. Also, knowledge of the dynamic changes in bacterial species under stressful conditions will add to our understanding of how interspecies relationships contribute to shifts in the gut microbial community.

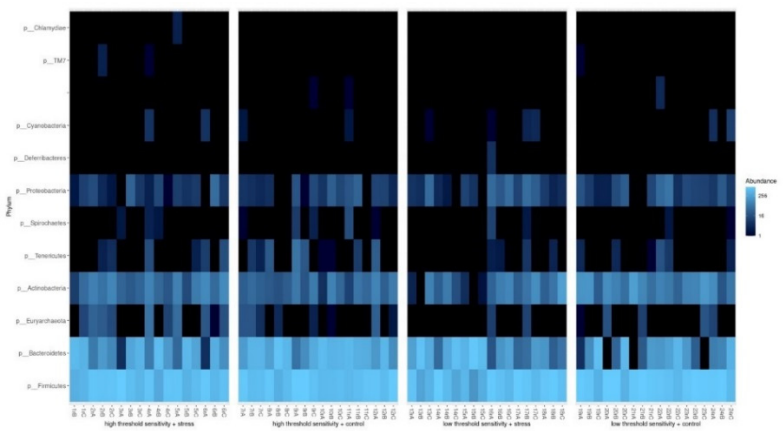

Fig. 1. Heatmap of the Phylum level of the gut microbiota of rats with high and low excitability of the nervous system. 1rA, 1rB, 1rC are the same animals at different time points

References

1. Rogers G.B., Kozlowska J., Keeble J., Metcalfe K., Fao M., Dowd S.E., Mason A.J., McGuckin M.A., Bruce K.D. Functional divergence in gastrointestinal microbiota in physically-separated genetically identical mice. *Sci Rep*. 2014;4:5437. doi: 10.1038/srep05437.
2. Vaido A., Shiryaeva N., Pavlova M., Levina A., Khlebaeva D., Lyubashina O., Dyuzhikova N. Selected rat strains Ht, Lt as a model for the study of dysadaptation states dependent on the level of excitability of the nervous system. *Laboratory Animals Sci*. 2018;3. doi: 10.29296/2618723X-2018-03-02. (in Russian)