Hippocampal Synaptic Electrophysiology in a Murine Model of Gut-Microbiota Depletion

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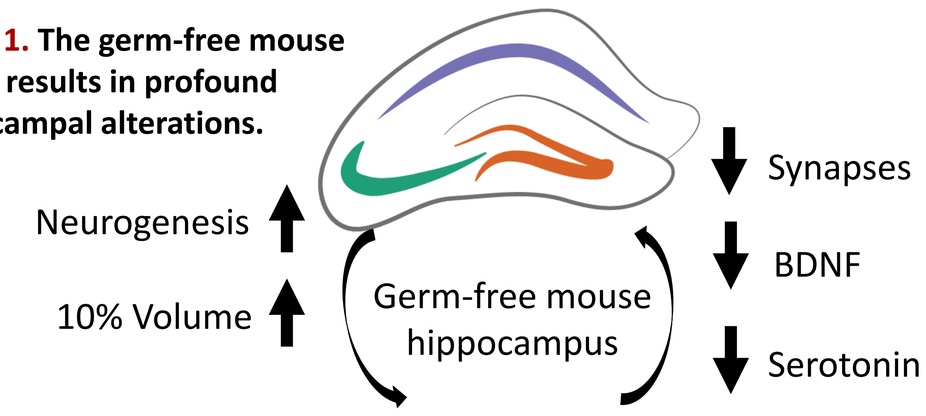
Introduction

Previous literature strongly suggests that neuronal circuits and neuronal activity can be influenced by microbiome factors.

- **Principally, altered behaviours seen with either supplementation or removal of gut microbiome factors (reviewed in Cryan et al., 2019) demands differential brain activity.**
- **Germ free mice exhibit altered synaptic plasticity alongside altered behavioural readouts including learning and memory processes (Darch et al., 2021; Gareau et al., 2011; Hoban et al., 2018; Lu et al., 2018; Luk et al., 2018; Pan et al., 2019).**
- **Morphological differences in germ-free (GF) hippocampus dendrites has been shown (Luczynski et al., 2016).**
- **Antibiotics decrease hippocampal neurogenesis and memory retention in 6-8 week old mice (Möhle et al., 2016).**

Figure 1. The germ-free mouse model results in profound hippocampal alterations.



Currently, it is unknown whether functional activity of the hippocampus is altered by acute microbiome perturbation in adulthood. Therefore, we used antibiotic-treated mice to determine whether the gut microbiome can exert influence on hippocampal synaptic properties in adulthood.

Methods

Multi-electrode array in-vitro hippocampal slice electrophysiology

Transverse hippocampal sections were taken from 3 groups of C57/Bl6 mice aged 10-11wks (**antibiotic cocktail-treated (**ampicillin 1g/L; vancomycin 0.5g/L and imipenem 0.25g/L) **(ABX)** (N female/male = 8/9), **vancomycin-treated (**vancomycin 0.5g/L) **(Vanc)** ((N female/male = 6/9**), vehicle control (CTRL)** (N female/male = 7/13)), as per standard protocols (Papouin and Haydon, 2018).: Antibiotics were delivered in drinking water for 2 weeks. Slices were placed in a multi-electrode array (MEA), stimulating the CA3/CA1 Schaffer collateral pathway. An adjacent electrode, 200um away from the stimulating electrode, recorded field Excitatory Post-Synaptic Potentials (fEPSPs) elicited by the Schaffer collateral axons.

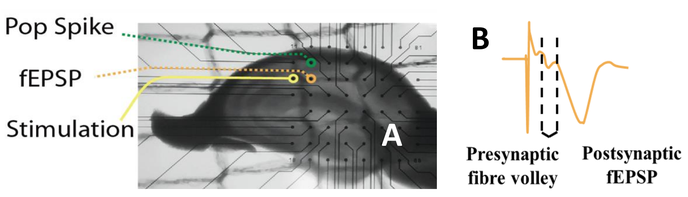


***Figure 2.** (A). Image of slice with recording and stimulation sites marked. (B). Graphic of neuronal population field Excitatory Post Synaptic Potential (fEPSP)*

Stimulation protocols:

Input/Output calibration

Slices were stimulated over a range of stimulus intensities between 1-150uA. Subsequent stimuli were calibrated to elicit fEPSP responses at ~40% of the maximum

Pared-Pulse Facilitation

Slices were given double stimuli with inter-pulse intervals ranging between 25 – 200ms. The percent change of the second pulse-response was computed.

Long-Term Potentiation

Post-Synaptic Long-Term Plasticity

After a minimum of 30 minutes single pulse stimulation with responses not varying more than 10%, a1x theta-burst induction stimulus was delivered. Single-pulse stimulation continued for 2-hours after the induction stimulus. The change in fEPSP or population spike size is then measured

Results

Acute Adult Microbiome Perturbation Leaves E-S Coupling Unchanged

Microbiome perturbation did not change the integration of synaptic excitation to generate neuronal output. F(2, 46) = 0.312, p = 0.733 - General Linear model

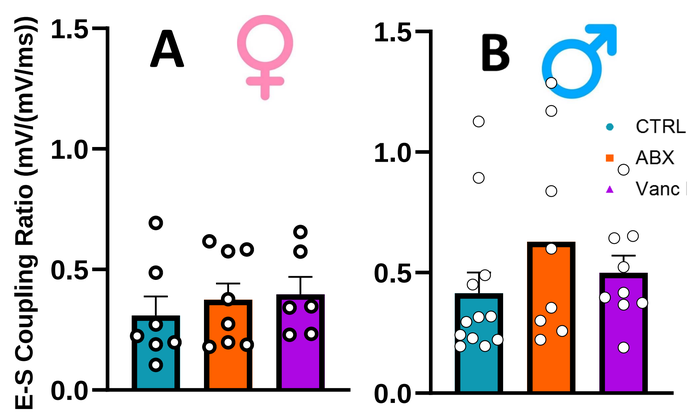


Figure 6. Bar Chart of EPSP-Spike Coupling 2 Hours post-LTP induction calculated as a ratio of Pop. Spike amplitude to fEPSP slopes for ABX, Vanc, and CTRL mice. **(A)** Female groups **(B)** Male Groups. Error bars represent SEM.

Results

Microbiome Depletion in Male Mice May Trend Toward Increase in Basal Synaptic Excitability

Vancomycin-treated male mice trend towards showing increased basal synaptic efficacy. Effect of treatment F(2, 46) = 1.608, p = 0.604 General Linear Model

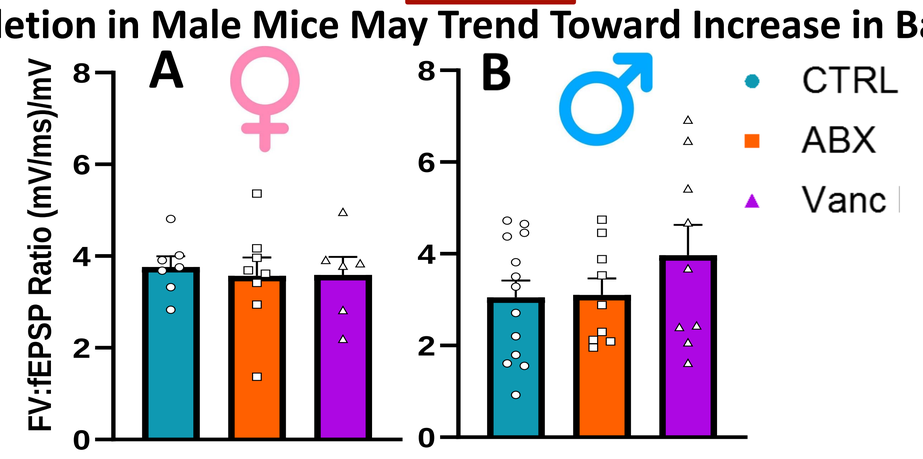


Figure 3. Ratio of evoked fibre volley amplitude to dendritic fEPSP slope is a measures synaptic efficacy in male and female mice. **(A)** Female groups **(B)** Male Groups. Error bars represent SEM

Microbiome Depletion in Male Mice Trends Toward Deficits in Short-Term Facilitation

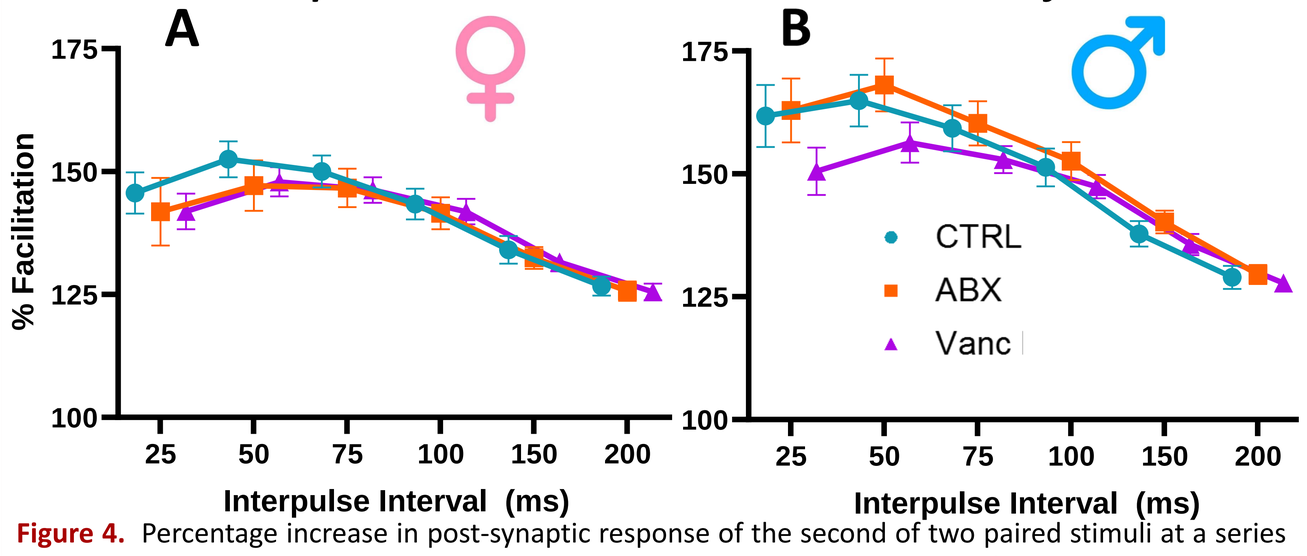


Figure 4. Percentage increase in post-synaptic response of the second of two paired stimuli at a series of interpulse intervals. Antibiotic-treated slices exhibited reduced short-term facilitation at 25ms and 50ms inter-pulse intervals. **(A)** Female groups **(B)** Male Groups. Error bars represent SEM.

Short-term plasticity, a process implicated in working memory, trends towards impairment particularly at pulse intervals of 25ms and 50ms in vancomycin-treated male mice

Effect of treatment F(2, 36) = 1.126, p = 0.335 – Linear Mixed Model with Repeated Measures

Acute Adult Microbiome Perturbation Leaves Long-Term Potentiation Unchanged

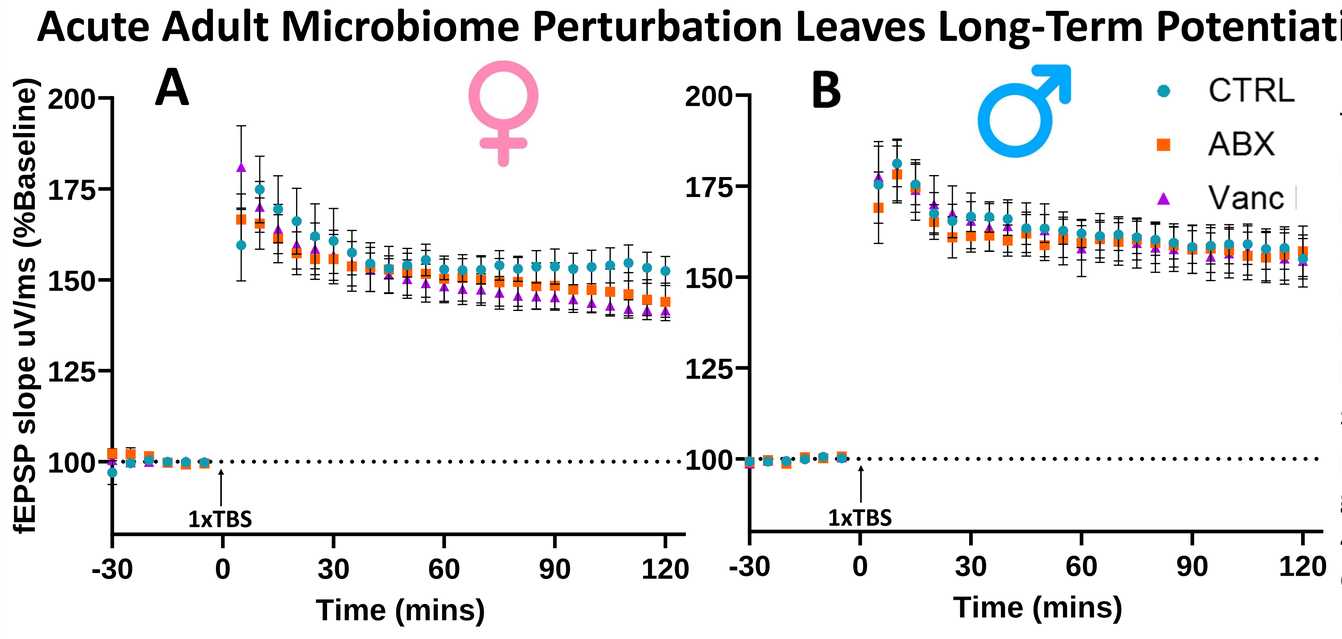


Figure 5. Timeline of CA1 dendritic fEPSP slopes for ABX, Vanc, and CTRL mice. A 30 minute baseline is followed by a potentiating 1x TBS stimulation. Responses are normalised to the average of the 30 minute baseline **(A)** Female groups **(B)** Male Groups. Error bars represent SEM

Theta-burst stimulation illicits long-lasting potentiation (~150-160%) of dendritic response which was measured up to two hours. No statistically significant difference in potentiation between groups was found. F(2, 46) = 0.574, p = 0.567 General Linear Model

Conclusions

Microbiome perturbation through vancomycin administration may have subtle effects on the hippocampal synaptic properties of adult male mice affecting basal synaptic excitability and short-term facilitation, a process associated with working memory.

However, synaptic properties seem to prove robust to a more widescale knockdown of the microbiome through antibiotic cocktail administration in adulthood.

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