mRNA EXPRESSION PROFILING OF NEURONAL SIGNALING MOLECULES IN MOUSE BRAINS

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/ CONTEXT

Recently, there has been growing evidence that the disruption of the gut microbial community impacts mental health. Several studies have shown that the gut microbiota may regulate brain functions through neuronal, metabolic, endocrine and immune pathways. Yet gut microbiota-brain communication remains poorly understood.

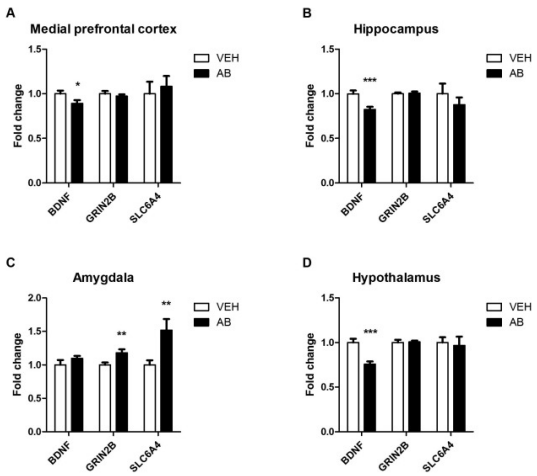
Antibiotic-induced short term disruption (dysbiosis) of the intestinal microbial community is a well documented model to investigate gun microbiota-brain communication. Here, using the Precellys homogenizer, we investigate the mRNA expression of several neuronal signaling molecules in brain tissues

from mice that have been treated with a mix of antibiotics (ampicillin, meropenem, neomycin, bacitracin, vancomycin) by oral gavage for 11 days.

/ MATERIALS AND PROTOCOL

- Brain tissues were microdissected on a cold plate set at -20°C. Working areas and dissection instruments had been previously cleaned to be RNAse free. The dissected brain tissues (medial prefrontal cortex, hypothalamus, amygdala, hippocampus) were stored at -70°C until homogenization.
- Samples were transferred on dry ice. 1mL Qiazol Lysis Reagent was immediately added into the tubes. Then, brain tissues and lysis reagent were pipetted into the Precellys homogenization tubes (Soft tissue homogenizing kit CK14 2mL P000912-LYSK0-A). The Precellys tubes were then placed on normal ice.
- Samples were homogenized with Precellys 24 homogenizer by running the following program twice: 6500rpm, 2x20s, 5s break. Between and after the two runs, tubes were placed on ice for a few minutes. [For a Precellys Evolution, the recommended program is: 8800rpm, 2x20s, 5s break]
- Lysates were pipetted into self-autoclaved Eppendorf tubes then placed on ice.
- RNA extraction was performed using the Rneasy lipid tissue mini kit (Qiagen).
- RT-PCR was performed in the Mastercycler gradient (Eppendorf, Hamburg, Germany) using the cDNA reverse transcription kit (Life Technologies).
- For relative quantification of mRNA levels, qPCR was performed on a LightCycler 480 System.

/ RESULTS



Antibiotic treatment alters the expression of neural signaling-related molecules in a brain region-specific manner.

Mice were treated with antibiotic (AB) mix or vehicle (VEH, control) by gavage for 11 days. The panels show the expression of brain-derived neurotrophic factor (BDNF), N-methyl-d-aspartate receptor subunit 2B (GRIN2B) and serotonin transporter (SLC6A4) mRNA in: the medial prefrontal cortex (A), hippocampus (B), amygdala (C), and hypothalamus (D).

mRNA expression is expressed as fold change relative to VEH-treated mice. Values represent means + SEM, n = 10–12; $^{*}p \leqslant 0.05$, $^{**}p \leqslant 0.01$, $^{***}p \leqslant 0.001$ compared to VEH-treated mice, *t*-test.

/ CUSTOMER

Medical University of Graz

/ CONCLUSION

The Precellys homogenizers coupled to the right set of lysing kits and parameters allow the evaluation of mRNA expression in mouse brain tissues from different regions such as the cortex, the amygdala, the hippocampus and the hypothalamus.

A short-term intragastric treatment with a mixture of antibiotics alters the expression of molecules relevant to neural signaling in a brain-tissue specific way. The cognitive impairment caused by gut dysbiosis results in part from changes in the expression of BDNF, GRIN2B, and the serotonin transporter SLC6A4.

PREC-026-DU144

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