**Optimization of THC-Ensure™ Gelatin Formulation for Cannabinoid Administration**

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Δ⁹-tetrahydrocannabinol (THC) is the primary psychoactive compound found in *Cannabis sativa*. In mice, intraperitoneal (i.p.) injections of THC, produce a characteristic triad of behavioral responses: hypolocomotion, hypothermia, and analgesia. However, injections of THC do not accurately represent how humans typically administer THC, which primarily consists of inhalation and oral consumption. Thus, we have developed and optimized a paradigm of oral THC consumption in mice to better model a typical route of administration used by humans. Our model balances an acute consummatory window with a highly palatable, chocolate-flavored gelatin. This incentivizes mice to voluntarily consume enough THC to produce measurable cannabimimetic behaviors. Over a 3-day exposure paradigm we habituated mice to the gelatin where they had *ad libitum* access for 2 hours each day. We introduced THC into the gelatin and measured the triad of behaviors immediately following consumption to determine whether voluntary oral consumption induces the acute cannabimimetic behaviors. We found significant hypolocomotion, hypothermia, and analgesia at our highest concentration. Next, to determine whether these behaviors are caused by THC’s action at the primary endocannabinoid receptor, CB₁R, we treated mice with the inverse agonist SR1 prior to the behavioral tests. SR1 blocked the cannabimimetic behaviors induced by the consumption of THC-gelatin, suggesting the effects are CB₁R-dependent. To finalize this model, we have adapted our oral consumption paradigm to an acoustic startle behavioral model. Following our consumption paradigm, mice are subjected to tones of varying decibels and their startle response is measured. Moving forward we will continue acoustic startle testing to confirm preliminary data and expand the doses tested. Overall, these data verify that our model effectively induces cannabimimetic behaviors and can be used for future behavioral studies investigating a more translational route of administration compared to i.p.

**A Role for Neuropeptide S in the Modulation of Reward**

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Substance use disorders are shockingly prevalent in the United States, with the Centers for Disease Control and Prevention estimating that in 2019 alone, nearly 50,000 people died from opioid-involved overdoses. Neuropeptide S and its receptor have been previously implicated in drug-seeking behavior, making it an important component in understanding the biological functions underlying addiction. However, such findings have not been localized to any specific region. We set out to investigate the connection between the locus coeruleus, a region with a known population of neuropeptide S producing cells, and the orbitofrontal cortex, a region known to express neuropeptide S receptors. By utilizing an NPSR1-cre mouse line and cre-dependent viral expression, we introduced GCaMP, a fluorescent calcium sensor, into NPSR1 expressing neurons in the orbitofrontal cortex. This enabled us to record calcium fluorescence in vivo as a proxy for neuronal activity. This technique was paired with various behavioral paradigms to explore the endogenous activity of these neurons in natural reward-seeking, social interaction, and fear conditioning. Our results demonstrate that these neurons are activated during cue and food reward-delivery, various social rewards, and foot shock. Aligning these findings with previous research that has demonstrated neuropeptide S’s involvement in drug reward-seeking behavior, we believe these neuropeptide S receptor-expressing neurons in the orbitofrontal...
cortex could be implicated in drug-seeking behaviors. These findings contribute to the understanding of the neural circuitry involved in substance use disorders, which is integral in continuing the development of treatment options for patients.

Decoding Neural Circuit Interactions between Stress and Binge Eating

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Binge eating disorder is a debilitating disease which can arise from many kinds of traumas, pains, and stresses of life. Previous characterization of a binge-eating model developed by our lab shows that mice will consume greater quantities of high palatable diet (HPD) following exposure to specific types of psychological stressors including forced swim and foot shock compared to mice exposed to such psychological stressors. It was found that the claustrum of the brain had increased neural activity following bouts of binge-eating. One aspect of my research required me to quantify the density of neural activation in the claustrum from its most rostral to caudal area. We found that stressed mice displayed significantly higher levels of claustrum neural activation compared to controls. For the behavioral pattern we wanted to rule out influence of energy expenditure in the stress paradigm. Mice were given access to running wheels for an hour and then received access to HPD. Mice who displayed high levels of running had similar food intake to that of mice who did not display running activity. This suggests that psychological stress is an underlying component in this model for stress eating. As an ongoing project we are utilizing 1-photon imaging in the claustrum to monitor single cell activity across no stress vs. stress sessions and subsequent feeding behavior. We have thus far found increased neural activity in response to onset of a feeding bout in no stress conditions and we are investigating how stress modulates the effect of neurons tracked across time. This research potentially has great impact on the scientific community’s knowledge behind why psychological stressors contribute to binge-eating behaviors and could one day have astounding translational benefits for treating humans with binge-eating disorder.

Elucidating the Role of Nociceptin Receptors in Aversive Memory Formation

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Understanding the neural circuit mechanism underlying aversive memory formation is important for developing new treatments for patients with post-traumatic stress disorder (PTSD). The nociceptive circuit involved in such aversive memory formation remains incompletely characterized. Nociceptin opioid peptide receptors (NOPRs) in this circuit are expressed throughout the brain and have been implicated in nociceptive responses, anxiety, and aversive fear memory. To model aversive memory formation, we used a cued fear conditioning paradigm in mice. A mouse first learns to associate a tone and foot shock on day 1 and is assessed by its behavioral freezing response to tone only on day 2. We observed that using a high dose of a NOPR agonist on day 1 of the cued fear conditioning was associated with decreased arousal and prevented associative learning on day 2. Then, using thermal pain assays, we demonstrated a weakly anti-nociceptive effect after administration of the NOPR agonist, suggesting the disrupted fear learning is likely not due to loss of sensory transmission. Because disruption of fear learning was associated with decreased arousal, we screened for brain regions involved in the sedation from NOPR activation by selectively expressing NOPRs using a viral vector in different brain areas in a mouse lacking NOPRs. Interestingly, we found activating NOPRs in the parabrachial nucleus (PBN) in the brainstem was sufficient to produce sedation. Additionally, in situ RNA hybridization showed strong co-localization of oprl1 (NOPR gene) expressing cells and calca, a genetic marker encoding CGRP of a small group of cells in the ventrolateral PBN. Given previous literature has shown disrupted fear learning by inhibiting neural activity of those CGRP neurons, we hypothesize that NOPR-expressing PBN neurons may play an important role in aversive memory formation as well. Future investigations will explore the necessity and sufficiency of NOPR-expressing PBN neurons in aversive memory formation.

Modulation of Wakefulness by Nociceptin Peptide Signaling in the Locus Coeruleus

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The locus coeruleus (LC) is a small nucleus of noradrenergic neurons in the pons, which, despite its size, has broad projections throughout the central nervous system (CNS). Functionally, the LC is believed to be involved in various critical functions, including in the physiological responses to stress and mediating arousal. Previous investigations have demonstrated that optogenetic activation of the LC using channelrhodopsin at a tonic frequency promotes wakefulness in rodents. While this observation causally implicates LC function in wakefulness, it is still not known how the LC is endogenously controlled to mediate arousal. One potential candidate in this control involves the peptide nociceptin and its cognate receptor, the nociceptin opioid peptide receptor (NOPR), both
highly expressed around the LC. To investigate, we conducted two pharmacological experiments using the NOPR agonist Ro64-6198 to investigate its effects on locomotion and on the activity of LC noradrenergic neurons. We found that Ro64-6198 (10 mg/kg) strongly reduced open-field locomotor activity compared to vehicle treatment. Using in vivo 2-photon calcium imaging (GCaMP6s), we found that Ro64-6198 (5 mg/kg) profoundly reduced LC noradrenergic neuron activity. Wakefulness appeared reduced in both in vivo experiments. To determine where the endogenous nociceptin signal to the LC originates, we performed an intracranial injection of a Cre-dependent retrograde virus (AAV2-DIO-eYFP) into the LC of a mouse expressing Cre recombinase in nociceptin-expressing neurons. We identified a long-range nociceptinergic projection from the bed nucleus of the stria terminalis (BNST). In order to evaluate how the activity of these BNST neurons are affected by wakefulness, we conducted homocage fiber photometry recordings. Together, these studies suggest that nociceptin acting on LC noradrenergic neurons reduces arousal, and that the endogenous sources of nociceptin come from the BNST. These experiments shed new light on an understudied endogenous opioid system that may be a druggable target for sleep disorders.

Epileptic Neural Activity Detection in Mouse Models of Epilepsies Using Machine Learning

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Epilepsy is a neurological disorder characterized by the presence of seizures (periods of abnormally synchronized neural hyperactivity) and interictal spikes (transient abnormal neural synchronization that occurs between seizures). Different genetic mutations and backgrounds lead to different forms of epilepsy, which in turn may lead to different manifestations of epileptiform neural activity. I used machine learning (ML) to detect interictal spikes in mouse models of different epilepsies. I used neural activity previously recorded in mice using two electrocorticographic (ECoG) electrodes and one electromyographic (EMG) electrode. I used data from mouse models of Dravet syndrome (DS; Heterozygous Scn1a gene deletion), focal cortical dysplasia (FCD; Pik3ca gene mosaic), Leigh Syndrome (LS; GABAergic Ndufs4 knockout) and, Alzheimer’s Disease (AD; Increased beta-amyloid production), as well as wild type (WT) control. I used recordings binned into 10 second interictal spikes. I then used a computer algorithm that extracted 96 features - events that characterize ECoG and EMG electrical signals. These features and the manually identified interictal spikes were used to train several ML models to score unidentified interictal spikes in the remaining recorded data. The best performing ML algorithm had a mean test accuracy between 60% and 80% for each of the different models of epilepsy, but the features it used were different in each epilepsy mouse model. These results suggest that, while our ML-based method may capture epileptic activity with high accuracy, its success relies on features that are characteristic of each type of epilepsy. These results suggest the potential need to utilize different ML models for different forms of epilepsy in order to attain the highest possible accuracy if used for real-time interictal spike detection and potential seizure forecasting.

The Impact of NDUFS4 Knockout on Neuronal Excitability in a Mouse Model of Leigh Syndrome

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Leigh syndrome (LS) is a progressive neurological disorder that manifests within the first year of life and is characterized by the loss of mental and movement abilities and is accompanied by epilepsy. LS has been associated with loss-of-function (LOF) mutations in genes that encode for proteins present in complex 1 of the electron transport chain. LOF mutations in one such gene, NADH dehydrogenase (ubiquinone) iron sulfur protein 4 (NDUFS4), are strongly associated with LS. Mice carrying an NDUFS4 knockout exhibit symptoms similar to those in humans, creating a relevant mouse model. I investigated the effects of an NDUFS4 knockout (KO) on the neuronal excitability of inhibitory and excitatory neurons across brain regions in LS mouse models. Two LS mouse models were generated by knocking out NDUFS4 in inhibitory or excitatory neurons utilizing LoxP/Cre technology. Mice carrying floxed alleles of NDUFS4 were crossed with Vglut2Cre or Gad2Cre driver mice, creating animals with excitatory and inhibitory neuron-specific NDUFS4 KO, respectively. NDUFS4 KO mutations in specific neuron types cause different phenotypes in these animal models, which together model various aspects of LS. I took the progeny with excitatory or inhibitory neuron-specific NDUFS4 KO (6 VglutCre, 16 GadCre, ages P90-120 & P60-70, respectively) and their control littermates (4 VglutCre, 11 GadCre, same age ranges), perfused them with phosphate buffered saline (PBS), and fixed with 4% paraformaldehyde (PFA). I took brains from these mice, sliced, and stained them with c-fos immunocytochemistry, then imaged them to quantify neuronal activity. Results show increased c-fos expression in GadCre mutant mice after spontaneous & thermally induced seizures, especially in the dentate gyrus and frontal cortex. In addition, there was decreased c-fos expression in the cerebellum and Pre-Bötzinger complex in VglutCre mutant mice. Findings from this study contribute to our understanding of the mechanisms for the development of seizures in LS.
Targeted *Ndufs4* Knockout in PV Interneurons is Sufficient to Produce a Mild LS-related Epilepsy Phenotype in Mice

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The conditional knockout (KO) of *Ndufs4* in only GABAergic interneurons leads to a severe epilepsy phenotype, suggesting GABAergic interneurons drive the severe and often fatal epilepsy phenotype commonly reported in Leigh Syndrome (LS) patients. Dysfunctions or loss of parvalbumin (PV) interneurons, a subtype of GABAergic interneurons, have been shown to play a key role in the mechanisms of various forms of epilepsy both in human and animal models. The present study aims to target PV interneurons. We hypothesized that KO of *Ndufs4* in PV interneurons will cause dysfunctions or loss of PV neurons leading to epilepsy in our cell-specific model of LS. Experimental mice models with *Ndufs4*^lox/flx^/PVCre^lox/+^ genotype for the mutants, and *Ndufs4*^lox/flx^/PVCre^lox/+^ genotype for the controls were used. For imaging experiments, *Ndufs4*^lox/flx^/Ai14^lox/+^/PVCre^lox/+^ were used for mutants and *Ndufs4*^lox/+^/Ai14^lox/+^/PVCre^lox/+^ were used for controls. Seizure susceptibility was assessed by recording occurrence, frequency and duration of seizures and epileptiform events. Mice susceptibility to provoked seizures was examined by the pentylenetetrazol (PTZ) challenge. Assessment of cell loss was tested in imaging studies. Ai14-labeled PV interneurons in key areas associated with epilepsy were counted between the two groups. Finally, to assess motor dysfunctions comorbid to epilepsy, I tracked the movement of mice of both genotypes. Our results showed PV mutants had an increase in the frequency of spontaneous myoclonic seizures and interictal spikes on electroencephalograms (EEGs). There was no difference in seizure susceptibility to PTZ seizures between mutants and controls, nor any major impairments in locomotor activity or anxiety like behavior in PV mutants. Finally, no cell loss changes in PV mutants were detected. In conclusion, PV mutants display a mild seizure phenotype with no cognitive or motor abnormalities, suggesting targeted *Ndufs4* KO in PV interneurons drives a small portion of the severe epilepsy phenotype observed in LS.