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Decision Making by the Prefrontal Cortex And the Role of Dopamine in Pyramidal Neuron Function

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DECISION MAKING BY THE PREFRONTAL CORTEX AND THE ROLE OF DOPAMINE IN PYRAMIDAL NEURON FUNCTION

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ABSTRACT

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There were two primary focuses of this research. The first aim was to investigate how students' perception toward the flipped classroom and video learning correlate to their characteristics including their demographics, first generation status, English language learner status, Grit level, motivation types, quality of peer collaboration, and social self-efficacy. Our data indicated that there is significant correlation between student's motivation status and attitude toward learning from video lectures. The intrinsically motivated students have a higher attitude toward learning from video. This study also demonstrates that participants with high Grit scores performed better than the participants with low Grit scores.

The second aim was to investigate the effect of D3R activation on resonance frequency and sag amplitude in type I layer V medial prefrontal cortical pyramidal neurons. Because dopamine D3R is a relatively hot area of research, I first completed an extended literature review on D3R cellular mechanisms and roles in many neuropsychiatric diseases. Then I explored the effect of D3R agonists on type I layer V pyramidal neurons. I used two types of novel Dopamine D3R agonists in this study. I found that D3R agonist application inhibited the sag amplitude and resonance frequency in type I layer V mPFC pyramidal neurons. This work shed light on previously unknown cellular mechanisms on the effect of dopamine D3R activation on intrinsic electrical properties of type I layer V pyramidal neurons. The concentrations of both agonists

used was 10 uM, at these concentrations; the drugs should saturate the D3R in our cortical slices. Further dose response experiments are needed to determine the concentration range of D3R agonists that could facilitate usage in future research.

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DEDICATION

I would like to dedicate this dissertation work to the beautiful soul of my beloved brother, Ebrahem, who unfortunately did not stay in this world long enough to see his sister achieving her dream.

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INTRODUCTION

Executive Function Definition and Examples

In humans, prefrontal cortex is responsible for executive functions (specifically, goal-directed behaviors) which include decision making, problem solving, planning and initiation of activities, working memory processes, social behavior, and targeted attention. In mice, these functions are mediated by homologous regions in the medial prefrontal cortex (mPFC; Heidbreder & Groenewegen, 2003). It is well known that the dopaminergic neurotransmitter system is a key regulator of mPFC executive function (Floresco, 2013; Goldman-Rakic, 1995). Insufficient dopamine release in PFC has been related to cognitive symptoms (Yang & Chen, 2005), and disturbance in the dopaminergic system has been reported in many conditions, including anxiety disorders (e.g., PTSD, Post Traumatic Brain Disorder [Hamner & Diamond, 1993]), and GAD, Generalized Anxiety Disorder (Madras et al., 2005)], attentional disorders (e.g., ADD, Attention Deficit Disorder [Solanto, 2002]), and schizophrenia (Kessler et al., 2009).

Prefrontal Cortex Function

As mentioned above, the prefrontal cortex is crucial for working memory, an important executive function. Working memory (WM), a form of short-term memory, entails the ability to maintain current events, objects, or locations in-mind in order to carry out a task (Baddeley, 2012). Working memory processes also include the ability to retrieve and use relevant information from long term memory (Goldman-Rakic, 1996). The common example for working memory is the ability to hold in mind a phone number for seconds until you dial the number,

then the number is forgotten. In oculomotor delayed response tasks (Goldman-Rakic et al., 1990), a monkey is trained to fixate their eyes on a central spot on a screen, and a visual cue is presented at one location for 0.5 second. Then the cue disappears for 3-5 seconds (the delay period). The monkey is trained to fixate their eyes when the cue appears as well as during the delay period. At the end of the delay period, the monkey is prompted to look at the cue location. If the monkey directs its gaze toward the correct cue location, they are rewarded with juice. The monkey learns to associate between making the right gaze and the juice reward during this training period. After the training period, recordings were performed from single neurons in the monkey's dorsolateral prefrontal cortex. Importantly, a population of neurons was found that fired during the entire delay period (this type of firing can be referred to as persistent activity). It has been hypothesized that the function of the persistent firing activity is to hold and manipulate the information related to the ongoing task for the delay period (Dudai, 2004). The neurophysiological researches have classified this type of persistent firing activity in dl-PFC during delayed response tasks (Compte et al., 2000; Fuster & Alexander, 1971; Kubota & Niki, 1971; Miller et al., 1996; Watanabe, 1981). It has been proved that a lesion in the dl-PFC results in dysfunction of the working memory (Mishkin & Manning, 1978). However, the neurophysiological basis of the underlying cellular mechanism of the persistent firing is still unclear.

Rodent As Model System for Studying Human Prefrontal Cortex

The rodent medial prefrontal cortex shares very small anatomical homology with certain regions of human and monkey medial frontal cortex. Further, both in humans and rodents the medial prefrontal cortex receives input from dorsomedial thalamus. Rodent PFC can be divided was the focus of this dissertation. Rodent mPFC is in turn divided into three anatomically

distinguishable areas, from dorsal to ventral: anterior cingulate, prelimbic, and infralimbic (Vertes, 2006). While it is well known that the medial prefrontal cortex receives inputs from different brain regions, including the amygdala, the mediodorsal thalamus (Little & Carter, 2012), the hippocampus, and the contralateral mPFC (Hoover & Vertes, 2007) it remains relatively unknown how the various inputs are integrated by the mPFC.

Cortical Structures; Cortical Layers, Cortical Columns (Feedforward/Feedback)

The human cerebral isocortex also called neocortex is the thin outermost layer. The inner white matter of the neocortex occupied by the myelinated axons while the outer grey matter is a five-to-six-layered structure, with layers arranged parallel to the cortical surface and contain different population of neurons that have different projections. Layer I is the most superficial layer also known as marginal zone (MZ), consisting mainly of the apical dendrites of pyramidal neurons whose cell bodies are in layers II and III, and layers V and VI (Llinás et al., 2002; Mitchell & Cauller, 2001; Vogt, 1991). Layer II also called external granular contains small pyramidal neurons and satellite cells (which is kind of glial cells). The axons of the neurons in both layers II and III cross the corpus collosum and project to the opposite hemisphere (called commissural fibers). Layer III known as external pyramidal layer contains mainly medium-sized pyramidal cells; layer IV called internal granular layer (if present) contains only satellite cells, whose axons collateralize locally (no layer IV is present in rodent PFC). Layer IV receives sensory information from the thalamus through the thalamo-cortical axons that project from the thalamus into layer IV (Agmon et al., 1993). Layer V (the focus of this dissertation) known as the internal pyramidal layer contains mainly large pyramidal neurons that project subcortically to spinal cord, brain stem, and basal ganglia to control behavior (Chen et al., 2005). Layer V is the major output layer therefore it is extensively studied by neuroscientists. Layer VI contains

various types of pyramidal and non-pyramidal cells that project to the thalamus, called the corticothalamic projection. The new cortex is also divided into four lobes according to the type of information that every lobe process and the pattern of the sulci and the gyri (Nolte & Sundsten, 2009). The occipital lobe processes the visual information; frontal lobe plays a very important role in cognitive function and controlling emotions, voluntary movement, and language; the parietal lobes are involved in processing all sensory information, and the temporal lobes are involved in processing of auditory information and memory encoding.

Focus on Layer V Subtypes of Pyramidal Neurons as Major Output Neurons

Pyramidal neurons can be found in the cortex and in the phylogenetically old cortical structures, the amygdala and hippocampus. The morphology of the pyramidal neurons differs from region to region and within region (e.g., Layers II/III vs. layer V) but generally they are all characterized by a soma (cell body) and apical and basal dendritic trees that arise from the base and apex of the soma. The reason behind giving this name to the pyramidal neuron is the shape of the soma, which resembles a pyramid. Layer V pyramidal neurons are characterized by having longer apical dendrites than the other pyramidal neurons present in the cortex (Spruston, 2008). Pyramidal neurons in layer V are the main neocortical output cells; a subset of layer V neurons send projection fibers to subcortical motor areas in the brainstem and spinal cord to ultimately control behavior. Because of this, many researchers have focused on characterizing layer V pyramidal neurons in terms of their connectivity, morphology, and electrophysiology (Gee et al., 2012). There are two subtypes of the layer V pyramidal neurons, type I and type II (Lee et al., 2014). Type I pyramidal neurons are also called corticopontine neurons (CPn), projecting to subcortical areas (brain stem, limbic systems, spinal cord); type II pyramidal neuron are also called commissural neurons (COM), projecting to the contralateral hemisphere (Molnár &

Cheung, 2006; Wang et al., 2006). In terms of morphology, type I pyramidal neurons have thick apical dendritic trees, wide tufts, and bigger soma, while type II neurons have thin apical dendritic trees, less complex tufts, and smaller soma (Reiner et al., 2003).

It is well known that the apical dendrites of layer V pyramidal neurons extend to layer I and receive input from various brain areas (Larkum et al., 2009). A hypothesis proposed by Larkum et al. (2009) suggested that, in layer V pyramidal cells, feed-forward information is delivered to synapses on the apical trunk (via layer IV or III neurons), while feed-back information is received by apical tuft synapses (in layer I). Finally, basal dendrites are thought to receive inputs primarily from other layer V cells. The information arrives at different layers and thus synapse on different locations on layer V pyramidal neurons, thus a major question arises as to how this information is processed by layer V pyramidal neurons. Larkum et al. (2009) suggested that the layer V pyramidal neurons act as coincidence detectors that generate a high frequency burst of action potentials at the soma when synaptic inputs to apical dendrites are activated concurrently with postsynaptic action potentials due to excitation impinging on the basal dendrites. This coupling is thought to be due to the fact that pyramidal neurons contain voltage gated Ca channels that contribute to spiking in apical dendrites (Larkum et al., 2009). Further, Na channels in the apical dendritic trunk are known to contribute to backpropagation of action potentials from soma to apical dendrites (Stuart et al., 1997).

Dopamine

Dopamine (DA) or 3,4-dihydroxyphenethylamine is a monoamine neurotransmitter produced by several areas of the brain and that has been related to many neurological processes in the brain including working memory, action-outcome association, reward processing, motivational drive, voluntary movement, learning, sleep, food intake, attention, olfaction, and

vision. The basic source of the dopamine in the brain are the midbrain nuclei, substantia nigra and ventral tegmental area; axons of these nuclei project to anterior regions, including medial prefrontal cortex and basal ganglia (Del Arco & Mora, 2009; Thierry et al., 1973). Damage to the dopaminergic neurons results in some psychiatric and neurological pathologies (Goldman-Rakic, 1997). Dopaminergic neurons project from the midbrain to innervate forebrain and basal ganglia through three pathways; the nigrostriatal pathway originates in substantia nigra and projects to basal ganglia, the mesolimbic pathway originates in ventral tegmental area (VTA) and projects to limbic structures, and the mesocortical pathway originates from VTA and projects mainly to the PFC.

Dopamine Receptors Subtypes and Signaling Mechanism

It is known that the dopamine system regulates various functions of the PFC through two types of receptors: D1 family and D2 family. Members of the same family share some homology. The D1 family includes D1R and D5R, while the D2 family includes D2R, D3R, and D4R (Ilani et al., 2001; Lachowicz & Sibley, 1997; Le Foll et al., 2009). Both subtypes of the dopamine receptors are G-protein coupled receptors (GPCR) that pass through the plasma membrane seven times with an intracellular carboxyl terminus and an extracellular amino terminus (Maramai et al., 2016). The G-protein coupled receptor performs its action through changing the permeability of ions channels or/and modulating other types of receptors (Lachowicz & Sibley, 1997). The D1-like subfamily is linked to G-protein G_s and it in turn stimulates adenylyl cyclase and increases the intracellular concentration of cyclic-AMP (adenosine 3′,5′-cyclic monophosphate), activating protein kinase A (PKA). Protein Kinase A in turn mediates phosphorylation of downstream proteins including the NMDAR and phosphorylation of NMDA receptors increase their activity (Snyder et al., 1998). While the D2-

like subfamily is linked to G-protein G_i that inhibits adenylyl cyclase and decreases cyclic AMP, inhibiting PKA. Also, D1-like and D2-like receptors can form heteromeric complexes that lead to activation of G_q, which leads to activation of the enzyme phospholipase-C (PLC). PLC increases the concentration of the second messengers, inositol 1,4,5, triphosphate (IP3) and diacylglycerol (DAG). IP3 binds to the IP3 receptor on the ER membrane and causes Ca^{2+} ion release from the ER; then DAG leads to activation of protein kinase C (PKC; Berridge, 2009). D1R and D5R share about 80% identity in their transmembrane domain, and the D2R and D3R share about 75% identity in their transmembrane domain (Missale et al., 1998). Essentially, dopamine has a high affinity to the GPCR when the G-protein is bound to the intracellular side of the GPCR, while the dopamine has a low affinity to GPCR if the G-protein is not bound to the GPCR. Dopamine binding to D1 family or D2 family receptors leads to β-arrestin recruitment (Neve et al., 2004). Furthermore, activation of the dopamine D2 family leads to activation of other signaling pathways like ion channels, phospholipases, and MAP kinase (Strange & Neve, 2013). It has been proposed that dopamine works through the D3R to activate the GPCR - G$\beta\gamma$ which is coupled to inward rectifier potassium channels (GIRKs), leading to inhibitory effects in many brain regions (Kuzhikandathil et al., 1998). A pioneering study revealed that dopamine activation of the D3R leads to activation of the Akt signaling pathway, where Protein kinase B (Akt) activation leads to phosphorylation and inactivation of Glycogen synthase kinase-3 (GSK-3).

Normal function of the PFC strongly depends on the appropriate dopaminergic input. As mentioned, damage to dopaminergic neurons results in some psychiatric and neurological pathologies (Goldman-Rakic, 1997). Therefore, it is important to understand the cellular mechanisms of how dopamine (specifically through the D3R) modulates PFC function to

understand mental illnesses like schizophrenia. The D2R and D3R share about 78% sequence identity in the transmembrane and binding domains. It has been proposed that dopamine has about 20 times higher affinity for the D3R than the D2R, and this indicates that at physiological dopamine concentrations, the dopamine D3R will be occupied with dopamine for a longer amount of time than the D2R (Richtand et al., 2001). Furthermore, because of the high affinity of D3R to dopamine, the D3R is very likely affected by tonic dopamine levels in the brain (Sokoloff et al., 1990). Finally, currently used antipsychotic medications have a high affinity to the D3R (Joyce & Millan, 2005).

Dopamine Receptor Expression in Medial Prefrontal Cortex

The dopamine receptor subfamilies differ in their anatomical expression in the brain. In mPFC the expression of both D1 and D2 receptors in deep layers (layers V and VI) is stronger than in the superficial layers (Santana et al., 2009). D1R is expressed more strongly in Layer VI, while D2R is exclusively expressed in layer V pyramidal neurons (Santana et al., 2009). In general, the expression of the D1-like subfamily is substantially greater than the D2-like subfamily in prefrontal cortical pyramidal neurons (Floresco, 2013; Puig et al., 2014). This is due to the fact that layer V type I pyramidal neurons express both D1 and D2 subfamilies, while type II pyramidal neurons express D1-like receptors only (Gee et al., 2012). This differential distribution of the dopamine receptors in layer V pyramidal neurons has a differential effect on regulating layer V pyramidal neuron subtypes (Leyrer-Jackson & Thomas, 2018). It has been proposed that dopamine acts on D1-like receptors in a dose dependent manner, with deficient or excessive dopamine receptor activation in PFC resulting in improper PFC executive function (Williams & Castner, 2006). Several studies revealed that dopamine binding to D1 receptors leads to phosphorylation and insertion of more AMPA receptors on the postsynaptic membrane

which leads to increases in excitatory transmission in the neurons (Chao et al., 2002; Leyrer-Jackson & Thomas, 2018).

Dopamine Role in Working Memory Tasks

Dopamine plays a very important role in working memory abilities. A study conducted by Watanabe et al., 1997 revealed that the level of dopamine increases during WM tasks in PFC dopamine acts mainly through the D1 receptor, modulating PFC electrical activity during WM tasks (Williams & Goldman-Rakic, 1995). It is well known that overstimulation or blockade of dopamine receptors in PFC impairs working memory function which then disrupts goal-directed behaviors (Zahrt et al., 1997). Also, depletion of PFC dopamine in monkey results in cognitive deficits (Brozoski et al., 1979). Depletion of dopamine in lateral PFC during a monkey oculomotor delay response task by using D1R and D2R antagonists led to impairment of learning ability, with the monkeys making more errors (Puig et al., 2014).

Modulation of Prefrontal Cortex Neurons: Intrinsic Properties and Synaptic Transmission

There are two types of firing patterns observed for dopaminergic neurons: tonic and phasic. The tonic state is defined as low frequency spontaneous firing which is related to changing tonic levels of extracellular dopamine in target structures (Grace & Onn, 1989). The phasic state (known as burst firing) is associated with high levels of DA released into the synaptic cleft in response to behaviorally related stimuli, for example reward presentation (Schultz et al., 1993). This large amount of dopamine that is released during burst firing acts transiently at the synaptic cleft and is then removed by uptake via dopamine transporters (DAT) into presynaptic dopamine neuron terminals (Chergui et al., 1994). Balance between these two firing patterns is thought to be important for proper PFC executive function, and disturbance in

tonic or phasic firing patterns have been related to some disorders, for example schizophrenia and ADHD (Durstewitz & Seamans, 2008).

Role of Dopamine in Neuropsychiatric Disorders

Prefrontal cortex receives dopamine input from ventral tegmental area (VTA) of the midbrain through the mesocortical dopaminergic fibers. The mesocortical dopamine input to the PFC plays a very significant role in PFC cognitive functions and dopamine dysfunction is implicated in neuropsychiatric disorders (Seamans & Yang, 2004). Dopamine replacement therapy is used to supply the areas of the brain when the primary pathology is related to dopamine deficiency. However, some brain areas have less dopaminergic input, and administration of dopamine to these areas may result in cognitive dysfunction (Narayanan et al., 2013). Furthermore, Angrist et al. (1974) found that the administration of substances that trigger dopamine release worsens the symptoms of psychosis. A theory implicating dopamine as a major factor in schizophrenia was first hypothesized by Carlsson and Lindqvist (2009), since administration of dopamine blocking agents like chlorpromazine reduces the symptoms of schizophrenia. Furthermore, some work has been done in psychotic patients suggesting that dopaminergic function is high in those patients (Howes et al., 2012). Remarkably, in patients with schizophrenia, a resting-state functional MRI study revealed reduced connectivity of VTA dopaminergic neurons that project to the PFC; however, the administration of antipsychotic agents restores this connectivity (Hadley et al., 2014). For about 60 years, the protocol for the treatment of patients with schizophrenia included administering the dopamine D2R antagonist, chlorpromazine. Dopamine also plays a crucial role in attention deficit hyperactivity disorders (ADHD). Amphetamine is a medication used to treat ADHD by increasing dopamine release and blocking dopamine uptake. These studies underscore the role of dopamine in various psychiatric

disorders (detailed more extensively in chapter 3), emphasizing the urgent need for more research on the mechanisms of dopamine's cellular actions.

Pyramidal Neuron Expression of Dopamine Receptors and Hyperpolarization and Cyclic Nucleotide Gated Nonspecific Cation Channels

Both subtypes of pyramidal neurons express a variable degree of hyperpolarization and cAMP activated cation channels (HCN) which are activated by membrane hyperpolarization (Gee et al., 2012). The activation of the HCN current (also called funny current- designated as If) is related to rhythmic activity in the theta range (Seong & Carter, 2012; Shah, 2014). The pyramidal neurons that project to subcortical areas express more HCN channels than the neurons projecting to contralateral cortex (Dembrow et al., 2010). Chapter 4 of this dissertation addresses the effects of D3R activation on this important ion channel in layer V pyramidal neurons.

CHAPTER II

HOW STUDENT PERCEPTION TOWARD THE FLIPPED CLASSROOM AND VIDEO LEARNING CORRELATES TO STUDENT CHARACTERISTICS

Introduction

The flipped classroom is a teaching innovation in which the didactic portion of a course occurs outside of the normal class period, often in the form of video lectures, and the class period is used for active, student-centered application of the material. Documented advantages of a flipped classroom include increased student engagement and attendance (Kurtz et al., 2014; Lage et al., 2000), providing opportunity for multiple learning styles (Gannod et al., 2008), putting students in the center of their own learning, and improved student performance and learning gains (Heyborne & Perrett, 2016). This project aim is to investigate how students' perception toward the flipped classroom and video learning correlates to their characteristics including their demographics, first generation status, English language learner status, Grit level, motivation types, quality of peer collaboration, and social self-efficacy.

Literature Review

The flipped classroom is a relatively new teaching innovation that has been used in the world of education. The simplest definition of the flipped classroom is a reversal of where the homework and lectures take place. During the class period, the students work on homework and projects, and what would be the usual class activities like lecture presentations are shifted to homework (Lage et al., 2000; Sohrabi & Iraj, 2016). The lecture contents are accessible to students outside of the classroom time and the students can write down questions as they arise.

During the class time, the students have already been exposed to the informational content and are prepared to ask questions and apply their knowledge. In this innovation, the students are in the center and responsible for their own learning. The teacher's classroom role is to plan activities to guide the students through application of the material to facilitate a deeper learning process (Bergmann & Sams, 2007; Johnson et al., 2016). According to Bloom's Taxonomy, this means the students are doing a lower level of Bloom's Taxonomy when they are gaining the knowledge outside of the classroom, and the instructor is able to be around and assist while the students engage in a higher level of Bloom's Taxonomy (apply the knowledge) during the class time (Krathwohl, 2002).

Two high school chemistry teachers in Colorado, Jonathan Bergmann and Aaron Sams, first coined the term "flipped classroom" in 2007 (Bergmann & Sams, 2014). They began to make YouTube lecture videos available to students to watch outside of class, and during class time, the students worked on faculty guided problem solving. The teachers' first aim was to make themselves accessible to students when they needed feedback. Shortly after they made the YouTube lecture videos accessible to all of their students in the classroom, they observed improvement in engagement and performance in the course (Abeysekera & Dawson, 2014). After Salman Khan's TED talks in 2011 "Let's Use Video to Reinvent Education," the flipped classroom innovation became more popular and obtained wider attention from educators (Khan, 2011).

A study conducted by Phillips and Trainor (2014) revealed that the students preferred to apply the knowledge during class rather than listening to lecture. Multiple studies conducted on how students and their instructors perceive the flipped classroom learning experience showed students' positive perception and teachers' higher satisfaction compared to traditional classroom

(Jensen et al., 2018; Kay & Kletskin, 2012; Tuna et al., 2018; Unal & Unal, 2017; Zhonggen, 2019). Several researchers indicated that the flipped classroom improved student's motivation to learn (Bhagat et al., 2016) and led to better course grades (Mason et al., 2013) than traditional lectures. Furthermore, a study done by Al-Zahrani (2015) indicated that flipped model improved the student's level of engagement with the course materials significantly as well as enhanced student's confidence and creativity.

The video lecture is not the only strategy for delivery of contents outside of class, however, it is the most commonly used strategy. He et al. (2016) revealed the advantage of using video tutorials in students' mastering of knowledge and improving their performance. A study conducted by Jensen et al. (2018) to investigate the students' performance in two different student groups (one group used textbook readings and another group used video lectures), found that the final summative assessment (where the student has to remember and apply the content) of the students who used video lectures raised scores by about eight points, which indicates there are students who benefit from using lecture videos in flipped classrooms. A study conducted by Leatherman and Cleveland (2019) investigated student attitudes toward this teaching innovation and found that a positive attitude toward the flipped classroom was correlated most closely with a positive attitude toward lectures on video. The students able to learn at their own pace, they can pause or replay the videos which is beneficial for the students who are struggling to understand the materials. Multiple studies have revealed that the flipped classroom strategy and specifically learning from video lecture provides learning gains to the students, however, we still do not know the characteristics of the students who benefit the most from flipped classroom video lectures.

Students' Background

One area of investigation in this research is which student demographic characteristics most closely correlate with satisfaction with the flipped classroom and video lectures. Relatively little research has focused on how the underrepresented racial/ethnic minority students, first generation students, and English language learner students perceive and benefit from the flipped classroom learning environment. According to Freeman et al. (2014) incorporation of active learning techniques enhances retention rates and work diversity among underrepresented minorities. Snyder's (2016) study revealed that in collaborative learning environments, underrepresented minorities in biology performed better than majority students or compared with themselves in another type of learning environment, and the failure rate significantly declined. Moreover, the English language learner students showed a higher level of engagement in flipped than non-flipped classrooms (Haghighi et al., 2018). Interestingly, increased course structure, typically found in flipped classrooms, led to reduced failure rate and an improvement in the performance of first-generation students (Eddy & Hogan, 2014). We are interested to know how first generation, underrepresented minority, and English language learner students perceive the benefits from a flipped classroom and how their perceptions correlate with their characteristics. We predicted that the flipped classroom strategy provides disproportional benefits to students who have additional challenges to their academic success. Therefore, a positive student perception toward the flipped classroom will be associated with first generation students, students of ethnic minorities, and English language learners.

Grit

Grit is a measure of perseverance and passion for long term goals (Duckworth et al., 2007). Grit is a non-cognitive skill defined as a personality trait that predicts success and performance. A student with a high level of grit shows lower levels of discouragement and is

able to face difficulties and accomplish short- and long-term goals (Duckworth & Quinn, 2009). The flipped classroom learning environment is different from the classical teacher-centered classroom. Since the flipped classroom requires more student-directed learning, both from the video-learning component and the in-class active peer-learning process, it is likely to be a challenging change for some students. We are interested to know if student's grit correlate with their perception toward the flipped classroom environment and video lectures. We predicted that higher levels of grit will be associated with greater ability to adapt to the different approaches taken in a flipped classroom, and as result, students with more grit will have more positive perceptions toward the flipped classroom experience.

Motivation

There are two types of motivation, intrinsic and extrinsic. Ryan and Deci (2017) defined intrinsic motivation as an individual's innate willingness to do or understand something, and extrinsic motivation as motivation originating outside of the individual and driven by external reward. Student performance is affected by their motivation, and students with higher intrinsic motivation have higher learning performance (Baumann & Harvey, 2018; Ryan & Deci, 2000). The students in flipped classrooms engage in active learning activities, and the primary goal of these activities is for the students to obtain a deeper understanding of the material. Another study done by Xiu and Thompson published in (2020) revealed that there is strong relationship between students' intrinsic motivation status and perception toward flipped designed courses. We predict that intrinsically motivated students will have more positive perceptions toward the flipped classroom than extrinsically motivated students in our study, since they recognize the value of deeper learning.

Peer Collaboration and Social Self-Efficacy

Peer collaboration is an active learning method often used in flipped classrooms. During class, the students collaborate in the activities and learn from each other. According to Keppell et al. (2006), students encourage meaningful learning when they teach and learn from each other. Peer interaction during class has been shown to contribute to students' motivation and higher academic performance (Summers, 2006); however, Cooper et al. (2018) found that group activities contributed to increased or decreased student anxiety according to how the student perceived the goal from working in groups. Stipek (1998) found that if students are not comfortable working with a group, they experience anxiety. A study done by Pintrich et al. (1993) concluded that the self-efficacy is significant indicator or students' performance. Furthermore, a pioneering study by Xiu and Thompson done in (2020) indicated that student's performance in flipped designed courses was strongly predicted by the student's level of self-efficacy. We are interested to find if student perceptions toward peer collaboration and their level of social self-efficacy correlates with a positive or negative attitude toward the flipped classroom. We think that the students with a positive perception toward peer collaboration will also have a positive perception toward the flipped classroom learning experience. Also, if the students have high social self-efficacy, they will prefer working in groups, and they will have a positive perception toward flipped classroom learning experience as a result.

Rationale

Past research on the flipped classroom revealed that some students like, and some students dislike the flipped classroom, and students' attitude about the flipped classroom correlated closely with their attitude about learning from videos, which may suggest that the students like the flipped classroom because they like to learn from recorded video lectures. We

know that the video lectures may provide learning gains to the students; however, we do not know the characteristics of the students who are more likely to gain the benefit from a flipped classroom and video lectures. I hoped to provide deeper insight into understanding the characteristics of the students who benefit the most from a flipped classroom experience in general and video lectures specifically. Understanding the student characteristics will help instructors provide better learning experience and customize to their students' needs.

Purpose

I am first interested to know how first generation, underrepresented minority, and English language learner students perceive the benefits from a flipped classroom and correlate their perception with their classroom performance and overall GPA. My second goal is to determine how a student's grit correlates with their perception toward the flipped classroom environment and video lectures. My third aim is to determine the type of student motivation (intrinsic or extrinsic) and correlate the student's motivation with their perception toward the flipped classroom. I am interested in interrogating students' attitudes toward peer collaboration and students' social self-efficacy and asking whether these student characteristics correlate with a positive or negative attitude toward videos and the flipped classroom. Since the flipped classroom I will be studying utilizes a great deal of student collaborative learning during the in-class activities, I think their perception toward the flipped classroom may hinge upon their attitude toward peer collaboration.

Method

The Participants

The participants in this study were 78 undergraduate college students enrolled in sophomore-level genetics flipped course. All participants were at least 18 years of age, and the

participants do not represent a vulnerable population. Very rarely, a minor may enroll in the course. Due to lab safety issues, the instructor is informed each semester about any student in the classroom who is under 18 years of age, and the instructor takes extra measures to ensure that survey data from minors are not used in any study. All survey questions received Institutional Review Board approval (1577391-2) shown in Appendix A, and the full survey is shown in Appendix B. The students who were enrolled in Spring 2020 and Fall 2021 courses were informed of the opportunity to participate in a survey on their attitudes about the flipped genetics classroom (Recruitment Email and Consent Form shown in Appendices C and D respectively). Data were collected during Spring semester 2020 and Fall semester 2021.

Instruments

Perception of the Flipped Classroom

Four questions were used to investigate students' perceptions about the flipped classroom in general. These were self-written by the researcher, the aim behind these four questions was to elicit participants' opinions regarding if they prefer a regular lecture or a flipped classroom (these are the same as the questions used in Leatherman and Cleveland (2019)). A sample item for this measure is "I would recommend the flipped version of Genetics compared to a traditional lecture to my peers." Response options for the first four items were on a 5-point rating scale, ranging from 1 (strongly agree) through 5 (strongly disagree). To obtain the perception of flipped classroom scores, the responses to the four items were summed; thus, the perception score could range from 4 to 20 points.

Perception of the Lecture Videos

Four questions were used to investigate students' perceptions toward learning from lecture videos in particular. These questions were self-constructed questions (written by the

researcher for the purpose of the study) to obtain the participants' opinion regarding whether or not they like the video lectures. A sample item to illustrate this measure is "Having the lectures on video helped me learn the material better than an in-class lecture would help me". Response options for the four items were a 5-point rating scale, ranging from 1 (strongly agree) through 5 (strongly disagree). To obtain the perception of lecture scores, the responses to the four items were summed for a possible score range from 4 to 20 points.

Grit

Eight items scale were used to characterize students' grit (Duckworth & Quinn, 2009). The Short Grit scale was composed of two subscales, each subscale formed of four items, and the total is eight items. The two subscales are: Consistency of Interest (i.e., Passion) and Persistence of Effort (i.e., Perseverance). A sample item from the Short Grit scale is "Setbacks don't discourage me." The items were rated on 1 to 7 points rating scale from 1 (not like me at all) through 7 (very true of me). The total Grit score was then divided by 8 to obtain the final score which ranges from 1 to 5; with 1 indicating low passion/perseverance and 5 indicating a high passion/perseverance (some of the items were reverse coded). I obtained the final grit score by computing the mean score across the eight items for a possible score range of 1 to 5. The maximum score on this scale is 5 (extremely gritty), and the lowest score on this scale is 1 (not at all gritty). The Grit scale is considered to be a reliable and valid scale with reliability estimate in previous research of 0.82 (Priyohadi et al., 2019).

Motivation

Six questions were used to characterize students' motivation, referred to as "motivation strategies for Learning Questionnaire" (MSLQ) from (Pintrich & De Groot, 1990). Response options were based on a 7-point Likert-type scale with responses ranging from 1 (not at all true

of me) to 7 (very true of me). The six items represent two value component subscales with three items for intrinsic and three items for extrinsic motivation. A sample item measuring intrinsic motivation is "When I have the opportunity in this class, I choose course assignments that I can learn from even if they don't guarantee good grades" whereas a sample item for extrinsic motivation is "Getting a good grade in this class is the most satisfying thing for me right now." Intrinsic and extrinsic motivation scores were created by summing responses to the respective subscale items resulting in subscale scores with a possible range from 8 (which indicates the student is highly extrinsically motivated) to 56 (which indicates the student is highly intrinsicly motivated). It's worth mentioning that prior psychometric evidence supporting reliability and validity of scores has been demonstrated for this six-item motivation scale (Pintrich & De Groot, 1990). For the intrinsic goal motivation, the coefficient alpha is 0.74, while for extrinsic goal motivation the coefficient alpha is 0.62 (Pintrich et al., 1993).

Social Self-Efficacy

Three questions were used to investigate students' social self-efficacy (adopted from (Ryan & Patrick, 2001) and one question was written by the researcher for the purpose of the study. A sample item to illustrate the content of the measure is "It's easy for me to start a conversation with another student about what we are learning in the class." Response options for the four items were based on a 5-point rating scale, ranging from 1 (strongly agree) through 5 (strongly disagree). To obtain total social self-efficacy scores we summed the four item responses for a possible score range from 4 to 20.

Attitude Toward Peer Collaboration

Eleven questions were used to investigate attitude toward peer collaboration (adopted from the Study of Collaboration Students Survey [CCSR 2009 my voice survey; Akey, 2006;

Fredricks et al., 2016; Patrick et al., 2007; Johnson et al., 1983; Micari & Drane, 2011). A sample item from the CCSR is "When doing group work, we feel comfortable disagreeing with each other." Response options for the CCSR are provided on a 5-point rating scale, ranging from 1 (never) through 5 (every time). Total scores for this measure were computed by summing the 11 item responses, for a possible score range from 11 to 55. Lower scores indicate negative attitude toward peer collaboration whereas higher scores indicate a positive attitude toward peer collaboration.

Demographic and Background Characteristics

Questions about the students' background characteristics included race/ethnicity, gender identity, and first-generation status (adapted from Gonzalez-Rodriguez et al., 2016). A student was considered a first-generation college student if neither parent obtained a bachelor's degree, and was considered a continuing generation student if at least one parent obtained a bachelor's degree. For race/ethnicities, the survey consisted of the following responses and the participants could mark more than one response to describe their race and ethnicity: Hispanic/Latinx, Black / African American, Asian/Pacific Islander, Native American, Middle Eastern, White/Caucasian, and multiracial where the participant had available space to identify their race/ethnicity if it was not listed in the list of response options. The gender identity question included the following choices: woman, man, transgender, non-binary, and other where the participant had an empty space to specify.

Data Collection Procedure

During the last four weeks of the semester, the students received a recruitment email (included in Appendix C) from the course instructor inviting them to participate in the online survey, and the link to the online Qualtrics survey was included in the email. When the students

clicked on the survey link, the consent form appeared first on which they were asked to type their name to verify their consent. In both the recruitment email and the consent form, students were informed that their individual responses (with their names attached) would not be read by their instructor. Their instructor only had access to de-identified responses, and even those were not viewed prior to the submission of grades for the semester. In addition, if students chose not to participate in the study, their non-participation did not affect their grade in the course. Students who chose to participate in the study earned 5 extra credit points. Students who chose not to participate in the study were offered an alternative opportunity to earn five extra credit points.

Data Analysis

For data analyses (for all of the research questions) I used (inferential statistic) Pearson chi square by IBM SPSS Statistics version 27. The Alpha level used to test all of the questions was 0.05. Cronbach alpha scores were calculated for each section of the survey to estimate the internal consistency (Table 1). Our Cronbach's Alpha indicate that we have moderate to high reliability, for my study, the reliability estimate using (Cronbach's alpha) is ranging between 0.50-0.90 which indicate the level of the scale reliability (Likert questions) is acceptable (Hinton et al., 2004, p. 364).

Table 1

Cronbach's Alpha for Each Instrument

Instrument Questions	Reliability Estimate (Cronbach's Alpha)
Perception toward Flipped Classroom	0.88
Perception toward Video lectures	0.88
Grit	0.69
Intrinsic Goal Motivation	0.84
Extrinsic Goal Motivation	0.51
Social Self-Efficacy	0.73
Attitude toward Peer Collaboration	0.89

Note. The reliability estimates for the used instrument range between 0.5-0.9 which indicate moderate to high reliability.

Results

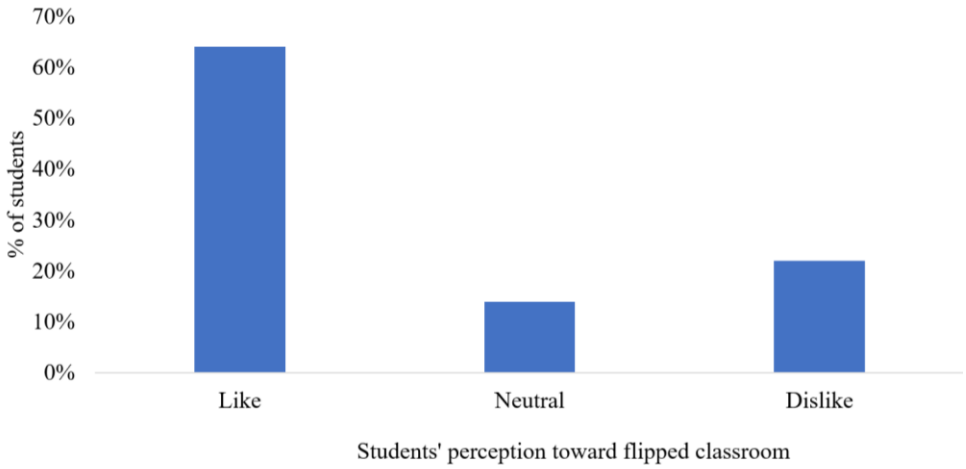
Student Attitude Toward Flipped Classroom and Video Lecture

Combining the responses from the Likert-scale survey to test student's satisfaction about the flipped classroom, we classified students with a Likert score of 4- 10 as "liked" the flipped classroom, students with a score of 11, 12,13 as "neutral" toward the flipped classroom, and students with a score of 14-20 as "disliked" the flipped classroom. Among the 78 participants 50 (64.1%) liked, (14.1%) neutral, and 17 (21.7%) were disliked regarding their satisfaction with the flipped classroom experience (Figure 1). For the students attitudes toward the learning from the video, we similarly classified students with a Likert score of 4-10 as "liked" learning from videos, students with a score of 11, 12, 13 as "neutral" toward learning from videos, and students with a score of 14-20 as "disliked" learning from videos. Among 78 participants (34.6%) liked, (23%) neutral, and (42.3%) were disliked (Figure 2). A Pearson chi-square test was used to determine the correlation between students' attitude toward the flipped classroom and learning from video lectures. The Chi-square test showed a statistically significant correlation between

students' attitudes toward the flipped classroom and their attitude toward learning from video lectures (Figure 3), as has been previously demonstrated ($\chi 2 = 24.2$, $df = 4$, $p = 0.00$; Leatherman & Cleveland, 2019).

Figure 1

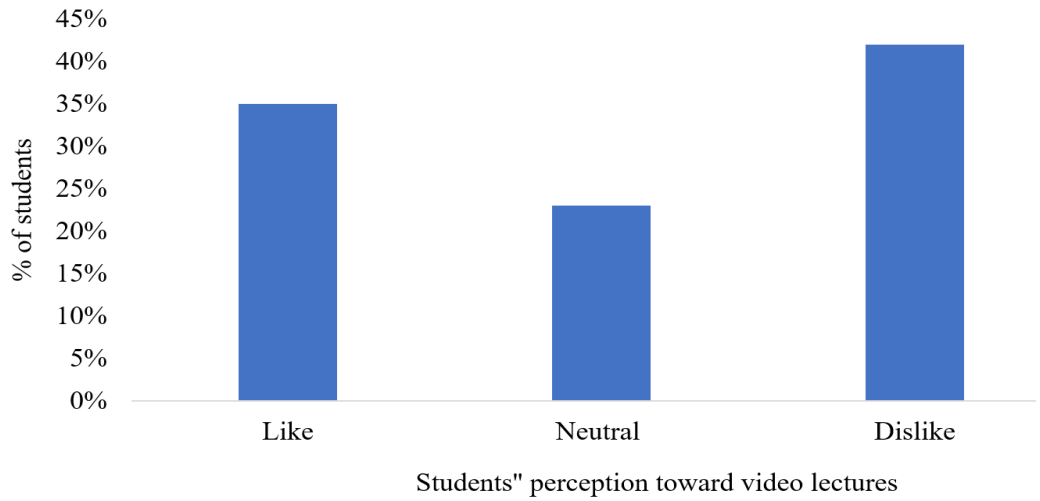
Students' Perception Toward Flipped Classroom.



Note. Among the 78 participants, 50 participants (64%) liked, 11 participants (14%) neutral, and 17 participants (22%) disliked the flipped classroom.

Figure 2

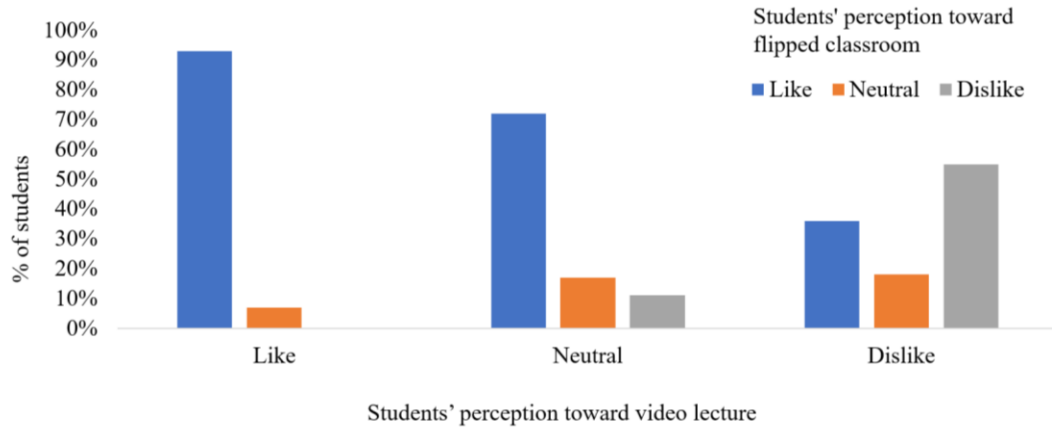
Students' Perception Toward Learning from Video Lectures



Note. Among 78 participants, 27 (35%) liked, 18 participants (23%) neutral, and 33 participants (42%) disliked the video lectures.

Figure 3

Students' Perception Toward the Flipped Classroom and Learning from Video Lectures



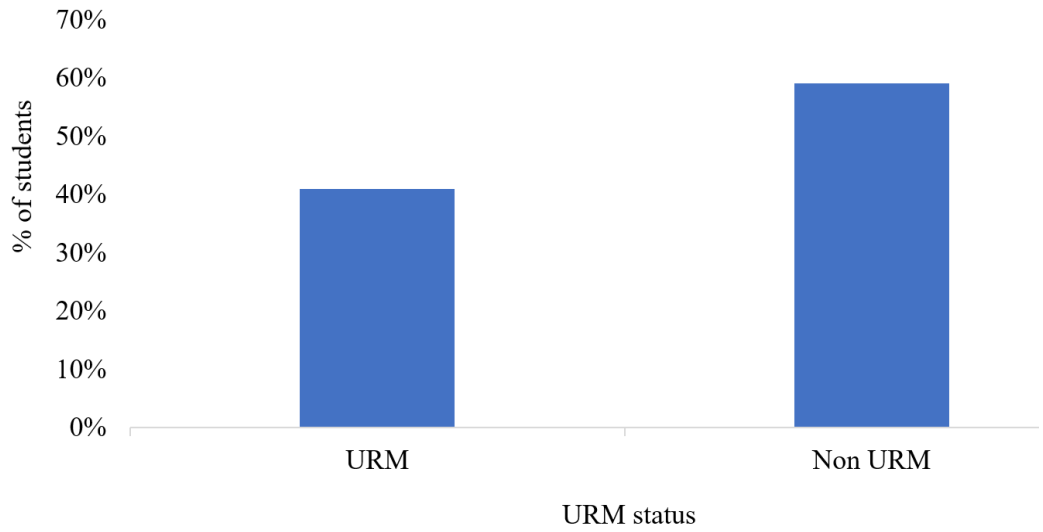
Note. There is a significant correlation between students' perception toward the flipped classroom and learning from video lectures.

Students' Attitude Toward the Videos and Students' Characteristics

To answer our first research question and see if there is correlation between student attitudes toward the video and student's characteristics Among the 78 participants, 32 (41%) of the participants identified themselves as underrepresented minorities (URM), and 46 (59%) of the participants were not underrepresented minorities (Figure 4). There were 57(73%) female participants, 20 (26%) male participants, and 1(1%) transgender participant (Figure 5). 14 (18%) of the participants identified as English as second language or what we called English language learners (ELL) and 64 (82%) of the participants identified English as their first language (Figure 6). 40 (51%) of the participants identified themselves as first generation students, and 38 (49%) of the participants were continuing generation students (Figure 7). We ran a Pearson chi-square test for every characteristic including ELL, first generation status, and URM status. The Chi-square statistical analysis did not show a significant correlation between student's attitude toward the video lectures and English language learning status ($\chi 2 = 1.319$, $df = 2$, $p = 0.517$). It also did not show a significant correlation between student's attitude toward the video lectures and student's first-generation status ($\chi 2 = 0.778$, $df = 2$, $p = 0.678$). Finally, it did not show a significant correlation between student's attitude toward the video lectures and student's URM status ($\chi 2 = 0.897$, $df = 2$, $p = 0.639$).

Figure 4

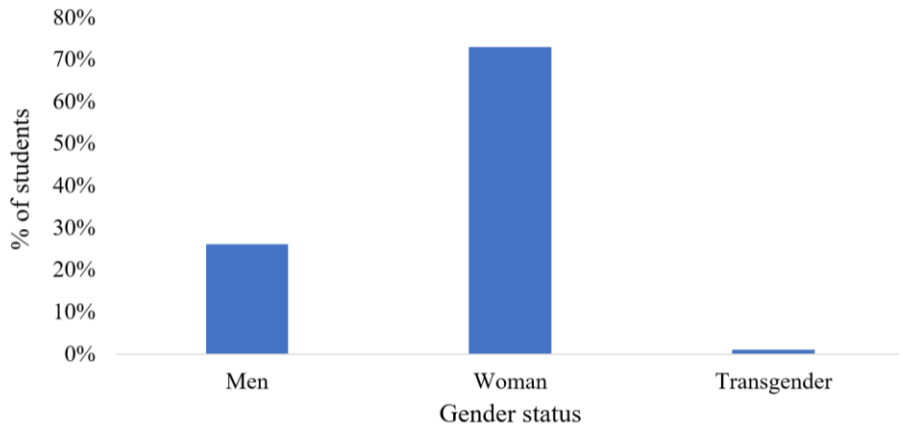
Students' Underrepresented Minorities Status



Note. Among the 78 participants, 32 (41%) of the participants identified themselves as Underrepresented Minorities (URM), and 46 (59%) of the participants were not Underrepresented Minorities.

Figure 5

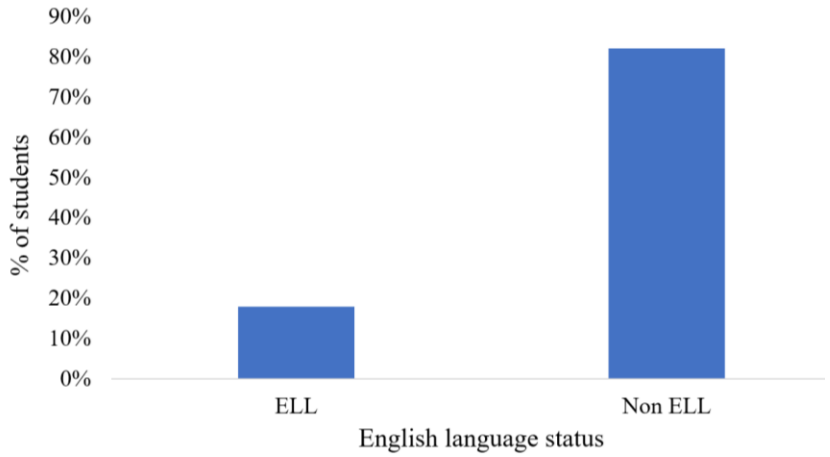
Students' Gender Status



Note. Among the 78 participants, 57 (73%) of the participants identified themselves as Woman, 20 (26%) as Man, and 1 (1%) as transgender participant.

Figure 6

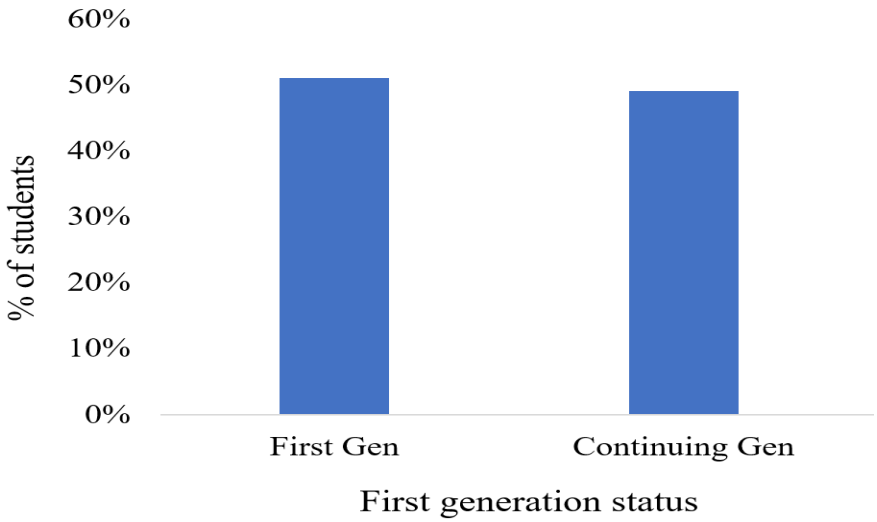
Students' English Language Status.



Note. 14 (18%) of the participants identified as English Language Learners (ELL) and 64 (82%) of the participants identified English as their first language Non-English Language Learners (Non ELL).

Figure 7

Students' First-Generation Status.



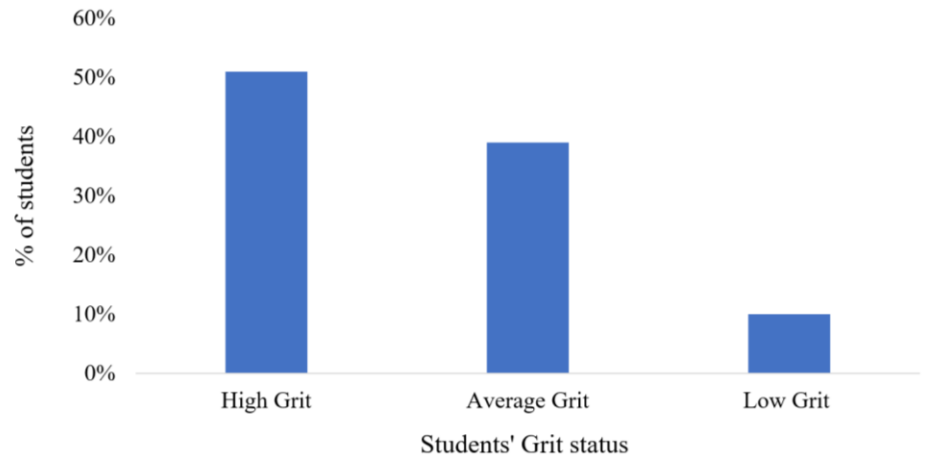
Note. Among 78 participants, 40 (51%) of the participants identified themselves as first generation (first gen) students, and 38 (49%) of the participants were continuing generation (continuing gen) students

Student's Grit Status and Student's Attitude Toward Flipped Classroom and Attitude Toward Video Lectures

We categorized students with a 3.5-5 score on Grit as high Grit status, and students with a 2.6-3.4 score as average Grit, and students with 1-2.5 as low Grit. Among 78 participants 40 (51%) high Grit, 30 (39%) average Grit, and 8 (10%) were low Grit (Figure 8) To answer our second research question and see if there is a correlation between student's attitude toward flipped classroom or attitude toward learning from video lectures and student's Grit status, we ran a Pearson chi-square test. The test did not show a significant correlation between student's attitude toward flipped classroom and the student's Grit status ($\chi 2 = 3.988$, $df = 4$, $p = .408$; Figure 9). Also, it did not show a significant correlation between student's attitude toward learning from the video lectures and student's Grit status ($\chi 2 = 2.035$, $df = 4$, $p = .729$; Figure 10). Overall, the student's Grit status did not correlate with their perception towards the flipped classroom nor perception toward learning from videos.

Figure 8

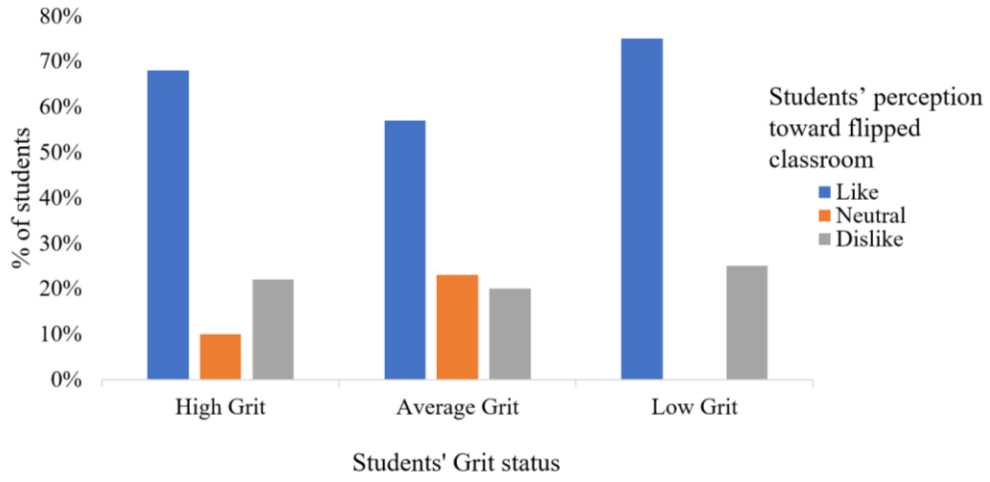
Students' Grit Status



Note. Among 78 participants 40 (51%) had high Grit, 30 (39%) average Grit, and 8 (10%) had low Grit.

Figure 9

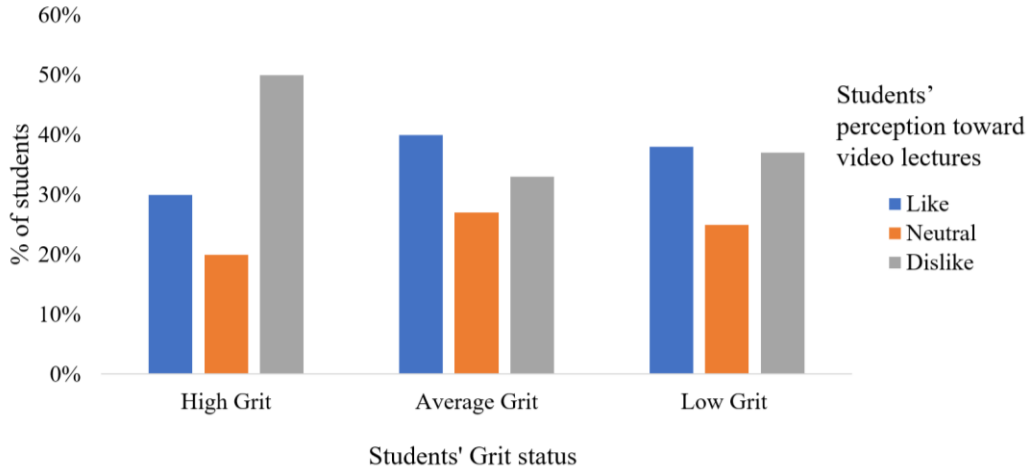
Students' Grit Status and Students' Attitude Toward Flipped Classroom



Note. No significant correlation between student's attitude toward flipped classroom and the student's Grit status.

Figure 10

Students' Grit Status and Students' Attitude Toward Video Lectures



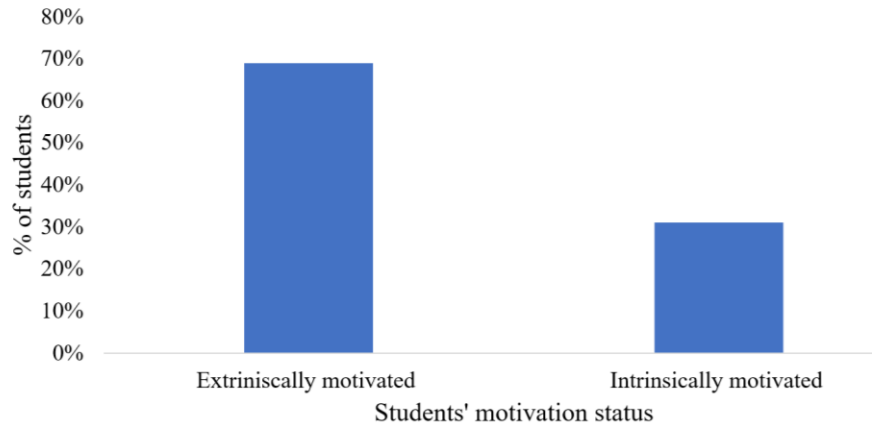
Note. No significant correlation between student's attitude toward learning from the video lectures and student's Grit status.

Student's Motivation Status and Student's Attitude Toward Flipped Classroom and Video Lectures

For our third research question, we categorized students with a 8-32 score on the motivation scale as extrinsically motivated, and students with a 33-56 score as intrinsically motivated. Among 67 participants, we found 54 (69%) of the students were extrinsically motivated, while 24 (30%) were intrinsically motivated (Figure 11). We ran a Pearson chi-square test to find if there is a correlation between student's motivation status and student's attitude toward the flipped classroom innovation or their attitude toward learning from video lectures. The test did not show a significant correlation between student's motivation status and student's attitude toward flipped classroom ($\chi 2 = 4.173$, $df = 2$, $p = 0.124$; Figure 12), however, the test did show a significant correlation between student's motivation status and attitude toward learning from video lectures, with students that were more extrinsically motivated more likely to Dislike Learning from videos ($\chi 2 = 6.169$, $df = 2$, $p = 0.04$; Figure 13).

Figure 11

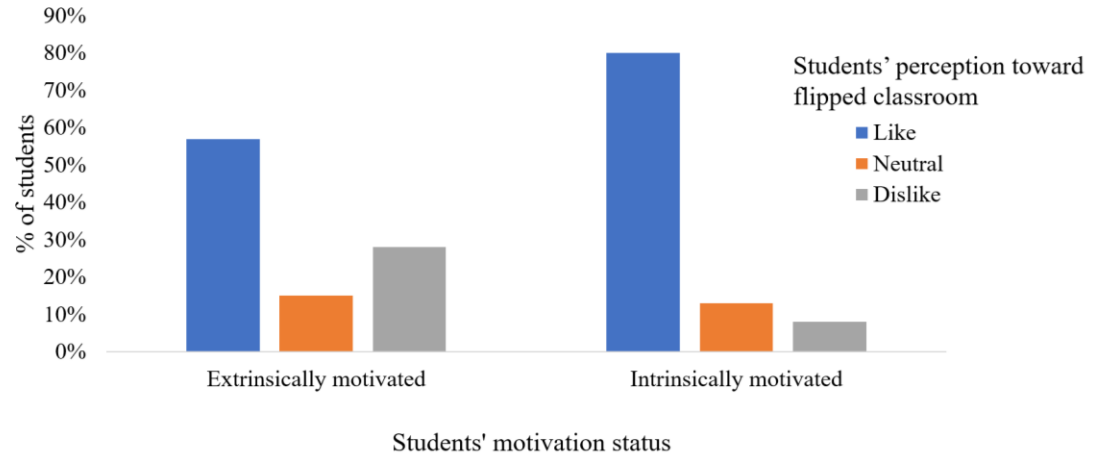
Students' Motivation Status



Note. Among 67 participants, 54 (69%) were extrinsically motivated, and 24 (30%) were intrinsically motivated.

Figure 12

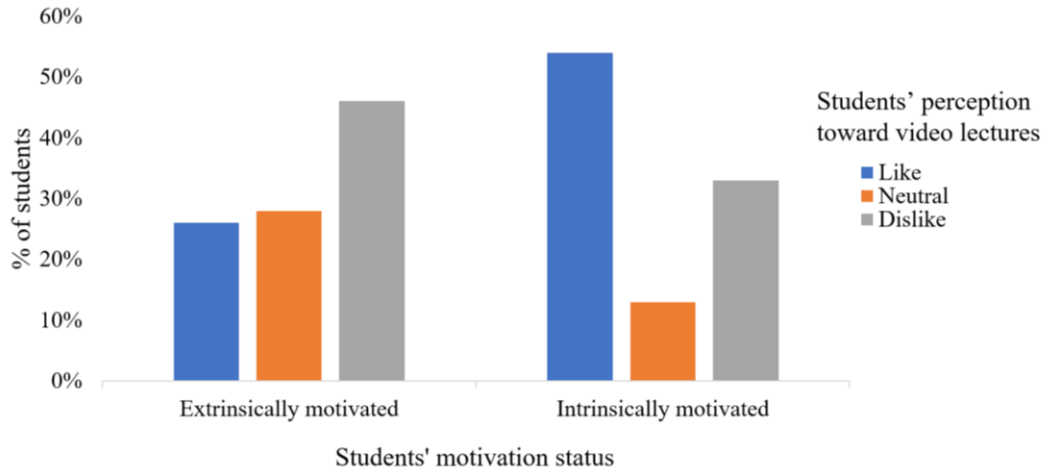
Students' Motivation Status and Attitude Toward Flipped Classroom



Note. No significant correlation between students' motivation status and students' attitude toward flipped classroom.

Figure 13

Students' Motivation Status and Attitude Toward Learning from Video Lectures



Note. There is a significant correlation between student's motivation status and attitude toward learning from video lectures. Students that were more extrinsically motivated more likely to dislike learning from videos.

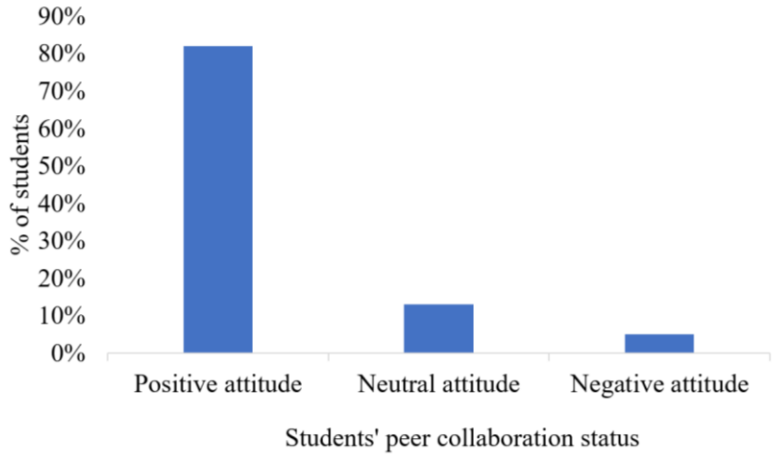
Student Attitude Toward Peer Collaboration and Students' Social Self-Efficacy Did Not Correlate with a Positive or Negative Attitude Toward the Flipped Classroom

For our last research question, we categorized students with 36-55 score on the Peer Collaboration questions as having a positive attitude toward peer collaboration, students with 28-35 score as having a neutral attitude toward peer collaboration, and students with 8-30 score as having a negative attitude toward peer collaboration. Among 67 participants, we found 64 (82%) of the students had a positive attitude, 10 (13%) had a neutral attitude, and 4 (5%) had a negative attitude toward peer collaboration (Figure 14). We ran a Pearson chi-square test to find if there is a correlation between student's attitude toward peer collaboration and student's attitude toward flipped classroom innovation. Again, the Chi-square test did not show a significant correlation between student's attitude toward flipped classroom and student's attitude toward peer

collaboration ($\chi 2 = 3.698$, $df = 4$, $p = 0.448$; Figure 15). For social self-efficacy status, we categorized the students having 14-20 scores as competent, students having 11-13 scores as neutral, and students with 4-10 scores as incompetent. Among 67 participants, we found 56 (72%) of the students were competent, 15 (19%) were neutral, and 7 (9%) were incompetent (Figure 16). Also, the test did not show a significant correlation between attitude toward flipped classroom and student's social self-efficacy status ($\chi 2 = 6.212$, $df = 4$, $p = 0.184$; Figure 17).

Figure 14

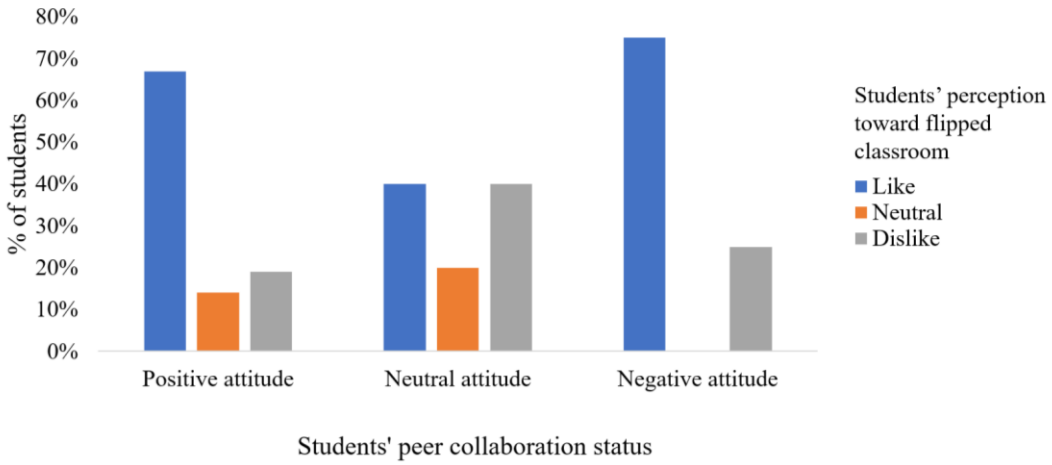
Students' Attitude Toward Peer Collaboration



Note. Among 67 participants, we found 64 (82%) of the students had a positive attitude, 10 (13%) had a neutral attitude, and 4 (5%) had a negative attitude toward peer collaboration.

Figure 15

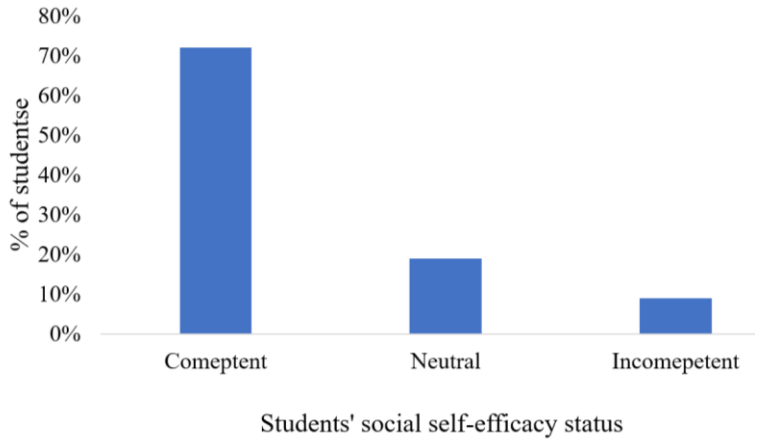
Students' Attitude Toward Peer Collaboration and Students' Attitude Toward Flipped Classroom



Note. No significant correlation between student's attitude toward peer collaboration and student's attitude toward flipped classroom.

Figure 16

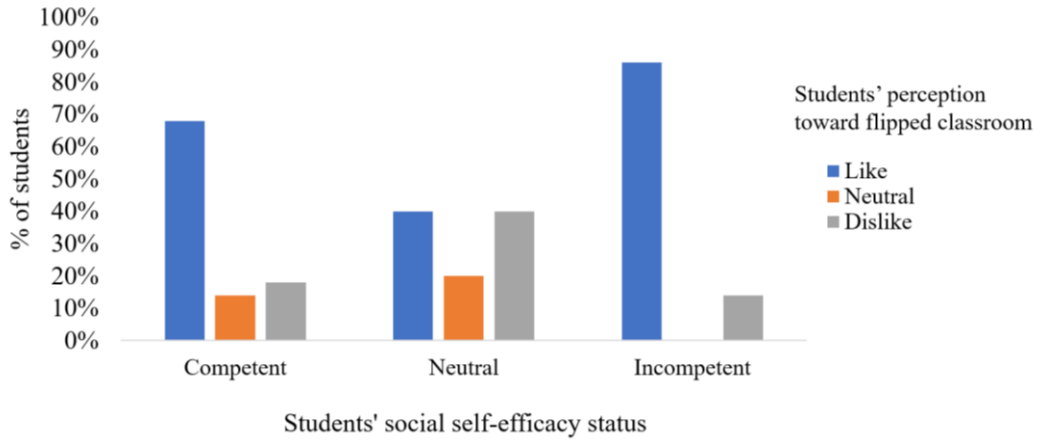
Students' Social Self-Efficacy Status



Note. Among 67 participants, we found 56 (72%) of the students were competent, 15 (19%) were neutral, and 7 (9%) were incompetent

Figure 17

Students' Social Self-Efficacy Status and Attitude Toward Flipped Classroom



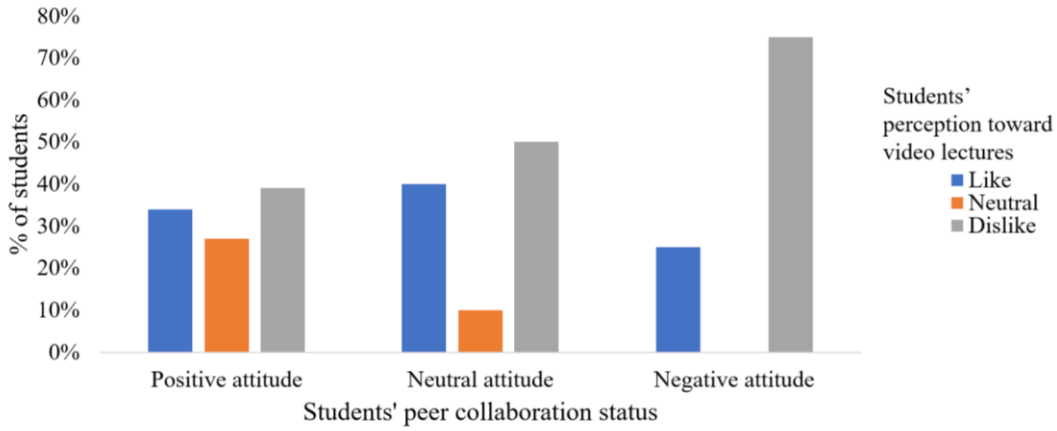
Note. No significant correlation between student's attitude toward flipped classroom and student's social self-efficacy status.

Student Attitude Toward Peer Collaboration and Students' Social Self-Efficacy Do Not Correlate with a Positive or Negative Attitude Toward Learning from Video Lectures

For our last research question, we ran a Pearson chi-square test to find if there is a correlation between student's attitude toward peer collaboration and student's attitude toward learning from video lectures. Again, the Chi-square test did not show a significant correlation between student's attitude toward peer collaboration and student's attitude toward learning from video lectures ($\chi 2 = 3.50$, $df = 4$, $p = .478$; Figure 18). Also, the test did not show a significant correlation between student's social self-efficacy status and attitude toward learning from video lectures ($\chi 2 = 4.65$, $df = 4$, $p = .325$; Figure 19).

Figure 18

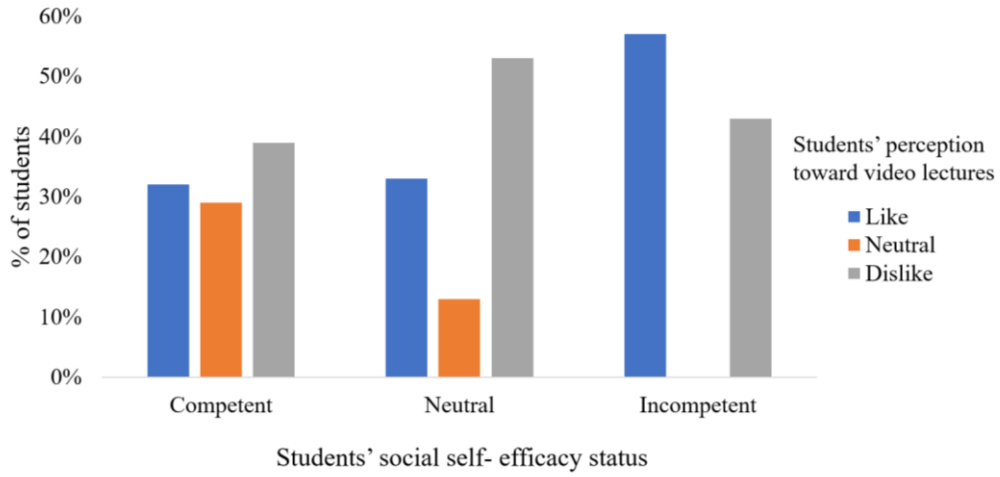
Students' Attitude Toward Peer Collaboration and Learning from Video Lectures



Note. Student attitudes toward peer collaboration do not correlate with student attitudes toward learning from video lectures.

Figure 19

Students' Social Self-Efficacy Status and Learning from Video Lectures



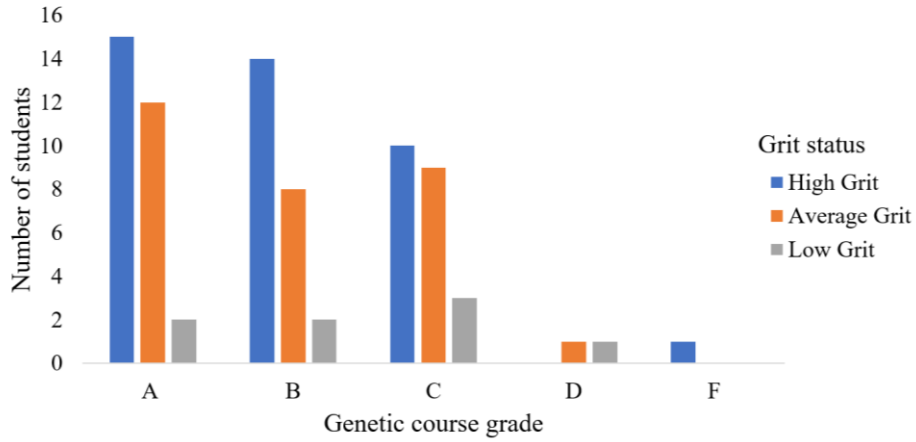
Note. Students' social self-efficacy status does not correlate with student attitudes toward learning from video lectures.

Student Grit Status and Their Final Genetics Flipped Course Scores

We did not see any significant correlation between participants' Grit status and attitude toward either flipped classroom learning innovation nor learning from video lecture; however, we did find a significant correlation between the participants' Grit status and their final Genetics flipped course scores ($\chi 2 = 66.5$, $df = 15$, $p = .000$; Figure 20). The participants with high and moderate Grit are most likely to obtain final grades of A and B. We also were able to see a significant correlation between students' perception toward flipped classroom and their final Genetic flipped classroom grade ($\chi 2 = 15.9$, $df = 8$, $p = .044$; Figure 21). The students who like the flipped classroom are most likely to obtain final grades of A and B.

Figure 20

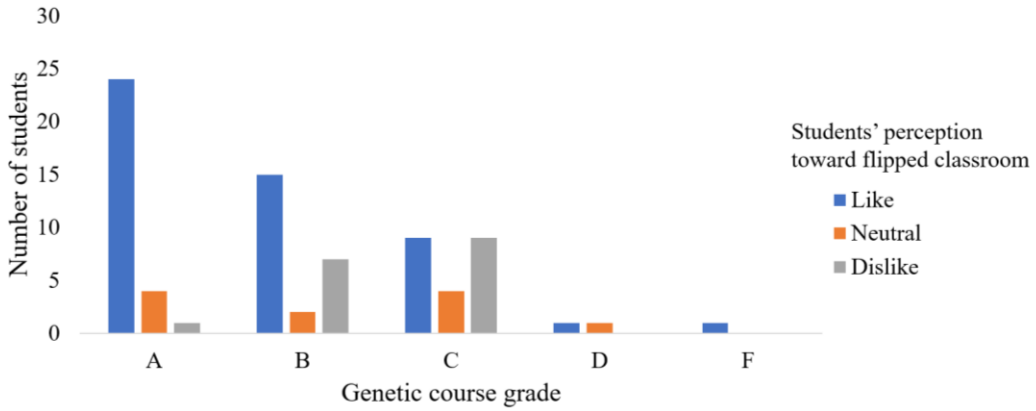
Students' Grit Status and Final Genetic Course Grades



Note. The participants' Grit status correlated with participant's final genetics flipped course scores. The participants with high and moderate Grit are most likely to obtain final grades of A and B.

Figure 21

Students' Perception Toward Flipped Classroom and Final Genetic Course Grades



Note. The participants' perception toward flipped classroom correlated with participant's final genetics flipped course scores. The participants who liked flipped classroom are most likely to obtain final grades of A and B.

Discussion and Conclusion

In this study, we were able to replicate our previous result that the students' attitude toward flipped classroom positively correlated to students' attitude toward learning from video lectures (Leatherman & Cleveland, 2019). Previous studies indicated that the implementation of flipped classroom design for teaching English as second language results in significantly improvement of the student performance (Chen Hsieh et al., 2016; Fresen, 2007). Our data did not show any significant correlation between the GPAs of the English language learner students before and after taking genetic flipped classroom. Also, our data did not support our current hypotheses and we found no correlation between any of the other students' characteristics that we tested and students' attitude toward the flipped classroom learning innovation nor students' attitude toward learning from video lectures.

Overall, the student's Grit status did not seem to correlate with their perception towards the flipped classroom nor perception toward learning from videos, however, the participants with

high Grit obtained final grades of A and B. This indicates that the participants with high Grit performed better than the participants with low Grit scores. This result supports the previous finding that higher Grit was associated with students' higher achievement (Tang et al., 2019).

Limited research has explored the motivation in flipped classroom. Studies have shown that student motivation status is an indicator for students' performance (Butler & Lumpe, 2008). Moreover, the literature mostly explored how the flipped classroom design affects students' learning motivation. Yough et al. (2017) found that flipped classroom design strength the students' motivation, however, another study done by Tse et al. (2017) revealed that flipped design did not increase the students' motivation to learn. There is limited research done to explore the effect of the type of the motivation status of the students on students' perception in flipped classroom. Our data indicated that there was significant correlation between student's motivation status and attitude toward learning from video lectures. The intrinsically motivated students have a higher attitude toward learning from video.

Our data indicated there was a significant correlation between students' social self-efficacy status and students' performance in flipped classroom. Students with higher self-efficacy status perform better in flipped classroom. Researcher also found that the students' performance is strongly speculated by students' self-efficacy status (Zimmerman, 2000) Our data, therefore, reinforce the finding of the previous research demonstrating the relationship between self-efficacy status and students' performance.

One factor that may play a role in our study results was that we were not able to get a large enough sample size to show significant correlations. Our sample size was only 78 participants from only two sections. Also, we collected our data during COVID, so there was a

potential confounding role for the COVID as an extraneous variable that might have contributed to our results.

Limitation and Future Research

Our sample was a convenient sample size, limited to the researcher's university where the genetic flipped classroom was implemented. That is why our study may not perfectly represent all the undergraduate population. We were planning to distribute the survey across three semesters (Spring 2020, Fall 2020, and Spring 2021). We obtained our data from 2020 spring and 2022 Fall semesters. However, we were not able replicate the experiment to get more data during spring 2021, and this is because the BIO 220 Genetic flipped classroom that we are using to recruit our participants was given in a different format during spring 2021. It was not implemented as a flipped classroom due to the limits of the classroom COVID capacity. For future research if we have the opportunity, we would replicate our experiment on a larger sample size in a renewed flipped classroom environment.

CHAPTER III

REVIEW OF DOPAMINE D3 RECEPTOR SUBTYPE EFFECTS ON CENTRAL NERVOUS SYSTEM AND SPECIFICALLY THE PREFRONTAL CORTEX

Introduction

In the introduction chapter of my dissertation, I reviewed dopamine, and the dopamine receptor subtypes in general. The purpose of this chapter is to review the current state of knowledge about dopamine D3R effects on the central nervus system (CNS) in general and on the mPFC specifically. I will develop a better understanding of this topic by critically discussing findings in the literature and evaluating the gaps in our current knowledge of this very significant aspect of dopamine receptor signaling, focusing on D3R-related behavior and pathology.

The D3R was detected first in 1990 by Sokoloff et al. (1990) and it was characterized by virtue of sharing some homology with the D2R. However, due to a lack of specific D3R agonists, researchers were not able to study the effects of specific D3R activation. Recently, promising selective D3R agonists have been developed that can be utilized to clarify the cellular mechanisms of D3R modulation, effects on neuropsychiatric disorders, and for development of treatment strategies (Kiss et al., 2021). Therefore, studies of the D3R have become a very active area of research, and a huge effort has been exerted in several directions. However, the role of the D3R in treatment of many neuropsychiatric disorders is still not clear and these selective agonists are lacking the factors that would make them candidate drugs to be used in research, for example gastro-intestinal absorption and the ability to penetrate the blood brain barrier (Doot et al., 2019; Kiss et al., 2021).

Dopamine D3R Expression in Limbic System and Medial Prefrontal Cortex

In humans, the D3R gene is localized to chromosome 3q13.3 (Le Coniat et al., 1991). The D1R and D2R are expressed in layer II and III pyramidal neurons (Clarkson et al., 2017). The D3R differs from D1R and D2R in terms of its expression pattern: the D3R has a restricted pattern of distribution and is expressed more in limbic areas, particularly nucleus accumbens (ventral striatum), islands of Calleja, bed nucleus of the stria terminalis, hippocampus and the mammillary nuclei (Bouthenet et al., 1991; Sokoloff et al., 1990). Dorsal striatum also expresses the D3R but at a low density (Nicola et al., 2000); moreover, the expression of D1R and D2R exceeds by 100-fold compared to D3R in striatum (Sun et al., 2012). It is worth mentioning that these limbic areas where the D3R is expressed also express D1R and D2R which suggests that these receptors cooperatively control these areas of the brain (Diaz et al., 1995; Sun et al., 2012). However, dopamine binds to the D3R with an affinity about 100-fold more than D1R and D2R (Sun et al., 2012). The D3R is expressed at low level in the PFC (Bouthenet et al., 1991; Hall et al., 1996); however, dysfunction of the D3R in the PFC has been related to some neuropsychiatric diseases (Newman et al., 2012). Interesting study done by Clarkson et al. (2017) used dopamine receptor reporter lines, and electrophysiology, they proposed that the D3R expressing layer V pyramidal neurons have distinct dendritic morphology, anatomical distribution, axonal projection patterns, and electrophysiological properties compared with D1R and D2R expressing pyramidal neurons. D1R expressing layer V pyramidal neurons have thin-tufted, less complex apical dendritic trees (Seong & Carter, 2012) and its axons project to contralateral cortex; D2R expressing layer V pyramidal neurons have thick-tufted, more complex apical dendritic trees (Gee et al., 2012) and their axons project to subcortical areas like thalamus and pons. Clarkson et al. (2017) proposed that D3R expressing layer V pyramidal neurons have

intermediate morphology between D1R and D2R expressing neurons and have projection patterns similar to D1R expressing layer V pyramidal neurons, which send axonal projections to the contralateral cortex, nucleus accumbens and basolateral amygdala. Since D1Rs are coupled to Gs and D3Rs are coupled to Gi, and they both have similar projection patterns to the contralateral cortex, this suggests that they both modify these commissural neurons, possibly in an opposing fashion (Clarkson et al., 2017). However, it is still unknown how the distribution of the dopamine receptors and the projection patterns of these neurons in PFC contribute to PFC function.

Signaling Mechanisms of the Dopamine D3R

Dopamine D3R belongs to the D2 like subfamily which is coupled to G-protein G_s and inhibits the activity of the AC enzyme and cAMP mediated cascade. Due to alternative splicing, D2-like receptors can be seen in two different forms: short D2-like and long D2-like receptors (Guiramand et al., 1995). Short D2-like receptors are thought to be located presynaptically, while long D2-like receptors are thought to be located postsynaptically (Monsma et al., 1989). Short D2-like receptors have a greater affinity to dopamine than long D2-like receptors, and antipsychotic medications like clozapine bind with 2 to 3-fold greater affinity to short D2-like receptors than long D2-like receptors (Malmberg et al., 1993). Moreover, short D2 isoforms are more potent than long D2 isoforms in terms of inhibiting the AC enzyme (Montmayeur & Borrelli, 1991).

Interestingly, D1-like and D2-like receptors can form heteromeric complexes and signal through G_q subunits of G-proteins to mediate the activity of phospholipase C (PLC). PLC converts PIP2 to the second messengers, DAG and IP3. IP3 increases cytosolic calcium by binding to and opening the IP3 gated calcium channel on the ER (Beaulieu & Gainetdinov,

2011). Independent of G-protein actions, D2-like receptors interact with a multifunctional scaffolding protein called β-arrestin resulting in recruitment of downstream Akt/GSK3 signaling pathways (Beaulieu et al., 2005). β-arrestin forms a complex with Akt and phosphatase 2A protein (PP2A); this complex mediates dephosphorylation and inactivation of Akt, which results in activation of glycogen synthase kinase-3 (both GSK3α and GSK3β; Beaulieu et al., 2004, 2005; Urs et al., 2012). Activation of GSK3 is also seen after application of the indirect dopamine agonist, amphetamine, which causes an increase in dopamine concentration in the extracellular space (Beaulieu et al., 2004). It is important to note that application of the D3R antagonist nafadotride exerts the opposite effect on Akt/GSK3 signaling (Sutton & Rushlow, 2012). Disturbance in GSK3 signaling has been linked to cognitive impairment in many psychiatric and neurodegenerative disorders such as schizophrenia, Alzheimer disease, and depression (O'Leary & Nolan, 2014). Therefore, GSK3 has been considered as a target in the development of treatment for many disorders associated with cognitive impairment. Furthermore, with GSK3 over-expression and a mutant GSK3 mouse where the GSK3 is constitutively active, both scenarios are strongly correlated with locomotor hyperactivity (Polter et al., 2010; Prickaerts et al., 2006). The targets of GSK3 are β-catenin and the NMDA receptor (Doble & Woodgett, 2003). β-catenin is a downstream substrate of Akt/GSK3 signaling, and in the absence of Wnt, β-catenin forms a complex with, and is phosphorylated by, GSK3 which causes its degradation. However, in the presence of Wnt, this complex is disturbed, and free β-catenin translocate to the nucleus and alters gene expression (Doble & Woodgett, 2003). It is important to note that some antipsychotic medications, such as haloperidol, risperidone, and clozapine, increase the level of expression of GSK3 and β- catenin in mPFC and striatum (Alimohamad et al., 2005). However, administration of dopamine receptor indirect agonists like

amphetamine revealed opposite effects on this signaling pathway (Sutton & Rushlow, 2012). The NMDA receptor is involved in synaptic plasticity (LTP and LTD) is also regulated by Akt/GSK3 signaling (Chen et al., 2007). The dopamine receptor signaling through GPCR is deactivated by a protein called G protein receptor kinase (GRK); GRK recruits β-arrestin and forms a complex with clathrin, leading to internalization of the GPCR (Beaulieu et al., 2011).

The Dopamine D2R family is also involved in regulation of dopamine release and uptake. Dopamine releasing neurons possess auto-receptors that contribute to regulation of dopamine signaling mechanisms, for instance, inhibiting dopamine release (Phillips et al., 2002), decreasing dopamine synthesis (Wolf & Roth, 1990), and controlling dopamine uptake at synaptic terminals (Truong et al., 2004). When dopamine is released from presynaptic release sites, the dopamine activates auto receptors on the same neuron to inhibit further dopamine release, and this action may contribute to the attenuation of dopamine during burst firing activity (Benoit-Marand et al., 2001). Dopamine also binds to the dopamine transporter (DAT) which is responsible for dopamine re-uptake into the terminal (Ford et al., 2010; Mayfield & Zahniser, 2001). Thus, during tonic firing of dopaminergic neurons, dopamine is released in large amounts and activates re-uptake by DAT (Benoit-Marand et al., 2011).

Functional Properties of the Dopamine D3R in Prefrontal Cortex and Limbic System

The Dopamine D3R has been linked to the pathophysiology of neuropsychiatric diseases that are associated with cognitive impairment, for example drug addiction, schizophrenia, depression, and Parkinson's disease (Miyamoto et al., 2012). Despite the fact that the PFC does not have a remarkable expression of D3Rs, a pioneering study suggests that the D3R modulates the cognitive function of the PFC (Loiseau & Millan, 2009). A study done by Glickstein et al. (2002) suggested that a PFC-dependent working memory task is disturbed in D3R knock-out

animals. However, the cellular mechanisms of how the D3R modulates the PFC pyramidal neuron's functions and circuits are still unclear. As mentioned above, the dopaminergic neurons in ventral tegmental area (VTA) and substantia nigra co-express D3R and D2R on their cell bodies and these receptors are thought be involved in regulating dopamine neuron firing rate and dopamine release (Sokoloff et al., 2006).

The D3R plays an important role in mPFC function and mental illness and currently used antipsychotic drugs work on both D2R and D3R but have greater affinity to D3R than D2R (Sokoloff et al., 1990). Therefore, the D3R has been treated as a crucial target for antipsychotic medications (Sokoloff et al., 2017). A study done by Clarkson et al. (2017; using transgenic reporter mice) revealed that dopamine acts on D3Rs of layer V pyramidal neurons, controlling the dynamics of the voltage gated calcium channels at the site of action potential initiation. Specifically, dopamine reduces the bursting behavior of the neurons and the authors suggested that this plays an important role in suppression of the action potential and regulation of the excitability of these neurons. Remarkably, D1R and D2R expressed in neighboring layer V pyramidal neurons do not show this suppression mechanism (Clarkson et al., 2017). This finding indicates that the D3R plays a crucial role in regulating an important PFC signaling mechanism by regulating cell bursting behavior.

The D3R antagonist has been considered as a possible therapeutic strategy to improve cognitive function mediated by the PFC. D3R-deprived genetically modified mice revealed enhancement of cognitive performance (Glickstein et al., 2002). Also, it has been demonstrated that D3R antagonists improve social memory in a rat model (Millan et al., 2007). Another pioneering study done by Loiseau and Millan (2009) revealed improvement in social recognition after blocking of PFC D3Rs. In an interesting study by Chang et al. (2020), where they knocked

out dopamine transporters to create a state of hyper-dopamine in mPFC, they tested performance in a novel object recognition memory. The test revealed a significant deficit, and this deficit was prevented by knock down of D3R expression or application of the D3R antagonist FAUC365. This finding indicates that the D3R antagonist might be a critical approach to treatment of neuropsychiatric disorders that are associated with cognitive impairment. Currently there are no effective drugs available to treat the cognitive dysfunction and improve the quality of life of the patients suffering from neuropsychiatric disorders.

Dopamine D3R Role in Cognition Associated with Psychiatric Disorders

As mentioned above, the D3R has been related to cognition modulation in many psychiatric disorders like schizophrenia, Parkinson's disease, substance use disorders, Alzheimer disease, depression, attention deficit hyperactivity disorders, and mania. However, effective D3R mediated treatment for cognitive dysfunction is still needed. A single nucleotide polymorphism (SNP) at the D3R involves substitution of serine to glycine at the N-terminal (rs6280), and this SNP has been linked to a number of neuropsychiatric disorders like autism spectrum disorder (ASD; Correia et al., 2010) and ADHD (Fageera et al., 2018).

Dopamine D3R and Schizophrenia

Schizophrenia (SCZ) is a neuropsychiatric disorder linked to alterations of dopamine in adult brain that results in loss of motivation and abnormalities in cognition and sensory functions of the effected individual (Simpson & Kellendonk, 2017; Sonnenschein & Grace, 2020). The D3R is thought to play a significant role in the pathophysiology of SCZ, but the data is conflicting. Buspirone is a non-selective dopamine receptor antagonist that binds to the D2R, D3R and D4R with higher affinity to D3R and D4R and with lower affinity to the D2R. (Kula et al., 1994; Tallman et al., 1997). A study by Torrisi et al. (2017) demonstrated that the buspirone

improved the cognitive dysfunction in patients with SCZ. However, data from other studies suggested that buspirone is ineffective in improving cognitive dysfunction (Maeda et al., 2014; Piškulić et al., 2009). Thus, it is clear that further studies are needed to demonstrate a definitive role for the D3R in the pathophysiology of SCZ.

Dopamine D3R and Parkinson's Disease

Dopamine is essential for normal locomotion. Parkinson's disease (PD) is an uncurable, progressive neurodegenerative disorders that affects about a million people in the USA; around 60,000 people are diagnosed yearly (Obeso et al., 2000). PD manifests primarily as a result of loss of substania nigra pars compacta dopaminergic neurons that project to the basal ganglia (Girault & Greengard, 2004; Kish et al., 1988). PD is mainly described as a type of movement disorder characterized by resting tremor, rigidity, and difficulty in initiation of voluntary movement. However, patients with PD also suffer from a group of cognitive impairments that are indistinguishable from patients who have lesions in the PFC, especially in late stages of the disorder (Dubois et al., 1994). Dopamine replacement therapy that enhances dopamine levels in basal ganglia structures (particularly striatum) is the standard and usually effective treatment of PD. The most used medication in treatment of the motor dysfunctions of PD are D2R and D3R agonists (Magnard et al., 2016). Levodopa (L-3,4-dihydroxy-phenylalanine) is a dopamine precursor that has been used as a standard treatment of PD since 1961 (Ciryam et al., 2014). However, the long-term use of levodopa is associated with a side effect called levodopa induced dyskinesia (LID) which is characterized by abnormal involuntary movement that starts mild and then progresses to negatively impact the patient's quality of life (Cotzias et al., 1967). Data indicated that the D3R is involved in the pathogenesis of PD and LID because it was found that D3R expression decreases during the onset of PD, and then increases with levodopa therapy and

LID (Bézard et al., 2003). Moreover, several pioneering studies demonstrated that the D3R is involved in cognitive and motor impairments in Parkinson disease (Sokoloff et al., 2006; Joyce & Millan, 2005). In vivo administration of the highly selective synthetic D3R agonist SK609 proved its efficiency in improving the cognition and dyskinesia in a Parkinson's animal model, with the monkeys performing less errors during task performance (Schneider et al., 2021; Simms et al., 2016). Furthermore, PD13R is a novel dopamine agonist that has high affinity and selectivity to D3R over D2R (about 1486-fold) and has shown promising results in the treatment of PD including a reduction in LID (Oh et al., 2022).

Dopamine D3R and Alzheimer Disease

Alzheimer disease (AD) is a neurodegenerative disease that is known to be associated with progressive decrease in brain cognitive function and memory. Despite extensive research focused on the pathophysiology of AD, there is still a big gap in etiology. It has been demonstrated that dopaminergic system activity decreases with age (Karrer et al., 2017). A recent study suggested that impairment in the dopaminergic system in general is associated with the pathology of AD (Nam et al., 2018). Another study done by Pan et al. (2019) revealed that dopamine levels and D2 like receptors are lower in AD than in controls, and the dopamine D3R ranked as the third dopamine receptor (after D1R and D2R) correlated with pathogenesis of AD.

Dopamine D3R and Depression

Depression is one of the devastating neuropsychiatric disorders affecting 350 million people worldwide (Mathers & Loncar, 2006). Depression is defined as persistent feeling of sadness, fatigue, loss of motivation and interest (WHO, 2016). Indeed, chronic stress and central nervus system inflammation are known to be associated with the pathology of depression (Troubat et al., 2020). However, the actual pathogenesis of depression is still under investigation.

Furthermore, about half of patients diagnosed with depression, their symptoms can be relieved by available antidepressant medications (Rush et al., 2006). Remarkably, D3R has been strongly implicated to depression like symptom in the rodents (Leggio et al., 2016). Furthermore, a behavioral study of D3R knockout mice revealed depression like symptoms (Moraga-Amaro et al., 2014). Also, administration of D3R antagonist alone revealed depression like symptoms in the mouse. A pioneering study was done where administration of lipopolysaccharides (which induce inflammation and significantly reduce the expression of the D3R in mPFC, NAc, and VTA of the mouse brain) resulted in the mice manifesting depression like symptoms that were relieved by administration of the D3R agonist PD128907 (Wang et al., 2018). Microglia are tissue resident immune cells in the CNS linked to inflammation-induced depression. Down regulation of D3R in the NAc triggered a proinflammatory condition and cytokine production in microglia which was thought to be mainly mediated through the Akt pathway (Wang et al., 2021). These studies shed light on the possibility of targeting the D3R in the development of treatment for depression.

Dopamine D3R and Attention Deficit Hyperactivity Disorders

Attention deficit hyperactivity disorders (ADHD) is a type of neurodevelopmental disorder that affects about 5% of children and 2.5% of adults all over the world (Barkley, 2006). The main symptoms of ADHD are low attention and impulsivity (Rube & Reddy, 2006). D3R has been related to the development of the symptoms of ADHD, moreover, a mouse lacking D3R revealed hyperactivity while administration of D3R agonist reduces the locomotor activity associated with ADHD (Daly & Waddington, 1993). A pioneering study was done by Fageera et al. (2018) to evaluate children diagnosed with ADHD and carrying D3R (rs3260) polymorphism.

The ADHD children revealed lower levels of attention during a continuous performance test (CPT) and Conner's Global Index Scale (valid scales to test children's behavior).

Dopamine D3R and Mania

Mania is one of the bipolar disorders (BD) phases characterized by impairment in working memory function (Bortolato et al., 2015). High dopamine level linked to development of manic symptoms (Swerdlow & Koob, 1987). Dopamine transporter knocked down animal revealed significant manic symptoms (Zhuang et al., 2001). High Glycogen synthase kinase 3 (GSK-3) protein is linked with cognitive impairment that appear in Bipolar disorder (O'Leary & Nolan, 2014). Liteium, anti-manic medication inhibits GSK-3 and reduce the manic symptoms (Freland & Beaulieu, 2012). Serine/threonine protein kinase AKT (also known as protein kinase B) phosphorylate the GSK-3 at inhibitory serine residue leads to its inhibition. GSK-3 is also modulated by dopaminergic activity. Activation of dopamine D3R leads to inhibition of Akt and abolish its inhibitory effect on GSK-3 (Chang, et al., 2020).

Dopamine D3R and Autism Spectrum Disorders

Autism spectrum disorder (ASD) is a neuropsychiatric disorder associated with cognitive, behavior, and social impairments (Bhat et al., 2014). A chromosomal micro assay analysis study done by Staal (2015) revealed that chromosomal deletion of 3q13.2-q13 of D3R raises the chance of autism in kids aged 1.5-2 years old. Another study conducted by Correia et al. (2010) to demonstrate the effect of serine glycine polymorphism on cognition in ASD patients, while the patients under risperidone treatment (Dopamine D2R antagonist), they performed Autism Treatment Evaluation Checklist (ATEC). They found that the test score of autistic patients with serine glycine polymorphism did not improve with administration of risperidone.

Conclusion

In the past decade, the D3R has been considered as a major focus in the field of neurology. A huge effort has been made in understanding the role of the D3R in brain disease that is associated with cognitive disorder. D3 receptors have a wide distribution in the limbic areas which are involved in cognitive function. A growing body of evidence suggests that the D3R is involved in many neurocognitive disorders such as schizophrenia, drug addiction, depression, mania, ADHD, ASD, Parkinson's disease, and Alzheimer's disease. In line with these findings, the pharmacological agents that modulate D3R have been considered as a potential target to treat these brain disorders. Understanding of D3R signaling mechanisms will contribute substantially to treatment of these dopamine related brain illnesses.

CHAPTER IV

EFFECTS OF DOPAMINE D3 RECEPTOR ACTIVATION ON MEDIAL PREFRONTAL CORTEX LAYER V PYRAMIDAL CELL RESONANCE

Abstract

Whole-cell patch clamp recordings were made from type I layer V pyramidal neurons in slices from mouse medial prefrontal cortex. Using current clamp mode, hyperpolarizing current pulses were applied and the "sag" amplitude measured before and after application of selective dopamine D3R agonists. The D3R agonists significantly inhibited the sag amplitudes of type I pyramidal neurons. In the same type I neurons, sinusoidal current was injected with the frequency swept from 0 Hz to 10 Hz over a 10-second period., Voltage responses were measured, and the resonant frequency determined, before and after the application of the D3R agonists. The D3R agonists significantly altered the resonant frequency in the type I layer V neurons.

Introduction

Depending on functional state and activity level, the brain displays different rhythms (Adrian & Matthews, 1934). These rhythms are generated by synchronized activity of a group of neurons and can be recorded as different brain waves by electroencephalogram (EEG). Rhythms are thought to play a crucial role in working memory and decision making, among other important prefrontal cortical functions. Brain waves are categorized according to their fundamental frequencies as beta (13-30 Hz), alpha (8-13 Hz), theta (4-8 Hz), delta (0.5-4 Hz), and gamma (40 – 80 Hz; Teplan, 2002). The intrinsic properties of individual neurons determine

their ability to oscillate at a preferred frequency (Wu et al., 2001), and this is defined as resonance. Resonance has been demonstrated in layer V pyramidal neurons (Felton et al., 2018). Resonance is thought to be important in routing and processing of neuronal information within and between neuronal networks; however, the dynamics of this synchronized rhythmic activity between neurons is still not fully clear.

Theta oscillations are observed in the EEG during memory processing in humans (Cashdollar et al., 2009) and monkeys (Lee et al., 2005), and it is crucial for proper PFC executive function (Huster et al., 2013; Klimesch, 1999). This theta oscillation is very crucial in determining the quality and dynamics of the communications between the PFC and various brain regions. Dopamine is known to modulate PFC neuronal activity during working memory tasks (which are associated with theta activity; Benchenane et al., 2011). It is well known that overstimulation or blockade of dopamine receptors in PFC impairs working memory function which then disrupts goal-directed behaviors (Zahrt et al., 1997). Furthermore, abnormal theta rhythm has been observed along with working memory deficits during working memory tasks (Brozoski et al., 1979). Also, depletion of dopamine in the lateral PFC during a monkey oculomotor delay response task by using D1R and D2R antagonists led to impairment of learning, with the monkeys making more errors (Puig et al., 2014). However, despite rigorous ongoing research, the mechanisms by which dopamine modulates the theta rhythm during working memory tasks are still not clear.

The ionic conductances present in the plasma membrane of pyramidal cells determine their ability to resonate at certain frequencies (Lörincz et al., 2002). The HCN channel is a hyperpolarization/cyclic nucleotide gated, nonspecific cation channel and the current generated by HCN channels is termed I_h (I_h -hyperpolarization, I_q - queer, or I_f - funny current). HCN

channels are activated experimentally mainly by application of hyperpolarizing current steps in current clamp mode, with the membrane potential held near the resting membrane potential (-60 to -70 mV). Activation of HCN channels generates an inward current, due to influx of Na ions. Na influx depolarizes the membrane and produces a pronounced hyperpolarization "sag" (ie. a depolarization; see (Figure 4.1). In this experiment, we used this sag as a proxy to study activation of the I_h current. HCN current has been observed in different types of cells including type I layer V pyramidal neurons of PFC (Lörincz et al., 2002). A growing body of evidence suggests that HCN current regulates the oscillatory behavior of layer V neurons (by generating spontaneous rhythmic activity; Pape, 1996), as well as their resonance in the theta frequency range (Erickson & Thomas, 2010).

There are four known isoforms of HCN, and all of these isoforms are expressed in mammalian brain (Monteggia, et al., 2000; Santoro et al., 2000). HCN2 is expressed strongly in most brain regions, HCN4 is expressed mostly in subcortical areas, HCN3 shows weak expression in the brain, and HCN1 shows selective expression in layer V pyramidal neurons (Craven & Zagotta, 2006; Santoro et al., 1997). This expression of HCN1 correlated with the presence of the I_h during patch clamp recordings from layer V pyramidal neurons. ZD-7288 specifically blocks the HCN current and blocks the resonance of layer V pyramidal neurons (Erickson & Thomas, 2010; Shin et al., 2001; Ulrich, 2002)

Rationale

Dopamine is known to play an important role in cognition, including working memory. Theta rhythms are observed in the mPFC during working memory tasks. Abnormal mPFC theta rhythms are observed in patients with working memory deficits. The purpose of this experiment is to understand whether dopamine alters the theta rhythm through activation of its cognate D3

receptor. In this study, we examined the effects of two selective D3R agonists on HCN currents (as measured by the hyperpolarization-induced sag in current clamp) and electrical resonance in layer V pyramidal neurons in mouse prefrontal cortical slices.

Materials and Methods

Slice Preparation

Slice preparation and whole-cell patch clamp recordings were performed according to previous studies (Leyrer-Jackson & Thomas, 2018). Tissue slices were prepared from 7-8 week-old mice (C57 BL/6 strain, UNC breeding colony). All animals used in this study were housed 1-4 animals per cage with a 12:12 light/dark cycle, on-site at the UNC animal facility and were given ad libitum access to food and water. Mice were anesthetized with carbon dioxide and rapidly decapitated following procedures outlined in an approved UNC Institutional Animal Care and Use Committee (IACUC) protocol. The brain was rapidly removed and immersed in ice-cold carbogen gas (95% O2/ 5% CO2) saturated artificial cerebrospinal fluid (aCSF, cutting buffer) containing (in mM): sucrose, 206; $NaHCO_3$, 25; KCl, 3.3; NaH_2PO_4, 1.23; $CaCl_2$, 1.0; $MgSO_4$, 4.0; dextrose, 10, with the osmolarity adjusted to 295 +/- 5 mOsm and pH adjusted to 7.40 +/- 0.03. The brain was then transferred to the cutting chamber of a vibrating tissue slicer (OTS500, Electron Microscopy Sciences, Hatfield, PA) and coronal slices of the PFC were prepared in ice-cold cutting aCSF. Four slices were cut 300 μm thick and taken from approximately 200 μm to 1400 μm caudal to the frontal pole. Slice were then placed in a holding chamber filled with recording artificial CSF solution containing (in mM): NaCl, 145; $NaHCO_3$, 25; KCl, 3.3; NaH_2PO_4, 1.2; $CaCl_2$, 0.9; $MgSO_4$, 2.0; dextrose, 10, with the osmolarity adjusted to 295+/-5 mOsm and pH adjusted to 7.40+/-0.03. The holding chamber aCSF was continuously bubbled with carbogen gas and the slices were incubated at 34 degrees Celsius for 45 minutes and then

allowed to cool to room temperature before slice recording. A slice was then transferred to the recording chamber and continuously perfused with carbogen-saturated recording aCSF at a flow rate of 1-2 mls/min. Throughout recordings, the recording chamber was held at 32+/- 1 degree Celsius with a temperature controller equipped with a chamber heater and in-line heater (CT-344B, Warner Instruments, Hamden CT).

Electrophysiology

Whole cell current clamp recordings were performed from layer V pyramidal neurons in slices from mouse mPFC. Recording pipettes were produced from thin-wall glass capillary tubes (1.5 mm OD, 1.12 mm ID, World Precision Instruments, Sarasota, FL) with a Narishige PC-10 pipette puller (Narishige, Tokyo, Japan). Recording pipettes (4-6MΩ tip resistance) were filled with an intracellular solution consisting of (in mM): $KMeSO_3$, 135; NaCl, 8; EGTA, 0.5; HEPES, 10; $MgCl_2$, 2; TrisATP, 2; TrisGTP, 0.3 (280 mOsm, pH 7.2). Recordings were made from the soma of layer V pyramidal neurons located within the anterior cingulate, prelimbic or infralimbic cortices after establishing a Giga-ohm seal (resistance range: 1-10 Gohm). Only neurons that exhibited thin, overshooting action potentials, and that consistently spiked throughout a 450 msec depolarizing current injection were used in this study. Access resistance (R_A) was monitored throughout experiments, and cells were excluded from analysis if the uncompensated R_A exceeded 20 MΩ. Cells were manually held at a holding potential of -70mV for all experiments in this study. Recordings were obtained with an A-M Systems model 2400 amplifier (A-M Systems, Sequim, WA) and digitized with a Digidata 1322a DAC (Molecular Devices, Sunnyvale, CA). Data were acquired at a 10 kHz sampling rate using pClamp 8.1 software.

Experimental Protocols

Sag amplitude was assessed in response to a 500 msec, 200 pA hyperpolarizing current injection. Then we determined the cells that were resonant by applying a constant peak-to-peak amplitude (100 pA) sinusoidal current swept linearly between 0 and 10 Hz over a 10 second period. Sag amplitude and resonance frequency were calculated (as described below) in control aCSF and again following a 5 minute application of each D3R agonist. Two selective D3R agonists were used in these experiments: PD128907 and SK609, purchased from Tocris Biosciences (Bristol, UK). The EC_{50} value for SK609 at the D3R is 24.8 nM while the EC_{50} value for PD128907 at the D3R is 7.4 nM (Cote & Kuzhikandathil, 2014). The concentrations of both agonists used were 10 uM. According to a study by Cote and Kuzhikandathil (2014), at these concentrations, the drugs should saturate the D3R in our cortical slices. We measured the resting membrane potential (RMP) in current clamp in control solution and directly following a 5-minute application of the D3R agonists. We also measured the membrane resistance (Rm) before and 5 minutes following application of agonists, using the voltage response to a 50 pA hyperpolarizing current to minimize activation of voltage-dependent currents.

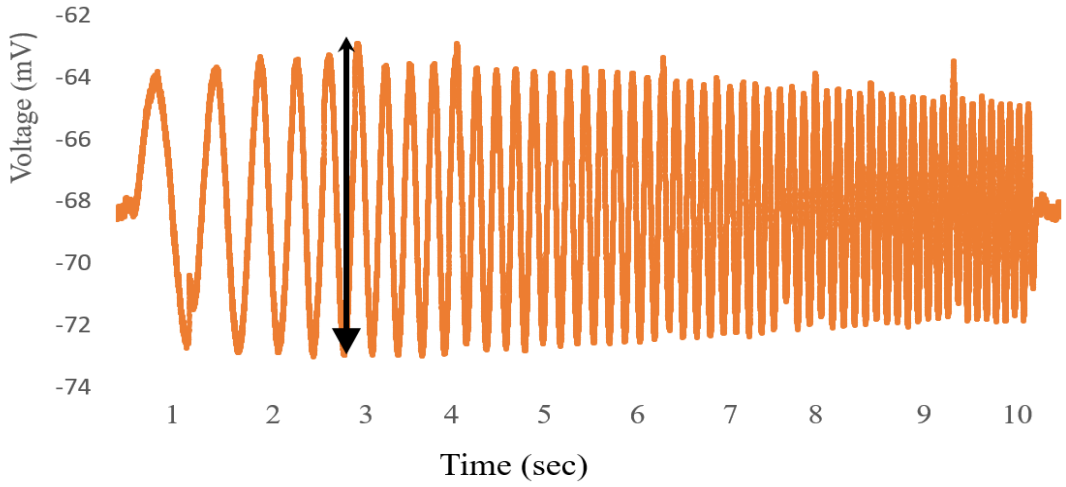
Data Analysis

Data were analyzed using Clampfit software (Axon Instruments Inc). Sag amplitudes were calculated as the differences between the maximal hyperpolarization amplitude and the amplitude at 400 msec into the 500 msec pulse. Sag amplitudes were expressed as a percentage of the maximal hyperpolarization. Neurons initially designated as type I displayed significantly larger hyperpolarization-mediated sags (18.2 ± 4. 4%) than neurons designated as type II (8.30 ± 5. 6%; Spindle & Thomas, 2014). The resonance frequency (F_R) was calculated by determining the time point where the maximum peak-to-peak amplitude of the cell's voltage response to the

swept sinusoidal current occurred (e.g., if the time point at maximum peak-to-peak amplitude was 3 seconds, the F_R was calculated as 3 Hz). This analysis is based on the fact that the sweep rate of the sinusoidal current was linear over the 10 second stimulus application. The resonance frequency was determined before and after application of the D3R agonists (Figure 22). All values were presented as mean +/- SEM. A paired *t*-test was used to analyze the effects of the D3 agonists.

Figure 22

Measurement of the Resonance Peak as a Function of Time



Note. The black arrow indicates the maximum peak-to-peak amplitude in a type I layer V pyramidal neuron. The time at the maximum peak-to-peak voltage response was used as a proxy for resonance frequency (F_R).

Result

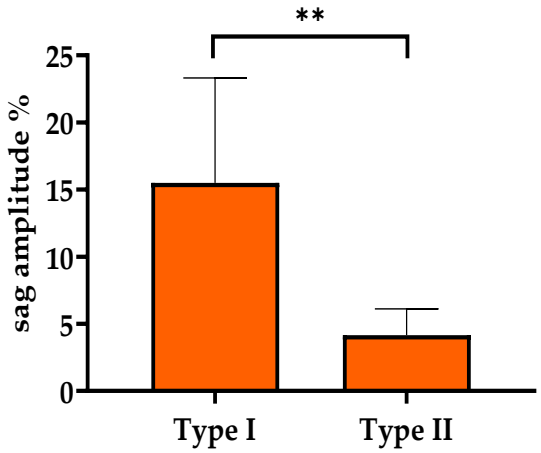
Sag Amplitude in Type I vs Type II Layer V Pyramidal Neurons

We initially discriminated type I and type II pyramidal neurons in terms of their HCN current expression, using the "sag" induced by membrane hyperpolarization as a proxy for the

magnitude of HCN expression levels. Neurons initially designated as Type I neurons displayed significantly larger hyperpolarization-induced sag amplitude than neurons designated as type II. As shown in Figure 23, the averages of the sag amplitude were 15.5 and 4.16 in “type I” and “type II” neurons, respectively (*t*-test $P = 0.007$, type I neurons n=6, type II neurons n=6).

Figure 23

Averages of the Sag Amplitude of Type I vs Type II Pyramidal Neurons in Percent



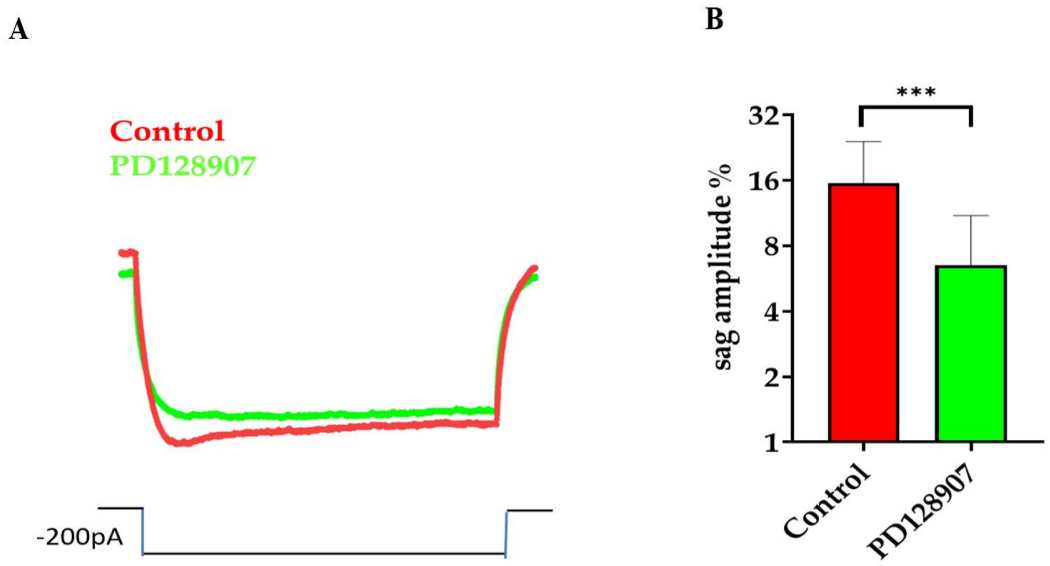
Note. Type I (n=6), type II (n=6), type I neurons display significantly larger hyperpolarization sag amplitude $p < 0.01$ one tailed, unpaired *t*-test).

Effect of the D3R Agonists PD-128907 and ES609 on Sag Amplitude in Layer V Pyramidal Neurons

D3R activation using both selective D3R agonists PD-128907 and ES609 inhibited the sag amplitude significantly in “type I” designated layer V pyramidal neurons. The average of the sag amplitude was 15.5% in control solution and 6.5% after application of PD-128907 (Paired *t*-test $p = 0.008$, n=6; Figure 24). The average of the sag amplitude was 17.3% in control solution and 9.8% after application of SK609 (Paired *t*-test $p = 0.0004$, n=5; Figure 25).

Figure 24

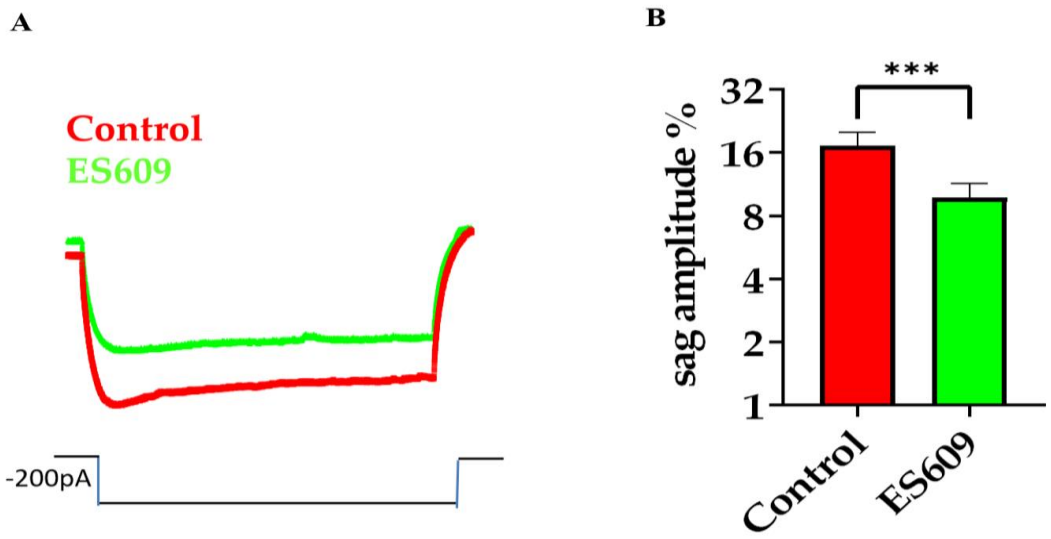
The D3R Agonist PD-128907 Inhibited the Sag in Type I Layer V Pyramidal Neurons



Note. (A) Averaged traces recorded in response to a 450 msec 150 pA hyperpolarization current injection (n=6). Traces from type I neurons before application of PD-128907 are depicted in red, whereas the green traces are after application of the PD-128907. (A) n=6, sag amplitude in type I pyramidal neurons the control vs after PD-128907 application. PD-128907 application inhibits the sag amplitude significantly (Paired *t*-test $p < 0.001$). Asterisks donate statistical significance: *** $p < 0.001$.

Figure 25

The D3R Agonist SK609 Inhibited the Sag in Type I Layer V Pyramidal Neurons



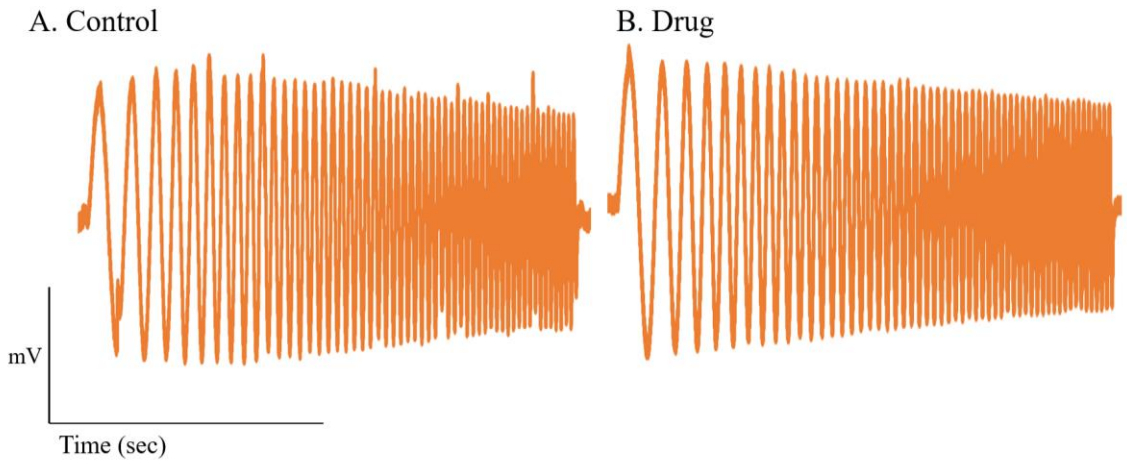
Note. (A) Averaged traces recorded in response to a 450 msec 150 pA hyperpolarization current injection (n=5). Traces from type I neurons before application of Sk609 are depicted in red, whereas the green traces are after application of the SK609. (B) n=5, sag amplitude in type I pyramidal neurons the control vs after SK609 application. SK609 application inhibits the sag amplitude significantly (Paired *t*-test $p < 0.001$). Asterisks donate statistical significance: *** $p < 0.001$.

Effect of the D3R Agonists PD-128907 and ES609 on Resonance Frequency in Type I Layer V Pyramidal Neurons

Interestingly, and consistent with the expression levels of the HCN current, both of the agonists shifted the resonance frequency significantly in type I layer V pyramidal neurons (Figure 26). Overall, the average resonant frequency measured in our type I pyramidal neurons was 2.07 Hz (n= 8). The average resonance frequency was 1.8 Hz in control solution vs 0.7 Hz after application of PD-128907 (Paired *t*-test $p = 0.02$, n=4; Figure 27A). The average resonance frequency shifted from 2.4 Hz in control solution to 0.5 Hz after application of ES609 (Paired *t*-test $p = 0.0002$, n=4; Figure 27B).

Figure 26

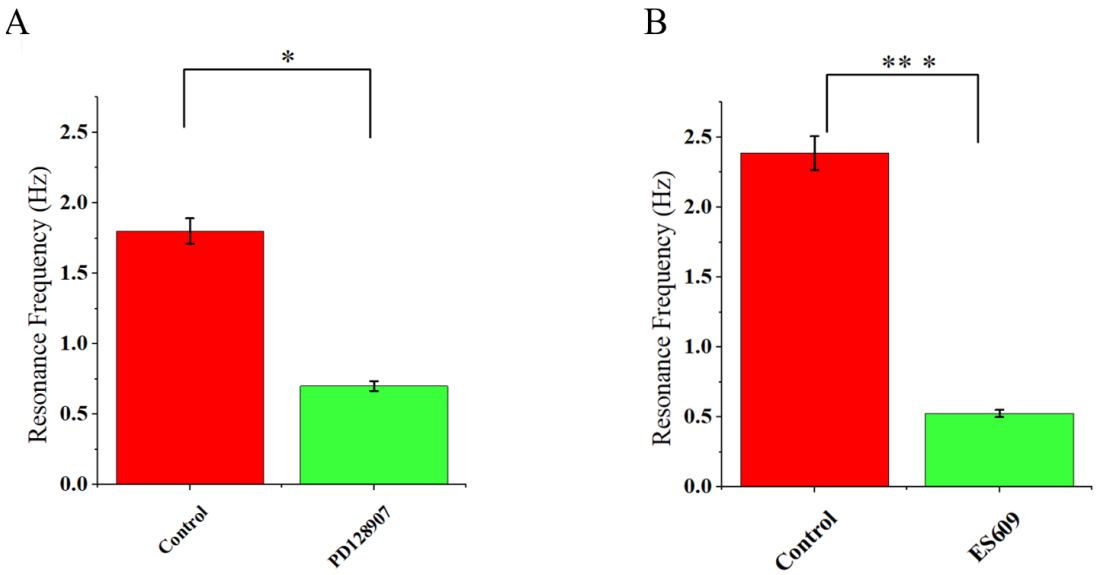
Examples of the Effect of D3R Agonist on Resonance in Type I Layer V Pyramidal Neurons



Note. Experimental data (Current Clamp) Showing the Voltage Response to ZAP Current Input of Type I Layer V Pyramidal Neuron during Resonance Test. A. Control Neuron. B. After D3R agonist application.

Figure 27

The D3R Agonist Inhibited the Resonance Frequency in Type I Layer V Pyramidal Neurons



Note. (A) n=4, resonance frequency in type I pyramidal neurond the control vs after PD-128907 application. PD-128907 (10uM) application shifted the resonance frequency significantly (Paired *t*-test $p < 0.05$). Asterisks donate statistical significance: * $p < 0.05$. (B) n=4, resonance frequency in type I pyramidal cells the control vs after ES609 application. SK609 (10uM) application shifted the resonance frequency significantly (Paired *t*-test $p < 0.001$). Asterisks donate statistical significance: *** $p < 0.001$.

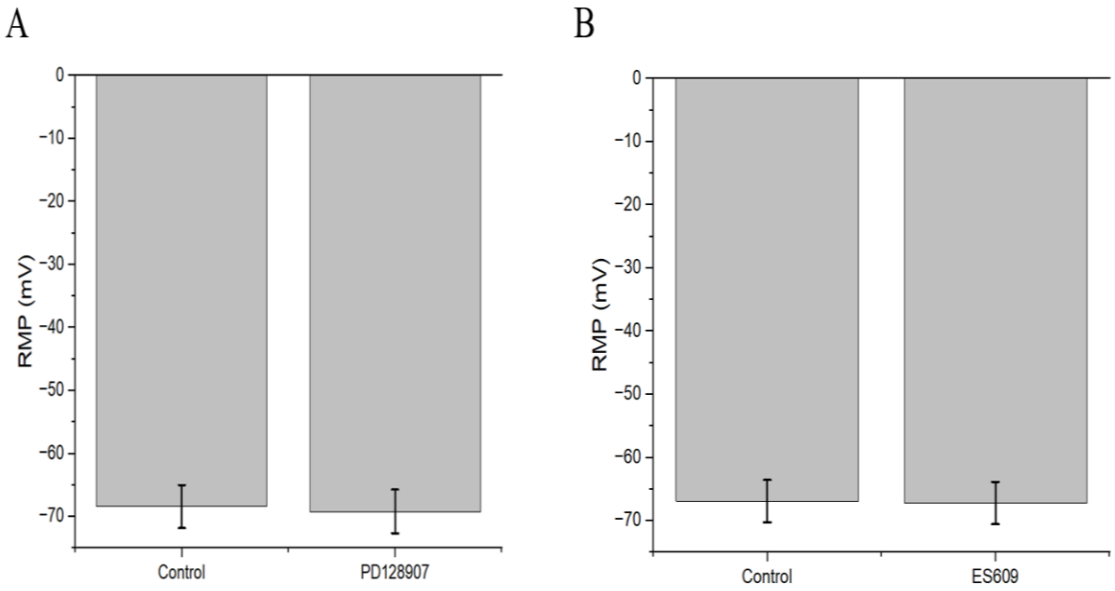
Effect of the D3R Agonists on the Resting Membrane Potential of Type I Layer V Pyramidal Neurons

To determine if the application of the D3R agonists altered the resting membrane potential (RMP) of type I pyramidal neurons, we compared the RMP measured in control solution with the RMP measured after 5 minutes in the agonist solution. The RMP was not significantly altered by application of both D3R agonists. The mean RMP in control solution was -68.4 mV and -69.2 mV after application of PD-128907 (Figure 28A; paired *t*-test, $p = 0.2$, n=6).

The mean RMP in control solution was -66.9 mV and -67.2 mV after application of SK609 (Figure 28B; paired *t*-test, $p = 0.9$, n=5).

Figure 28

Effect of D3R Agonists on Resting Membrane Potential of Type I Layer V Pyramidal Neurons



Note. (A) Average of RMP of type I layer V pyramidal neurons control and after application of PD128907 (n=6). There is no significant difference in the RMP before and after PD128907 application (means= -68.8 ± 0.2 mV, paired *t*-test $p > 0.05$). (B) Average of RMP of type I layer V pyramidal neurons control and after application of SK609 (n=5). There is no significant difference in the RMP before and after SK609 application (means= -67 ± 0.2 mV, paired *t*-test $p > 0.05$).

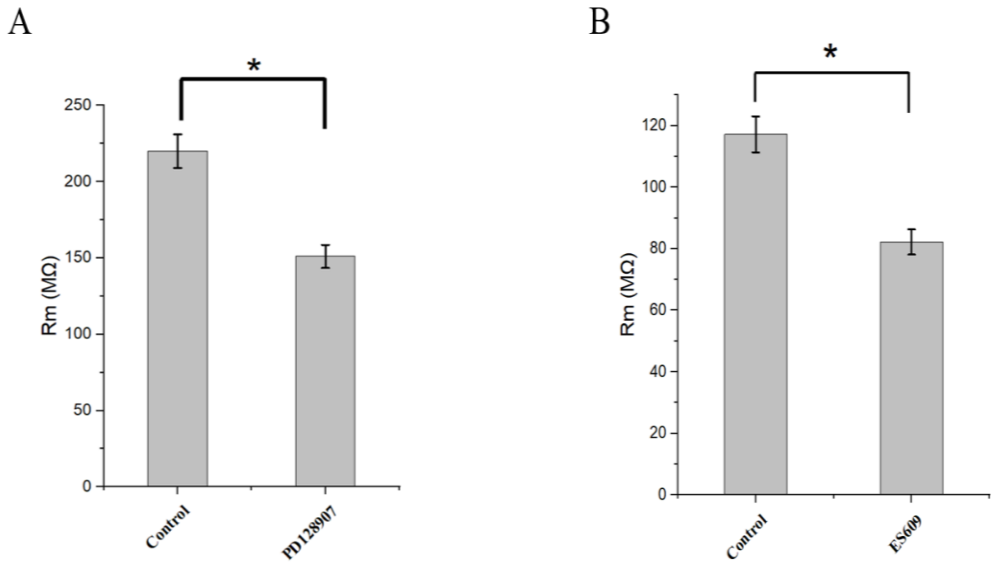
Effect of D3R Agonists on Membrane Resistance of Type I Layer V Pyramidal Neurons

We also determined whether the application of the D3R agonists altered the membrane resistance (Rm) of type I layer V pyramidal neurons. The D3R agonists had a significant effect on Rm. The mean Rm in control solution and after application of PD128907 were 220 MΩ and 151.6 MΩ respectively (paired *t*-test, $p = 0.02$, n=5; Figure 29A). The mean Rm in control

solution and after application of SK609 were 117.2 MΩ and 82.2 MΩ respectively (Figure 32B; paired *t*-test $p = 0.01$).

Figure 29

Effect of D3R Agonists on Membrane Resistance of Type I Layer V Pyramidal Neurons



Note. (A) Average of Rm of type I layer V pyramidal neurons control and after application of PD128907 (n=6). There is significant difference in the Rm before and after PD128907 application (paired *t*-test $p > 0.05$). (B) Average of Rm of type I layer V pyramidal neurons control and after application of SK609 (n=5). There is significant difference in the Rm before and after SK609 application (paired *t*-test $p > 0.05$).

Discussion and Conclusion

Effect of Dopamine D3 Receptor Activation on Sag Amplitude and Resonance Frequency

Dopamine plays a very important role in working memory tasks (Brozoski et al., 1979). Theta rhythmic activity is observed from the onset and continues until the offset of focused attention (working memory) tasks (Raghavachari et al., 2006; Tafakori et al., 2020; Yi et al., 2015). It has been demonstrated that patients with working memory deficits exhibit abnormal

theta rhythms during task performance (Brozoski et al., 1979). In this study, resonant behavior was characterized in type I pyramidal neurons, and the average resonance frequency (2.07Hz.) was close to the theta frequency (4-7 Hz) at the normal rodent core temperature of 37 degrees Celsius, accounting for the fact that our experiments were recorded at 32 degrees Celsius. The electrical resonance observed in type I pyramidal neurons requires HCN current (Erickson & Thomas, 2010; Shin et al., 2001; Ulrich, 2002). We tested for the presence of HCN current by applying hyperpolarizing current steps and observed the hyperpolarization-induced voltage sag. After application of two selective D3R agonists, the hyperpolarization-induced sag was significantly inhibited. We measured the resonance frequency by passing constant amplitude sinusoidal current swept linearly between 0 and 10 Hz over a 10 second period. Along with inhibition of the HCN current, application of the two D3R agonists virtually abolished resonance in type I layer V cells. Overall, our data demonstrate that D3R activation inhibits resonance in Type I layer V pyramidal cells, at least in part, by inhibiting HCN current.

Effects of D3R Activation on Resting Membrane Potential and Membrane Resistance

A study done by Yang et al. (2018) revealed that blocking the HCN current in pyramidal neurons using ZD7288 led to hyperpolarization of the RMP. Our data indicated that the RMP was not significantly altered after inhibition of the HCN current in type I layer V prefrontal pyramidal cells. We hypothesize that, near the RMP, HCN channels are not active in prefrontal pyramidal cells, and only open when the membrane is strongly hyperpolarized. This is supported by the absence of a significant depolarizing sag in response to a small (50 pA) hyperpolarizing stimulus. On the other hand, HCN channels are known to contribute to neuronal excitability by modulation of membrane resistance (Shah et al., 2010), and a previous study suggested that

activation of HCN channels reduces the membrane resistance (He et al., 2014). Our findings showed that application of D3R agonists significantly decreased the Rm of type I layer V cells, consistent with published reports. However, the D3R effects on Rm are unlikely due to HCN blockade (since there was no evidence of HCN current activation near the RMP), and thus are probably due to a separate effect of the D3R agonists on other steady-state currents active near the RMP.

Significance of Dopamine D3 Receptor Effects on Working Memory

Abnormal theta rhythms are associated with working memory deficits (Brozoski et al., 1979). A study by Nolan et al. (2003) revealed that HCN channel knock-out animals showed impairment in learning and memory. However, the opposite effect was observed in another study by Wang et al. (2007) which found that blocking of the HCN channel by ZD2788 improved working memory performance. Our results certainly support the idea that overstimulation or blockade of dopamine receptors in PFC impairs working memory function, disrupting goal-directed behaviors (Zahrt et al., 1997). Our findings suggest that modulation of HCN channels by D3R activation might be a useful pharmacological target to treat disorders associated with cognitive and working memory impairments. Our study contributes another piece to the puzzle of how non-optimal dopamine levels contribute to PFC working memory deficits.

CHAPTER V

SUMMARY

The flipped classroom is a student-centered learning strategy in which students are able to control their own learning through the opportunity to explore the pre-class learning materials at their own pace. The instructor's role is to support the students by providing some learning content, and by monitoring and addressing students' weaknesses during group work. Students' learning performance has been shown to improve significantly with implementation of the flipped classroom innovation. Students in a flipped classroom learn how to make decisions to manage their personal study time effectively before the class. The flipped classroom innovation provides learning gains to students; however, we do not know the characteristics of the students who benefit the most from implementation of flipped classroom. We investigated how students' perceptions towards the flipped classroom and video learning correlate to their characteristics. Characteristics we tested including demographics, first generation status, English language learner status, Grit level, motivation types, quality of peer collaboration, and social self-efficacy. Our data indicated that there is a significant correlation between students' motivation status and attitudes towards learning from video lectures. The intrinsically motivated students have a more positive attitude towards learning from video lectures. This study also demonstrates that students with high Grit scores performed better than those with low Grit scores.

The prefrontal cortex is layered into six layers. We focused on layer V because it is the major output layer. There are two major subtypes of neurons in layer V: type I pyramidal neurons, projecting to subcortical areas and type II pyramidal neurons, projecting to the

contralateral hemisphere. The prefrontal cortex is responsible for executive function (goal directed behavior) which includes decision making, problem solving, planning and initiation of activities, working memory processes, social behavior, and targeted attention.

It is well known that the dopaminergic neurotransmitter system is a key regulator of medial prefrontal cortex executive function. Dopamine is produced in the midbrain nuclei substantia nigra and ventral tegmental areas. Dopaminergic fibers extend from the ventral tegmental area mainly to the prefrontal cortex to ultimately control the executive function. The dopamine system regulates the executive function of the PFC through two types of receptors: the D1-like subfamily and the D2-like subfamily. Both subtypes of the dopamine receptors are G-protein coupled receptors. The D1-like subfamily includes D1R and D5R, while the D2-like subfamily includes D2R, D3R, and D4R. The D1-like subfamily couples to the G-protein G_s and it in turn stimulates adenylyl cyclase and increases the intracellular concentration of cyclic-AMP. The D2-like subfamily, meanwhile, couples to the G-protein G_i which inhibits adenylyl cyclase and decreases cyclic AMP. We focused on D3R because it is involved in many neurocognitive disorders such as schizophrenia, drug addiction, depression, mania, attention deficit hyperactivity disorders, autism spectrum disorder, Parkinson's disease, and Alzheimer's disease. In line with these findings, the pharmacological agents that modulate D3R have been considered as a potential target to treat these brain disorders.

Rhythmic activity is thought to play a role in the executive function of the prefrontal cortex by synchronization of activity within and between neural networks. Neurons generate rhythmic activity involving intrinsic electrical resonance. Specifically, the theta rhythm (4-7 Hz) has been observed from onset and continues until offset of working memory tasks. Layer V pyramidal neurons throughout the cortex have been shown to be resonant at theta frequency.

This resonance has been shown to be limited to type I layer V pyramidal neurons. Several ionic currents have been identified that could contribute to these resonant properties. Hyperpolarization and cAMP activated nonspecific cation (HCN) current is essential for resonance in medial prefrontal cortex layer V type I pyramidal neuron.

In this dissertation, we initially discriminated type I and type II pyramidal neurons in terms of their HCN current expression, using the "sag" induced by membrane hyperpolarization as a proxy for the magnitude of HCN expression levels. Neurons initially designated as type I neurons displayed significantly larger hyperpolarization-induced sag amplitudes than neurons designated as type II. We applied two highly selective D3R agonists on type I pyramidal neurons and examined their effect on sag amplitude and resonance frequency. D3R agonist application significantly inhibited the sag amplitude in type I layer V medial prefrontal cortex pyramidal neurons. We measured the resonance frequency by passing a constant amplitude sinusoidal current swept linearly between 0 and 10 Hz over a 10 second period. Along with inhibition of the HCN current, application of the two D3R agonists virtually abolished resonance in type I layer V cells. Overall, our data demonstrate that D3R activation inhibits resonance in Type I layer V pyramidal cells, at least in part, by inhibiting HCN current. We also found that D3R agonists inhibit the membrane resistance of type I pyramidal neurons, however, it had no effect on resting membrane potential. Our findings suggest that modulation of HCN channels by D3R activation might be a useful pharmacological target to treat disorders associated with cognitive and working memory impairments

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APPENDIX A

INSTITUTIONAL REVIEW BOARD APPROVALS

UNIVERSITY OF
NORTHERN COLORADO

Institutional Review Board

DATE:	April 6, 2020
TO:	Nesrien Mohamed, PhD
FROM:	University of Northern Colorado (UNCO) IRB
PROJECT TITLE:	[1577391-2] How student perception toward the flipped classroom and video learning correlates to student characteristics.
SUBMISSION TYPE:	Revision
ACTION:	APPROVAL/VERIFICATION OF EXEMPT STATUS
DECISION DATE:	April 6, 2020
EXPIRATION DATE:	April 6, 2024

Thank you for your submission of Revision materials for this project. The University of Northern Colorado (UNCO) IRB approves this project and verifies its status as EXEMPT according to federal IRB regulations.

We will retain a copy of this correspondence within our records for a duration of 4 years.

If you have any questions, please contact Nicole Morse at 970-351-1910 or nicole.morse@unco.edu. Please include your project title and reference number in all correspondence with this committee.

This letter has been electronically signed in accordance with all applicable regulations, and a copy is retained within University of Northern Colorado (UNCO) IRB's records.

APPENDIX B

SURVEY QUESTIONS

General perception toward flipped class

1. I prefer a traditional lecture course compared to a flipped classroom.
2. I would recommend the flipped version of Genetics compared to a traditional lecture to my peers.
3. Overall, I believe the way class time was used in Genetics this semester was MORE beneficial for my learning than typical lecture format.
4. If given the option, I would participate in a flipped class compared to a traditional lecture course.

Perception toward the video lectures:

1. I prefer to listen to an online lecture more than listening to a lecture in class.
2. Having the lectures on video helped me learn the material better than an in-class lecture would help me.
3. I prefer listening to a lecture directly from the professor over watching a lecture video.
4. I can learn difficult concepts better by attending an in-class lecture than from watching a lecture video.

Short Grit scale includes the following 8 items:

1. New ideas and projects sometimes distract me from previous ones.
2. Setbacks don't discourage me.
3. I have been obsessed with a certain idea or project for a short time but later lost interest.
4. I am a hard worker.
5. I often set a goal but later choose to pursue a different one.
6. I have difficulty maintaining my focus on projects that take more than a few months to complete.
7. I am diligent.
8. I finish whatever I begin.

Motivation question:

1. Getting a good grade in this class is the most satisfying thing for me right now.
2. I prefer class work that is challenging, so I can learn new things.
3. When I have the opportunity in this class, I choose course assignments that I can learn from even if they don't guarantee good grades.
4. The most important thing for me right now is improving my overall grade point average, so my concern in this class is getting good grades.
5. If I can, I want to get a better grade in this class than most of the other students.
6. The most satisfying thing for me in this course is trying to understand the content as thoroughly as possible.

Social self-efficacy questions:

1. It's easy for me to start a conversation with another student about what we are learning in the class.
2. I can explain my point of view about what I am learning with other students in my class.
3. I can work well with other students in my class.
4. I prefer to work by myself during in class activities.

The quality of peer collaboration questions:

1. My classmates give compliments to each other on their ideas or solutions.
2. Classmates with different ethnicities get along well.
3. We build each other's ideas.
4. We talk about different solutions or points of view.
5. We feel comfortable disagreeing with each other.
6. When my classmates share their ideas, the other students ask questions or give them feedback.
7. My classmates ask for my opinion.

8. I feel comfortable asking questions if I don't understand something.
9. I am comfortable sharing my idea with the group.
10. I feel as if it's ok to make mistakes in front of the group.
11. My classmates carefully listen to each other’s point of view.

APPENDIX C

CONSENT FORM

UNIVERSITY OF
NORTHERN COLORADO

Informed Consent Form for Participation in Research
UNIVERSITY OF NORTHERN COLORADO

Project Title: How student perception toward the flipped classroom and video learning correlates to student characteristics including background, grit, classroom performance, motivation, peer collaboration, social self-efficacy and overall GPA.

Student Researcher: Nesrien Mohamed
Corresponding Student Email: nesrien.mohamed@unco.edu

Faculty Research Advisor: Judith Leatherman, Ph.D., School of Biological Sciences
Phone: 970-351-1511 **Email:** judith.leatherman@unco.edu

Purpose: The purpose of this study is to examine factors associated with Biology undergraduate students' perceptions of the flipped classroom and associate these factors with their background, grit, motivation, social self-efficacy, peer collaboration, and quizzes scores.

Procedures: If you participate in this study, you will be asked to complete one in-class survey, which will take approximately 30 minutes. Additionally, we are seeking your permission to link your survey responses with your assignments, quiz scores. Students will receive five points of classwork extra credit for participation.

Eligibility: You must be 18 years of age or older and an undergraduate student enrolled in BIO 220.

Confidentiality: Although students' names will be requested on the survey, only an investigator who is NOT your instructor, will know the name connected with your responses. We will take every precaution to protect your anonymity. All student names will be replaced with codes by the investigator, so that specific responses cannot be linked to an individual by the instructor.

All digital data will be stored on password-protected computers that are only accessible by members of the research team and IT staff.

Questions: If you have any concerns about your selection or treatment as a research participant, please contact Nicole Morse, Research Compliance Manager, University of Northern Colorado at nicole.morse@unco.edu or 970-351-1910.

Voluntary Participation: Please understand that your participation is voluntary. You may decide not to participate in this study, and if you begin participation you may still decide to stop and withdraw at any time. Your decision will be respected and will not result in loss of benefits to which you are otherwise entitled.

Please take all the time you need to read through this document and decide whether you would like to participate in this research study.

If you decide to participate in this survey, please sign below. You can only participate in this survey once.

_____

Research Participant's name (please print)

_____

Research Participant's Signature Date

_____

Researcher's Signature Date

APPENDIX D

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE APPROVAL

UNIVERSITY OF
NORTHERN COLORADO

Institutional Animal Care and Use Committee

Date:	April 4, 2022
Principal Investigator:	Dr. Mark Thomas
Committee Action:	**IACUC Protocol- Third Year Protocol Approval**
Action Date:	April 4, 2022
Protocol Number:	2209C-MT-M-25
Protocol Title:	*Brain Tissue Harvest for an In Vitro Mouse Model of Schizophrenia*
Previous Number:	1915C-MT-M-22
Expiration Date:	April 4, 2025

The University of Northern Colorado Institutional Animal Care and Use Committee (IACUC) APPROVED animal use protocol ***Brain Tissue Harvest for an In Vitro Mouse Model of Schizophrenia*** – **2209C-MT-M-25** on April 4, 2022 as a new protocol.

The committee's review was based on the requirements of the Government Principles, Public Health Policy, USDA Animal Welfare Act and Regulations, the Guide for the Care and Use of Laboratory Animals, as well as university policies and procedures related to the care and use of animals at the UNC. Based on the review, the IACUC has determined that all review criteria have been adequately addressed. The PI is approved to perform the experiments or procedures as described in the protocol as approved by the committee. It is the responsibility of the PI to be familiar with and comply with the protocol and all pertinent institutional, state, and federal rules and policies. The PI must confirm and document that all personnel complete the required training in the care and use of laboratory animals and acquire specific training in all assigned procedures prior to beginning their work on this protocol.

During the three-year approval period, annual IACUC review of the protocol is required for animal use to continue. These annual reviews, known as Continuations, must be submitted by the Principal Investigator before the anniversary date of the initial approval date noted above. To continue this research beyond the three-year approval period, a new protocol submission is required for IACUC review. To avoid a lapse in IACUC approval, it is essential that the completed protocol be **submitted and approved by the IACUC prior to the expiration date of April 4, 2025**.

It is also the responsibility of the Principal Investigator to notify the IACUC of any:

- Proposed changes regarding the work described within this protocol. The PI agrees that no such changes will be implemented until an amendment has been approved by the IACUC or is encompassed under veterinary care.

Ross Hall 0295 | Campus Box 110 | Greeley, CO 80639 | Office 970-351-2842 | Fax 970-351-1934

- The IACUC regarding any adverse events or unexpected study results that impact the animals or personnel. Any unanticipated pain or distress, morbidity or mortality must be immediately reported to the attending veterinarian and the Director of Compliance and Operations, ACUP.

If you have any questions, please contact the UNC Animal Care and Use Program (ACUP) Director, Laura Martin, at 734-730-6631 or via e-mail at laura.martin@unco.edu. Additional information concerning the requirements for the welfare and use of animal subjects can be found at the websites for the University of Northern Colorado ACUP https://www.unco.edu/research/research-integrity-and-compliance/iacuc/, the NIH's Office of Laboratory Animal Welfare https://olaw.nih.gov/, and the USDA's Animal Plant and Health Inspection Services https://www.aphis.usda.gov/aphis/home/.

Sincerely,

Laura W. Martin
Director of Compliance and Operations
Animal Care and Use Program

OLAW Assurance: D16-00579
USDA Registration: 84-R-0008