Effects of gut microbiota depletion on anxiety-like behaviour and synaptic protein expression in rodent models

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Introduction

- Anxiety is one of the most prevalent psychiatric conditions and there is a pressing need for translational research.
- The gut microbiota can modulate host behaviour, including anxiety-related behaviour.
- Antibiotics are often used as a model of gut microbiota depletion.
- The aim of this study is to investigate the role of the gut-microbiota in anxiety-like behaviour and brain physiology.

Methods

1 Gut Microbiota Depletion, Anxiety-like Behaviour and Neuronal Activation

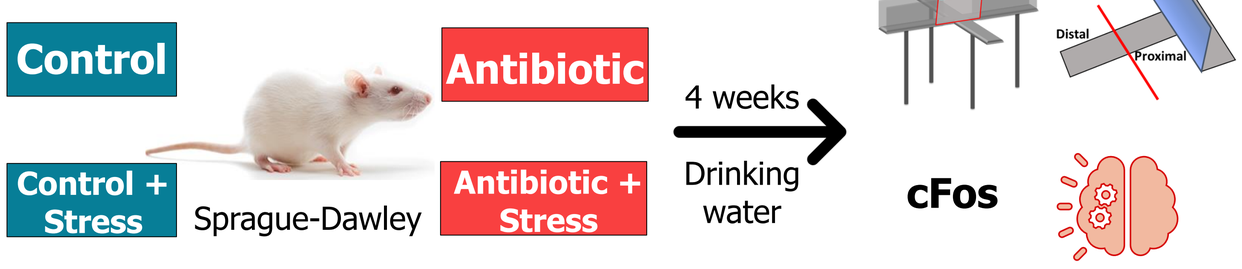


Figure 1. Experimental Design. Adult male Sprague-Dawley rats where exposed to an antibiotic cocktail in drinking water for 4 weeks. The antibiotic cocktail consisted of ampicillin 1 g/L, vancomycin 0.5 g/L, and imipenem 0.25 g/L. At the end of the treatment, rats were exposed to the elevated open arm test, which is a mild anxiogenic stimulus (stress). After 90 minutes animals were perfused and brains were collected to perform immunohistochemistry to detect cFos.

2 Evaluating Hippocampal Plasticity in mice with Depleted Microbiota

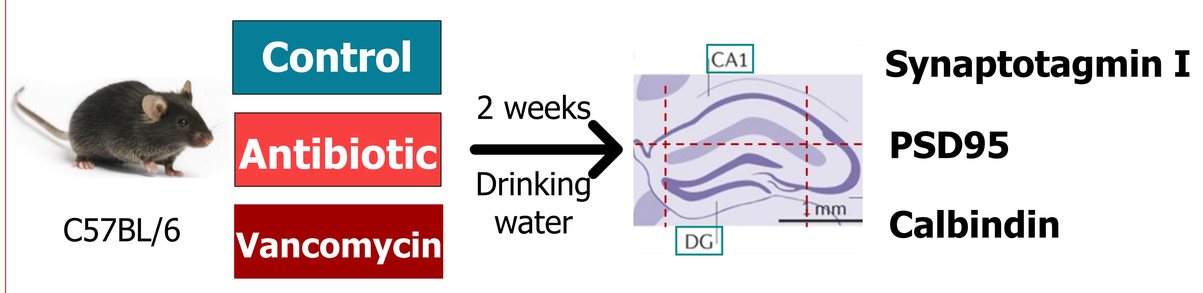


Figure 2. Experimental Design. Adult male and female C57BL/6 mice received an antibiotic cocktail or vancomycin in drinking water for 2 weeks. The antibiotic cocktail consisted of ampicillin 1 g/L, vancomycin 0.5 g/L, and imipenem 0.25 g/L; vancomycin group was at 0.5 g/L. Animals were euthanized and bilateral hippocampii were collected. CA1 and Dentate Gyrus (DG) were dissected. The synaptic proteins, Synaptotagmin I, PSD95, and Calbindin were measured using western blot.

Results

1 Gut Microbiota Depletion Induces Anxiety-Like behaviour but not changes in neuronal activation

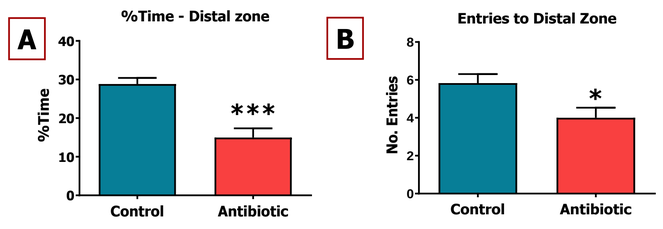


Figure 3. Increased anxiety-like behaviour following 4 weeks of antibiotic cocktail treatment. **A.** Percentage of time spent in the distal zone of the platform. **B.** Number of entries to the distal zone of the platform. N=7, t-test (*p < 0.05, ***p < 0.001).

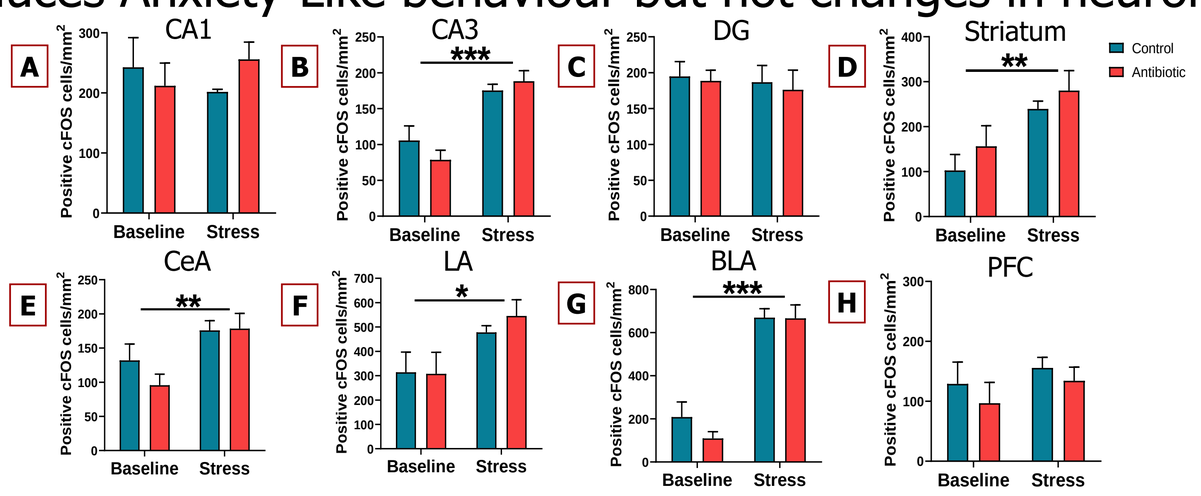


Figure 4. Neuronal activation is driven by stress but not antibiotics. cFos expression, an indicator of neuronal activation, was measured in different brain regions, including CA1, CA3, and dentate gyrus of the hippocampus (**A-C**); **D.** striatum; central, lateral, and basolateral amygdala (**E-G**); **H.** prefrontal cortex. The analysis was performed by using immunohistochemical detection in brain slices. N=7, Two way ANOVA (*p < 0.05, **p < 0.01, ***p < 0.001).

2 Synaptic protein expression in a rodent model of microbiota depletion

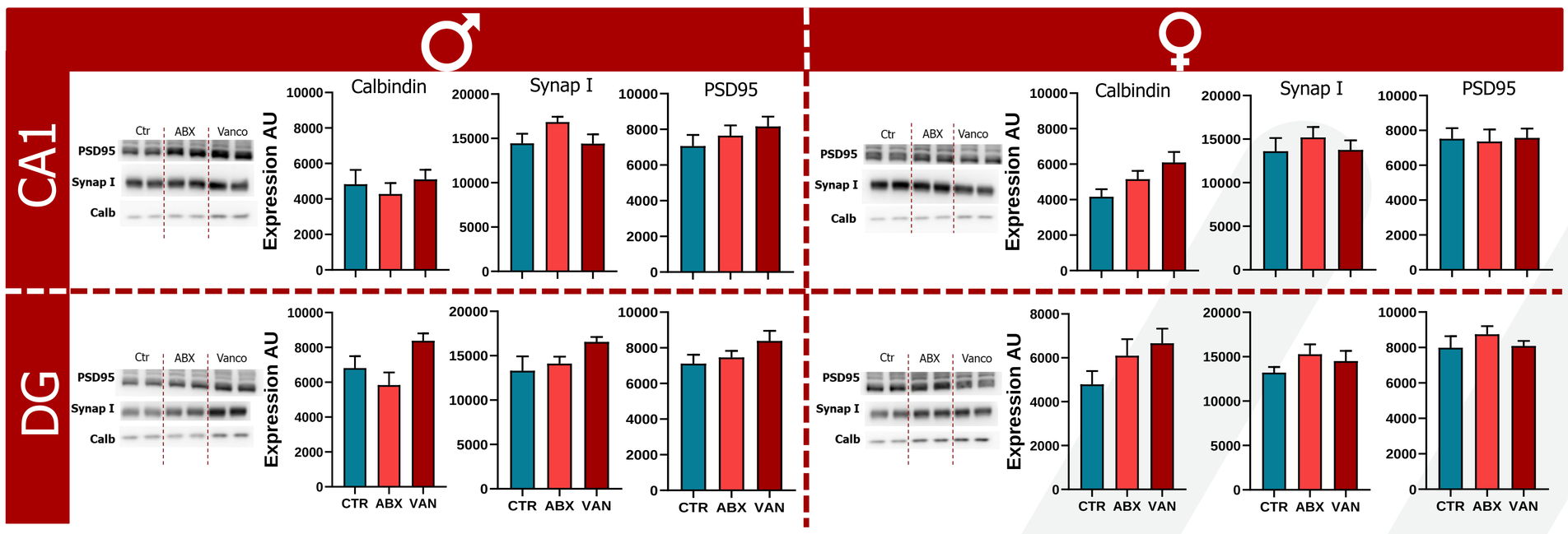
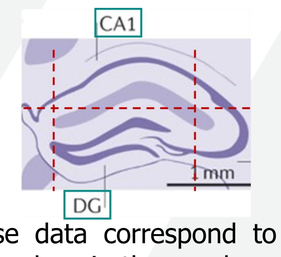


Figure 5. Potential effects of antibiotics on the expression of synaptic proteins in hippocampus of male and females mice. The expression of calbindin, synaptotagmin I, and PSD95 were measured through western blot



Note: These data correspond to preliminary results. The values in the graphs correspond to intensity of each band. 20 μg of total protein was loaded into each lane, the samples were analysed in duplicates.

Conclusions

- Gut microbiota depletion induced anxiety-like behaviour in male rats.
- Stress increased neuronal activation in several brain regions. In contrast, no changes were observed as a result of antibiotic delivery.
- Preliminary data about synaptic protein expression suggest potential sex-dependent effects resulting from a course of 2 weeks of antibiotics delivery.

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