Fasting Modulates Synaptic Plasticity in the Dentate Gyrus of Male Rats

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1 INTRODUCTION

- Intermittent metabolic switching (IMS) describes the transition from utilizing one major cellular fuel source to another (between carbohydrates and glucose to fatty acids and ketones). IMS may be associated with cellular and molecular adaptations leading to enhanced synaptic plasticity and neurogenesis, performance in motor function, and resistance to neuronal degeneration (Mattson *et al.* 2018).
- The dentate gyrus is thought to be involved in the formation of new episodic memories and is located in the hippocampus, a structure that plays a pivotal role in neuroplasticity in the adult brain
- The objective of this study was to evaluate the effects of intermittent fasting (IF) on synaptic plasticity, namely, on the levels of long-term potentiation (LTP) in the lateral and medial perforant pathways of the dentate gyrus in the hippocampus.

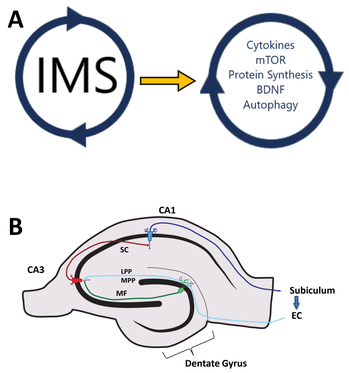
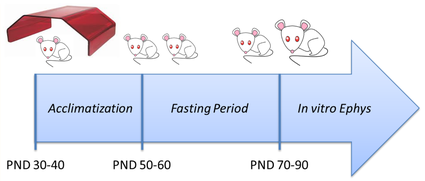
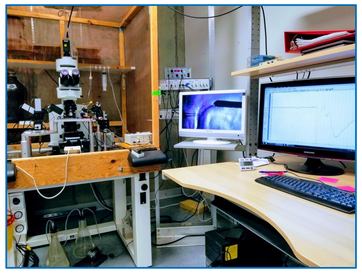


Figure 1 – Intermittent metabolic switching and its connection to the dentate gyrus. (A) Intermittent metabolic switching leads to an alternating cycle between increasing and decreasing levels of cytokines, mTOR, protein synthesis, BDNF, and autophagy which is thought to contribute to enhanced cellular stress resistance, growth, and plasticity pathways (Mattson *et al.* 2018). (B) Simplified illustration of a cross-sectional slice of the hippocampus showing the tri-synaptic circuitry involved in communicating information to and from the Entorhinal cortex (EC). The primary route of communication is via the perforant pathway, with both the medial perforant pathway (MPP) and lateral perforant pathway (LPP), projecting to both the cornu ammonis 3 (CA3) pyramidal neurons, directly, and to the granule cells of the dentate gyrus, in an en passant fashion. These granule cells also project to the CA3 pyramidal neurons via their axons, the mossy fibers (MF). The CA3 neurons project to the cornu ammonis 1 (CA1) pyramidal neurons via their schaffer collateral (SC) axons. Lastly, the CA1 pyramidal neurons project efferently from the hippocampus to the subiculum, which projects to the EC.

2 EXPERIMENTAL PROCEDURES

Intermittent Fasting Protocol Paradigm

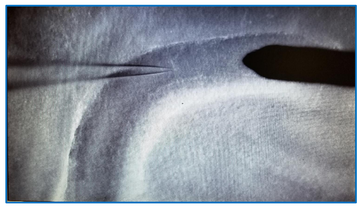




- Adult male Sprague Dawley rats, 30-40 days old, were randomly assigned to either a control or food restriction (FR) group
- Control group had access to food *ad lib;* FR group had access to food in a 2 hour window every 24 hours for 3 weeks

In vitro Electrophysiology

- Transverse hippocampal sections (400µM) in regular artificial cerebrospinal fluid (aCSF)
- field excitatory postsynaptic potentials (fEPSP) were measured using a stimulating electrode placed in the medial or lateral perforant paths and a recording electrode placed within 200 µm of the recording electrode.
- Slices with stable baselines were exposed to a GABA antagonist—bicuculine methiodide (20µM; 15 min), to reduce inhibition and facilitate LTP induction.
- High-frequency stimulation (HFS) of 4 trains of 50 pulses at 100 Hz, 30 seconds apart was used to induce PTP/LTP



Recording paradigm

Pre-conditioning (baseline) (20 min) → High-Frequency Stimulation (4x50 @100Hz) → Post-Conditioning (Decay) (60 min)

3 RESULTS

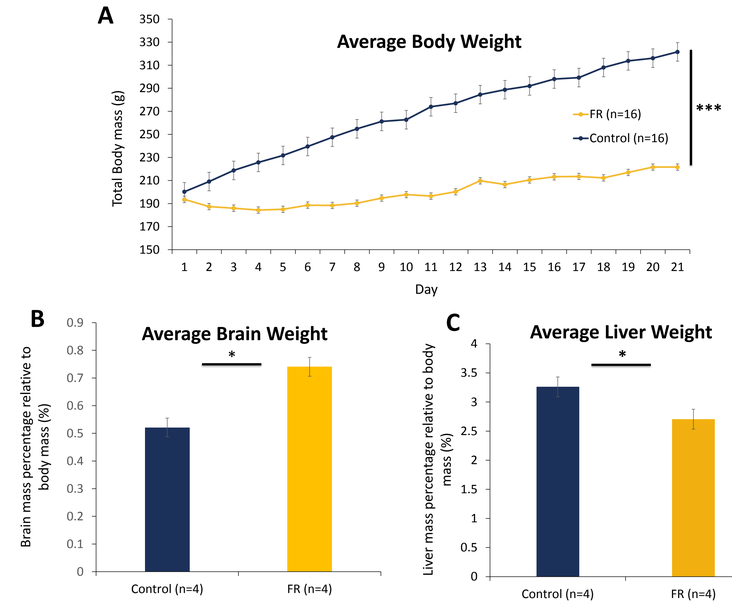


Figure 2. Body mass, brain, and liver weight of FR and Control rats after 3-week fasting protocol. (A) Comparison of average total body mass between control and food restricted male Sprague Dawley rats over a 3-week fasting protocol (n=16 total rats; 8 control, 8 FR). ***p<0.001 (Student's t-test). (B) Comparison of average brain weights between FR and control animals. Measurements were recorded by weighing isolated brains and converting it to a percentage of animal's total body mass (n=8 total rats; 4 control, 4 FR). *p<0.05 (Student's t-test). (C) Comparison of average liver weights. Isolated livers were weighed and converted to a percentage of animal's total body mass (n=8 total rats; 4 control, 4 FR). *p<0.05 (Student's t-test).

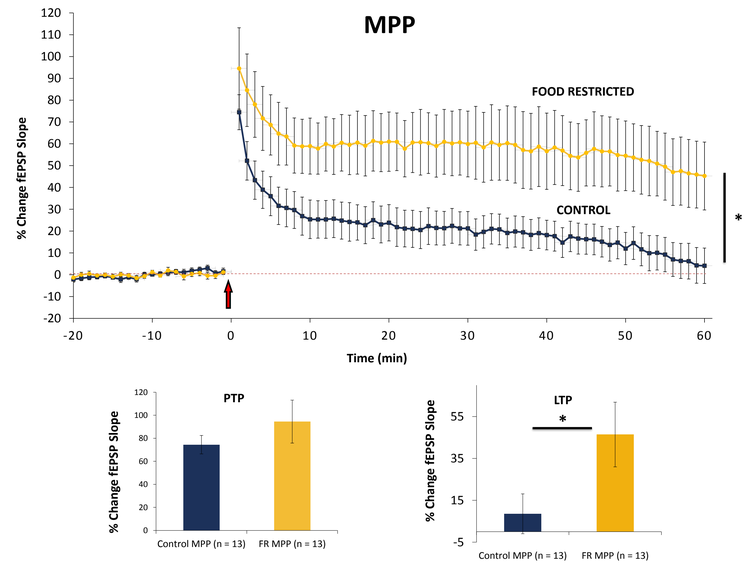


Figure 3. Comparison of post-tetanic potentiation (PTP) and long-term potentiation (LTP) in the medial perforant path between control and food restricted (FR) rats. Red arrow indicates end of pre-conditioning baseline and start of HFS (4x50 @ 100 Hz). PTP was measured as first minute of post-condition decay. Between control and FR groups, no significant difference in the percent change in fEPSP slope was observed between control and FR groups, with 74.5 ± 8.01% and 94.5 ± 18.7% respectively. LTP was measured as average of percent change in fEPSP slope between minutes 55-60 of post-condition decay. A significantly higher magnitude of LTP was observed in FR rats with an average of 46.4 ± 15.44% change in fEPSP slope versus the average of 5.6 ± 8.0 % in control rats (n=26 slices *p<0.05 (Student's t-test).

4 RESULTS CONTINUED

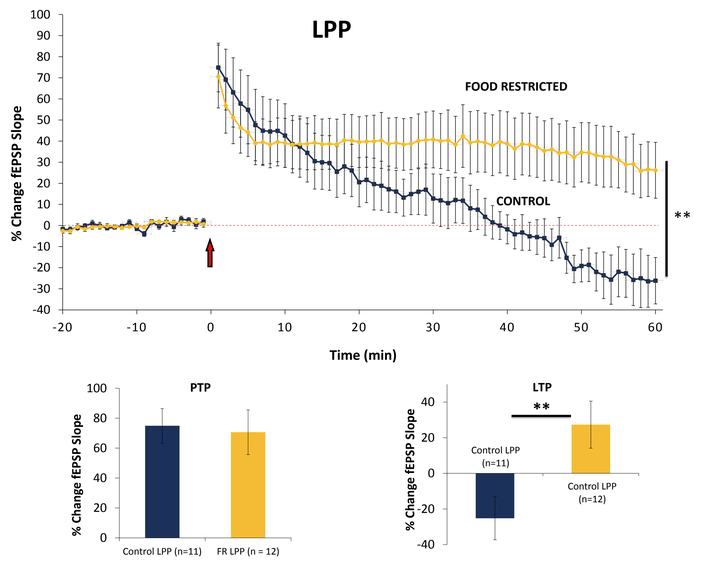


Figure 4. Comparison of post-tetanic potentiation (PTP) and long-term potentiation (LTP) in the lateral perforant path between control and food restricted (FR) rats. Red arrow indicates end of pre-conditioning baseline and start of HFS (4x50 @ 100 Hz). PTP was measured as first minute of post-condition decay. Between control and FR groups, no significant difference in the percent change in fEPSP slope was observed between control and FR groups, with 74.9 ± 11.5% and 70.62 ± 14.89% respectively. LTP was measured as average of percent change in fEPSP slope between minutes 55-60 of post-condition decay. A significantly higher magnitude of LTP was observed in FR rats with an average of 27.35 ± 13.2% change in fEPSP slope versus the average of -25.2 ± 12.1 % in control rats. **p<0.01 (Student's t-test).

5 CONCLUSIONS

Conclusions

- No significant differences in PTP between control and FR rats were observed in the MPP and LPP. A significantly higher level of LTP was found in FR rats when compared to controls in both the MPP and LPP, perhaps suggesting that food restriction may lower the threshold for LTP induction.
- When taken as a percentage of total body mass, FR rats had livers that accounted for a smaller percentage but had brains that accounted for a higher percentage.

Future Considerations

- Analyze blood and liver samples to assess levels of blood glucose, ketones, and glycogen.
- Immunoblots to analyze levels of different receptors and BDNF. Staining to observe dendritic spine density and morphology.
- Behavioural tests to assess memory, problem solving, and exploring abilities.

6 ACKNOWLEDGEMENTS

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