Do Pair-Fed diets have Sex-Specific Influences on Bi-Directional Plasticity in the Medial Perforant Path of the Dentate Gyrus?

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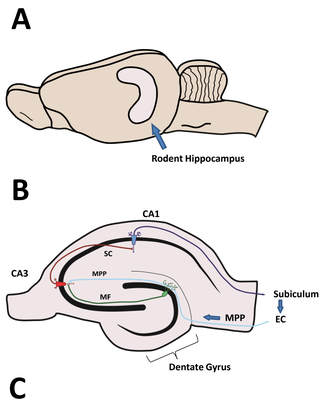
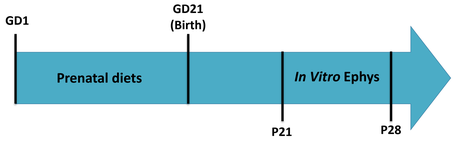
1. Introduction



- Ethanol is a teratogen that can negatively impact gestational development, resulting in fetal alcohol spectrum disorders (FASD) (Fontaine et al., 2016).
- We can study deficits of synaptic plasticity in the hippocampus, caused by prenatal ethanol exposure (PAE), using animal modelling.
- Liquid diets are considered an appropriate method of ethanol administration as they mimic human consumption (Gil-Mohapel et al., 2010).
- Pair-fed diets are often used as an isovolumetric/isocalometric control for the potential confound of caloric consumption deficits seen in Ethanol conditions, which may be causing additional deficits in plasticity in the hippocampus (Patten et al., 2014).
- Though the usual premise of pair-fed experiments is to compare differences in long-term potentiation (LTP) and long term depression (LTD) to control and ethanol conditions, little research has been conducted looking at sex-specific differences within the pair-fed condition itself, especially within the dentate gyrus (DG).
- Here, we examine differences in LTP, LTD, post-tetanic potentiation (PTP), and short-term depression (STD) within male and female pair-fed rats to see if sex plays a role in how caloric restriction, as a result of pair-fed diets, may affect synaptic plasticity within the medial perforant path (MPP) of the DG.

Figure 1 – Schematic depiction of hippocampal structure and function from a tissue to synaptic level. (A) Simplified sagittal illustration of the rodent brain, showing the relevant positioning of one hippocampi relative to the rest of the brain. (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 the medial perforant pathway (MPP) illustrated here, 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. (C) Basic illustration of the cellular process behind long-term potentiation (LTP) and long-term depression (LTD) at the synaptic level (Fontaine et al., 2016). In comparison with baseline neurotransmission, LTP elicits stronger excitatory postsynaptic potentials (EPSP) while LTD elicits weaker EPSPs. LTP and LTD is also commonly associated with the recycling of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors to and from the post-synaptic membrane surface as a function calcium concentrations fluxed by N-methyl-D-aspartate (NMDA) receptors in DG and CA1 neurons. Pre-natal ethanol exposure (PAE) is thought to affect LTP and LTD by acting as a NMDAR antagonist at these synapses.

2. Materials and Methods



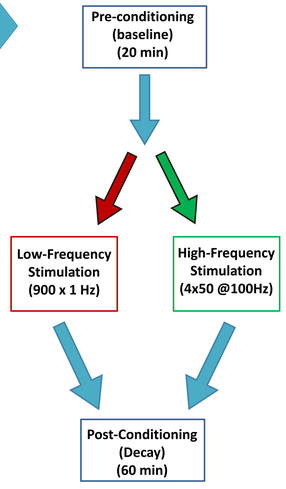
Pair-fed Control paradigm

- Pair-fed animals' diets are isovolumetrically/isocalometrically matched to a previous Ethanol condition mother's diet consumption.
- Pair-fed animals are provided with a Weinberg/Keiver high protein liquid dietcontrol (no. 710109), similar to that of the ethanol diet except with a maltose-dextrin carbohydrate substitute for the ethanol (Weinberg, 1985).

***In Vitro* Electrophysiology**

- Transverse hippocampal sections (400μM) are cut.
- Using current clamp electrophysiology, field excitatory postsynaptic potentials (fEPSP) are measured with a stimulating electrode placed in the medial perforant path and a recording electrode placed in the proximal path of the dendritic arbor of the granule cells in the DG.
- High-frequency stimulation (HFS) and Low-frequency stimulation (LFS) protocols are run to measure properties of PTP/LTP and STD/LTD, respectively.
- 10μM of Bicuculine, a GABA antagonist, is used in LTP recordings during pre-conditioning and high-frequency stimulation.

Recording paradigm



3. Results

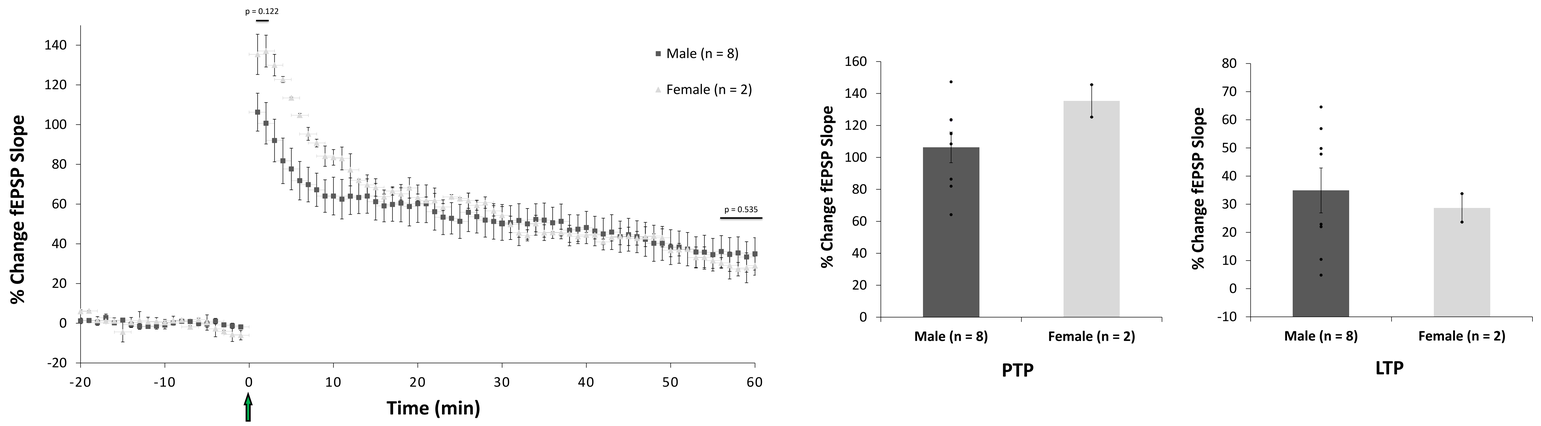


Figure 2 – Sex-specific results of post-tetanic potentiation (PTP) and long-term potentiation (LTP) of pair-fed rats (P21-28) following high-frequency stimulation of the medial perforant path of the dentate gyrus within the hippocampus. Seen in the left figure, following stable pre-conditioning baseline (-20-0 min) and HFS (4x50 @ 100Hz) stimulation, indicated by the green arrow at time 0, PTP, within the first minute of post-conditioning, was noted to have a 106 ± 9.55 % change in fEPSP slope relative to pre-conditioning, for males (n = 8), while PTP was noted to have a 135 ± 10.1 % change for females (n = 2). A 34.9 ± 7.98 % change was found for LTP within the males, while a 28.7 ± 5.05 % change was noted for female LTP. Individual points in the left figure represent the average % change from initial pre-conditioning fEPSP slope for each minute and error bars represent the standard error of the mean. Sex-specific differences in PTP and LTP were not found to be statistically different at α = 0.05 with p = 0.122 and p = 0.535, respectively. The middle and right figures compare PTP and LTP in males and females, respectively. The individual points represent the magnitude of % change from the initial pre-conditioning fEPSP slope for the first minute of post-conditioning (0-1 min) of each slice for the PTP figure, and the last five minutes (55-60 min) of post-conditioning % change from the initial pre-conditioning fEPSP slope of each slice for the LTP figure. Error bars represent the standard error of the mean.

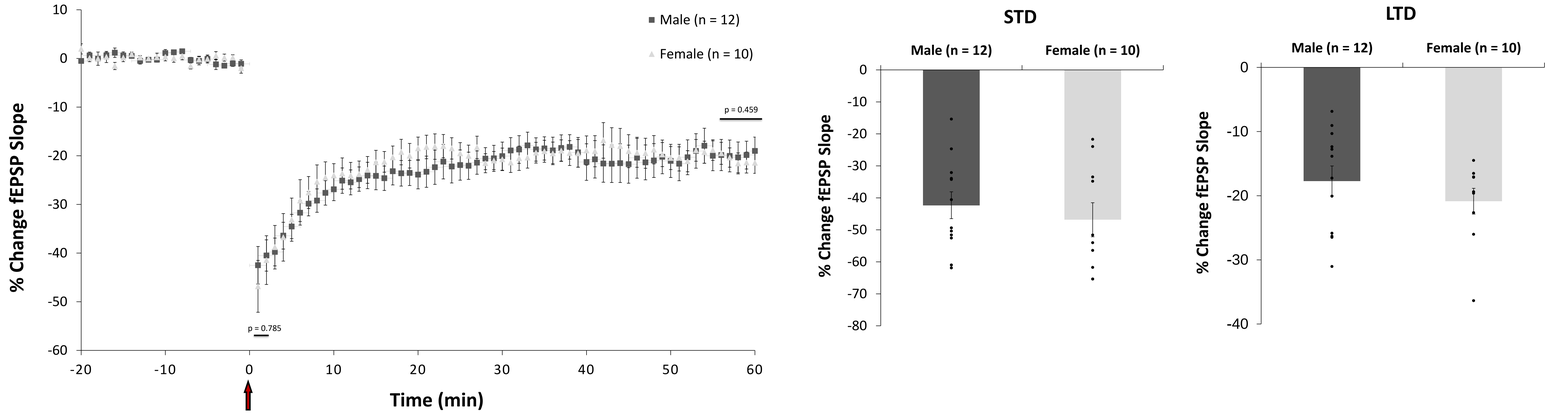


Figure 2 – Sex-specific results of short-term depression (STD) and long-term depression (LTD) of pair-fed rats (P21-28) following low-frequency stimulation of the medial perforant path of the dentate gyrus within the hippocampus. Seen in the left figure, following stable pre-conditioning baseline (-20-0 min) and LFS (900 x 1Hz) stimulation, indicated by the red arrow at time 0, STD ,within the first minute of post-conditioning, was noted to have a -42.3 ± 4.19 % change in fEPSP slope relative to pre-conditioning, for males (n = 12), while STD was noted to have a -46.8 ± 5.32 % change for females (n = 10). A -17.7 ± 2.33 % change was noted for LTD within the males, while a -20.86 ± 2.01 % change was noted for female LTD. Individual points in the left figure represent the average % change from initial pre-conditioning fEPSP slope for each minute and error bars represent the standard error of the mean. Sex-specific differences in STD and LTD were not found to be statistically different at α = 0.05 with p = 0.785 and p = 0.459, respectively. The middle and right figures compare STD and LTD in males and females, respectively. The individual points represent the magnitude of % change from the initial pre-conditioning fEPSP slope for the first minute of post-conditioning (0-1 min) of each slice for the STD figure, and the last five minutes (55-60 min) of post-conditioning % change from the initial pre-conditioning fEPSP slope for the LTD figure. Error bars represent the standard error of the mean.

4. Conclusions and Considerations

Conclusions

- Pair-fed diets do not elicit sex-specific differences in PTP, LTP, STD, and LTD.
- This differs from previous findings in our research which have found sex-specific differences of LTP and LTD in pre-natal alcohol exposed (PAE) rats.

Future Considerations

- More data will need to be collected for the pair-fed female HFS paradigm to make stronger conclusions on sex-specific differences in LTP.
- Determine reason for why sex-specific differences in plasticity are seen in PAE animals but not Pair-fed animals.

5. References

Fontaine, C.J., Patten, A.R., Sickmann, H.M., Helfer, J.L., and Christie, B.R. 2016. Effects of pre-natal alcohol exposure on hippocampal synaptic plasticity: Sex, age and methodological considerations. Neurosci. Biobehav. Rev. 64, 12-34. doi: 10.1016/j.neubiorev.2016.02.014.
Gil-Mohapel, J., Boehme, F., Kainer, L., and Christie, B.R. 2010. Hippocampal cell loss and neurogenesis after fetal alcohol exposure: Insights from different rodent models. Brain Research Reviews 64, 283-303. doi: 10.1016/j.brainresrev.2010.04.011.
Patten, A. R., Fontaine, C. J., & Christie, B. R. 2014. A comparison of the different animal models of fetal alcohol spectrum disorders and their use in studying complex behaviors. Frontiers in Pediatrics. 2, 93. http://doi.org/10.3389/fped.2014.00093
Weinberg J. 1985. Effects of ethanol and maternal nutritional status on fetal development. Alcohol. Clin. Exp. Res. 9, 49–55.

6. Acknowledgments

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