

1. We have developed a chronic stress protocol for mice, which results in anxiety and anhedonia. Related publications: PMID: 28736330; PMID: 31821847; PMID: 35880047). This model is a two hit protocol in which mice pups are separated from their mothers for 3 hours daily for 12 days (maternal separation, first hit) then exposed to chronic variable stressors for 3 weeks as adults (second hit). Using various behavioral and hormonal tests, we have validated the model.
2. We have compared the hormonal-metabolic and behavioral profiles of mice exposed to acute (restraint and hypoglycemia) chronic repeated (restraint) and chronic variable stress schedule. Related publications: PMID: 28736330; PMID: 30996341; PMID: 35163282; PMID: 35880047. All acute stressors elevated blood corticosterone concentration and all types of stress (acute and chronic) resulted in increased energy expenditure. Exposure to acute stress induces locomotion, while chronically stressed mice moved less and utilised differential fuels during the post-stress period. Among the stressors investigated only chronic variable stress resulted in anxiety, anhedonia and anti-social behavior in mice. Based on these findings, the chronic variable stress was used in further experiments.
3. We have found that chronic variable stress alters the composition of gut microbiome Related publications: PMID: 31821847 and DOI: 10.1016/S0924-9771(7)30114-1. Colon microbiome diversity was analyzed at the phylum level and the results showed an increased abundance in the phylum of *Bacteroidetes* over Firmicutes in chronically stressed mice. There was a special increase in the concentration of *Clostridium* species in stressed samples. These results highlighted stress-induced specific changes in the mouse gut microbiome.
4. In addition to microbiome changes we found increased gut permeability in chronically stressed mice, which results in systemic bacterial load as measured by increased LPS levels in the blood and appearance of bacterial DNA in the mesenteric lymph node, elevated proinflammatory cytokine levels and macrophage infiltration into the submucosal layer of the colon.
5. Along the changes in microbiome composition, gastrointestinal abnormalities we also detected increased anxiety, anhedonia and increased locomotor activity in stress-exposed mice.
6. Rifaximin is a non-absorbable, non-systemic, GIT-specific antibiotic, which selectively eliminates pathogenic bacteria. Using this antibiotic in chronically stressed mice most of the stress-induced microbial, gastrointestinal and immune-related changes were reversed.
7. By contrast, stress-induced behavioral abnormalities (anxiety, anhedonia and increased locomotor activity) remained in spite of restoration of gut microbiome. These new results do not support the generally accepted hypothesis on the direct regulatory loop between the gut microbiome and behavior.
8. We hypothesized that the application of certain probiotic bacteria, such as anti-inflammatory *E.coli Nissle 1937* strain as a psychobiotic in stressed mice would prevent stress-induced changes in behavior. In spite of the expectations, this probiotic strain was not effective in modulating physiological and behavioral adverse effects in stressed mice. (Related thesis work: Laura Svajda Budapest University of Technology and Economy, 2017).
9. Next, we investigated the potential probiotic effect of certain newly isolated Lactobacilli. Among the Lactobacilli (Lb) strains we tested, one strain, referred to as *Lactiplantibacillus plantarum* TV45 (formerly known as *Lactobacillus plantarum*) displayed strong probiotic,

psychobiotic and reprobiotic features. Oral administration of Lb TV45 for 4 weeks resulted in decrease of body fat and increased lean body mass, decreased anxiety and anhedonia. In stress situation Lb treated mice secrete significantly less corticosterone and display pro-social behavior in social stress tests. Based on these findings we have filed this Lb strain in the Hungarian Intellectual Property Office as a new probiotic.

10. During this period two PhD thesis (Zsuzsanna Winkler and Dániel Kuti) was completed in conjunction with this OTKA grant. Three MSc thesis (Laura Svajda, David Jenő Takács University of Technology and Economy and Maryam Gazeran, Eötvös Lorand University) have been written. We have published 11 papers covered by this grant.