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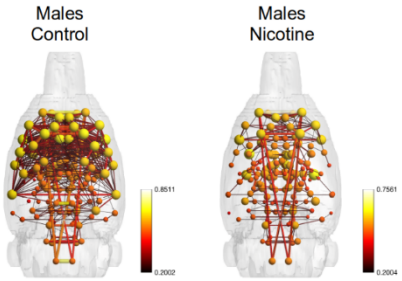
The role the gut-microbiome-brain axis in cardiovascular disease following prenatal exposure to nicotine

Briefly, in Aim 3 of the FLDOH-funded project we proposed to determine whether prenatal nicotine exposure (PNE)-associated changes in gene expression **can be reversed** by fecal matter transfer (FMT). We hypothesized that unfavorable physiological outcomes caused by PNE such as gut dysbiosis, epigenetic modifications in HPA axis neuropeptides linked to obesity, and an increased cardiovascular response to ANGII (high blood pressure and heart rate) are reversible by FMT from healthy offspring. This aim of the study is important, as (to the best of our knowledge) whether FMT from healthy offspring can reverse or attenuate adverse obesity and cardiovascular related effects associated with PNE has never been explored.

To this end, the females will be bred at 8 weeks of age and undergo nicotine or vehicle exposure during pregnancy. At 7 weeks of age animals from different PNE litters and all vehicle treated animals will be euthanatized and the cecal content will be collected. These animals will serve as FMT donors. At 10 weeks of age, all animals will be placed on a cocktail of antibiotics via gavage to allow full gut microbiota depletion for 3 days. Following two days of recovery, all rats will receive FMT via oral gavage from either vehicle or PNE donors. Fecal microbiota will be tested prior to FMT and at several time points post-FMT to ascertain the success and longevity of the FMT procedure. Alternatively, PNE offspring could be cross-fostered by unexposed dames and used for the same experiments. At 11 weeks of age all telemetry-instrumented rats will be assigned to receive either ICV vehicle, acetate or leptin challenge for the next 7 days throughout the induction period. Body weight, food intake and fecal content will be monitored once a week. 24-hour blood pressure, heart rate and activity will be continuously monitored in telemetry rats. Following testing all animals will be euthanized and selected tissues (brain, cecum, pituitary, adrenal, and white adipose tissue), plasma, and cecal contents will be collected for analysis.

Given the timeline, The Florida Veterinary Scholar will be participating in the last stage of the project, analyzing gene expression in the selected tissues, comparing control, PNE offspring and FMT offspring. We have already determined a number of candidate genes in the offspring cecal tissue (e.g., *Igf2r, Hsd2*, *Tph2*, *Enac*, *Ocln*), plasma (*Leptin*, *IGF2*), and brain (e.g., *Igf2r*, *Ffar2*, *Ffar3*, *Dnajc6*, *Gabrb3*, *Ht2rc* and *Hapin4*).

In this respect the FVSP project will consist of 2 aims:



1. Analyzing expression of already identified candidate genes in brain and cecal tissue that are affected in the PNE offspring and evaluating these genes in the FMT (reversed) offspring.
2. Discovering novel candidate genes in the pituitary, adrenal and white adipose tissue that are affected in the PNE offspring and evaluating these genes in the FMT (reversed) offspring. Specifically, our results demonstrate a dramatic reduction in brain connectivity in PNE male offspring compared to controls (**Fig**). In this respect, brain-connectivity genes that have been recently described by Zador laboratory ("*BRICseq Bridges Interregional Connectivity to Neural Activity and Gene Expression in Single Animals*", Huang et al., 2020, Cell 182, 177–188) will be explored.

Briefly, RNA will be isolated and purified; cDNA will be generated and gene expression will be queried by qRT-PCR and differential expression will be calculated using the ΔΔCt method. Differences in gene expression will be analyzed using Student's t-test on 5 or more independent samples.