2016-01-01

Characterization Of A Medial Prefrontal Cortex - Caudal Pontine Reticular Nucleus Relevant To Sensorimotor Gating

Sebastian Andres Pace
University of Texas at El Paso, sapace@miners.utep.edu

CHARACTERIZATION OF A MEDIAL PREFRONTAL CORTEX – CAUDAL PONTINE RETICULAR NUCLEUS CONNECTION RELEVANT TO SENSORIMOTOR GATING

SEBASTIAN A. PACE

Master's Program in Biology

APPROVED:

_____

Karine Fénelon, Ph.D., Chair

_____

Arshad M. Khan, Ph.D

_____

Laura O'Dell, Ph.D

_____

Charles H. Ambler, Ph.D
Dean of the Graduate School

Dedicated to my Grandfather, Jose D. Alva, M.D

CHARACTERIZATION OF A MEDIAL PREFRONTAL CORTEX – CAUDAL PONTINE RETICULAR NUCLEUS CONNECTION RELEVANT TO SENSORIMOTOR GATING

By

Sebastian A. Pace, B.S.

THESIS

Presented to the Faculty of the Graduate School of

The University of Texas at El Paso

in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF SCIENCE

Department of Biological Science

THE UNIVERSITY OF TEXAS AT EL PASO

December 2016

Acknowledgments

Firstly, I want to thank my P.I. Dr. Karine Fénelon for her mentorship, support and help throughout my time with her. I am grateful that she accepted me into her new lab and let me be a part of the exciting research she is performing. The project I completed in her lab was challenging and difficult yet rewarding. I am thankful for the opportunity I had to work and learn from her; her enthusiastic and encouraging approach to research was both genuine and contagious.

I also want to thank my thesis committee members: Dr. Arshad M. Khan and Dr. Laura O'Dell; their time, guidance and support is very much appreciated. Additionally I want to thank Dr. Manuel Miranda for allowing me to use much of his equipment to produce the results presented in this thesis.

I am sincerely thankful to my labmates and lab tech who made my graduate experience enjoyable and easier: Vanessa Navarro, Eduardo Peru, Anahis Tena, Laura Montes, Luis Martinetti, and Carla Loyola. I want to offer special thanks to Jose C. Cano, who helped me during every phase of this project.

Lastly I want to acknowledge my support away from lab: my parents, Adriana and Tracy; my sisters, Heather, Lindsey, Lauren, Adriana, and Daria; girlfriend, Kayla; and my friends, Jesse, Christin, and Bianca. All of my friends and family have shown me immense love and support; and all have contributed to who I am today.

Abstract

Focusing attention depends on filtering irrelevant sensory stimuli to prevent brain overload. This ability of our brain to block or "gate" such information is a pre-attentive neuronal mechanism known as sensorimotor gating (SG). SG abnormalities have been linked to a variety of psychiatric disorders, including schizophrenia. Initial studies related to the SG circuitry were performed more than four decades ago (Graham, 1975). However, SG is still the subject of numerous current studies involving both humans and animal models. Despite these research efforts, knowledge gaps persist regarding the identity neurons involved, their locations in different brain regions and their precise functional roles in SG. Here, our objective was to further identify and functionally characterize the neural structures and brain circuits that contribute to SG. The caudal pontine reticular nucleus (PnC) is a central structure within the SG circuitry (Davis et al., 1982, Koch et al., 1993; Fendt and Koch, 1999). We hypothesized that direct connections originating from various brain regions exist and innervate the PnC, yet remain to be identified. Such key experiments are crucial to later determine whether such brain regions exert an alternate gating mechanism by acting directly onto the PnC.

To test our hypothesis, we first used a retrograde neuronal tracer to identify brain regions anatomically connected to the PnC. Retrogradely labeled cells were found in the medial prefrontal cortex (mPFC). Therefore next, we used an *in vitro* Optogenetics approach combined to extracellular field electrophysiological recordings in brain slices by injecting Channelrhodopsin-2 (ChR-2) into the mouse mPFC. This was done to further confirm that this brain region (ie., the mPFC) is synaptically connected to the PnC. Photostimulation of ChR2-expressing mPFC fibers in acute PnC slices produced synaptic

responses confirming the integrity of the connection. Our preliminary data suggest for the first time that excitatory afferent fibers originating from the mPFC project onto PnC neurons. Future work will determine if this functional connection contributes to or modulates sensorimotor gating.

Table of Contents

List of Figures

I. Introduction

I.1. Sensorimotor gating

Sensorimotor gating (SG) is a neuronal inhibitory mechanism that helps us filter out irrelevant sensory information to focus attention (Perry and Braff, 1994; Swerdlow et al., 1996; Swerdlow et al., 1999). SG is a pre-attentive process, and thereby an innate mechanism that does not require conscious effort (Treisman et al., 1992). Without the ability to filter irrelevant sensory information, an individual's attention is easily diverted, resulting in reduced academic performance or diminished job success. SG deficits are seen in various neurological disorders and diseases such as: Attention-deficit/hyperactivity disorder (ADHD), obsessive compulsive disorder (OCD), Tourette's syndrome, post-traumatic stress disorder (PTSD), autism spectrum disorders (ASD) Huntington's disease and schizophrenia (SCZ) (Geyer and Braff, 1987; Swerdlow et al., 1993; Swerdlow et al., 1995; Grillon et al., 1996; Swerdlow et al., 2001; Perry et al., 2007). According to the World Health Organization, SCZ alone affects 2.6 million people throughout the United States and 21 million people worldwide. Currently, there is no cure for the pre-attentive impairments associated with SCZ. The numerous disorders associated with SG deficits, coupled with the financial burden of treatment, provide ample motivation to better understand the neural circuitry underlying SG.

I.2. Prepulse inhibition of the startle reflex: a measure of SG

In both humans and animal models, SG can be assessed using the prepulse inhibition (PPI) of the startle reflex task (Graham, 1975). The startle reflex is a physiological, highly

protective or survival reflex elicited in response to a startling sensory stimulation (Swerdlow et al., 1992). PPI is the reduction or gating of that startle response when a non-startling stimulus is presented prior to the startling stimulus. As a result, when a non-startling stimulus precedes a startling stimulus in a healthy subject, the startle response is decreased, resulting in a measurable PPI effect (as a percent of the non-attenuated initial startle response) (**Figure 1**).

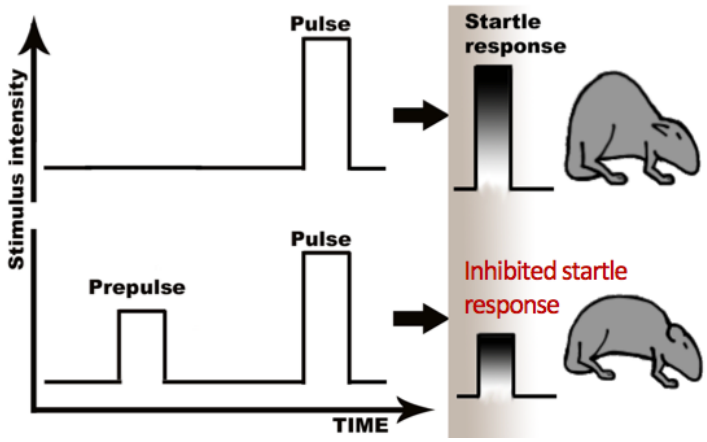


Figure 1. *Prepulse inhibition of the startle reflex. Adapted from Kohl et al., 2013.*

Although somewhat variable, previous studies have measured values of PPI in healthy subjects. However, as expected, decreased PPI values have been observed in human subjects and animals models of psychiatric and neurological disorders such as OCD, bipolar disorder, panic disorder, Tourette's syndrome, PTSD, HD, ASD and SCZ. In fact, compared to healthy individuals, decreased PPI values are a hallmark of SCZ (Swerdlow et al., 1995; Castellanos et al., 1996; Grillon et al., 1996; McAlonan et al., 2002; Perry et al., 2001; Ahmari et al., 2012). For example, in 1978, Braff and colleagues observed that SCZ-affected individuals experienced a reduction of 34% to 55% in PPI compared to a healthy control group. Since then, numerous studies have been performed using human and animal models to confirm the link between abnormal PPI values and SG deficits (Geyer and Braff, 1982; Swerdlow et al., 1991, Swerdlow et al., 2006, Li et al., 2009).

PPI studies have a high translational value in research utilizing animal models due to the capability of measuring PPI in numerous animal models as well as humans. The startle reflex is exhibited across a wide variety of species therefore allowing PPI to be studied. Many animal models have been used for PPI research including: mice, rats, zebrafishes, goldfishes, guinea pigs, pigs, marine mollusks and primates. (Vaillancourt and Boksa, 2000; Linn et al., 2003; Lind et al., 2004; Frost et al., 2003; Curtin and Preuss, 2015; Bergeron et al., 2015). Additionally, PPI of the startle reflex test can be tested by using auditory, tactile and/or vestibular modalities (Weike et al., 2000).

I.3. Brain regions involved in acoustic startle reflex and PPI

Tactile and vestibular modalities can be used to elicit a startle response and PPI; however, the most commonly used stimulus is an auditory stimulation. From previous work describing the brain regions activated during the acoustic startle reflex (ASR) test, it is known that a loud acoustic stimulus first activates the cochlear nuclei located in the brainstem. From there, the cochlear neurons will activate the giant glutamatergic neurons in the caudal pontine reticular nucleus (PnC) also in the brainstem. Such giant neurons project directly onto spinal and cranial motor neurons causing a startle response (**Figure 2**). On the other hand, when a milder acoustic stimulus is presented prior to a startling stimulus, a separate neural pathway is activated that will ultimately inhibit the PnC. That is, the mild sound will activate the inferior colliculus, which will activate the superior colliculus leading to the activation of the pedunculopontine tegmental area (PPTg) (Davis et al., 1982) (**Figure 2**). The PPTg will in turn inhibit the PnC. Because the PnC is inhibited by the PPTg (and therefore, by the mild sound), the subsequent application of a strong

startling stimulus that normally directly activates the PnC will lead to a reduced startle response and to the measurable PPI phenomenon (Koch et al., 1993). Although the underlying neuronal circuitry for ASR and PPI has been the focus of abundant research that started more than four decades ago, knowledge gaps still exist regarding how inhibition of PnC neuronal activity occurs.

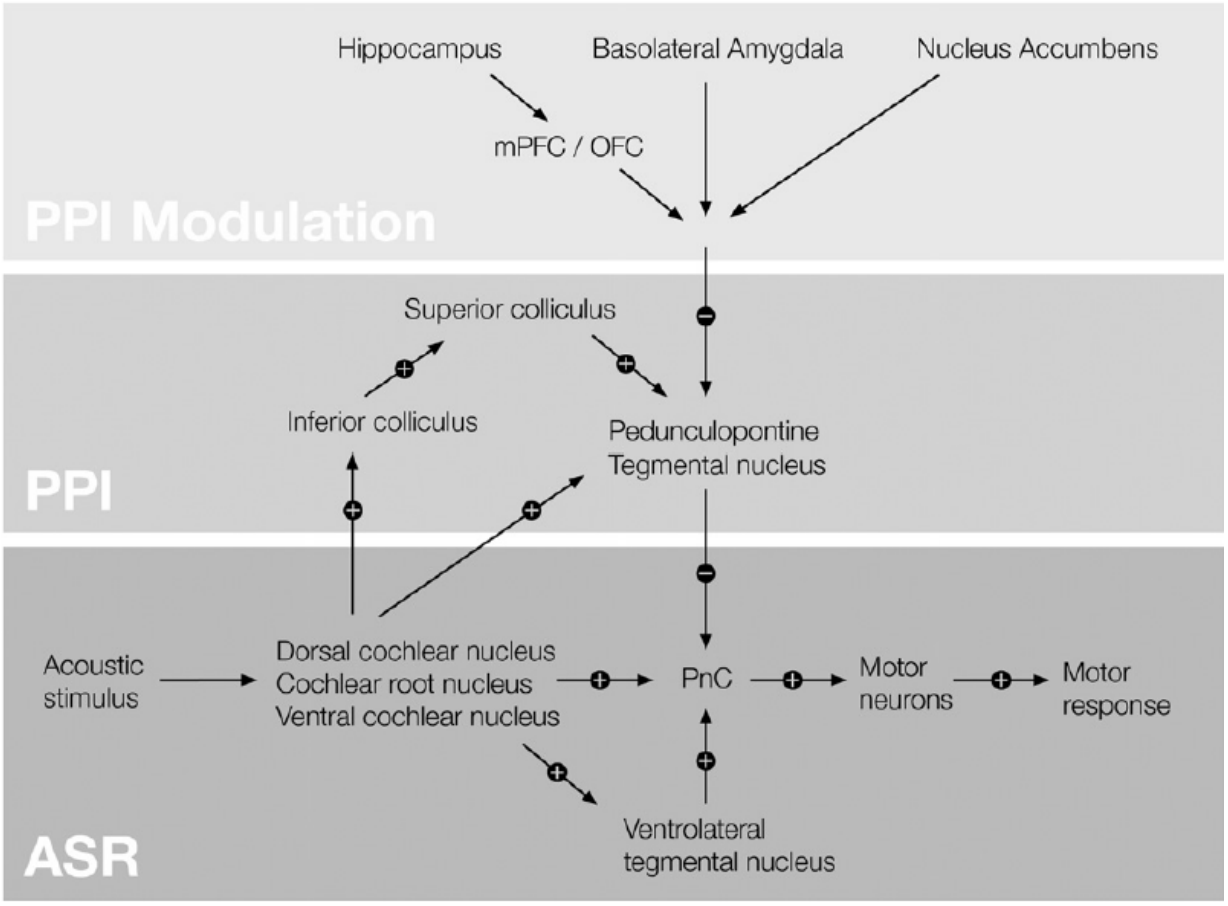


Figure 2. ASR pathway, PPI circuitry and modulatory neural structures. The bottom portion of the diagram illustrates the circuitry underlying the acoustic startle reflex (ASR). The middle portion of the diagram illustrates the known circuitry involved for prepulse inhibition (PPI). The top portion of the diagram shows neuroanatomical structures implicated in modulating PPI. (Kohl et al., 2013.)

I.4. PnC neurotransmitters and membrane receptors contributing to the startle reflex and PPI

Initially, cholinergic PPTg projections to the PnC were thought to be *entirely* responsible for the PPI phenomenon. This is because PPI values were greatly decreased (ie.. indicative of a decreased inhibition of the startle response) when PPTg cholinergic projections were inhibited *in vivo* (Koch et al., 1993; Swerdlow and Geyer, 1993). In addition, application of cholinergic receptor antagonists on PnC slices during *in vitro* PPTg stimulations modulated the activity of PnC giant neurons (Bosch and Schmid, 2008). However, other membrane receptors located on PnC giant neurons have been shown to affect PPI. For example, activation of mGluRIIIs *in vitro* was shown to inhibit the activity of PnC giant neurons, a result that was replicated *in vivo*, using local injections of mGluRIII agonist, L-AP4 (Schmid et al., 2010). Furthermore, GABAergic projections from the substantia nigra have been seen to mediate PPI *in vivo*, at the level of the PnC. More specifically, $GABA_B$ receptors neurons have been proposed to play a predominant role at long interstimulus intervals between the non-startling and the startling stimulation (Koch et al., 2000), whereas $GABA_A$ receptors were shown to play a role at short interstimulus intervals (Yeomans et al., 2010) during PPI.

Interestingly, the PnC was shown to also contain glycinergic interneurons, along with giant glutamatergic neurons, (Lingenhöhl and Friauf, 1992, 1994; Koch et al., 1993), but these PnC glycinergic interneurons are not directly activated by an auditory stimulation and their role in PPI remain to be investigated. In fact, *in vivo* studies using glycine receptor antagonists in the PnC as well as glycine receptor genetic knockout mice showed potentiated startle responses without significantly affecting PPI (Davis, 1988; Koch

and Friauf, 1995; Andrew and Owen, 1997; Plappert et al., 2001). Despite these previous findings, the exact mechanism of inhibition at the level of the PnC is not fully understood since applying a cocktail of glutamatergic, cholinergic and other neurotransmitter receptor antagonists/inhibitors in the PnC never completely abolished PPI *in vivo* and *in vitro* (for review see Fendt et al., 2001).

I.5. Neuronal PnC afferent regions involved in PPI

The PnC is a central brainstem structure that functions as a relay between sensory stimuli and motor responses. Multiple sensory inputs coming from the cochlear nuclei, vestibular nuclei, trigeminal nerve and dorsal columns modify PnC neuronal activity. These direct anatomical connections allow the PnC to be activated by acoustic, vestibular, and tactile stimuli. In fact, previous studies using retrograde neuronal tracer injected into the PnC confirmed direct synaptic connections projecting to the PnC and originating from the: medullary, pontine and mesencephalic reticular formation, cochlear nucleus, superior olivary complex, central gray, deep mesencephalic nuclei, substantia nigra, zona incerta, central nucleus of the amygdala and PPTg (Koch et al., 1993). Specifically, the PPTg was previously thought to be a predominant structure projecting to the PnC and contributing to PPI. The PPTg is a midbrain tegmentum structure known to project to numerous areas such as the thalamus, basal ganglia, inferior colliculus, limbic structures and the PnC. Additionally, the PPTg is implicated in many processes such as reward learning, visual orienting, sensory-motor patterns and sleep regulation. While much research had initially supported the inhibitory role of the PPTg cholinergic neurons in the PnC neurons, subsequently rat studies have shown that PPI persists when PPTg cholinergic projection

neurons are lesioned both *in vivo* and *in vitro* (Koch et al., 1993; Bosch and Schmid, 2008). Additionally, other structures such as the hippocampus (HPC), amygdala (AMG), medial prefrontal cortex (mPFC) and nucleus accumbens (NAC), have all been found to have a functional role within the PPI circuitry. Despite these previous research efforts, the neuronal mechanisms by which these regions contribute to PPI are still not yet entirely characterized. Therefore overall, research needs to be completed to further identify the brain circuits and neuronal mechanisms underlying PPI.

I.6. Modulatory neuronal pathways involved in PPI

In addition to the PPTg, early *in vivo* studies on PPI identified forebrain neural structures suggested to exert a "down-stream" or regulatory influence (Davis and Gendelman, 1977). Presently, brain areas such as the HPC, AMG, mPFC and NAC are considered to be PPI modulatory areas (Figure 2) (for review see Fendt, 2001 or Kohl et al., 2013; Wan and Swerdlow, 1997; Miller et al., 2010; Saint Marie et al., 2010). The modulatory effect of these regions on PPI will be briefly described here.

Hippocampus. In regards to the HPC, rat studies infusing cholinergic receptor agonist carbacol into the HPC partially inhibited PPI (Caine et al., 1991). Additionally, application of NMDAR (NMDA receptor) agonist into the ventral HPC also reduced PPI (Wan et al., 1996). The same result was obtained with the infusion of the NMDAR antagonist, AP-V, into the dorsal HPC (Bakshi and Geyer, 1998). No direct anatomical connection from the dorsal or ventral HPC to the PnC has currently been described.

Amygdala. Quinolinic acid lesions of the basolateral amygdala (BLA) reduced PPI in rats (Wan and Swerdlow, 1997). Additionally, GABA(A) receptor antagonist picrotoxin and

NMDAR antagonist dizocilpine infusion into the BLA reduced PPI (Fendt et al., 2000). This effect was reversed when dopamine antagonist haloperidol was systemically administered (Fendt et al., 2000).

Nucleus accumbens. Much research thus far has looked at dopamine (DA) receptors as being the key to the role of the NAC in PPI. Application of DA infused into the NAC caused a decrease in PPI in rats (Swerdlow et al., 1990a). This result was replicated when DA receptor agonist amphetamine was infused into the NAC (Davis et al., 1990), causing a DA overflow was found to lead to loss of PPI in rats. However, when 6-hydroxydopamine lesions were used to deplete DA in the NAC, PPI returned to normal (Swerdlow et al., 1990b). Knowledge of subcortical projections from the HPC, AMG and prefrontal cortex to the NAC, prompts the speculation that the NAC may be an important structure between forebrain and limbic regions within the PPI circuitry (Swerdlow et al., 2001).

Medial prefrontal cortex. Disrupted PPI due to NMDAR antagonist injected in the PnC were reversed with NMDAR agonist injections in the mPFC *in vitro* (Valsamis et al., 2014). In addition, it was only recently that a direct connection from the anterior cingulate cortex of the mPFC to the oral pontine reticular nucleus of the pontine reticular formation, a larger formation that includes the PnC, was described in locomotor context (ie., behavioral arrest) (Giber et al., 2015). Prior to that, in rats cortical connections were investigated from the PnC and projections were shown from the mPFC to the PnC (Torigoe et al., 1986b). Additional research is still needed to determine if and how the mPFC-PnC connection contributes to SG.

To date, we still do not have an exact understanding of how each of these brain regions are anatomically and synaptically connected within the PPI circuitry. Therefore, functional connections must be studied further to understand their contribution to PPI.

I.7. PFC neurotransmission and SCZ

The PFC is not only a modulatory structure within PPI circuitry, but moreover is well known for the role it plays in decision-making, executive control and working memory (Dalley et al., 2004; Chudasama and Robbins, 2006). Not surprisingly, PFC dysfunction is associated with various cognitive deficits that are symptoms of a number of neuropsychiatric disorders such as: autism spectrum disorders, depression and SCZ (Knable and Weinberger, 1997; Mann et al., 2000; von dem Hagen et al., 2013; Pezze et al., 2014). It is now well established that SG deficits are a hallmark of SCZ, and PFC-related abnormalities have been described in animal models of this disease (Fénelon et al., 2011, 2013). Therefore, better understanding the role of the PFC in PPI will have an international impact on patients worldwide. Interestingly, deficits in glutamate neurotransmission are linked to both SCZ and SG. Interest in glutamatergic neurotransmission in relation to SCZ began with observations after systemic administration of the non-competitive NMDAR antagonist, phencyclidine (PCP), in rats (Javitt and Zukin, 1991). Specifically, PCP application caused SCZ-like clinical observations such as inhibited cognition, hallucinations and working memory deficits (Javitt and Zukin, 1991). Further studies utilizing the NMDAR antagonist ketamine in rats also resulted in increased glutamate and DA levels in the PFC (Moghaddam et al., 1997). When a pretreatment of AMPA/kainate receptor antagonist LY293358 was given systemically prior to the ketamine, DA levels remained normal

(Moghaddam et al., 1997). Additional studies focusing on glutamate receptors showed that the administration of mGluR2 agonists was able to reverse the SZC-like behavioral effects of PCP although DA levels remained increased in the PFC (Moghaddam and Adams, 1998). Additionally, increased glutamate levels in the PFC of postmortem schizophrenic brain samples have previously been reported (Deakin et al., 2006).

In regards to PPI, deficits were also seen during the administration of PCP and other NMDAR antagonists. In rats, systemic application of PCP and ketamine effectively inhibited PPI (Mansbach and Geyer, 1989; Mansbach and Geyer, 1991). Moreover, dizocilpine locally injected in limbic areas such as the amygdala and ventral hippocampus showed a decrease of PPI in rats (Bakshi and Geyer, 1998). Likewise, mice showed a decrease in PPI after systemic PCP administration (Dalawa and Geyer, 1996). In primates, specifically brown capuchins (Cebus apella), again PPI of the ASR was exhibited prior to PCP-induced PPI deficits.

More recently, localized injections of a NMDAR antagonist, MK-801, into the PFC in rats induced PPI deficits (Valsamis et al., 2014). Activation of mGluR2/3 receptors through localized injections of LY354740 in the ventral-rostral mPFC reversed the PPI disruptions (Valsamis et al., 2014). As mentioned above, in animal studies, activating or blocking glutamatergic neurotransmission in both the mPFC and the PnC, two structures thought to be located within the SG circuitry, modulated PPI. Given the fact that mPFC deficits are linked to both SG abnormalities and SCZ, the exact role of the PFC within the PPI circuitry must be explored further.

I.8. mPFC-PnC synapses: an excitatory connection that could lead to inhibition

Our laboratory and others have been motivated to explore the involvement other inhibitory or gating structures may play a role during PPI. Previous studies utilizing lesions and receptor antagonists in animal models did not prevent PPI entirely leading us to hypothesize that other neural structures connected to the PnC are implicated in PPI. The focus of my project was the potential connection between the mPFC to the PnC within the PPI circuitry. As mentioned before, this connection has recently been shown to play a role in behavioral arrest in mice (Giber et al., 2015). In addition, the PnC is at the core of the SG circuitry while deficits in the mPFC are linked to SG deficit, a hallmark of SCZ. Therefore, we hypothesized that mPFC afferent fibers may play a direct role in PPI by directly modulating PnC neuronal activity.

I.9. Specific Aims

Two aims were used to investigate a mPFC-PnC connection. Specific Aim 1: Confirm a direct anatomical connection between the mPFC-PnC. Few previous studies have established that direct connections exist between the mPFC and the PnC (Torigoe et al., 1986b)(Giber et al., 2015). However, no studies have produced data concerning the prelimbic (PrL) and infralimbic (IL) areas of the mPFC projecting to the PnC in mice. Understanding which areas of the mPFC project onto the PnC is the first step to understanding if an mPFC-PnC connection is relevant in SG. To do this, the retrograde tracer Fluoro-Gold (FG) was injected into the PnC to identify which brain regions at the level of the mPFC send projections to the PnC. I used confocal microscopy and adjacent nissl stained sections to understand this connection.

Specific Aim 2: Functionally characterize mPFC-PnC synaptic properties. For the first time, the synaptic properties that underlie the mPFC-PnC connection will be investigated. To accomplish this, a viral construct consisting of ChR2-eYFP was injected into the mPFC and *in vitro* extracellular electrophysiological field recordings were performed on acute PnC slices. A blue optic fiber was used to photostimulate the ChR2-eYFP expressing projections from the mPFC in the PnC. Subsequently, electrophysiological protocols assessing synaptic transmission and neurotransmitter release were completed. This data regarding mPFC-PnC characteristic synaptic properties can be used as a baseline for comparison when looking at the connection in models of diseases associated to SG deficits.

II. Materials and Methods

II.1. Mice

Adult C57BL/6 mice (Jackson Laboratories) were housed in the University of Texas – El Paso (UTEP) Vivarium, a facility offering controlled temperatures and a 12:12 light/dark cycle. All protocols were approved and performed under the authority of the UTEP Institutional Animal Care and Use Committee.

II.2. Retrograde tracing

Six to ten week-old-mice received a unilateral injection Fluoro-Gold (FG) to the caudal pontine reticular nucleus (PnC). The mice were anesthetized with aerosolized 2.0% isoflurane via inhalation through a docked nose cone attached to a mouse stereotaxic (Kopf Instruments) for the duration of the surgery. A glass micropipette pulled with a Flaming Brown Micropippette Puller, Model P-1000 (Sutter Instrument). This micropipette was filled with mineral oil and used to inject 70 nL of 2% of the retrograde neuronal tracer FG (dissolved in saline solution, Molecular Probes) into the PnC, at the following injection coordinates: AP -5.33, ML -0.5, DV -5.12 (Paxinos and Franklin, 2004). After a seven-day recovery period during which the tracer was retrogradely transported, the mice were anesthetized with isoflurane and exsanguinated via transcardial perfusion with 0.8% saline solution and 4% paraformaldehyde (PFA). After perfusion, the brains were removed and placed in 4% PFA. After 24 hours, the perfused brains were washed three times with a 20% sucrose solution, and post-fixed in it. The brains were then sectioned with a microtome at a thickness of 30μm and mounted onto slides for microscopic viewing. Glycerol and

coverslips were placed atop the dried slides and sealed with fingernail polish. All slides were properly stored at -4^{0}C for future imaging. Additional sections were stored for future nissl staining.

II.3. Nissl staining

Nissl staining allowed us to examine the cell bodies in our areas of interest. Initially we mounted our thin-sectioned tissue on gel-coated slides. These slides are then placed in a holder and dipped into ascending then descending concentrations of alcohol and xylene to undergo dehydration and demyelination, staining in a Thionine solution, then rehydration before being cover slipped with DPX.

II.4. Viral Injections

Six to ten week-old-mice received unilateral injections of 200nL of AAVDJ-CaMKIIα-ChR2-eYFP in the medial prefrontal cortex (mPFC) at known injection sites (Paxinos and Franklin, 2004). mPFC injection coordinates were as follows: AP -1.94, ML -0.4, DV -2.65; AP -1.824, ML -0.4, DV -2.3; AP -1.9, ML -0.4, DV -3.12. The mice were anesthetized with 2.0% isoflurane via inhalation through a docked nose cone. Six to eight weeks' post-viral injection, these mice were used for *in vitro* electrophysiological/Optogenetics experiments.

II.5. Brain slice preparation for *in vitro* electrophysiological/Optogenetics experiments

Mice previously injected with a ChR2 (see above) were deeply anesthetized with isoflurane then decapitated. After a skull incision, the brain was removed and placed in oxygenated, 94.99% O_2, 5.01% CO_2, ice-cold dissecting solution (in mM): 195 sucrose, 10 NaCl, 2.5 KCl, 1 NaH_2PO_4, $NaHCO_3$, 10 glucose, 4 $MgSO_4$ and 0.5 $CaCl_2$. Coronal brain sections at 300µm were cut utilizing a vibratome (Leica VT1200S) filled with ice-cold dissecting solution allowing for acute brain slices containing the PnC to be collected. These freshly cut PnC slices recovered in the recording chamber for two hours at 34-36^{o}C and were constantly perfused with oxygenated artificial cerebrospinal fluid (aCSF) (in mM): 124 NaCl, 2.5 KCl, 1 NaH_2PO_4, 25 $NaHCO_3$, 10 glucose, 1 $MgSO_4$ and 2 $CaCl_2$ at a rate of 2-3mL/min.

II.6. *In vitro* electrophysiological/Optogenetics experiments

Extracellular field electrophysiological recordings were performed on acute PnC coronal slices from mice previously injected with ChR2-eYFP in the mPFC. Acute slices were kept in a recording chamber under an upright microscope (Zeiss Stemi 2000C) while being perfused with aCSF at 2-3mL/min. Field electrophysiological recording were performed using a glass microelectrode filled with aCSF and placed within the PnC. A bare blue (473nm) high performance optical fiber (Plexon) was placed in close proximity to the PnC slice. Extracellular field synaptic responses were triggered by photostimulation. All electrophysiology recordings were amplified with an extracellular amplifier (Cygnus

Technology) and were digitized using a Digidata 1440A (Axon Instruments). The data was filtered with a 1-kHz low-pass filter with a sampling rate of 10,000-kHz. Clampex 10.6 software was used for data acquisition and Clampfit 10.6 software was used for analysis.

All electrophysiology experiments went through a set of protocols. The first protocol is a 0.5ms photostimulation to determine the best placement for the recording electrode and the optic fiber. Once an optimal synaptic response is achieved, basic synaptic transmission was evaluated using a protocol consisting of gradually raising the optic fiber light intensity, every 30 seconds. Such "input-output" protocol was repeated 10 times to ensure that the averaged final traces/responses yielded minimal background noise. The input-output protocol allowed us to choose the intensity that would be fixed for the remaining sets of protocols. The light-evoked synaptic responses were quantified by measuring the initial negative slope of the voltage signal. Next, a paired-pulse protocol was used at varying interstimulus intervals (ISI) (repeated 10 times) to indirectly assess the probability of neurotransmitter release. A paired-pulse ratio (PPR) was then calculated by measuring the slope of the second synaptic response divided by the slope of the first synaptic response. PPR analysis can help determine if the synapses show short-term facilitation or short-term depression, both beign presynaptic mechanisms that are impaired in many disease states, highlighting the importance of measuring such plastic events under physiological conditions in healthy animals. At the end of the electrophysiological experiments, the acute slices were placed in 4.0% PFA overnight. The next day, the brain slices were froze in super-cooled hexane and were stored in a -80^{0}C fridge. The brain slices were then re-sectioned with a microtome at a thickness of 20-30µm and mounted onto slides for microscopic viewing.

II.7. Microscopy

Brain slices used for the FG experiments and that used for the electrophysiological experiments that were sectioned and mounted were examined using a Zeiss Observer.Z1 florescence microscope equipped with eYFP and FG filter sets. The brain slices containing the injection site and projection sites were imaged. The Axio software allowed me to acquire hi-resolution images and to place them each together with a 10% overlay to form a mosaic image.

III. Results

Understanding the mPFC-PnC connection requires both visualizing connected neurons and functionally characterizing it. To visualize the mPFC neurons connected to the PnC, both retrograde and anterograde neuronal tracers were used. Then, to investigate the synaptic characteristics of this connection, in vitro extracellular field electrophysiological/ Optogenetics experiments were performed. The results of such experiments are described below.

III.1. mPFC-PnC anatomical connection

To visualize mPFC projections to the PnC, retrograde neuronal tracing experiments and anterograde tracing experiments were performed using different sets of mice. A total of 20 mice were injected with the retrograde tracer Fluoro-Gold (FG) and 20 mice were injected with AAVDJ-CaMKIIα-ChR2-eYFP (ChR2-eYFP). An injection summary for both of these experiments is in Appendix 1 and 2, respectively.

III.1.1. Fluoro-Gold injections in the PnC retrogradely labels neurons in the mPFC

FG tracer was injected in a medial location of the PnC (**Figure 3**)(n=3). The injection sites were evaluated using the Paxinos and Franklin mouse brain atlas (Paxinos and Franklin, 2004). The 'halo effect' produced by FG injections was taken into consideration when examining photomicrographs of the FG deposited sections. Nissl staining was performed on the adjacent sections. Examination of those Nissl stained sections allowed for approximated brain region boundaries. When evaluating each injection site, the size of the

injection was taken into consideration when determining its location. This required looking at multiple sections to determine the size of the injection, rostro-caudally. Through understanding if we injected in the correct area, we are able to look at backfilled neurons with confidence.

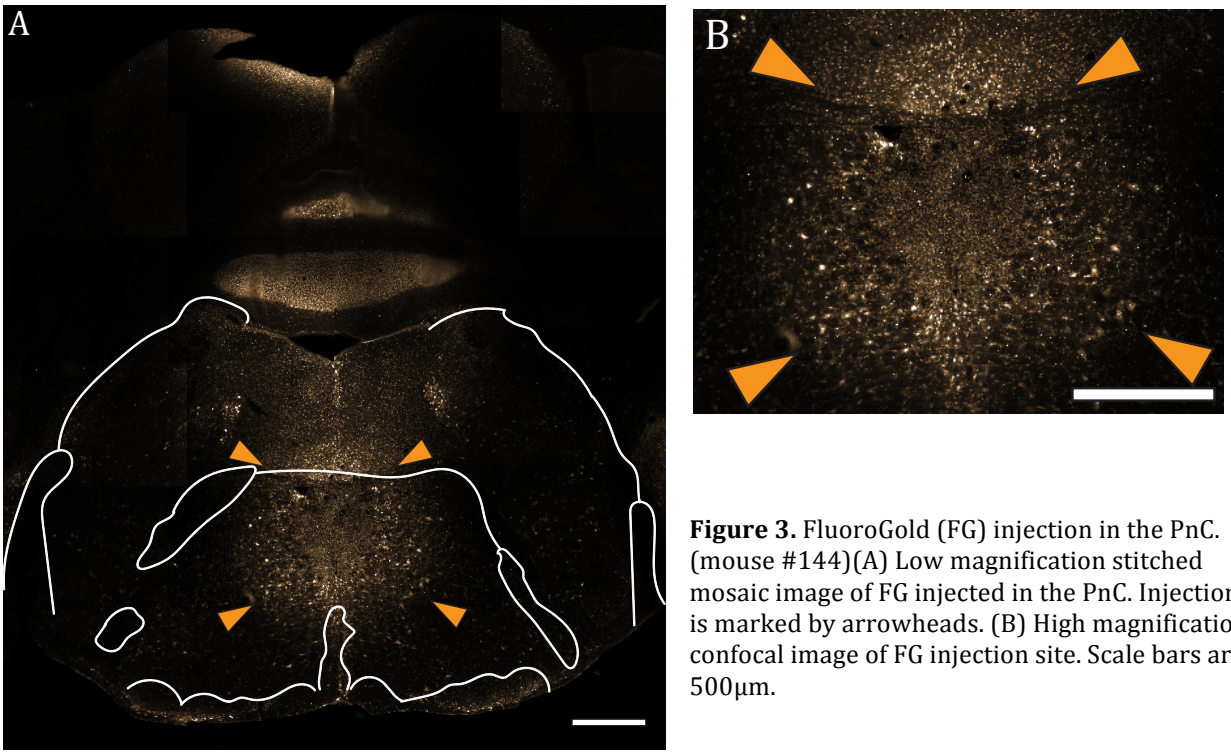


Figure 3. FluoroGold (FG) injection in the PnC. (mouse #144)(A) Low magnification stitched mosaic image of FG injected in the PnC. Injection is marked by arrowheads. (B) High magnification confocal image of FG injection site. Scale bars are 500µm.

When looking at sections containing mPFC areas, retrogradely labeled neurons were observed throughout the coronal sections that contain the mPFC; specifically in the subregions that make up the mPFC: prelimbic area (PrL), infralimbic area (IL) and anterior cingulate cortex (ACC)(**Figure 4**)(n=3). Literature concerning cytoarchitecture characterization of the prefrontal cortex was used as a reference when differentiating regions (Van de Werd et al., 2009). The motor cortex was also labeled in all sections. Cell

bodies backfilled with FG were observed bilaterally. In the case of mouse #144, we observed labeling in the PrL and IL in a mPFC rostral section, +1.98mm to bregma (**Figure 4A)**. Additional labeling was observed in the ventral tenia tecta in most experiments, and again labeling was observed in the motor cortex across all injections. In a more caudal section, +1.54mm to bregma, there was labeling in the PrL, IL and ACC (**Figure 4B**). Differential labeling between the layers that make up the mPFC was also observed. Specifically retrogradely labeled neurons in the PrL tended to be in lower layers, while labeled neurons in the IL tended to be in higher layers (**Figure 4B**).

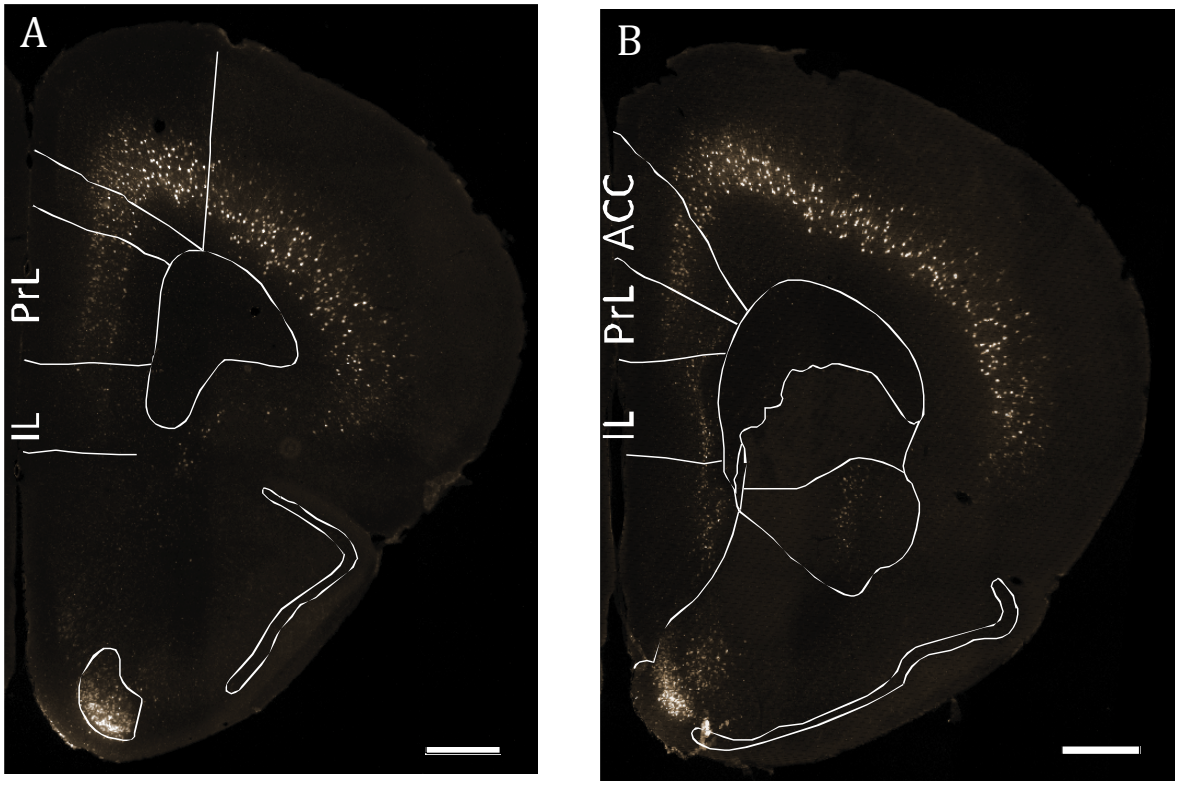
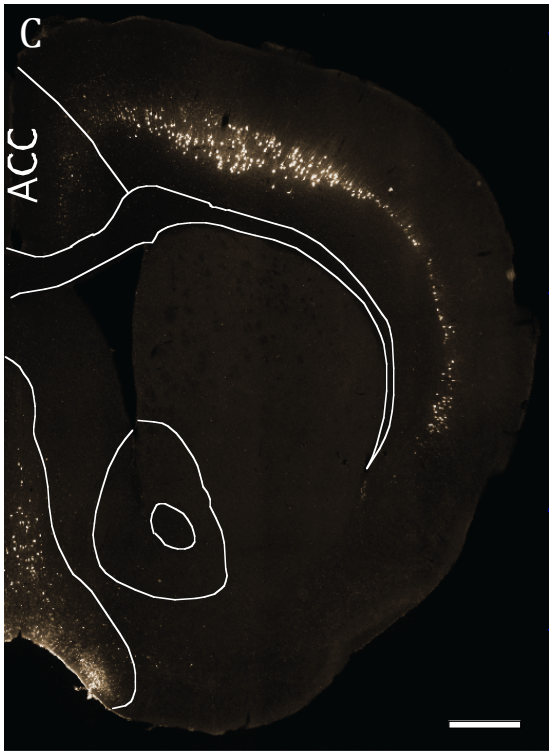


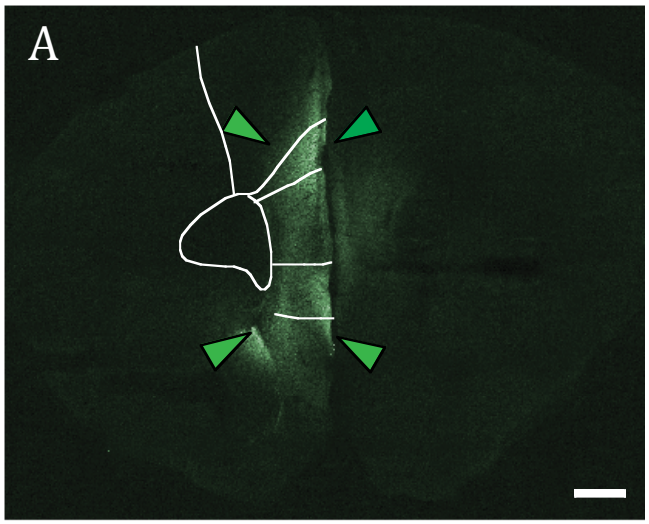
Figure 4. FG retrograde labeling in the mPFC (mouse #144). (A) Low magnification stitched mosaic image of a coronal brain section at +1.98mm to bregma. FG-labeled cell bodies were found in the PrL and IL of the mPFC. (B) Low magnification stitched mosaic image of a coronal brain section at +1.51mm to bregma. FG-labeled cell bodies were found in the PrL, IL and ACC of the mPFC. (C) (below) Low magnification stitched mosaic image of coronal brain section at +1.10mm to bregma. FG-labeled cell bodies were found in the ACC of the mPFC. Scale bars are 500μm.



Additional labeling was seen in the ventral palladium and nucleus accumbens core in some experiments, and again in the motor cortex. Lastly in a section +1.10mm to bregma, labeling was observed in the ACC (**Figure 4C**), with other labeling in the medial septal nucleus and the motor cortex.

III.1.2. ChR2-eYFP injections in the mPFC anterogradely projects to the PnC

A viral construct containing ChR2-eYFP was injected into the PrL and IL of the mouse mPFC (**Figure 5A**). This brain tissue was used for the electrophysiological experiments, which will be described in more details in the following section. At the end of the electrophysiological experiments, the brain tissue was re-sectioned and mounted for imaging the expression of ChR2-eYFP at the projection site (ie., the mPFC). The projection and injection sites were mapped to the Paxinos and Franklin atlas (Paxinos and Franklin, 2004). Additionally, literature pertaining to the cytoarchitectonic characterization of the prefrontal cortex was used as a guide (Van de Werd et al., 2009). Fibers expressing ChR2-eYFP were found in the PnC, 8-10 weeks post-injection (**Figure 5B,C**).



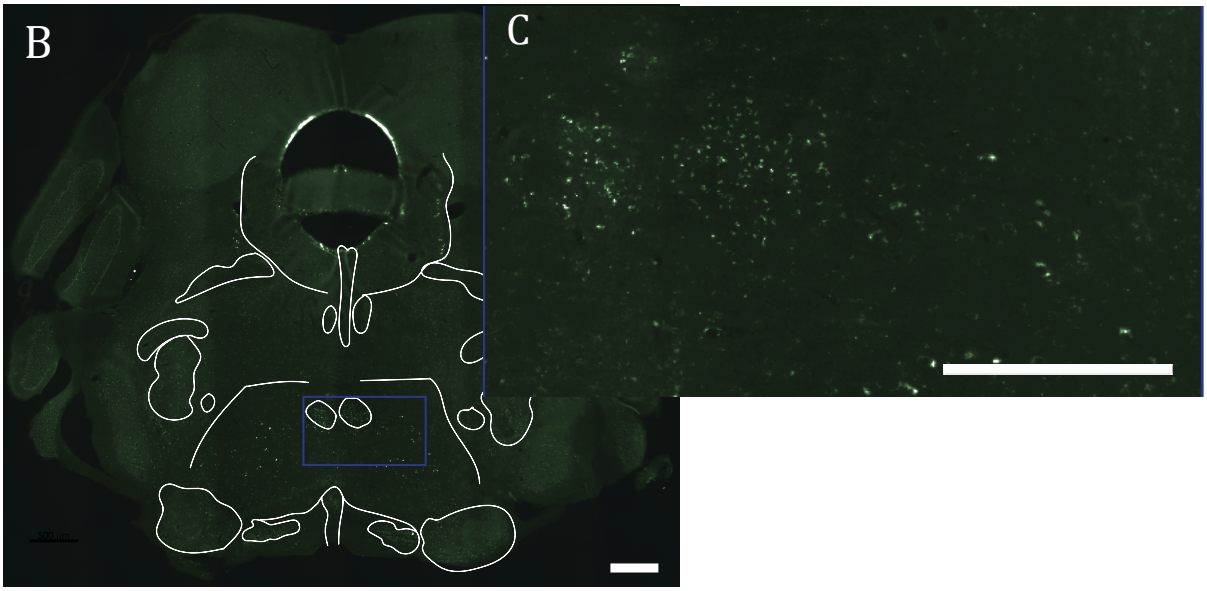


Figure 5. A viral construct containing ChR2-eYFP was injected into the mPFC; fibers from the mPFC expressing CHR2-eYFP were found in the PnC (mouse #279). (A) Low magnification stitched mosaic image of ChR2-eYFP injected in the mPFC. Injection is marked by arrowheads. (B) Low magnification stitched mosaic image of ChR2-eYFP expressing fibers in the PnC. (C) High magnification confocal image of ChR2-eYFP expressing fibers in the PnC. Scale bars are 500μm.

III.2. Electrophysiological characterization of the mPFC-PnC synapse

To further characterize the mPFC-PnC connection, we performed *in vitro* extracellular field electrophysiologial recordings from acute PnC slices. Previously, these mice were injected with AAVDJ-CaMKIIα-ChR2-eYFP in the PL and IL areas of the mPFC. 8 to 10 weeks post injection, the brains were obtained and 300μm acute brain slices containing the PnC were cut. Light-evoked field synaptic responses were recorded with a glass microelectrode

placed in the PnC of the acute brain slice (**Figure 6**). These light-evoked synaptic responses recorded by activating ChR2-eYFP expressing fibers originating in the mPFC and projecting to the PnC, again suggest a direct, monosynaptic mPFC-PnC connection. After electrophysiological experiments, the brain slices were further sectioned and imaged to verify ChR2-eYFP expression as described in the previous section (see III.1.2.).

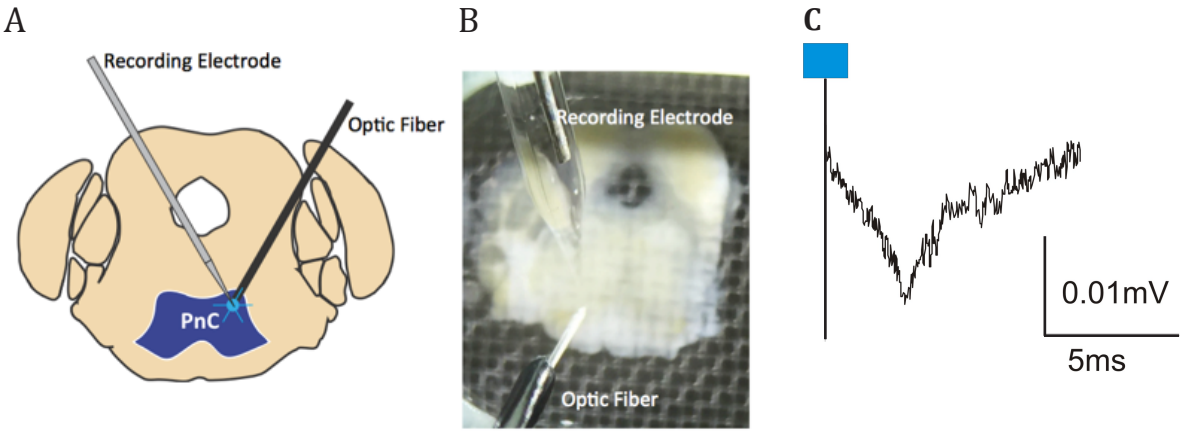


Figure 6 Extracellular field recordings in the PnC. (A) Schematic represenation of *in vitro* electrophysiology experiments performed on an acute PnC slice. (B) Photograph of an acute PnC slice with both the recording electrode and optic fiber placed in the PnC during photostimulation. (C) Example of a photostimulation-induced synaptic response recorded in the PnC of an acute brain slice.

Electrophysiological experiments began with positioning the recording electrode in the PnC and trying to get a synaptic response from the photostimulation. Once an optimal synaptic response was attained, we performed electrophysiological protocols to better characterize the synaptic properties of this connection. We first performed an Input-Output protocol, by gradually increasing photostimulation intensity as synaptic responses were recorded. This protocol was used to assess basal synaptic transmission which was

quantified by measuring the initial negative slope of these light-evoked synaptic responses. We observed an intensity-dependent increase of the response slope (**Figure 7**)(n=3).

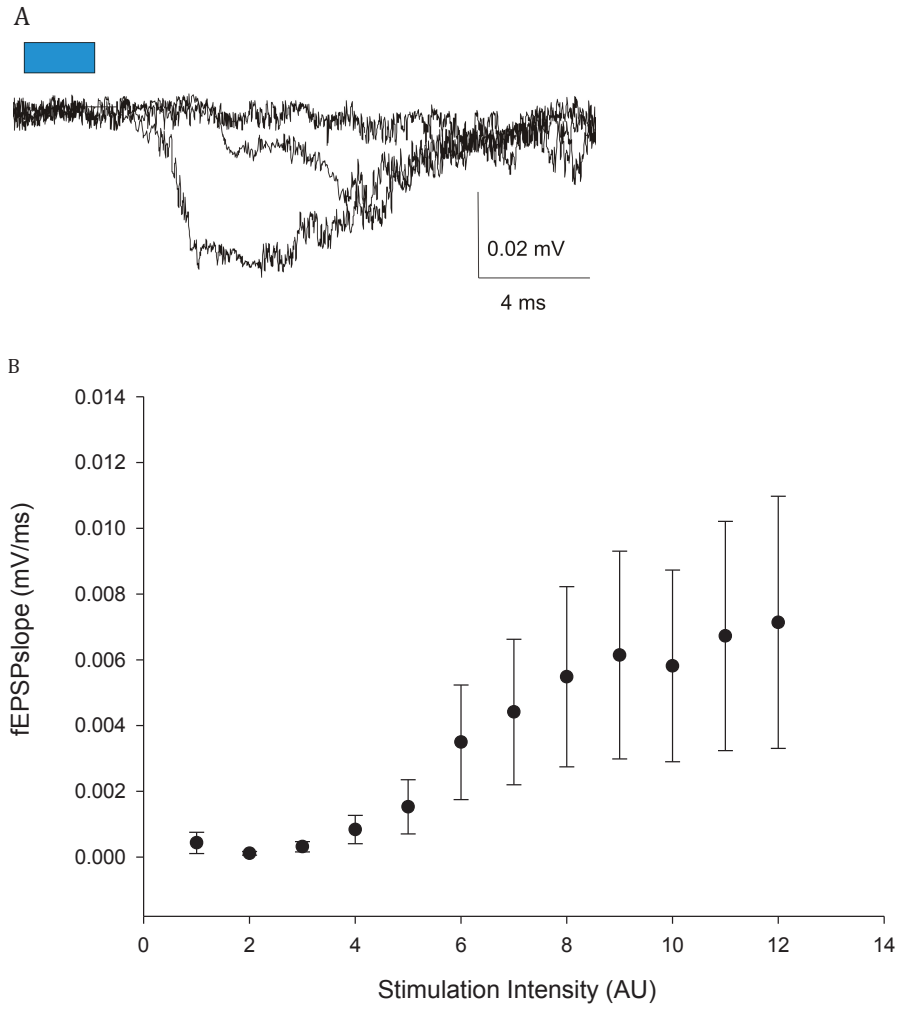


Figure 7. The input/output relation. (A) The initial trace recorded after a photostimulation at basal simulation intensity is shown in green. The last trace recorded after a photostimulation at the highest stimulation intensity is shown in purple. (B) Each resulting trace is plotted after measuing it's respective fEPSP slope (n=3).

Next, the probability of neurotransmitter release was assessed using a paired pulse protocol, which applies two photostimulation pulses at various interstimulus intervals (ISIs)(**Figure 8. A,B,C**). A paired-pulse ratio (PPR) was then calculated by measuring the slope of the second synaptic response divided by the slope of the first synaptic response

(**Figure 8.D**) (n=5). PPRs < 1 are defined as short-term depression, which was observed at short ISIs such as 10ms – 50ms. PPR > 1 are defined as short-term facilitation, was seen at ISIs 100ms – 200ms. At ISIs 400ms – 1500ms, the PPR were spproximately 1, meaning the synapse neither depressing or facilitating.

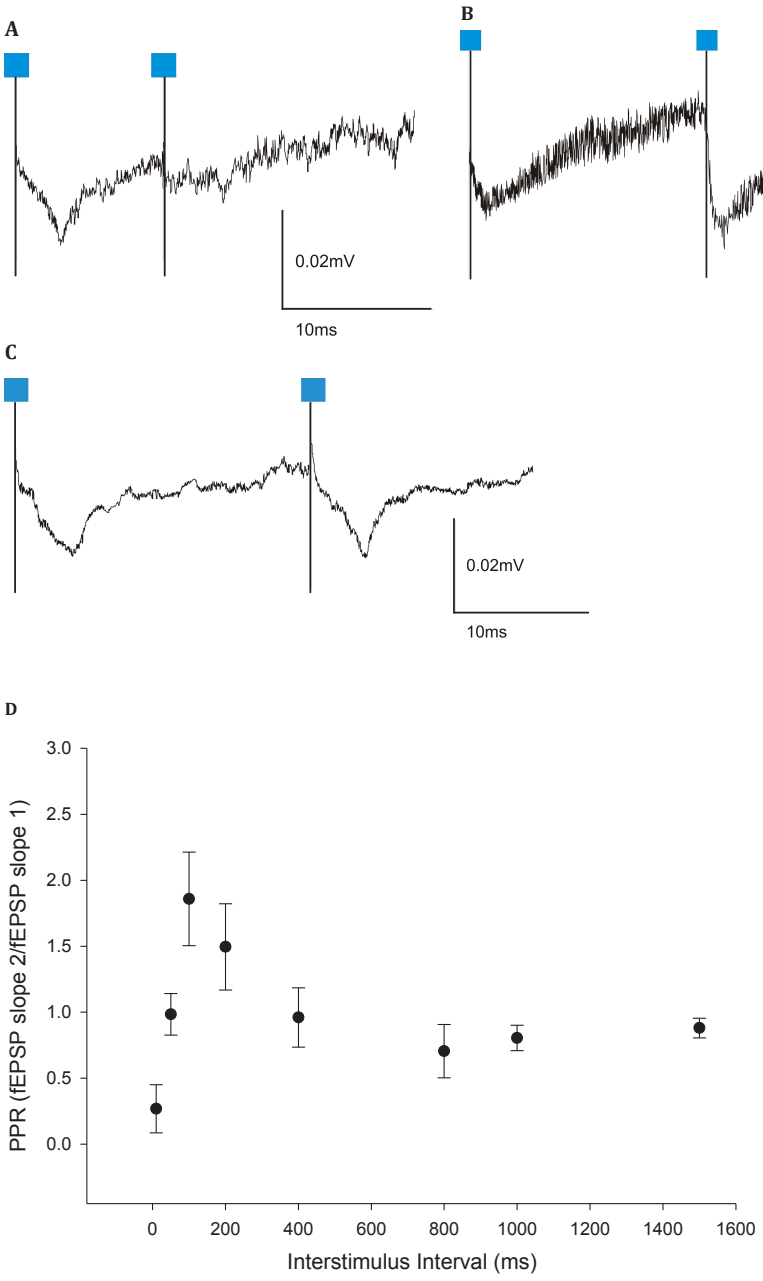


Figure 8 Paired-pulses at different interstimulation intervals (ISIs). Recorded traces of paired-pulse photostimulations are recorded at ISIs of (A)10ms, (B)100ms, (C)1000ms. (D) Paired-pulse ratios (PPRs) are graphed at different Interstimulus Intervals (ISIs). If the PPR is <1, synaptic depression is ocurring. If the PPR is >1, synaptic facilitation is ocurring (n=5).

IV. Discussion

The goal of my research was to understand a mPFC-PnC connection by first, confirming an anatomical connection and second, characterizing the synaptic properties of the mPFC-PnC synapse. Tract-tracing experiments and extracellular electrophysiology with Optogenetics were performed separately to accomplish these goals. First, FG was injected into the PnC and shown to label various mPFC areas. Few previous studies focusing on the pontine reticular formation (PRF) have shown a connection from the mPFC (Torigoe et al., 1986b; Giber et al., 2015). However, their data either did not focus on the PnC or was performed on rats. Our research focuses specifically on the mPFC-PnC connection in mice with the appeal to understand the connection within the PPI circuitry. This research is the initial step in a long-term goal of better understanding PPI, an operational measure of SG. Due to the PnC being at the core of PPI circuitry, it's indefinite afferent connections can potentially be part of this circuitry, and therefore may play a role in SG.

Our data suggests that a mPFC-PnC connection does exist in mice. Separately, a viral construct containing ChR2-eYFP was injected in the mPFC of mice. Imaging of these mice again, suggested a mPFC-PnC. Extracellular electrophysiological experiments were used to characterize synaptic transmission and neurotransmitter release at the mPFC-PnC synapse. Data from these experiments provide a novel synaptic profile of the synapse. Electrophysiological properties of the mPFC-PnC synapse in healthy adult mice may be compared to the mPFC-PnC synapse of diseased mice, specifically utilizing animal models of diseases associated with SG deficits.

IV.1. Retrograde tracing experiments

IV.1.1. Injecting FG in the PnC

FG was used as the retrograde tracer in our experiments. FG is a tracer commonly used to investigate afferent structures to regions of interest. However, FG does have limitations. FG is taken up by axon terminals to label afferent regions, but may also be taken up by damaged fibers. Therefore, an anterograde tracer must be used to complement these retrograde labelling and further confirm anatomical connections.

FG was injected in the PnC; a fairly large brain region located in the ventral portion of the brainstem. Following the Franklin and Paxinos mouse atlas, when examining coronal sections; the PnC encompasses -5.02mm through -5.68mm from bregma. The PnC is surrounded by the 7th facial cranial nerve laterally from -5.40mm through -5.68mm from bregma. The 7th facial cranial nerve can be used as a landmark for posterior portions of the PnC, but anterior parts are harder to differentiate. This is because the PnC is largely uncharacterized in regards to chemoarchitecture, microcircuits and particularly anatomical boundaries. Consequently, differentiation between the anterior parts of the PnC and posterior parts of the adjacent PnO (oral pontine reticular nucleus) is difficult.

The PnC and PnO are subparts of the pontine reticular formation (PRF). Cytoarchitectural studies of the PRF have been performed in rats and yet no anatomical boundary between the PnO and PnC was described (Torigoe et al., 1986a). The only cytoarchitectural differences observed was described as a central and pericentral division encompassing the entire pontine reticular formation (PRF)(Torigoe et al., 1986a). The central subdivision positioned in the medial portion of the PRF is composed of large

multipolar cells, small spherical and fusiform neurons. The pericentral subdivision located lateral to the central portion is composed of loosely packed fusiform and spherical neurons (Torigoe, et al., 1986a). A potential key to understanding a boundary between the areas may be neuron size (ie., soma diameter). Careful analysis of Nissl stained brain sections shows that the PnC has more large multipolar cells in comparison to the PnO (Paxinos and Franklin, 2004; Liang et al., 2015). This rational is observed in recent studies concerning the PnC which identity the area by giant neurons with a soma diameter of >35μm (Weber et al., 2008, Schmid et al., 2010).

When looking at another mouse brain atlas: the Allen Brain Atlas, differentiation between the PnO and PnC is unclear as well. Interestingly, the PnO and PnC boundary is further rostral in the Allen Brain Atlas than the Paxinos and Franklin mouse brain atlas thus adding this anatomical uncertainty. Albeit this ambiguity between these two regions can be easily overlooked, further analysis of the PnO and PnC is necessary especially in regards to PPI neural circuitry. The core of SG and PPI is specifically the PnC; therefore if we are to use rodent models to study underlying neuroanatomical circuitry, it is important to understand these discrepancies regarding the PnC.

IV.1.2. Observing backfilled neurons in the mPFC

A FG injection in the PnC caused neurons in the mPFC to backfill. These neurons were found throughout the areas that encompass the mPFC; notably, the PrL, IL and ACC. Labeling of these mPFC regions was fairly consistent through the injections performed. PrL labeling at superficial lower layers in some sections and IL labeling at higher layers suggested specific connections from neurons in those regions. Typically, higher layers such

as layer 5 pyramidal neurons serve as integrated outputs for the mPFC. This result is expected, especially when considering the distal connection to the PnC currently being assessed. The interesting notion to this mPFC-PnC connection is not just the fact that they are so far apart, but also the implicated functions of both regions. The mPFC is known as the executive center of the brain with implications in reward, working memory and cognition. Oppositely, the PnC is a brain stem region with the principle task of startle reflex. The contrast between both regions implies an extreme "top-down" relationship that must be investigated more to be understood.

IV.2. Extracellular electrophysiology and Optogenetics

IV.2.1. Extracellular electrophysiological field recordings

Extracellular electrophysiological field recordings were performed *in vitro* on acute PnC slices. I ran two synaptic protocols after finding an optimal synaptic response. The first protocol was an input-output relation to assess basal synaptic transmission. The second protocol was a paired pulse protocol, which applies two photostimulations with a specific time increment between them called an interstimulation interval (ISI). A paired-pulse ratio (PPR) was then calculated by analyzing the field excitatory post-synaptic potential (fEPSP) slope of the second synaptic response divided by the slope of the first synaptic response. PPR indirectly measures fractional neurotransmitter release thus demonstrating short-term facilitation or depression at specific ISIs. If the PPR is < 1 the synapse is in depression. If the PPR is > 1 the synapse is in facilitation. Understanding these types of use-dependent

presynaptic plasticity offers a novel perspective of how the mPFC-PnC synapse behaves in healthy animals.

Characteristically, presynaptic mechanisms underlying synaptic plasticity involve several factors such as: intracellular calcium, cationic channels, readily releasable pool size and neurotransmitter vesicular recycling; all of which can be altered during disease states (Crabtree and Gogos, 2014). The alterations of molecular entities responsible for intracellular calcium and such, cause plasticity dysfunction, which can rework information flow in neural circuits. Therefore, it may result in behavioral changes seen in neuropsychiatric disorders. My project focuses on understanding a mPFC-PnC connection relevant to SG; a neural process implicated in the neuropsychiatric disorders with dysfunctional presynaptic qualities. By measuring such plastic events under physiological conditions in healthy animals, future comparisons of the presynaptic mechanisms may be impaired in many disease states. By doing so, the mPFC-PnC connection may prove to be a future target in therapeutic therapies.

IV.2.2. mPFC fibers expressing ChR2-eYFP in the PnC

eYFP expressing mPFC fibers were found in the PnC after electrophysiology experiments. Tissue processing of acute brain slices for further confocal microscopy after electrophysiology experiments does not have much precedent. Initially, I attempted to image the PnC brain slices at the thickness used to complete electrophysiology experiments, 300μm. Unfortunately, these brain slices were too large to obtain any useable imaging data. After more trial-and-error, I was able to develop a reliable protocol to obtain better quality ChR2-eYFP imaging. By first placing the PnC brain slice in 4% PFA overnight,

then hexane freezing the brain slice and lastly thin-sectioning the individual brain slices to a smaller thickness, 30μm, I was able to obtain optimal brain sections for microscopy. From these thin PnC brain sections, I was able to obtain images of ChR2-eYFP expressing fibers in the PnC. The ChR2-eYFP expressing fibers were spread throughout the PnC with no localization to any specific subregion of the PnC. Further experimentation analyzing which PnC neurons the mPFC fibers synapse onto is needed.

IV.3. Future studies

The original motivation of this project was to better understand afferent PnC projections due to knowledge gaps underlying PPI and therefore SG neural circuitry in healthy mice. The results of this project suggest a novel mPFC-PnC connection and provide a baseline synaptic characteristic of the connection in healthy mice. In the future, looking at this connection in animal models of diseases associated with SG deficits may lead us to different results. For an example, 22q11.2 microdeletions, a genetic model used heavily in schizophrenia research, has been shown to cause alterations in cognition and synaptic plasticity (Fenelon et al., 2011, 2013). Working memory is strongly linked to the mPFC and coincidently is deficient in mice with the 22q11.2 microdeletions. Additionally, anatomical abnormalities such as an increased volumetric size of the PFC are seen in mice with 22q11.2 microdeletions (Ellegood et al., 2014). Understanding the differences between healthy mice and animal models of diseases could potentially lead to a therapeutic target to help those afflicted with SG deficits. Even so, much work lies ahead to fully understand the mPFC-PnC connection within neuronal, molecular and functional levels.

Previous studies have shown the pedunculopontine tegmental area (PPTg) to be the foremost inhibitory structure on the PnC within the PPI circuitry (Davis et al., 1982). Due to studies that ablate or inhibit PPTg function yet showed PPI persisting (Swerdlow and Geyer, 1993), speculation arose concerning what other neural structures could have an inhibitory effect on the PnC. Our FG studies backfilled neurons in the mPFC, and viral injections in the mPFC specific for excitatory fibers were shown to project to the PnC. These studies suggest a direct monosynaptic connection from the mPFC-PnC. Due to previous studies showing that the application of glutamatergic receptor antagonists *in vivo* in the mPFC modulates PPI (Valsamis et al., 2014), there may be a potential for the mPFC to have an inhibitory effect on the PnC as well. To test this theory, different experiments can be performed in the future.

A previous study analyzing PPTg activation that inhibits startle mediating giant PnC neurons, used angled brain slices containing both the PPTg and the PnC in a single acute brain slice (Bosch and Schmid, 2008). Having both regions on a single slice allowed them to activate the PPTg and observe the inhibitory effect it had on giant PnC neurons previously activated by terminal auditory fibers. In a future study, we can simply inject our current viral construct in the mPFC and perform *in vitro* electrophysiological experiments (ie., whole-cell patch clamp recordings) to record from giant PnC neurons; thus recreating a similar experiment with the goal of understanding the mPFC-PnC connection. If the mPFC-PnC connection is similar to the PPTg-PnC connection, then we may see a similar to result to the earlier study - an inhibitory effect on PnC giant glutamatergic neurons (Bosch and Schmid, 2008).

To understand how mPFC fibers projecting to the PnC cause an inhibitory effect, we must better understand the functional microcircuit within the PnC. We speculate glycinergic interneurons in the PnC may play a functional role due to bath application of glycine inhibiting PnC activity *in vitro* (Geis & Schmid, 2011). Performing patch recordings on glycinergic PnC neurons may elucidate some intrinsic properties of these neurons and potential inputs. This is but one example of how previous studies investigating the PPTg-PnC connection can be used as precedent for future studies exploring the mPFC-PnC connection, especially since we speculate that the mPFC may have a similar inhibitory role as the PPTg onto the PnC.

To go further, intricate tracing experiments utilizing retrograde and anterograde tracers simultaneously serve as refined means to exhibiting brain connections. Again, FG may be injected in the PnC as the retrograde tracer. An anterograde tracer such as *Phaseolus vulgaris*-leucoagglutinin (PHA-L) in the mPFC should garner labeled neurons in the PnC. Following successful tracing experiments, immunohistochemistry can be used to elucidate the identities of filled neurons in both brain regions. In combination, tracing and immunohistochemistry can show the connection as well as the chemoidentities of the neurons involved.

Lastly, it is still imperative to show if the mPFC-PnC connection is relevant to SG. To confirm this, an *in vivo* experiment beginning with injecting a viral construct with a silencing opsin such as halorhodopsin in the mPFC, before installing a cannula with a yellow optic fiber is applicable. With this approach, we can test PPI paradigm *in vivo*. The PPI paradigm begins with subjecting an animal model to a starting pulse and measuring the resulting startle reflex. Next, an application of a less intense pulse followed by the same

startling pulse should yield a measurable inhibited response, thus PPI. If our speculations are correct concerning the mPFC as an inhibitory structure upon the PnC, photostimulation of the mPFC prior to applying the prepulse then pulse, should lead to disinhibition of PPI. Such a result would prove that the mPFC serves as an inhibitory structure onto the PnC within the PPI circuitry.

IV.4. Conclusions

SG is a pre-attentative neuronal filtering process regulated by a central inhibitory mechanism that helps us focus attention. SG deficits have been associated with several neuropsychiatric disorders and diseases such as schizophrenia, obsessive compulsive disorder, Huntington's disease and anxiety. Nevertheless, there are limited treatment options for those diseases and disorders. The absence of treatment is somewhat due to the knowledge gaps concerning the neural circuitry underlying SG, even under normal conditions. Previous studies have suggested that a brainstem region known as the PnC is at the core of the SG circuitry. However, the afferent brain regions connected to the PnC are not well known.

Our objective is to confirm and characterize monosynaptic connections between the mPFC-PnC. To test this hypothesis, we used FG to identify PnC afferent brain regions. Backfilled neurons were found in the mPFC. Subsequently, viral injections of ChR2-eYFP into the mPFC showed that it projects to the PnC. *In vitro* photostimulation of ChR2-expressing fibers in acute PnC slices produced synaptic responses verifying the mPFC-PnC connection. Electrophysiological protocols were performed to further characterize the basic synaptic properties and presynaptic mechanisms of the mPFC-PnC synapse. Our

preliminary data suggest for the first time, that excitatory afferent fibers originating from the mPFC project onto PnC neurons. Future work will determine if this functional connection contributes to or modulates sensorimotor gating and how it compares to animal models of diseases associated with SG deficits.

V. References

Ahmari SE, Risbrough VB, Geyer MA, Simpson HB. Impaired sensorimotor gating in unmedicated adults with obsessive-compulsive disorder. Neuropsychopharmacology. 2012 Apr;37(5):1216-23. PubMed PMID: 22218093; PubMed Central PMCID: PMC3306882.

Allen Mouse Brain Atlas. 2015. Allen Institute for Brain Science. Allen Brain Atlas API. Available from: brain-map.org/api/index.html

Andrew M, Owen MJ. Hyperekplexia: abnormal startle response due to glycine receptor mutations. Br J Psychiatry. 1997 Feb;170:106-8. PubMed PMID: 9093496.

Bakshi VP, Geyer MA. Multiple limbic regions mediate the disruption of prepulse inhibition produced in rats by the noncompetitive NMDA antagonist dizocilpine. J Neurosci. 1998 Oct 15;18(20):8394-401. PubMed PMID: 9763482.

Bergeron SA, Carrier N, Li GH, Ahn S, Burgess HA. Gsx1 expression defines neurons required for prepulse inhibition. Mol Psychiatry. 2015 Aug;20(8):974-85. PubMed PMID: 25224259; NIHMSID: NIHMS619141; PubMed Central PMCID: PMC4362800.

Bosch D, Schmid S. Cholinergic mechanism underlying prepulse inhibition of the startle response in rats. Neuroscience. 2008 Jul 31;155(1):326-35. PubMed PMID: 18571866.

Braff D, Stone C, Callaway E, Geyer M, Glick I, et al. Prestimulus effects on human startle reflex in normals and schizophrenics. Psychophysiology. 1978 Jul;15(4):339-43. PubMed PMID: 693742.

Broersen LM, Uylings HB. Visual attention task performance in Wistar and Lister hooded rats: response inhibition deficits after medial prefrontal cortex lesions. Neuroscience. 1999;94(1):47-57. PubMed PMID: 10613496.

Bubser M, Koch M. Prepulse inhibition of the acoustic startle response of rats is reduced by 6-hydroxydopamine lesions of the medial prefrontal cortex. Psychopharmacology (Berl). 1994 Jan;113(3-4):487-92. PubMed PMID: 7862864.

Caine SB, Geyer MA, Swerdlow NR. Carbachol infusion into the dentate gyrus disrupts sensorimotor gating of startle in the rat. Psychopharmacology (Berl). 1991;105(3):347-54. PubMed PMID: 1798831.

Castellanos FX, Fine EJ, Kaysen D, Marsh WL, Rapoport JL, et al. Sensorimotor gating in boys with Tourette's syndrome and ADHD: preliminary results. Biol Psychiatry. 1996 Jan 1;39(1):33-41. PubMed PMID: 8719124.

Chudasama Y, Robbins TW. Functions of frontostriatal systems in cognition: comparative neuropsychopharmacological studies in rats, monkeys and humans. Biol Psychol. 2006 Jul;73(1):19-38. PubMed PMID: 16546312.

Crabtree GW, JA Gogos. Synaptic plasticity, neural circuits, and the emerging role of altered short-term information processing in schizophrenia. Front Syn Neuro. 2014 Nov 25;6;21.

Curtin PC, Preuss T. Glycine and GABAA receptors mediate tonic and phasic inhibitory processes that contribute to prepulse inhibition in the goldfish startle network. Front Neural Circuits. 2015 Mar 24;9:12. PubMed PMID: 25852486; PubMed Central PMCID: PMC4371714.

Dalley JW, Theobald DE, Bouger P, Chudasama Y, Cardinal RN, et al. Cortical cholinergic function and deficits in visual attentional performance in rats following 192 IgG-saporin-induced lesions of the medial prefrontal cortex. Cereb Cortex. 2004 Aug;14(8):922-32. PubMed PMID: 15084496.

Davis M, Gendelman DS, Tischler MD, Gendelman PM. A primary acoustic startle circuit: lesion and stimulation studies. J Neurosci. 1982 Jun;2(6):791-805. PubMed PMID: 7086484.

Davis M, Gendelman PM. Plasticity of the acoustic startle response in the acutely decerebrate rat. J Comp Physiol Psychol. 1977 Jun;91(3):549-63. PubMed PMID: 874121.

Davis M. Apomorphine, d-amphetamine, strychnine and yohimbine do not alter prepulse inhibition of the acoustic startle reflex. Psychopharmacology (Berl). 1988;95(2):151-6. PubMed PMID: 3137590.

Dulawa SC, Geyer MA. Psychopharmacology of prepulse inhibition in mice. Chin J Physiol. 1996;39(3):139-46. PubMed PMID: 8955560.

Ellenbroek BA, Budde S, Cools AR. Prepulse inhibition and latent inhibition: the role of dopamine in the medial prefrontal cortex. Neuroscience. 1996 Nov;75(2):535-42. PubMed PMID: 8931016.

Fendt M, Koch M. Cholinergic modulation of the acoustic startle response in the caudal pontine reticular nucleus of the rat. Eur J Pharmacol. 1999 Apr 9;370(2):101-7. PubMed PMID: 10323257.

Fendt M, Li L, Yeomans JS. Brain stem circuits mediating prepulse inhibition of the startle reflex. Psychopharmacology (Berl). 2001 Jul;156(2-3):216-24. PubMed PMID: 11549224.

Fénelon K, Mukai J, Xu B, Hsu PK, Drew LJ, et al. Deficiency of Dgcr8, a gene disrupted by the 22q112 microdeletion, results in altered short-term plasticity in the prefrontal cortex. Proc Natl Acad Sci U S A. 2011 Mar 15;108(11):4447-52. PubMed PMID: 21368174; PubMed Central PMCID: PMC3060227.

Fénelon K, Xu B, Lai CS, Mukai J, Markx S, et al. The pattern of cortical dysfunction in a mouse model of a schizophrenia-related microdeletion. J Neurosci. 2013 Sep 11;33(37):14825-39. PubMed PMID: 24027283; PubMed Central PMCID: PMC3771024.

Frost WN, Tian LM, Hoppe TA, Mongeluzi DL, Wang J. A cellular mechanism for prepulse inhibition. Neuron. 2003 Dec 4;40(5):991-1001. PubMed PMID: 14659097.

Geir HR, S Schmid. Glycine inhibits startle-mediating neurons in the caudal pontine reticular formation but is not involved in synaptic depression underlying short-term habituation of startle. Neuro Res. 2011;71:114-123.

Geyer MA, Braff DL. Startle habituation and sensorimotor gating in schizophrenia and related animal models. Schizophr Bull. 1987;13(4):643-68. PubMed PMID: 3438708.

Geyer MA, Braff DL. Habituation of the Blink reflex in normals and schizophrenic patients. Psychophysiology. 1982 Jan;19(1):1-6. PubMed PMID: 7058230.

Giber K, Diana MA, Plattner VM, Dugué GP, Bokor H, et al. A subcortical inhibitory signal for behavioral arrest in the thalamus. Nat Neurosci. 2015 Apr;18(4):562-8. PubMed PMID: 25706472; NIHMSID: EMS61926; PubMed Central PMCID: PMC4885661.

Graham FK. Presidential Address, 1974 The more or less startling effects of weak prestimulation. Psychophysiology. 1975 May;12(3):238-48. PubMed PMID: 1153628.

Grillon C, Southwick SM, Charney DS. The psychobiological basis of posttraumatic stress disorder. Mol Psychiatry. 1996 Sep;1(4):278-97. PubMed PMID: 9118351.

Japha K, Koch M. Picrotoxin in the medial prefrontal cortex impairs sensorimotor gating in rats: reversal by haloperidol. Psychopharmacology (Berl). 1999 Jun;144(4):347-54. PubMed PMID: 10435407.

Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry. 1991 Oct;148(10):1301-8. PubMed PMID: 1654746.

Knable MB, Weinberger DR. Dopamine, the prefrontal cortex and schizophrenia. J Psychopharmacol. 1997;11(2):123-31. PubMed PMID: 9208376.

Koch M, Kungel M, Herbert H. Cholinergic neurons in the pedunculopontine tegmental nucleus are involved in the mediation of prepulse inhibition of the acoustic startle response in the rat. Exp Brain Res. 1993;97(1):71-82. PubMed PMID: 8131833.

Koch M. The neurobiology of startle. Prog Neurobiol. 1999 Oct;59(2):107-28. PubMed PMID: 10463792.

Koch M, Fendt M, Kretschmer BD. Role of the substantia nigra pars reticulata in sensorimotor gating, measured by prepulse inhibition of startle in rats. Behav Brain Res. 2000 Dec 20;117(1-2):153-62. PubMed PMID: 11099769.

Koch M, Friauf E. Glycine receptors in the caudal pontine reticular formation: are they important for the inhibition of the acoustic startle response?. Brain Res. 1995 Feb 6;671(1):63-72. PubMed PMID: 7728534.

Kohl S, Heekeren K, Klosterkötter J, Kuhn J. Prepulse inhibition in psychiatric disorders--apart from schizophrenia. J Psychiatr Res. 2013 Apr;47(4):445-52. PubMed PMID: 23287742.

Lacroix L, Spinelli S, Heidbreder CA, Feldon J. Differential role of the medial and lateral prefrontal cortices in fear and anxiety. Behav Neurosci. 2000 Dec;114(6):1119-30. PubMed PMID: 11142644.

Li L, Du Y, Li N, Wu X, Wu Y. Top-down modulation of prepulse inhibition of the startle reflex in humans and rats. Neurosci Biobehav Rev. 2009 Sep;33(8):1157-67. PubMed PMID: 19747594.

Lind NM, Arnfred SM, Hemmingsen RP, Hansen AK. Prepulse inhibition of the acoustic startle reflex in pigs and its disruption by d-amphetamine. Behav Brain Res. 2004 Dec 6;155(2):217-22. PubMed PMID: 15364480.

Lingenhöhl K, Friauf E. Giant neurons in the caudal pontine reticular formation receive short latency acoustic input: an intracellular recording and HRP-study in the rat. J Comp Neurol. 1992 Nov 22;325(4):473-92. PubMed PMID: 1281843.

Lingenhöhl K, Friauf E. Giant neurons in the rat reticular formation: a sensorimotor interface in the elementary acoustic startle circuit?. J Neurosci. 1994 Mar;14(3 Pt 1):1176-94. PubMed PMID: 8120618.

Linn GS, Negi SS, Gerum SV, Javitt DC. Reversal of phencyclidine-induced prepulse inhibition deficits by clozapine in monkeys. Psychopharmacology (Berl). 2003 Sep;169(3-4):234-9. PubMed PMID: 12845408.

Mann JJ, Huang YY, Underwood MD, Kassir SA, Oppenheim S, et al. A serotonin transporter gene promoter polymorphism (5-HTTLPR) and prefrontal cortical binding in major depression and suicide. Arch Gen Psychiatry. 2000 Aug;57(8):729-38. PubMed PMID: 10920459.

Mansbach RS, Geyer MA. Effects of phencyclidine and phencyclidine biologs on sensorimotor gating in the rat. Neuropsychopharmacology. 1989 Dec;2(4):299-308. PubMed PMID: 2692589.

Mansbach RS, Geyer MA. Parametric determinants in pre-stimulus modification of acoustic startle: interaction with ketamine. Psychopharmacology (Berl). 1991;105(2):162-8. PubMed PMID: 1796122.

McAlonan GM, Daly E, Kumari V, Critchley HD, van Amelsvoort T, et al. Brain anatomy and sensorimotor gating in Asperger's syndrome. Brain. 2002 Jul;125(Pt 7):1594-606. PubMed PMID: 12077008.

Miller EJ, Saint Marie LR, Breier MR, Swerdlow NR. Pathways from the ventral hippocampus and caudal amygdala to forebrain regions that regulate sensorimotor gating in the rat. Neuroscience. 2010 Jan 20;165(2):601-11. PubMed PMID: 19854244; NIHMSID: NIHMS155656; PubMed Central PMCID: PMC2796367.

Moghaddam B, Adams B, Verma A, Daly D. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. J Neurosci. 1997 Apr 15;17(8):2921-7. PubMed PMID: 9092613.

Moghaddam B, Adams BW. Reversal of phencyclidine effects by a group II metabotropic glutamate receptor agonist in rats. Science. 1998 Aug 28;281(5381):1349-52. PubMed PMID: 9721099.

Paxinos, Franklin. *The mouse brain in stereotaxic coordinates.* Academic Press, San Diego, CA. 2004.

Perry W, Braff DL. Information-processing deficits and thought disorder in schizophrenia. Am J Psychiatry. 1994 Mar;151(3):363-7. PubMed PMID: 8109644.

Perry W, Minassian A, Lopez B, Maron L, Lincoln A. Sensorimotor gating deficits in adults with autism. Biol Psychiatry. 2007 Feb 15;61(4):482-6. PubMed PMID: 16460695.

Perry W, Minassian A, Feifel D, Braff DL. Sensorimotor gating deficits in bipolar disorder patients with acute psychotic mania. Biol Psychiatry. 2001 Sep 15;50(6):418-24. PubMed PMID: 11566158.

Pezze M, McGarrity S, Mason R, Fone KC, Bast T. Too little and too much: hypoactivation and disinhibition of medial prefrontal cortex cause attentional deficits. J Neurosci. 2014 Jun 4;34(23):7931-46. PubMed PMID: 24899715; PubMed Central PMCID: PMC4044251.

Plappert CF, Pilz PK, Becker K, Becker CM, Schnitzler HU. Increased sensitization of acoustic startle response in spasmodic mice with a mutation of the glycine receptor alpha1-subunit gene. Behav Brain Res. 2001 Jun;121(1-2):57-67. PubMed PMID: 11275284.

Rampon C, Peyron C, Petit JM, Fort P, Gervasoni D, et al. Origin of the glycinergic innervation of the rat trigeminal motor nucleus. Neuroreport. 1996 Nov 25;7(18):3081-5. PubMed PMID: 9116245.

Saint Marie RL, Miller EJ, Breier MR, Weber M, Swerdlow NR. Projections from ventral hippocampus to medial prefrontal cortex but not nucleus accumbens remain functional after fornix lesions in rats. Neuroscience. 2010 Jun 30;168(2):498-504. PubMed PMID: 20338222; NIHMSID: NIHMS191748; PubMed Central PMCID: PMC2887283.

Schmid S, Brown T, Simons-Weidenmaier N, Weber M, Fendt M. Group III metabotropic glutamate receptors inhibit startle-mediating giant neurons in the caudal pontine reticular nucleus but do not mediate synaptic depression/short-term habituation of startle. J Neurosci. 2010 Aug 4;30(31):10422-30. PubMed PMID: 20685984.

Swerdlow NR, Braff DL, Geyer MA. Cross-species studies of sensorimotor gating of the startle reflex. Ann N Y Acad Sci. 1999 Jun 29;877:202-16. PubMed PMID: 10415651.

Swerdlow NR, Bakshi V, Geyer MA. Seroquel restores sensorimotor gating in phencyclidine-treated rats. J Pharmacol Exp Ther. 1996 Dec;279(3):1290-9. PubMed PMID: 8968353.

Swerdlow NR, Geyer MA. Prepulse inhibition of acoustic startle in rats after lesions of the pedunculopontine tegmental nucleus. Behav Neurosci. 1993 Feb;107(1):104-17. PubMed PMID: 8447948.

Swerdlow NR, Geyer MA, Braff DL. Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. Psychopharmacology (Berl). 2001 Jul;156(2-3):194-215. PubMed PMID: 11549223.

Swerdlow NR, Caine SB, Geyer MA. Opiate-dopamine interactions in the neural substrates of acoustic startle gating in the rat. Prog Neuropsychopharmacol Biol Psychiatry. 1991;15(3):415-26. PubMed PMID: 1862221.

Swerdlow NR, Caine SB, Braff DL, Geyer MA. The neural substrates of sensorimotor gating of the startle reflex: a review of recent findings and their implications. J Psychopharmacol. 1992 Jan;6(2):176-90. PubMed PMID: 22291349.

Swerdlow NR, Paulsen J, Braff DL, Butters N, Geyer MA, et al. Impaired prepulse inhibition of acoustic and tactile startle response in patients with Huntington's disease. J Neurol Neurosurg Psychiatry. 1995 Feb;58(2):192-200. PubMed PMID: 7876851; PubMed Central PMCID: PMC1073317.

Swerdlow NR, Mansbach RS, Geyer MA, Pulvirenti L, Koob GF, et al. Amphetamine disruption of prepulse inhibition of acoustic startle is reversed by depletion of mesolimbic dopamine. Psychopharmacology (Berl). 1990;100(3):413-6. PubMed PMID: 2315438.

Swerdlow NR, Braff DL, Masten VL, Geyer MA. Schizophrenic-like sensorimotor gating abnormalities in rats following dopamine infusion into the nucleus accumbens. Psychopharmacology (Berl). 1990;101(3):414-20. PubMed PMID: 2114026.

Torigoe Y, Blanks RH, Precht W. Anatomical studies on the nucleus reticularis tegmenti pontis in the pigmented rat II Subcortical afferents demonstrated by the retrograde transport of horseradish peroxidase. J Comp Neurol. 1986 Jan 1;243(1):88-105. PubMed PMID: 3512625.

Torigoe Y, Blanks RH, Precht W. Anatomical studies on the nucleus reticularis tegmenti pontis in the pigmented rat I Cytoarchitecture, topography, and cerebral cortical afferents. J Comp Neurol. 1986 Jan 1;243(1):71-87. PubMed PMID: 3512624.

Treisman A, Vieira A, Hayes A. Automaticity and preattentive processing. Am J Psychol. 1992 Summer;105(2):341-62. PubMed PMID: 1621885.

Vaillancourt C, Boksa P. Birth insult alters dopamine-mediated behavior in a precocial species, the guinea pig Implications for schizophrenia. Neuropsychopharmacology. 2000 Dec;23(6):654-66. PubMed PMID: 11063921.

Valsamis B, Chang M, Typlt M, Schmid S. Activation of mGluR2/3 receptors in the ventro-rostral prefrontal cortex reverses sensorimotor gating deficits induced by systemic NMDA receptor antagonists. Int J Neuropsychopharmacol. 2014 Feb;17(2):303-12. PubMed PMID: 24067361.

von dem Hagen EA, Stoyanova RS, Baron-Cohen S, Calder AJ. Reduced functional connectivity within and between 'social' resting state networks in autism spectrum conditions. Soc Cogn Affect Neurosci. 2013 Aug;8(6):694-701. PubMed PMID: 22563003; PubMed Central PMCID: PMC3739917.

Waldvogel HJ, Baer K, Eady E, Allen KL, Gilbert RT, et al. Differential localization of gamma-aminobutyric acid type A and glycine receptor subunits and gephyrin in the human pons, medulla oblongata and uppermost cervical segment of the spinal cord: an immunohistochemical study. J Comp Neurol. 2010 Feb 1;518(3):305-28. PubMed PMID: 19950251.

Wan FJ, Swerdlow NR. The basolateral amygdala regulates sensorimotor gating of acoustic startle in the rat. Neuroscience. 1997 Feb;76(3):715-24. PubMed PMID: 9135045.

Wang HL, Morales M. Pedunculopontine and laterodorsal tegmental nuclei contain distinct populations of cholinergic, glutamatergic and GABAergic neurons in the rat. Eur J Neurosci. 2009 Jan;29(2):340-58. PubMed PMID: 19200238; NIHMSID: NIHMS527256; PubMed Central PMCID: PMC3833361.

Weber M, Schmitt A, Wischmeyer E, Döring F. Excitability of pontine startle processing neurones is regulated by the two-pore-domain K+ channel TASK-3 coupled to 5-HT2C receptors. Eur J Neurosci. 2008 Sep;28(5):931-40. PubMed PMID: 18691333.

Weike AI, Bauer U, Hamm AO. Effective neuroleptic medication removes prepulse inhibition deficits in schizophrenia patients. Biol Psychiatry. 2000 Jan 1;47(1):61-70. PubMed PMID: 10650450.

Wible CG, Anderson J, Shenton ME, Kricun A, Hirayasu Y, et al. Prefrontal cortex, negative symptoms, and schizophrenia: an MRI study. Psychiatry Res. 2001 Nov 30;108(2):65-78. PubMed PMID: 11738541; NIHMSID: NIHMS186611; PubMed Central PMCID: PMC2845854.

Yee BK. Cytotoxic lesion of the medial prefrontal cortex abolishes the partial reinforcement extinction effect, attenuates prepulse inhibition of the acoustic startle reflex and induces transient hyperlocomotion, while sparing spontaneous object recognition memory in the rat. Neuroscience. 2000;95(3):675-89. PubMed PMID: 10670435.

Yeomans JS, Bosch D, Alves N, Daros A, Ure RJ, et al. GABA receptors and prepulse inhibition of acoustic startle in mice and rats. Eur J Neurosci. 2010 Jun;31(11):2053-61. PubMed PMID: 20497471.

Zavitsanou K, Cranney J, Richardson R. Dopamine antagonists in the orbital prefrontal cortex reduce prepulse inhibition of the acoustic startle reflex in the rat. Pharmacol Biochem Behav. 1999 May;63(1):55-61. PubMed PMID: 10340524.

Zeilhofer HU, Studler B, Arabadzisz D, Schweizer C, Ahmadi S, et al. Glycinergic neurons expressing enhanced green fluorescent protein in bacterial artificial chromosome transgenic mice. J Comp Neurol. 2005 Feb 7;482(2):123-41. PubMed PMID: 15611994.

Appendix 1: FG injection summary

Mouse Identification	Imaging
112	Leaking
123	Leaking, Manual Injector
027	Miss
817	Miss
308	Miss, leaking
311	Miss, leaking
273	Miss, leaking
274	Partial hit, leaking, no expression in mPFC
862	Miss, leaking
865	Miss
866	Partial hit; leaking in cerebellum, expression in cortex regions, IL, PrL, ACC
299	Partial hit; leaking in cerebellum, expression in cortex regions, IL, PrL, ACC
298	Partial hit; leaking in cerebellum, expression in cortex regions, IL, PrL, ACC
785	Partial hit; leaking in cerebellum, expression in cortex regions, IL, PrL, ACC
786	Partial hit; leaking in cerebellum, expression in cortex regions, IL, PrL, ACC
866-2	Hit; part of halo is rostral and dorsal to PnC. Expression in cortex regions, IL, PL
075	Partial hit; part of injection is rostral to PnC. Expression in cortex regions, IL, PL, ACC
079	Partial hit; part of injection is anterior to PnC. Expression in cortex regions, IL, PL
144	Hit, part of halo is rostral to PnC. Expression in cortex regions, IL, PrL, ACC
281	Partial hit, rostral to PnC. Expression in cortex regions, IL, PrL, ACC
282	Hit, part of halo is rostral to PnC. Expression in cortex regions, IL, PrL, ACC

Appendix 2: AAVDJ-CaMKII-ChR2-eYFP experiment summary

Mouse Identification	Synaptic Response	Input-Output	Paired Pulse
275	No	No	No
376	No	No	No
861	No	No	No
858	No	No	No
857	No	No	No
303-1	Yes	Yes	Yes
304	No	No	No
305	No	No	No
301	No	No	No
793	No	No	No
794	Yes	Yes	Yes
073	No	No	No
278	No	No	No
300	No	No	No
272	No	No	No
279	Yes	No	Yes
302	No	No	No
303-2	Yes	No	Yes
304-2	Yes	Yes	Yes
305-2	No	No	No

Curriculum Vita

Sebastian A. Pace was born in El Paso, Texas as the fourth child to Tracy and Adriana Pace. He graduated from Capt. John L. Chapin High School in 2009, and subsequently his undergraduate studies at the University of Texas at El Paso. He received his Bachelor of Science in Biology in 2013 and was accepted into graduate school in 2014. Sebastian worked as a Teacher's Assistant for biology laboratories: Topics in the Study of Life and Anatomy and Physiology. Additionally, he was a graduate assistant in the Work With A Scientist Program.

Contact Information: sapace711@gmail.com

Sebastian Pace typed this thesis.