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Interaction of gut microbiota and the brain in Anorexia Nervosa
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Aim

The purpose of the study was to investigate alterations of gut microbiota and the gut-brain axis in a translational animal model for anorexia nervosa (AN) during chronic starvation and after refeeding (bodyweight rehabilitation). The number of different bacterial species (α-diversity) and the composition of the gut microbiota (β-diversity) of the different groups were analyzed and associations between these parameters and brain volume, gut morphology, and behavioral alterations were analyzed.

Background

Until recently the role of gut bacteria was underestimated in the pathogenesis of psychiatric disorders. Current studies of the gut-brain axis, however, indicate an association between microbiota and psychiatric impairments such as anxiety and depression as well as bodyweight regulation. Since a dysregulation of bodyweight is a core symptom in AN and anxious and depressive symptoms are common comorbidities, research studying the role of microbiota in AN is warranted.

Method

The activity-based anorexia (ABA) paradigm is a well-established and commonly used animal model to study anorexia nervosa. The ABA model in the present study was conducted with female adolescent rats and is a combination of food restriction and access to a running-wheel. To analyze the gut microbiota fecal samples were collected from the animals at different time points (before starvation, after acute starvation, after chronic starvation, and after refeeding (if applicable)). Gut microbiota composition and the relative abundances of the individual bacteria were identified by a 16S rRNA gene amplicon sequencing (Illumina MiSeq). Before and after starvation all animals underwent two behavioral tests. The forced swimming test was used to analyze depressive-like behavior and the elevated plus maze was used to analyze anxiety-like behavior in the rats. At the end of the study protocol, brain and gut tissue were collected for molecular and cellular experiments. The brain tissue was used to measure brain volume alterations, to perform mRNA expression studies of the astrocyte marker GFAP and to stain and count GFAP-positive astrocytes. The gut tissue was mainly used to perform histological analysis.

Results and Discussion

We found significant alterations in gut microbiota composition between food restricted rats and control animals. One the one hand the number of different species (α-diversity) was significantly greater in ABA rats compared to controls and on the other hand the relative abundance of specific bacteria was different in the various groups. After refeeding, no differences were present anymore regarding the α-diversity, however, the bacterial composition was still different comparing samples from before starvation and after refeeding. These findings support the hypothesis that gut microbiota alterations are not purely an epiphenomenon of food starvation. There were no clear statistical changes in the brain volume of food-deprived rats as seen in our previous study. Nevertheless, we observed again reduced numbers of GFAP-positive astrocytes and less mRNA expression of GFAP in ABA rats compared to normally-fed rats. Several associations between the relative abundance of gut bacteria and brain volume or astrocyte parameters could be observed, for example: the higher the relative abundance of Lactobacillus the relatively higher the volume of the cerebral cortex and the corpus callosum, making it a possible candidate for future targeted probiotics-studies.

Morphological analysis of the gut tissue revealed a clear atrophy (smaller crypts in the colon and smaller villi in the small intestine) in ABA animals compared to controls. Moreover, increased numbers of lymphatic plaques were observed in ABA animals. Interestingly, the number of lymphatic plaques was lower in ABA animals that received short-term treatment with multi-strand probiotics during the starvation period.

Regarding the behavioral tests, no differences were observed in the forced swim test. The elevated plus maze showed that anxious animals were more susceptible to food restriction in the ABA paradigm and lost bodyweight faster. This association was not present anymore after refeeding.

These results show distinctive changes in the gut microbiome in the ABA animal model that did not completely normalize with weight gain supporting a role of microbiota and the gut-brain axis in anorexia nervosa (AN). Next, stool transplantation from patients with AN into antibiotics-treated animals will be used to further study the potential causal role of microbiota in the etiology and maintenance of AN. Nutritional interventions, supplements, individualized pre- and probiotics and maybe even stool transplantations might be helpful microbiome-targeted interventions to support the therapy of AN in the future.

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