4-10-2017

Observing the Functional Maturation of the Female Prefrontal Cortex Using Ventral Hippocampal Stimulation

Lily E. Veldran
Lake Forest College, veldranle@lakeforest.edu

Observing the Functional Maturation of the Female Prefrontal Cortex Using Ventral Hippocampal Stimulation

Abstract
Adolescence is a time when the brain continues to mature. Specifically the prefrontal cortex (PFC) goes through a process of disinhibition to inhibition as we age. Inputs to this region have a frequency dependent maturation pattern in male rats, but this has not been studied in females. We focused on frequency dependent responses in the medial PFC that are evoked by stimulation to the ventral hippocampus. This was conducted by measuring Local Field Potential responses in the medial PFC via in vivo electrophysiology. We found that stimulating with a 10 Hz train response showed no difference across age groups. In contrast, a 20 Hz train of stimulation revealed an age-dependent shift through increasing suppression of the signal. Finally, a 40 Hz train stimulation evoked suppression in all three age groups, but in different magnitudes. With these established patterns we can compare a normal PFC maturation with factors such as chronic drug use and stress.

Document Type
Thesis

Degree Name
Bachelor of Arts (BA)

Department or Program
Neuroscience

First Advisor
Douglas B. Light

Second Advisor
Jean-Marie N. Maddux

Third Advisor
Nicholas L. Wallin

Fourth Advisor
Kuei Y. Tseng, Rosalind Franklin University of Medicine and Science

Subject Categories
Developmental Neuroscience | Neuroscience and Neurobiology

LAKE FOREST COLLEGE

Senior Thesis

Observing the Functional Maturation of the Female Prefrontal Cortex Using Ventral Hippocampal Stimulation

by

Lily E. Veldran

April 10, 2017

The report of the investigation undertaken as a
Senior Thesis, to carry two courses of credit in
the Neuroscience Program

_____	_____
Michael T. Orr Krebs Provost and Dean of the Faculty	Douglas B. Light, Chairperson
	_____ Jean-Marie N. Maddux
	_____ Nicholas L. Wallin
	_____ Kuei Y. Tseng Rosalind Franklin University of Medicine and Science

Abstract

Adolescence is a time when the brain continues to mature. Specifically the prefrontal cortex (PFC) goes through a process of disinhibition to inhibition as we age. Inputs to this region have a frequency dependent maturation pattern in male rats, but this has not been studied in females. We focused on frequency dependent responses in the medial PFC that are evoked by stimulation to the ventral hippocampus. This was conducted by measuring Local Field Potential responses in the medial PFC via *in vivo* electrophysiology. We found that stimulating with a 10 Hz train response showed no difference across age groups. In contrast, a 20 Hz train of stimulation revealed an age-dependent shift through increasing suppression of the signal. Finally, a 40 Hz train stimulation evoked suppression in all three age groups, but in different magnitudes. With these established patterns we can compare a normal PFC maturation with factors such as chronic drug use and stress.

Acknowledgments

The past year and a half on this project has certainly felt much longer. The Tseng lab has many people with extraordinary talents, and I had the incredible opportunity to work with. I am sure there was hesitation when I said I wanted to learn *in vivo* electrophysiology the first time Kuei and I talked face to face. Each one of those apparatuses cost more than I can fathom, but he let me near it anyway. The skills I learned in that lab were things I never thought I would do until I was well into graduate school. Luckily Kuei had some faith in me and this project was born.

I cannot thank Dan Thomases enough as well. Watching me learn to conduct recordings, slice brains, mount tissue, and stain every last slice probably cut time out of his work, but he always answered every questioned, encouraged me that I would get better, and everyone in the lab was in the same boat at one point in their lives.

The amount of support I have gotten from Rosalind Franklin University, Lake Forest College, and friends and family during this processes has been more than I could even imagine. Kuei, Adriana, Dan, and Eden showed me what a hardworking, dedicated academic lab looks like. I was pushed to an intellectual level that I never thought was possible. Whether it was sitting in Kuei's office trying to figure out how to interpret my results and learning was plasticity actually meant, to painstakingly learning Dan's mastery of mounting tissue, and asking Adriana everything there is to known about PV, fast-spiking neurons, to being in complete awe at Eden's *in vitro* skills.

Lake Forest College was one of the best choices I have made in my life. I was fortunate to have two amazing advisors throughout my college career, Dr. Light and Dr. DebBurman, who gave me more than enough guidance through this process. Whenever I

doubted myself, whether in classes, this thesis, or life in general, both of them helped me pick myself up and continue on this path.

My experience during this processes has made me excited for my future in science. Finishing this project has made me realize that I may doubt myself in finishing something this daunting, but looking back on the journey makes it all worthwhile.

With infinite gratitude,

Lily

Table of Contents

Abstract i

Acknowledgments ii

List of Figures v

List of Abbreviations vi

Introduction 1

Brain anatomy and development 1

The Hippocampus 6

Neurotransmitters 8

Brainwaves 16

Methods 23

Animals 23

Experimental Groups 23

Local Field Potential (LFP) Recordings of PFC Responses to Ventral Hippocampal Stimulation In Vivo 23

Data Analysis and Statistics 28

Results 29

Electrode Placement Confirmation and Stimulation 29

Medial Prefrontal Cortex Response to Stimuli of Increasing Intensity 31

Medial Prefrontal Cortex Response from Train Stimuli at Different Frequencies 32

Female Rodent mPFC Response from vHipp Stimulation at Different Frequencies 34

Discussion 38

Future Studies 46

Conclusion 50

References 52

List of Figures

Figure 1: Human versus rodent brain 3

Figure 2: Outline of four major brain lobes 4

Figure 3: Brodmann Areas outlining the prefrontal cortex 5

Figure 4: Three Major divisions of the prefrontal cortex 6

Figure 5: Dorsal and Ventral Streams of the Hippocampus 7

Figure 6: Steps during an action potential 9

Figure 7: Glutamate, GABA, and Dopamine Pathways 12

Figure 8: Overview of cellular pathway changes in the prefrontal GABAergic system . 16

Figure 9: The five major brainwave patterns 17

Figure 10: The four wave patterns generated in the hippocampus. 21

Figure 11: Medial prefrontal cortex coordinates 24

Figure 12: Ventral hippocampal coordinates 25

Figure 13: Verification of electrode placement with Nissl staining 30

Figure 14: Example of a single pulse given to the vHipp and recorded in the medial prefrontal cortex 30

Figure 15: Example of input-output curve to determine optimal amplitude 31

Figure 16: Classification of LFP response patterns from 10, 20, and 40 Hz stimulation trains 33

Figure 17: 10 Hz frequency stimulation elicits facilitation in all ages 34

Figure 18: 20 Hz frequency stimulation shows an age dependent shift from a transient facilitation to a transient suppression 35

Figure 19: 440 Hz frequency stimulation elicits differences in the strength of suppression based on age group 36

List of Abbreviations

ANOVA: Analysis of Variance

CAS: Conative Assessment System

CB: Calbindin

CR: Calratinin

EEG: Electroencephalogram

FDA: Food and Drug Administration

fMRI: Functional Magnetic Resonance Imaging

FSI: Fast-Spiking Interneurons

GABA: gamma-Aminobutyric acid (γ-Aminobutyric acid)

Hz: Hertz

IACUC: Institutional Animal Care and Use Committee

LFP: Local Field Potential

LSD: Least Significant Difference

LTD: Long Term Depression

LTP: Long Term Potentiation

mPFC: Medial Prefrontal Cortex

MRI: Magnetic Resonance Imaging

ms: millisecond

NIH: National Institute of Health

NMDA: N-Methyl-D-aspartic acid

PBS: Phosphate Buffered Saline

PFA: Paraformaldehyde

PV: Parvalbumin

vHipp: Ventral Hippocampus

VTA: Ventral Tegmental Area

REM: Rapid Eye Movement

SEM: Standard Error of the Mean

Introduction

The World Health Organization (2015) defines adolescence as the time after childhood, but before adulthood, which is about ages 10-19 years old. As we grow out of childhood, we increase our engagement with peers and yearn for acceptance from them, and we think about who we are as a person, as well as self-analyze (Guyer, Silk, & Nelson, 2016). During adolescence, many life events can evoke strong emotional responses that are not typically seen in adulthood. For example, many adolescents engage in behaviors to better themselves (e.g., making friends and gaining independence), but also engage in risky behaviors (e.g., drugs, unsafe driving, and sexual activity). Charles Darwin proposed in his book *Expression and Emotion in Man and Animals* that emotion helps us cope and respond properly to major events in life (Darwin, 1872).

Brain anatomy and development

Experts who study adolescent health and development agree that the greatest threats to young people are self-inflicted accidents like automobile and other accidents, violence, drugs and alcohol use, and sexual risk-taking (Williams, Hombeck, & Greeley, 2002). Early adolescence in humans is associated with a transformation of cognitive thought, which develops into abstract reasoning, as well as cognitive control (Graber & Petersen, 1991). This extensive emotional network is mediated by the prefrontal cortex (PFC) (Miller & Cohen, 2001). A 1997 study done by Casey et al. looked at the development of this behavior and its corresponding activation in the PFC between children ages 7-12, and young adults ages 21-24. Each participant was put in a magnetic resonance imaging (MRI) machine to see how their brain responded to go/no-go tasks. Participants were

shown a sequence of letters, and were instructed that if they saw an "X," they should not press the button. However, if they saw any other letter besides "X" then they were to press the button. This task was designed to test attention and the ability to override a response. They found that children had a more scattered pattern of activation in the PFC than adults. Specifically children activated the dorsolateral region of the PFC as they inhibited the action to press the button when an "X" flashed on the screen. The adults did not have activation of the dorsolateral PFC when an "X" flashed and they did not press the button as much during this presentation. They speculated that the dispersed pattern may relate to the fine-tuning process in the PFC in which relevant connections are not made until maturity. As this network matures during development, less activation is needed to overcome new material presented (Hare & Casey, 2005). Most developmental brain research has focused on the prefrontal cortex (PFC), amygdala, insula, and anterior cingulate cortex regions, because these regions work together to promote learning, assign meaning to certain life events, and integrate sensory information to guide behavior in a certain context (Ernst, Torrisi, Balderston, Grillon, & Hale, 2015).

One might argue that what makes us human is that we have a well-developed cerebrum (cerebral cortex) that allows for executive function (Fuster, 2002). Some of these functions include our ability to store and recall events as memories, inhibit certain emotions, and the ability to solve problems (Frith & Dolan, 1996). There is a clear morphological difference, for example, when comparing the human brain with one from a rat (Fig. 1). In many respects, the rat brain has many similarities to ours. For instance, both structures have a cerebrum to control movements, an olfactory bulb for smell, and a hippocampus to help us with learning and memory functions (Purves et al., 2001)

However, the rat cerebrum is much simpler than a human's. Our cerebrum is significantly larger, and has numerous folds (gyri) and valleys (sulci) to increase its total surface area (Fig. 1). The larger cerebral cortex of a human allows for higher cognitive function (Toro et al., 2008). In addition, the human cerebral cortex is divided into four major lobes; the occipital, parietal, temporal, and frontal lobes (Fig. 2).

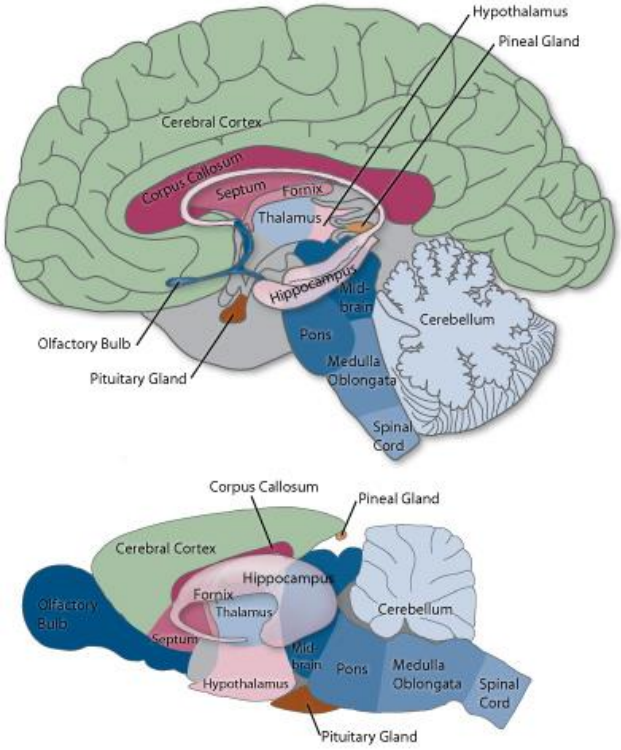

Figure 1: *Human versus rodent brain*. Comparison of the adult human brain (top) and rat brain (bottom). The major difference is not only the overall organ size but most notably the size of the cerebral cortex. Note that in the human brain there are numerous folds (gyri and sulci), which increase surface area and presumably allow higher function to occur (Genetic Science Learning Center, 2013).

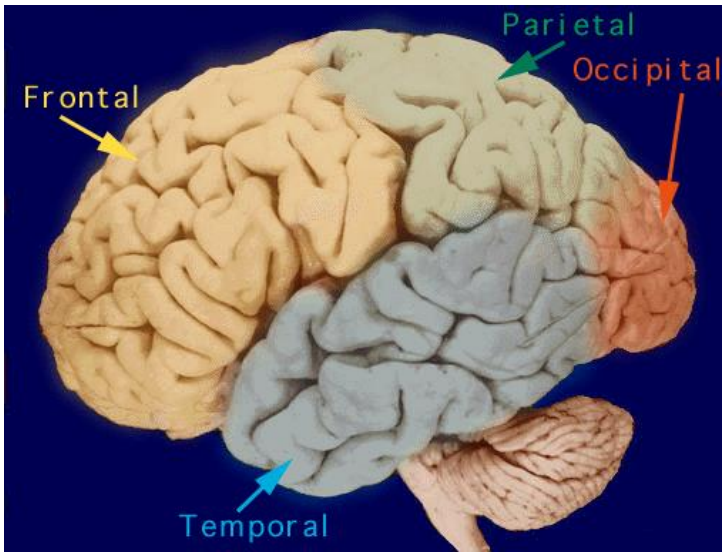

Figure 2: *Outline of four major brain lobes.* The domains and outlines of the four major lobes of the human brain. These regions include the occipital, parietal, temporal, and frontal lobes (Kinser, 2000).

For this study we will focus on the frontal lobe, which deals with reasoning, planning, parts of speech, movement, emotions, and problem solving (Kimberg & Farah, 1993). For this study, we focused on the frontal cortex because it is the last region of the brain to develop (Casey et al., 2005). Specifically we examined Brodmann areas, 8, 9, 10, 11, 44, 45, 46, and 47 (Fig. 3), which define the prefrontal cortex (PFC).

The PFC has three major divisions: the lateral, medial, and orbitofrontal (Fig. 4) (Masterman & Cummings, 1997). Each of these has important connections from the amygdala, hypothalamus, midbrain, and pons (Chatterjee, Kumar, Siddiqui, & Goyal, 2008). These specific regions are associated with certain higher-order functions, such as attention control, inhibitory control, working memory, and problem solving (Diamond, 2013). For instance, the lateral prefrontal cortex is shown to play a role in language, attention, memory, and novelty processing, which is important for learning about new

contexts (Daffner et al., 2000). In contrast, the orbitofrontal cortex of the PFC plays a significant role in social and emotional behaviors (Gold, Berman, Randolph, Goldberg, & Weinberger, 1996). Specifically, it anticipates reward by processing the possible outcomes of the present context (Stuss & Benson, 1986). Finally, the medial prefrontal cortex (mPFC) is important for attention to cognitive tasks, spatial memory, and conflict resolution. In particular, the lower region of the mPFC (ventral-medial PFC) helps us make decisions (Spinella, Yang, & Lester, 2004).

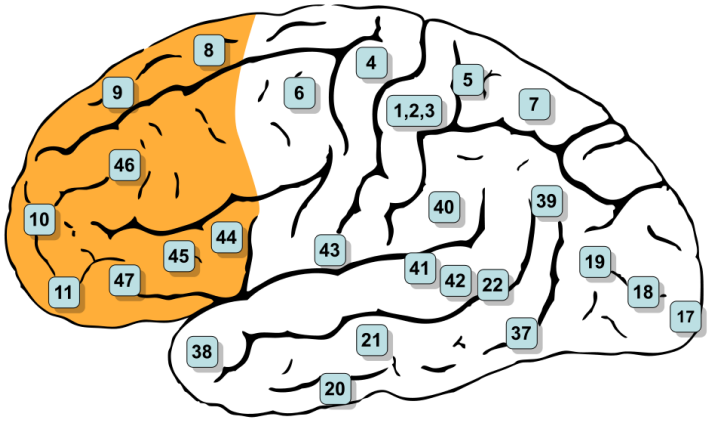

Figure 3: *Brodmann Areas outlining the prefrontal cortex.* Areas of the human brain as defined by the anatomist K. Brodmann (Finger, 2001). The regions in orange define the prefrontal cortex (Carter & Gray, 1918).

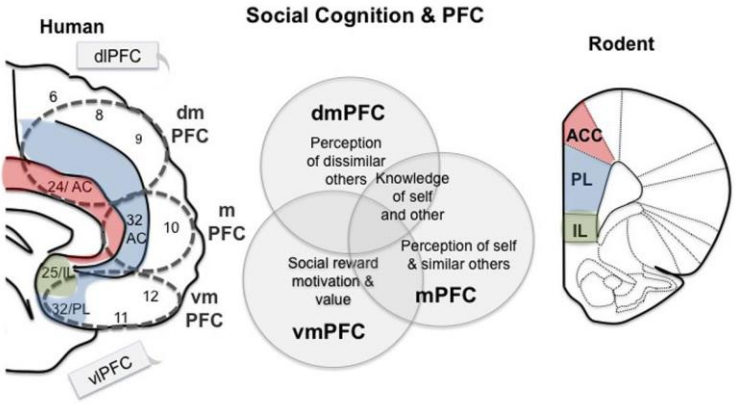

Figure 4: *Three Major divisions of the prefrontal cortex.* A comparison of the three major divisions of the prefrontal cortex (PFC) in human and rat brains. The medial portion of the PFC is related to social behaviors between organisms, such as interacting with peers and showing empathy. Both the dorsal and ventral portions of the lateral PFC (dlPFC and vlPFC) are related to social tasks. The vlPFC specifically encodes information of social reward and punishment to encode emotional value to objects and ideas. Both the dorsal and medial portions of the PFC are active when we judge the trustworthiness of someone (Bicks, Koike, Akbarian, & Morishita, 2015).

The Hippocampus

Many studies have shown that certain disorders such as anxiety, depression, apathy, and disruptive behavioral disorders involve prefrontal lobe dysfunction (Chatterjee et al., 2008). Additional studies have shown that as all of these regions develop, they receive signals from dopaminergic terminals in the cortex (Thierry, Blan, Sobel, Stinus, & Glowinski, 1973), hippocampal (Carr & Sesack, 1996), and amygdala neurons (Bacon, Headlam, Gabbott, & Smith, 1997). In fact, recent evidence has confirmed that the PFC receives significant input from the amygdala and the ventral hippocampus (Caballero, Granberg, & Tseng, 2016). For this study, we only focused on the medial prefrontal cortex, because this region receives the most innervation during maturation (Benes et al., 1993). In particular, for this preliminary study we only focused on the ventral hippocampal-prefrontal cortex pathway.

The hippocampus resides in the medial temporal lobe (Gray, 1918). The entire hippocampal region helps with memory and cognition (Scoville & Milner, 1957), as well as emotional processing (Gray & McNaughton, 2000). However, lesion studies have shown that the hippocampus has two streams of processing, the dorsal and ventral streams (Swanson & Cowan, 1977).

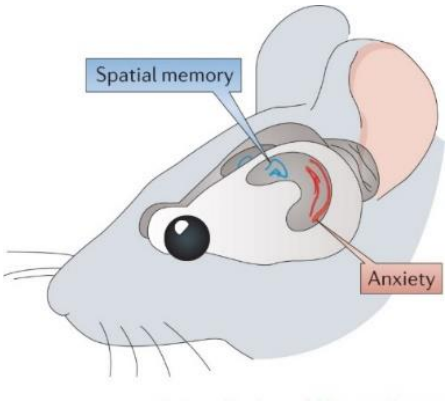

Figure 5: *Dorsal and ventral streams of the hippocampus*. The hippocampus can be separated functionally into dorsal (upper) and ventral (lower) pathways (Bannerman, et al., 2014). The dorsal stream helps gather and retain information about the environment around us (Moser et al., 1995). The ventral stream helps process spatial (environmental) memory (Ferbinteanu & McDonald, 2001). The ventral stream projects to the prefrontal cortex.

The dorsal stream processes spatial memory (Moser, Moser, Forrest, Andersen, & Morris, 1995), which is memory that retains information about your environment, like your neighborhood layout. The ventral stream processes the emotional significance of certain environments (Ferbinteanu & McDonald, 2001). For example, normally, a rat would be cautious and show stress before entering an unprotected region of a maze. However, Ferbinteanu and McDonald (2001) showed that rats with a lesioned ventral hippocampus did not fear entering into an unprotected arm of a maze to get to a food reward. As previously stated the ventral hippocampus plays a critical role in innervating

the PFC. One of the main functions of the ventral hippocampus (vHipp)-PFC pathway is to help process our working memory (Freidman & Goldman-Rakic, 1988). However this pathway does not fully develop until adulthood (Luna, Garver, Urban, Lazar, & Sweeney, 2004).

Neurotransmitters

The driving factor mediating this vHipp-PFC pathway are small chemical molecules called neurotransmitters. Neurotransmitters allow for communication between neurons in the brain, and neurons and muscles in the periphery. The neurotransmitter molecules start in one neuron end (pre-synaptic terminal), are released into a gap called the synapse, and are accepted by the next neuron at a site called a receptor (Lodish, et al., 2000). These chemicals can have two different effects on the postsynaptic membrane: hyperpolarization (inhibitory effect) and depolarization (excitatory effect) (Trautwein, 1963). These effects are measured by the difference in voltage between the inside and outside of a neuron and the change in this voltage caused by postsynaptic receptor binding to a neurotransmitter (Purves, Augustine, & Fitzpatrick, 2001) (Fig. 6). This difference in voltage is known as the membrane potential. Most neurons have a resting potential of about -70 mV (Lewis, et al., 2011).

If hyperpolarization occurs, it makes the membrane potential more negative. This is caused by a shift of ions in and out of the cell, specifically if positive potassium ions leave the cell, or negative chloride ions enter the cell (Hille & Catterall, 1999). With depolarization, the cell becomes less negative because positive sodium ions rush into the cell (Hille & Catterall, 1999). If the neuron membrane potential reaches threshold about -

55 millivolts, the neuron's axon will transmit all-or-none action potentials, signals that travel the length of the neuron's axon (Burke, Kiernan, & Bostock, 2001). When hyperpolarization occurs, the post-synaptic membrane voltage increases in magnitude, thereby making the neuron less likely to reach threshold and initiate action potentials (Purves et al, 2001).

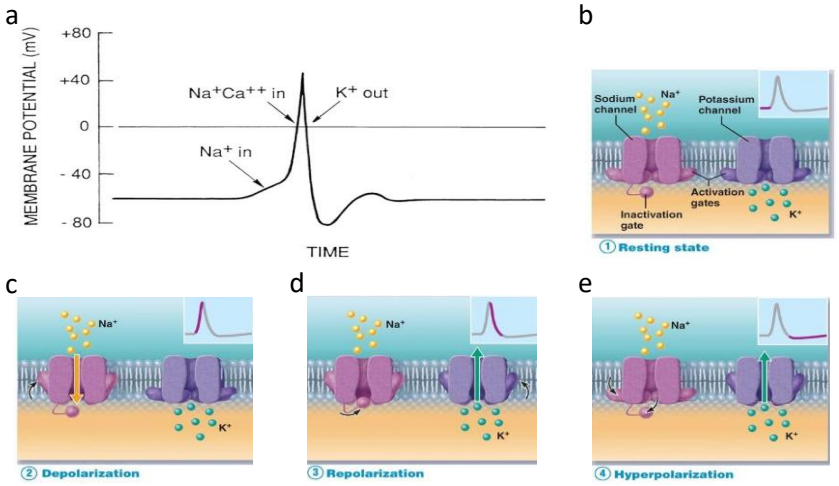

Figure 6: *Steps during an action potential*. There are three major steps in an action potential after the membrane potential reaches threshold, depolarization, repolarization, and hyperpolarization. **a)** The electrical potential changes that occur in the cell during an action potential (Institute of Medicine (US) Committee on a National Neural Circuitry Database, 1991). **b)** A cell rests at a negative membrane potential of about -60 millivolts which means the inside of the cell is more negative than the outside. **c)** If the cell reaches -55mV, due to the Na+ voltage gated channels being activated to open, Na+ ions rush in the cell down its electrochemical gradient causing the membrane voltage to become positive. **d)** Eventually voltage gated K+ channels are activated and K+ ions leave the cell, causing a negative potential inside the cell once more. **e)** Na+ channels close, but K+ channels are still open, causing a slightly more negative potential than resting (Byrne, 2011).

Action potentials are voltage changes by which neurons transmit signals, thereby stimulating the release of neurotransmitter to communicate with another neuron it innervates (Siegel et al, 1999). There are a number of different neurotransmitters, and some of the major ones in the brain include glutamate, γ-aminobutyric acid (GABA),

acetylcholine, dopamine, serotonin, norepinephrine, epinephrine, and histamine (Purves et al., 2001). In this study we focused on glutamate, GABA, and dopamine because these neurons project to the rat PFC and change during peri-adolescence (Caballero et al., 2016) (Fig 7).

Glutamate is an amino acid neurotransmitter that specializes in producing an excitatory response (Purves et al., 2001). If glutamate concentrations become too high, excitotoxity can occur, which causes neurons to become overexcited and die (Rothman, 1983). On the other hand, GABA inhibits neurons so they are less likely to transmit action potentials (Purves et al., 2001). GABA is created through the breakdown of glutamate with vitamin B_6 (Nikolaus, Antke, Beu, & Müller, 2010). GABA is also important because one of its precursors is derived from vitamin B_6 Without vitamin B_6, glutamate cannot be broken down into GABA and can cause adults to experience anxiety and infants can experience seizures (Petty, 1995). Dopamine plays a major role in executive function (Logue & Gould, 2014), motor control (Brooks, 2001), motivation arousal (Ikemoto & Panksepp, 1999), reinforcement (Holroyd & Coles, 2002), and reward (Berridge & Robinson, 1998) in the brain.

There are three major dopaminergic pathways in the brain: the mesocorticolimbic projection, the nigrostriatal pathway, and the tuberoinfundibular pathway (Sian, Youdim, Riederer, & Gerlach, 1999). The mesocorticolimbic projection has two sub-regions: the mesocortical pathway, and the mesolimbic pathway (Sian et al., 1999). The mesocortical pathway projects from the ventral tegmental area (VTA) to the prefrontal cortex, and dopamine in this pathway plays a role in cognitive control, motivation, and emotional response (Malenka, Nestler, & Hyman, 2009).

In this study, we examined the hippocampus-PFC pathway because it is modulated by at least three neurotransmitters: dopamine (Goldman-Rakic, Leranth, Williams, Mons, & Geffard, 1989), glutamate (Floresco, Seamans, & Phillips, 1997), and GABA (Caballero, Thomases, Flores-Barrera, Cass, & Tseng, 2014). When the PFC has fully matured, dopamine has full control over functions such as working memory, inhibitory control, and attention (Horvitz, 2000). Dopamine's control is mediated by the amount of excitatory and inhibitory neurons innervating the PFC (Tseng, Chambers, & Lipska, 2009).

The excitatory effect of dopamine is mediated by dopamine receptor D1, which enhances glutamatergic transmission via the NMDA glutamate receptor, and therefore can have different behavioral effects (O'Donnell, 2010). If D1 receptors in the PFC are activated, memory retrieval and working memory are enhanced (Seamans, Floresco, & Phillips, 1998). If both the D1 and NMDA receptors are activated in the PFC, appetitive behaviors such as searching for food when you are hungry are exhibited in adult rats (Baldwin, Sadeghian, & Kelley, 2002).

When the D1 receptors are activated in the mature adult PFC, there is a lasting depolarization effect in the pyramidal neurons. However, pyramidal neuron transmission is mediated by NMDA receptors and calcium-dependent signaling. Although, this long-lasting effect is not fully present until late adolescence, or around P45 in rats (Tseng & O'Donnell, 2005). Therefore, the excitatory effects seen in the PFC are a product of D1 and NMDA receptor interactions (Baldwin et al., 2002). The major importance of this effect is its strong impact on plasticity, and the emergence of adult behaviors during the transition from adolescence to adulthood.

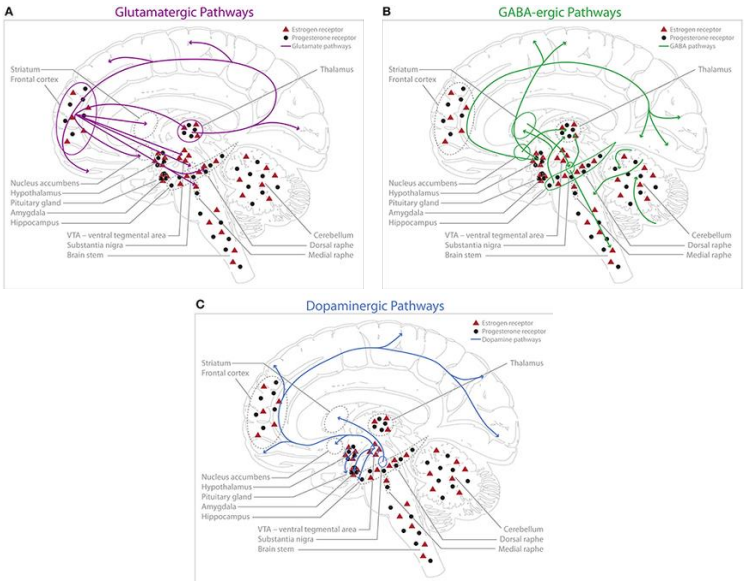

Figure 7: *Glutamate, GABA, and Dopamine Pathways.* Above are the three major neurotransmitters we focused on and where they project in the brain (Barth, Villringer, & Sacher, 2015). **A)** The glutamatergic pathway is mainly excitatory (Purves et al., 2001). **B)** The GABAergic pathway is opposite in the sense that it is mainly inhibitory (Purves et al., 2001). **C)** The dopaminergic pathway has many roles including executive function (Logue & Gould, 2014), motor control (Brooks, 2001), motivation arousal (Ikemoto & Panksepp, 1999), reinforcement (Holroyd & Coles, 2002), and reward (Berridge & Robinson, 1998).

The inhibitory effect of dopamine involves D2 receptors (Tseng & O'Donnell, 2004) and GABAergic interneurons in the PFC that contain D1 and D2 receptors (Vincent, Khan, & Benes, 1993). Inhibition in the PFC can also be caused by a facilitation in GABAergic neurons (Gorelova, Seamans, & Yang, 2002). However, during the juvenile age, P25-35, recordings show that the GABAergic interneurons only have D1 receptors, which increases the excitability of fast-spiking interneurons (FSI) (Tseng et al., 2006). Although fast-spiking interneurons can maintain an intense inhibitory signal to GABAergic neurons, this is not as effective later in life. After late adolescence (around P50), there is a strong excitability of D2 receptors, which in turn synapse onto

GABAergic neurons in the PFC (Kalsbeek, Voorn, Buijs, Pool, & Uylings, 1988). The development of both D1 and D2 receptor control over GABAergic cells in the PFC allows for greater inhibitory control, which can also affect the timing of local neuron populations for skills like working memory (Lewis & Gonzalez-Burgos, 2006).

Another important neurotransmitter in adolescent development is glutamate (Gleich et al., 2015). Neurons that contain glutamate carry contextual and emotional information from the ventral hippocampus (Floresco et al, 1997) and amygdala (Garcia, Vouimba, Baudry, & Thompson, 1999). These hippocampal-PFC and amygdala-PFC pathways also continue to develop during adolescence (Cressman et al., 2010; Seamans et al, 1998). In a 2013 study, Thomases et al. found that in three different age groups (juvenile, adolescence, and adulthood), there is a distinct difference in PFC response due to ventral hippocampal (vHipp) stimulation. This pattern shows that at a frequency of 20 Hz and higher, male rats P30-40 show a facilitation of the vHipp signal in the medial PFC. However, male rats P45 and older show a slight inhibition of this signal (Thomases, Cass, & Tseng, 2013).

These studies show that as the brain, specifically the hippocampal-PFC pathway, develops from childhood to adulthood, there is an increase in the intensity of an overall inhibitory effect. This pathway is mediated by increased expression of a specific subunit of the NMDA receptor, called GluN2B (Caballero et al, 2016). Recent studies have shown that NMDA receptors with GluN2B are important for adult PFC-dependent functions like working memory (Wang et al., 2013) and fear conditioning (Gilmartin, Kwapis, & Helmstetter, 2013). Another important factor of GluN2B in NMDA receptors

is its ability to selectivity amplify important information from events originating in the ventral hippocampus (Flores-Barrera et al., 2014).

All of the studies above have shown that the excitatory neurotransmitter glutamate plays an important role in how we perceive our world. Working memory and emotional context help our PFC make decisions about our environment based on past experience (Kensinger, Clarke, & Corkin, 2003). On a molecular level, we can see that this skill develops as we age and is seen by increased amounts of GluN2b subunits, and the strengthening of the vHipp-PFC pathway to control neuronal timing.

The final main neurotransmitter that affects PFC development is GABA. As previously mentioned, dopamine regulates GABAergic modulation, but the local GABAergic system in the PFC also undergoes significant changes (Uhlhaas & Singer, 2011). Specifically, the number of GABA interneurons that contain the proteins parvalbumin (PV), calratinin (CR), and calbindin (CB) change significantly in the prefrontal cortex (Caballero, Flores-Barrera, Cass, & Tseng, 2014). The number of GABAergic interneurons in a particular neuron population can be measured by looking at levels of PV, CR, and CB, as these proteins make up more than 80% of GABAergic cells (Gabbot, Dickie, Vaid, Headlam, & Bacon, 1997).

Changes in these proteins can impact the functional characteristics of their interneurons, which in turn can affect the inhibitory control of the PFC output (Kinney et al., 2006). This alteration in PFC output is mainly seen in PV-positive interneurons. Kinney et al. (2006) found that if glutamatergic transmission is blocked, PV levels decrease in the PFC. As discussed, glutamate is crucial in the development of a functional PFC. Based on evidence from the studies described above, glutamate will transmit a

signal to GABAergic interneurons in the PFC, which will produce an inhibitory effect. However, PV-positive, FSIs are between the glutamate input signals to the GABAergic neuron. Therefore, changes in PV levels alters the effects of inhibitory control in the PFC.

Caballero et al. (2014) reported that PV levels follow a developmental pattern. They stained sections of the mPFC in juvenile, adolescent, and adult rats. Fluorescent immunostaining revealed that PV expression in neurons was most abundant in adolescence and adulthood, compared to juvenile rats. Another characteristic of the stain was the pattern of expression.

In juvenile rats (P25-35), PV-positive interneurons showed a small concentrated area of fluorescence in the mPFC. In the adolescents and adults (P45-55 and P65-75 respectively), there was a wider and more intense stain representation. Caballero et al. (2014) also examined signal recordings from a single PV-positive cell. They showed there was a stronger glutamatergic signal in PV-positive cells after P35 than in the P25-35 group. Linking these observations together, it can be said that the developmental increase in PV expression correlates with the increase in PV signaling and glutamatergic input during adolescent development. Subsequently, the GABAergic system plays a crucial role in producing an inhibitory effect in the PFC, but the system itself undergoes developmental changes as well. This is seen by an increase in glutamatergic transmission on PV, fast-spiking interneurons, which produce a strong signal onto GABAergic interneurons to the PFC.

Overall, the mediation of glutamatergic neurons, PV-positive cells, and GABAergic transmission from adolescence to adulthood is summarized in Figure 8. This shows how

upregulation of these factors allows for proper inhibitory control when higher frequency inputs arrive at these neurons.

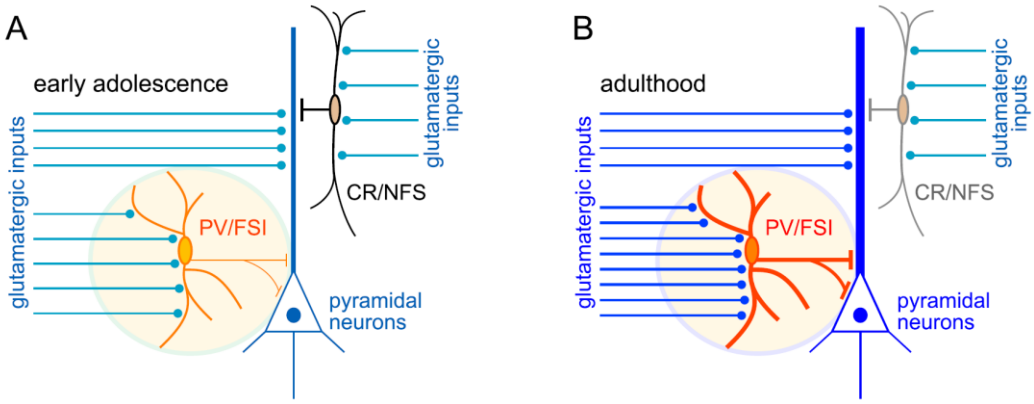

Figure 8: Overview of cellular pathway changes in the prefrontal GABAergic system. Here Caballero et al. (2016) summarized what we know thus far about the development of inhibitory control through the male prefrontal GABAergic system. Early adolescence shows less glutamatergic input, to fewer FSIs, onto minimal GABAergic interneurons. In adulthood, the number of GABAergic interneurons increases in the PFC, PV expression increases, causing an increase in FSIs, and glutamatergic inputs increase to these FSI cells. Overall, this creates a more efficient system to handle excitatory inputs, and be able to suppress the signal if needed.

Brainwaves

The action of neurotransmitters to alter membrane potential in the brain is the underlying cause of regular electrical patterns known as brainwaves (Tsien & Barrett, 2013). Brainwaves were first recorded in 1929 as cyclic wave-like changes in voltage, and thus were named brainwaves (Berger, 1929). These waves are categorized as delta, theta, alpha, beta, and gamma (Purves et al, 2001) (Fig 9). The slowest wave pattern is the delta wave, which has a frequency of 0.2-3 Hertz (Hz). This pattern is seen when we are in deep sleep (Botella-Soler, Valderrama, Crepon, Navarro, & Le Van Quyen, 2012). The next wave, theta, oscillates at a frequency of 3-8 Hz, and are present during light

sleep and extreme relaxation (Wickramasekera, 1977). Alpha waves have a frequency of 8-12 Hz, and they are evoked when one is in a relaxed state. They are most present when an individual wakes up in the morning (Huang & Charyton, 2008). Beta brainwaves oscillate at a frequency of 12-30 Hz. They are present when a person is wide-awake and active during the day (Walter & Matthews, 1934). The final brainwave pattern, gamma, oscillate at 30-120 Hz, and are important for the formation of ideas, language, memory processing, and learning (Miltner, 1999).

Many human brainwave studies use a technique called an electroencephalography (EEG). This method measures brain activity using electrodes attached to the skin on the scalp (Britton et al., 2016). Results from this machine show the amplitude and frequency of brain waves (Timofeev, Bazhenov, & Seigneur, 2012). Although you can calculate where a signal is the strongest to see which brain region it is in, this does not tell you exactly where that signal originated. (NeuroCognitive Imaging Lab, 2017).

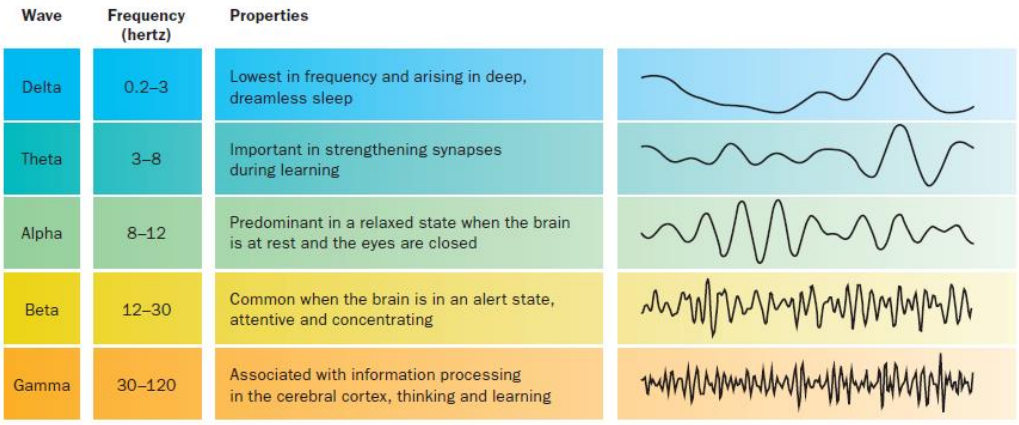

Figure 9: *The five major brainwave patterns.* Brainwave patterns were determined through EEG examinations (Fields, 2016). In this study we stimulated the hippocampus at alpha, beta, and gamma frequencies.

It has been shown that brainwave electrical activity early in one's life controls several developmental processes, such as the differentiation of neurons, migration of neurons to their appropriate place in the brain, creating specific synapses, neurotransmitter specification, and strengthening and weakening of synapses (Zhou & Poo, 2004; Moody & Bosma, 2005). Immediately following birth, a neonate displays intermittent bursts of delta waves, and this phenomenon is suppressed as we age (Drefus-Brisac & Larroche, 1971). In the adolescent phase of life (age 10-19), it has been shown that during sleep the brain produces delta brain waves at a lower amplitude than in adulthood (Feinberg, Higgins, Khaw, & Cambell, 2006).

In a recent review, studies have shown that the hippocampus can generate three distinct wave patterns: theta, sharp-wave ripples, and gamma waves, all of which correlate with specific behaviors (Colgin, 2016) (Fig 10). For example, theta waves in the rat hippocampus have been shown to not only be present during REM sleep, but also play an essential role in learning and memory (Landfield, McGaugh, & Tusa, 1972). On a cellular level, theta waves are rhythmically mediated by GABAergic neurons projecting into the hippocampus (Freund & Antal, 1988).

The second wave pattern in the hippocampus is the sharp wave-ripple pattern. These waves have large amplitudes (height of wave) and occur irregularly (Schlingloff, Kali, Freund, Hajós, & Gulyas, 2014). This pattern emerges during slow-wave sleep (Buzsaki, 1986). They also have a functional role of consolidating significant memories and erasing traces that are insignificant during sleep (Lee & Wilson, 2002). The sharp part of the wave represents excitatory neural activity, and starts in the CA3 region, transmitting its signal to the CA1 region (Buzsaki et al., 1986), whereas the ripples are

locally generated in the CA1 region (Schlingloff et al., 2014). It has been proposed that the ripple formation plays a role in memory consolidation, because during a sharp wave-ripple, CA1 cells are depolarized by the sharp wave and are also inhibited by the ripples (English et al., 2014). This inhibition raises the threshold for initiation of action potentials, thereby preventing most neurons from firing. Therefore, the ripples may only select cells that encode memories for consolidation to then be moved to long-term storage (Colgin, Kubota, Jia, Rex, & Lynch, 2004).

Finally, gamma waves are present in the hippocampus. This specific pattern correlates with a variety of behaviors like preparatory motor movements (Kristeva-Feige, Feige, Makeig, Ross, & Elbert, 1993), auditory detection (Jokeit & Makeig, 1994), auditory attention (Bertrand, 1998), and complex task processing (Spydell & Sheer, 1982). Gamma waves also have lower amplitudes than the theta and sharp wave-ripple rhythms (Buzsaki, Leung, & Vanderwolf, 1983).

The gamma frequency in the CA1 has a large range, from 30-120 Hz (Colgin et al., 2009). In fact, this range has been divided into two distinct frequencies. At the 30-55 Hz range, low gamma waves occur and is driven by CA3 input (Schomburg et al., 2014). The other type of gamma wave, fast gamma, has a higher frequency range, from 60-120 Hz and are mediated by inputs from the medial entorhinal cortex onto the hippocampus (Schomburg, et al., 2014).

The role fast gamma waves play in memory is still poorly defined. Some studies have concluded that since these waves are in the entorhinal cortex, which processes sensory information, the fast gamma waves encode sensory information in memory (Kemere, Carr, Karlsson, & Frank, 2013). In contrast, other studies have found that fast

gamma rhythms are involved in working memory rather than memory encoding (Yamamoto, Suh, Takeuchi, & Tonegawa, 2014).

The slow gamma waves are thought to be involved with memory retrieval because one of the functions of the CA3 region is to store and retrieve memories (Nakazawa, et al., 2002). It is also known that slow gamma waves in the CA1 are synchronized by inputs from the CA3 region (Colgin et al., 2009). However, there is more than one finding relevant to the memory retrieval hypothesis. Some have shown that slow gamma waves are involved with memory retrieval when they are reactivated for memories of earlier experiences (Pfeiffer & Foster, 2015), whereas others have concluded that the memory retrieval from CA3 to CA1 is more intense when an animal is in a new environment (Kitanishi et al., 2015).

Knowing the range of hippocampal frequencies allows us to pick specific frequencies to stimulate in the vHipp. Specifically, it is know that at alpha waves (8-12 Hz), NMDA receptors are activated (Flint & Connors, 1996). When beta waves at 20 Hz, and low gamma waves around 50 Hz are elicited, fast-spiking interneurons projecting to GABAergic neurons become active (Berke, 2011).

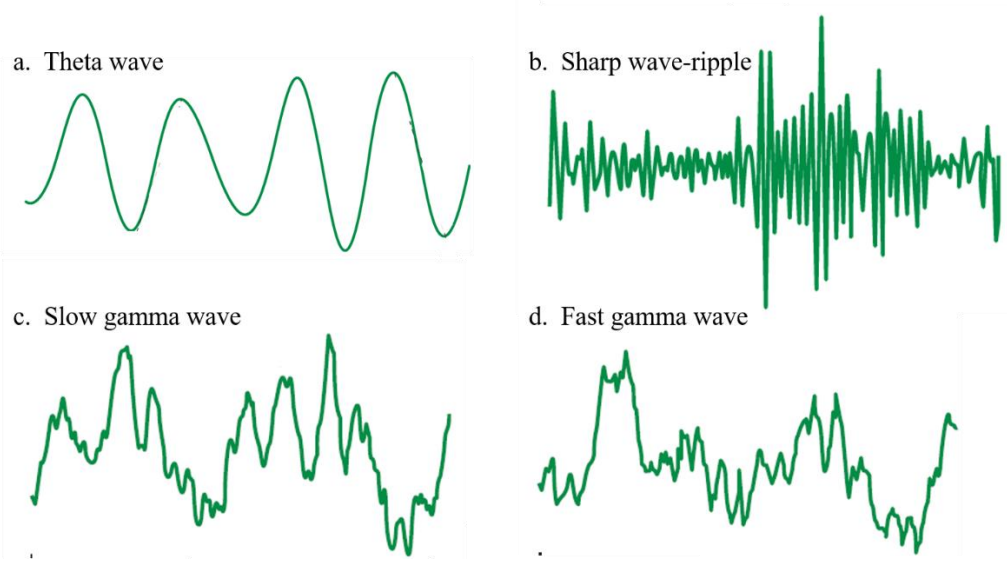

Figure 10: *The four wave patterns generated in the hippocampus*. Colgin (2016) defines the four wave patterns generated in the hippocampus as **a)** theta waves, which have control in REM sleep and learning and memory (Landfield, McGaugh, & Tusa, 1972). **b)** Sharp wave-ripples appear in large amplitudes and irregularly. They are important for consolidating important memories, and erasing others (Lee & Wilson, 2002). **c)** Slow gamma waves are a subset of the gamma wave pattern. The function is still debated between memory retrieval and reactivation of memories (Pfeiffer & Foster, 2015). **d)** The function of fast gamma waves is also controversial between encoding sensory information (Kemere et al., 2013) and working memory processing (Yamamoto et al., 2014).

Adolescence is an important time to study brain function, because it is vulnerable in this period. That is, if brain development is impaired, disorders such as schizophrenia, bipolar disorder, and anxiety can occur (Hoffman & Lewis, 2011). Related to this, it has been shown that the PFC can be impaired if cannabinoids (Cass et al., 2014), ketamine (Thomases, Cass, Meyer, Caballero, & Tseng, 2014), or cocaine (Cass, Thomases, Caballero, & Tseng, 2013) are used during the adolescent period in rats (about 35-60 days after birth). Compared to control rats, those who received chronic drug treatment show disinhibition in the PFC at hippocampal stimulation frequencies of 20 and 40 Hz (Cass et al., 2013). However, these studies have only been conducted in male rats. Given

the importance of understanding how the brain matures, it would be helpful to determine whether it is the same in both sexes.

Understanding brain function in both sexes also is important when prescribing drugs. In 2013, the FDA released a safety announcement for the public about the insomnia drug zolpidem (Ambien, Edluar, and Zolpimist). Although this drug is not related to brain maturation, it is significant to mention because it has been shown that zolpidem remains in the bloodstream in females longer than males (Food and Drug Administration, 2013). As a consequence, a normal dose prescribed for women has a higher probability of remaining in the bloodstream, thereby potentially increasing the likelihood of a motor vehicle accident (Hansen, Boudreau, Evel, 2015). Accordingly, the NIH will create appropriate policies to take into account differences between male and female subjects in future studies (Clayton & Collins, 2014).

Given that studies with female brain maturation have not been described, we used female rats in three different age categories: preadolescence, adolescence, and adulthood to determine how the medial prefrontal cortex develops with ventral hippocampal stimulation. Based on what we have seen in male rats for their normal development, we hypothesized that as female rats age from preadolescence to adulthood, more inhibitory control will be seen in the medial prefrontal cortex at higher ventral hippocampal stimulation frequencies.

Methods

Animals

All experimental procedures followed guidelines set by the National Institutes of Health *Guide for the Care and Use of Laboratory Animals.* In addition, methods were approved by the Rosalind Franklin University Institutional Animal Care and Use Committee (IACUC). In this study female Sprague Dawley (Envigo, Indianapolis, IN) rats from different age groups were used. They were housed two to three animals per cage and maintained under conditions of constant temperature (21º C - 23º C). Females were kept in a 12-hour light/dark cycle with food and water available *ad libitum*.

Experimental Groups

All animals were allowed to habituate for at least 5 d before being subjected to any experimental manipulation. Rats were separated into three age groups: postnatal day 30-35 (P30-35) (n=8), P40-45 (n=9), and P50 or older (n=9).

Local Field Potential (LFP) Recordings of PFC Responses to Ventral Hippocampal Stimulation In Vivo

Measurements of prefrontal cortex maturation came from local field potential recordings from this region. Responses were recorded from stimulation to the ventral hippocampus *in vivo*. Animals were anesthetized with 8% chloral hydrate (400 mg/kg, intraperitoneal). Females were placed in a stereotaxic apparatus (ASI Instruments, Warren, MI) and maintained at 37-38° C using a Physitemp PCAT-2LV Controller (Physitemp Instruments, Clifton, NJ). Lidocaine (2% lidocaine hydrochloride with 1:100,000 epinephrine; Cooke-Waite) was applied subcutaneously under the skull before any incisions in the skin were made. After the rat was properly placed in the frame,

anesthesia was maintained with 8% chloral hydrate at a rate of 400 µl/h. This was delivered through an intraperitoneal cannula attached to an automated syringe system (BASi Baby Bee Syringe Drives, West Lafayette, IN). The level of general anesthesia was set based on the overall cortical electroencephalogram (EEG) activity. A small hole in the skull was drilled for electrode placement in the medial prefrontal cortex (mPFC) and ventral hippocampus (vHipp). Coordinates were determined based on coronal brain sections depicted in a rat brain atlas (Paxinos & Watson, 1998). Coordinates for the mPFC (Fig. 11) were 3.2-2.7 mm anterior to bregma, 0.8 mm lateral from the midline of the brain, and the electrode was placed 4.2-5.2 mm below the brain surface.

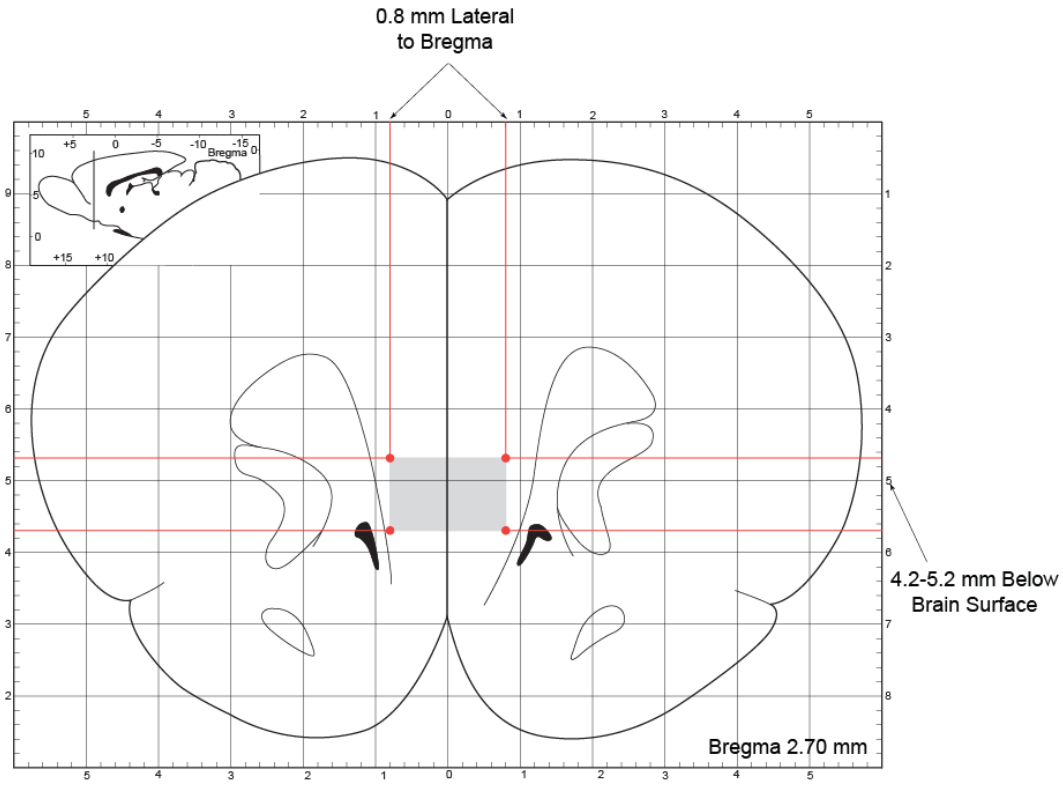

Figure 11: *Medial prefrontal cortex coordinates*. Medial prefrontal cortex coordinates from Paxinos and Watson's rat brain atlas (1998), which was used as a guide for experiments in this study.

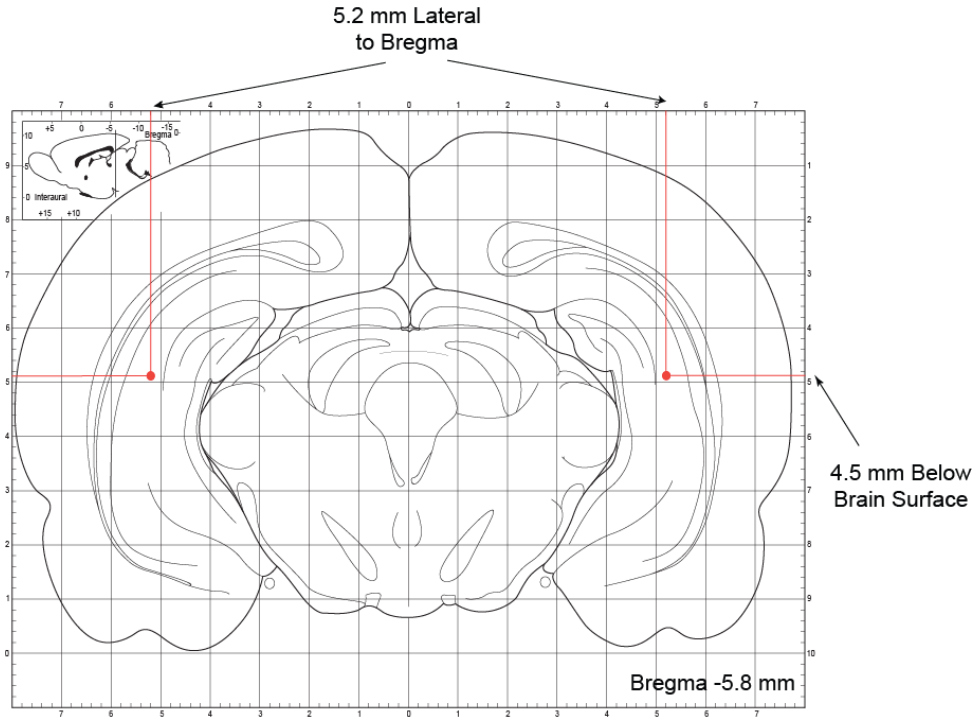

Figure 12: *Ventral hippocampal coordinates*. Ventral hippocampal coordinates from Paxinos and Watson's rat brain atlas (1998).

For the vHipp (Fig. 12), a hole was drilled 5.8 mm posterior to bregma, 5.2 mm to the left or right of the midline, and the electrode was placed 4.5 mm below the brain surface. LFPs were recorded in the mPFC and stimulation was applied in the vHipp using concentric bipolar electrodes (SNE-100x, 50 mm extension from mounting point, Rhodes Instruments, Tujunga, CA). The current coming from the cells was amplified (IR283A, Cygnus Technology, Delaware Water Gap, PA), filtered to remove signals with a bandwidth of 1-100 Hz, and sampled at 10 kHz (Digidata 1440A, Molecular Devices, Sunnyvale, CA). After electrodes were properly placed, prefrontal LFPs from hippocampal stimulation were generated by a computer-controlled pulse generator (Master 9 Stimulator, A.M.P.I., Israel). The intensity of the stimulation was chosen from

a current range of 0.25-1.0 milliamps (mA). The specific optimal intensity was chosen to evoke a reliable prefrontal response within a 15% variability in amplitude and slope. Once determined, single and train stimulations (multiple single stimulations) were delivered every 15 s to vHipp. Each set of trains was comprised of 10 pulses delivered at 10, 20, and 40 Hz, and changes from the onset to the peak amplitude of the evoked responses were measured.

Tissue processing and histological confirmation of the electrode placements

Upon completion of recordings, rats were deeply anesthetized with 8% chloral hydrate and decapitated. The brain was removed, and incubated in 4% paraformaldehyde (PFA) at 4° C. Once the brain was saturated in PFA by sinking to the bottom of the tube, it was stored in phosphate-buffered saline (PBS) containing 30% sucrose. The brain remained in this solution until it again sunk to the bottom of the tube, which took approximately 24-72 hours. The purpose of this step was to have the PFA stop tissue decay, and preserve the state of the cells. The sucrose in PBS allowed us to freeze the tissue for cutting, but not destroy it in the process. If the tissue was just in water, the water would expand during freezing, causing the tissue to expand with it. However, a saturated sucrose solution allows proper freezing, but will not expand. The tissue was then cut into 50 µm coronal sections on a freezing stage (PFS-30MP controller, Physitemp Instruments, Clifton, NJ) by a sliding microtome (HM430, Thermo Scientific, Miami, FL) to obtain the mPFC and vHipp regions.

Neurons contain a substance called Nissl, which is a unique stack of rough endoplasmic reticulum. This pattern is only found in neurons (Byrne & Roberst, 2009).

Cresyl violet is a type of dye that specifically stains Nissl bodies (Purves et al., 2001). The mPFC and vHipp sections were stained with cresyl violet in order to determine where the electrode was in the brain.

Tissue was mounted on Superfrost Plus slides (VWR, Batavia, IL). The tissue was then exposed to formyl ethanol (95% ethanol, formalin, and deionized water), dehydrated in 95% ethanol and then 100% ethanol to remove water from the tissue for 3 minutes per solution. Dehydration was followed by treatment with xylene to remove residual ethanol, again for 3 minutes. This step is crucial not only for replacing water in the tissue with ethanol, but to also drain the color of myelin and other cell components, except the cell body (Harrington, 2016).

Slides were then rehydrated with decreasing ethanol concentrations (100%, 95%, 70% and finally distilled water) for three minutes each. Once rehydrated the tissue was stained with cresyl violet for 2 minutes. After stain was applied, the tissue was briefly rinsed in distilled water to wash away excess stain. Brain tissue was then dehydrated again and treated with a final bath of xylene. Slides were then covered in a mounting medium called Permount (Thermo Fisher Scientific). This specific mounting medium has a low viscosity, allowing for a thin and bubble-free application. A coverslip was applied, slides were allowed to dry, and then were viewed with a light microscope (Nikon Optiphot, 4X magnification, Japan) for electrode tracks.

Data Analysis and Statistics

Once verification of the electrode was completed, analysis of the corresponding data were analyzed. All reported measurements are expressed as a mean ± standard error of the mean (SEM). Instead of using standard deviation, which tells us the degree to which individuals in the sample differ from our sample mean, we used standard error, which estimates how far our sample mean is from the population mean (Barde & Barde, 2012). Analyses were conducted using Statistica (Dell Inc.). A p value < 0.05 was considered significant. To determine whether there were significant differences between the age groups tested, we used a one-way analysis of variance (ANOVA), which permitted us to compare all three groups for a significant difference. Fisher's LSD was used because it allows us to find possible differences more easily than other post-hoc tests. Two types of comparisons were made. The first was to examine a possible difference in the mPFC LFP response, as a function of individual pulses delivered to vHipp. The other comparison was the average LFP response from pulses 2 to 10 as a function of age, for each frequency stimulation.

Results

Electrode Placement Confirmation and Stimulation

We first confirmed that the placement of the stimulating electrode was correctly lowered into the ventral hippocampus, and the recording electrode in the medial prefrontal cortex. This step was important because we used a best estimate for coordinates from a rat brain atlas (Paxinos & Watson, 1998), and previous experiments (Cass, et al., 2014, Thomases et al., 2013, and Cass et al., 2013). If our coordinates were inaccurate, and post-experiment histology showed our electrode was placed incorrectly, we would not use data from that animal. Figure 13a shows an example of the medial prefrontal cortex (mPFC, right) and ventral hippocampus (vHipp, left), as well as the desired placement of electrodes. From this study only one rat was excluded, which is why the juvenile group only has 8 rats unlike the other two who have 9 rodents. After the brains were cut and stained, we were able to determine the lowest mark of the electrode track to confirm whether it was placed correctly (Figure 13b).

In particular, we examined the local field potential (LFP) response in the mPFC. Figure 14 shows an example of this response. In general, stimulation to a population of neurons around an electrode allows voltage-gated channels (channels opened by a certain electrical potential across a cell membrane) to open, thereby allowing positive ions (cations) in the extracellular fluid to flow into the cell. This creates an overall positive potential inside the cell, and negative outside. This is why the LFP value moved in the downward (negative) direction. In a complete response, we were interested in obtaining the change in amplitude from the stimulus onset to the maximum LFP response (the amplitude of the peak, Fig. 14).

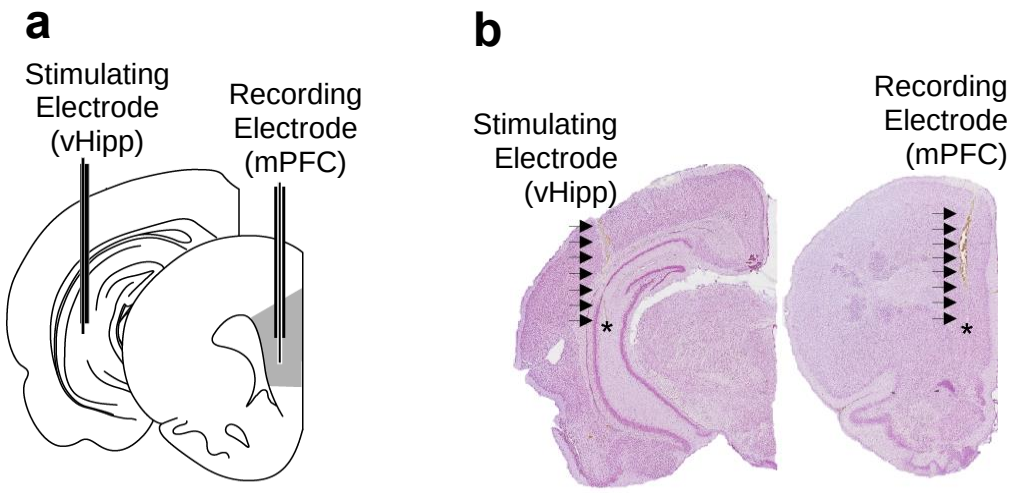

Figure 13: *Verification of electrode placement with Nissl staining*. After experiments were conducted, brain tissue was stained to verify that electrodes were placed in the correct regions. **(a)** Correct regions were determined from a rat brain atlas (Paxinos & Watson, 1998; Thomases et al., 2013). **(b)** We used cresyl violet to stain for Nissl substance in the neuronal cell bodies to visualize tracks.

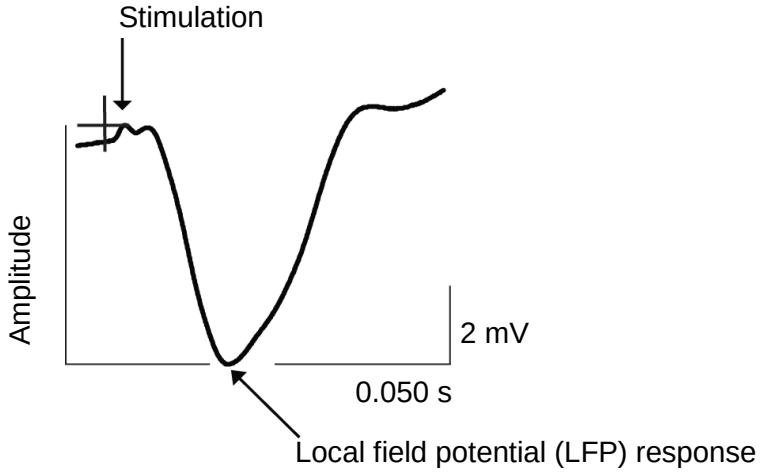

Figure 14: *Example of a single pulse given to the vHipp and recorded in the medial prefrontal cortex.* A single pulse was stimulated in the vHipp and recorded in the mPFC. The amplitude was measured from the point of stimulation to the peak of the overall current in the neuron population (local field potential).

Medial Prefrontal Cortex Response to Stimuli of Increasing Intensity

To examine how a single animal's mPFC would respond to increasing intensity of stimulation applied to the vHipp, we generated an input-output curve. A typical example of this is shown in Figure 15a. For this procedure, we gave a single stimulation at different electrical intensities: 0.25 mA, 0.5 mA, 0.75 mA, and 1.0 mA. Figure 15b illustrates that as the intensity of stimulation increased, so did the amplitude of the response. In order to have meaningful comparisons, the slope of the input-output curves were normalized to the 1 mA intensity slope for each intensity (i.e., the value of the slope of each intensity was divided by the slope of the 1 mA value). By doing so, the optimal intensity for a strong response was determined based on it having less than 15% variability in amplitude and it falling in the 75-80% range of the intensity curve.

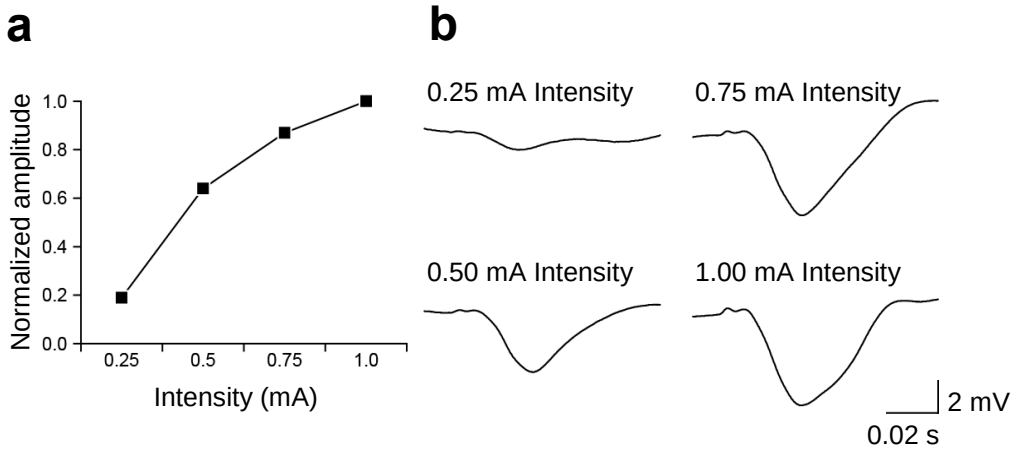

Figure 15: *Example of input-output curve to determine optimal amplitude*. **(a)** Example of a typical input-output curve. This measurement was used to determine the optimal intensity stimulation required to elicit a reliable response. Each amplitude was divided by the one at 1.0 mA (normalized amplitude). **(b)** Example of a single pulse stimulation at four intensities. The optimal intensity was chosen based on it having less than 15% variability in amplitude and it falling in the 75-80% range of the intensity curve.

Medial Prefrontal Cortex Response from Train Stimuli at Different Frequencies

Once this curve was established, we next examined a response to stimulation trains of different frequencies. This involved ten stimulations that were given at specific time intervals; the stimulations were applied in the vHipp and then the response was recorded in the mPFC. For this part of the study, we used three different frequencies: 10 Hz, 20 Hz, and 40 Hz (Figure 16).

At 10 Hz, a stimulation was given every 0.1 sec (100 ms). As shown in the graph on the left in Figure 16a, the 10 Hz response often elicited facilitation; that is, an overall increase in the response amplitude with repeated stimuli. This can be seen in the response from the first pulse compared to the tenth pulse. As illustrated in Figure 16b and 16c, each pulse after the first one was greater in amplitude and remained at an elevated amplitude similar to the second pulse.

For the 20 Hz frequency, a stimulation was given every 50 ms. In this case, the responses were variable, with some showing a transient suppression, and at other times there was a transient facilitation. During a transient suppression, usually pulses 2 and 3 were smaller than the first pulse, however, the remaining pulses tended to waver back towards the amplitude of the first pulse, thereby creating a transient suppression (Figure 16 a, c). In a transient facilitation, pulses two and three moved towards a facilitation, however, the subsequent pulses decreased in amplitude back near the size of first pulse (Figure 16 a, c).

Finally, for the 40 Hz stimulation, current was passed through the neuron population every 25 ms. The response was suppressed as indicated by a large decrease in

amplitude seen when comparing the first pulse to each succeeding pulse (Figure 16a, c).

During suppression, pulses two through ten resulted in a sustained decrease in amplitude.

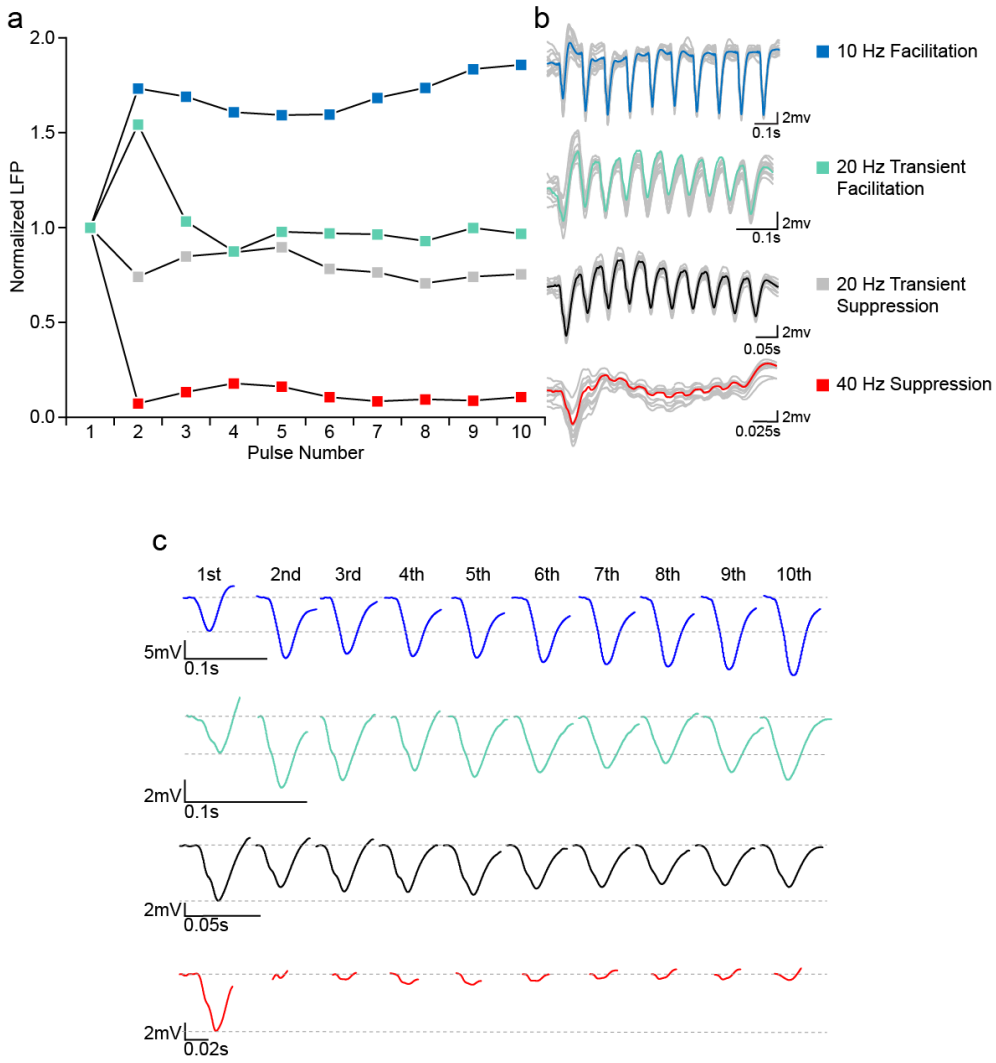

Figure 16: *Classification of LFP response patterns from 10, 20, and 40 Hz stimulation trains*. **(a)** Examples of LFP responses after ten consecutive pulses (train). Each amplitude was normalized and compared to the first pulse. **(b)** Average of traces corresponding to the normalized values to the left. **(c)** Visual comparison of amplitude change from pulses one to ten.

Female Rodent mPFC Response from vHipp Stimulation at Different Frequencies

The main goal of the current experiments was to examine the pattern of maturation in the female medial prefrontal cortex. Using LFP recordings *in vivo*, ventral hippocampal stimulation was done to assess the mPFC response. We first looked at adult females because the mPFC is fully matured at this stage. At 10 Hz there was a sustained facilitation across all age groups (Figure 17). Of note, there were no significant age group differences.

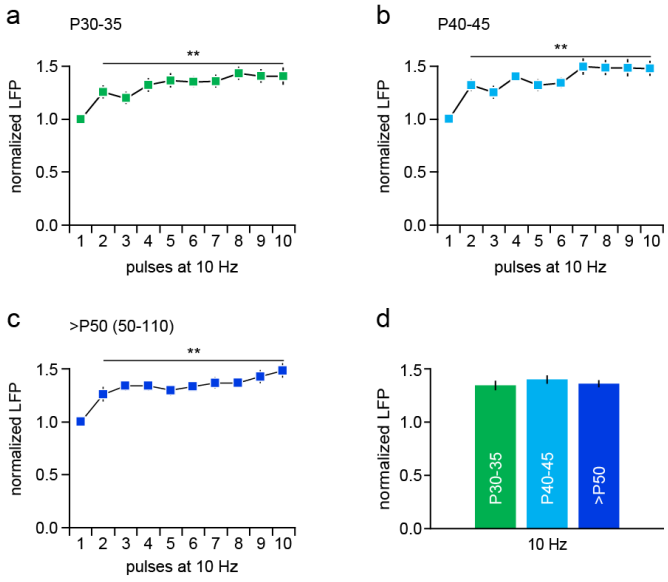

Figure 17: *10 Hz frequency stimulation elicits facilitation in all ages.* **(a)** Young rats showed an overall local field potential (LFP) facilitation in response to a hippocampal train stimulation at 10 Hz (main effect of pulse number, $F(9, 70) = 5.8630$, $p < 0.0001$, one-way ANOVA, $^{**}p < 0.005$ vs. first pulse, Fisher LSD post-hoc). **(b)** At 10 Hz, young adolescent rats at ages P40-45 showed LFP facilitation (main effect of pulse number, $F(9, 70) = 7.9205$, $P < 0.0001$, one-way ANOVA, $^{**}p < 0.005$ vs. first pulse, Fisher LSD post-hoc.). **(c)** Adult rats P50 and older also showed facilitation following vHipp stimulation (main effect of pulse number, $F(9, 70) = 7.7736$, $p < 0.0001$, one-way ANOVA, $^{**}p < 0.005$, Fisher LSD post-hoc). **(d)** A comparison of the average normalized response amplitudes for pulses 2-10 at 10 Hz. There were no significant group differences.

At 20 Hz the P30-35 age groups showed facilitation after the first pulse (Figure 18a)**.** In contrast, the P40-45 age group showed no overall suppression or facilitation (Figure 18 a, d). Additionally, in the >P50 group there was an overall suppression after the first pulse (Figure 18c). The juvenile age group was significantly different from both the adolescent, and adult age groups. The adolescent group was not different from the adult group.

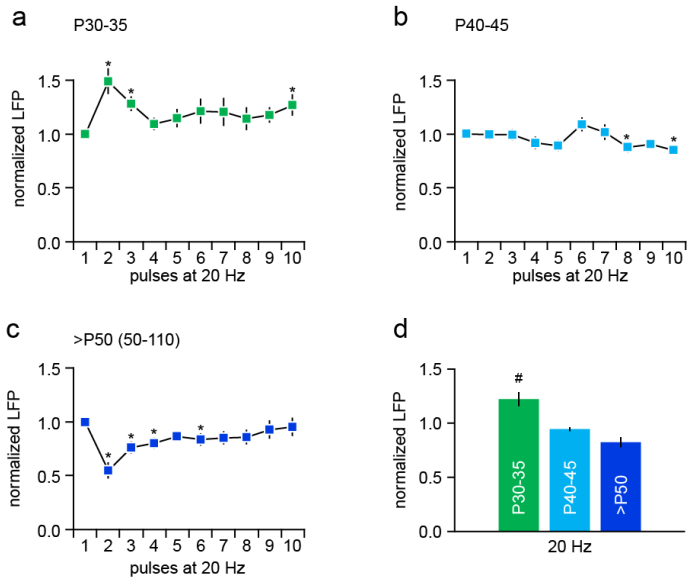

Figure 18: *20 Hz frequency stimulation shows an age dependent shift from a transient facilitation to a transient suppression*. **(a)** Young rats showed LFP facilitation response to hippocampal train stimulation at 20 Hz (main effect of pulse number, $F(9, 70) = 2.2812$, $p < 0.05$, one-way ANOVA, $^{*}p < 0.05$ vs. first pulse, Fisher LSD post hoc). **(b)** At 20 Hz rats at P40-45 overall showed a slight suppression (main effect of pulse number, $F(9, 70) = 2.9428$, $p < 0.005$, one-way ANOVA, $^{*}p < 0.05$, Fisher LSD, post hoc). **(c)** In rats that are P50 and older, there was a LFP suppression response (main effect of pulse number, $F(9, 70) = 4.8721$, $p < 0.0001$, one-way ANOVA, $^{*}p < 0.05$ vs. first pulse, Fisher LSD post hoc. **(d)** Summary of the average normalized value from pulses 2-10. The P30-35 group shows a LFP facilitation response not seen in the P40-45 group, or >P50 group ($^{\#}p < 0.0005$ P30-35 vs. P40-45 and P30-35 vs. >P50, LSD post hoc, one-way ANOVA, $F(2, 21) = 18.499$, $p < 0.0001$).

At 40 Hz the P30-35 and P40-45 groups both showed suppression (Figure 19a, b), but not as much suppression as the >P50 group (Figure 19c, d). In fact, both the P30-35 and the P40-45 age groups were statistically different from the >P50 group, but not significantly different from each other.

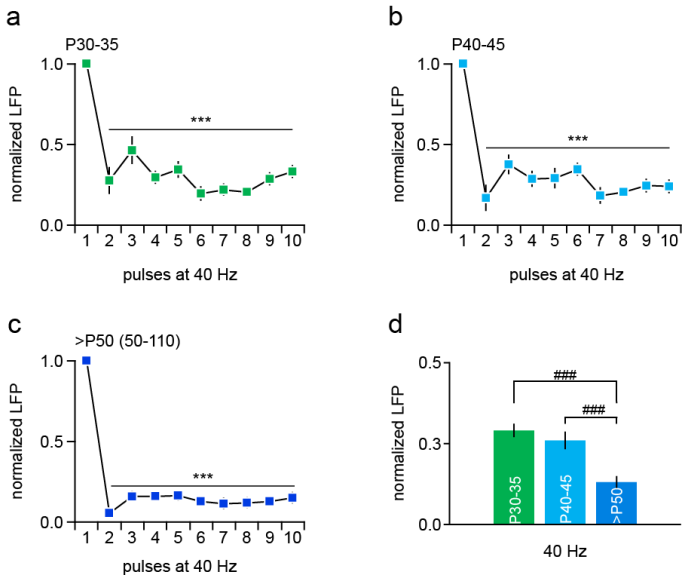

Figure 19: *40 Hz frequency stimulation elicits differences in the strength of suppression based on age group*. **(a)** Young rats showed an LFP suppression response to hippocampal train stimulation at 40 Hz (main effect of pulse number, $F(9, 70) = 24.478$, $p < 0.0001$, one-way ANOVA, ***$p < 0.0005$ vs. first pulse, Fisher LSD post hoc). **(b)** In the P40-45 age group a suppression of the response was also seen (main effect of pulse number, $F(9, 70) = 27.219$, $p < 0.0001$, one-way ANOVA, ***$p < 0.0005$ vs. first pulse, LSD post hoc). **(c)** In rats that are P50 and older, there was a LFP suppression response (main effect of pulse number, $F(9, 70) = 126.81$, $p < 0.0001$, one-way ANOVA, ***$p < 0.0005$ vs. first pulse, Fisher LSD post hoc. **(d)** Summary of the average normalized values from pulses 2-10. The P30-35 and P40-45 groups both showed suppression, but not as much as the P50 ($^{###}p < 0.0005$ P30-35 vs >P50 and $^{###}p < 0.0005$ P40-45 vs. >P50, LSD post hoc, one-way ANOVA, $F(2, 21) = 14.507$, $p < 0.0001$.

Overall these results show the time course of the maturation of the mPFC. Specifically maturation, as assessed through age related changes, was seen with ventral hippocampal stimulation at 20 and 40 Hz frequencies. At 20 Hz there was a facilitated response only in P30-35 age, but after 40 postnatal days there was a slight transient suppression, and after 50 days there was a suppression of this signal. Similarly, there was a suppression of the signal at the 40Hz stimulation, and the signal was significantly more suppressed after P50.

Discussion

The present study was designed to observe the normal developmental pattern of the female rodent medial prefrontal cortex. There are numerous reports showing differences in the developmental rates of males and females, with females developing faster (Lenroot et al., 2007). Although the rates are different, the end result of a fully developed brain appears to be the same for both sexes (Lim, Han, Uhlhaas, & Kaiser, 2015). Additionally, there is much in the literature about how this development occurs in males (Benes, Vincent, & Molloy, 1989; Gee et al., 2013). However, we cannot assume the pattern of development will be the same in females as it is in males because recent evidence has shown that females have different cortical area sizes (Goldstein et al., 2001), some innate social preferences (Alexander & Hines, 2002), and stress coping mechanisms (Cahill, Gorski, & Le, 2003; Shors, Falduto, & Leuner, 2004) compared to males.

Therefore it is reasonable to explore the question of sex differences in brain development. Using *in vivo* local field potential (LFP) recordings from the mPFC we hypothesized that as a female rat ages from preadolescence to adulthood, there is an age dependent shift from disinhibition to inhibition in a matured mPFC. This occurs because as the brain matures, there is an increase in GABAergic function, producing a more robust inhibitory control in the mPFC.

As previously stated, the pattern of disinhibition to inhibition occurs due to an increase in GABAergic function in the PFC and an increase in glutamatergic inputs to those neurons (Thomases et al., 2013; Caballero et al., 2014 a, 2014b). A strong excitatory response on the inhibitory GABAergic neurons thereby results in a large

inhibitory effect on the PFC (Caballero et al., 2016). In contrast, the variation in the amount of inhibitory control is caused partly by low levels of GABAergic neurons in the developing brain, allowing for a more active PFC (Erickson & Lewis, 2002). However, as one reaches adulthood, there is inhibitory control because the GABAergic system in the PFC has gone through maturation and is able to better control excitatory input from other brain regions (Lew & Tseng, 2014). Knowing that both male and female brains are able to fully mature in adulthood suggests there will be a pattern of disinhibition to inhibition in females based on prior male studies (Thomases et al., 2013; Gogtay et al., 2004; Erickson & Lewis, 2002). In my present study, I did see this age-dependent shift, but only at the 20 and 40 Hz frequency stimulations.

The age dependent shift from a transient facilitation to a transient suppression was most likely due to an increase in GABAergic interneurons, PV-positive fast-spiking interneurons, and glutamatergic inputs as females aged from juvenile to adulthood. This observation is consistent with a report that there is an increase in GABAergic function, during adolescence in the PFC, leading to a stronger inhibition with the increasing input activity to the PFC (Caballero et al., 2014b). At 10 Hz the glutamatergic cells are mainly activated (Flint & Connors, 1996). This is why we see an excitatory, facilitation of the signal at 10 Hz. As previously stated, glutamate does change during adolescence, its projections to the GABAergic system are what produce the robust shift to an inhibitory control system in adulthood.

At 40 Hz my results showed that all age groups had a suppressed LFP response, but the adolescent females (P30-45) showed a weaker suppression of the signal than the adults. According to previous developmental models (Tseng & O'Donnell, 2007a; Tseng

& O'Donnell, 2007b), dopamine receptors 1 and 2 (D1 and D2) change during maturation. At a high frequency rate (40 Hz), juvenile rodents rely on D1 receptors to produce an inhibitory effect. However, in adulthood, both D1 and D2 receptors are active in facilitation of GABAergic activity, causing a profound and prolonged inhibitory effect. This was seen in my results (Fig. 19), where from P30-45 there was suppression of the mPFC LFP response, but it was sporadic in amplitude changes. On the other hand, females ages P50 and older showed a sustained suppression, with very little variability in amplitude of each subsequent response after the first pulse. Accordingly, the differences seen with 20 Hz compared to 40 Hz could have been caused by an increase in D1 and D2 receptor control over the GABAergic system.

As previously stated, a 20 Hz frequency correlates with attention and alertness (Walter & Matthews, 1934), and this beta stimulation in the vHipp results in activation of the PFC GABAergic system through subsequent activation of the PV-positive FSIs in the PFC (Berke, 2011). A transient facilitation at this frequency would then indicate prefrontal excitation, whereas a transient suppression would indicate inhibition. At a cellular level, the adolescents showing a transient facilitation may have less glutamatergic input, or fewer FSIs to handle the increased excitation to the mPFC, which is why this pattern occurs, rather than the transient suppression as seen in the adults.

Assuming my results correlate with changes in the glutamate and dopamine systems and their effects on the PFC, this shift may be seen behaviorally as children age through adolescence. In fact, this claim is supported by Casey et al. (1997) who looked at prefrontal activation in adolescent and adult males and females using a go/no-go task. Their fMRI results showed overall greater activation in the entire prefrontal cortex in

adolescents than the adults, whereas adults showed less activation, but the pattern of activation was centered in the mPFC. The goal of the task was to inhibit button pushing when an "X" appeared on the screen. The adolescent PFC activation pattern correlated with increased button pushing.

It should be noted here that an fMRI measures overall changes in excitation of specific brain regions (Singleton, 2009). One of the major limitations to fMRI studies is interpreting its signal. In an fMRI, a signal corresponds to changes in blood flow. The consensus is that if a brain area is in use, there will be an increase in blood flow to that region (Logothetis, Pauls, Augath, Trinath, & Oeltermann, 2001). This also can be used to calculate the summation of excitatory and inhibitory responses (Logothetis, 2008). If there is a strong signal seen with fMRI, an initial interpretation would be that there is an excitatory response originating in that area of the brain, related to the task being performed.

Overall, the Casey et al. (1997) go/no-go task study showed similar findings to my results at 20 Hz. The adolescents in their study could not inhibit the action of pressing the button when "X" appeared on the screen. I interpreted these results to indicate that the children were not as attentive as the adults, who showed better inhibition during this task. The pattern of activity was also different between the age groups. The children had a scattered pattern of activation in the PFC, whereas the adults had a concentrated signal around the medial PFC. In the results of that study, Casey et al. (1997) quantified the volume of activation, which showed the children had more PFC activity. Similarly, at a 20 Hz frequency, the preadolescent female rodents I examined showed higher excitation response in the PFC compared to the adult females.

Casey et al. (1997) observed similar findings to my 20 Hz results because adolescents showed overall more activation in the entire PFC, but very little in the mPFC. An active PFC would indicate activation of the inhibitory control output of the PFC. In the Casey et al. study, adults who had active mPFCs were better at stopping themselves from pressing the button when "X" appeared on the screen. For children, they had to recruit other brain areas to perform the same task. Therefore, a less active mPFC exhibits an overall excitatory output, which is what the adolescent rodent response was when a 20 Hz stimulation arrived at this region.

One difference between my study and the one conducted by Casey et al. (1997) is the use of human subjects versus female rodents. Nonetheless, the findings of Casey et al. (1997) are consistent with my results where adolescent female rats showed a more excitatory PFC response than adults at a 20 Hz stimulation. However, in a study done by Davis et al. (2011), they showed an increased PFC signal from fMRI results, which correlated with participants who had better attention and planning. Attention and planning is processed in our dorsolateral PFC, a region that is functionally homologous to the rodent mPFC (Uylings, Groenewegen, & Kolb, 2003). In mature adult brains, Newman, Carpenter, Varma, & Just, (2003) found that as a planning task became more difficult, the dorsolateral PFC is being recruited, and becomes increasingly more active. However, in adolescents, they use the dorsolateral PFC to make primary decisions, instead of recruiting the mPFC like adults do (Blakemore, & Choudhury (2006). Knowing that the dorsolateral PFC undergoes changes during adolescence makes this another region of interest in brain development, but this becomes complicated since functionally this region is similar to the rodent mPFC. It is worth noting that Pratt &

Mizumori, (2001) found that the rodent mPFC produces both inhibitory and excitatory responses depending on the task performed. Therefore, I would postulate signals that correlate to attention and planning show and age dependent change in the mPFC, since my results show that the mPFC undergoes inhibitory processing changes as well. The results of my study are consistent with those reported by Casey et al. (1997) because they solely focused on inhibitory tasks, but knowing the rat mPFC can also processes excitatory information related to attention and processing, the results of Davis et al. (2011) may be relevant as well.

My other major finding was that at the 40 Hz frequency stimulation, a greater suppression of the signal was only seen in adulthood, which again correlates with the age dependent increase in GABAergic interneurons and the increase in glutamatergic inputs to these cells to accommodate an increase in inhibitory control. As noted, the general focus of my study was to examine whether there is an age-dependent shift from disinhibition to inhibition in female rats, which has previously been seen in male rodents from preadolescence to adulthood (Thomases et al., 2013). Others have shown that high frequency stimulation arriving at the PFC from the vHipp produces inhibitory effects in this region of the brain (Tseng & O'Donnell, 2007a). It is important to note that a 40 Hz frequency arriving at the PFC correlates with behaviors like thinking and learning (Miltner, 1999) and emotional control (Tang et al., 2011). Therefore, a PFC with proper inhibition allows us to develop into highly cognitive, emotional stable adults.

This stability in adulthood is clearly seen with a decrease in foot-stomping to get what we want and irrational fears of the dark. Gee et al. (2013) noted that in the transition from childhood to adulthood there is a decrease in specific emotional processes like

tantrums and separation anxiety. These authors specifically focused on the amygdala-medial prefrontal cortex pathway, because this region of the brain undergoes major changes during adolescence (Banks, Eddy, Angstadt, Nathan, & Phan, 2007). Most studies on these pathways have been conducted in non-human animals. In contrast, Gee et al. (2013) ran an fMRI study using participants of ages 4-22 to examine whether there was a developmental decline in amygdala reactivity to fearful and happy faces. They accomplished this by having participants view faces in two different sessions. During the first session, participants had to view happy and neutral faces. During the second session, participants viewed fearful and neutral faces. To make sure participants were paying attention, they had them press a button when they were viewing a neutral face.

During their analysis, they found significant results for the fearful face reactions. Specifically they found an age-dependent decrease in amygdala activation when viewing fearful faces. To measure amygdala-mPFC connectivity, they used a psycho-physiological interaction (PPI) analysis to see if the amygdala activity co-varies with the mPFC activity, and if the connectivity between these regions changes with age. The PPI analysis revealed different correlations between amygdala and mPFC activation when viewing fearful faces. They found that the children had highly active amygdalas and mPFCs when viewing fearful faces. In contrast, adolescents and young adults showed low amygdala activity and high PFC activity when viewing fearful faces. All in all, Gee et al. (2013) were able to show that as we age our amygdala activity in relation to viewing fearful images declines. However, the mPFC continues to respond the same even though the amount of amygdala reactivity decreases. The adult participants in this study showed better emotional control by not strongly reacting to fearful faces, as the children

did. This study looked at the amygdala-mPFC pathway, whereas I looked at the vHipp-mPFC pathway during adolescence to adulthood. Since Gee et al. (2013) saw no overall change in mPFC activity from adolescence to adulthood, the conclusion can be made that the ventral hippocampal region has more of an effect during this transitional period than the amygdala.

The amygdala plays an important role in adolescence because it projects both glutamatergic and GABAergic input onto the PFC (Krettek & Price, 1977). Therefore, the finding of an active PFC by Gee et al. (2013) makes sense, as there may be active glutamatergic signals projecting from the amygdala to the PFC, creating an excitatory response. However, amygdala neurons also can provide GABAergic input to the PFC (Cunningham, Bhattacharyya, & Benes, 2008). Therefore a strong excitatory output on the glutamatergic neurons would strongly activate the GABAergic neurons, creating an overall inhibitory effect. The fact that there is an age dependent decline in amygdala activity, but maintenance of a highly active PFC, indicates there may be an age-dependent difference with other types of fear inducing behaviors. For example an amygdala-PFC inhibitory effect has been seen in rats during conditioned fear inhibition where they learn to reduce their level of fear to a previous stimulus related to fear (foot shock) (Garcia et al., 1999). Overall, the activation of the amygdala to the PFC is age dependent, but its effect on the PFC varies depending on the stimulus.

I would like to point out that I have not discussed studies that looked at hippocampal development and the underlying behaviors that correlate with it. It is known that a developed hippocampus is important for maintenance of proper working memory (Luna et al., 2004). However, most of these studies do not go into detail about the

underlying mechanisms of this behavior, or they simply looked at hippocampal volume changes from adolescence to adulthood. Therefore, my results showing an age dependent, inhibitory shift in the ventral hippocampal to medial prefrontal cortex pathway begins to build on a possible mechanism.

Future Studies

Cass et al. (2014) has provided a framework for the normal developmental pattern of the prefrontal cortex. They have also shown the importance of how fragile this pattern is. For example, they have studied the effect of adolescent drug exposure and stress, which can in turn manipulate the mature PFC later in adulthood. However, this work has yet to be replicated in females to determine whether there are similarities or differences with what has been found for males. Accordingly, a relevant future study would be to examine the effects of adolescent cannabinoid use on the overall development of the prefrontal cortex. Studies have shown that adolescent cannabis use can lead to adult psychosis (Caspi et al., 2005; Moore et al., 2007). However, an underlying mechanism for this dysfunction remains unclear.

As the number of states legalizing marijuana increases, the possibility of adolescent use also increases. However, the use of marijuana has declined amongst 8th and 10th graders, and has remained unchanged in 12th graders compared to the last five years (National Institute on Drug Abuse, 2016). Although the drug is becoming legal, it is not without consequences. However, survey data indicates that adolescents do not associate much risk with marijuana. In 2000, 58.8% of adolescents surveyed, associated

risk with smoking marijuana, in 2016, only 31.1% did (Johnston, O'Malley, Miech, Bachman, & Schulenberg, 2016). With adolescents taking risks, the need for a clear mechanism of damage to the brain with drugs during development is needed. Consequently, a study by Cass et al. (2014) is appropriate. In this case, adolescent female rats P35-50 received one injection per day, of the cannabinoid receptor type 1 CB1 agonist, WIN 55, 212-2 (WIN) for five consecutive days. For a control group, the ages remained the same, but they received the vehicle solution. WIN activates the cannabinoid receptor, CB1, which in turn reduces the neuronal oscillations at the beta and gamma wave frequencies (20-100 Hz) in cortical GABAergic interneurons (Hajós, Hoffmann, & Kocsis, 2008). The decrease in oscillations is thought to be caused by a decrease in the transmission of the GABAergic interneurons (Uhlhass et al., 2006). Therefore, adolescent exposure to WIN should show a disinhibition at 20 and 40 Hz as these fall in the range of its effects on the normally inhibitory GABAergic system. However, if WIN is given during adulthood, it should not have as much of a profound effect on the GABAergic system, because it should be fully developed by this age.

This effect could be observed by recording LFP responses in the mPFC in adulthood (P65-85) after chronic adolescent drug use. As a comparison I would use the adulthood pattern from 10 to 40 Hz stimulation to the ventral hippocampus and its corresponding response in the medial prefrontal cortex as observed from my present results. Following the *in vivo* LFP recording protocols described in this study, I would expect the vehicle treated group to show normal facilitation, transient suppression, to suppression, as the stimulations moved from 10 to 40 Hz. In the adolescents treated with WIN (Cass et al., 2014) adult males showed similar patterns to normal adolescents: 10 Hz

would elicit a facilitation, but at 20 Hz there would be a transient facilitation rather than suppression, and at 40 Hz a weaker suppression of the LFP response compared to the vehicle treated adults would appear. I would expect to see the same results pattern in females because the final developed pathways in the brain are shown to be the same in human males and females (Joel et al., 2015). However, we cannot make direct conclusions unless testing this in other species. To truly see if there are timing differences in age development, I would add two adolescent age groups to see if there is a specific female age range during which WIN has a negative impact on the PFC.

In designing this future study I would also add *in vitro* experiments. When male rats were exposed to WIN during adolescence, their GABAergic synaptic transmission decreased. *In vitro* electrophysiology utilizes a single cell response from the brain. If GABAergic transmission decreases, the overall inhibitory control would not be as strong as a healthy developed PFC. Less inhibitory control is seen at 20 and 40 Hz in the chronic use animals (Cass et al., 2014). These types of studies have also been repeated with cocaine (Cass et al., 2013) and MK-801 (an NMDA receptor antagonist that mimics the effects of ketamine; Thomases et al., 2013), but have only been studied in male models.

In vivo electrophysiological studies can also be used to study long-term plasticity in a developing brain (Thomases et al., 2014). Having this method would show whether WIN affects the switch from an excitatory to inhibitory controlled PFC. When a high frequency stimulation is given, usually at 100 Hz for 50 consecutive pulses, the strength of the response changes (Caballero et al., 2014). If high frequency stimulation is applied to the vHipp and recorded in the mPFC, the LFP responses shows a sustained response that is decreased in strength, which is called a Long Term Depression (LTD). However, if

high frequency stimulation is given in the basolateral amygdala, an increase in the strength of the mPFC LFP response is stronger and sustained. This sustained increase is called Long Term Potentiation (LTP).

Additionally, it would be helpful to study possible developmental differences in the hippocampus itself. This can be tested using the Morris Water Maze which is a hippocampal dependent task in which rats learn to locate a platform in water (D'Hooge & De Deyn, 2001). This method allows for the rodent to use cues in the maze to locate the platform situated in opaque water. Ideally, this type of study would produce a formed memory using spatial cues. However, doing this *in vivo* would be impractical because even though one can record while an animal is awake and learning, this cannot be done when rats are in water. Instead, an *in vitro* methodology could be used to examine how cells respond (brain slices would be obtained after the animal goes through training). If so, I postulate there will be stronger responses in the hippocampus after spatial memories have been formed due to LTP effects. Gazova et al. (2013) used a human analogous version of the Morris Water Maze, to see how spatial learning is affected as an individual ages. They found that egocentric (body-centered) navigating did not change with age. However, allocentric (world-centered) navigation performance was significantly impaired in older adults. Allocentric navigation is a hippocampus dependent form of navigation (Grön, Wunderlich, Spitzer, Tomczak, & Riepe, 2000). Therefore, on a cellular level, there should be changes in hippocampal cell response before and after the Morris Water Maze Task in adolescence, but they may use it differently than older rodents.

On a cellular level, both GABA and glutamate are involved in learning and memory (Myher, 2003), however, an increase in GABA would help inhibit information

we do not need about the environment. This was seen, for example, by Garske et al. (2013) when adolescent rats had a difficult time staying focused on task to find a Froot Loop (breakfast cereal) buried in sand. Adolescent rats would start to dig for the Froot Loop, but as the distance in which it was buried underground increased, they gave up quicker. The ability to focus on a task at hand not only keeps us productive when working, but also keeps us safe during other tasks like driving. By studying the vulnerability of pathways that affect this attention during adolescence gives us a picture on how easily it can be manipulated with substances like drugs and alcohol, and stress.

Conclusion

Adolescence is a critical time period in our life. We expand our knowledge of our world and how we can further contribute to it. We learn to self-analyze, yearn for acceptance from our peers, and make executive decisions. My study has shown that as female rodents age from preadolescence (P30-35), to adolescence (P40-45) to adulthood (P50 and older) there is a shift from a disinhibitory to inhibitory effect as stimulation is applied in the ventral hippocampus and evoked-responses are recorded in the medial prefrontal cortex. This in turn suggests an increase in GABAergic function providing a higher magnitude of inhibition, with the help of glutamatergic inputs to these cells to handle incoming information more efficiently. This effect was specifically seen with a 20 and 40 Hz frequency. At 20 Hz, preadolescent rats showed a transient facilitation, adolescents showed no real change in amplitude after the first pulse, and adults showed a transient suppression of the LFP response. At 40 Hz, all ages showed a suppression of the signal. However, the adults showed a greater magnitude of LFP inhibition over the P30-35 and P40-45 age groups. This pattern shows an age dependent modulation of the

vHipp-mPFC pathway, and can be used in future studies to examine topics such as adolescent drug use, which have thus far been mainly examined in male models. The work I have presented is a step towards this goal, and can easily be manipulated to replicate past studies to add to the current knowledge of adolescent brain development.

References

Alexander, G. M., & Hines, M. (2002). Sex differences in response to children's toys in nonhuman primates (*Cercopithecus aethiops sabaeus*). *Evolution and Human Behavior, 23*(6), 467-479.

Andersen, R. A., & Cui, H. (2009). Intention, action planning, and decision making in parietal-frontal circuits. *Neuron, 63*(5), 568-583.

Andersen, S. L. (2003). Trajectories of brain development: point of vulnerability or window of opportunity? *Neuroscience & Biobehavioral Reviews, 27*(1), 3-18.

Bacon, S. J., Headlam, A. J., Gabbott, P. L., & Smith, A. D. (1996). Amygdala input to medial prefrontal cortex (mPFC) in the rat: A light and electron microscope study. *Brain Research, 720*(1), 211-219.

Baldwin, A. E., Sadeghian, K., & Kelley, A. E. (2002). Appetitive instrumental learning requires coincident activation of NMDA and dopamine D1 receptors within the medial prefrontal cortex. *The Journal of Neuroscience, 22*(3), 1063-1071.

Banks, S. J., Eddy, K. T., Angstadt, M., Nathan, P. J., & Phan, K. L. (2007). Amygdala–frontal connectivity during emotion regulation. *Social Cognitive and Affective Neuroscience, 2*(4), 303-312.

Bannerman, D. M., Sprengel, R., Sanderson, D. J., McHugh, S. B., Rawlins, J. P., Monyer, H., & Seeburg, P. H. (2014). Hippocampal synaptic plasticity, spatial memory and anxiety. *Nature Reviews Neuroscience, 15*(3), 181-192.

Barnet, R. C., & Hunt, P. S. (2005). Trace and long-delay fear conditioning in the developing rat. *Learning & Behavior, 33*(4), 437-443.

Barth, C., Villringer, A., & Sacher, J. (2015). Sex hormones affect neurotransmitters and shape the adult female brain during hormonal transition periods. *Frontiers in Neuroscience,* 9, Article 37, 70-89.

Bechara, A., & Van Der Linden, M. (2005). Decision-making and impulse control after frontal lobe injuries. *Current Opinion in Neurology, 18*(6), 734-739.

Behrens, M. M., Ali, S. S., Dao, D. N., Lucero, J., Shekhtman, G., Quick, K. L., & Dugan, L. L. (2007). Ketamine-induced loss of phenotype of fast-spiking interneurons is mediated by NADPH-oxidase. *Science, 318*(5856), 1645-1647.

Benes, F. M. (1989). Myelination of cortical-hippocampal relays during late adolescence. *Schizophrenia Bulletin, 15*(4), 585-593.

Benes, F. M., Vincent, S. L., & Molloy, R. (1993). Dopamnine-Immunoreactive axon varicosities form nonrandom contacts with GABA-immunoreactive neurons of rat medial prefrontal cortex. *Synapse, 15*(4), 285-295.

Berger, H. (1929). Über das Elektrenkephalogramm des Menschen. *European Archives of Psychiatry and Clinical Neuroscience, 87*(1), 527-550.

Berke, J. D. (2011). Functional properties of striatal fast-spiking interneurons. *Frontiers in Systems Neuroscience, 5*, Article 45, 80-86.

Berridge, K. C., & Robinson, T. E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Research Reviews, 28*(3), 309-369.

Bertrand, O. (1998). Auditory induced 40-Hz activity during a frequency discrimination task. *Neuroimage, 7*, P-0370.

Bicks, L. K., Koike, H., Akbarian, S., & Morishita, H. (2015). Prefrontal cortex and social cognition in mouse and man. *Frontiers in Psychology, 6*, 1805.

Blakemore, S. J., & Choudhury, S. (2006). Development of the adolescent brain: implications for executive function and social cognition. *Journal of Child Psychology and Psychiatry*, *47*(3-4), 296-312.

Britton, J. W., Hopp, J. L., Korb, P., Lievens, W. E., Pestana-Knight, E. M., & Frey, L. C. (2016). The Normal EEG. In E. K. Louis, & L. C. Frey (Eds.), *Electroencephalography (EEG): An Introductory Text and Atlas of Normal and Abnormal Findings in Adults, Children, and Infants.* Chicago: American Epilepsy Society.

Botella-Soler, V., Valderrama, M., Crepon, B., Navarro, V., & Le Van Quyen, M. (2012). Large-scale cortical dynamics of sleep slow waves. *Public Library of Science, 7*(2), e30757.

Brooks, D. J. (2001). Functional imaging studies on dopamine and motor control. *Journal of Neural Transmission, 108*, 1283-1298.

Burke, D., Kiernan, M. C., & Bostock, H. (2001). Excitability of human axons. *Clinical Neurophysiology*, *112*(9), 1575-1585.

Buzsaki, G. (1986). Hippocampal sharp waves: their origin and significance. *Brain Research, 398*, 242-252.

Buzsaki, G., Leung, L. W., & Vanderwolf, C. H. (1983). Cellular bases of hippocampal EEG in the behaving rat. *Brain Research, 287*, 139-171.

Byrne, J. H. (2011). *Propagation of the Action Potential.* Retrieved January 27, 2017, from Neuroscience Online: http://neuroscience.uth.tmc.edu/s1/chapter03.html

Byrne, J. H., & Roberst, J. L. (2009). *From Molecules to Networks: An Introduction to Cellular and Molecular Neuroscience* (2nd ed.). (M. N. Ruth Heidleberger, Ed.) Burlington, MA: Academic Press.

Caballero, A., & Tseng, K. Y. (2012). Association of cannabis use during adolescence, prefrontal CB1 receptor signaling, and schizophrenia. *Frontiers in Pharmacology, 3*, 101.

Caballero, A., Flores-Barrera, E., Cass, D. K., & Tseng, K. Y. (2014a). Differential regulation of parvalbumin and calretinin interneurons in the prefrontal cortex during adolescence. *Brain Structure and Function, 219*(1), 395-406.

Caballero, A., Granberg, R., & Tseng, K. Y. (2016). Mechanisms contributing to prefrontal cortex maturation during adolescence. 70, 4-12. *Neuroscience & Biobehavioral Reviews, 70*, 4-12.

Caballero, A., Thomases, D. R., Flores-Barrera, E., Cass, D. K., & Tseng, K. Y. (2014b). Emergence of GABAergic-dependent regulation of input-specific plasticity in the adult rat prefrontal cortex during adolescence. *Psychopharmacology, 231*(8), 1789-1796.

Cahill, L. (2005). His brain, her brain. *Scientific American, 292*(5), 40-47.

Cahill, L., Gorski, L., & Le, K. (2003). Enhanced human memory consolidation with post-learning stress: interaction with the degree of arousal at encoding. *Learning and Memory, 10*(4), 270-274.

Carr, D. B., & Sesack, S. R. (1996). Hippocampal afferents to the rat prefrontal cortex: synaptic targets and relation to dopamine terminals. *Journal of Comparative Neurology, 369*(1), 1-15.

Carter, H. V., & Gray, H. (1918). *Anatomy of the Human Body* (20th ed.). New York: Lea and Febiger.

Casey, B. J., Trainor, R. J., Orendi, J. L., Schubert, A. B., Nystrom, L. E., Giedd, J. N., & Forman, S. D. (1997). A developmental functional MRI study of prefrontal activation during performance of a go-no-go task. *Journal of Cognitive Neuroscience, 9*(6), 835-847.

Caspi, A., Moffitt, T. E., Canon, M., McClay, J., Murray, R., Harrington, H., & Poulton, R. (2005). Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene. *Biological Psychiatry, 57*(10), 1117-1127.

Cass, D. K., Flores-Barrera, E., Thomases, D. R., Vital, W. F., Caballero, A., & Tseng, K. Y. (2014). CB1 cannabinoid receptor stimulation during adolescence impairs the maturation of GABA function in the adult rat prefrontal cortex. *Molecular Psychiatry, 19*(5), 536-543.

Cass, D. K., Thomases, D. R., Caballero, A., & Tseng, K. Y. (2013). Developmental disruption of gamma-aminobutyric acid function in the medial prefrontal cortex by noncontingent cocaine exposure during early adolescence. *Biological Psychiatry, 74*(7), 490-501.

Charles River Laboratories. (n.d.). *Sprague Dawley Rat*. Retrieved March 1, 2017, from Charles River Laboratories: http://www.criver.com

Chatterjee, U., Kumar, D., Siddiqui, A., & Goyal, N. (2008). Neuropsychology of prefrontal cortex. *Indian Journal of Psychiatry, 3*(50), 302.

Chiou, W. L., & Barve, A. (1998). Linear correlation of the fraction of oral dose absorbed of 64 drugs between humans and rats. *Pharmaceutical Research, 15*(11), 1792-1795.

Clarke, P., & Kumar, R. (1983). The effects of nicotine on locomotor activity in non-tolerant rats. *British Journal of Pharmacology* (78), 329-337.

Clayton, J. A., & Collins, F. S. (2014). NIH to balance sex in cell and animal studies. *Nature, 509*(7500), 282-283.

Colgin, L. L. (2016). Rhythms of the hippocampal network. *Nature Reviews Neuroscience, 17*, 239-249.

Colgin, L. L., Denninger, T., Fyhn, M., Hafting, T., Bonnevie, T., Jensen, O., & Moser, E. I. (2009). Frequency of gamma oscillations routes flow of information in the hippocampus. *Nature, 462*(7271), 353-357.

Colgin, L. L., Kubota, D., Jia, Y., Rex, C. S., & Lynch, G. (2004). Long-term potentiation is impaired in rat hippocampal slices that produce spontaneous sharp waves. *Journal of Physiology, 558,* 953-961.

Cressman, V. L., Balaban, J., Steinfeld, S., Shemyakin, A., Graham, P., Parisot, N., & Moore, H. (2010). Prefrontal cortical inputs to the basal amygdala undergo pruning during late adolescence in the rat. *Journal of Comparative Neurology, 518,* 2693-2709.

Cunningham, M. G., Bhattacharyya, S., & Benes, F. M. (2008). Increasing interaction of amygdalar afferents with GABAergic interneurons between birth and adulthood. *Cerebral Cortex, 18*(7), 1529-1535.

Daffner, K. R., Mesulam, M. M., Scinto, L. M., Acar, V., Calvo, R., Faust, A., . . . Holcomb, P. (2000). The central role of the prefrontal cortex in directing attention to novel events. *Brain, 123*(5), 927-939.

Darwin, C. (1872). *The Expression of the Emotions in Man and Animals.* John Murray.

Davis, C. L., Tomporowski, P. D., McDowell, J. E., Austin, B. P., Miller, P. H., Yanasak, N. E., & Naglieri, J. A. (2011). Exercise improves executive function and achievement and alters brain activation in overweight children: a randomized controlled trial. Health Psychology. *Health Psychology, 30*(1), 91-98.

D'Hooge, R., & De Deyn, P. P. (2001). Applications of the Morris water maze in the study of learning and memory. *Brain Research Reviews, 36*(1), 60-90.

Diamond, A. (2013). Executive Functions. *Annual Review of Psychology, 64,* 135.

Drefus-Brisac, C., & Larroche, J. C. (1971). Discontinuous electroencephalograms in the premature newborn and at term. Electro-anatomo-clinical correlations. *Electroencephalography and Clinical Neurophysiology, 1*, 95-99.

English, D. F., Peyrache, A., Stark, E., Roux, L., Vallentin, D., Long, M. A., & Buzsaki, G. (2014). Excitation and inhibition compete to control spiking during hippocampal ripples: intracellular study in behaving mice. *The Journal of Neuroscience, 34*(49), 16509-16517.

Erickson, S. L., & Lewis, D. A. (2002). Postnatal development of parvalbumin-and GABA transporter-immunoreactive axon terminals in monkey prefrontal cortex. *Journal of Comparative Neurology, 448*(2), 186-202.

Ernst, m., Torrisi, S., Balderston, N., Grillon, C., & Hale, E. A. (2015). fMRI Functional Connectivity Applied to Adolescent Neurodevelopment. *Annual Review of Clinical Psychology, 11*, 361-377.

Evans, J. S., & Watson, P. C. (1976). Rationalization in a reasoning task. *British Journal of Psychology, 67*(4), 479-486.

Feinberg, I., Higgins, L. M., Khaw, W. Y., & Cambell, I. G. (2006). The adolescent decline of NREM delta, an indicator of brain maturation, is linked to age and sex but not to pubertal stage. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 291*(6), R1724-R1729.

Ferbinteanu, J., & McDonald, R. J. (2001). Dorsal/ventral hippocampus, fornix, and conditioned place preference. *Hippocampus, 11*(2), 187-200.

Fields, R. D. (2016). Learning When No One Is Watching. *Scientific American Mind, 27,* 56-63.

Finger, S. (2001). *Origins of Neuroscience: A History of Explorations into Brain Function.* Oxford University Press, USA.

Flint, A. C., & Connors, B. W. (1996). Two types of network oscillations in neocortex mediated by distinct glutamate receptor subtypes and neuronal populations. *Journal of Neurophysiology, 75*(2), 951-957.

Floresco, S. B., & Phillips, A. G. (2001). Delay-dependent modulation of memory retrieval by infusion of a dopamine D_1 agonist into the rat medial prefrontal cortex. *Behavioral Neuroscience, 115*(4), 934.

Floresco, S. B., Seamans, J. K., & Phillips, A. G. (1997). Selective roles for hippocampal, prefrontal cortical, and ventral striatal circuits in radial-arm maze tasks with or without a delay. *The Journal of Neuroscience, 17*(5), 1880-1890.

Flores-Barrera, E., Thomases, D. R., Heng, L. J., Cass, D. K., Caballero, A., & Tseng, K. Y. (2014). Late adolescent expression of GluN2B transmission in the prefrontal cortex is input-specific and requires postsynaptic protein kinase A and D1 dopamine receptor signaling. *Biological Psychiatry, 75*(6), 508-516.

Food and Drug Administration (FDA). (2013, Jan 12). FDA requiring lower recommended dose for certain sleep drugs containing zolpidem: Reminder about risk of impaired activities the morning after use for all insomnia drugs. *FDA News Release*. Retrieved Jan 27, 2017, from: www.fda.gov/newsevents/ newsroom/pressannouncements/ucm334798.htm

Freidman, H. R., & Goldman-Rakic, P. S. (1988). Activation of the hippocampus and dentate gyrus by working-memory: a 2-deoxyglucose study of behaving rhesus monkeys. *The Journal of Neuroscience, 8*(12), 4693-4706.

Freund, T. F., & Antal, M. (1988). GABA-containing neurons in the septum control inhibitory interneurons in the hippocampus. *Nature, 336*, 170-173.

Frith, C., & Dolan, R. (1996). The role of the prefrontal cortex in higher cognitive functions. *Cognitive Brain Research, 5*(1), 175-181.

Fuster, J. M. (2002). Frontal lobe and cognitive development. *Journal of Neurocytology, 31*(3-5), 373-385.

Gabbot, P. L., Dickie, B. G., Vaid, R. R., Headlam, A. J., & Bacon, S. J. (1997). Local-circuit neurons in the medial prefrontal cortex (areas 25, 32 and 24b) in the rat: morphology and quantitative distribution. *Journal of Comparative Neurology, 377*(4), 465-499.

Garcia, R., Vouimba, R. M., Baudry, M., & Thompson, R. F. (1999). The amygdala modulates prefrontal cortex activity relative to conditioned fear. *Nature, 402*(6759), 294-296.

Garske, A. K., Lawyer, C. R., Peterson, B. M., & Illig, K. R. (2013). Adolescent changes in dopamine D1 receptor expression in orbitofrontal cortex and piriform cortex accompany an associative learning deficit. *PLoS One, 8*(2), e56191.

Gathercole, S. E. (1999). Cognitive approaches to the development of short-term memory. *Trends in Cognitive Sciences, 3*(11), 410-419.

Gee, D. G., Humphreys, K. L., Flannery, J., Goff, B., Telzer, E. H., Shapiro, M., & Tottenham, N. (2013). A developmental shift from positive to negative connectivity in human amygdala–prefrontal circuitry. *Journal of Neuroscience, 33*(10), 4584-4593.

Genetic Science Learning Center. (2013). *Animal Models for Addiction Research.* Retrieved November 19, 2016, from Genetic Science Learning Center: http://learn.genetics.utah.edu/content/addiction/mice

Giedd, J. N. (2004). Structural magnetic resonance imaging of the adolescent brain. *Annals of the New York Academy of Sciences, 1*(1021), 77-85.

Gilmartin, M. R., Kwapis, J. L., & Helmstetter, F. J. (2013). NR2A- and NR2B-containingNMDA receptors in the prelimbic medial prefrontal cortex differentially mediate trace, delay, and contextual fear conditioning. *Learning and Memory, 20*, 290-294.

Gleich, T., Lorenz, R. C., Pöhland, L., Raufelder, D., Deserno, L., Beck, A., & Gallinat, J. (2015). Frontal glutamate and reward processing in adolescence and adulthood. *Brain Structure and Function, 220*(6), 3087-3099.

Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaituzis, A. C., & Rapoport, J. L. (2004). Dynamic mapping of human cortical development during childhood through early adulthood. *Proceedings of the National academy of Sciences of the United States of America, 101*(21), 8174-8179.

Gold, J. M., Berman, K. F., Randolph, C., Goldberg, T. E., & Weinberger, D. R. (1996). PET validation of a novel prefrontal task: Delayed response alternation. *Neuropsychology, 10*(1), 3.

Goldman-Rakic, P. S., Leranth, C., Williams, S. M., Mons, N., & Geffard, M. (1989). Dopamine synaptic complex with pyramidal neurons in primate cerebral cortex. *Proceedings of the National Academy of Sciences, 86*(22), 9015-9019.

Goldstein, J. M., Seidman, L. J., Horton, N. J., Makris, N., Kennedy, D., Caviness, V. S., & Tsuang, M. T. (2001). Normal sexual dimorphism of the adult human brain assessed by in vivo magnetic resonance imaging. *Cerebral cortex, 11*(6), 490-497.

Graber, J. A., & Petersen, A. C. (1991). Cognitive changes in adolescence: biological perspectives. In K. R. Gibson, & A. C. Peterson, *Brain maturation and cognitive development: comparative and cross cultural perspectives* (pp. 253-297). New York, NY: Aldine de Gruyter.

Gray, H. (1918). *Anatomy of the Human Body* (20 ed.). Lea and Febiger.

Gray, J. A., & McNaughton, N. (2003). *The neuropsychology of anxiety: An enquiry into the function of the septo-hippocampal system* (No. 33). Oxford university press.

Grön, G., Wunderlich, A. P., Spitzer, M., Tomczak, R., & Riepe, M. W. (2000). Brain activation during human navigation: gender-different neural networks as substrate of performance. *Nature Neuroscience, 3*(4), 404-408.

Gullone, E., & King, N. J. (1997). Three-year follow-up of normal fear in children and adolescents aged 7 to 18 years. (111, Ed.) *British Journal of Developmental Psychology, 15*(1), 97.

Guyer, A. E., Silk, J. S., & Nelson, E. E. (2016). The neurobiology of the emotional adolescent: From the inside out. *Neuroscience and Biobehavioral Reviews, 70*, 74-85.

Hajós, M., Hoffmann, W. E., & Kocsis, B. (2008). Activation of cannabinoid-1 receptors disrupts sensory gating and neuronal oscillation: relevance to schizophrenia. *Biological psychiatry, 63*(11), 1075-1083.

Hansen, R. N., Boudreau, D. M., Evel, B. E., Grossman, D. C., & Sullivan, S. D. (2015). Sedative hypnotic medication use and the risk of motor vehicle crash. *Journal Information, 105*(8).

Hare, T. A., & Casey, B. J. (2005). The neurobiology and development of cognitive and affective control. Cognition, Brain and Behavior. *Cognition, Brain and Behavior, 9*(3), 273-286.

Harrington, D. (2016, August 31). *Tissue Processing in Histology.* Retrieved from Online Laboratory Continuing Education: https://www.labce.com/tissue_processing_in_histology.aspx

Hille, B., & Catterall, W. A. (1999). Electrically Excitable Cells. In G. J. Sigel, B. W. Agranoff, & R. W. Albers, *Basic Neurochemistry: Molecular, Cellular and Medical Aspects.* Philadelphia: Lippincott-Raven.

Hoffman, G. D., & Lewis, D. A. (2011). Postnatal developmental trajectories of neural circuits in the primate prefrontal cortex: identifying sensitive periods for vulnerability to schizophrenia. *Schizophrenia bulletin, 37*(3), 493-503.

Holroyd, C. B., & Coles, M. G. (2002). The neural basis of human error processing: reinforcement learning, dopamine, and the error-related negativity. *Psychological review, 109*(4), 679-709.

Horvitz, J. C. (2000). Mesolimbocortical and nigrostriatal dopamine responses to salient non-reward events. *Neuroscience, 96*(4), 651-656.

Huang, T. L., & Charyton, C. (2008). A comprehensive review of the psychological effects of brainwave entrainment. *Alternative therapies in health and medicine, 14*(5), 38.

Ikemoto, S., & Panksepp, J. (1999). The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. *Brain Research Reviews, 31*(1), 6-41.

Illig, K. R. (2005). Projections from orbitofrontal cortex to anterior piriform cortex in the rat suggest a role in olfactory information processing. *Journal of Comparative Neurology, 488*(2), 224-231.

Institute of Medicine (US) Committee on a National Neural Circuitry Database. (1991). Overview of Neuroscience Research: A Closer Look at the Neural Hierarchy. In C. M. Pechura, & J. B. Martin (Eds.), *Mapping the Brain and its Functions: Integrating Enabling Technologies into Neuroscience Research.* Washington, DC: National Academic Press.

Joel, D., Berman, Z., Tavor, I., Wexler, N., Gaber, O., Stein, Y., & Liem, F. (2015). Sex beyond the genitalia: The human brain mosaic. *Proceedings of the National Academy of Sciences, 112*(50), 15468-15473.

Johnston, L. D., O'Malley, P. M., Miech, R. A., Bachman, J. G., & Schulenberg, J. E. (2016). *Monitoring the Future national survey results on drug use, 1975-2015: Overview, key findings on adolescent drug use.* The University of Michigan, Ann Arbor: Institute for Social Research.

Jokeit, H., & Makeig, S. (1994). Different event-related patterns of gamma-band power in brain waves of fast- and slow-reacting subjects. *Proceedings of the National Academy of Sciences, 91*(14), 6339-6343.

JoVE Educational Database. (2016). Essentials of Neuroscience. Cambridge, MA.

Kahneman, D., & Tversky, A. (1984). Choices, values, and frames. *American psychologist, 39*(4), 341-350.

Kalsbeek, A., Voorn, P., Buijs, R. M., Pool, C. W., & Uylings, H. M. (1988). Development of the dopaminergic innervation in the prefrontal cortex of the rat. *Journal of comparative neurology, 269*(1), 58-72.

Kemere, C., Carr, M. F., Karlsson, M. P., & Frank, L. M. (2013). Rapid and continuous modulation of hippocampal network state during exploration of new places. *PloS one, 8*(9), e73114.

Kensinger, E. A., Clarke, R. J., & Corkin, S. (2003). What neural correlates underlie successful encoding and retrieval? A functional magnetic resonance imaging study using a divided attention paradigm. *Journal of Neuroscience, 23*(6), 2407-2415.

Kimberg, D. Y., & Farah, M. J. (1993). A unified account of cognitive impairments following frontal lobe damage: the role of working memory in complex, organized behavior. *Journal of Experimental Psychology: General, 122*(4), 411.

Kinney, J. W., Davis, C. N., Tabarean, I., Conti, B., Bartfai, T., & Behrens, M. M. (2006). A specific role for NR2A-containing NMDA receptors in the maintenance of parvalbumin and GAD67 immunoreactivity in cultured interneurons. *Journal of Neuroscience, 25*(6), 1604-1615.

Kinser, P. A. (2000). *Brain structures and their functions.* Retrieved November 19, 2016, from Serendip Studios: http://serendip.brynmawr.edu/bb/kinser/Structure1.html

Kitanishi, T., Ujita, S., Fallahnezhad, M., Kitanishi, N., Ikegaya, Y., & Tashiro, A. (2015). Novelty-induced phase-locked firing to slow gamma oscillations in the hippocampus: requirement of synaptic plasticity. *Neuron, 86*(5), 1265-1276.

Klüver, H., & Bucy, P. C. (1937). “Psychic blindness" and other symptoms following bilateral temporal lobectomy in Rhesus monkeys. *American Journal of Physiology, 119*, 352-353.

Krettek, J. E., & Price, J. L. (1977). Projections from the amygdaloid complex to the cerebral cortex and thalamus in the rat and cat. *Journal of Comparative Neurology, 172*(4), 687-722.

Kristeva-Feige, R., Feige, B., Makeig, S., Ross, B., & Elbert, T. (1993). Oscillatory brain activity during a motor task. *Neuroreport, 4*(12), 1291-1294.

Landfield, P. W., McGaugh, J. L., & Tusa, R. J. (1972). Theta rhythm: a temporal correlate of memory storage processes in the rat. *Science, 175*(4017), 87-89.

Leary, S., Underwood, W., Anthony, R., Cartner, S., Corey, D., Grandin, T., & Miller, D. (2013). *AVMA guidelines for the euthanasia of animals: 2013 edition.* Schaumburg, Illinois: American Veterinary Medical Association.

Lee, A. K., & Wilson, M. A. (2002). Memory of sequential experience in the hippocampus during slow wave sleep. *36*, 1183-1194.

Lenroot, R. K., Gogtay, N., Greenstein, D. K., Wells, E. M., Wallace, G. L., Clasen, L. S., & Thompson, P. M. (2007). Sexual dimorphism of brain developmental trajectories during childhood and adolescence. *Neuroimage, 36*(4), 1065-1073.

Lew, S. E., & Tseng, K. Y. (2014). Dopamine modulation of GABAergic function enables network stability and input selectivity for sustaining working memory in a computational model of the prefrontal cortex. *Neuropsychopharmacology, 339*(13), 3067-3076.

Lewis, D. A., & Gonzalez-Burgos, G. (2006). Pathophysiologically based treatment interventions in schizophrenia. *Nature Medicine, 12*(9), 1016-1022.

Lewis, R., Asplin, K. E., Bruce, G., Dart, C., Mobasheri, A., & Barrett-Jolley, R. (2011). The role of the membrane potential in chondrocyte volume regulation. *Journal of cellular physiology, 226*(11), 2979-2986.

Lim, S., Han, C. E., Uhlhaas, P. J., & Kaiser, M. (2015). Preferential detachment during human brain development: age-and sex-specific structural connectivity in diffusion tensor imaging (DTI) data. *Cerebral Cortex, 25*(6), 1477-1489.

Lodish, H., Berk, A., Zipursky, S. L., Matsudaira, P., Baltimore, D., & Darnell, J. (2000). Section 21.4, Neurotransmitters, Synapses, and Impulse Transmission. In *Molecular Cell Biology* (4th ed.). New York: W. H. Freeman.

Logothetis, N. K. (2008). What we can do and what we cannot do with fMRI. *Nature, 453*(7197), 869-878.

Logothetis, N. K., Pauls, J., Augath, M., Trinath, T., & Oeltermann, A. (2001). Neurophysiological investigation of the basis of the fMRI signal. *Nature, 412*(6843), 150-157.

Logue, S. F., & Gould, T. (2014). The neural and genetic basis of executive function: Attention, cognitive flexibility, and response inhibition. *Pharmacology Biochemistry and Behavior, 123*, 45-54.

Luna, B., Garver, K. E., Urban, T. A., Lazar, N. A., & Sweeney, J. A. (2004). Maturation of cognitive processes from late childhood to adulthood. *Child Development, 75*(5), 1357-1372.

Malenka, E. J., Nestler, S. E., & Hyman, R. C. (2009). Chapter 13: Higher Cognitive Function and Behavioral Control. In A. Sydor, & R. Y. Brown, *Molecular Neuropharmacology: A Foundation for Clinical Neuroscience* (2nd ed., p. 218). New York: McGraw-Hill Medical.

Masterman, D. L., & Cummings, J. L. (1997). Frontal-subcortical circuits: the anatomic basis of executive, social and motivated behaviors. *Journal of Psychopharmacology, 11*(2), 107-114.

Mayo Clinic Staff. (2014). *EEG (electroencephalogram)*. Retrieved January 23, 2017, from Mayo Clinic: http://www.mayoclinic.org/tests-procedures/eeg/basics/definition/prc-20014093

Miller, E. K., & Cohen, J. D. (2001). An integrative theory of prefrontal cortex function. *Annual Review of Neuroscience, 24*, 167-202.

Miltner, W. H. (1999). Coherence of gamma-band EEG activity as a basis for associative learning. *Science, 397*(6718), 434-436.

Moody, W. J., & Bosma, M. M. (2005). Ion channel development, spontaneous activity, and activity-dependent development in nerve and muscle cells. *Physiology Reviews, 85*, 883-941.

Moore, T. H., Zammit, S., Lingford-Huges, A., Barnes, T. R., Jones, P. B., Burke, M., & Lewis, G. (2007). Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *The Lancet, 370*(9584), 319-328.

Moser, M. B., Moser, E. I., Forrest, E., Andersen, P., & Morris, R. G. (1995). Spatial learning with a minislab in the dorsal hippocampus. *Proceedings of the National Academy of Sciences, 92*(21), 9697-9701.

Myher, T. (2003). Neurotransmitter systems involved in learning and memory in the rat: a meta-analysis based on studies of four behavioral tasks. *Brain Research Reviews, 41*(2), 268-287.

Nakazawa, K., Quirk, M. C., Chitwood, R. A., Watanabe, M., Yeckel, M. F., Sun, L. D., & Tonegawa, S. (2002). Requirement for hippocampal CA3 NMDA receptors in associative memory recall. *Science, 297*(5579), 211-218.

National Institute on Drug Abuse. (2016). *Monitoring the Future Survey: High School and Youth Trends*. Retrieved March 2017, from https://www.drugabuse.gov/publications/drugfacts/monitoring-future-survey-high-school-youth-trends

National Institute on Drug Abuse. (2016). *National Survey of Drug Use and Health.*

National Research Council. (2010). *Guide for the care and use of laboratory animals* (8th ed.). Washington, D.C.: National Academic Press.

Neath, K. N., Limebeer, C. L., Reilly, S., & Parker, L. A. (2010). Increased liking for a solution is not necessary for the attenuation of neophobia in rats. *Behavioral neuroscience, 124*(3), 398-404.

Nelson, C. A., Monk, C. S., Lin, J., Carver, L. J., Thomas, K. M., & Truwit, C. L. (2000). Functional neuroanatomy of spatial working memory in children. *Developmental Psychology, 36*(1), 109-116.

NeuroCognitive Imaging Lab. (n.d.). *ERP/EEG*. Retrieved January 27, 2017, from NeuroCognitive Imaging Lab: http://www.ncilab.ca/erpeeg

Neurotransmitter release and removal. (2001). In D. Purves, G. J. Augustine, & D. Fitzpatrick, *Neuroscience.* Sunderland: Sainauer Associates.

Newman, S. D., Carpenter, P. A., Varma, S., & Just, M. A. (2003). Frontal and parietal participation in problem solving in the Tower of London: fMRI and computational modeling of planning and high-level perception. *Neuropsychologia, 41*(12), 1668-1682.

Nikolaus, S., Antke, C., Beu, M., & Müller, H. W. (2010). Cortical GABA, striatal dopamine and midbrain serotonin as the key players in compulsive and anxiety disorders—results from in vivo imaging studies. *Reviews in the Neurosciences, 21*(2), 119.

O' Donnell, P. (2003). Dopamine gating of forebrain neural ensembles. *European Journal of Neuroscience, 17*(3), 429-435.

O'Donnell, P. (2010). Adolescent maturation of cortical dopamine. *Neurotoxicity Research, 18*, 306-312.

Pandya, D. N., & Seltzer, B. (1982). Intrinsic connections and architectonics of posterior parietal cortex in the rhesus monkey. *Journal of Comparative Neurology, 204*(2), 196-210.

Paulus, M. P. (2007). Decision-making dysfunctions in psychiatry—altered homeostatic processing? *Science, 318*(5850), 602-606.

Paxinos, G., & Watson, G. (1998). *The Rat Brain in Stereo Taxic Coordinates.* Academic Press.

Petty, F. (1995). GABA and mood disorders: a brief review and hypothesis. *Journal of Affective Disorders, 34*(4), 275-281.

Pfeiffer, B. E., & Foster, D. J. (2015). Autoassociative dynamics in the generation of sequences of hippocampal place cells. *Science, 349*, 180-183.

Purves, D., Augustine, G. J., & Fitzpatrick, D., et al. (2001). GABA and Glycine. In *Neuroscience.* Sunderland: Sinauer Associates.

Purves, D., Augustine, G. J., & Fitzpatrick, D., et al. (2001). Glutamate. In *Neuroscience.* Sunderland: Sinauer Associates.

Purves, D., Augustine, G. J., & Fitzpatrick, D., et al. (2001). Stages of Sleep. In *Neuroscience.* Sunderland: Sinauer Associates.

Purves, D., Augustine, G. J., & Fitzpatrick, D., et al. (2001). The ionic basis of action potentials. In *Neuroscience.* Sunderland: Sinauer Associates.

Purves, D., Augustine, G. J., Fitzpatrick, D., et al. (2001). The long-term storage of information. In *Neuroscience* (2nd ed.). Sunderland, Sinauer Associates.

Pratt, W. E., & Mizumori, S. J. (2001). Neurons in rat medial prefrontal cortex show anticipatory rate changes to predictable differential rewards in a spatial memory task. *Behavioural brain research, 123*(2), 165-183.

Quin, S., Cho, S., Chen, T., Rosenberg-Lee, M., Geary, D. C., & Menon, V. (2014). Hippocampal-neocortical functional reorganization underlies children's cognitive development. *Nature Neuroscience, 17*(9), 1263-1269.

Rothman, S. M. (1983). Synaptic activity mediates death of hypoxic neurons. *Science* (220), 536-537.

Schematic of an Action Potential. (2007, June). Retrieved January 27, 2017, from WikiMedia Commons: https://en.wikipedia.org/wiki/User:Chris_73

Schlingloff, D., Kali, S., Freund, T. F., Hajos, N., & Gulyas, A. I. (2014). Mechanisms of sharp wave initiation and ripple generation. *Journal of Neuroscience, 34*, 11385-11398.

Schomburg, E. W., Fernandez-Ruiz, A., Mizuseki, K., Berenyi, A., Anastassiou, C. A., Koch, C., & Buzsaki, G. (2014). Theta phase segregation of input-specific gamma patterns in entorhinal-hippocampal networks. *Neuron, 84*(2), 470-485.

Schultz, W. (1999). The reward signal of midbrain dopamine neurons. *Physiology, 14*(6), 249-255.

Scoville, W. B., & Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *Journal of Neurology, Neurosurgery & Psychiatry, 20*(1), 11-21.

Seamans, J. K., Floresco, S. B., & Phillips, A. G. (1998). D1 receptor modulation of hippocampal-prefrontal cortical circuits integrating spatial memory with executive functions in the rat. *Journal of Neuroscience, 18*, 1613-1621.

Semple, B. D., Blomgren, K., Gimlin, K., Ferriero, D. M., & Noble-Haeusslein, L. J. (2013). Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Progress in neurobiology*, *106*, 1-16.

Shors, T. J., Falduto, J., & Leuner, B. (2004). The opposite effects of stress on dendritic spines in male vs. female rats are NMDA receptor-dependent. *European Journal of Neuroscience*, *19*(1), 145-150.

Sian, J., Youdim, M. B., Riederer, P., & Gerlach, M. (1999). Biochemical Anatomy of the Basal Ganglia and Associated Neural Systems. In G. J. Siegel, B. W. Agranoff, & R. W. Albers (Eds.), *Basic Neurochemistry: Molecular, Cellular and Medical Aspects* (6th ed.). Philadelphia: Lippincott-Raven.

Siegel, G. J., Agranoff, B. W., & Albers, R. W. (1999). Electrically Excitable Cells. In *Basic Neurochemistry: Molecular, cellular and medical aspects.* Lippincott-Raven.

Singleton, M. J. (2009). Functional Magnetic Resonance Imaging. *The Yale Journal of Biology and Medicine, 82*(4), 233.

Sohal, V. S., Zhang, F., Yizhar, O., & Deisseroth, K. (2009). Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature, 459*(7247), 698-702.

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neuroscience & Biobehavioral Reviews, 24*(4), 417-463.

Spinella, M., Yang, B., & Lester, D. (2004). Prefrontal system dysfunction and credit card debt. *International Journal of Neuroscience, 114*(10), 1323-1332.

Spydell, J. D., & Sheer, D. E. (1982). Effect of problem solving on right and left hemisphere 40 hertz EEG activity. *Psychophysiology, 19*(4), 420-425.

Stuss, D. T., & Benson, D. F. (1986). *The frontal lobes.* Raven Press.

Swanson, L. W., & Cowan, W. M. (1977). An auto radiographic study of the organization of the efferent connections of the hippocampal formation in the rat. *Journal of Comparative Neurology, 172*(1), 49-84.

Tang, Y., Li, Y., Wang, J., Tong, S., Li, H., & Yan, J. (2011). Induced gamma activity in EEG represents cognitive control during detecting emotional expressions. *Annual International Conference of the IEEE Engineering in Medicine and Biology Society. 2011,* pp. 1717-1720. IEEE Engineering in Medicine and Biology Society.

Tanji, J., & Hoshi, E. (2008). Role of the lateral prefrontal cortex in executive behavioral control. *Physiological Reviews, 88*(1), 37-57.

Tarpert, S. F., & Brown, S. A. (2000). Substance dependence, family history of alcohol dependence and neuropsychological functioning in adolescence. *Addiction, 95*(7), 1043-1053.

Thierry, A. M., Blan, G., Sobel, A., Stinus, L., & Glowinski, J. (1973). Dopaminergic terminals in the rat cortex. *Science, 182*(4111), 499-501.

Thomases, D. R., Cass, D. K., & Tseng, K. Y. (2013). Periadolescent exposure to the NMDA receptor antagonist MK-801 impairs the functional maturation of local GABAergic circuits in the adult prefrontal cortex. *Journal of Neuroscience, 33*(1), 26-34.

Thomases, D. R., Cass, D. K., Meyer, J. D., Caballero, A., & Tseng, K. Y. (2014). Early adolescent MK-801 exposure impairs the maturation of ventral hippocampal control of basolateral amygdala drive in the adult prefrontal cortex. *Journal of Neuroscience, 34*(27), 9059-9066.

Timofeev, I., Bazhenov, M., & Seigneur, J. (2012). Neuronal Synchronization and Thalamocortical Rhythms in Sleep, Wake and Epilepsy. In J. L. Noebels, M. Avoli, & M. A. Rogawski (Eds.), *Jasper's Basic Mechanisms of the Epilepsies* (4th ed.). Bethes, MD, (US); 2012. National Center for Biotechnology Information.

Toro, R., Perronq, M., Pike, B., Richer, L., Veillette, S., Pausova, Z., & Paus, T. (2008). Brain size and folding of the human cerebral cortex. *Cerebral Cortex, 18*(10), 2352-2357.

Trautwein, W. (1963). Generation and conduction of impulses in the heart as affected by drugs. *Pharmacological reviews, 15*(2), 277-332.

Tseng, K. Y., & O'Donnell, P. (2004). Dopamine–glutamate interactions controlling prefrontal cortical pyramidal cell excitability involve multiple signaling mechanisms. *The Journal of Neuroscience, 24*(22), 5131-5139.

Tseng, K. Y., & O'Donnell, P. (2005). Post-pubertal emergence of prefrontal cortical up states induced by D1–NMDA co-activation. *Cerebral Cortex, 15*(1), 49-57.

Tseng, K. Y., & O'donnell, P. (2007a). D2 dopamine receptors recruit a GABA component for their attenuation of excitatory synaptic transmission in the adult rat prefrontal cortex. *Synapse, 61*(10), 843-850.

Tseng, K. Y., & O'donnell, P. (2007b). Dopamine modulation of prefrontal cortical interneurons changes during adolescence. *Cerebral Cortex, 17*(5), 1235-1240.

Tseng, K. Y., Chambers, R. A., & Lipska, B. K. (2009). The neonatal ventral hippocampal lesion as a heuristic neurodevelopmental model of schizophrenia. *Behavioral Brain Research, 204*(2), 295-305.

Tseng, K. Y., Mallet, N., Toreson, K. L., LeMoine, C., Gonon, F., & O'Donnell, P. (2006). Excitatory response of prefrontal cortical fast-spiking interneurons to ventral tegmental area stimulation in vivo. *Synapse, 59*(7), 412-417.

Tsien, R. W., & Barrett, C. F. (2013). A Brief History of Calcium Channel Discovery. In P. Santamaria, *Madame Curie Bioscience Database.* Austin, TX: Landes Bioscience.

Uhlhaas, P. J., & Singer, W. (2011). The development of neural synchrony and large-scale cortical networks during adolescence: relevance for the pathophysiology of schizophrenia and neurodevelopmental hypothesis. *Schizophrenia Bulletin, 37*(5), 514-523.

Uhlhass, P. J., Linden, D. E., Singer, W., Haenschel, C., Lindner, M., Maurer, K., & Rodriguez, E. (2006). Dysfunctional long-range coordination of neural activity during Gestalt perception in schizophrenia. *Journal of Neuroscience, 26*(31), 8168-8175.

Uylings, H. B., Groenewegen, H. J., & Kolb, B. (2003). Do rats have a prefrontal cortex? *Behavioural brain research, 146*(1), 3-17.

Vincent, S. L., Khan, Y. U., & Benes, F. M. (1993). Cellular distribution of dopamine D1 and D2 receptors in rat medial prefrontal cortex. *The Journal of Neuroscience, 13*(6), 2551-2564.

Walter, V. J., & Matthews, B. (1934). The central effects of rhythmic sensory stimulation. *Electroencephalography & Clinical Neurophysiology, 1*(1-4), 57-86.

Wang, M., Yang, Y., Wang, C. J., Gamo, N. J., Jin, L. E., Mazer, J. A., . . . Arnstein, A. F. (2013). NMDA receptors sub serve persistent neuronal firing during working memory in dorsolateral prefrontal cortex. *Neuron, 77*, 736-749.

Wang, X. J. (1999). Synaptic basis of cortical persistent activity: the importance of NMDA receptors to working memory. *Journal of Neuroscience, 19*, 9578-9603.

Wickramasekera, I. (1977). On attempts to modify hypnotic susceptibility: Some psychophysiological procedures and promising directions. *Annals of the New York Academy of Sciences, 296*, 143-153.

Williams, P., Hombeck, G., & Greenley, R. (2002). Adolescent health psychology. *Journal of Consulting and Clinical Psychology, 70*, 828-842.

World Health Organization. (2015). Core competencies in adolescent health and development for primary care providers: including a tool to assess the adolescent health and development component in pre-service education of health-care providers. *World Health Organization*.

Yakovlev, P. I., & Lecours, A. R. (1967). The myelogenetic cycles of regional maturation of the brain. In A. Minkowski, *Regional development of the brain in early life* (pp. 3-65). Blackwell: Oxford.

Yamamoto, J., Suh, J., Takeuchi, D., & Tonegawa, S. (2014). Successful execution of working memory linked to synchronized high-frequency gamma oscillations. *Cell, 157*(4), 845-857.

Young, P. T. (1949). Food-seeking drive, affective process, and learning. *Psychological Review, 56*(2), 98-121.

Zhou, Q., & Poo, M. M. (2004). Reversal and consolidation of activity-induced synaptic modifications. *Trends in Neuroscience, 27*, 378-383.