DEBI FADOOL LABORATORY

Dr. Debra Ann Fadool, Dr. Douglas Storace, Louis Kolling, Daniel Landi Conde, Ashley Loeven, Meizhu Qi, Destinee Gatlin, Ryan Ochoa, Alexis Cox, Tyla Dolezel, Camilla May, Claudia Silver, and Nicholas Maykut

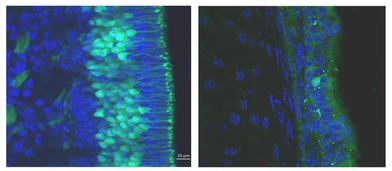
program in NEUROSCIENCE

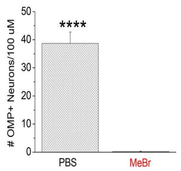
FLORIDA STATE UNIVERSITY

Introduction

We are researching learning, memory, and neural plasticity at the level of the ion channel protein. In order to do this, we combine skills in electrophysiology with those of protein biochemistry (phosphorylation assays; protein-protein interactions), molecular biology (creating mutant ion channels and signaling proteins), and molecular genetics (genetically targeted "knock-out" mice to study cell signaling by loss of function). Our mainstay in the laboratory is biophysics, specifically a technique called patch-clamp electrophysiology, where we can measure single conformational changes in ion channel proteins that elicit electrical signals, essentially the language of the brain. We have discovered that hormones and neurotrophins (glucagon-like peptide 1 (glp-1), insulin, and brain-derived neurotrophic factor (BDNF)) modulate electrical activity in the brain at the level of the ion channel via phosphorylation. These types of signaling molecules are also involved in maintaining energy homeostasis. **Currently, we hypothesize that the olfactory system not only senses our external chemical environment (odors) but is a detector of internal chemistry, the chemistry of metabolism.** As such, we are studying the neuropathology of diabetes and obesity - how sensory systems, such as the olfactory system are perturbed by diet-induced obesity at the level of the ion channel.

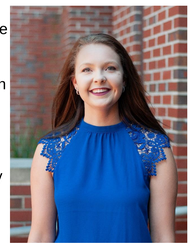
Systemic Metabolic Changes as a Result of Ablating Olfactory Sensory Neurons in the Main Olfactory Epithelium and the Bulb





Chemosensory cues help animals detect changes in their environments to assist with reproduction and survival. Previously, our lab discovered that diet-induced obesity causes an inadequate ability to detect chemosensory signals due to a malfunctioning in olfactory sensors and overall loss of olfactory sensory neurons.

However, the link between the subsequent loss of olfactory sensory neurons and metabolism has yet to be understood. My project evaluates metabolic effects of olfactory sensory neuron ablation in the main olfactory epithelium using methimazole, unilateral naris occlusion and genome editing. Comprehensive Lab Monitoring System (CLAMS$_{TM}$) chambers and behavioral assays such as the buried cookie assay were used to facilitate data collection. Our lab has also previously discovered that excess nutrition leads to a decrease in total energy expenditure. Thus, we hypothesize that the manipulation of olfactory sensory neurons might result in similar decreases in energy expenditure.

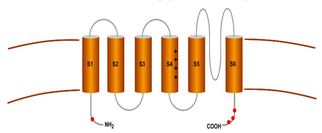


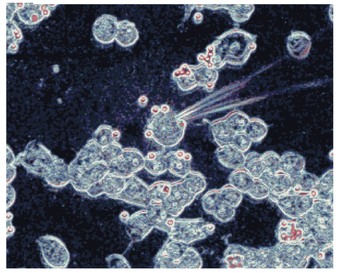
The Effects of Cannabinoid Agonists and Antagonists on a Mouse Model of Anxiety

Cannabidiol (CBD) is a non-psychoactive ingredient of cannabis that has demonstrated changes in anxiety, chronic pain, sleep, and prevention of substance abuse in adult mouse and human subjects. Hemp and hemp metabolites have developed high commercial value in the state of Florida. Our project investigates the therapeutic potential of CBD and WIN 55, 212-2-mesylate for treatment of anxiety and ADHD. We use a newly found mouse model of anxiety and attention deficit (Kv1.3-/- mice) to investigate possible reversal of these behaviors using acute and chronic treatment with the above antagonist and agonist, respectively, of the cannabinoid receptors (CB1/CB2). Use of the Kv1.3-/- mouse is advantageous because it has trait anxiety, therefore we can study chronic drug therapy using behavioral tests on three stages of development: fetal, young adult, and aged mice. We can pair these behavioral studies with metabolic assessment, (body weight, energy consumption, food ingestion) and brain recordings to determine the compound's effect on neuronal excitability.

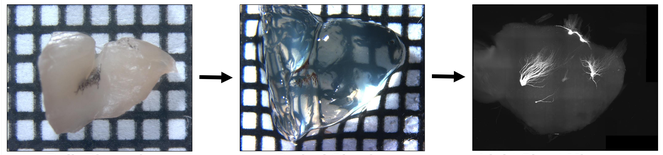
Kv1.3 Modulation by GLP-1

Glucagon-like peptide 1 (GLP-1) is a hormone that it reduces blood glucose levels released by intestinal cells following a meal. This hormone, which exerts strong effects on the control of metabolism, is also found locally in the CNS, and the receptor for it can be located in mitral cells of the olfactory bulb. We know that activation of GLP-1 signaling reduces the activity of Kv1.3 ion channels in these cells. Studying the intricacies of native ion channels coming straight from a mouse's brain can be tricky. For this I use an alternative approach: taking cultured human embryonic kidney (HEK293) cells and inserting in them the necessary DNA to express Kv1.3 ion channels and the receptor for GLP-1. This allows me to simplify the system and examine with detail the interaction between these molecules using patch-clamp electrophysiology. I want to examine how down-stream signaling of PKA messenger pathways may be phosphorylating the ion channel due to GLP-1 activation. For this I am using site-directed mutagenesis of amino acids predicted to be a substrate in this biochemical interaction.

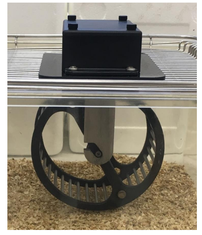




The Effects of Diet and Exercise on the Olfactory System



Our lab has shown that mice fed a fatty diet lose a significant number of olfactory sensory neurons in the nose that are responsible for detecting odors). I am seeking to uncover whether the detrimental effect of fatty diet on olfaction in mice can be prevented or reversed via modulation of diet/feeding or by allowing the mice to participate in voluntary wheel running exercise. Or more generally, how do these two metabolic factors alter olfactory structures and function.



Fatty Diet-induced Neuroinflammation

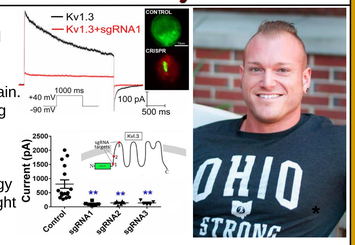
Our research has shown that mice fed a moderately high-fat diet exhibit a loss of olfactory sensory neurons. I have found that mice that are isocalorically matched on a diet that prevents obesity but contains 32% fat, still exhibit neuronal loss in the olfactory epithelium. This narrowed down my research focus from diet-induced obesity to dietary fat. The molecular process behind this loss is unknown, and the irreversible nature of the neuronal loss is interesting to me. It is known that dietary fat leads to whole-body inflammation and gut microbiome changes. I suspect that the gut microbiome shift induces systemic inflammation, which leads to an inflammatory state of the olfactory system and that this is responsible for the imbalance in neuroregeneration and cell death. I am exploring the connectivity of dietary fat, the gut microbiome, circulating inflammatory factors, neuroinflammation, and olfactory sensory neuronal loss.

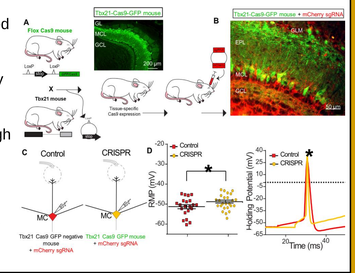
Composition*	Control diet	Moderately high-fat diet	High-fat diet
Carbohydrates	57	51	20
Protein	30	17	20
Fat	13	32	60

Thiebaud, N., Johnson, M. C., Butler, J. L., Bell, G. A., Ferguson, K. L., Fadool, A. R., et al. (2014). Hyperlipidemic diet causes loss of olfactory sensory neurons, reduces olfactory discrimination, and disrupts odor-reversal learning. *Journal of Neuroscience* 34, 6970–6984. doi:10.1523/JNEUROSCI.3366-13.2014.

The Role of the Olfactory Bulb in Metabolism as Revealed by CRISPR

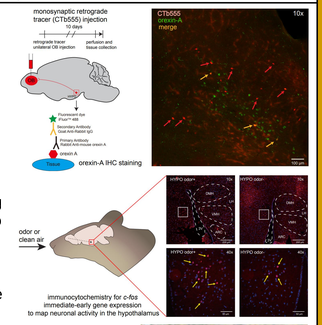
We are exploring how energy homeostasis can be maintained by the olfactory system as a means to prevent diet-induced obesity and unwanted weight gain. I am using CRISPR gene editing and chemogenetics (called DREADDs) to up- and down-regulate output neurons of the olfactory bulb to modulate energy expenditure, adiposity, and weight gain when mice are challenged with a fatty diet. I explicitly used CRISPR to generate a conditional knockout of the potassium channel, Kv1.3, because these proteins regulate neuronal excitability and the resting membrane potential. I developed a technique to retro-orbitally deliver my designed CRISPR sgRNAs in AAV9 viral particles into genetically-engineered mice that express Cas9 in only mitral/tufted cells of the olfactory bulb. I used methodologies such as slice electrophysiology, odor habituation/dishabituation behavior assays, protein biochemistry, serum analysis, histology, and systems physiology (metabolic chambers) to characterize my CRISPR mice that demonstrated improved olfactory & health metrics compared to control littermates. I discovered that my engineered DREADDs mice oppositely demonstrated an obesogenic phenotype. Through my research, I have uniquely discovered that output neurons of the olfactory bulb communicate olfactory coding information and simultaneously participate in metabolic regulation.





The lateral hypothalamic projections to the olfactory bulb constitute an anatomically distinct subpopulation of orexin neurons

Recent work has shown that inducing an imbalanced metabolism in mice via the consumption of a high-fat diet can drive functional and structural changes in the olfactory bulb (OB). I wanted to understand the underlying mechanism and judged that brain areas known to be involved in homeostatic regulation could be directly projecting to the OB. Therefore, I have been injecting monosynaptic retrograde tracers into the bulb to discover their hypothalamic connections. The connections I have discovered are part of the signaling pathways for the hormone, orexin.



I am currently using immunocytochemistry approaches to explore this anatomically distinct subpopulation to uncover how this signaling pathway might be driving metabolic modulation of the olfactory system during feeding or changed metabolic states. I am also using a technique called immediate-early gene (IEG) activation to explore how odor activation of mice under varying nutritional states can change hypothalamic c-fos labeling to give us clues as to networked areas of regulation to the olfactory bulb. I work in two laboratories – jointly with Drs. Storace and Fadool.

Recently Published

1. Role of olfaction for eating behavior. **Kolling LJ and Fadool DA.** *The Senses: A Comprehensive Reference. Elsevier, Netherlands*, ed. Bernd Fritzsch and Wolfgang Meyerhoff. (2020)
2. Olfactory bulb-targeted quantum dot (QD) bioconjugate and Kv1.3 blocking peptide improve metabolic health in obese male mice. **Schwartz AB…Huang Z…Fardone E**, King GF, Mattoussi H, and **Fadool DA**. *J Neurochemistry* 157(6):1876-1896 (2021).
3. A unique olfactory bulb microcircuit driven by neurons expressing the precursor to glucagon-like peptide 1. **Thiebaud N**, Gribble F, Reimann F, Trapp S, **Fadool DA**. *Scientific Reports* 2019. 9(1):15542. (2019).
4. Consumption of dietary fat causes loss of olfactory sensory neurons and associated circuitry that is not mitigated by voluntary exercise in mice. **Chelette BM, Loeven AM, Gatlin DN, Landi Conde DR, Huffstetler CM, Qi M, Fadool DA.** *Journal of Physiology* epub ahead of press. (2021).
5. Modulation of neural microcircuits that control complex dynamics in olfactory networks. **Huang Z, Tatti R, Loeven AM, Landi Conde DR, Fadool DA.** *Frontiers Cellular Neuroscience* 15:662184. (2021).

Funding & Following

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