# Modulation of Sensorimotor Gating through PnC Afferent Brain Regions

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## Introduction

Sensorimotor gating is a neural process in which irrelevant sensory information is filtered out preserving a primary input of information to sustain focus. This operation is handicapped in a variety of ailments such as: ADHD, Schizophrenia and post-traumatic stress disorder (PTSD). According to the Center for Disease Control (CDC), approximately 11% of children (4-17 years of age) within the United States (6.4 million) have been diagnosed with ADHD as of a study done in 2011. These diseases impair attention processes required to perform simple everyday tasks, much of which include social interactions, labor, and critical thinking skills. As of today there are no cures for these neurological disorders and the knowledge pertaining to the underlying neural mechanisms and brain regions affected by these diseases are much unknown. Our objective is to identify these unknown regions and understand the mechanism of how they interact with the caudal pontine reticular nucleus (PnC), a brain region within the brainstem that is the core processor of the sensorimotor gating process, a key element in proper attention activity. In addition, we also want to determine if certain neural antagonists will impair this pathway. There are two processes to which we plan to achieve this objective: an injection of retrograde tracer of fluorogold and a coupled process of optogenetic injections with extracellular electrophysiology which will afterwards be perfused with AP-5 and CNQX. With these processes we hope to better understand the underlying neurological circuitry of sensorimotor gating.

## Methods/Materials

### Tracing

Mice will receive a unilateral injection of Fluorogold (FG) to the caudal pontine reticular nucleus (PnC). The mice will be anesthetized with isoflurane by inhalation through a docked nose cone attached to stereotaxic head holder of which the duration of the surgery will take place. Using glass micropipettes (inner tip diameter 40 µm), mechanical injections of 0.05µL of 2% FG in saline will be applied to the PnC by means of Stereotaxic coordinates (-0.5, -5.33, -5.12) (Paxinos and Watson, 2004). After a 5-day survival period the mice are anesthetized with isoflurane and perfused with phosphate -buffered saline (PBS; pH 7.4) by 4.0% paraformaldehyde. After perfusion the brain is removed and placed in 4.0% paraformaldehyde. The brains are sectioned on a vibratome set to a thickness of 50 µm and docked onto slides for microscopic viewing.

### Optogenetic Injections

Mice will receive unilateral injections of 0.2µL of AAV2-ChR2-eYFP-CaMKIIα in the medial prefrontal cortex (mPFC) (-0.4, 1.94, -2.65 ; -0.4, 1.82, -2.3; -0.4, 1.9, -3.12) and the hippocampus (-2.65, -3.05, -4.55; -2.65, -3.05, -1.4; -3.0,-3.05, -4.25; -3.0, -3.05, -1.71; -3.35, -3.05, -3.9; -3.35, -3.05, -2.3; -3.7, -3.05, -3.3; -3.7, -3.05, -3.17; -3.7, -3.05, -2.92). The mice will be anesthetized with isoflurane by inhalation through a docked nose cone attached to stereotaxic head holder of which the duration of the surgery will take place. After 4-6 weeks of proliferation, the mice will be ready for optogenetic experiments.

### Slice Preparation

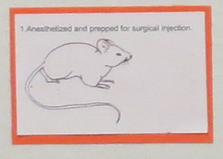
4-8 week-old-mice are anesthetized with isoflurane and decapitated. After skull a incision, the brain is removed and placed in ice cold dissecting solution. The PnC is removed and coronal brain sections are cut in the vibratome; these freshly cut PnC slices are allowed to recover for 2 hours at 34-36□ and are fed artificial cerebrospinal fluid at a rate of 2-3ml/min.

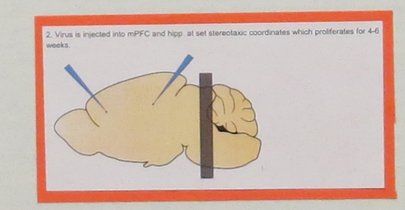
### Electrophysiology and *in vitro* Optogenetic Stimulation

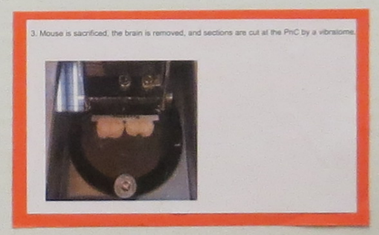
Mice infected with the AAV2-ChR2-eYFP-CaMKIIα virus are recorded from. Field EPSPs were recorded via a glass microelectrode (3-5MΩ) filled with a aCSF and placed in the PnC. A blue LED is placed near the PnC slice and stimulates any present opsin proteins. Afterwards an electrode stimulates the auditory fibers near lateral superior olive(LSO). Lastly both procedures are combined.

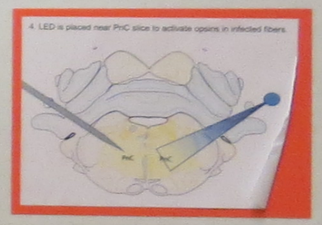
## Experimental Plan

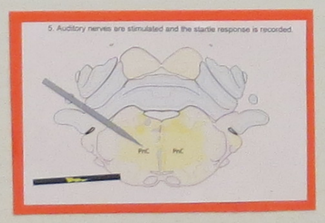
### *in vitro* Electrophysiology and Optogenetic Stimulation

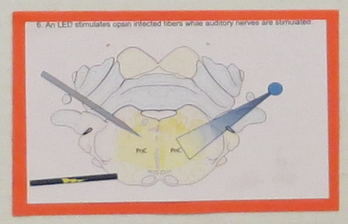


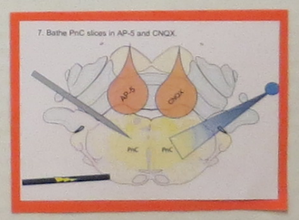



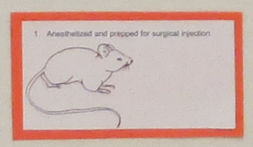



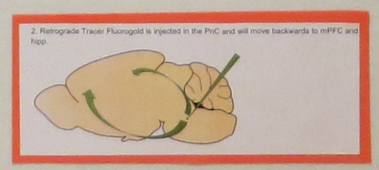



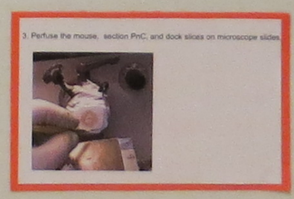




### Retrograde Tracing




## Expected Results

### Tracing

After docking the slices that contain FG and viewing them under a microscope, we should observe expression in regions such as the medial Prefrontal Cortex (mPFC) and the Hippocampus (hipp).

### Optogenetics/Electrophysiology

The first stimulation should reveal opsin proteins within the PnC showing a presence of neural fibers from the mPFC or hipp regions. The second stimulation should simulate a normal functioning Acoustic Startle Response (ASR) so that we would see a depolarization in our recorded data. The combined stimulation should show an inhibited ASR than what would normally be measured, revealing that either the mPFC or the hipp interacts with the PnC. Lastly we will perfuse the PnC slices with the pharmaceutical antagonist AP-5 and CNQX to hopefully knockout any glutamatergic activity.