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EFFECTS OF OPTOGENETIC ACTIVATION AND PHARMACOLOGICAL MODULATION OF DOPAMINE NEURONS

BY

REMINGTON J RICE

THESIS

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This thesis by Remington J Rice is recommended for approval by the student's Thesis Committee and Department Head in the Department of Psychology and by the Assistant Provost of Graduate Education and Research.

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ABSTRACT

THE ACTIVATION OF DOPAMINERGIC NEURONAL GROUPS

By

Remington J Rice

This study corroborates with evidence that increased activity in dopamine pathways can cause hyperactive locomotion and other behaviors related to psychosis. However, the involvement of these specific cell types in schizophrenia is not fully understood. The psychostimulant amphetamine is one of the numerous compounds that can alter dopamine concentrations all over the brain, leading to psychotic effects in healthy humans. Administration of amphetamine to experimental animals has been used to create models of schizophrenia; producing a unique array of behavioral effects, such as hyper-locomotion and a collection of behaviors referred to as stereotypy. In contrast, certain antipsychotic drugs that act directly upon dopaminergic neurons have been shown to alleviate schizophrenic symptoms. Three weeks after the infusion of an adeno-associated viral vector into transgenic TH: Cre Sprague Dawley rats, a light sensitive channel, will be expressed on dopaminergic neurons. Depolarization of the neuron rapidly occurs after pulses of 465 nm wavelengths of light, blue light, are sent through a fiber optic cable to the injection site. These dopaminergic cells exist in relatively high concentrations throughout multiple brain regions. Optogenetic tools have been used to selectively activate dopaminergic neurons in the ventral tegmental area (ventral tegmental area) during behavioral assessment sessions. These findings validate past ideals about the nature of dopaminergic pathways in the brain and their relation to mental illness.

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Schizophrenia

Schizophrenia is a mental disorder that encompasses a wide range of behavioral patterns that can result in a severely reduced ability to function in everyday life. Since its first description in the mid to late 1800s, the numerous life-altering symptoms have been cataloged. However, the causes of these symptoms are incompletely understood. Advancements in research techniques and pharmaceutical compounds have enhanced humanity's understanding of how the mental disorder affects the brain. The continuing toil from scientists around the world will continue to decrease the suffering in patients diagnosed with schizophrenia (Frith, 1987; Friston, 1992; Knapp, 2004; Shenton, 2001).

The term itself is a product from the Greek roots *skhizein* "to split" and *phrën* "mind" (Kuhn & Cahn, 2004). Eugen Bleuler first used the term during a lecture in Berlin on April 24th, 1908. In 1911, Bleular expanded on schizophrenia in his seminal study *Dementia Praecox, oder Gruppe der Schizophrenien* (Dementia Praecox, or the Group of Schizophrenias). It would be another 40 years until it was translated into English. During this time, the ideas tested by Emil Kraepelin and Kurt Schneider also added into the modern sense of the word (Andreasen, 1989). All three of these researchers heavily influenced the definition of schizophrenia described by the American Psychiatric Association in the Diagnostic and Statistical Manual (DSM-III).

The latest edition of the manual (DSM-V), carries over six different criteria (A-F) from previous editions, to assist in the evaluation of patients. Typically, a diagnosis is given when two symptoms from criteria A and at least one other are present for a

significant amount of time (Tandon et al., 2013). Characteristic symptoms of schizophrenia (Criterion A), are divided into positive and negative symptoms. Positive symptoms are feelings or behaviors that are not usually present. The list of positive symptoms includes delusions, hallucinations, disorganized speech, grossly disorganized, and catatonic behavior. Negative symptoms are a lack of feelings or behaviors. Examples of this include affective flattening, anhedonia, and alogia (American Psychiatric Association, 2013). The DSM V continues and describes criterion B as social/ occupational dysfunctional. A duration of symptoms with no change for 6 months defines criterion C. The remaining criteria are meant to exclude other explanations for the symptoms. Criteria D-F attempt to ensure that the patient is not suffering from a mood disorder, substance abuse, and/or autism spectrum disorder.

Schizophrenia occurs in about 7 people per 1000 (Saha, Chant, Welham, & McGrath, 2005). A 2004 cost-of-illness study analyzed 62 peer-reviewed articles in an attempt to calculate the societal burden of schizophrenia. The researchers summed up direct, indirect, and intangible costs. Direct costs include payments made for various therapies and treatments. When a disorder causes an individual to not be able to work in society, it is categorized as an indirect cost; in other words, a resource is lost. The intangible costs are described as pain or depression. Knapp (2004) collected past estimates of cost and demonstrated that in the year 1990, this mental disorder put a $65 billion burden on the United States economy.

The societal costs of schizophrenia are severe, in part due to reduced life expectancy for patients. The life expectancy for men with schizophrenia is below the national average by 14.6 years (Chang et al., 2011). Chang et al. (2011) explained that the impact of this serious mental illness on life expectancy is significant and commonly

higher than the corresponding impact of well-recognized adverse exposures such as smoking, diabetes and obesity. Strategies to isolate and prevent causes of premature death are urgently necessary.

In addition to a higher rate of suicide, 15% of patients also suffer from Type II diabetes (Dixon et al., 2000). Dixon et al. (2000) demonstrated that this is significantly higher than the national average and the patients' health suffered in other areas; such as decreased life satisfaction and an overall diminished quality of life. However, these authors commented that 87% of the patients in the study were prescribed antipsychotic compounds. This made determining if the poor health observed was due to the mental illness or medication, the latter has been shown to have a host of adverse effects (described below).

The causes of schizophrenia are unknown. Research has produced evidence that environmental variables, brain structure, and genetic components factor into the complex issue. Environmental factors such as drug use and prenatal stressors have all been correlated with a portion of schizophrenic patients (Picchioni & Murray, 2007). In addition to that premise, magnetic resonance imaging (MRI) studies have shown less grey matter volume in the frontal cortex and temporal lobes (Hirayasu et al., 1998). There is a 1% prevalence in the general population and near 50% for monozygotic twins or having two biological parents with schizophrenia
(DiLalla & Gottesman, 1991). Finally, the increased odds of diagnosis between relatives that have never met, shows that there is a strong heritability factor in the mental illness (Kendler & Diehl, 1993). Many of the numerous potential causes have also been connected with specific neurotransmission abnormalities. One of the theories put forth to describe the causal mechanisms of schizophrenia is the dopamine hypothesis.

The Dopamine Hypothesis of Schizophrenia

In regard to proposals put forth to explain what dysfunctional mechanisms are causing schizophrenia, the dopamine hypothesis has been one of the most prevailing. The earliest versions of this hypothesis proposed that dopamine producing neurons in the brain were hyperactive, leading to increased extracellular levels of dopamine (Meltzer & Stahl, 1976, Haracz, 1982). The dopamine hypothesis was put forth after the discovery of antipsychotic drugs and the observations that they could relieve some psychotic symptoms (Delay, Deniker, & Harl, 1952). It was found that some of these drugs accelerated the breakdown of dopamine in the brain (Carlsson & Lindqvist, 1963). In addition to that, other compounds, namely reserpine, was also effective in treating psychosis and it was discovered that it blocks the storage of monoamine vesicles and essentially depletes dopamine in neurons (Carlsson, Lindqvist, & Magnusson, 1957). The dopamine enhancing drug levodopa, which is used to treat Parkinson's disease symptoms, can also cause psychotic side effects similar to schizophrenia (Seaman, 1998).

Illicit substances such as amphetamine and cocaine increase dopamine release and prevent recycling of the neurotransmitter in the synapse; this action has been shown to induce psychotic symptoms (Lieberman, Kane, & Alvir, 1987). Lieberman discovered that "amphetamine psychosis" is, in part due to an increase of synaptic monoamine levels. With this evidence, the first version of the dopamine hypothesis put forth the idea that increased dopamine levels would cause schizophrenia-like positive symptoms.

Later in the 20th century, a modified dopamine hypothesis of schizophrenia was introduced (Davis, 1991). By this time, imaging data had narrowed down the effects into

specific regions. Evidence that the overactive neurons may be a mechanism of the disorder, comes from positron emission tomography data (Friston, Liddle, Frith, Hirsch, & Frackowiak, 1992). In 30 schizophrenic patients, a significant portion of the patients had increased regional cerebral blood flow in left medial temporal region, mesencephalic, thalamic and left striatal structures; with the highest concentration in the left parahippocampal region. Similar MRI studies have confirmed the intense neuron activity (Shenton, Dickey, Frumin, & McCarley, 2001).

Neuroimaging studies also support the dopamine hypothesis for schizophrenia. Lim et al. (2012) confirmed dopamine type 2 (D_2) receptor antagonists produce reduced activity in some of the aforementioned areas. Eight male volunteers were given oral doses of haloperidol once daily for seven days. Comparisons of PET scans before and after treatment indicated more D_2 receptor blockage with treatment. This knowledge has been helpful in administrating "optimal" dosages to patients.

Dopaminergic Pathways of the Brain

Dopamine is released in the brain by neurons in the central nervous system and functions as a neurotransmitter. Dopamine is a monoamine neurotransmitter, meaning that it contains a single "amine" molecule. Amines are comprised of a basic nitrogen atom with a pair of non-binding valence electrons. Dopamine is an amine derived from the decarboxylation of L-DOPA. A widespread diversity of physiological function is mediated by the D_1, D_2, D_3, D_4, and D_5 G-protein couple receptors (Beaulieu et al., 2015). Beaulieu's (2015) review of dopamine receptor literature shows that dopamine receptor actions extend past the action of cAMP signaling and influences numerous other cellular responses, such as receptor desensitization.

Dopamine has been strongly implicated in the numerous physiological functions, including the control of body temperature (Chaperon et al., 2003). Chaperon (2003) demonstrated that a selective D_1 antagonist SCH 23390 and selective D_2 antagonist L-741,626 would elicit hypothermia in experimental rats. Other research conducted with D_3 knock-out rats demonstrated the same hypothermia as well as decreased locomotion (Boulay, 1999). Other physiological functions will be described below, in conjunction with the individual dopaminergic pathways responsible.

There are four major dopamine pathways in the human brain. The mesolimbic, mesocortical, nigrostriatal, and tuberoinfundibular pathways.

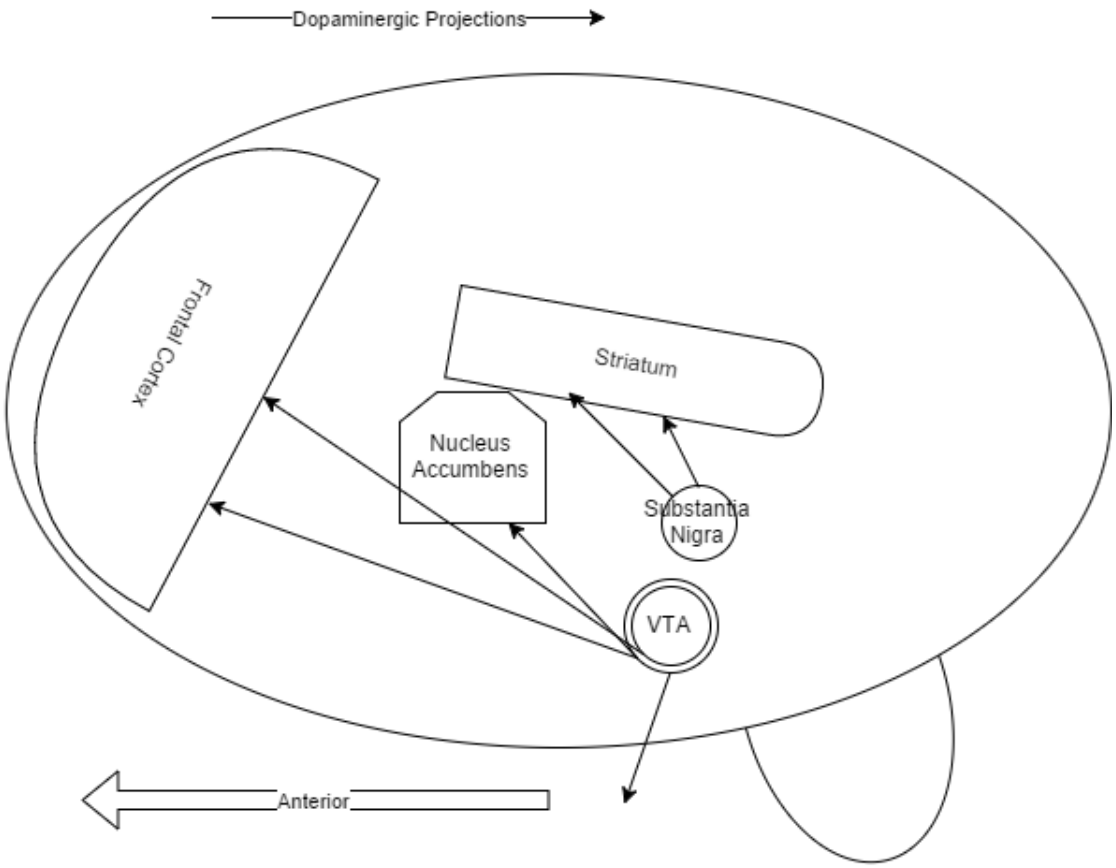

Figure 1. The ventral tegmental area has dopaminergic projections to the frontal cortex (prefrontal cortex and other frontal lobe regions), the nucleus accumbens, the striatum, and the tuberoinfundibular pathway extends through the hypothalamus to the pituitary gland.

Current electrophysiological techniques have been challenged in discerning which ventral tegmental area dopamine neurons project to the nucleus accumbens (and elsewhere in the limbic system) or the prefrontal cortex (PFC). However, dual-probe micro dialysis has been more enlightening about the nature of these neurons (Westerink, Kwint, & deVries, 1996. German & Manaye, 1993). Researchers discovered that less than 10% of the A10 (the identification of the ventral tegmental area in rats) dopamine neurons project to the PFC. Florescent staining has confirmed this hypothesis as well. Multiple fluorescent staining procedures have demonstrated that the projections to the

nucleus accumbens could account for up to 80% of dopaminergic neurons (Swanson, 1982). However, a wide diversity of neurons exists along with dopaminergic neurons. Both glutamatergic neurons (Yamaguchi et al., 2007) and GABAergic neurons (Carr & Sesack, 2000) exist and communicate with dopaminergic projections.

As noted earlier, the mesolimbic pathway has been shown to produce the "positive" symptoms of schizophrenia (Gray et al., 1995). Gray (1995) demonstrated that excess dopamine from the mesolimbic pathway contributes to difficulties in processing incoming stimuli. The researcher reviewed studies that disrupted this pathway in animal models with either surgical damage or systemic drug delivery and tested them with a behavioral paradigm called latent inhibition. "Negative" symptoms such as reduced emotional responsiveness (affective blunting) or social withdrawal has been connected to diminished mesocortical pathway activity (Meltzer & Stahl, 1976; Gold et al., 2013). Antipsychotic drug effects in the nigrostriatal pathway have been implicated in being responsible for a type of drug-induced movement dysfunctions known as extrapyramidal symptoms (Pierre, 2012). Finally, the tuberoinfundibular pathway is a group of dopamine neurons that regulate the secretion of prolactin from the anterior pituitary gland (described further below).

The "meso" prefix means "middle" in Greek, and refers to the midbrain or "middle brain." The mesolimbic and mesocortical pathways both stem from midbrain regions, specifically the ventral tegmental area. This brain region overlaps in both pathways, but there are distinct differences between the two. The differences can be simply ascertained from looking at where the ventral tegmental area projects too. The mesolimbic pathway consists of mid brain regions connected to the limbic system and the mesocortical consists of midbrain regions communicating with the cerebral cortex.

The largest group of dopaminergic neurons in the mesolimbic pathway stems from the ventral tegmental area through the medial forebrain bundle to the nucleus accumbens (German & Manaye, 1993). The ventral tegmental area also communicates with the basal lateral amygdala in this pathway. The nucleus accumbens plays a major role in pleasure, reward, motivation, and reinforcement learning (Wenzel, Rauscher, Cheer, & Oleson, 2015). Wenzel (2015) found that dopamine signaling from the ventral tegmental area influences motivated behavior and response to environmental stimuli. The group of researchers used fast-scan cyclic voltammetry to show real-time increases in nucleus accumbens dopamine concentrations when animals were presented with predictors of aversion and its avoidance. This evidence clearly shows that dopamine activity in the nucleus accumbens plays a role in moderating positive and negative stimuli during adaptive behaviors. He continues and states that the nucleus accumbens is composed of medium spiny neurons. Along with the dopaminergic neuronal projections from the ventral tegmental area, the nucleus accumbens receives input from glutamatergic neurons in the amygdala, hippocampus, and medial prefrontal cortex.

The mesocortical pathway connects the ventral tegmental area to the prefrontal cortex. The prefrontal cortex carries out executive functions such as regulating voluntary movement and planning for actions and is essential for working memory. These functions have been strongly linked to the prefrontal cortex by analyzing deficits during various behavioral paradigms such as the radial-arm maze, water maze, and attention tasks after experimental animals received lesions or injections of excitotoxin (Romanides, et al., 1999; Sakurai & Sugimoto, 1985; Viggiano, et al., 2002).

Recent fMRI experiments with healthy humans have shown neuronal activity to increase in the PFC during periods of manic like behaviors (Passamonti et al., 2015).

These behaviors have been linked to disorders associated with dopamine dysfunction (Post, Jimerson, Bunney, & Goodwin, 1980). fMRI data in human beings collected by Passamonti et al. (2015) has validated that this critical component of the prefrontal cortex, is responsible for certain behaviors attributed with dopamine function. However, fMRI results lack the specificity to determine exactly what cell types were active in those areas.

Specific gene mutations that produce abnormal protein function in the mesocoritcal pathway has been implicated in schizophrenia. A recent study has shown that abnormal gene activity in the frontal lobe, specifically a functional polymorphism of the Val and Catechol-*O*-methyltransferase (COMT) gene, can slightly increase risk for schizophrenia (Egan et al., 2001). COMT is an enzyme that breaks down dopamine. The researchers state that high activity of the COMT and Val alleles can compromise the postsynaptic impact of dopamine response in the frontal cortex.

Furthermore, evidence is now demonstrating how both the mesolimbic and mesocortical pathways communicate with each other (Laviolette, 2007). The image below details how dysfunction in the pathways can give rise to symptoms of schizophrenia. Laviolette (2007) makes it apparent that dysfunction of dopamine from the ventral tegmental area plays a role with functions necessary for every day behaviors, such as the encoding of emotional, motivational, and sensory inputs.

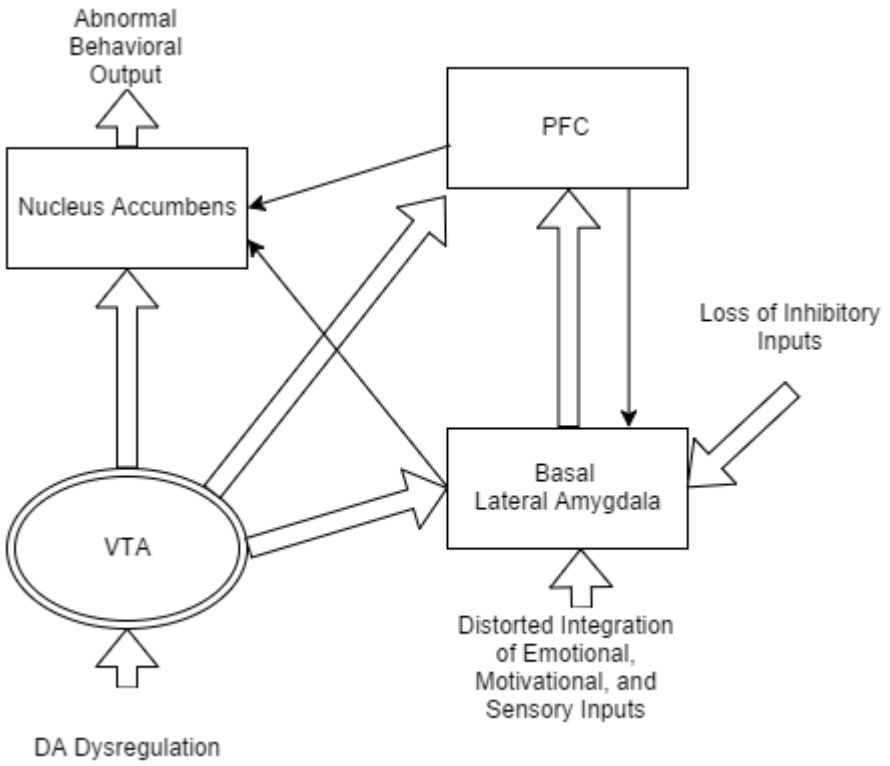

Figure 2. When dopamine production in the ventral tegmental area is dysregulated, this can have varying effects in multiple brain regions that are held responsible for schizophrenic symptoms. The large arrows represent forward dopaminergic projections that exist in the mesolimbic and neocortical pathways. The small arrows represent feedback loops that exist in both pathways. This image demonstrates that each pathway is intimately involved with each other. See the main text for descriptions of specific regions and paths involved with individual pathway.

The nigrostriatal pathway is mostly involved in the production of movement and loss of these dopamine neurons is one of the foremost pathological features of Parkinson's disease (Diaz, 1996). As noted earlier, antipsychotic compounds have been shown to induce extrapyramidal side effects (EPS) in patients with schizophrenia. EPS are drug induced movement disorders that include tardive dyskinesia, rigidity, and restlessness. These symptoms are typically caused by antipsychotic drugs that antagonize dopamine D_2 receptors (Pierre, 2005). This notion will be explored further in latter portions of this document.

The last major dopamine pathway, the tuberoinfundibular pathway, is important for the regulation of the release of prolactin. Prolactin is a luteotropic hormone that enables mammals to produce milk and is involved in a large number of other hormonal processes. The entire dissection of this endocrinology topic is outside the scope of this document. However, it is important to note that the tuberoinfundibular pathway may not be linked to the symptoms of schizophrenia, but antipsychotic drugs definitely exert effects here (Clemens, Smalstig, & Sawyer, 1974). Clemens (1974) produced evidence that prolactin concentrations increased after the administration of dopamine antagonizing antipsychotic drugs. This excess of prolactin levels can cause hyperprolactinemia, which can include infertility, loss of libido, erectile dysfunction, hypoestrogenism, and other life altering symptoms (Melmed et al., 2011).

Pharmacotherapy for Schizophrenia

Beginning in the 1950s, the scientific community has been gaining substantial amounts of knowledge about which neurotransmitters are associated in schizophrenia. In part, due to the development of selective drugs that allowed researchers to infer which neurotransmitters are involved in schizophrenia. The development of compounds used to treat Parkinson's disease and the development of classical antipsychotic drugs advanced our understanding which neurotransmitters are involved with schizophrenic symptoms. Dopamine replacement therapy has helped patients with Parkinson's disease gain some of their motor function loss (Hornykiewicz et al., 1973). This therapy includes administration of a precursor to dopamine, L-DOPA, and it effectively increases dopamine concentrations. In contrast, some of the first antipsychotic drugs developed have induced deficits in motor function similar to the motor symptoms of Parkinson's disease, which again is referred to as extra pyramidal symptoms. The first drugs that caused this motor dysfunction, primarily chlorpromazine and haloperidol, are now classified as "typical," "classical," or "first-generation" antipsychotic drugs; referred to as typical antipsychotics for this document. Along with these side effects, these drugs alleviate the positive symptoms of schizophrenia by blocking dopamine receptors (Seaman, 1975) (Crow, 1980). As mentioned before, the decrease of dopamine and decrease of psychotic symptoms caused by antipsychotic drugs, led to the concept that an increase of dopamine activity caused psychosis. This was essential to the foundation of the dopamine hypothesis of schizophrenia.

Since the development of the first antipsychotic drugs, new compounds have been manufactured that produce a lower risk of extra pyramidal symptoms at therapeutically-

effective doses (Pierre, 2012). These are known as second generation antipsychotics, also referred to as “atypical” or “novel” antipsychotic drugs. Atypical antipsychotics can still produce other severe side effects as well including tardive dyskinesia (involuntary repetitive body movements) and neuroleptic malignant syndrome (muscle rigidity, fever, and cognitive deficits).

The World Health Organization (WHO) recommends the administration of atypical antipsychotics as first line treatments for patients with schizophrenia. The effects and reduction of symptoms may take as long as 6-8 weeks (Emsley et al., 2006). There are several health organizations that offer a schematic and information to provide the optimal drug administration in varying conditions (Taylor, Paton, & Kapur, 2015).

It is important to note at this time that most, if not all, antipsychotic drugs exert effects on several different types of receptors and neurons all over the brain. However, every antipsychotic drug does bind to D_2 receptors, with the exception of one that is claimed to be a partial agonist (aripiprazole). All other typical antipsychotic drugs are D_2 antagonists and the typical antipsychotic drug haloperidol, like many others. also has a binding affinity for noradrenergic and serotonin receptors (Schotte, Janssen, Megens, & Leysen, 1993). Schotte (1993) put forth that the antagonism of the serotonin receptor 5-HT_{2A} could contribute to haloperidol’s reduction of hallucinations, delusions, and other positive symptoms of schizophrenia. However, most atypical antipsychotic drugs have greater affinity the antagonism of 5-HT_{2A} receptors than D_2 receptors (Meltzer, 1989; Schotte et al., 1996). These series of experiments have made it clear that dopamine receptors are important, but are not the only component involved with schizophrenia. Diminished levels of glutamate release in the same areas that dopamine pathways communicate with may also cause symptoms of schizophrenia (Paz, Tardito, Atzori, &

Tseng, 2008). Elevated concentrations of dopamine, has been shown to worsen positive symptoms of schizophrenia (Laruelle et al., 1996). Laruelle (1996) administered amphetamine to drug-free patients with schizophrenia and collected computerized tomography data that detailed striatal dopamine release. Their data indicates that schizophrenic patients had more D_2 occupancy after amphetamine administration, when compared to healthy individuals. This would imply that the patient's neurons were hypersensitive to dopamine. The dopamine hypothesis and other evidence provides a better understanding of the mental illness. However, there is still important questions to be answered within each individual hypothesis.

Animal models of schizophrenia have been vital in gaining knowledge to help patients. It has been demonstrated that the dopamine antagonist raclopride, when co-administered with other antipsychotic drugs, can increase the dopamine concentrations in the medial prefrontal cortex (Westerink et al., 2001, Farde et al., 1992). This synergism of drugs is meant to reduce psychotic symptoms. Farde (1992) used D_2 radioligands and positron emission tomography (PET) to determine that raclopride quickly occupies D_2 receptors in the basal ganglia. The basal ganglia is mainly comprised of dopaminergic neurons that synapse onto gamma–Aminobutyric acid (GABA) producing neurons (Tritsch, Ding, & Sabatini, 2012).

Optogenetics

Francis Crick articulated in 1979 that in order to better understand the brain, scientists would need to be able to control specific types of cells or individual neurons (Crick, 1979). He stated that if this were possible, one could activate a single neuron and watch the cascade of other neurons being activated. Or, alternatively, inhibit a neuron and see what other cells around it followed. Crick continues and believed that this would someday be possible. His knowledge of the visual system, a system of the brain that responds to light, may have led to his thoughts that we could control specific types of neurons in any part of the brain.

It is now possible to control specific cell types with high temporal and spatial precision in the central nervous system (CNS) of mammals. In the past, electrical stimulation has been able to target areas of the brain with temporal precision, but was not cell specific; electric current will stimulate every type of cell in the area of effect (You, 1998). Drug research in the past has been able to target specific types of cells, but lacks the ability to target specific areas. An example of a drug exhibiting these characteristics is haloperidol. Haloperidol blocks dopamine transmission on D_2 receptors located on many different types of neurons throughout the brain (Seeman, 2002). The modern procedure described in the coming pages, optogenetics, attempts to overcomes both of these obstacles.

The development of optogenetics was cultivated from several different fields of science. The microbial opsins involved in optogenetics are similar to the photoreceptor cells produced in the human eye. Photoreceptor cells contain a pigment called retinal, which transforms electromagnetic radiation (light) into electrical signals that are sent

further into the brain for processing. A photon of light is captured and causes isomerization of retinal; an alteration in the molecular structure initiating the surge of information. Using the technology of optogenetics, it is possible to induce expression of similar photoreceptors onto targeted neurons in the brain, creating neurons that are sensitive to light (Deisseroth, 2011).

However, humans are not the only species to have evolved machinery to sense electromagnetic energy in their setting. For example, channelrhodopsin-2 (ChR2) is a light-gated ion channel present in green algae Chlamydomonas reinhardtii (Nagel, 2003). Unicellular green alga uses these photoreceptors to orient themselves in response to sunlight. The photoreceptors belong to a family of rhodopsins that function as a channel by absorbing a photon of light to create permeability for monovalent and divalent cations. Researchers discovered the ChR channel in 1971 (Foster, 1980). Not until three and a half decades later was the potential of this biological machine brought to light.

In 2005, Karl Deisseroth and Ed Boyden discovered a way to make mammalian hippocampal cells sensitive to light through implantation of ChR2 (Boyden, 2011). Shining blue light (473 nm wavelength photons) on these cells in vitro would cause them to send nerve impulses 1-2 milliseconds later. Once the light was turned off, the cells returned to normalcy. In their resting state, an electrical potential exists between the interior of the cell and outside of the cell. When ChR2 was activated, its channel would open and cause an influx of positive ions, acting to increase the internal cellular charge and cause cellular depolarization. This process of neurons changing their electrical charge and sending action potentials is a similar to the normal electrical communication that occurs all over the brain during every emotion, action, and sensory input we perceive.

In order to produce ChR2 in a mammalian brain, the genetic codes for the light sensitive protein must be injected into each neuron with the help of a vector. The primary vectors used in optogenetics, the adeno-associated viruses (AAVs), were discovered in the 1960s. AAVs are infectious and non-pathogenic and have been used in various other gene therapy techniques.

Another vital piece of the optogenetics puzzle is the light source. ChR2 is activated about 1 mW of blue light, ~470 nm wavelengths (Lin, 2011). Diode-pumped solid-state lasers have been the prevalent equipment to activate ChR2. Lasers can output thousands of mWs into rotary components, brain matter, and long fiber optic cables. This is important because each junction of the light path, such as the rotary component, can cause a 50% loss of power. New light-emitting diode (LED) technology has been used successfully. Several images in Appendix A show one LED arrangement. The advent of LED fiber coupled equipment, with higher numerical aperture fiber, has made it possible to use LEDs that are significantly less expensive than a laser setup.

Through lasers, genetic engineering, and the technology to use viral vectors, it is now possible to produce and control these channels in mammalian brains. Optogenetics creates neurons that can be excited or inhibited instantly (50-250 fS) and with great precision (Nagel et al., 2003). Since the discovery of ChR2, several other channels have been discovered. In general, Channelrhodopsins are excitatory, halorhodopsins silence neurons by hyperpolarizing them, and archearhodposin works on similar principles as halorhodopsin, but with higher affinity for mammalian neurons (Xue 2012).
The tools for optogenetic studies keep being refined and new tools are found constantly. The ChR2 protein has also had some genetic mutations happen that scientists have isolated for different variations. Zhao (2010) made use of the mutation ChR2(H134R).

This mutation allows the channel to stay open longer and is more sensitive to lower mWs of light. This could benefit behavioral studies, where the effect of neural activation is effective, making it easier to distinguish. These light gated ion channels also have an EYFP florescent protein attached; this allows confirmation of the existence of these channels.

Optogenetic tools are often used in conjunction with other genomic technologies, a common pairing includes Cre/Lox recombination. Cre/Lox recombination is a site-specific recombinase technology and is used to delete, insert, translocate, and/or invert at specific sites in DNA. This was made possible by researchers that were able to introduce bacteriophage recombination systems into mammalian cells, typically a mouse or rat (Sauer & Henderson, 1988). The enzyme "Cre recombinase" uses a mechanism that cuts one of the DNA strands between two DNA recognition sites (loxP sites), carries out the specific recombination event, and then reanneal the strand. Cre inducible viral vector technology has been used to insert chanelrhodopsin DNA into sites that also produce the enzyme tyrosine hydroxylase (Madisen et al., 2012). This specific targeting system effectively expresses chanelrhodopsin on only catecholaminergic neurons (epinephrine, norepinephrine, and dopamine).

In order to activate ChR2, 470 nm (blue) wavelengths of light must be pulsed onto it (Zhang et al., 2010). As mentioned previously, Bass et al. (2003) discovered that 250 light pulses at 5 Hz directed into the ventral tegmental area of experimental animals would increase dopamine in the rat's nucleus accumbens. The researchers used fast-scan cyclic voltammetry to confirm dopamine concentration levels. This data collection tool utilizes carbon-fiber microelectrodes to detect dopamine and numerous other

neurotransmitter concentrations. The experiment also established that ethanol consumption would significantly drop during periods of increased dopamine activity.

Researchers have recently used optogenetic tools to delve into general anxiety disorder (GAD), which has long been a chronic and recurrent mental health issue with high prevalence (Kessler, 2009). Kessler describes GAD as disproportionate worries in the lack of urgent threats. GAD has a 28% lifetime prevalence, and a high concordance rate with other mental illnesses that display anxiety. Twin studies (Roy, 1995) have shown that a significant portion of patients inflicted by GAD also develop major depression (MD). A study using functional magnetic resonance imaging (fMRI) technology has shown that amygdala activity plays a role in anxiety (Etkin, 2009). The study concludes that decreased oxygen levels in the amygdala are correlated with GAD; the fMRI finding still lacks more precise details, such as cell type. At Stanford University, a study used optogenetics to excite basolateral amygdala glutamate terminals in the central terminal of the amygdala. Excitation of these neurons would increase anxiety and the inverse action, inhibition of these neurons, would reduce anxiety (Tye et al., 2011). Behavioral assessment of animal models in conjunction with optogenetics has given researchers a more transparent glimpse into mental health.

GAD has been the focus of several animal behavioral assessments. Two such assessments used in the previously mentioned study (Tye et all, 2011), are named the elevated-plus maze and open-field test. The elevated-plus maze consists of a plus shaped raised platform in which two of the arms have high walls. It is believed that rodents will spend more time in the exposed area when they are less stressed. A reduction in stress, as measured through increased time spent in the open arms of the elevated-plus maze, has been demonstrated in rodents who have been given pharmacological agents designed to

reduce stress (Fernandes & File, 1996). Scientists knew that these anti-anxiety drugs would reduce anxiety in humans, but the mechanism of action remained unknown.

Major depressive disorder is characterized as a persistent low mood accompanied by loss of pleasure in customarily enjoyable activities. Another way to look at major depressive disorder is that an individual with depression is less motivated to escape stressors compared to a healthy individual. Lack of motivation has been emulated in animal models with the tail suspension test. In the tail suspension test, healthy mice are restrained by their tail and will struggle to get away; 'depressed' mice will not. The causes of depression are complex and multiple brain regions, as well as different classes of neurotransmitters, have been linked to the disorder. Optogenetics has been used to explore the underlying mechanisms as well as how anti-depressant drugs exert their effects.

Using a mouse model of depression called chronic social defeat stress and optogenetics, researchers determined that excitation of the medial prefrontal cortex (medial prefrontal cortex) would produce anti-depressant effects (Covington et al., 2010). A mouse was placed in a chamber with another aggressive mouse that caused chronic stress. According to Covington, mice subjected to social defeat stress will have decreased levels of the immediate early genes, c-fos, and arc; which are known to be cellular indicators of clinical depression in humans' postmortem. Optogenetic stimulation of the medial prefrontal cortex would produce strong effects similar to anti-depressant compounds and bring IEG levels to normal, without the known side effects of anti-depressant drugs such as reduction in locomotor behavior and memory (Covington et al., 2010).

Dopaminergic neurons in the ventral tegmental area are crucial for the brains reward circuitry, but are also important in depression. Patients with MDD exhibit anhedonia, or an inability to enjoy pleasurable activity. Optogenetic tools and the aforementioned chronic social defeat stress model have linked activity of specific types of neurons in the ventral tegmental area to depression (Chaudhury et al., 2013). Chaudhury established that stimulation of dopaminergic neurons in the ventral tegmental area would cause an increased susceptibility to social defeat stress and anhedonia, as measured through social avoidance and decreased sucrose preference. Furthermore, the researchers showed that inhibition of ventral tegmental area projections would induce resilience to stressors. These studies reveal novel neural and circuit-specific mechanisms of depression.

Addiction, clinically known as substance dependence, is a chronic condition that grows from repetitive drug intake that results in withdrawal symptoms once drug intake ceases. Substances that cause dependence are thought to activate the mesocorticolimbic pathway. As already mentioned, the ventral tegmental area in the midbrain extends to the nucleus accumbens through the medial forebrain bundle. The ventral tegmental area also projects to the prefrontal cortex (PFC), where additional information is integrated into the motivated behavior. Food, water, sex, and other pleasurable daily normal activities activate this mesocorticolimbic circuit. Simply put, drugs of abuse "hijack" this circuitry to cause addiction. Activation of the circuit in response to drugs of abuse can be demonstrated in animal models.

According to Tzschentke (2007) conditioned place preference is a type of Pavlovian conditioning applied to quantify the motivational effects of objects or experiences. A research animal is placed into a container that consists of two distinct

chambers. Typically, the animal will receive a drug of abuse that has been known to produce pleasurable effect in only one of the chambers. In the other chamber, the animal receives the vehicle to act as a control. conditioned place preference has been able to show repeatedly that the animal will develop a preference for the chamber where they receive the drug.

Hsing-Chen Tsai (2009) implanted ChR2 into dopaminergic neurons of the ventral tegmental area and tested animals performing in a conditioned place preference environment. The experimental group of animals was placed in a container that had two chambers. The first day of testing, mice received 1 hertz of optical excitation in chamber one. The following day, mice received 50-Hz stimulation in chamber two. Mice developed a clear preference for the chamber with 50 Hz stimulation ($P < 0.001$ t test; $n = 13$ mice). Mice would prefer a chamber with 1 Hz over no stimulation, but 50 Hz produced the strongest preference. These findings sufficiently demonstrated that dopamine could establish place preference.

Rationale

This study was conducted to evaluate locomotor behavior during activation of brain regions linked to the positive symptoms of schizophrenia and the actions of antipsychotic drugs. This study corroborates with evidence that increased activity in mesolimbic dopamine pathways can cause hyperactive locomotion in animals and psychosis in humans. The basis of these findings mostly comes from electrophysiology and pharmacological techniques. The recent development of optogenetic tools have allowed researchers to re-examine the aforementioned notion. The recent development of optogenetic tools allow researchers a level of specificity for cell types not previously available…As previously mentioned, the ventral tegmental area is comprised of a wide diversity of neurons that produce GABA, glutamate, and dopamine. When an area of brain is electrically stimulated, all types of neurons are activated. Optogenetic tools overcome this wide-spread activation with the use of Cre/Loxp systems, which was also previously mentioned. The pairing of these technologies allow for the targeting of specific neurotransmitter groups. This study used these technologies to evaluate D_2 receptor antagonism in rats who had mesolimbic and mesocortical dopaminergic neurons activated with optogenetic tools. The tools used in this study could make it easier to evaluate the involvement of these specific cell types, in regards to behavior and the effects of antipsychotic drugs. Questions about the causal mechanisms behind the symptoms still remain. This study does not attempt to answer those immense questions, instead it is a simple demonstration that light shown onto genetically modified neurons elicits a behavioral response. This study builds the foundation for future experiments, asking similar questions, to grow upon.

This study used highly spatial and temporally precise tools to investigate how neuronal activity can affect locomotor behavior. Video tracking equipment allows us to visually track their movements, from where they traveled to their velocity to how close they got to the walls, and other behaviors. The expectation is that they'll be more active with increased dopamine activity, to a certain point. There is also a record of body temperature, which can increase when dopamine production is elevated. These studies will allow us to specifically associate behavioral and physiological effects with activation of dopamine neurons and allow us to examine the ability of D_2 receptor antagonism to reverse these effects or pharmacological enhancement of dopamine release to augment these effects. This study took the first steps in using optogenetic tools for evaluating antipsychotic drug effects.

Methods

Subjects

All experiments were performed on adult Male Sprague Dawley rats. 10 wildtype animals and 10 Tyrosine Hydroxylase (TH)-Cre animals were purchased from Sage Laboratories. Surgical procedures on the animals began when they were between 350-400 g and were between 10-14 weeks old. Behavioral procedures occurred at least four weeks after viral injection. Subjects were housed at 22-24*C with freely–available food and water. All procedures were approved by the Northern Michigan University Institutional Animal Care and Use Committee (IACUC #257) (see Appendix A).

Stereotactic injection and cannula implantation materials

pAAV-EF1a-double floxed-hChR2(H134R)-EYFP-WPRE-HGHpA was a gift from Karl Deisseroth (Addgene plasmid # 20298), (Image 9 Appendix B). Anesthesia, NMU Biology department's isoflurane vaporizer to anesthetize mice or rats. Isoflurane is volatile and proper ventilation is needed. Nonsteroidal anti-inflammatory drug (NSAID) Meloxicam, 1.0 mg per kg, Penicillin G, 30% Bleach in beaker, to dispose materials in contact with virus, Lubricant eye ointment, Anti-Septic-Chlorhexidine & Ethanol, C&B metabond, Dental cement, ~10-week old WT and TH: Cre rats, Surgical tools. Scissors, forceps, and scalpel blades. Small animal stereotactic frame, Cannula Holder (custom made by Kale Polkinghorne, Northern Michigan University). Programmable micro syringe pump, 10 micro liter Hamilton micro syringe and tubing, Cotton swabs, High-speed micro drill with charger. 0.5 mm and 0.9 mm Micro drill stainless steel burrs. 1 ml Syringes with subcutaneous needles, Surgical suture, Heating blanket, and Epoxy glue.

Material for optogenetic stimulation

4 channel PlexBright system driver with Radiant 2.0 and pattern generation. Drives LEDs or Lasers; 8 digital inputs and 16 digital outputs. Includes controller, 4 colored BNC cables, Radiant 2.0 software, USB license key and electronic user guide., Dual LED rotary commutator with 2 Magnetically mounted compact LED modules for use with commutator, terminating in an LC connector for use with an LC patch cable. Blue, 465nm, 300mA max current. 2 200/230µm fibers; LC connector to compact LED module; LC ferrule with polished tip; 1 m length with stainless steel jacketing LC-LC coupler, Bronze mating sleeves, Refractive gel, and an Optical cleaning kit with cleaning solution, lint free wipes, and lens paper. Light Measurement Kit; includes ThorLabs PM100D Digital optical power meter with 4-inch LCD display & USB interface; ThorLabs S140C integrating sphere photodiode optical power sensor, 350-1100 nm, 1 µW-500 mW; and Plexon PlexBright adaptor set.

Materials for behavioral assessment

Noldus Ethovision Hardware and XT 7.0 software (Noldus Information Technology, Leesburg, VA), PC, Locomotor Enclosure, and Infrared Thermometer

Apparatus

The locomotor enclosure was a wooden box with four separate identical compartments measuring 45.72 cm long x 45.72 cm wide and 27 cm tall. The side walls were painted white and a black rubber mat was placed in the bottom to provide a contrast in color to better track movement. An infrared thermometer (model # 153IRB, Bioseb, Pinellas Park, FL) was used to collect temperature from the base of the animal's tail.

Drugs

Saline, raclopride, and amphetamine were administered subcutanteously. intraperitoneally in 1.0 mg/kg, 0.1, or 0.3 mg/kg doses. Drugs were dissolved in a ringer's solution containing 145 mM NaCl 2.7 mM KCl, 1.0 mM MgCl2, and 1.2 mM CaCl2. Drugs were purchased from Sigma Aldrich.

Procedures

Proper target coordinates (Paxinos, 1997) were confirmed prior to when the experimental animals underwent surgery. Image 1 (Appendix B) was processed and collected by Dr. Erich Ottem; he utilized an Olympus Fluoview confocal laser-scanning microscope here at Northern Michigan University. This image demonstrated proper needle placement in the ventral tegmental area, the visible needle tract aligned with the same coordinates described in Image 2 (Appendix B).

Implant Testing and Fabrication

The fiber optic light guides were custom made in-house, following protocols from ThorLabs's *Guide to Connectorization and Polishing Optical Fibers*, this PDF is freely available online (ThorLabs, 2006). After fabrication, all implants were tested to ensure proper assembly and that enough light would be emitted. When 50 mWs were pulsed from a LED, each implant used during experiments emitted at least 5 mWs. 5 mWs is above the activation threshold for ChR2 and this was deemed sufficient for implantation.

At this activation strength and wavelength, light was emitted in a cone shape that did not extend past the ventral tegmental area (assuming the implantation hit the coordinates).

Surgical Procedures

The procedures to inject 0.5 ul of the viral vector and to implant light guides that terminate at the same coordinates were similar to procedures described by Deisseroth (2011) and Pehrson et al. (2012). First, all surgical tools were cleaned and sterilized. The surgical table was disinfected with 70% ethanol. Aseptic conditions are necessary to prevent infection and to guarantee survival for the following weeks. Underneath a sterile absorption gauze, a heating pad was placed and kept at 35˚ C. Then the AAV was thawed on ice; AAV can be kept at 4 Cº for 1 month. Meloxicam (1.0 mg/kg) and penicillin (0.25 ml) were given subcutaneously to minimize pain and discomfort, it is important to administer one hour before causing pain and to prevent infection. Rats were anesthetized using 1.5-2.5% isoflurane. The injection needle was slowly lowered to the proper depth (A/P -4.9mm, M/L +2.1mm; D/V -8.6mm; Paxinos et al., 1997) and the virus was injected the virus with a microsyringe through an internal cannula at a rate of 0.1 ul/min. We waited one additional minute to allow time for the virus to spread, and then slowly removed needle. Next, the light guide was placed in the cannula holder and slowly lowered into the brain to the same coordinates as the injection needle. Then the skull was dried with cotton swabs. After it was dry, a thin layer of C&B metabond was applied to the skull and around the cannula pedestal to fix the pedestal in place. Both groups (THCre and WT) underwent the surgery and viral injection, in order for the WT rats to serve as controls. Follow up injections of 0.5 mg/kg were given every 24 hours for the first three days of recovery. Behavioral testing began four weeks after surgical procedures.

Behavioral Procedures

The rats were brought into the behavioral testing room 30 minutes prior to video recording; which was comprised of three five-minute long tracking sessions per animal. Ten minutes before a recording session, the rat would be connected to the fiber optic cable. Each experiment was designed in an A/B/A pattern (baseline/treatment/baseline), meaning that the first five-minute session they would receive no light, the second 5-minute session began with 50 seconds of light pulses from the blue LED diodes, and the final five-minutes there was no light. At the end of each 5-minute session, body temperature would be recorded. The first experiment was conducted to determine if light would produce a significant increase of movement and/or an increase of body temperature while the animals received no drugs. The second experiment included treatment with amphetamine. The third and final experiment included treatment with raclopride.

Data Analysis

The dependent variables measured for the locomotor test were total cm travelled and body temperature. A repeated measures one-way analysis of variance was used to analyze the effects of light and each drug on each measure. Any significance was analyzed further with a Tukey's multiple comparisons test in order to determine which group means were significant from each other. Missing data points were filled in with the group mean. All analyses were conducted using GraphPad Prism for Windows Version 6.0 (GraphPad Software, La Jolla, CA).

Results

Locomotor Behavior Experiments

Total cm travelled: 5 minute trials

Figure 3 represents the mean path length for 5 minutes before (M = 1506.55, SD = 493.56), during (M = 1893.16, SD = 705.12), and after trials of light pulses into the transgenic rats. Pulses of light significantly increased path length $F(2, 16) = 6.284$, $p < 0.01$. A significant increase of path length was found between the "during" session compared to the "before" or "after" trials.

Figure 4 represents the mean path length in 5 minutes, before (M = 1560.92, SD = 377.17) and during (M = 1636.35, SD = 502.65) and after trials of light pulses in wild type rats. Pulses of light had no significant effect on path length in the wildtype rats, $F(2, 12) = 0.09793$, $p = 0.9074$.

Body Temperature

Figure 5 represents the mean body temperature, which was assessed at the end of each locomotor activity trial, for the in transgenic rats. Pulses of light significantly increased body temperature $F(2, 16) = 21.78$, $p < 0.01$. A significant increase of body temperature was found between the "during" session compared to the "before" or "after" trials.

Figure 6 represents the mean body temperature for the wild type rats. Pulses of light had no significant effect on body temperature $F(2, 12) = 0.9141$, $p = 0.42$.

Amphetamine Experiments

Figure 7 represents the mean path length in 5 minutes, before and during trials of light pulses in transgenic rats during saline and amphetamine treatments. Amphetamine significantly increased path length $F(3, 21) = 67$, $p < 0.01$. A significant path length increase was found between the "during" session compared to the "before" or "after" trials.

Figure 8 represents the mean body temperaturefor the transgenic rats treated with amphetamine. Pulses of light had a significant increase on body temperature $F(3, 21) = 5.2$, $p < 0.01$. Amphetamine treatment had no significant effect on body temperature.

Raclopride Experiments

Figure 9 represents the mean path length in 5 minutes, before and during trials of light pulses in transgenic rats during saline and raclopride 0.1 mg/kg and 0.3 mg/kg treatments. Significant differences across the testing conditions was found, $F(5, 30) = 3.0$, $P < 0.05$. Post hoc results demonstrated that there was significant path length increase was found between the means of saline treatments and 0.1 mg/kg raclopride+ treatments. There was no significant increase found between saline treatments and 0.3 mg/kg+.

Figure 10 represents the mean body temperature for the transgenic rats during saline and raclopride 0.1 mg/kg and 0.3 mg/kg treatments. 0.3 mg/kg raclopride treatment compared to saline significantly decreased body temperature $F(5, 35) = 6.1$, $p < 0.01$. Post hoc results showed a significant decrease in body temperature at 0.1 mg/kg treatment and a significant increase from 0.1 mg/kg to 0.3 mg/kg.

All figures represent the mean plus the standard error of the mean.
"+" =trial with pulses of light and "*" = significance.

Figure 3: **THCre Baseline/Distance Travelled**

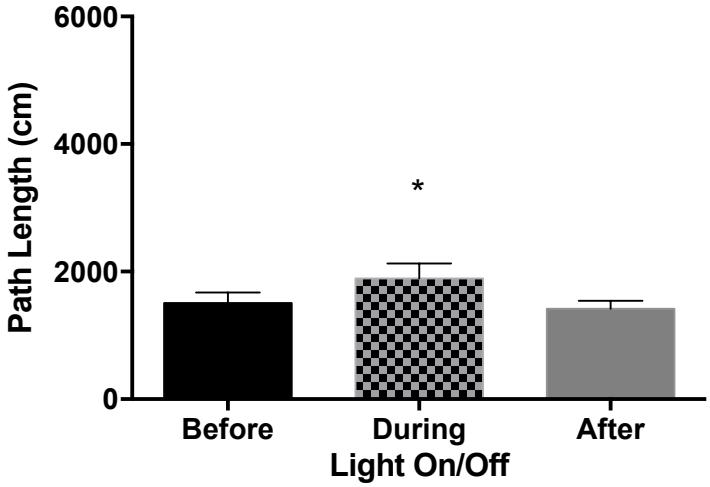

Figure 3 represents the post hoc results comparing means for the total cm travelled in 5 minutes, before, during, and after trials of light pulses into the transgenic rats. Treatment with light pulses significantly increased the path length *$p < 0.05$ versus "before" and +$p<0.05$ versus "after."

Figure 4: **Wild Type Control Group/Distance Travelled**

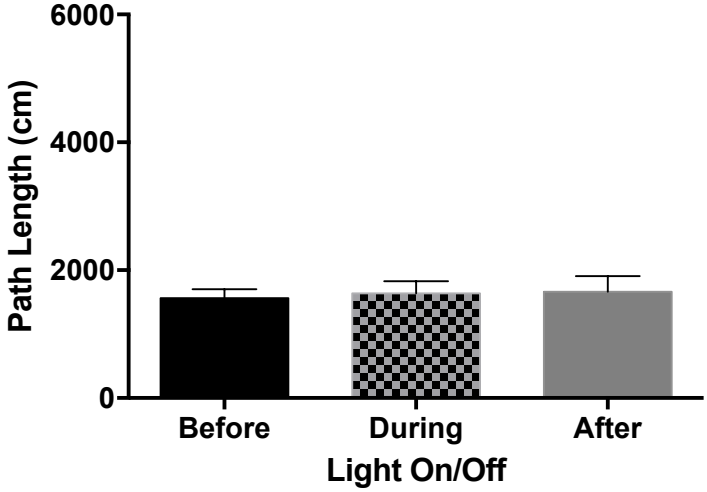

Figure 4 represents the mean for the total cm travelled in 5 minutes before, during, and after light pulses into the WT rats. Treatment of light pulses into wild type rats had no significant effect.

Figure 5: **THCre Baseline/Body Temperature**

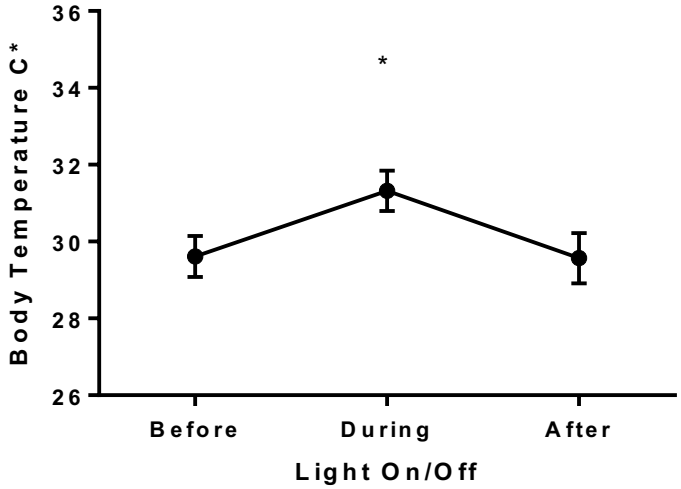

Figure 5 represents the mean for body temperature before, during, and after trials of light pulses into transgenic rats. Treatment with light pulses significantly increased body temperature *$p < 0.05$ versus "before" and +$p<0.05$ versus "after."

.

Figure 6: **Wild Type Temperature/Control Group**

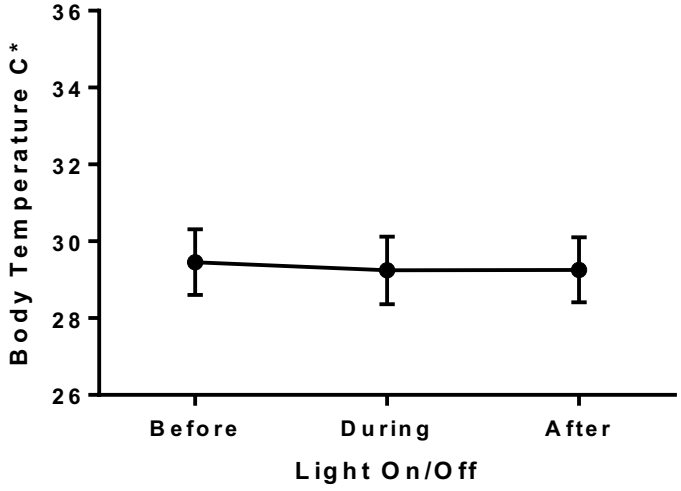

Figure 6 represents the mean for body temperature travelled before, during, and after trials of light pulses into wild type rats. Treatment with light pulses had no significant effect on body temperature.

Figure 7: **Amphetamine and Distance Travelled**

"+" = Light

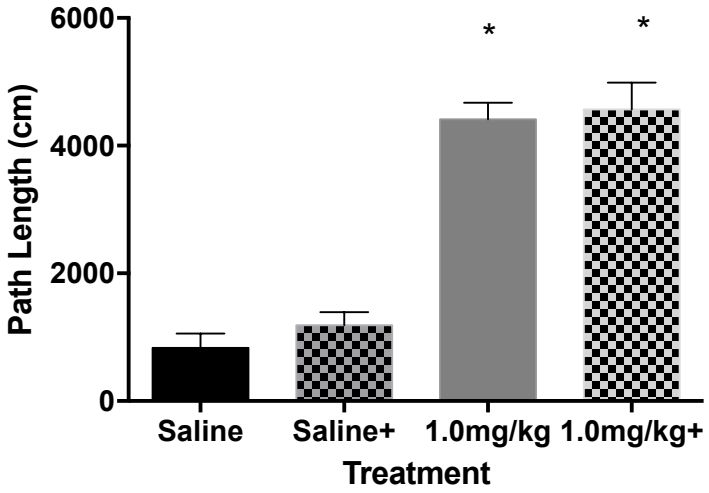

Figure 7 represents the mean for the total cm travelled in 5 minutes, before, during, and after trials of light pulses into saline and amphetamine treated transgenic rats. Treatment with amphetamine significantly increased the path length *p < 0.05 versus "Saline" and +p<0.05 versus "1.0mg/kg".

Figure8: **Amphetamine and Temperature**

"+" = Light

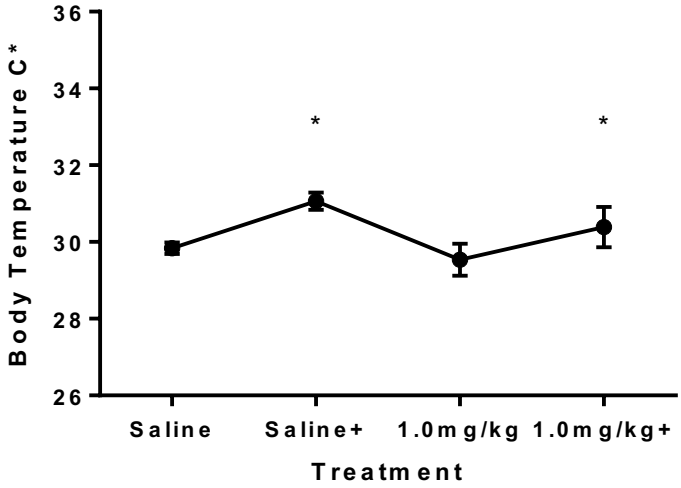

Figure 8 represents the mean for body temperature before, during, and after trials of light pulses into saline and amphetamine treated transgenic rats. Treatment with light pulses significantly increased body temperature *p < 0.05 versus "Saline+" and +p<0.05 versus "1.0mg/kg+".

"+" = Light

Figure 9: **Raclopride and Distance Travelled**

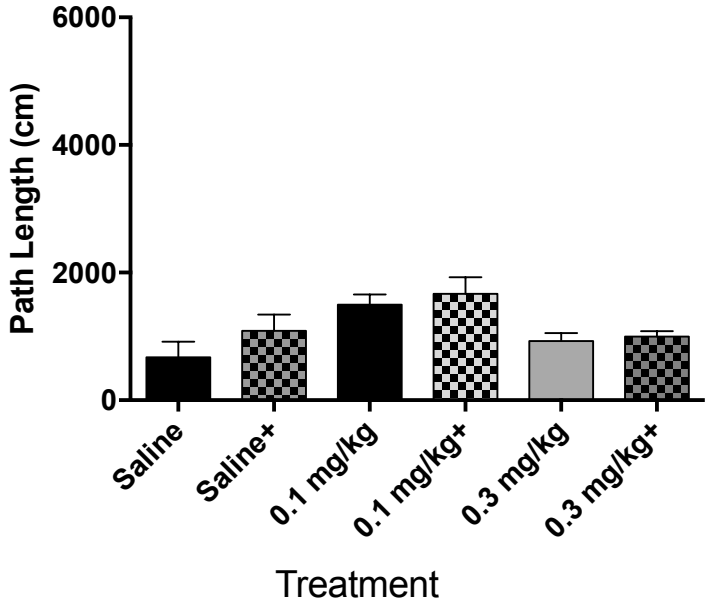

Figure 9 represents the mean path length in 5 minutes, before and during trials of light pulses in transgenic rats during saline and raclopride 0.1 mg/kg and 0.3 mg/kg treatments.

Figure 10: **Raclopride and Temperature**

"+" = Light

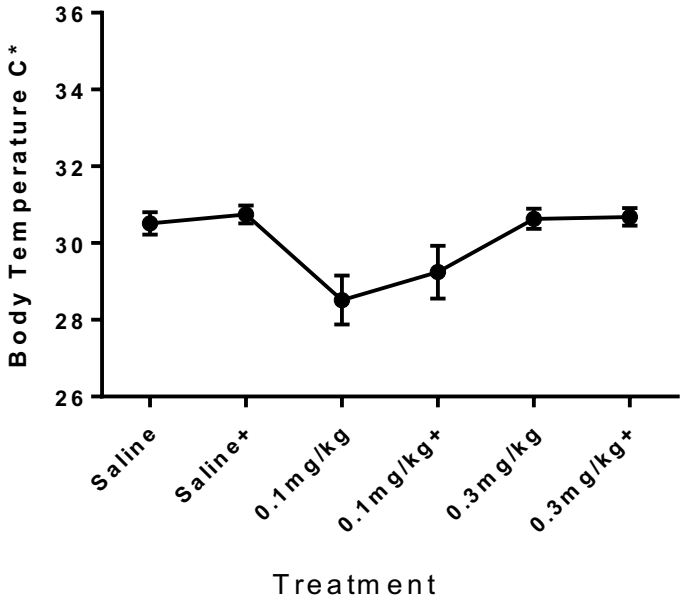

Figure 10 represents the mean body temperature for the transgenic rats during saline and raclopride 0.1 mg/kg and 0.3 mg/kg treatments.

Discussion

This study explored the use of optogenetic tools to better understand treatments used for schizophrenia. The "positive" symptoms (e.g., hallucinations, paranoia, etc.) of schizophrenia may come from overexpression of dopamine in mesolimbic dopamine neurons. Positive symptoms can be produced in healthy volunteers treated with amphetamine, a psychostimulant drug and dopamine releaser. Conversely, antipsychotic drugs may reduce positive symptoms by blocking receptors for dopamine. This study used optogenetics to explore how drugs that alter dopamine neurotransmission might alter behaviors occurring from activation of mesolimbic dopamine neurons in the ventral tegmental area. Light-induced activation of dopaminergic neurons in the ventral tegmental area in male rats produced a significant increase in total distance traveled in an open field and body temperature. Animals treated with amphetamine, a dopamine releaser, also had a significant increase in total distance travelled and body temperature. Light-induced activation of dopaminergic neurons during trials of amphetamine did not show a significant change, it is logical to think amphetamine depleted neurons of dopamine. A $D_{2/3}$ receptor antagonist did block these effects. These results demonstrate that selective activation of mesolimbic dopamine neurons produces effects similar to amphetamine. Further, they coincide with previous findings that used different research techniques to link amphetamine's effects to mesolimbic dopamine neurons. This proof of concept study was the first to be conducted at Northern Michigan University, and also serves as the first to report the effects of an antipsychotic drugs used with optogenetic techniques. A significant amount of trial and error managed to successfully implement this research tool for future NMU students to utilize.

Optogenetic tools have increased locomotor behavior before, optogenetics was recently used to selectively activate neurons expressing Calcium/calmodulin-dependent protein kinase type II alpha chain (Commonly known as CaMKIIa) in the ventral tegmental area of Sprague Dawley rats (Guo et al., 2014). CamKIIa is an enzyme is crucial for calcium signaling involved with hippocampal long-term potentiation and spatial learning. Guo (2014) demonstrated that excitation with light into the ventral tegmental area would increase activity in free roaming tasks.

Pulses of light significantly increased path length in the transgenic rats. As previously shown, increased dopamine concentrations can cause an increase of activity within the sympathetic nervous system (Tritsch, Ding, & Sabatini, 2012). The mean for the total cm travelled in 5 minutes, before and during. Adding to this increase of locomotor behavior, pulses of light significantly increased body temperature. A significant increase of body temperature was found between the means of the session with light compared to before and after. As mentioned earlier, dopamine antagonists have been shown to cause hypothermia (Chaperon et al., 2003). This study demonstrated that dopamine plays an intimate role with body temperature regulation.

The control group was comprised of wild type Sprague Dawley rats that were bred and raised from the same strain as the transgenic rats and in the same location. As expected, Pulses of light had no significant effect on path length.

In five minute trials of the testing sessions, amphetamine at a 1.0 mg/kg dose produced a significant increase of distance travelled compared to baseline data. These results are comparable to previous experiments (Westerink et al., 2001) (Griffith et al., 1972) (Lieberman et al., 1987) (Laruelle et al., 1996).

This study raises several intriguing research questions, such as what would occur when a treatment of a D_1 receptor antagonist treatment is implemented? Or an atypical 5-HT receptor antagonist? What would these drugs do to activity? The addition of electrophysiological. Fast-scan cyclic voltammetry, or microdialysis equipment would be immensely helpful in answering more detailed questions. Optogenetics is commonly paired with these technologies and allow researchers to more thoroughly understand neuronal communications. As previously mentioned, fast-scan cyclic voltammetry could report specific neurotransmitter concentrations in regions connected to a region that is being activated with optogenetics.

Optogenetics has been used in a variety of animals, rodents mostly, but also zebrafish (Douglass et all, 2008), fruit flies (Drosophila melanogaster) (Lima & Miesenböck, 2005), nematode worms (Caenorhabditis elegans) (Blaxter, 2010) and non-human primates (Han et al., 2011). With a research tool as powerful as optogenetics, the logical next step is producing it in humans for therapeutic effects on mental illnesses. Chow and Boyden (2013) recently published an article with a discussion of obstacles that would have to be overcome to bring optogenetics to clinical trials. The largest issue is the use of viral vectors and the subsequent immune response (Chow & Boyden, 2013). Human opsins, such as rhodopsin in the human eye, could be inserted into neurons, but these cells are slower than the microbial ones currently in use for optogenetics. Gene therapy is advancing every day and it is possible that someday humans could reap the benefits of the precision actions optogenetics has to offer.

Even if optogenetics remains as purely a research tool and does not give rise to beneficial effects directly in our species, it has still offered humans an unparalleled look into the function and dysfunction of the nervous system. Optogenetics has been adopted

in laboratories around the world and has enabled scientists to increase or decrease the activity of exclusive brain regions on command. Great insight has been obtained through the use of optogenetics and a great deal more has yet to be discovered.

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Appendix A

Application to Use Vertebrate Animals in Research, Testing or Instruction

Northern Michigan University

Project Title (If using external funds, enter the title used on the grant application): Assessment of dopamine effects using optogenetics

General Instructions
Please check the IACUC website to ensure you are using the current version of the form. All parts of this form *must be submitted electronically* to the Institutional Animal Care and Use Committee (email: IACUC@nmu.edu) and the relevant Department Head or other departmental designee. Review of this application will commence upon receiving the electronic application, but the project may not begin until all required approval signatures are obtained via Right Signature. Please contact the IACUC chair (email: IACUCChr@nmu.edu) if you have any questions.

Shaded area for IACUC use only.

Application Number: 257
Date Application Received: February 16, 2015
XApproved ☐ Denied on May 26, 2015

Review Dates:
Designated Member Review of applications (appropriate for USDA Use Categories B and C) will be completed within two weeks after receipt of the electronic application.

Full Committee Review of applications will take place on the last Friday of every month. Applications for Full Committee Review must be electronically received by the first Friday of the month. Full Committee Review is required for applications that fall under USDA Use Categories D and E. Applications that fall under USDA Use Categories B and C will receive Full Committee Review if requested by an IACUC member. Detailed procedures on the IACUC review processes are located at the IACUC website.

I. Principal Investigator (Must be a faculty member or Department Head): Dr. Adam Prus

Co- Investigator: Remington Rice

Department: Psychology

Phone number: 2941

II. Funding Sources/Course Information and Dates
If the proposed work is for a course, please include the number of the course and title of the course

Funding Sources (External & Internal, if applicable) Internal

Additional Funding Pending (click on the correct box)? ☐Yes XNo

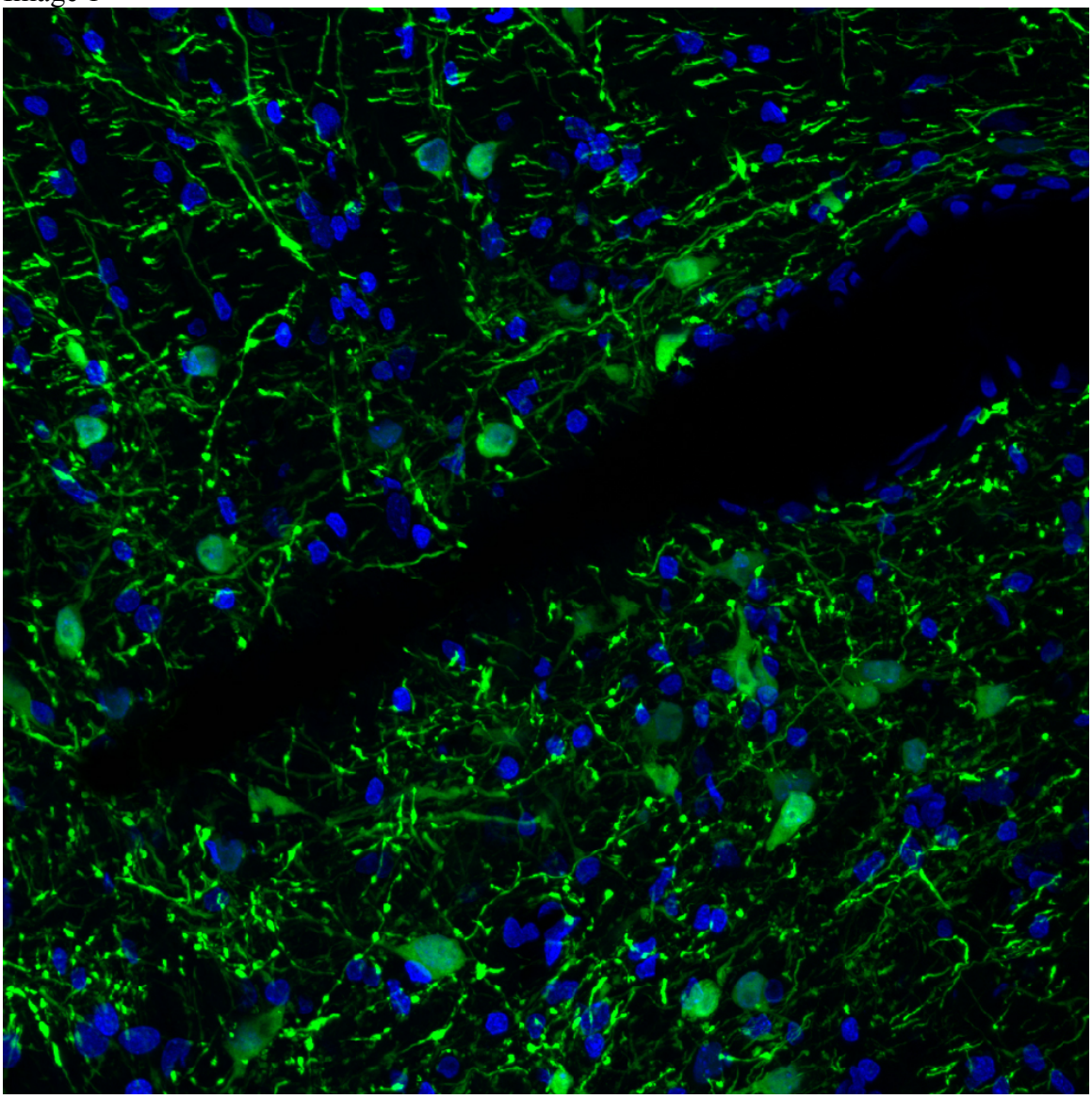
Project/Course Start Date: August 3, 2015
End Date (three year maximum): 8/ 2/ 2018

This application is (check one) XNew ☐Modification of an application currently approved by the Institutional Animal Care and Use Committee (a **new** protocol must be submitted after three years)

III. SPECIES, NUMBER OF ANIMALS, AND USE CATEGORY

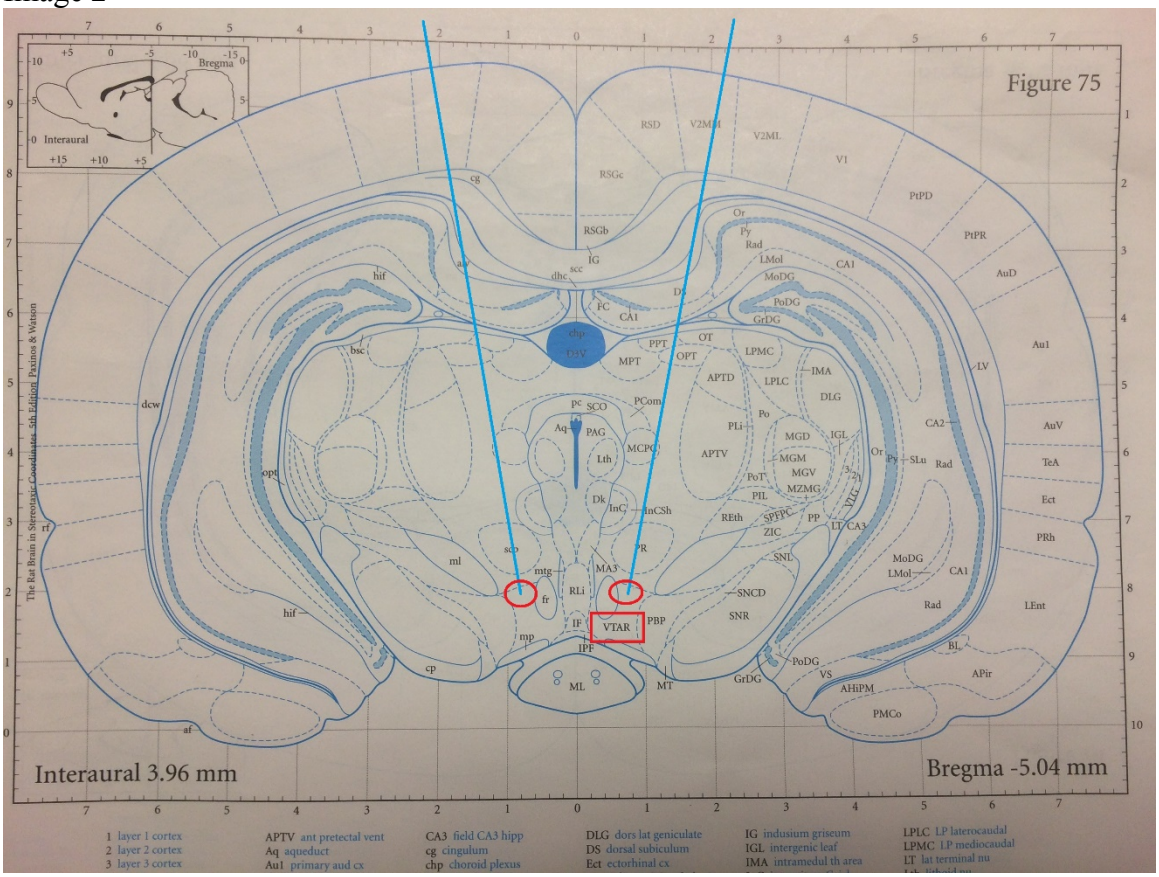
Appendix B

Image 1



This image demonstrates the coordinates were in a dopamine rich ventral tegmental area. The green color in the image is a florescent marker bound to tyrosine hydroxylase. The blue color is named 4',6-diamidino-2-phenylindole (DAPI), it is a florescent DNA-binding protein that stains cellular nuclei. This effectively illuminates where the DNA, nucleus, and cell body of neurons exist. The large black path in the center is the tract left by the injection needle.

Image 2



This image shows the ventral tegmental area coordinates from a sterotaxic map (Paxinos, 1997).

Image 3

Image 3 shows the light testing equipment in action, each implant passed the minimum requirement of 5 mWs of 465 nm light wavelength emittion.

Image 4

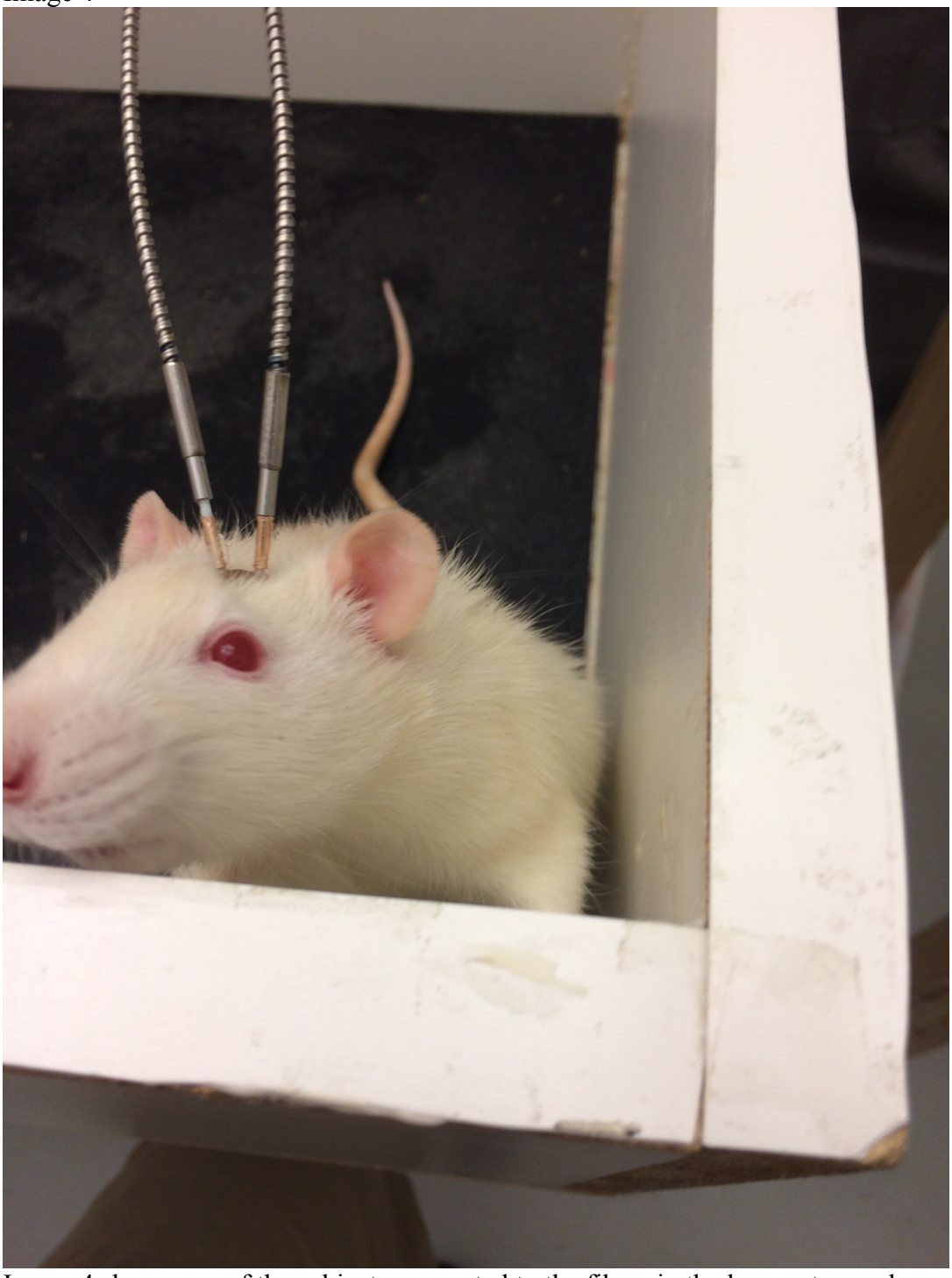


Image 4 shows one of the subjects connected to the fibers in the locomotor enclosure.

Image 5

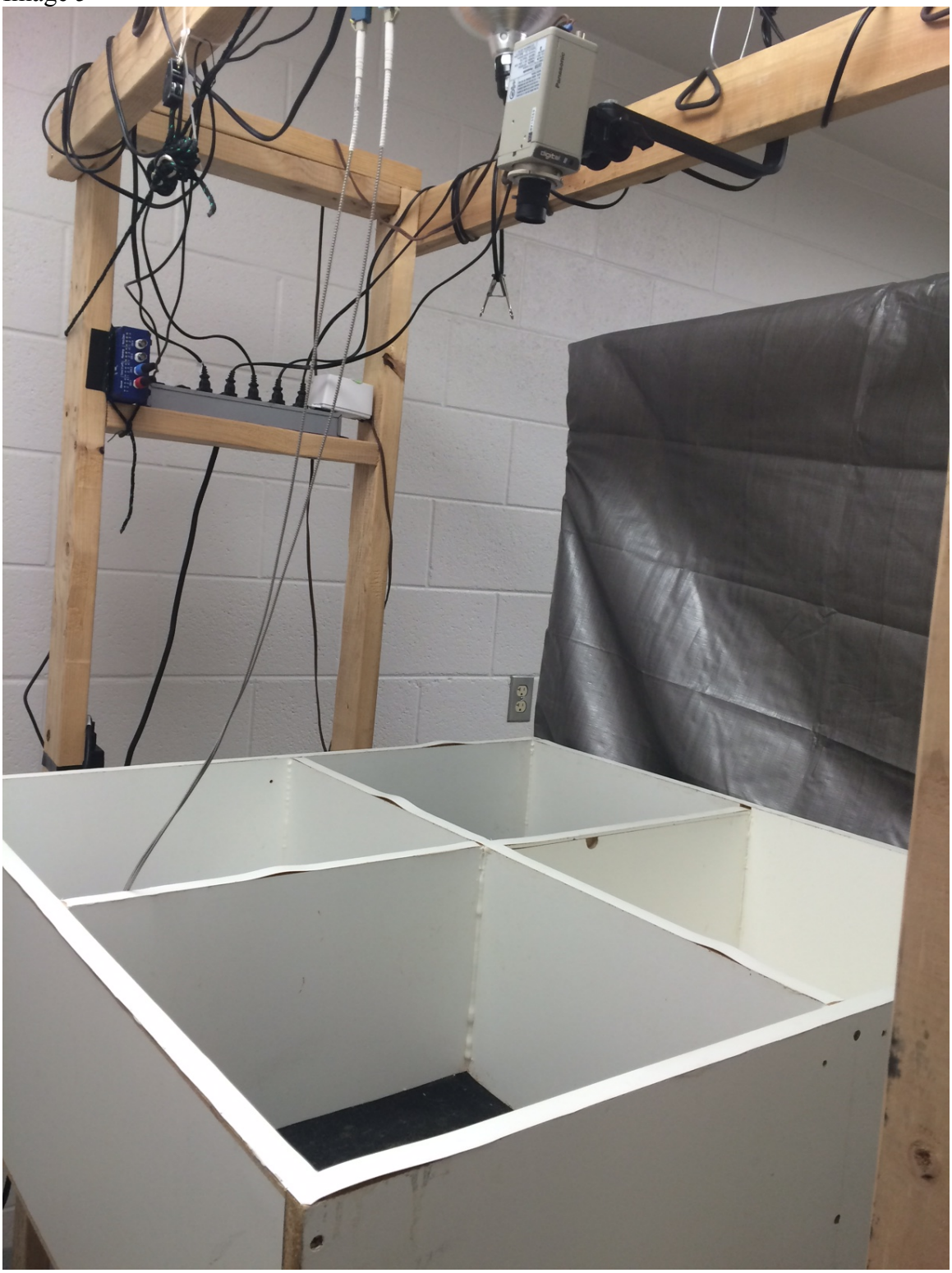


Image 5 shows the video camera above the locomotor enclosure.

Image 6

Image 6 shows the fibers in the locomotor enclosure.

Image 7

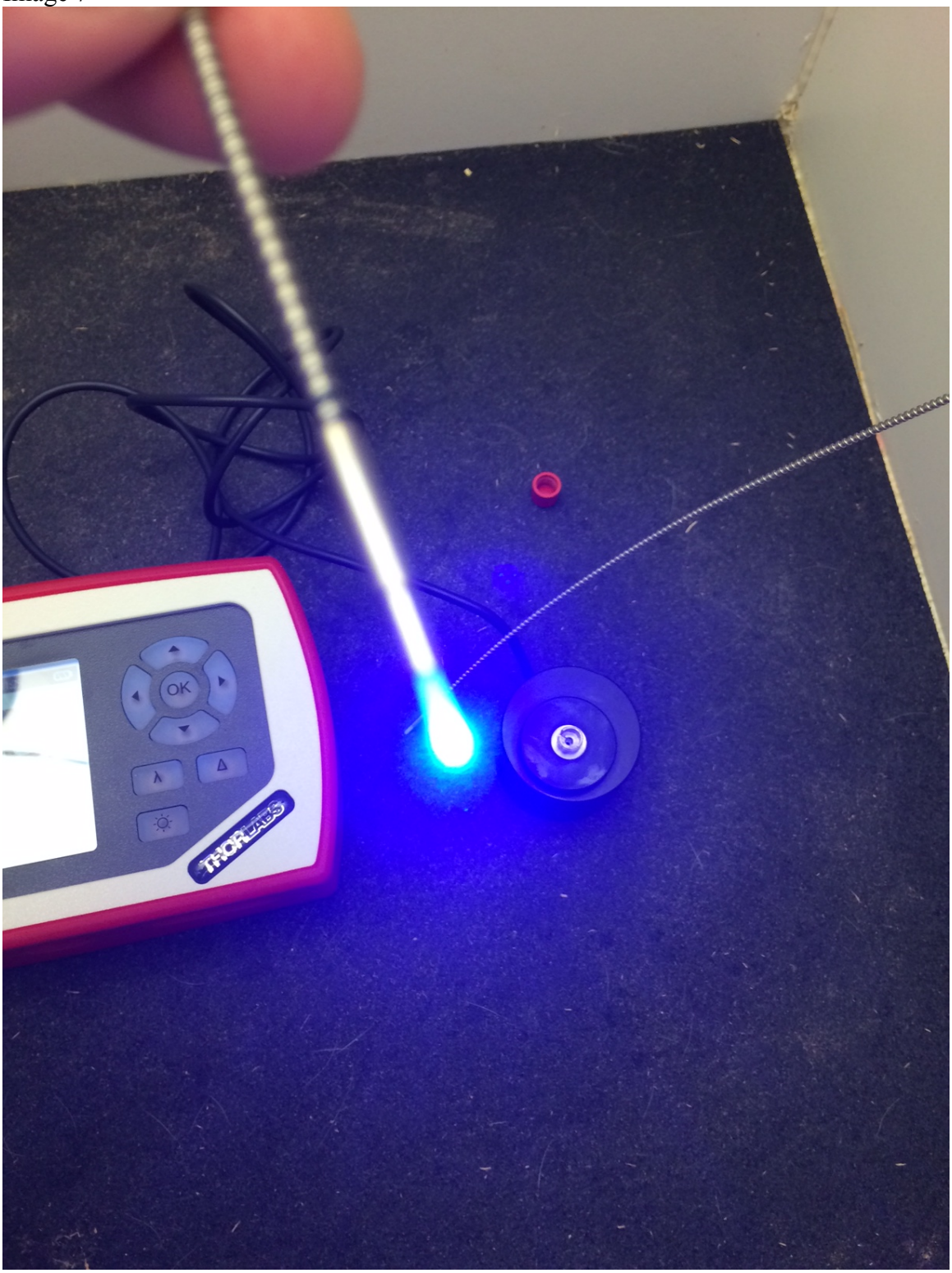


Image 7 shows the end of a fiber lit up by 465 nm wavelengths of light.

Image 8

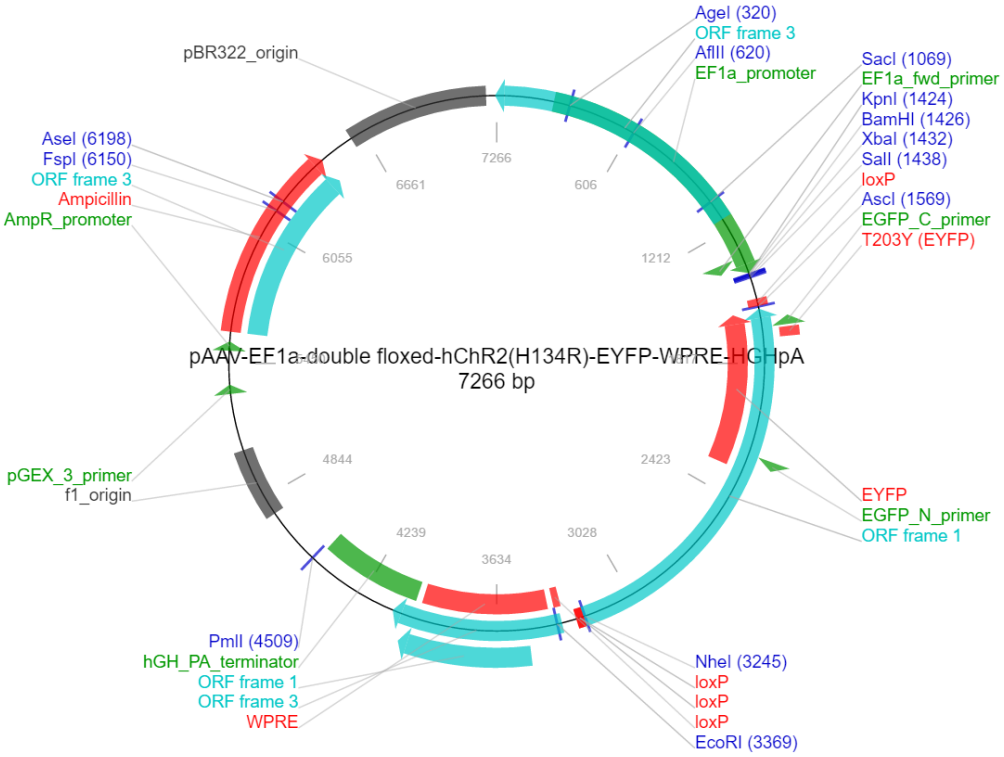


Image 8 Full Sequence Map for pAAV-EF1a-double floxed-hChR2(H134R)-EYFP-WPRE-HGHpA. This was a gift from Karl Deisseroth (Addgene plasmid # 20298). Map generated by Addgene from full sequence supplied by Karl Deisseroth.

Image 9

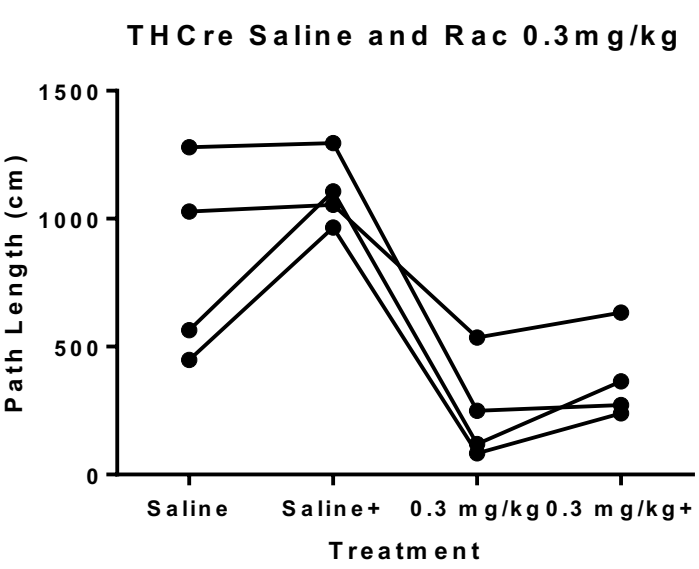


Image 9 shows individual data points during the final series of raclopride.