ABSTRACT

Title of Document: THE IMPACT OF PRENATAL NICOTINE EXPOSURE ON IMPULSIVITY AND NEURAL FIRING IN THE MEDIAL PREFRONTAL CORTEX

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Prenatal nicotine exposure (PNE) is linked to a large number of psychiatric disorders, including attention deficit hyperactivity disorder (ADHD). Current literature suggests that core deficits observed in ADHD reflect abnormal inhibitory control governed by the prefrontal cortex (PFC) of the brain. The PFC is structurally altered by PNE, but it is still unclear how neural firing is affected during tasks that test behavioral inhibition, such as the stop-signal task, or if neural correlates related to inhibitory control are affected after PNE in awake behaving animals. To address these questions, we recorded from single medial PFC (mPFC) neurons in control rats and PNE rats as they performed our stop-signal task. We found that PNE rats were faster for all trial types and were less likely to inhibit the behavioral response on STOP trials. Neurons in mPFC fired more strongly on STOP trials and were correlated with accuracy and reaction time. Although the number of neurons exhibiting significant modulation during task performance did not differ between groups, overall activity in PNE was reduced. We conclude that PNE makes rats impulsive and reduces firing in mPFC neurons that carry signals related to response inhibition.

THE IMPACT OF PRENATAL NICOTINE EXPOSURE ON IMPULSIVITY AND NEURAL FIRING IN THE MEDIAL PREFRONTAL CORTEX

BY

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(Research in Testing ADHD's Link to Impulsivity in Neuroscience)

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List of Abbreviations

ACC - Anterior Cingulate Cortex
ACd - Dorsal Anterior Cingulate Cortex
ADHD - Attention Deficit Hyperactivity Disorder
BOLD fMRI - Blood Oxygen Level-Dependent Functional MRI
5-CSRTT - 5-Choice Serial Reaction Time Test
DNA – Deoxyribonucleic Acid
DP - Dorsal Peduncular Cortex
DSM-V - The Diagnostic and Statistical Manual of Mental Disorders V
IL - Infralimbic Cortex
LO - Lateral Orbital Cortex
mPFC - Medial Prefrontal Cortex
mRNA- Messenger Ribonucleic Acid
MRI - Magnetic Resonance Imaging
NAcc - Nucleus Accumbens
nAchR - Nicotinic Acetylcholinergic Receptor
PET - Positron Emission Tomography
PFC - Prefrontal Cortex
PL - Prelimbic Cortex
PNE - Prenatal Nicotine Exposure
PO - Ventral Orbital Cortex
SHR - Spontaneously Hypertensive Rat
SSD - Stop-signal Delay
SSRT - Stop-signal Reaction Time
SST - Stop-signal Task
SWM - Spatial Working Memory

Introduction

Attention deficit hyperactivity disorder (ADHD)

Attention deficit hyperactivity disorder (ADHD) is a psychiatric disorder characterized by impulsivity[1], hyperactivity, and inattention that influences one's ability to concentrate and regulate behavior (National Institute of Mental Health, 2008). Impulsivity is a behavioral trait characterized by a tendency toward rapid, unplanned actions without considering the negative consequences of these actions (International Society for Research on Impulsivity, 2014). Hyperactivity is generally defined as high or excessive levels of motion. Inattention generally presents as difficulty concentrating, distractibility, and problems completing tasks (Milich, Balentine, & Lynam, 2001). These symptoms usually appear in early stages of life and in many cases persist through adulthood. Children with ADHD are more likely to encounter academic difficulties, such as scoring poorly on exams and withdrawing prematurely from school (Biederman & Faraone, 2005; Karande & Kulkarni, 2005). According to the American Psychological Association, 3.0-7.0% of school-aged children have ADHD (2013). Estimates of adult prevalence of ADHD in the United States vary greatly but are projected to be between 1.0-7.3% (Simon, Czobor, Balint, Meszaros, & Bitter, 2009).

Controversy of ADHD diagnosis

This disorder has caused controversy due to disagreements over its diagnostic criteria, its frequency of diagnosis, and its method of treatment. Currently, there is no well established and experimentally verified neurological basis for ADHD, so the disorder has been diagnosed based on subjective, behavioral observations rather than

[1] For definitions of this and other terms, please see the glossary.

objective, neurobiological identifiers of the disorder. This ineffective method of diagnosing ADHD has led to numerous misdiagnoses and over-prescribed medications, which can be detrimental to the health of patients because of possible harmful side effects. For example, methylphenidate, a commonly prescribed drug for ADHD treatment, may cause insomnia, headaches, increased blood pressure, and increased heart rate (Evans, Morrill, & Parente, 2010). Additional dangers associated with ADHD medications include suicidal ideation, psychosis, heart attack, and even sudden death (Ruggiero et al., 2012; Lakhan & Kirchgessner, 2012). Such diagnostic methods have also contributed to rising medical costs. Between $36 and $52 billion (in 2005 dollars) is spent annually for ADHD associated medical expenses (Centers for Disease Control and Prevention, 2013). Combined research examining the neural basis of ADHD and its behavioral observations will help create a more concrete method for diagnosing and treating this disorder.

Research demonstrates that ADHD is linked to failure of the brain to control or inhibit behavior. The stop-signal task (SST), a popular method used in psychology to measure impulsivity, has shown that those with ADHD tend to have slower inhibition response times (Eagle & Baunez, 2010). Poor performance on these trials of the SST is observed after pharmacological manipulation of the prefrontal cortex (PFC), which suggests that there is an association between this brain area and impulsivity (Aron, Fletcher, Bullmore, Sahakian, & Robbins, 2003).

Animal model of ADHD

In general, animal models of impulsivity disorders are critical because they allow one to isolate certain causal factors from other developmental, genetic, or environmental factors which may also impact behavioral and neural deficits involved in these types of

disorders. Even if an animal model is not directly related to ADHD, the behavioral and brain deficits observed in the model could still provide insights into how the brain governs inhibitory control and how prescription drugs act. The research suggesting a causal link between prenatal nicotine exposure (PNE) and ADHD demonstrates that the PNE model has the potential to be a valid clinical animal model.

To fully understand the neural basis of ADHD in humans, it is necessary to first establish a valid animal model of ADHD. Sontag, Tucha, Walitza, and Lange state that the best animal model should combine face validity, construct validity, and predictive validity (2010). Face validity is based primarily on similarities in symptoms; therefore, an effective animal model should demonstrate three core symptoms of ADHD to be present: attention deficit, hyperactivity, and impulsivity. Sontag et al. also assert that construct validity shows that the model corresponds to an established pathophysiological basis of the disorder. In addition, predictive validity is the ability to predict unknown characteristics of the neurobiology and pathophysiology of a disorder to provide potential new treatments. Numerous animal models, such as the Spontaneously Hypertensive Rat (SHR) and the Naples High-Excitability Rat, have been suggested for ADHD, but the validity of these models remains debatable. SHR has been criticized as a model for ADHD because of the high variability in impulsiveness among these rodents and the presence of hypertension, a symptom rarely seen in ADHD. These factors reduce the SHR's viability as a model for ADHD due to poor face and construct validity, respectively (Garcia & Kirkpatick, 2013). Additionally, although research suggests that Naples High-Excitability rats demonstrate inattentiveness, they do not exhibit hyperactivity or impulsivity, thus they lack face validity (Sagvolden, Russell, Aase,

Johansen, & Farshbaf, 2005). A third animal model demonstrates impulsivity, as PNE might, through disruption of the superior colliculus, an area, which integrates sensory inputs from multiple modalities. This model demonstrated face validity through impaired performance of the Go/No-Go task, but still lacks construct and predictive validity (Mathis et al., 2014). According to Sontag et al., even though there are many different animal models that have been used to study ADHD, no model has shown all three types of validity that are not limited by potential confounding variables.

Although a thoroughly validated animal model of ADHD has not yet been established, another potential model, which has not yet been thoroughly examined, is the PNE rat. This rat model highlights the relationship noted between pregnant mothers who smoke cigarettes and the 2 to 4 fold increased risk that their children will be diagnosed with ADHD (Wasserman, Liu, Pine, & Graziano, 2001; Heath & Picciotto, 2009). In 2005, approximately 10.7 to 12.4% of pregnant women in the United States reported smoking (Martin et al., 2007). Research has demonstrated that PNE leads to a dysfunction in the development of dopaminergic and noradrenergic pathways in the brain; this dysfunction has been attributed to notable decreases in attention span and increases in impulsivity (Muneoka et al., 1997; Slotkin et al., 1987). Our study serves to suggest and study the PNE rat as a plausible model of ADHD by examining behavioral and neural deficits during performance of a SST. We focused on the medial prefrontal cortex (mPFC) because it has been disrupted in ADHD and PNE, and several studies have provided a clear link between the mPFC and inhibitory control.

Our study

In this study, we examined the relationship between PNE and mPFC activity to determine the validity of the PNE rat as a suitable model to study ADHD-like impulsivity. To do this, we examined the correlation between neural firing in the mPFC and impulsivity while characterizing neural firing and behavioral differences between PNE rats and control animals. We hypothesized that increased neural activity in mPFC mediates response inhibition. In addition, we hypothesized that PNE rats would show increased impulsivity during a task where behavioral inhibition is necessary, due to reduced neural firing in the mPFC.

If abnormal neural firing in the mPFC is correlated with impulsivity in PNE rats, this will further validate the PNE rat as an acceptable animal model of ADHD. Firing in the PFC is thought to be disrupted in ADHD patients (Aron et al., 2003). Demonstrating that neural firing patterns in mPFC neurons are associated with impulsivity is fundamental for health professionals and pharmaceutical companies because they can potentially use an empirical basis of diagnosis to develop more effective treatments for ADHD. The precise temporal and spatial resolution of single neuron recordings will allow us to pinpoint the signals involved in impulsive action, which might enable drug development that better incorporates the activity of the mPFC. A full and proper understanding of mPFC circuitry is essential to the development of more effective treatment solutions and diagnostic strategies related to impulse disorders such as ADHD.

Literature Review

From 2003 to 2007, ADHD diagnoses increased by an average of 5.5% yearly,

which may be attributed to diagnoses being based on qualitative observations of an individual's behavior (Centers for Disease Control and Prevention, 2013). The lack of a clinically significant and verified neurological basis has resulted in significant increases in misdiagnoses (Kim & Miklowitz, 2002). Understanding the brain regions associated with the pathology of ADHD is instrumental in diagnosing patients in a consistent manner. Research on the role of the mPFC in ADHD can help formulate a concrete diagnosis of the disorder.

Currently, the long-term efficacy of stimulant use to treat ADHD is unclear. Studies have shown that commonly prescribed drugs, such as Ritalin® and Adderall®, may be effective short-term treatment options for children with ADHD, but do not have any long-term effects on academic performance of adult college students with ADHD (Advokat, 2010; Blase et al, 2009). In a study examining the cognitive effects of stimulants, the same academic impairment in children and adolescents with ADHD was shown to be present in college students with the disorder as well Advokat & Scheithauer, 2013). Furthermore, ADHD undergraduates were shown to be capable of performing just as well as students without ADHD, provided they practiced effective study habits. While stimulants have been shown to reduce frustration and improve self-regulation without impairing attention, they have also been shown to promote risky behavior and increase the likelihood of becoming distracted (Campbell-Meiklejohn et al., 2012; Advokat & Scheithauer, 2013). Further research on the neural basis of ADHD can therefore help expand the existing database of treatment for the disorder.

In order to elucidate the neural basis of impulsivity as observed in ADHD, we must choose a valid rat model of the disorder and integrate it with neural recording of the

mPFC. Our literature review addresses various aspects of ADHD. First, we discuss the clinical components of ADHD to assess the deficiencies in the current system of diagnosis. Next, we analyze the multiple behavioral factors of ADHD, one of which is response inhibition as measured by the SST. Then, we review relevant research on the neurophysiology of ADHD, focusing on the mPFC and the neurotransmitters dopamine and noradrenaline. Following this, we examine the PNE rat model, which has been shown to have ADHD-like symptoms, but requires further study to validate it as an accurate model of ADHD. Finally, we review the results of imaging studies on the neurological presentation of ADHD.

Clinical Components: Difficulties in diagnosing and treating ADHD

The Diagnostic and Statistical Manual of Mental Disorders V (DSM-V)

The Diagnostic and Statistical Manual of Mental Disorders V (DSM-V) is the American Psychiatric Association's most recently produced guide for the standard criteria for the classification of mental disorders. According to the manual, there are 18 symptoms associated with the ADHD, the most common of which are impulsivity, inattention, and hyperactivity (American Psychiatric Association, 2013). These symptoms also overlap with symptoms for other psychiatric disorders, such as Obsessive-Compulsive Disorder, Post-Traumatic Stress Disorder, or Learning Disorder (Spiro, 2013). Although the DSM-V is the most reputable source to use for ADHD diagnosis, the DSM-V may not actually result in a more accurate diagnosis of ADHD (Ghanizadeh, 2013). Furthermore, some of these symptoms may not be applicable to all children with ADHD. Because it is still difficult to identify symptoms that are specific to ADHD, there

is still a great need for finding more accurate ways to diagnose and treat the disorder. Indeed, EEG combined with task performance has already been show to predict ADHD diagnosis with high accuracy (Lenartowicz et al., 2014; Heinrich, Hoegl, Moll, & Kratz, 2014), suggesting that a better understanding of the neurobiological basis of the disorder would make diagnosis more accurate by coupling DSM criteria with imaging during behavioral tasks.

The 18 symptoms presented in the DSM-V are separated into two categories or symptom domains: inattention and hyperactivity-impulsivity. The inattention domain includes symptoms such as the inability to pay attention on tasks, to listen when spoken to directly, and to complete homework or work-related duties. The hyperactivity-impulsivity domain includes behaviors such as fidgeting with the hands and feet, being unable to participate in leisure activities quietly, and interrupting others often. These symptoms, which have been shown to impair the ability to function at school, work, or in social environments, must be present in at least two different settings. To be diagnosed with ADHD, an individual must have experienced the onset of several of these symptoms prior to the age of 12. Children 16 years of age and younger must display at least six symptoms from one of these domains, whereas adults and adolescents 17 years and older must display at least five symptoms (Centers for Disease Control and Prevention, 2013).

Based on the symptoms that an individual expresses, he or she is considered to be predominantly hyperactive-impulsive, predominantly inattentive, or combined hyperactive-impulsive and inattentive. If he or she displays enough symptoms from the hyperactivity-impulsivity category but not enough from the inattention category, he or she is predominantly hyperactive-impulsive. Similarly, a predominantly inattentive

individual expresses enough symptoms in the inattention category, but not from the hyperactivity-impulsivity category. Combined hyperactive-impulsive and inattentive individuals show enough symptoms from both categories. In all three cases, symptoms must have been present for six months prior to diagnosis (Centers for Disease Control and Prevention, 2013). Although all of these characteristics are fully discussed in the DSM-V, these criteria still have limitations. Most children diagnosed with ADHD have a combined hyperactive-impulsive character; due to the overlapping nature of these symptoms, it is rather difficult to clearly distinguish the different symptoms present in each child.

Diagnosis of ADHD

The diagnosis process consists of mostly behavioral observations. Licensed health professionals gather information about the child's behavior along with the environment that he or she is in. First, the health professional tries to rule out other possible disorders based on the symptoms the child displays. They will generally look for learning disabilities, depression, or sudden changes in lifestyle, such as a death in the family (National Institute of Mental Health, 2012). The second part of the process consists of checking school and medical history. The health professional will gather information about the child's behavior from teachers, parents, babysitters, and other adults who know the child well. According to the National Institute of Health, some possible questions include, "Are behaviors a continuous problem in response to the temporary situation?" and "Are behaviors excessive and long lasting? Do they affect all aspects of the child's life?" (2012). Finally, the health professional will observe the child's behavior in a psychiatric setting, evaluating his or her ability and academic achievement. Overall, the

diagnostic process is rather qualitative; it is mostly up to the health professional to accurately diagnose the disorder based on vague behavioral observations and questionnaires. Although health professionals rely on information from teachers, many of these instructors are often untrained on behavioral disorders such as ADHD and are unable to correctly identify symptoms in children. The type of questionnaires used for diagnosis may also affect a teacher's report on a child. Some questionnaires may be broader and based more on subjective observations, while others may be based more on DSM criteria (Kieling et al., 2010; Dias et al., 2013). Thus, observations of the child in non-clinical settings, such as in school, do not always provide accurate information about the child's behavior.

Increases in ADHD diagnoses

Over the past ten years, the diagnosis of ADHD has increased by 66% (Galéra et al., 2011). While the DSM-V outlines the current methods of diagnosing ADHD, these methods rely solely on behavior observations. Thus, it is possible to infer that many inaccurate diagnoses are possible under this current system. Furthermore, the DSM-V guidelines do not consider that individuals within a specific subtype can have symptoms that vary in severity. Certain factors, such as gender, age, and cultural background must also be taken into account when making the diagnosis (Frick & Nigg, 2011). Even assessments such as the Conners' Continuous Performance Test, which produces response patterns based on a patient's reaction time to letters on a computer screen, are not always accurate indicators of ADHD (IPS Information Circular, 2009). Results of these tests can be confounded by the presence of other contributing factors such as reading disorders and other learning disabilities (McGee, 2000). In college-aged students,

current tests used to identify ADHD symptoms do not distinguish between persons with actual symptoms of ADHD and persons who were coached to malinger, suggesting a great need for more accurate diagnostic tools (Sollman, Ranseen, & Berry, 2010).

A Test of Behavioral Inhibition: The Stop-Signal Task (SST)

Stop-Signal Task Assesses Impulsivity in Humans

In numerous studies, the SST is a method that is used to measure impulsivity across several species (Dagenbach & Carr, 1994). The task gauges how quickly an already-initiated response to a stimulus is inhibited (Eagle & Baunez, 2010), a behavior that is repressed in ADHD patients and is correlated with other measures of impulsivity (Oosterlaan, Logan, & Sergeant, 1998). The SST enables one to determine if poor inhibition is due to dysfunctional executive processing (Oosterlaan, Logan, & Sergeant, 1998). In this task, the subject is trained to respond to a conditioned stimulus, known as the go-signal, such as a tone. After this initial training, the subject practices restraining his or her response to the go-signal and responding to a second conditioned stimulus, known as the stop-signal. All of the trials begin with the go-signal; however, on a minority of trials (~20%), the stop-signal appears after the go-signal. Because the subject becomes accustomed to reacting habitually to the go-signal, it is more difficult to inhibit his or her response on stop-signal trials. The race model describes how performance on the SST might be controlled (Liddle et. al, 2009; Logan, Cowan, & Davis, 1984). The model suggests a race between the processes that underlie response execution (responding to the go-signal) and inhibition (responding to the stop-signal) (Logan, 1981; Alderson, Rapport, & Kofler, 2007). Stop and go processes compete with each other to alter the behavior of the subject, and the one that finishes first determines the subject's

response. From these trials, one can measure the subject's ability to stop the initiated go-response and obtain a measure of impulsivity (Logan, 1981; Alderson, Rapport, & Kofler, 2007).

The SST provides a quantitative measure of motor inhibition and impulsivity, which are measured by the stop signal reaction time (SSRT) and stop accuracy. The SSRT is the time needed by the subjects to inhibit the initiated response to the go-signal and change their behavior to the conditioned response of the stop-signal. Stop accuracy is the percent of STOP trials during which the subject correctly inhibits a response and completes the appropriate behavior (Bari et al., 2011). The SST is most widely used in studying the behavior of children with ADHD. In a review by Verbruggen & Logan, they found that children with ADHD have slower SSRTs than individuals without the disorder (2008). By obtaining and analyzing a subject's SSRT values upon completion of the SST, it is possible to use these values as a basis for measuring inhibitory control and impulsivity.

Experts in the field of clinical psychology have made extensive use of SSRTs to study response inhibition in persons deemed generally impulsive, such as those with ADHD (van Boxtel, van der Molen, Jennings, & Brunia, 2001). A study published in 2008 used a SST to measure inhibition in children that were 4-12 years old with and without ADHD. The researchers found that levels of inhibitory control improve with age, meaning high impulsivity at age four or five can be ameliorated by age 12. They also concluded that the SST and resulting data, such as the SSRT, is useful for diagnosing ADHD (Tillman et al., 2008). Longer SSRTs, which suggest that a greater amount of time is needed to inhibit an initiated response, have been associated with both children

and adults with ADHD (McAlonan et al., 2009). Similar results have been demonstrated in animal models of ADHD (Bari & Robbins, 2011). A high SSRT value, therefore, is correlated to a lower level of inhibitory control and higher level of impulsivity in subjects (Verbruggen & Logan, 2009).

The SST can also be used to determine the severity of ADHD. In 2013, Crosbie et al. used the SST to determine if factors such as deficits in response inhibition, increased variability, and slower latency were endophenotypes of ADHD. An endophenotype is a stable behavioral symptom that is rooted in genetics. For example, a deficit in behavioral inhibition may be an endophenotype of ADHD. The study focused on subjects with ADHD varying in severity according to scores from the Strengths and Weaknesses of ADHD-symptoms and Normal-Behavior scale, which uses diagnostic criteria for ADHD from the DSM-IV. Researchers then compared the SSRT data with diagnoses of ADHD and found that those with longer SSRTs had more severe cases of ADHD (2013).

Finally, ADHD-diagnosed children exhibit difficulty inhibiting behavior on STOP trials and reengaging their responses after inhibition (Nigg, 1999; Schachar, Tannock, Marriott, & Logan, 1995). Furthermore, several studies have also reported increased variance in movement times in general. This variance may be attributed to deficits in responding to visual stimuli and then processing the second stimulus. These results suggest that children with ADHD have deficits not only in response inhibition but also in attentional and cognitive control (Hooks, et al., 1994; Alderson, Rapport, & Kofler, 2007; Lijffijt et al., 2005; Alderson, Rapport, Sarver & Kofler, 2008).

Stop-Signal Task Assesses Impulsivity in Rats

The SST has been used extensively not only in humans, but also in animal models.

In these studies, the animal subjects are required to push a lever or enter a well after illumination of a light on 80% of trials to obtain a reward (Ajarem & Ahmad, 1998). On 20% of trials, a stop-signal, which is either a tone or a second light, is presented which instructs the animal to stop its ongoing movement. Thus, this animal analog of the human stop-signal task captures the same behavioral functions, namely the ability to inhibit habitual prepotent tendencies, such as to stop following the go signal.

Importance of using an animal model for neurobiological research

Animal models allow researchers to pose neurobiological questions that cannot be addressed via human research. The rat is the most widely used animal model for studying impulsivity as observed in ADHD, taking advantage of the structural and functional homology of the brains between humans and rats and the simplicity of the tasks evaluating behavioral inhibition in both species. Species that show homology have structures that have a common ancestry, behavioral purpose, and mechanisms of action. These factors allow researchers to investigate psychiatric dysfunction with invasive techniques that are not possible with human subjects, such as single neuron recordings and changes in neurotransmitter concentrations during behavioral tasks.

Humans and rats share similar mechanisms and structures in brain function. Both species have a conserved structure of basal ganglia, which is actively involved in behavioral decisions. They also share similar ascending neurotransmitter systems such as acetylcholine, noradrenaline, and dopamine. This structural and functional homology between humans and rats allows for comparative studies. In addition, the basic forms of SSTs can be used in both species without significant alterations in experimental design (Eagle, Bari, & Robbins, 2008). Rats can be used to show relationships between brain

activity and behavior that cannot be achieved in human subjects.

Prefrontal Cortical Circuit: Impulse Control and Attention

Basic Functions and Connectivity

The PFC is a region of the brain in both human and rats that functions as an executive control center important for decision-making, learning, and memory and is disrupted in many psychiatric disorders, including ADHD. Executive functions can be defined as processes that regulate or control cognitive circuits that govern behavior (e.g., response selection, attention, inhibitory control, working memory, etc). Here, we review connectivity that supports these functions with specific focus on circuits that may be related to performance on the SST.

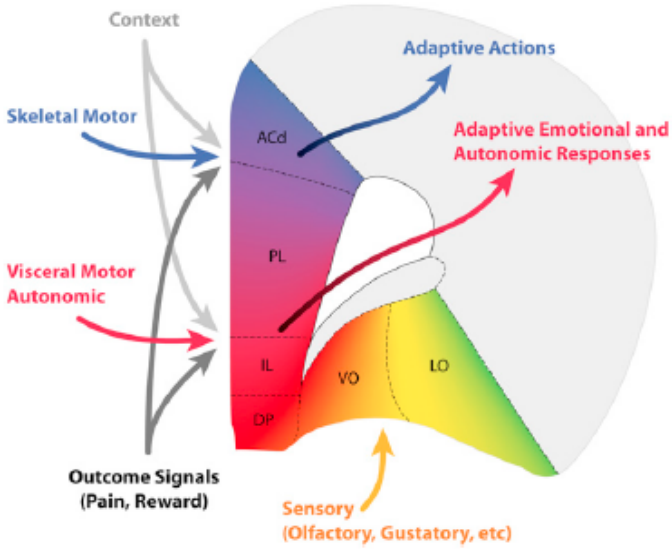

Figure 1.1. Overview of mPFC circuitry. (Euston, Gruber, & McNaughton, 2012). Abbreviations: dorsal anterior cingulate cortex (ACd), dorsal peduncular cortex (DP), infralimbic cortex (IL), lateral orbital cortex (LO), prelimbic cortex (PL), ventral orbital cortex (VO).

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The mPFC is divided into several interconnected regions that appear to have unique functions. Euston et al. conclude that these different parts of the mPFC form functional subunits in which dorsal anterior cingulate and prelimbic cortices receive skeletal motor input, infralimbic and dorsal peduncular cortices receive autonomic input, and ventral orbital and lateral orbital cortices receive sensory input (2012). These subsets of mPFC form a network that executes action or emotional response depending on each subset's location in the cortex, as shown in Figure 1.1.

The afferent (input) and efferent (output) connections of the mPFC suggest a clear distinction between the ventral and dorsal portions in the mPFC. Ventral mPFC projects to the piriform cortex, nucleus accumbens (NAcc), amygdala, hypothalamus and hippocampus (Vertes, 2006), areas associated with the limbic system. It receives information (afferents) from insular areas and the piriform cortex. Dorsal mPFC innervates sensorimotor areas in the frontal cortex, parietal lobes, and dorsal striatum, areas critical for executive function and attention, and receives input from secondary visual and posterior agranular cortices. Consistent with these connections, lesions in the ventral and dorsal pathways produce distinguishable deficits in limbic processing and executive function (e.g., response inhibition) (Euston et al., 2012).

Further examination of the connectivity of the mPFC and its associated functions reveals the complex role the mPFC has in modulating cognition and behavior. Lesions of the ventral projections to the hippocampus, a brain area responsible for memory formation, emotion, navigation, and spatial orientation, have been shown to correlate with an increased likelihood of impulsive decision-making (Cheung & Cardinal, 2005; Eagle & Baunez, 2010). The neural connection between the hippocampus and mPFC is

also critically involved in spatial working memory (SWM), the part of memory that is responsible for the spatial environment. In animals, SWM is necessary in performing tasks that are vital to survival, such as foraging for food.

In addition to SWM, the dorsal portion of the mPFC is also involved in coordinating stimulus-dependent processing necessary to perform the SST. Both the SSRT and SWM are impaired in individuals with ADHD. Clark et al. administered both a SST and a SWM task to 20 adults with ADHD. In the SWM task, the participants were given visual cues to reproduce in the correct orientation and space. They found that the SSRT was significantly associated with the SWM capacity, suggesting a link between the two processes. This link could be explained by the fact that both processes rely on the mPFC (2007).

The role of mPFC in modulating behavior is also evident in its dorsal projections to the cerebellum, an area that is, in addition to other motor regions, responsible for fine motor control and associative learning. A 2013 study by Chen et al. showed through the use of the trace eyeblink conditioning test in guinea pigs that connections between the cerebellum and the mPFC are necessary for an organism to learn a conditioned response. The eyeblink conditioning test works by using a puff of air to stimulate the eye to blink. The guinea pig is then conditioned to blink to conditioned stimuli (e.g., tone) that predict the air puff. If the mPFC is inhibited or connections between mPFC and cerebellum are disrupted, this conditioned response is impaired (2013).

Although research suggests that the mPFC may play an extensive role in cognitive and behavioral functions, researchers continue to debate whether the mPFC in humans is homologous to the mPFC in rat models. Anatomical evidence suggests that the mPFC in

rodents is similar to that of primates, as shown in Figure 1.2 (Euston, Gruber & McNaughton, 2012). Similar connectivity patterns are observed in rats and humans; the dorsal mPFC connects to sensorimotor and association neocortical areas, whereas the ventral areas connect to the amygdala and temporal and limbic association cortices. The rat mPFC has been implicated in working memory, attention, response initiation and management of autonomic control and emotion, which may be attributed to these connections (Heidbreder & Groenewegan, 2003). This suggests that the mPFC may serve a similar function in the rat as it does in humans. Thus, by examining the mPFC in rat models, which has both structural and functional homology with the human mPFC, we may elucidate further functions of the mPFC in humans and its role in various pathologies.

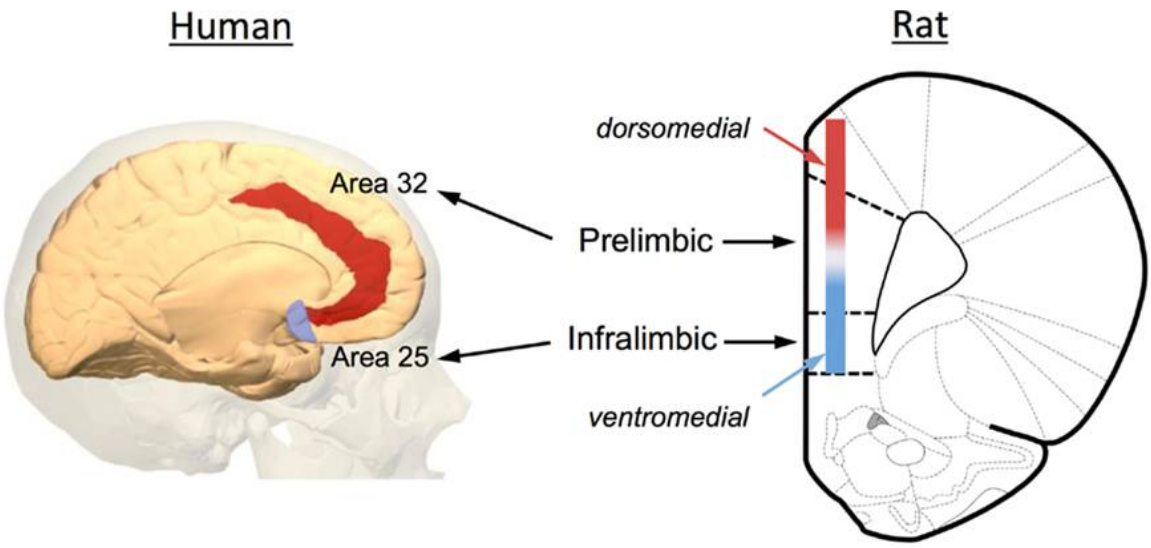

Figure 1.2. Homology between human and rat mPFC. Human mPFC has a structurally and functionally homologous area in the rat brain (Gass & Chandler, 2013).

Role of mPFC and Connected Areas in Attention and Impulsivity in Rats

Areas strongly connected with mPFC are clearly involved in functions pertaining

to performance on the SST. Striatum, one of the efferents of the mPFC, plays a crucial role in regulating attention, decision-making, and motivation/reward processing (Liljeholm, 2012). In a study conducted by Eagle et al., lesions of the medial striatum resulted in significant deficits on SST performance and longer stop-signal reaction times (SSRTs) (2003). In another study utilizing the 5-choice serial reaction time task (5-CSRTT), a task capable of measuring different aspects of performance such as attention, inhibition, and impulsive responses, medial striatal lesions induced increased premature responding, which is similar to what has been described after PNE (Rogers et al., 2001). These results signify that the circuit connecting the mPFC with the striatum is involved in controlling inhibition and loss of its function results in impulsive decision-making. In a study conducted by Christakou et al., the circuit comprising the mPFC and the dorsomedial striatum was disconnected in rats performing the 5-CSRTT. In these rats, there was a persistent deficit characterized by a reduction in accuracy and speed in responding to the visual stimulus in the task, suggesting the circuit's role in regulating visual attention as well (2001).

Other studies have shown that the NAcc, another efferent of the mPFC, is critical for regulating impulsivity related to delayed gratification. In one 2001 study, rats performed the delay-discounting task, where the subject chooses between a small, immediate reward or a large, delayed reward. At the beginning of each training session, rats choose the larger reward, but as the delay for the large reward increases, animals act impulsively and start choosing the smaller, more immediate reward. Rats with lesions in the NAcc were more likely to select the smaller, immediate reward than control rats, thus exhibiting increased impulsivity, a clinical feature of ADHD (Cardinal, Pennicott,

Sugathapala, Robbins, & Everitt, 2001).

Role of Neurotransmitters in PFC and Impulsivity

Neurotransmitters play key roles in information processing within brain structures such as the mPFC. The dopaminergic and noradrenergic neurotransmitter pathways are both integral to the control of mPFC-dependent cognitive processes such as behavioral inhibition and impulsivity. In rats, there is a significant positive correlation between impulsive choice and levels of the dopamine receptors D1 and D5 in the mPFC of rats (Loos et al., 2010). In ADHD patients, who have abnormal behavioral inhibition processing and greater levels of impulsivity, Positron Emission Tomography (PET) scans show decreased dopamine and noradrenaline activity in frontostriatal circuits. This decrease in neuronal activity results from the potential combination of imbalances in neurotransmitter synthesis, release, receptor activation, and neuronal responsiveness (del Campo et al., 2011). These neurotransmitter systems are acted upon by amphetamine (Adderall®) and methylphenidate (Ritalin®). A 2006 study showed that the effects of these drugs on the brain were mimicked by a dopamine reuptake inhibitor, which increases the amount of neurotransmitter available in the synapse, and attenuated by a dopamine D1 receptor antagonist and an adrenergic α2 receptor antagonist, which blocks activity of these neurotransmitters. This suggests that both dopamine and noradrenaline are involved in regulating impulsive choice (van Gaalen, van Koten, Schoffelmeer, & Vanderschuren, 2006).

Human Imaging Studies and the Stop-Signal Task

During the SST, various functional neuroimaging techniques have been used to perform localization of executive functions, such as response inhibition within the mPFC

(Kelly, Margulies, & Xavier, 2007). These neuroimaging techniques have enabled researchers to suggest a neurological basis of symptoms of ADHD.

Quantitative neuroimaging analysis of ADHD has demonstrated decreased brain volume in patients with ADHD (McAlonan et al., 2007; Kelly, Margulies, & Xavier, 2007). A healthy individual normally attains 90% of total brain volume by the age of five and reaches a maximum in total cerebral volume by early adolescence. Also, grey matter volumes in the frontal and parietal lobes peak at approximately 12 years. In one study, children with ADHD showed cerebral volumes that were 3.2% less than those of controls, and their decreased cerebral volumes were correlated with increased ADHD symptoms (Durston et al., 2004; Krain & Castellanos, 2006). In ADHD patients, certain brain regions, such as the lateral PFC, the basal ganglia, and the cerebellum, show significantly reduced volumes compared to those of control groups (Emond, Joyal, & Poissant, 2009). Children with ADHD have shown defects in cortical development as well. The peaks of grey matter maturation primarily in the prefrontal area in ADHD patients occur three years later than in controls (Shaw et al., 2006; Curatolo, D'Agati, & Moavero, 2010). Similarly, individuals with ADHD exhibit reductions in white matter volumes, midsagittal corpus callosum regions, and cortical thickness (Castellanos et al., 2002; Curatolo, D'Agati, & Moavero, 2010).

Magnetic resonance imaging (MRI) is the most common method to analyze anatomical differences and observe distinct neuroanatomical characteristics between ADHD patients and control subjects. MRI allows studies to quantify volumes of specific brain areas and differences in grey and white matter (Krain & Castellanos, 2006). The most common form of MRI used in ADHD pathology analysis is blood oxygen level-

dependent functional MRI (BOLD fMRI). When the neurons in a specific brain region are active, the amount of oxygen present in the blood increases because the blood flow is locally increased. The increased blood flow compensates for the use of oxygen by the tissue. Thus, a BOLD signal associated with increased neural activity reflects an increase in oxygen. Because BOLD fMRI measures the ratio of oxygenated to deoxygenated blood, a change in blood oxygenation levels is representative of a change in local neural activity, which then appears as a change in signal on the fMRI scan. This method also enables assessment of the neurobiology underlying the disorder by comparing the different task-aroused brain activity patterns between ADHD patients and controls (Tian et al., 2007). For example, Hart et al. investigated the relationship between inhibition and attention in ADHD patients during performance of the SST. They discovered that patients with ADHD had reduced activation during inhibition in the right inferior frontal cortex, mPFC, supplementary motor area, anterior cingulate cortex (ACC), striatum, and thalamic areas (2013).

The meta-analysis of resting-state fMRI studies reveals widespread differences between ADHD patients compared to control groups in a number of regions, including visual, somatomotor, dorsal attention, ventral attention, limbic, frontoparietal, and default networks. Overall, ADHD patients exhibit significant hypoactivation in visual and frontoparietal regions, two areas involved in selection of sensory contents of attention and hyperactivation in regions associated with the default network, a brain circuit involved in internal tasks such as daydreaming instead of external tasks. In children, the meta-analysis also revealed hypoactivation in frontal regions and hyperactivation in the posterior cingulate cortex and midcingulate cortex (Cortese et al., 2012). Prefrontal

hypoactivity has the potential to dysregulate dopaminergic function, which leads to reduced responsivity to reward-related cues and thus global disruption in reinforcement and motivation (Kosobud, Harris & Chapin, 1994; Kollins et al, 2014).

Drugs used to treat ADHD, such as methylphenidate, have been shown to modulate brain activity in certain regions. fMRI studies in children demonstrate that methylphenidate causes increased frontal lobe activation (Vaidya et al., 1998; Czerniak et al., 2013). In addition, individuals with lesions to the mPFC demonstrated reduced performance on the SST in addition to deficits on neuropsychological testing (Lovstad et al., 2012). Additional evidence that supports the mPFC's role in the SST is the observed increased activation of the mPFC during the task as recorded by fMRI. It is believed that this increase is associated with intentional inhibition (Schel et al., 2014). fMRI studies have also demonstrated that shorter SSRTs are correlated with increased mPFC activity, which is believed to be associated with stop-signal inhibition (Li, Yan, Sinha, & Lee, 2008).

Despite its advantages, MRI has some limitations. The biggest concern is its high cost, which makes obtaining large sample sizes difficult. Due to possible confounding variables such as gender, age, and clinical setting, obtaining an accurate comparison between ADHD patients and controls using only MRI is also challenging (Rossi, 1990; Krain & Castellanos, 2006). MRI has limited spatial and temporal resolution. However, it can be used to detect activity in specific regions across the entire brain over time, thus it is still widely used in investigation of neurophysiology of various mental illnesses.

Prenatal Nicotine Exposure as a Model for ADHD

To better understand the neurobiology of ADHD, scientists have adopted the use

of animal models. Animal models enable scientists to directly measure symptoms of a disorder and identify areas of the brain that may be responsible for these symptoms in humans. Unfortunately, the scientific community has yet to agree upon a single experimentally validated animal model of ADHD. Many proposed models exist, but none have met all validation criteria. Such a model is needed to examine the origin of the disorder symptoms and the effectiveness and long-term consequences of pharmacological treatments.

Developing animal models of ADHD is difficult due to the combined genetic and environmental causes of ADHD. However, several studies correlate PNE in children to a high incidence of ADHD and other behavioral deficits later in life (Nomura et al., 2010). PNE rats could be used to further study the neurological basis of ADHD if this model were thoroughly validated.

Maternal smoking is correlated with higher rates of child diagnosis of ADHD and other behavioral disorders. Several studies have found correlations between maternal smoking during pregnancy and behavioral deficits in children, including ADHD (Thapar et al., 2003; Wasserman et al., 2001). Children exposed to smoking prenatally had a two- to four-fold increased risk of developing ADHD (Ernst, Moolchan & Robinson, 2001; Heath & Piccotto, 2009). Through animal model research, nicotine has been implicated as causing these disorders via long-term changes to a child's brain structure and behavior (Ajarem & Ahmad, 1998). Nicotine is a teratogen; when a mother is exposed to nicotine, it can cross the placental blood barrier from the mother’s blood to the fetuses and affect fetal development. In particular, it disrupts fetal development of central neurotransmitter systems, including dopaminergic and monoaminergic systems (Slotkin et al., 1987;

Navarro et al., 1989; Oliff & Gallardo, 1999). PNE causes a multitude of neurochemical changes, including reduced DNA synthesis, altered neurotransmitter function, and cortical morphogenesis (the formation of cortical structures) (Wickström, 2007). These changes occur during critical periods of neonatal brain development, leading to changes in brain area volumes, firing patterns, neurotransmitter concentrations, and receptor density. These alterations are present in areas important for impulse inhibition and cognitive focus, leading to behavioral deficits in children. These behaviors show considerable similarities with those of ADHD, making PNE rats suitable candidates for an animal model of the disorder.

Research has demonstrated a positive correlation between the magnitude of nicotine exposure and the severity of attentional control (Motlagh et al., 2011; Schmitz et al., 2006) and hyperactivity-impulsivity (Langley et al., 2007) symptoms in children with ADHD. Furthermore, children diagnosed with ADHD who were exposed to nicotine prenatally are more likely to have higher ADHD symptom scores and be less responsive to symptom intervention (Vujik et al., 2006). This correlation appears even when controlling for socioeconomic status, parental IQ, and parental ADHD status (Milberger et al., 1998; Biederman et al., 2009; Mick et al., 2002). Additionally, several longitudinal studies have demonstrated that children born to smoking mothers were more likely to be diagnosed with ADHD (Romano et al., 2006; Heath & Picotto, 2009; Galéra et al., 2011). At the pharmacological level, chronic nicotine exposure followed by acute withdrawal leads to significant reductions in tonic dopamine activity and in reward-related brain functions, further supporting the correlation (Epping-Jordan, Watkins, Koob, & Markou, 1998; Zhang, Dong, Doyon, & Dani, 2012).

One meta-analysis that examined 24 studies assessing the relationship between PNE and the risk of developing behavioral problems related to ADHD also found that maternal consumption of tobacco during pregnancy was suspected to be associated with higher ADHD risk. However, the same study found that ADHD risk resulting from other maternal lifestyle factors, including alcohol and caffeine consumption and psychological stress during pregnancy, were too inconsistent to draw results from (Linnet et al., 2003). Meta-analyses of other environmental teratogens on behavioral deficits have drawn similar conclusions (Langley et al., 2005).

Several studies have elucidated the effect that genetics may have on the incidence of ADHD, in particular that prenatal environmental effects and parental genetics may be inherently linked. A 2009 study examining maternal and paternal smoking habits as linked to attention deficits found that paternal smoking rates serves as a proxy for genes that contribute to attentional deficits, and that maternal smoking rates and child attention deficits are not linked (Atlink et al., 2009). This study is useful in suggesting that genetic factors should also be examined, as parental smoking may be caused by a genetic predisposition to impulsive behavior. However, it cannot be concluded that maternal smoking causes attention deficits, as their sample contained a low percentage of mothers who smoked, and of those who smoked, only 2.5% smoked 10 or more cigarettes per day during pregnancy.

Impact of Prenatal Nicotine Exposure on Neurotransmitter Systems

Prenatal nicotine exposure impacts several neurotransmitter systems including the acetylcholinergic and dopamine systems, which are reviewed below. During prenatal neural development, acetylcholine binds to the nicotinic acetylcholinergic receptor

(nAchR), stimulating dopamine release (Chen et al., 2005). During development, dopamine is critical for normal cell division, differentiation into their specialized cell types, and migration to their permanent location (Chen et al., 2005). Overstimulation of nAchR also leads to a long lasting dopamine deficiency, which leads to problems with attention, impulse control, and hyperactivity (Chen et al., 2005).

Nicotinic Acetylcholinergic Receptor (nAchR)

PNE changes the expression of nAchR in areas of the brain involved in dopamine neurotransmission. PNE affects neurons with nAchR receptors in the NAcc, PFC, ventral tegmental area, and substantia nigra in 14-day-old rats. Furthermore, mRNA assays show decreased expression of nAchR mRNAs in the ventral tegmental area for all receptor subtypes and in the NAcc and the PFC for one receptor subtype (Chen et al., 2005), suggesting that PNE leads to widespread downregulation of nAchR in areas previously deemed important for inhibition. Another study found similar results when measuring nAchR mRNAs in the thalamus, hypothalamus, and basal forebrain, areas responsible for wakefulness and arousal (Frank et al., 2001). Findings from these two studies demonstrate that nAchR are downregulated in the dopaminergic reward pathway in PNE rats with similar symptoms to humans diagnosed with ADHD, suggesting that there may be a correlation in neurophysiology between the two conditions. During development, however, PNE can upregulate nAchRs and alter the sensory processing, which may underlie the several behavioral characteristics observed in ADHD (Tizabi, Popke, Rahman, Nespor & Grunberg, 1997; Heath & Picciotto, 2009).

DAT gene & D2/D3 receptors

Dopamine receptor and transporter downregulation in ADHD can mostly be

explained by genetic factors. ADHD is highly heritable, as demonstrated by family, twin, and adoption studies yielding estimates around 76% heritability; thus, the majority of variance in ADHD diagnoses between members of the same family can be explained by genetic rather than environmental factors (Crosbie et al, 2013). The genes implicated in the etiology of ADHD are dopamine receptor genes DRD4 and DRD5, dopamine transporter gene DAT, dopamine beta-hydroxylase gene DBH (converts dopamine into noradrenaline), serotonin transporter gene 5-HTT, serotonin receptor gene HTR1B, and t-SNARE gene SNAP-25 (allows neurotransmitters to enter the synaptic space) (Faraone et al., 2005). However, these genes do not fully explain the phenotypic manifestation and developmental course of the disease, suggesting an environmental interaction. For instance, although some dopamine receptors (D2 and D3) and transporters (DAT) are down-regulated in ADHD, an examination of genes for these receptors (DRD4 and DRD5) and transporter (DAT1) show no gene and environment interaction with maternal smoking significantly correlated with ADHD symptoms (Langley et al., 2008). Thus, D2 and D3 downregulation might be explained by environmental factors.

The results of this study match those of a human PET study, which found that D2 and D3 were less expressed in the NAcc, midbrain, caudate, and hypothalamus of children with ADHD than in controls. The study further found that the DAT was downregulated in the midbrain of subjects with ADHD. The amount of downregulation of the dopamine receptors and transporters was correlated with the amount of attentional deficits demonstrated by the subject (Volkow et al., 2009). The same patterns of downregulation were further found in rats prenatally exposed to nicotine (Slotkin et al., 1987). Additional research suggests that dopamine receptors may be downregulated only

after a period of increased dopamine turnover following nicotine exposure. Similarly, In children with ADHD, presynaptic dopamine storage in the prefrontal cortex and midbrain was significantly reduced and negatively correlated with ADHD symptom severity (Ernst et al., 1999). Disruption in the dopamine system has been correlated with hyperactivity in rats (Richardson & Tizabi, 1994; Heath & Picciotto, 2009).

PNE as a rodent model of ADHD

Ajarem and Ahmad first proposed that a PNE model be used for exploring behavioral disorders. They administered nicotine to pregnant mice via injections and examined the pups' righting reflex, cliff avoidance, rotating reflex, locomotion, and anxiety, all measures of motor and cognitive development. The rotating and righting reflexes of the nicotine-exposed mice were significantly delayed, showing motor delays. Furthermore, PNE mice were more active in the locomotion task, suggesting increased hyperactivity, a symptom of ADHD. Since mice have brain areas controlling motor skills and cognition that are homologous to those in humans, this article suggested that PNE in humans would retard growth during a critical prenatal period of brain development. The study further identified nicotine as the causative agent in cigarettes that lead to behavioral deficits (1998).

Another study by Zhu et al. took a similar approach to propose the PNE mouse as an animal model of ADHD (2012). Zhu et al.'s research found that mice that present ADHD-like symptoms after PNE have dopamine deficits in brain structures homologous to the ACC and mPFC. These structures also show reduced volumes. The behavioral component of the study found that PNE mice were more hyperactive, suggesting that they

had an executive control dysfunction. Furthermore, methylphenidate decreased hyperactivity and increased dopamine in the ACC, suggesting another link between PNE and ADHD (Zhu et al., 2012).

Thus, although there is no perfect model of ADHD, PNE has been linked to ADHD. It has started to gain acceptance because of its parallels to ADHD at behavioral, neuroanatomical, and neuropharmacological levels and its responsiveness to methylphenidate treatment. Some studies have gone as far to say that children born to mothers who smoke cigarettes during pregnancy show symptoms of ADHD that are indistinguishable from the ADHD symptoms that arise from other etiologies (Biederman et al., 2012). Despite the growing amount of research on impulsivity disorders such as ADHD, the neurophysiological mechanisms that mediate them are not completely understood.

Summary

Focused research on the neural correlates of ADHD can create a validated method for diagnosing the disorder and reduce the costs associated with numerous misdiagnoses. Current diagnostic methods consist primarily of behavioral observations and other tests that are subjective and lack standardization. This is mainly due to our lack of knowledge on frontal brain areas and its association with behavioral control. Understanding this link can be achieved through animal models, such as the PNE model. In PNE and ADHD, the mPFC is a crucial brain area. The brain regions association with impulsivity, a defining characteristic of ADHD, can be evaluated using the SST. Further investigation into the role of mPFC and its underlying correlates are essential to reducing the number of misdiagnoses and developing better pharmacological treatments.

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Methodology

Animal Care

All procedures were approved by University of Maryland Institutional Animal Care and Use Committee (see Appendix A). The rats used for breeding during this study were Long-Evans Rats obtained through Charles River Laboratory. Throughout the study, we adhered to the procedures outlined in the *Guide for the Care and Use of Laboratory Animals* (National Research Council of the National Academy of Sciences, 2011). Using these guidelines, we housed our rats in appropriate cages with proper room temperature, ventilation, and feeding. At the end of the study, rats were perfused with saline and fixative after being rendered unconscious via isofluorane overdose. Brains were collected and stored in fixative.

Prenatal Nicotine Exposure

Rats were prenatally exposed to nicotine by administering a nicotine solution in the pregnant mothers' drinking water. Ten females were obtained from Charles River Laboratory. Five of those were acclimated to nicotine solution (0.2 mg/mL). The five remaining had free access to water. Each mother's total water consumption was measured twice a week (see Appendix B1 for sample water log). While the water consumption of the mothers exposed to nicotine was significantly lower than the water consumption of the control mothers, there was still continuous weight gain before and during pregnancy (t-test, $p < 0.05$). The average nicotine mother fluid consumption was 128 mL per week and the average control mother fluid consumption was 174 mL per measurement. There

was a significant difference between the weights of the nicotine mothers and the control mothers (t-test, $p < 0.05$). The average nicotine mother weight was 289 grams and the average control mother weight was 314 grams. These differences are consistent with previous research on prenatal nicotine exposure of rats (Schneider et al., 2010). All 20 rats (five nicotine mothers, five control mothers, and ten mating males) were weighed twice a week. After two weeks on the 0.02 mg/mL dosage, the dose was increased to 0.04 mg/mL, and then increased again two weeks later to 0.06 mg/mL, where it remained until the nicotine mothers gave birth. Nicotine administration was halted after the mothers gave birth. In all, this final nicotine exposure of 0.06 mg/mL was equivalent to human mothers smoking two to three packs of cigarettes per day according to a previous study (Schneider, Bizarro, Asherson, & Stolerman, 2010).

Each time a nicotine mother gave birth to a litter, the pups were cross-fostered to a control mother on postnatal day three. This time period ensured that any handling of the pups by humans did not cause the mothers to reject the new pups. Cross fostering is the process by which pups are raised by the surrogate mothers. Cross fostering was done for nicotine pups to ensure that they were not exposed to the nicotine expressed through the nicotine mother's milk. The control pups were taken care by other control mothers to ensure consistency. All cross fostering was successful, and we obtained 40 PNE pups and 44 control pups from the four control dams and the three nicotine dams that were pregnant. We chose to use only male pups because decision-making circuits have been more extensively studied in males and PNE has been shown to have more dramatic effects on males than females (Romero & Chen, 2004). Additionally, ADHD is more prevalent in males than females (Evans, Morrill, & Parente, 2010).

The pups were weaned from their foster mothers on postnatal day 21 based on IACUC protocol. The pups were weighed once a week, and there was no significant difference between control male pups and PNE male pups (t-test, $p = 0.5$, see Appendix B2 for sample weight log). At postnatal day 40, control male pups weighed 198 grams on average and PNE male pups weighed 195 grams on average. To test for any preliminary behavioral deficiencies, the male pups performed a locomotion task on postnatal day 30. In this task, rats were individually placed into boxes with eight infrared beams across the box. When the rat crossed the beam, it was recorded as a crossing. The analysis showed that there was no significant difference in locomotor activity between the control rats and the PNE rats (t-test, $p = 0.81$). The control rats averaged 87 crossings and the PNE rats averaged 93 crossings. We created a cohort of 18 rats for the recording experiment described below. We randomly selected 11 control males from 4 control mothers. Two male offspring from two nicotine mothers were randomly selected and three male offspring from the remaining nicotine mother were randomly selected.

Behavioral Task

On postnatal day 49, adolescent rats (~12 years of age in human years, which is the prepubescent years when ADHD is most prevalently diagnosed) were introduced to the behavioral boxes. For the training procedure and the SST, recording was conducted in aluminum chambers approximately 18” on each side with downward sloping walls narrowing to an area of 12” × 12” at the bottom. On one wall, a central odor port was located above two adjacent fluid wells. Directional lights were located next to fluid wells. House lights were located above the panel, as shown in Figure 2.1. Task control was

implemented via computer. Port entry and licking was monitored by disruption of photo beams.

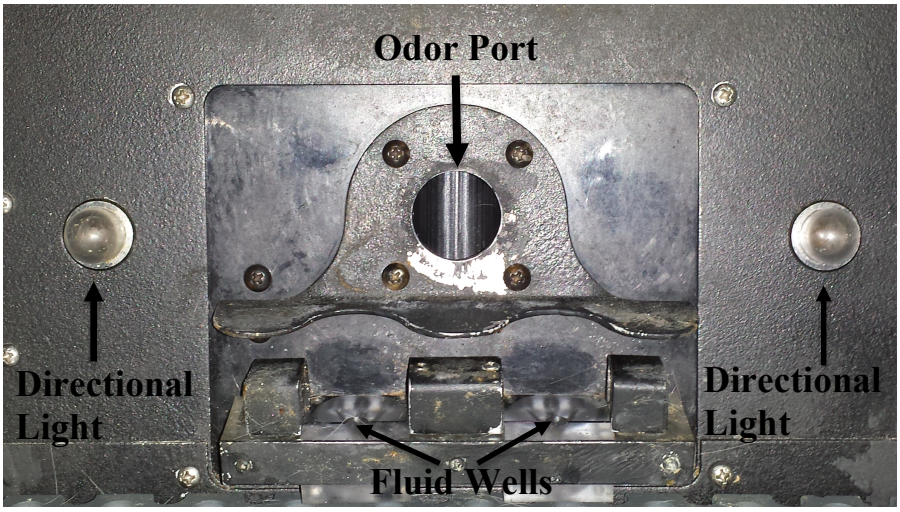

Figure 2.1. Inside the behavioral boxes. Rats nose poke into the odor port above, follow the directional lights to the right or left, and then enter the respective left or right fluid well to receive reward.

Before the final version of the SST was introduced, rats were shaped to perform the basis of the task. Each rat began with a free period where nose pokes were paired with reward delivery at the fluid well. After two days of sessions with this procedure, the basic GO task was introduced. Rats had to nose poke to initiate a trial, then one directional light flashed on, and then reward could be collected by entering the correct fluid well. After 18 days with the GO task, all rats responded correctly on at least 70% of trials and the stop-signal trial type was introduced (see Appendix B3 for a sample behavior log and Appendix C3 for complete task training protocol).

The basic design of this complete task procedure is illustrated in Figure 2.2. Each trial began by illumination of house lights that instructed the rat to nose poke into the central port. Nose poking initiated a 1000 ms delay period, after which a directional cue

light either to the left or right of the nose poke flashed for 100 ms, indicating the direction in which the animal must respond to receive reward in a fluid well. These trials will be referred to as GO trials and occurred on 80% of trials. On a randomly interleaved 20% of trials, after exiting the central port, a second cue light illuminated opposite the first, instructing the animal that they must stop the already initiated movement and respond in the opposite direction (i.e. toward the second light). Illumination of the second light occurred between 0-100 ms after port exit (stop-signal delay; SSD). These trials will be referred to as "STOP-change" or "STOP" trials for short. The STOP cue was illuminated only after the movement had been initiated, thus we are examining the rats ability to inhibit a behavior already set in motion. Trial types are illustrated in Figure 2.3. For both GO and STOP trials, animals were required to wait between 800 and 1000ms in the fluid well before receiving reward. There were a total of four different trial-types: GO-left, GO-right, STOP-left-GO-right, and STOP-right-GO-left; however, for the remainder of the paper, response direction (i.e. left and right) will be referenced to the directional preferences of individual neurons (preferred or non-preferred) as determined by the direction of the behavioral response that produced the strongest firing (averaged over STOP and GO trial-types during the response epoch). Trials were presented in a pseudorandom sequence such that left and right trials were presented in equal numbers (±1 over 250 trials).

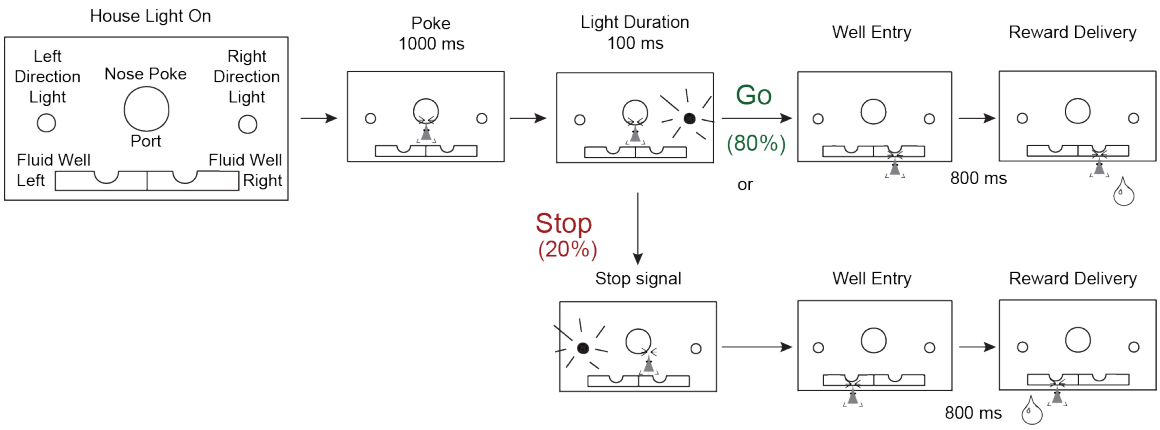

Figure 2.2. The stop-signal task. The figure shows an overview of task procedure (Bryden et al., 2012).

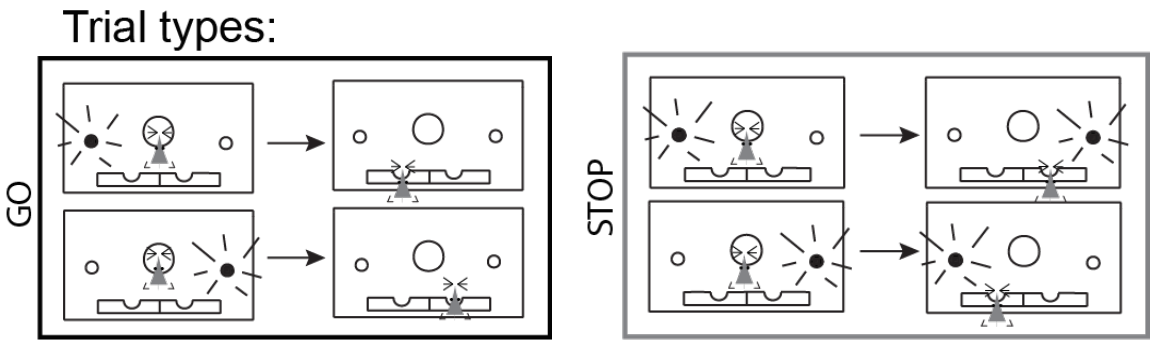

Figure 2.3. Trial types in the stop-signal task. The two main trial types discussed here are GO and STOP, illustrated here for both left and right directional signals (Bryden et al., 2012).

Surgical Procedure

Surgical procedures followed guidelines for aseptic technique. Electrodes were manufactured and implanted as in prior recording experiments (see Appendix C1 for complete protocol). Rats had a drivable bundle of 10 25-µm diameter FeNiCr wires (Stablohm 675, California Fine Wire, Grover Beach, CA) chronically implanted in the mPFC (+3.3 mm anterior to bregma, +0.6 mm lateral to bregma, 2 mm ventral to brain). Immediately prior to implantation, these wires were freshly cut with surgical scissors to

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extend ~1 mm beyond the cannula and electroplated with platinum (H_2PtCl_6, Aldrich, Milwaukee, WI) to an impedance of ~300 kOhms, as illustrated in Figure 2.4 (see Appendix C1 for complete electrode protocol).

To begin the surgeries to implant the electrodes, the rats were first anesthetized with isoflurane, an inhaled veterinary anesthetic, and fixed within ear bars to ensure stability throughout the surgery. An incision was then made into the scalp to expose the periosteum and skull. Based on an atlas of the rat's brain, holes were drilled in the rat's skull in order to install anchor screws that hold the electrode in place. A larger central hole was made for the insertion of the electrode itself. With the use of a microscope, the dura, the outermost layers of the membranes that cover the brain, were cut away from this central hole, and the microelectrode was inserted into the brain tissue. The electrode was driven further into the brain at a rate of 100 microns/minute until the region of interest was reached. The electrode was then fastened to the skull using grip cement. The incision was then stapled together, and the rat was administered buprenorphine and placed into a recovery chamber. Buprenorphine was administered twice during the 24-hour period following surgery for acute pain relief. The rats needed to recover postoperatively for one to two weeks (Bari et al., 2011; Acheson et al., 2006). Cephalexin (15 mg/kg) was administered twice daily for two weeks post-operatively to prevent infection (see Appendix B4 for a sample surgery log and Appendix A for complete surgery protocol).

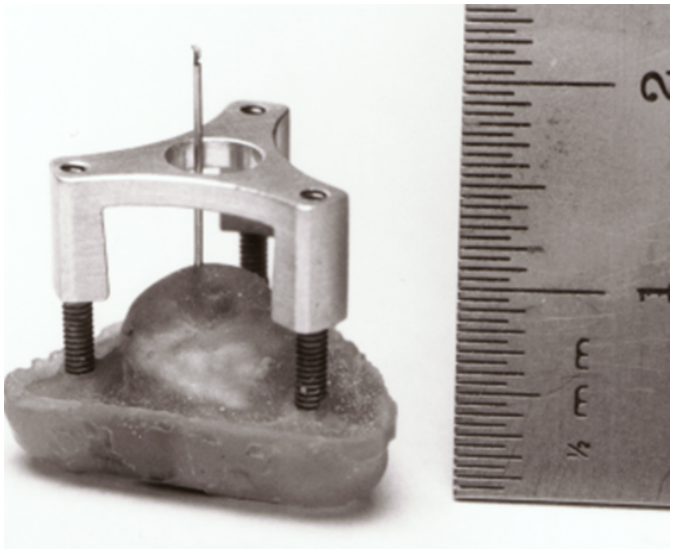

Figure 2.4. Drivable recording electrode. The fine wires at the tip (top left) are inserted into the brain. Photo courtesy of Schoenbaum lab, part of the National Institute on Drug Abuse (NIDA).

Single-Unit Recordings

Procedures were the same as described previously (Bryden et al., 2011). Electrode wires were screened for activity daily during SST sessions. If no activity was detected, the rat was removed from the behavioral box, and the electrode assembly was advanced 40 or 80 μm. When activity was detected, a session was conducted, and the electrode was advanced 40 μm at the end of the session (see Appendix B6 for sample electrode advancement log). Extracellular neural activity was recorded each day from each animal using the four identical Plexon Multichannel Acquisition Processor systems (Dallas, TX), which interfaced with stimulus-response training chambers (see Appendix B5 for sample single-unit recording log). Signals from the electrode wires were amplified 20X by an op-amp headstage located on the electrode array. Immediately outside the training chamber, the signals were amplified 50X and filtered at 150-9000 Hz. The single unit signals were then sent to the Multichannel Acquisitions Processor box, where they were further

filtered at 2500-8000 Hz, digitized at 40 kHz and amplified at 1-32X. Waveforms, the shape of the electrical potential change as collected by the electrode during a single action potential which also had greater than a 2.5:1 signal-to-noise ratio, were extracted from active channels and recorded to disk by an associated workstation with event timestamps from the behavior computer.

Data Analysis

Units were sorted using Offline Sorter software from Plexon Inc. (Dallas, TX) using a template-matching algorithm. Sorted files were processed in Neuroexplorer to extract unit time-stamp and relevant event markers. These data were subsequently analyzed in Matlab (Natick, MA). Baseline firing was taken during a 1 second epoch starting 2 seconds prior to trial initiation (nose poke). For the majority of the analysis, activity was examined during the period between nose poke exit and well entry (response epoch), while the movement was being made and/or canceled. Wilcoxon tests, t-tests, ANOVA, and Pearson Chi-square tests was implemented to compare and measure relevant statistics (Bryden et al., 2011). Examples of analyses include comparing histograms of neural firing patterns across the trial time-course as well as observing the relationship between SSRT and neural firing. When a rat's session was analyzed, the intensity and timing of its neural firing was compiled and aggregated with other sessions to provide an informative comparison of neural activity of the mPFC in all groups of rats (see Appendix D for complete MATLAB data analysis script).

Histology

The histological analysis was performed to confirm that the electrodes were

placed in the correct region of the brain during surgery. The final locations of the electrodes are illustrated in Figure 2.5.

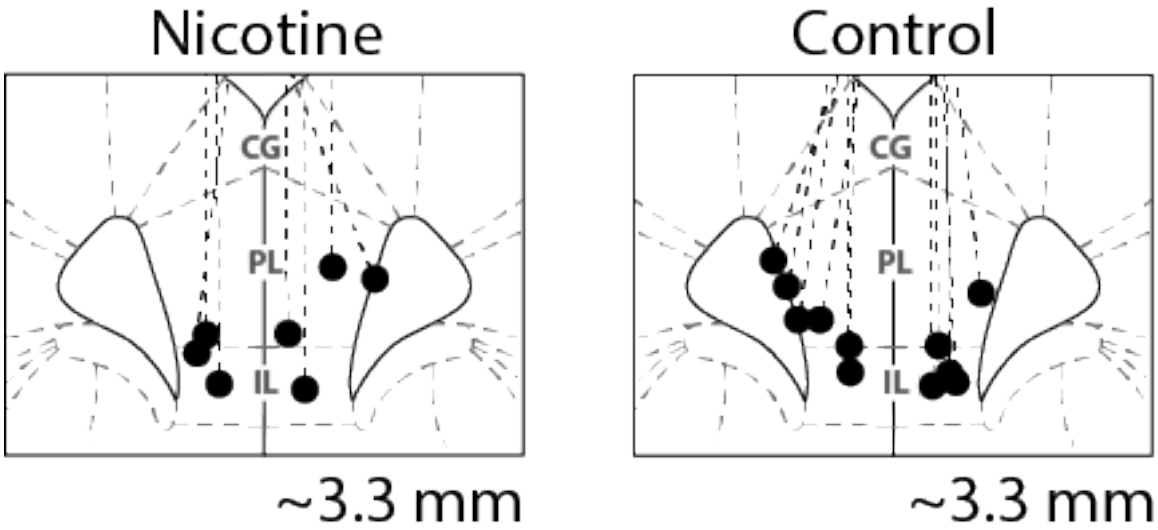

Figure 2.5. Coronal slice approximately 3.3 mm from bregma. Each dot represents the final position of the recording electrode for each animal based on histology. Dashed lines reflect the estimated track that the electrode traversed through the brain. Recording was contained to the mPFC, including anterior cingulate cortex, prelimbic prefrontal cortex, and dorsal aspects of the infralimbic prefrontal cortex. (CG: cingulate gyrus, PL: prelimbic cortex, IL: infralimbic cortex)

The following is the histology procedure that was used: First, distilled water, sodium hydroxide, and acetic acid were mixed and heated until the solution was just boiling. Then, thionin was added, and solution was refluxed for 45 minutes, while stirring. After cooling the solution to room temperature, 1000 mL of the solution was decanted into a dark bottle, while the rest of the solution was decanted into another bottle and stored as excess. They were kept at 37 °C and filtered out before each use. In order to perform a Nissl stain, a histological stain procedure used to view neural tissues, the sliced

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tissue was mounted on a slide. It was placed in a solution of equal parts of concentrated chloroform and ethanol for one hour under a fume hood. After soaking the tissue in 100% ethanol twice for two minutes each, the tissue was then soaked in 95% ethanol, 70% ethanol, and 50% ethanol each for two minutes at a time; then, it was dipped in distilled water twice. In order to create the stain, the tissue was soaked for 20 s in 0.25% thionin and again dipped in distilled water twice to remove excess stain. Finally, the tissue was dipped in 50% ethanol, 70% ethanol, 95% ethanol twice, and 100% ethanol twice for four minutes each to remove excess water. Afterwards, the tissue was soaked for four minutes in ortho-dimethylbenzene, meta-dimethyl benzene, and para-dimethylbenzene. Once soaked in the above solutions, the tissue was dried thoroughly and cover-slipped.

Study Limitations

Attrition effects were a main concern for our study. Factors such as fatigue, hunger, and thirst altered the rats' motivation levels, which forced us to disregard trials that were adversely affected by these conditions; that is, when the rat did not complete the entire trial (National Research Council of the National Academy of Sciences, 2011). We were able to control for these variables by ensuring that the rats were not subjected to exhaustive tests and that they were allowed ample rest time between days of task performance. In order to ensure that trials were executed efficiently, we mildly deprived rats of water prior to completing the trials and used a thirst-based reward system. The rats received 35 mL of water per day. Several hours prior to running the task, the rats did not receive water. This lack of water acted as an incentive to motivate the rats to perform the task in order to receive water as a reward.

There was also the possibility of experimenter error in our study. A small group of team members built the electrodes and implanted them into the rats' brains. If the building or implanting of the electrode differed between members, this may have affected the validity of our results. To compensate for any differences, we followed a set of consistent procedures and electrodes and surgeries were divided evenly between control and experimental groups. In addition, post-mortem histology revealed whether or not electrode placement was correct.

An additional experimenter limitation was that our study was not conducted in a double-blind fashion, as the experimenters were aware of whether each rat belonged to the control or PNE group during data collection or analysis. To account for this, all rats were handled identically according to established procedures (see Methods). In addition, all analyses were performed on data sets from both groups simultaneously, and any data removed from analysis was done so based on the behavioral session being incomplete, not on the group to which the data belonged.

In order to account for a possible influence of gender on impulsivity, we only used male rats for several reasons. First, we opted not to use both genders first due to structural differences in the PFC in male rat brains as compared to female rat brains (Diamond, Johnson, Young, & Singh, 1983). Second, we chose to use male rats instead of female rats because male rats show higher impulsivity than female rats due to the effects of testosterone on brain development (Bayless, Darling, & Daniel, 2013). Furthermore, female rats have shown changes in learning strategies (Warren & Juraska, 1997) and in impulsivity (Fuchs, Evans, Mehta, Case, & See, 2005) over the course of their estrous cycle, which would have affected how they learned and performed the task

and may have prevented us from comparing all trials to each other. Finally, decision-making circuits have been more extensively studied in males and PNE has been shown to have more dramatic effects on males than females (Romero & Chen, 2004). Future work should examine the behavioral and physiological differences between male and female PNE rats.

By accounting for these variables across both experimental and control groups, we preserved the internal validity of our research.

Results

Prenatal nicotine exposure impairs inhibitory control

Rats in both control and PNE groups exhibited significantly slower movement speeds from port exit to well entry (Figure 3.1A) and reduced accuracy (Figure 3.1B) on STOP trials as compared to GO trials. Within each trial type, a slower latency resulted in better task performance. This is consistent with a speed-accuracy trade off in both groups. This is illustrated in Figure 3.2, which plots movement times (well entry minus port exit) against average percent correct scores for each recording session of all trial types. During sessions in which the rat was slower, performance was better. Consistent with this finding, STOP trial error movement times were significantly faster than movement times on correctly performed STOP trials (Figure 3.1A; t-test; $p < 0.05$). These results suggest that rats were planning and generating a movement prior to illumination of the stop-signal, in response to illumination of the first cue light, and that inhibition and redirection of the behavioral response was necessary to correctly perform STOP trials.

When comparing control and PNE rats, we found that PNE rats were significantly

faster over all trial-types (Figure 3.1A; black versus grey; t-test; $p < 0.05$). Although the two groups did not differ significantly during performance of GO trials, PNE rats made significantly more errors on STOP trials than did control rats (Figure 3.1B; black versus grey; t-test; $p < 0.05$). We conclude that PNE makes rats less able to suppress movement on STOP trials but were unimpaired on GO trials, suggesting that deficits were limited to trial types during which rats had to inhibit their movement.

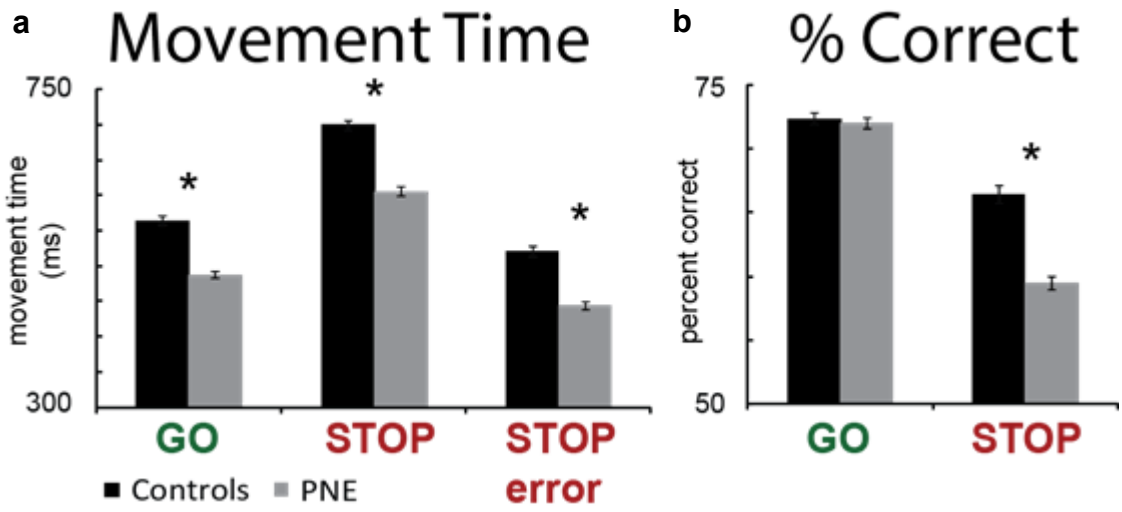

Figure 3.1. Average movement times **(a)** and average percent correct **(b).** Both PNE and control groups are shown. Behavior was taken from neural recording sessions. Error bars are SEM. Asterisks indicate $p < 0.05$.

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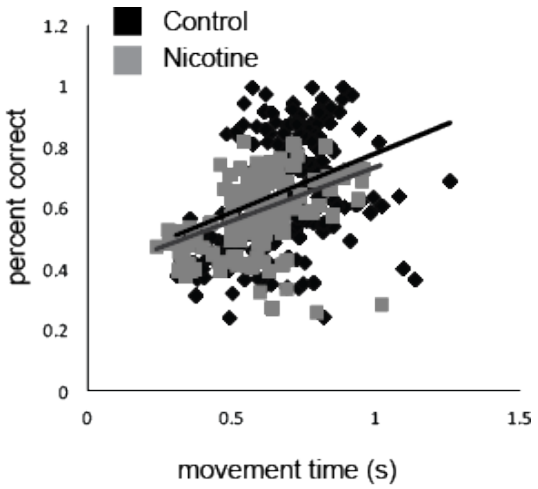

Figure 3.2. Scatter plot of movement time versus percent correct. Each dot represents one recording session. Both the PNE and control groups are shown. ($p < 0.001$, $r^2 = 0.95$ and $p < 0.001$, $r^2 = 0.13$, respectively)

Counts of task-related neurons were similar across control and PNE rats

We recorded 631 and 552 neurons from mPFC in control and PNE rats, respectively. The recording locations are illustrated in Figure 2.5. Use of the SST in the context of behavioral neurophysiology allows us to examine activity related to response inhibition and redirection of behavior. STOP trials, during which the movement had to be stopped and redirected, are directly compared to responses made in the same direction, which cannot be done with more typical SSTs. Our first analysis was to determine the number of neurons in each group exhibiting task-related firing that was significantly modulated from baseline during the response epoch (port exit to well entry) relative to baseline (1 s prior to trial initiation; t-test; $p < 0.05$).

We performed an analysis of the single-units and did not find any significant difference across the four groups. In controls, 20% and 31% of neurons significantly increased and decreased firing during the response epoch relative to baseline, respectively.

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In PNE rats, 25% and 34% of neurons exhibited significant increases and decreases during the response epoch, respectively. These are defined as increasing- and decreasing-type cells, respectively. In both areas the number of significant neurons were more than expected from chance alone ($p < 0.001$) and the proportion of 'increasing' and 'decreasing' type cells did not significantly differ between the two groups. We conclude that the counts of neurons showing significant task-related increases and decreases in firing were not significantly different between groups.

Activity of increasing-type neurons was attenuated after PNE

A single cell example of increasing-type neuron firing is illustrated in Figure 3.3. This particular neuron was selective for trial-type and response direction in that activity was stronger for STOP trials and for movements made to the right. The direction that elicited the strongest average firing (in this case, right) will be referred to as the cell's preferred direction.

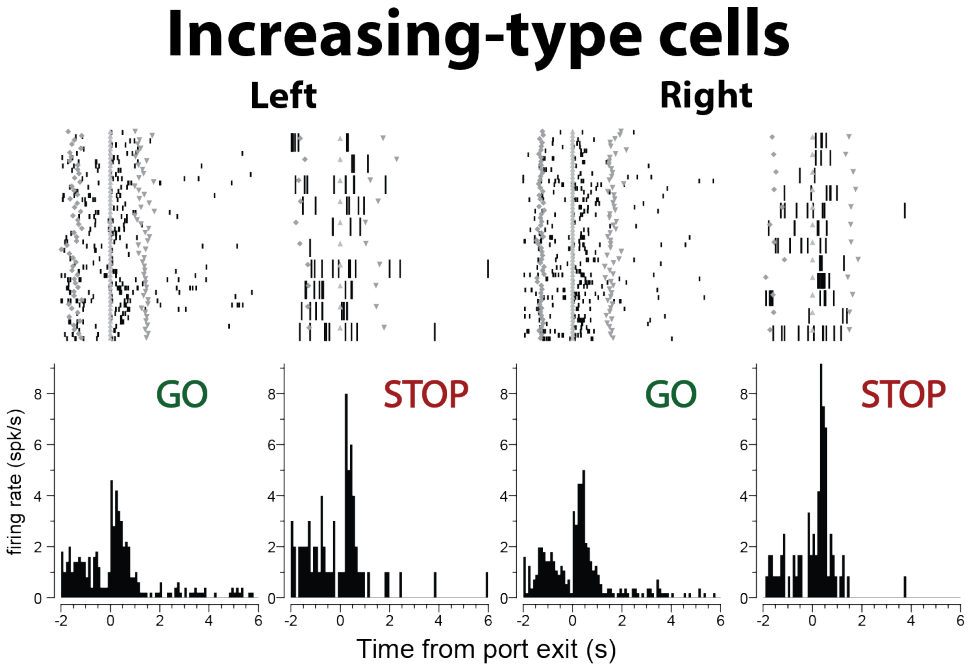

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Figure 3.3. Neural firing of characteristic increasing-type cells on correct trials. Firing for both left and right directions are shown. Shown above, each histogram is a raster plot. On these plots, rows represent trials and columns represent times during the trials. The histogram is a sum of the spikes during each individual time point across all trials of that trial-type during the session. The direction indicates the final well.

The average firing over all increasing-type neurons, broken down by trial-type, is illustrated in Figure 3.4A and 3.4B for controls and PNE rats, respectively. Neural activity is aligned to port exit (stop-signal onset) and fluid well entry. Since roughly equal numbers of neurons fired more or less strongly for left and right movements, population activity was divided into each cell's preferred and non-preferred direction for these plots. As defined by our analysis, activity in the preferred direction (Figure 3.4A and 3.4B, thick) is stronger than activity in the non-preferred direction (Figure 3.4A and 3.4B, thin).

When comparing average firing between control and PNE rats (Figure 3.4A versus 3.4B), the most striking difference between them is the overall reduction in mPFC firing regardless of trial-type or direction. This is apparent during early baseline firing and during the 2 s after initiation of the behavioral response (port exit). Average firing from 4 to 2 s prior to the initiation of the movement was 4.77 spikes/s and 4.07 spikes/s for controls and PNE rats, respectively (t-test; $p < 0.05$). Average firing during the 2 s after initiation of the movement was 6.28 spikes/s and 4.86 spikes/s for controls and PNE, respectively (t-test, $p < 0.05$). Thus, the mPFC in PNE rats was hypoactive compared to that of control rats.

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Stop-signal encoding in mPFC was not disrupted after PNE

Although neural firing in PNE rats was attenuated as compared to control rats, the average population histograms suggest that the strength of selectivity for different trial-types was unaffected. For both control and PNE rats, activity appeared to be slightly higher for STOP relative to GO trials for responses made in the preferred direction (Figure 3.4A and 3.4B; solid red versus solid green). Although overall activity was reduced in PNE rats, the difference between STOP and GO trials in the preferred direction did not appear to be weaker in PNE rats. To quantify differences between STOP and GO trials, we computed a stop index defined as the difference between STOP and GO trial activity ((STOP - GO)/(STOP + GO)) for each neuron. The distributions of these indices for preferred and non-preferred directions are plotted in Figure 3.4C-3.4F.

In these plots, a shift in the positive direction indicates that more neurons fired more strongly for STOP than for GO trials compared to those showing the opposite effect (i.e. stronger firing for GO relative to STOP trials). In the preferred direction, the shift was significant and positive for PNE rats only; however, the two distributions did not significantly differ from each other (Figure 3.4C versus 3.4E; control versus PNE). In PNE rats, the counts of neurons that fired significantly more strongly for STOP relative to GO trials outnumbered those showing the opposite effect (Figure 3.4, black bars; χ^2, $p < 0.05$); however, this difference in control rats was not more than expected by chance, and the frequency of effects were not significantly different across groups. In the non-preferred direction, stop indices for control and PNE rats were not significantly shifted from zero or from each other. We conclude that PNE attenuates firing of increasing-type

neurons but does not alter selectivity related to STOP and GO trial-types.

Directional selectivity of increasing-type neurons was not disrupted after PNE

As described previously, firing of neurons in mPFC was highly directional. 44% and 27% of increasing-type neurons in control rats and PNE rats, respectively, exhibited significantly different firing between left and right on GO trials. Although there was a 17% reduction in the number of neurons that exhibited activity that was directionally selective, this reduction was not significant (χ^2; $p = 0.058$). To further assess the directional encoding for each trial-type, we computed a directional index ((preferred - non-preferred)/(preferred + non-preferred)) during the response epoch independently for STOP and GO trials. By defining preference based on the average over STOP and GO trials, this analysis allows us to ask if the distribution of directional indices is different between the two trial types. During both GO and STOP trials, the directional index distribution was shifted significantly above zero in both groups, and there was no significant difference between the control and PNE distributions. We conclude that PNE attenuates firing of increasing-type neurons but does not alter selectivity related to response direction on correct trials.

Directional responding implies that mPFC is involved in executive functions pertaining to the direction of the response. If directional signals in mPFC are important for directing behavior, then they should be attenuated on errors. Consistent with this hypothesis, the mean of the distribution was significantly reduced on STOP errors compared to correct STOP trials, suggesting that without substantial directional selectivity, errors were made ($p < 0.001$). The reduction in directional selectivity during error trials was present in both groups and there was no significant difference between

them. Although weaker, the means of the distributions were still positive, suggesting that activity in mPFC better reflected the nature of the movement, not the sensory stimulus that triggered it.

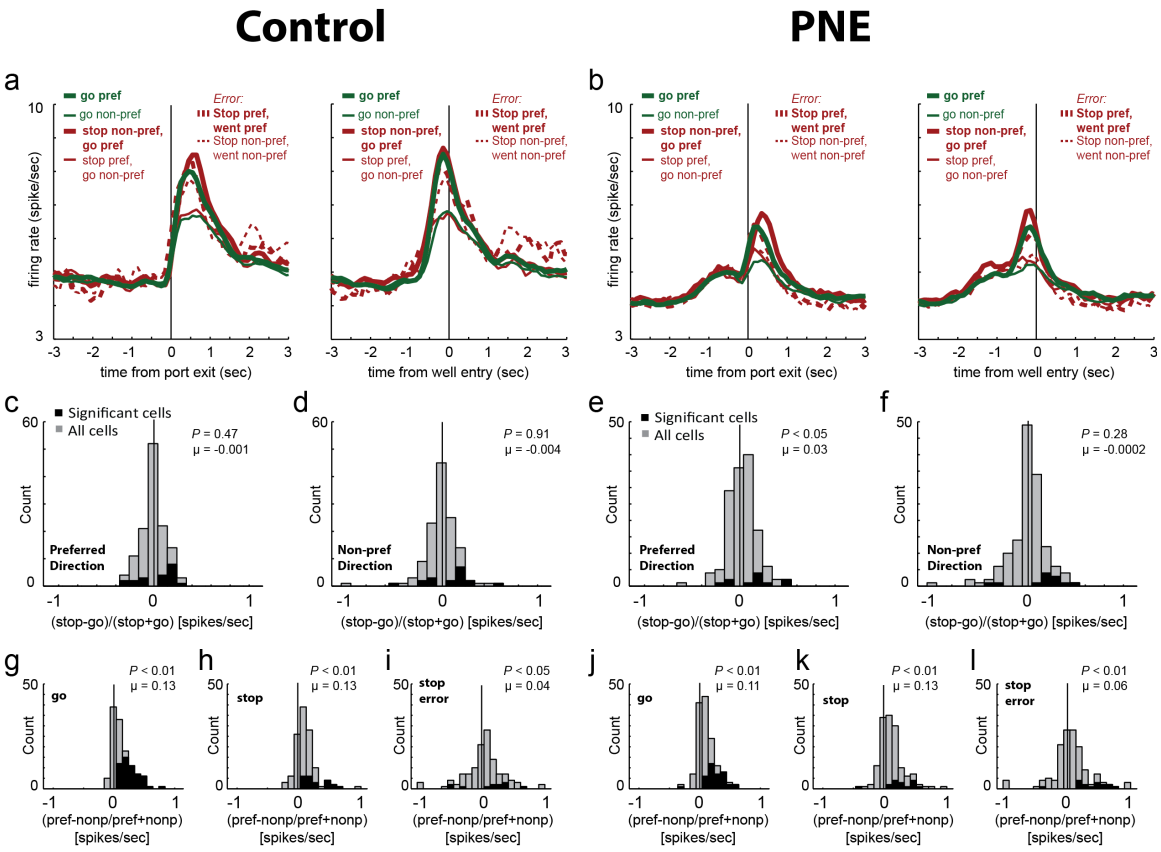

Figure 3.4. (a,b) Average firing rate over time aligned on port exit **(a)** and well entry **(b)** 20% and 25% of neurons from control and PNE rats significantly increased firing above baseline, respectively (χ^2; p = 0.08). Activation during the response was significantly reduced in PNE rats (4.85 versus 6.28 spikes/s; Wilcoxon; $p < 0.001$). **(c-f)** Histograms of the stop index are shown for each group. Activity was slightly stronger on STOP trials in the preferred direction as indicated by a positive shift in stop index distributions. The shift was significant only in PNE rats **(e)**, but the two groups did not significantly differ between **(c)** and **(e)**. **(g-l)** Histograms of the directional index versus normalized firing are shown. 44% and 27% (χ^2; p = 0.06) of increasing-

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type neurons from control and PNE rats, respectively, exhibited significantly different firing between preferred and non-preferred (black bars). In both groups, the strength of the directional response was significantly reduced on STOP errors as shown in **(h)** versus **(i)** and **(k)** versus **(l)**.

Activity of decreasing-type neurons was attenuated after PNE, but encoding was unaffected.

The average firing over all decreasing-type neurons, delineated by trial-type, is illustrated in Figure 3.6 for controls and PNE rats. As for increasing-type neurons, average firing appeared to be attenuated for PNE rats. This was significant for activity after the response (4.64 spikes/s versus 3.79 spikes/s; Wilcoxon; $p < 0.05$) but not during the 2 s epoch preceding initiation of the trial (6.16 spikes/s versus 5.19 spikes/s; Wilcoxon; $p = 0.16$).

As for increasing-type neurons, neural activity appeared higher for STOP compared to GO trials for responses made in the preferred direction for both controls and PNE rats (Figure 3.5: example; Figure 3.6A and 3.6B; population firing). As above, stop indices (Figure 3.6C-F) and directional indices (Figure 3.6G-L) were computed for each decreasing-type neuron during the response epoch for both controls and PNE rats and compared against each other.

The shift in the stop index was significant and positive for both control and PNE rats. Furthermore, neurons that fired significantly more strongly under STOP trials were in the significant majority (χ^2; $p < 0.05$). Stop index distributions and frequency of effects did not significantly differ between groups. We conclude that PNE attenuates firing of decreasing-type neurons but does not alter selectivity related to trial-type (i.e., STOP versus GO).

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The strength of directional encoding for decreasing-type neurons was unaffected by PNE. 28% and 23% of decreasing-type neurons in controls and PNE exhibited significantly different firing between left and right response directions, respectively. The counts of neurons that were directionally tuned were more than expected from chance alone and did not differ significantly between control and PNE rats (χ^2; $p = 0.46$). For both groups, the positive shift in the directional index was significantly shifted for both GO and STOP trial-types, and there was no difference between control and PNE distributions (Figure 3.6C-F). Finally, as with increasing-type neurons, the strength of directional tuning was significantly reduced on error trials (Figure 3.6I and 3.6L). We conclude that overall firing of decreasing-type cells in mPFC was attenuated after PNE, but directional encoding remained intact.

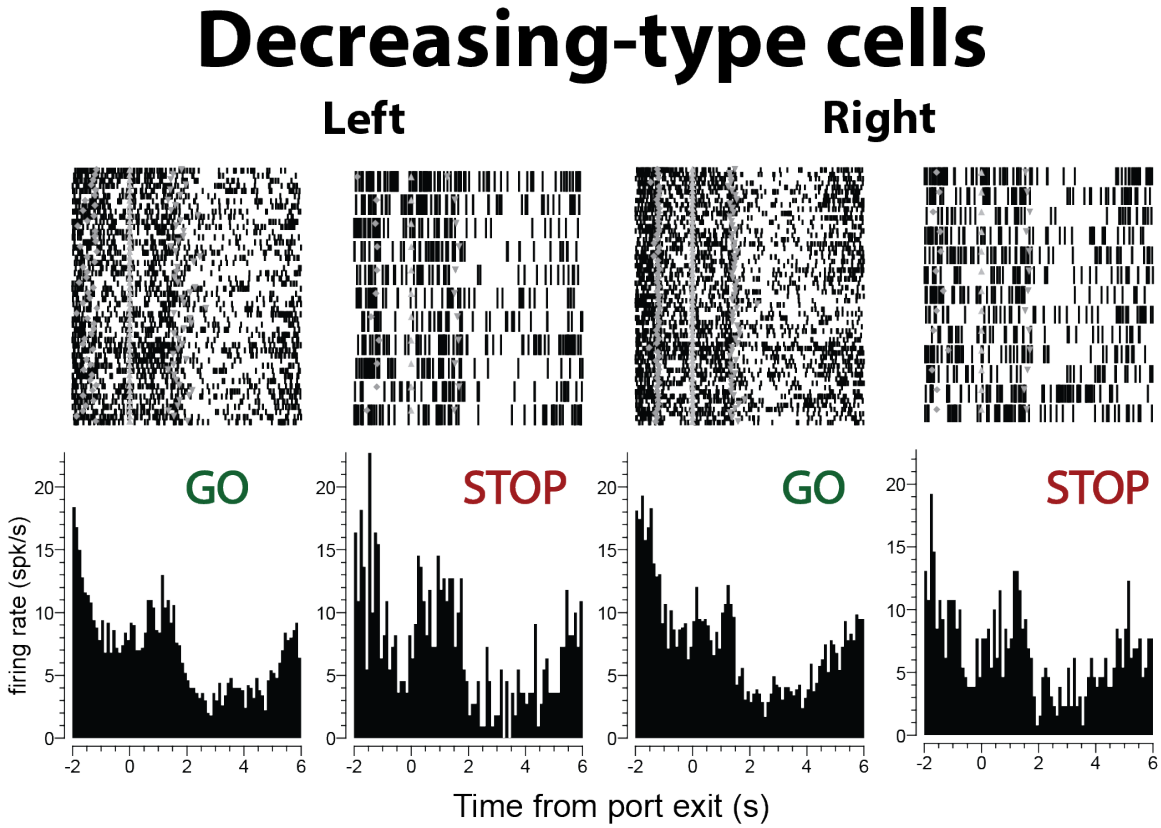

Figure 3.5. Plots of neural firing of characteristic decreasing-type cells. Firing for both left and

right directions are shown. Shown above, each histogram is a raster plot. On these plots, rows represent trials and columns represent times during the trials. The histogram is a sum of the spikes during each individual time across all trials of that trial-type during the session. The direction indicates the final well.

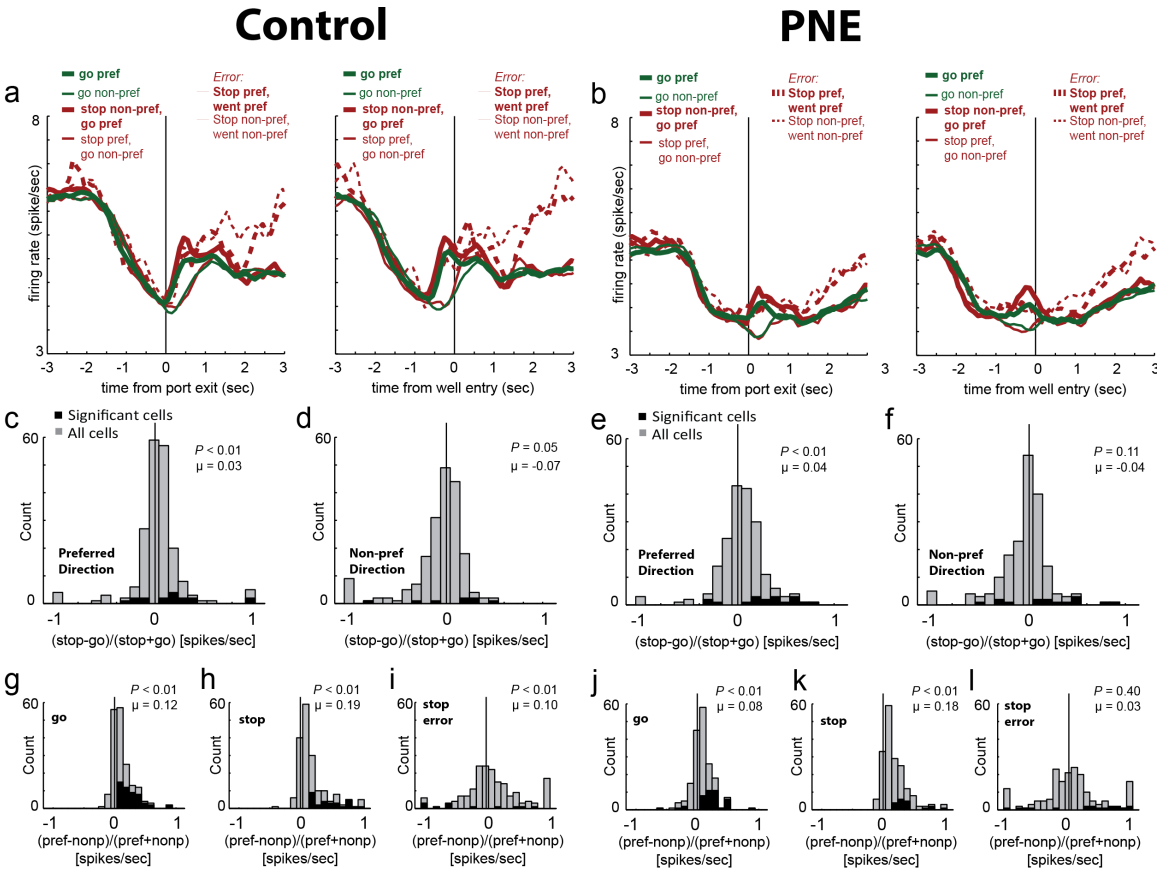

Figure 3.6. Average firing over all decreasing type neurons. (a,b) 31% and 34% of neurons from control and PNE rats significantly decreased firing below baseline, respectively (χ^2; p = 0.47). Activation during the response was significantly reduced in PNE rats (3.79 versus 4.64 spikes/s; Wilcoxon; $p < 0.05$). **(c-f)** Activity was stronger on STOP trials in the preferred direction as indicated by a significant positive shift in stop index distributions for both control **(c)** and PNE rats **(e)**. The two groups did not significantly differ from each other as shown in **(c)** versus **(e)**. **(g-l)** 28% and 23% (χ^2; p = 0.46) of decreasing-type neurons exhibited significantly

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different firing between left and right response directions (black bars). In both groups, the strength of the directional response was significantly reduced on STOP errors as shown in **(h)** versus **(i)** and **(k)** versus **(l)**.

Activity in mPFC was positively correlated with movement time and percent correct

The data described above demonstrates that mPFC is hypoactive after PNE. Remarkably, even with reduced activation, neural encoding of task parameters was not significantly altered. The fact that activity is generally reduced after PNE and that PNE rats are faster and less accurate on STOP trials suggests that firing in mPFC should be correlated with movement time and accuracy. To address this question, we asked if neural activity was correlated with movement time and percent correct separately for increasing- and decreasing-type neurons from both groups.

Response-related activity of increasing-type neurons was positively correlated with percent correct but not movement time. Significant firing correlations with percent correct were present in both control (Figure 3.7A) and PNE rats (Figure 3.7B), but was weaker in PNE rats. Activity of decreasing-type neurons was positively correlated with movement time (Figure 3.7G) but not percent correct (Figure 3.7E) during the same response period. The correlation was not significant in PNE rats. The results suggest that higher firing in mPFC is linked to slower responses and better performance, and that when mPFC activity is reduced, rats are faster and less accurate as observed after PNE.

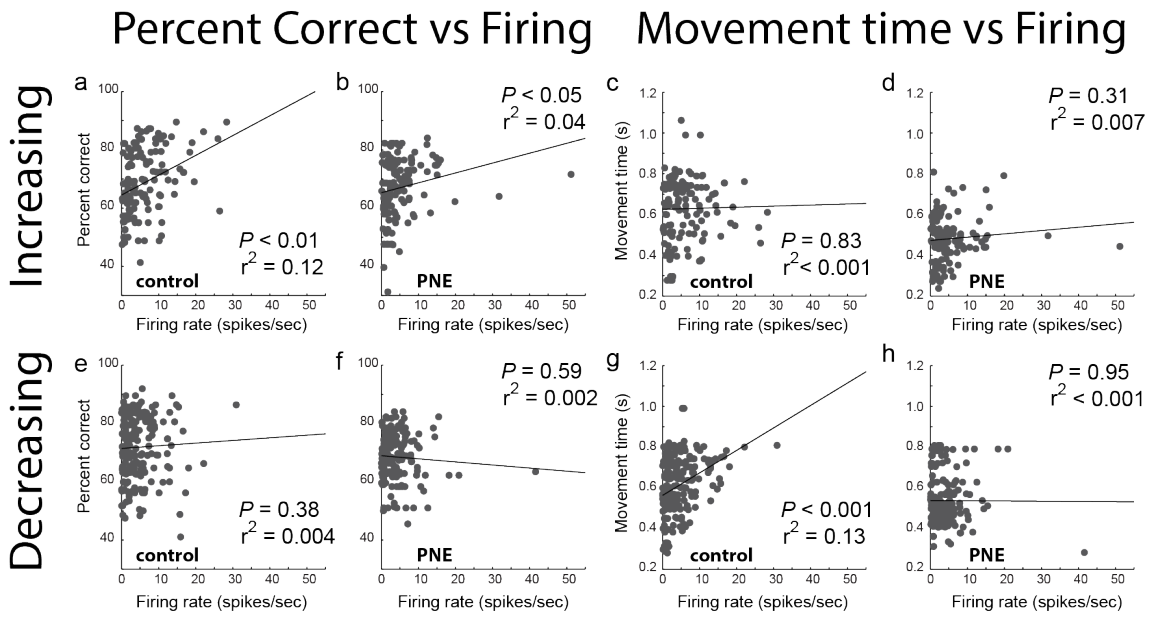

Figure 3.7. Percent correct and movement time versus firing. Plots of firing rate versus percent correct for all trial types are shown for increasing **(a,b)** and decreasing **(e-f)** type cells. Each dot represents a single neuron. Plots of firing rate versus movement time for increasing **(c,d)** and decreasing **(g,h)** type cells are shown. Activity was positively correlated with percent correct **(a,b)** but not movement time **(c,d)** for increasing-type cells. The correlation between increasing-type cell firing rate and percent correct for all trial types was weaker in PNE rats **(b)**. Activity was positively correlated with movement time **(g,h)** but not percent correct **(e,f)** for decreasing-type cells. There was no significant correlation between firing and movement time in PNE rats **(h)**.

Discussion

This discussion aims to provide context for the results, substantiate them with evidence from previous research, and elicit further questions to help understand the processes of impulsivity and ADHD. This section delves into the significance of the PNE rats' behavior during the SST and alterations to physiology as compared to controls,

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discusses the importance of the mPFC during task performance, and describes the link between PNE, ADHD, and the multitude of neural circuits involved in impulsivity.

In this study, we show that PNE rats were faster than controls over all trial-types and made more errors on STOP trials (i.e., they were less able to inhibit behavior when instructed). Although neural activity in the mPFC was hypoactive after PNE, the number of increasing- and decreasing-type neurons did not significantly differ between controls and PNE rats and the ability to complete the task was not lost in PNE rats. Firing of mPFC correlated with executive function necessary to perform this behavioral task. Activity was significantly stronger on STOP trials relative to GO trials and the majority of neurons exhibited a directional preference. On errors, the strength of the directional signal was attenuated, suggesting that the strong directional signal on correct trials was necessary for accurate performance. Furthermore, activity of increasing and decreasing-type neurons was positively correlated with percent correct and movement time, respectively. Thus, rats were more accurate and slower during performance of this task when activity in the mPFC was higher. When activity was low, rats tended to be faster and performance tended to be poor. We conclude that PNE makes rats more impulsive, most likely due to hypoactivation of neurons in mPFC that are important for executive control and response inhibition.

Comparison between PNE and ADHD

Here, we compare our PNE results with relevant work done in the human ADHD population. We will first focus on behavior during stop-signal performance, and then discuss neural components relevant to executive function and response inhibition. Finally, we will summarize how these parameters establish validity for PNE as an animal model

of ADHD.

Behavior

In the present study, we show that PNE rats were more impulsive on a SST. Notably, our PNE rats showed no signs of deficits beyond performance on our behavioral task. They performed the same number of trials and were actually faster to perform trials relative to controls. In contrast to these findings regarding PNE rats' performance on GO trials, most, but not all, stop-signal studies of humans with ADHD demonstrate slower reaction times on GO trials to humans without the disorder (Schachar et al., 2000; Alderson et al., 2008). This discrepancy may arise from several factors. One fundamental difference between the rat and human versions of the task is that the rat task chamber presents a single light stimulus against a backdrop of deprived stimulation (almost complete darkness and constant background noise from the computer) while the human SST is administered on a computer in an environment that is more stimulating. Perhaps distractions outside the task at hand may slow performance on GO trials, whereas when in isolation, attention-grabbing stimuli are more salient promoting faster reaction times. In addition, we administered immediate, external reinforcement following each successful trial, which has been shown to improve performance to the same degree as an administration of methylphenidate in children with ADHD and to a greater degree in children with ADHD than in unaffected children (Strand et al, 2012). Thus, this reward could have accounted for the decreased movement times on all trial types as a function of motivation. There is a need to analyze the behavior of humans with ADHD and rats with ADHD-like symptoms on the SST with specific task design choices that test this hypothesis, such as inclusion of distraction tones or lights.

Another difference between rats and humans is the nature of the learning process for this task. Human participants learn the SST via verbal instruction while rats learn this task through operant conditioning. There may be something about the difference in learning approach that is captured in these behavioral results. Another difference involves the motivation behind the performance of the task. The rats are highly motivated to complete the task because of their water deprivation and are playing for immediate reinforcement. Humans completing a SST do not have these same conditions and so their motivation will probably differ. Testing these predictions in human subjects might shed light to underlying mechanisms that may be disrupted in patients with ADHD-like symptoms.

If further research into human stop-signal performance was tailored to match the conditions used in rat studies (motivational factors, extended training periods, and operant learning procedures), then the nature of these behavioral differences could be clarified. An alternative explanation of the difference in trial accuracy and speed could relate to the level of PNE. The level of nicotine dosage in our study could have been a "sweet spot" of exposure, in which rats' behavior was impaired but not so much as to affect the ability to perform the task. Therefore, the offspring of mothers exposed to higher concentrations of nicotine could show greater behavioral deficits, while mothers exposed to lower concentrations of nicotine could show fewer or no behavioral deficits. Additional studies in which mothers are exposed to varying concentrations of nicotine would help elucidate the possible dose-dependency of these behavioral deficits. Potentially interesting findings of such a study would be threshold or ceiling effects from the varying levels of PNE.

Neural Activity

Here, we show that mPFC is hypoactive in PNE rats during performance of the SST. Although many brain areas are disrupted with PNE and in ADHD as discussed below and in the literature review, mPFC activity is consistently reduced in ADHD patients (Emond, Joyal, & Poissant, 2009) as well as in animal models that use PNE. In biology, there is a strong correlation between anatomical structure and functionality. Thus, anatomical abnormalities, from cellular to organ scale, often surface as changes in functionality. Anatomical analysis of ADHD has focused on the frontal lobe because of its crucial role in decision-making, reward, attention, and memory tasks. Past anatomical studies using MRI have revealed structural differences in the frontal lobe, including PFC, between ADHD patients and non-ADHD subjects. One study that compared 12 children with ADHD to age-matched controls observed a decreased volume of PFC in the children with ADHD compared to the controls (Mostofsky et al., 2002; Krain & Castellanos, 2006). Several functional studies (fMRI) have demonstrated that these prefrontal areas, including mPFC, play a critical role in response inhibition and are hypoactive in children and adults with ADHD (Emond, Joyal, & Poissant, 2009).

Similar results have been described in adolescents performing a GO/NO-GO task, another task that assesses impulsivity. Like the SST, the GO/NO-GO task builds a habitual prepotent response by having the large majority of trials be GO trials. In this version of the task, letters were presented one at a time on a computer monitor and subjects were told to push a button for all letters except the letter "V." The majority letters were not V's, thus it was difficult to inhibit the behavior, similar to "stopping" on STOP trials. Consistent with ADHD, activation in several brain areas was disrupted in

children exposed to nicotine. Most relevant to our results was the finding that significantly more voxels, or equal volumes of brain displaying activation, analogous to pixels on a screen, were present in unexposed children relative to nicotine exposed children during response inhibition (i.e., activation on correct NO-GO trials minus correct GO trials) (Bennett, 2009).

Thus, overall behavioral and neural effects appear to be fairly consistent between PNE and ADHD, further suggesting the PNE is a useful animal model to better understand the neural underpinnings of ADHD-like behaviors and develop new treatments.

Validity

Here, we present further evidence that the PNE rat could be a valid model of ADHD symptoms, specifically symptoms of impulsivity. ADHD is prevalent in the children of mothers who smoke tobacco during pregnancy and in controlled fetal nicotine trials using animal models, which suggests a causal link between developmental nicotine exposure and impulsivity (Wasserman et al., 2001). We first maintained construct validity by administering nicotine in a manner and dosage that mimics human mothers smoking during pregnancy. Furthermore, we focused on nicotine rather than other elements ingested from tobacco due to this causal link. Next, we maintained face validity by demonstrating a quantifiable significant increase in impulsive behavior in PNE rats as compared to controls. This model has been previously shown to have hyperactivity in PNE mice; though our own results did not show significant difference in hyperactivity (Zhu et al., 2012). This simply suggests that different doses of nicotine exposure may be needed to induce quantifiable differences in each ADHD symptom. Finally, we

established predictive validity by recording from individual neurons in mPFC. The patterns of hypoactivity across the mPFC during SST performance as measured by single-unit recordings is consistent with patterns of hypoactivity measured by fMRI studies of human ADHD subjects during the same task (Cortese et al., 2012). This suggests that PNE rats could be further used to predict single neuron activity in children with ADHD during impulsivity tasks.

Relation to other rodent prenatal nicotine studies

Our work is consistent with a previous rat study using the same dose of nicotine in drinking water as the method of drug administration. In that study, rats also performed a battery of sensorimotor tasks at different developmental milestones to further assess the impact of nicotine exposure. PNE rats in this study exhibited deficits during performance on the 5-CSRTT, which assesses attention and impulse control, as well as lower birth weights and delayed sensorimotor development. Importantly, these developmental differences were not apparent later in life when cognitive testing was performed (Schneider et al., 2011). Thus, it is unlikely that other developmental problems beyond those related to attention and impulse control can account for the differences observed during performance of our SST.

Studies in mice have shown that PNE groups were significantly more active than controls in a locomotor activity test, suggesting increased hyperactivity even into adulthood (Ajarem & Ahmad, 1998). A 2012 study by Zhu et al. similarly showed a relationship between PNE and the development of hyperactivity that was correlated with decreased cingulate volume and increased sensorimotor volume, as well as decreased dopamine turnover in the frontal cortex. Interestingly, elevated levels of hyperactivity as

measured by locomotor tests can be restored to normal levels with methylphenidate, suggesting the reversibility of the effects of PNE (2012).

Although our PNE rats were significantly faster on both GO and STOP trials, suggesting that they might also have exhibited increases in hyperactivity, we saw no effects on locomotion. All rats, not only those used for the SST and neural recordings, were tested at postnatal day 30 for their locomotion activity. There was no significant difference between the two groups. This finding is inconclusive specifically with regard to hyperactivity. For the most part, increased locomotion has been correlated with PNE (Ajarem & Ahmad, 1998). However, studies have also demonstrated no significant change in locomotion after PNE (Martin & Becker, 1970). While it is tempting to use an apparent increase in locomotion to support the association of PNE with ADHD-like symptoms, the varying findings do not fully support this correlation. Rather, cognitive, attentional, and response inhibition deficits associated with PNE should be the basis for this claim (Ernst, Moolchan, & Robinson, 2001). At the very least, our work suggests that cognitive deficits can be observed in the absence of hyperactivity.

Lastly, it is worth mentioning recent work demonstrating that hyperactivity observed in mice after PNE transmits across generations. As in our study, pregnant animals were given nicotine via drinking water. Remarkably, the study found that hyperactivity, caused by PNE, was transmitted from one generation to the next through the maternal line. This suggests that transgenerational transmission can result in propagation of environmentally induced ADHD-like behaviors in the human population and fits well with proposed genetic and environmental factors associated with the etiology of ADHD (Zhu, Lee, Spencer, Biederman, & Bhide, 2014).

Race Model and mPFC

A model that can potentially explain why hypoactivity in mPFC can result in increased impulsivity is the race model. The race model considers the STOP and GO processes (inhibiting and initiating movements, respectively) to be independent from each other. In this study, the GO process is the impetus for the rat to go to the first well following the flash of light indicating direction. The STOP process, which occurs on 20% of trials, is the process by which the rat reacts to the second light, or stop-signal. These two processes that control a movement or the inhibition of a movement compete with each other and the one that reaches threshold first controls the movement (Logan, Cowan, & Davis, 1984). If neurons in mPFC are hypoactive, then they are most likely going to lose the race with the other areas that are unaffected by PNE. SSRT is a measure of this race model; a greater SSRT indicates that the GO process was stronger than the STOP process and thus the STOP process took longer to override the GO process to correctly inhibit movement. This fits with the finding that PNE rats are worse at inhibiting an already initiated movement and that activity in mPFC is correlated with accuracy and movement time.

This work is consistent with the mPFC's critical role during performance of standard SSTs. Specifically, previous research that temporarily inactivated mPFC showed that dorsal mPFC areas are crucial for inhibiting an already initiated response during STOP trials. They further showed that injection of noradrenaline reuptake inhibitor atomoxetine, a drug approved to treat ADHD, into mPFC improved task performance (Bari et al., 2011). Our results can provide context for these findings. One possible

mechanism by which this drug improved response inhibition is by increasing the activity of hypoactive neurons in mPFC necessary for performance of this task. Indeed, it has been shown that administration of atomoxetine, a selective noradrenaline reuptake inhibitor used to treat ADHD, increases *fos*-like immunoreactivity, a histological marker indicating neural activity (Bymaster, 2002). In addition, prolonged cocaine-self administration leads to mPFC hypoactivation which can be rescued through optogenetic stimulation, further suggesting that mPFC hypoactivation causes a loss of inhibitory control (Chen et al, 2014).

Other brain areas involved

Although our work points to disruption of mPFC function, it is also necessary to consider the impact of PNE on other neural circuits and systems. A review of the effects of nicotine on the development of the nervous system further suggests that a relationship exists between PNE and deficits in attentional control and sensory processing (Heath & Picciotto, 2009). Nicotine is a teratogen that crosses the placental blood barrier and disrupts fetal development of central neurotransmitter systems, including dopaminergic and monoaminergic systems (Slotkin et al., 1987; Navarro et al., 1989; Oliff & Gallardo, 1999). PNE causes a multitude of neurochemical changes, including reduced DNA synthesis, altered neurotransmitter function, and cortical morphogenesis (Wickström, 2007). These changes occur during critical periods of neonatal brain development, leading to changes in brain area volumes, firing patterns, neurotransmitter concentrations, and receptor density. These alterations are present in areas responsible for impulse inhibition and cognitive focus, such as the ACC (Nomura et al., 2010; Zhu et al., 2012).

PNE can result in disruption of these mechanisms critical for normal division, differentiation, and migration of neurons via binding to nAchR, leading to several behavioral abnormalities (Represa & Ben-Ari, 2005; Heath & Picciotto, 2009). Even if PNE is not the perfect model of ADHD-like symptoms, it gives us insight into how the brain controls these types of behaviors and what mechanisms are disrupted in animals with poor impulse control.

PNE was also found to increase the expression of dopamine receptors genes in striatum and dopamine turnover in frontal cortex (Schneider et al., 2011). During prenatal neural development, acetylcholine binds to the nAchR, stimulating the release of dopamine. This dopamine guides neurons to divide, differentiate into their specialized cell types, and migrate to their permanent location. However, nicotine competitively binds to nAchR, such that when the fetal brain is exposed to nicotine during gestation, the neurons are guided improperly and sensory processing may be impaired, leading to behavioral alterations (Heath & Picciotto, 2009). Furthermore, this overstimulation of nAchR leads to a dopamine deficit later in development due to downregulation of dopamine receptors. Dopamine is important for executive control in the PFC, and a deficit leads to problems with attention, impulse control, and hyperactivity (Robbins & Everitt, 1987: Eagle, Bari & Robbins, 2008). Thus, PNE can impact many components of brain function, both inside and outside the mPFC circuit. However, our work points to mPFC as being a critical node in the development of poor impulse control.

Our study clearly shows that PNE makes rats more impulsive. Although many other brain mechanisms are likely to be involved, our work points to mPFC as a critical component. Based on this finding and the existence of a positive correlation between

activity and behavioral performance, this work suggests that global increases in mPFC firing may improve performance in animals performing tasks that assess executive control and response inhibition. Further studies using pharmacological or optogenetic stimulation methods are necessary to test this hypothesis. Despite the multitude of effects that occur due to exposure to nicotine during pregnancy, restoring firing in the mPFC may be an effective method of ADHD treatment.

Conclusions

Is PNE a good model of ADHD?

The literature provides a strong argument for PNE's role as a model of ADHD and the research conducted in this study helps to further cement this role. Numerous studies on the behavioral, genetic, anatomical, and pharmacological effects of PNE demonstrate how it may be the closest model of ADHD available today. The research performed in this current study emphasizes the effects of PNE on impulsivity and the neural activity of mPFC. PNE rats were significantly more impulsive on the SST; they performed all trial types more quickly and were less accurate on STOP trials. PNE rats also demonstrated global hypoactivity in mPFC, specifically during performance of the SST. Thus, this study serves as additional evidence that PNE has potential as a model of ADHD.

The limitations of this study are that the genetic and pharmacological aspects of the model are unassessed and that the behavior involving performance on GO trials does not completely match that seen typically in humans with ADHD. This might reflect a number of factors including task design, learning, motivation, and nicotine doses, as laid

out above. Future research should attempt to augment these findings by including genetic, neurophysiological, and pharmacological evidence for the model's validity. Specifically, analysis should delve into the effects of PNE on the genetics and expression of nicotinic acetylcholine receptors and dopamine receptors. It should also investigate how drugs like methylphenidate and amphetamine affect physiology, task performance, and neural activity. This research needs to be conducted in mPFC as well as in brain areas that it is connected to such as orbitofrontal cortex, ventral striatum, and basal ganglia. Optogenetic experiments can help determine how circuits between areas like mPFC and ventral striatum are encoded to produce task-relevant behavior; they can also be employed to investigate whether stimulation of mPFC can reverse the effects of PNE on task performance. These studies will provide further support and context for the PNE model and validate its connections to the causation and manifestation of ADHD.

Appendix A: Institutional Animal Care and Use Committee Protocol

UMCP Institutional Animal Care & Use Committee
Animal Study Protocol

***Please note that 'animal' is used to generically define all vertebrates covered by AWR and PHS Policy.**

PART I: CORE INFORMATION (Sections A - F required for all protocols)
SECTION A: ADMINISTRATIVE

A1. PERSONNEL INFORMATION

Principal Investigator: Matthew Roesch | Dept/Div: Psychology | Bldg/Office #: BPS 2201, 2205
Office Phone: 443-226-1583 | Lab Phone | E-mail Address: mroesch@umd.edu
Project Title: Measuring Impulsivity as Correlated with dPL Activity and Mediated by Adderall Administration in Fetal Nicotine Rats
Funding Source(s): Gemstone | Has a grant been submitted for this work? (•) NO ( ) YES (It is optional to attach proposal.)
Protocol Number: (Assigned by IACUC) | Previous Protocol Number: (Renewals Only):

A2. INTERACTION WITH ANIMALS

( ) None. This is an observational study. No animal holding, housing, or management will occur under this protocol. (Section O is not required.)
(•) This is a study where animals will be held, housed, managed, or controlled. (Section O is required.)

A3. KEY PERSONNEL

NOTE: A Personnel Qualification Form (Part I: Section F) must be completed for every member of the research group listed on this protocol. The form should illustrate skills or training necessary for the roles specified below (e.g., surgeon, anesthetist, phlebotomist, breeder, provides husbandry, observer, etc.).

	NAME	ROLE IN PROJECT
Add Name	Matthew Roesch	Principal Investigator- breeder, surgeon, anesthetist
Add Name	Brian Barnett	Breeder, surgeon, anesthetist
Add Name	Valerie Cohen	Breeder, surgeon, anesthetist
Add Name	Taylor Hearn	Breeder, surgeon, anesthetist
Add Name	Emily Jones	Breeder, surgeon, anesthetist
Add Name	Reshma Karilyl	Breeder, surgeon, anesthetist
Add Name	Alice Kunin	Breeder, surgeon, anesthetist
Add Name	Sae In Kwak	Breeder, surgeon, anesthetist
Add Name	Jessica Lee	Breeder, surgeon, anesthetist
Add Name	Brooke Lubinski	Breeder, surgeon, anesthetist
Add Name	Gautam Rao	Breeder, surgeon, anesthetist
Add Name	Ashley Zhan	Breeder, surgeon, anesthetist
Add Name	Daniel Bryden	Breeder, surgeon, anesthetist
Add Name	Greg Bissonette	Breeder, surgeon, anesthetist
Add Name	Vadim Kashtelyan	Breeder, surgeon, anesthetist
Add Name	Brandon Goldstein	Breeder, surgeon, anesthetist

Version 2009.01

Add Name	Amanda Burton	Breeder, surgeon, anesthetist
Add Name		

A4. COLLABORATING (INCLUDES SUB-CONTRACTING) INSTITUTIONS

Will any facilities other than University of Maryland facilities (main campus or Research and Education Centers) be used for animal use activities (e.g., housing, experimentation, observation, or procedures)?

- (•) No. All work will be performed in University of Maryland facilities.
- ( ) Yes. Work will be performed at another institution. **STOP.** Please contact the IACUC Manager before you fill out this form.

A5. CONTINUING RESEARCH

Does this application continue research conducted on a current or previous protocol?

(•) No. This is a new protocol. ( ) Yes. This protocol is the 3 year *de novo* review of a currently active protocol.

A6. SUPPLEMENTAL SECTIONS INCLUDED IN THIS APPLICATION (Select all that apply.)

(Please fill out those supplemental sections that pertain to the nature of your research.)

- [x] Section G: Hazardous Agent Use* - i.e. biological, chemical, radiological, rDNA (*only those agents used on animals)
- [ ] Section H: Chemical Restraint for Non-surgical procedures
- [x] Section I: Survival Surgical Procedures
- [x] Section J: Non-Survival Surgical Procedures
- [x] Section K: Non-surgical Procedures
- [ ] Section L: Field Capture / Field Studies
- [x] Section M: Breeding colonies (Including Genetically Engineered Animals)
- [ ] Section N: Antibody Production
- [x] Section O: Special Instructions for Emergency Animal Care REQUIRED for all animals held on campus.
- [x] Section P: PI Managed Animal Facility (holding longer than 12 hours)
- [ ] Section Q: Exemptions to The Guide or Exemptions to Routine Animal Care Procedures

Previous Protocol Number: (Renewals Only):

SECTION B: ANIMAL USE JUSTIFICATION

B1. PURPOSE

Describe in lay terms the purpose and goals of this animal use study. Discuss the potential scientific benefit with respect to human or animal health, the advancement of knowledge, or the good of society.
Avoid the use of jargon and define ALL acronyms/abbreviations.

Many brain areas are thought to be critical for goal-directed behavior, in which the motivational or incentive value of learned outcomes are used to guide decisions. In past work, we have employed brain lesion/inactivation techniques, which experimentally remove the influence of these specific brain regions, along with techniques to record neural activity of single cells in awake rats to elucidate how different brain areas interact to support goal-directed behavior. Here we propose to further this investigation in normal animals and in prenatally manipulated models such as fetal nicotine.
Attention-deficit/hyperactivity disorder appears to alter the ability of subjects to bridge the gap in time between predictive cues and delayed rewards. This is evident behaviorally in that subjects act impulsively by choosing a small or poor reward that is available immediately over a more valuable reward that is delayed. The behavioral task used in this protocol, termed delayed discounting, systematically measures impulsivity, or the choice of less valuable rewards delivered immediately over more valuable rewards delivered in the future. The mechanisms underlying this behavior is not fully understood. By understanding these mechanisms in normal animals, we can better understand what neural substrates may be altered in disease and how to better treat patients with this disease.

B2. LITERATURE SEARCH FOR ALTERNATIVES TO PAINFUL PROCEDURES

Does the study include procedures that have the potential for producing pain (see instructions)?

- ( ) No. There is no potential for pain.
- (•) Yes. There is potential for pain.

A literature search for alternatives to the potentially painful procedures is required. Provide the following details:

Date (day, month, year) literature search was performed: 2/15/2012

Years Covered By The Search (From - To): (i.e. 1995-2009) 2000-2011

Keywords used in the search: fetal nicotine, Adderall, delay discounting, dorsal prelimbic cortex, cannula

At least two (2) Databases Searched (check all that apply):

- ☐ BIOSIS
- ☐ AGRICOLA
- ☒ PUBMED
- ☒ CAB Abstracts
- ☐ Animal Welfare Information Center
- ☐ Other (define):

Did the literature search reveal less painful alternatives to the potentially painful procedures that are proposed?

- ○ No alternatives were found.
- ◉ Yes, alternatives were found but they cannot replace the procedures that are proposed for the following reason(s):

Alternatives included other species such as monkeys, humans, mice and pigeons. Other techniques included fMRI, in vitro techniques, computer models and microdialysis. Computer models would not work because little is known about the activity in the brain areas within this protocol during discounting behavior, we would have no grounds for assuming what aspects of signaling these areas is critical or necessary for signaling in another area, therefore making attempts at designing computation models invalid or unjustified. In vitro techniques would not suffice, because behavioral context is necessary for eliciting the neural and behavioral endpoints of interests. The animal must be awake and engaged in the behavior in order to investigate the role of these regions during decision making.

B3. ANIMAL USE JUSTIFICATION (check all that apply)

The justification for using live vertebrate animals rather than alternative means of achieving the research goal is: (check all that apply.)

☒ The complexity of the processes being studied cannot be duplicated or modeled in non-vertebrate systems because:

It would be inappropriate to use a different species because our understanding of the limbic brain structures and the interactions between them (as outlined in this proposal) is based upon neuroanatomical work done almost entirely in rat species and usually in Long-Evans rats. Parallels between the architecture, physiology and functions of circuits in rats and primates are now well established. Imaging in humans and microdialysis in rats can be used to study goal-directed behavior however it does allow the precise temporal resolution necessary for these types of experiments.

☐ There is not enough information known about the processes being studied to design nonliving models. Explain:

☐ Other (explain):

B4. SPECIES JUSTIFICATION (address each species individually)

Species: Rattus norvegicus

This species was selected for the study because of the following attributes (select all that apply):

☒ A large database exists allowing comparisons with previous data.

Explain: Long-Evans rats are a popular choice for laboratory use because they act as a multipurpose model organism and can be adapted for different types of behavioral research.

☒ The anatomy or physiology is uniquely suited to the study proposed.

Explain: As stated above, Long-Evans neural circuits have been well-studied and the correlations between areas of the rat brain and areas of human brains are better established than in other species.

☐ This is the lowest species on the phylogenetic scale that is suitable for the proposed study.

☐ Other attributes. *(details required)*.

Add Species

B5. NUMBER JUSTIFICATION (address each species individually)

Species: Rattus norvegicus

The number of animals requested for this protocol is based on the following (select all that apply):

☒ A statistical estimate of the number required to achieve statistical significance.

Explain: The number of rats required for the individual experiments is based upon what we have found to be the minimum that will achieve statistically significant or publishable data from our 10+ years of experience with these behavioral protocols. These group sizes are similar to those we have employed to demonstrate statistically significant effects of lesions and/or other manipulations on behavior and neurophysiology in these

behaviors. The experiments here are identical those previously published, except here, we are examining the function of other brain areas in similar tasks.

☐ The estimated minimum number necessary to achieve the goals of the study in the absence of a statistical estimate.

☒ The number necessary to obtain sufficient tissue or other material for testing or analysis.

Explain: The number of rats required is also based upon studies that have shown that rat mothers may kill approximately one-half of their fetal nicotine offspring. Due to this high number of deaths, it is important that we account for accidental deaths in our number of animals so that enough data and tissue may be collected at the end of the study to verify the effects of the fetal nicotine and Adderall (R).

☐ The number required to provide sufficient technical training or practice for the number of trainees expected.

☐ Other *(details required)*.

Add Species

B6. ANIMAL NUMBERS

Adult and/or juvenile/larval animals only:

NOTE: If this is a 3 year renewal protocol and there are animals remaining on the expiring protocol, the number of animals remaining on the expiring protocol (and transferring to the new protocol) must included in the number of animals requested under the new protocol. Animals on the expiring protocol will be transferred to the new protocol upon activation of the new protocol.

Species: Rattus norvegicus | Age or weight range: 150-750 g | Sex and strain/stock: 20 females, 10 males

TOTAL# required for 3-year protocol: 30

Categorize animals by the most severe pain they will experience using USDA Categories: *See IACUC website for examples of painful procedures.*

Category C: (Non-Painful Procedures)	Category D: (Procedures using anesthesia/analgesia)	Category E: (Painful procedures without anesthesia/analgesia)
0	100	0

Source of the animals:
NOTE: If transferred from another protocol, provide PI name and protocol number. Charles Rivers Laboratory

Add Species

Will embryonic and/or neonate animals be used at any time during this protocol?

◉ No. Only censused animals will be used. See IACUC policy on *Accounting for Animals in Census*.

○ Yes. Embryos (avian, aquatic, or mammal) and/or preweaning neonates will be used.

B7. OVERVIEW OF EXPERIMENTAL DESIGN AND ANIMAL USE TIMELINES

Provide a brief (250 words or less) summary of the overall experimental design of this proposal. The description should define animal groups, group sizes, and how each group will be tested or used. It should not include a detailed review of surgery or other activities, but should include the use of any unique drugs or practices: *Animal numbers described in timeline must be consistent with section B.6.*

First, fetal nicotine rats will be bred from Long-Evans rats using nicotine-infused water dosages. Next the rats will be trained on the stop-signal behavioral task and then a survival surgery will be performed to insert an electrode into the dorsal prelimbic cortex to measure neural activity. We will measure behavior and/or neural activity while rats perform the behavioral task. If we are manipulating the brain (lesion or inactivation) then we require two groups, experimental (n = 15) and control (n =15). If we are interested only in neural activity in normal (control) animals then we only require 15 animals (no experimental group). In both scenarios, rats only undergo one survival surgery. Fetal nicotine rats will then receive Adderall (R) administrations to determine how the drug alters behavior and neural activity. Afterwards, a nonsurvial surgery will be performed to analyze tissues.

Describe the anticipated sequence of experimental events (timeline) such as breeding, preparation of animals, surgery, testing procedures, collection of tissues, euthanasia, etc.:

Breeding the fetal nicotine rats will take approximately one month, followed by a period of waiting until the rats are mature enough to take part in the behavioral study. During this time, the rats will be trained to perform the stop-signal task for 2-3 months. Surgical procedures are anticipated to take approximately one hour in the case of lesions, intravenous surgery, or electrode implantation and 2-4 hours when a single animal will undergo multiple procedures. Rats will be assessed daily and handled briefly for a period of 2 weeks following surgery. Data will be collected from the stop-signal task described above for up to 6 months. During this time, the experimental group will be administered Adderall and will also be evaluated on the behavioral task. At the end of training, rats will be euthanized and perfused in order to fix the brain tissue, so that the location of the electrodes/cannula and the extent of the lesions can be verified. Several months will be allotted to analyze the data and examine brain tissue.

SECTION C: SPECIAL CONCERNS FOR ANIMAL USE

C1. ANIMAL HOUSING and PROCEDURE LOCATIONS (UMCP)

Specify building and room number for each planned activity below (address each species individually). Section O required.

Species: **Rattus norvegicus**

ACTIVITY		BUILDING	ROOM
Housing/Holding	Greater than 12 hours	Bio-psych	Animal Facilitation Room
	Less than 12 hours	Bio-psych	2201, 2205
Survival Surgery	Pre-operative preparation	Bio-psych	2201, 2205
	Surgery	Bio-psych	2205
	Post-operative care	Bio-psych	2201, 2205
Non-Survival Surgery	Pre-operative preparation	Bio-psych	2205
	Surgery	Bio-psych	2205
Nonsurgical Procedures (ie. Behavior testing)		Bio-psych	2201, 2205
Euthanasia (including Tissue Harvesting)		Bio-psych	2201, 2205
Imaging			
Breeding Colony Activities		Bio-psych	2205

Add Species

C2. SPECIAL HUSBANDRY REQUIREMENTS

Are there any special husbandry needs? Note that special husbandry needs that are approved must be implemented through direct arrangements with DLAR or Facility Manager of the relevant housing facility.

- (•) No. There are no special husbandry requirements.
- ( ) Yes. There are special husbandry needs. (Section Q must be filled out.)

C3. PHYSICAL RESTRAINT

Will the proposed research require the use of physical restraint (other than short-term hand-held) of awake animals?

- (•) No. Physical restraint of awake animals will not exceed short-term hand restraint.
- ( ) Yes. Physical restraint must be used.

C4. WITHHOLDING OF ANESTHETICS OR ANALGESICS

Does this protocol involve procedures that are expected to cause pain, but for which pain-relieving anesthetics and/or analgesics will not be provided? (A literature search must be conducted for any painful procedures.) *All numbers must be consistent with B.6 and B.7.*

- ( ) No. There are no painful procedures (i.e., no greater pain than would be expected from simple injections).
- (•) No. Anesthetics and/or analgesics will be provided for pain relief.
- ( ) Yes. This protocol includes painful procedures for which anesthetics and/or analgesics must be withheld.

C5. ANTICIPATED COMPLICATIONS (This section required for all LD/ID/MI studies.)

Do you anticipate any animal health complications (e.g. local or systemic infection, physical or physiological impairment, heavy tumor burden, tumor necrosis, malnutrition, dehydration, etc.) arising from the experimental procedures or animal manipulations in this protocol?

- (•) No. Animal health complications are not expected.
- ( ) Yes. Animal health complications may occur. Plan of care must include humane endpoints.

C6. ADMINISTERED SUBSTANCES

- ( ) No. No other substances will be given to animals.
- (•) Yes. Administered substances are listed below. Justification is needed for all non-pharmaceutical grade substances.

List substance, dose or concentration, route (to include osmotic pumps, via headposts, etc), volume, frequency, site and needle size.

Substance 1

N-methyl-D-aspartate (0.05-0.5 µl of 20 µg/µl PBS solution or 0.1-1.0 µl of 10 µg/µl PBS solution) , quinolinic acid (0.1-1.0 µl of 15 µg/µl PBS solution), and 6 µg/µL 6- hydroxydopamine (6-OHDA) in PBS in 0.1% w/v ascorbic acid vehicle will be infused once locally into a particular brain site via a 26 guage cannula.

Additional Substance

SECTION D: EUTHANASIA & DISPOSITION

D1. EUTHANASIA: (consistent with AVMA Guidelines for Euthanasia)

Please indicate the role of euthanasia in the proposed activity:

- [x] Animals will be euthanized as part of the experimental protocol. (Specify method below.)
- [ ] Euthanasia is not planned, but will be performed to prevent animal distress. (Specify method below.)
- [ ] Euthanasia will not be performed.

Please specify the method(s) of euthanasia below. Use a separate line for each species studied under this protocol.

Generally Acceptable Methods Of Euthanasia *(injection, inhalation, physical under anesthesia)*

	Species	Method	Anesthetic or Injectable Agent / Dose / Route of Administration or MS222 Concentration
Add Species	Rattus norvegicus	Inhalant anesthetic gas (not ether)	Inhalation of isoflurane (2-5%) in an asphyxiation chamber.

NOTE: If CO2 or isoflurane euthanasia was selected for any species, a secondary method to ensure non-recovery is required. Please select the secondary method(s) that will be used to ensure euthanasia:

☐ Bilateral thoracotomy ☒ Decapitation ☐ Tissue / organ collection.

☐ Other method to ensure death Specify:

Conditionally Acceptable Methods Of Euthanasia *(physical without anesthesia)*

	Species	Method	Scientific Justification for Procedure without Anesthesia
Add Species			

D2. FINAL DISPOSITION OF ANIMALS

Indicate the method(s) of terminating responsibility for the live animals (select all that apply):

☒ Euthanized by methods specified in section D1 above (Euthanasia).

☐ Live animals returned to production / breeding unit.

☐ Live animals transferred to alternate protocol #

☐ Other (specify):

D3. FINAL DISPOSITION OF TISSUES, FLUIDS, OR CARCASSES

Indicate the method(s) of disposing of the carcasses and surplus tissues or fluids (select all that apply):

☒ Carcasses of non-biohazardous dead animals will be disposed of by DES.

☐ Non-radioactive tissues or fluids will be disposed of by the PI.

☐ Radioactive carcasses, tissues, or fluids will be disposed of by DES.

☐ Other (specify):

E1. TRANSPORTATION

Indicate the method(s) of disposing of the carcasses and surplus tissues or fluids (select all that apply):

☒ No transportation of animals will occur once they are on campus.

☐ All transportation will conform to UMCP transportation guidelines.

☐ Other (specify):

PRINCIPAL INVESTIGATOR AGREEMENT

This agreement signifies that you (as PI) have read and understood your responsibilities to operate as the Principal Investigator. The concurrent signatures signify that the appropriate individuals have been contacted as to conducting this research on campus. The agreement may be signed electronically as part of this form or a copy may be signed manually and sent separately from an electronically submitted protocol application.

- ☐ I acknowledge responsibility for the procedures and care of animals used in this protocol. I will conduct all work in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, USDA regulations (9 CFR Parts 1, 2, 3), the Federal Animal Welfare Act (7 USC 2131 et. Seq.), the *Guide for the Care and Use of Laboratory Animals*, and policies set forth by the University of Maryland IACUC.
- ☐ I have determined that the research proposed is not unnecessarily duplicative.
- ☐ I confirm that all individuals on this protocol are participating in an appropriate Occupational Health & Safety Program. (Note: The UMCP Animal Handler Health Review forms are located at http://www.health.umd.edu/forms/animalhandlerform609.pdf; participation in an Occupational Health Program is mandatory for those with direct animal contact). Labs should also have DES Chemical Hygiene Plan.
- ☐ I authorize individuals listed on this application to conduct procedures involving animals and I accept responsibility for their oversight in the conduct of this proposal.
- ☐ I confirm that all individuals listed on this protocol as working with animals have completed the Animal User training or will be required to do so before being permitted to begin work with animals. Further, I certify that those individuals are properly trained, or will receive such training prior to working with animals, in all areas relevant to their assigned work with animals
- ☐ For animals held in a UMCP operated facility, I understand that in cases of necessary medical treatment, UMCP University veterinarians are authorized to provide the treatment required to sustain life, or if that is not possible, to prevent distress and pain by humane euthanasia. I recognize that the veterinary staff will contact me as soon as possible using the emergency contact information that I provide in this application, but I understand that such contact may not always be possible prior to providing treatment or performing euthanasia.
- ☐ I will notify the IACUC regarding any unexpected study results that negatively impact the welfare of the animals, including but not limited to those that require veterinary care or treatment not described in the approved protocol.
- ☐ For animals held in a UMCP operated facility or used on the UMCP campus, I will notify a University veterinarian and the IACUC when unanticipated pain or distress, unexpected morbidity, or unanticipated mortality occurs with animals approved for use under this protocol.
- ☐ I will obtain approval from the IACUC before initiating any change in the study design or procedures by submitting a request for minor or significant change as appropriate. I understand that work performed without IACUC approval cannot be published with certification of IACUC approval and may result in federally-required reporting of non-compliance.
- ☐ For all USDA Category D (anesthesia / analgesia provided to relieve potential pain) and USDA Category E (pain not relieved by anesthesia / analgesia) animal use procedures, I certify that I have reviewed the pertinent scientific literature and the sources and/or databases noted in this application and found no scientifically acceptable alternative to any of those procedures that would result in less pain or distress.

PI Name: Matthew Roesch Date:

Project Title: Measuring Impulsivity as Correlated with dPL Activity and Mediated by Adderall Administration in Fetal Nicotine Rats

Principal Investigator signature: Date:

Chair Signature: Date:

DES Signature (If applicable): Date:

Facility Manager Signature (If applicable): Date:

SECTION F: PERSONNEL QUALIFICATIONS FORM (PQF)(Cover each individual listed in Section A3)

F1. PERSONAL INFORMATION

Name (Last, First): Roesch, Matthew Day Phone #: 443-226-1583

Dept / Div: Psychology Office/Lab #: Bio-psych 2201, 2205 E-mail Address: mroesch@umd.edu

Highest Degree Earned:

☐ High School ☐ Associate ☐ BA / BS ☐ MA / MS ☐ MD / DVM / DDS ☒ PhD ☐ Other (specify):

UMCP Relationship:

☒ Faculty ☐ Staff ☐ Post-doctoral ☐ Visiting Scientist ☐ Off campus Associate

☐ Graduate Student ☐ Undergraduate Student ☐ Other (specify):

PI statement: ○ This individual **WILL NOT HAVE** animal contact. (No further information is required.)

● This individual **WILL HAVE** animal contact. (Complete the remainder of this form.)

If this individual WILL HAVE animal contact, have they completed the PI/Animal Users training class?

○ No. Animal users must schedule and complete training before initiating any animal activities.

● Yes. The individual completed the PI/Animal Users training class.

What experience do you have to perform the procedures and use the techniques required of you in this protocol? (Be specific.)

10 years experience working with rats and 7 years with primates. 8 years experience with the animal procedures listed above.

If the protocol requires **specific skills that are not listed above** please detail those here along with the training received and whether or what level of proficiency the individual has with the specific techniques.

If training in specific skills is needed (as checked above) or if you will be engaged in procedures for which you are not presently proficient, indicate who will provide the necessary training and who will provide oversight until you have achieved proficiency.

Add another PQF form

PART II: PROTOCOL SPECIFIC INFORMATION (Append to Part I, if applicable)
SECTION G: HAZARDOUS AGENT USE (Only those hazardous agents used in animals.)

G1. HAZARDOUS AGENTS

Please indicate the type of hazardous agent(s): (check all that apply)

- ☐ Human cells or fluids
- ☐ Virus / bacteria / prion
- ☐ Radioactive materials
- ☐ Recombinant DNA
- ☐ Carcinogens / mutagens*
- ☐ Toxicological agents*
- ☒ Other hazardous chemicals*
- ☐ Tissue fixatives (formalin, paraformaldehyde)
- ☐ Laser / irradiator / x-ray machines
- ☐ Generation of transgenic animals
  - embryo injection
  - breeding

If already approved by the IBC, provide approval #

G2. DETAILS OF HAZARDOUS AGENT USE

	Agent (identify)	Dose and Frequency of Administration	Route of Administration	Duration of Treatment
Add Agent	Nicotine			
Add Agent	Paraformaldehyde			
Add Agent	Adderall	1.6-6.0 mg/kg	consumed through a chocolate drink in a needle-free syringe	Given once daily over a period of fourteen days
Add Agent				
Add Agent				
Add Agent				
Add Agent				
Add Agent				

For each agent listed above, please address the following issues:

Agent (identify): Nicotine

Personnel protection precautions to be used by laboratory personnel and individuals performing animal husbandry:

Mask, gloves, goggles, lab coat, scrub pants, all chemicals mixed in the fume hood

Length of time the agent remains a threat to the health of the animals or humans working with the animals:

No threat if the agent is mixed in the hood.

Duration of animal survival between exposure to the agent and euthanasia:

3-6 months

Means of caging and equipment decontamination:

CARF will maintain the sterile cages using cage washing.

Method of animal waste disposal:

Waste will be disposed in the garbage.

Method of animal carcass disposal:

All animals will be decapitated and placed in a carcass freezer.

Add Information for another Agent

Agent (identify): Paraformaldehyde

Personnel protection precautions to be used by laboratory personnel and individuals performing animal husbandry:

Mask, gloves, goggles, lab coat, scrub pants, all chemicals mixed in the fume hood

Length of time the agent remains a threat to the health of the animals or humans working with the animals:

No threat if the agent is mixed in the hood.

Duration of animal survival between exposure to the agent and euthanasia:

3-6 months

Means of caging and equipment decontamination:

CARF will maintain the sterile cages using cage washing.

Method of animal waste disposal:

Waste will be disposed in the garbage.

Method of animal carcass disposal:

All animals will be decapitated and placed in a carcass freezer.

Add Information for another Agent

Agent (identify): Adderall

Personnel protection precautions to be used by laboratory personnel and individuals performing animal husbandry:

Mask, gloves, goggles, lab coat, scrub pants, all chemicals mixed in the fume hood

Length of time the agent remains a threat to the health of the animals or humans working with the animals:

No threat if the agent is mixed in the hood.

Duration of animal survival between exposure to the agent and euthanasia:

3-6 months

Means of caging and equipment decontamination:

CARF will maintain the sterile cages using cage washing.

Method of animal waste disposal:

Waste will be disposed in the garbage.

Method of animal carcass disposal:

All animals will be decapitated and placed in a carcass freezer.

Add Information for another Agent

PART II: PROTOCOL SPECIFIC INFORMATION (Append to Part I, if applicable)
SECTION I: SURVIVAL SURGERY PROCEDURES

NOTE: Repeat items I1 through I15 *for each species* that will have survival surgery.

The following items I1 - I15 apply to *(identify species)*:

I1. MULTIPLE SURVIVAL SURGERY

Will any of the animals have undergone survival surgery prior to being entered into this study (e.g., by the vendor or under a different protocol)?

- (•) No. Animals will not have had prior survival surgery.
- ( ) Yes. Animals will have had prior surgery before entering into this study.

Will any of the animals experience more than one survival surgery, including surgery prior to entering the study?

- (•) No. Animals will have only one survival surgery procedure.
- ( ) Yes. Animals will have more than one survival surgery procedure.

I2. NARRATIVE OF SURVIVAL SURGERY PROCEDURES UNDER THIS PROTOCOL

Description of survival surgery procedures:

The experiments covered under this protocol will include stereotaxic surgeries to make lesions or implant hardware to allow neural recordings to be made and for reversible inactivation of brain regions. Stereotaxic surgery will be performed according to the procedures for aseptic technique in survival surgery from The Experimental Animal in Biomedical Research, Vol 1, Ed. B. Rollin and M.L. Kesel, CRC Press, 1990. All surgical instruments will be sterilized in a steam autoclave or by bead sterilizer before each surgery. The experimenter will be masked and gloved to prevent contamination. Prior to surgery, the animal will be anesthetized with isoflurane (3-5% for induction and 2-3% for maintenance via nosecone) via a gas anesthesia system. Depth of anesthesia will be monitored via the respiration pattern and the suppression of reflex responses to tail- and foot-pinch. Surgical procedures are anticipated to take approximately one hour in the case of lesions, intravenous surgery, or electrode implantation and 2-4 hours when a single animal will undergo multiple procedures. After adequate anesthesia has been obtained, the animal will be shaved over the surgical site, and the field will be sterilized with a betadine scrub and alcohol. An incision will be made along the midline of the rats skull to expose the skull, and the underlying fascia will be removed via blunt dissection.

When lesions are to be made, holes will be drilled in the skull at appropriate positions using a small hand drill with a sterilized drill bit mounted on a stereotaxic arm, and the dura will be slit to permit needle passage. A Hamilton syringe will be advanced through the holes to stereotaxic coordinates for each specific lesion, then a neurotoxic agent will be delivered via a microinjection unit to create a lesion. Neurotoxic agents include N-methyl-D-aspartate (0.05-0.5 µl of 20 µg/µl PBS solution or 0.1-1.0 µl of 10 µg/µl PBS solution) or quinolinic acid (0.1-1.0 µl of 15 µg/µl PBS solution), which act through glutamate receptors to cause neuronal cell death, thus sparing non-neuronal cells and fibers of passage and 6 µg/µL 6-hydroxydopamine (6-OHDA; Sigma, St. Louis, MO, USA) in PBS and 0.1% w/v ascorbic acid vehicle to cause neuronal cell death in dopamine neurons. All vehicles will be autoclaved.

To implant a 26g cannula to allow inactivation, burr holes will be drilled above the target area, and anchoring screws will be secured in 3 places on the skull. The cannula will be lowered to a predetermined location and held in place with a stereotaxic arm. The cannula and anchoring screws will then be secured to the skull with dental acrylic. To prevent foreign materials from entering the brain, an obdurator/stylus will be screwed into the cannula and remain in place until immediately before administering any neural inhibitor via Hamilton syringe. Once the hardware has been installed and/or lesions have been completed, any open holes will be filled with gel-foam, and the incision will be closed using sutures as much as permitted by any recording hardware affixed to the skull. Inactivation will be caused by slow infusion (0.1 µl/10 sec) of the gaba agonist muscimol (0.05-1 µg in 0.5 µl PBS solution) or the NMDA antagonist AP-5 (0.05-1 µg in 0.5 µl PBS solution) into each cannula. All vehicles will be autoclaved.

To implant a microelectrode, burr holes will be drilled in predetermined positions for anchoring screws and a somewhat larger central hole will be made for insertion of the microelectrode bundle. Under stereomicroscopic guidance, the dura will be cut away from this central hole and the microelectrode bundle will be visualized entering the cortical tissue. The electrode assembly will then be advanced at a rate of 100 microns/minute until appropriately positioned for subsequent recording. The assembly will then be affixed to the skull and anchoring screws via grip cement and dental acrylic.

In the event we chose to implant IV catheters, the rat will be momentarily removed from of the stereotaxis (just after initial incision has been made on the head, and fascia has been cleared). The open incision will be covered with a sterile gauze to prevent contamination, then the rat will plugged into an acccessory isoflurane nosecone (our standard nosecones are affixed to the stereotaxis device), and placed on its back to expose the catheter insertion site. An incision will be made lateral to the midline to expose the jugular vein. The catheter will be inserted into the jugular vein and secured using two sterile silk sutures. The catheter will then pass subcutaneously to the top of the skull where it is connected to the modified cannula head mount which sits to the side during the electrode portion of the surgery. Next, the rat will be removed from the accessory isoflurane line and placed back in the stereotaxic apparatus. Any additional procedures, lesion, cannula or microelectrode implantation will be completed. Once additional hardware are cemented in place, the catheter, attached to the appropriate head mount will be cemented anterior and lateral to the electrode or cannula. The wound areas will be sutured with sterile silk and rats will be

given 2 ml of saline (injected s.c) to replace fluids lost during surgery. A plastic blocker will be placed over the open end of the cannula head mount. All rats will be placed on a thermal heating pad before and during the surgery and will be placed under heat lamps following the surgery. There will be a thermometer placed at the level of the animal under the lamp to ensure that the temperature under the lamp does not exceed 80-85 degrees F.

Specify the method of wound closure: Sutured with sterile silk or staples

Will all sutures and/or wound clips be allowed to remain in place beyond the 7th post-operative day?

- (•) No. All sutures and/or wound clips will be removed on or before the 7th day after surgery.
- ( ) Yes. Sutures and/or wound clips will remain in place for more than 7 days.

I3. PRE-OPERATIVE ANIMAL SUPPORT (NOT ANESTHESIA)

Specify pre-operative actions that will be taken to prepare the animals for survival surgery *(select all that apply)*:

- [ ] Physical exam
- [ ] Overnight food withdrawal
- [x] Body temperature support
- [ ] Drugs (other than anesthetics and sedatives) or fluids *(List below)*:
- [x] Ophthalmic ointment to eyes
- [x] Iodine (or Chlorhexidine) + alcohol skin scrub, 3 alternating cycles
- [x] Clipping of fur

I4. PRE-OPERATIVE ANESTHESIA / SEDATION / TRANQUILIZATION

Will pre-operative anesthesia, sedation or tranquilization be provided to the animals?

- (•) No. Drugs will not be administered to the animals prior to surgical anesthesia.
- ( ) Yes. Pre-operative drugs will be used to calm the animals. *(List below)*

I5. INTRA-OPERATIVE ANIMAL SUPPORT (NOT ANESTHESIA)

- [ ] Mechanical ventilation
- [ ] Intravenous fluids
- [ ] Ophthalmic ointment to eyes
- [ ] None (explain):
- [ ] Heat to prevent hypothermia
- [ ] Cooling to prevent hyperthermia
- [ ] Other (specify):

I6. INTRA-OPERATIVE ANESTHESIA

Please list all agents and dosing regimens to be used for intra-operative anesthesia.

	Anesthetic Agent	Dose	Route of Administration	Frequency of Administration	Duration of Treatment
Add Agent					

I7. NEUROMUSCULAR BLOCKING AGENTS (PARALYTICS)

Will neuromuscular blocking agents (paralytics) be used at any time during the procedure?

- (•) No. Neuromuscular blocking agents will not be used for the procedure.
- ( ) Yes. Neuromuscular blocking agents will be used. *(Provide details below)*

I8. MONITORING DURING ANESTHESIA

Indicate below the indices that will be used for intra-operative monitoring of animal condition and depth of anesthesia.

- [x] Respiratory rate / effort
- [x] Mucous membrane color
- [ ] Body temperature
- [ ] Oxygen saturation
- [ ] Heart rate
- [ ] Capillary refill time
- [ ] EKG
- [x] Reflex (specify): tail and foot pinch
- [ ] Other (specify):
- [ ] Other (specify):

Specify the frequency at which the above indices will be recorded: Every 10 minutes.

I9. POST-OPERATIVE ANIMAL SUPPORT DURING RECOVERY FROM ANESTHESIA

Indicate care that will be provided to animals during post-operative recovery from anesthesia: *Select all that apply.*

- [x] Heat to prevent hypothermia
- [ ] Cooling to prevent hyperthermia
- [ ] Other (specify):
- [ ] None (explain):
- [ ] Intravenous fluids
- [ ] Ophthalmic ointment to eyes
- [ ] Other (specify):

I10. MONITORING DURING RECOVERY FROM ANESTHESIA

Indicate below the indices that will be used for post-operative monitoring of animal condition during recovery from anesthesia.

☒ Respiratory rate	☐ Heart rate	☐ Reflex (specify):
☒ Mucous membrane color	☐ Blood pressure	☒ Other (specify): Responsiveness to touch
☐ Body temperature	☐ Capillary Refill Time	☐ Other (specify):
☐ Oxygen saturation	☐ EKG	

Specify the frequency at which the above indices will be recorded: Every 15-30 minutes.

I11. PAIN MANAGEMENT

NOTE: The UMCP IACUC encourages the use of pre-emptive analgesia for pain management. Analgesia should be provided as early in the procedure as possible, ideally before it begins.

Will analgesia be provided to the animal for relief of post-operative pain?

○ No. Post-operative analgesia will not be provided. (justify the omission of analgesia below)

◉ Yes. Analgesia will be provided. (specify details below)

Please list analgesics and dosing regimens below:

	Analgesic	Timing of Administration	Dose	Route of Administration	Frequency of Administration	Duration of Treatment
Add Analgesic	Analgesic buprenorphine	Pre-procedure	0.03 mg/kg	subcutaneous	Once before and once the day after surgery	Two days

I12. POST-OPERATIVE ANTIBIOTIC OR DRUG THERAPY

Will antibiotics or drugs other than experimental agents be provided to animals during the post-operative period?

○ No. Such treatment is not planned and will be provided only if medically advised.

◉ Yes. Antibiotics and/or drugs will be administered. (specify details below)

	Agent	Dose	Route of Administration	Frequency of Administration	Duration of Treatment
Add Agent	Topical triple antibiotic ointment with lidocaine	Cover wound	Applied to all wound edges	Once	After surgery
Add Agent	Cephalexin	15 mg/kg po bid	oral	Twice daily	Fourteen days
Add Agent	Gentamicin/Saline solution	0.16% gentamicin	Flushed through intravenous catheters	Every 24-48 hours	Fourteen days

I13. MONITORING AFTER POST-OPERATIVE RECOVERY UNTIL TERMINATION OF THE STUDY

Select parameters from the list below that will be used to detect pain, distress, or discomfort and promote the general well-being of the animals. Monitoring of five or more parameters is recommended.

Monitoring Parameter	Frequency of Observation
☒ Not eating or drinking (requires individual housing)	Daily
☐ Fecal and urine output (requires individual housing)	
☒ Body weight (requires frequent weight checks)	Weekly (if sick, then everyday)
☒ Wound healing (checking at least daily until suture removal)	Daily
☐ Behavioral change (aggression, guarding, hiding)	
☒ Licking, biting, scratching or shaking of operative site	Daily
☒ Hair coat (ruffled fur, lack of grooming, piloerection)	Daily
☒ Posture or ambulation (tense, tucked-up, stiff gait)	Daily
☒ Activity level (restlessness, pacing, reluctance to move)	Daily
☒ Facial expression (eyes dull, pupils dilated, pinning of ears)	Daily
☒ Sweating or salivation (stressed rodents salivate excessively)	Daily
☐ Oculonasal discharge (rats shed porphyrin pigment)	
☐ Teeth grinding (rabbits, livestock)	

☒ Other, Specify:	Performance on behavioral tasks	Daily
☐ Other, Specify:		

For each monitoring parameter checked above, indicate the action you will take if observations suggest pain, distress, or a decline in animal well-being.

Rats will be taken off water restriction, wet food will be place in cage, antibiotics started and vet notified.

I14. SPECIMEN COLLECTION FROM LIVE ANIMALS

Will specimens be collected from living animals during or after the survival surgery?

◉ No. Specimens will not be collected from living animals.

○ Yes. Specimens will be collected from living animals.

I15. HUMANE ENDPOINTS THAT WILL PROMPT INTERVENTION

Select those humane endpoints from the list below that are appropriate within the context of the proposed study for determining when intervention for humane reasons will occur. For each endpoint selected, indicate the action that will be taken should the endpoint be reached.

	Humane Endpoint that will prompt: >>>	Veterinary Consultation	Euthanasia	Other Action *(define)*
☐	Infection unrelated to the protocol.	☒	☒	Or put on antibiotics an monitor
☐	Signs of moderate to severe pain or distress that was not anticipated by the study plan.	☒	☒	
☐	Body weight loss exceeding 15% of free-feeding body weight relative to an age-matched reference.	☒	☒	Put wet food in cage, give antibiotics and monitor closely.
☐	Mutilation of operative site or other self mutilation.	☒	☒	Give break from task and put on free water. Monitor closely.
☐	Neurological disorders (e.g., seizures, blindness, ataxia) that were not anticipated by the study plan.	☐	☒	
☐	Cardiopulmonary disorders (e.g. sudden weakness, vascular collapse, coma) that were not anticipated by the study plan.	☐	☒	
☐	Abnormal feeding or defecation for 48 hours (e.g., decreased feed or water intake and/or decreased fecal production that is unrelated to the study plan).	☒	☒	
☐	Non-weight bearing for 72 hours (e.g., difficulty walking, inability to maintain upright posture)	☐	☒	
☐	Other (specify):	☐	☐	
☐	Other (specify):	☐	☐	

Add Sections I1 through I15 for another species.

PART II: PROTOCOL SPECIFIC INFORMATION (Append to Part I, if applicable)
SECTION K: NON-SURGICAL PROCEDURES

NOTE: Repeat items K1 through K13 ***for each species*** that will have non-surgical procedures.

K1. NARRATIVE OF NON-SURGICAL PROCEDURES

Species: Rattus Norvegicus

Description of non-surgical procedures:

Stop-signal task

K2. PRE-PROCEDURE ANIMAL SUPPORT (NOT ANESTHESIA)

Will special pre-procedural care be provided?

○ No. The procedures do not require special pre-procedural care.

◉ Yes. Specify pre-procedural actions that will be taken to prepare animals for the procedure(s) *(select all that apply)*:

- ☐ Physical exam
- ☐ Overnight food withdrawal
- ☐ Clipping of fur
- ☐ Ophthalmic ointment to eyes
- ☐ Body temperature support
- ☐ Iodine (or Chlorhexidine) + alcohol skin scrub, 3 alternating cycles
- ☐ Drugs (other than anesthetics and sedatives) or fluids (list agents below):

	Agent	Dose	Route of Administration	Frequency of Administration	Duration of Treatment
Add Agent	Adderall	1.6-6.0 mg/kg	consumed through a chocolate drink in a needle-free syringe	Given once daily	Fourteen days

K3. PRE-PROCEDURE ANESTHESIA / SEDATION / TRANQUILIZATION

Will pre-procedure anesthesia, sedation or tranquilization be provided to the animals?

◉ No. Drugs will not be administered to the animals prior to the procedure(s).

○ Yes. Pre-procedure drugs will be used to calm the animals. *(List below)*

	Drug	Dose	Route of Administration	Frequency of Administration	Duration of Treatment
Add Drug					

K4. INTRA-PROCEDURE ANIMAL SUPPORT (NOT ANESTHESIA)

Will special intra-procedure care be provided?

◉ No. The procedures do not require special intra-procedural care.

○ Yes. Specify intra-procedure care that will be provided to animals during the procedure(s) *(select all that apply)*:

- ☐ Heat to prevent hypothermia
- ☐ Ophthalmic ointment to eyes
- ☐ Cooling to prevent hyperthermia
- ☐ Other (specify):
- ☐ Other (specify):
- ☐ Drugs (other than anesthetics and sedatives) or fluids (list agents below):

	Agent	Dose	Route of Administration	Frequency of Administration	Duration of Treatment
Add Agent					

K5. INTRA-PROCEDURE ANESTHESIA OR CHEMICAL RESTRAINT

Will intra-procedure anesthesia or chemical restraint be provided?

◉ No. The procedures do not require intra-procedural anesthesia or chemical restraint.

○ Yes. The procedure requires chemical restraint as described in Section J or anesthesia as described below:

	Anesthetic Agent	Dose	Route of Administration	Frequency of Administration	Duration of Treatment

Add Agent					

K6. NEUROMUSCULAR BLOCKING AGENTS (PARALYTICS)

Will neuromuscular blocking agents (paralytics) be used at any time during the procedure?

- ◉ No. Neuromuscular blocking agents will not be used for the procedure.
- ○ Yes. Neuromuscular blocking agents will be used. *(Provide details below)*

	Paralytic Agent	Dose	Route of Administration	Frequency of Administration	Duration of Treatment	Reversal Agent (if appropriate)
Add Agent						

Please state why the use of paralytic agents during the procedure is necessary.

Please describe how adequate anesthesia will be ensured during the time the animal is undergoing neuromuscular blockade:

K7. MONITORING DEPTH OF ANESTHESIA DURING PROCEDURES

Indicate below the indices that will be used for monitoring animal condition and depth of anesthesia.

- ☐ Respiratory rate / effort
- ☐ Mucous membrane color
- ☐ Body temperature
- ☐ Oxygen saturation
- ☐ Heart rate
- ☐ Capillary refill time
- ☐ EKG
- ☐ Reflex (specify):
- ☐ Other (specify):
- ☐ Other (specify):

Specify the frequency at which the above indices will be recorded:

K8. POST-PROCEDURE ANIMAL SUPPORT

Will special post-procedure care be provided?

- ◉ No. The procedures do not require special post-procedural care.
- ○ Yes. Specify post-procedure care that will be provided to animals after the procedure(s) *(select all that apply)*:
  - ☐ Heat to prevent hypothermia
  - ☐ Cooling to prevent hyperthermia
  - ☐ Other (specify):
  - ☐ Intravenous fluids
  - ☐ Ophthalmic ointment to eyes
  - ☐ Other (specify):

K9. MONITORING DURING RECOVERY FROM ANESTHESIA *(if used)*

Indicate below the indices that will be used for post-procedure monitoring of animal condition during recovery from anesthesia (i.e., until sternal recumbancy is regained and maintained):

- ☐ Respiratory rate
- ☐ Mucous membrane color
- ☐ Body temperature
- ☐ Oxygen saturation
- ☐ Heart rate
- ☐ Capillary Refill Time
- ☐ EKG
- ☐ Reflex (specify):
- ☐ Other (specify):
- ☐ Other (specify):

Specify the frequency at which the above indices will be recorded:

K10. PAIN MANAGEMENT INTRA- OR POST-PROCEDURE

NOTE: The UMCP IACUC encourages the use of pre-emptive analgesia for pain management. Analgesia should be provided as early as possible in the procedure if it is expected to be painful or result in residual pain, ideally before the procedure begins.

Is the procedure expected to cause pain or result in residual pain?

- ◉ No. The procedure is not expected to cause pain.
- ○ Yes. Pain during and/or after the procedure is likely.

If pain is expected, will analgesia be provided for pain relief?

- ○ No. Analgesia will not be provided. (justify the omission of analgesia below)

○ Yes. Analgesia will be provided. (specify details below)

If pain is expected and analgesia will not be provided, please explain why pain relief will be withheld:

If analgesia will be provided for pain relief, please list analgesics and dosing regimens below:

	Analgesic	Timing of Administration	Dose	Route of Administration	Frequency of Administration	Duration of Treatment
Add Analgesic						

K11. MONITORING DURING THE STUDY UNTIL TERMINATION

Select parameters from the list below that will be used to detect pain, distress, or discomfort and promote the general well-being of the animals during the course of the study. Monitoring of five or more parameters is recommended.

Monitoring Parameter		Frequency of Observation
☒ Not eating or drinking (requires individual housing)		daily
☐ Fecal and urine output (requires individual housing)		
☒ Body weight (requires frequent weight checks)		daily
☒ Behavioral change (aggression, guarding, hiding)		daily
☒ Licking, biting, scratching or shaking of procedure site		daily
☒ Hair coat (ruffled fur, lack of grooming, piloerection)		daily
☒ Posture or ambulation (tense, tucked-up, stiff gait)		daily
☒ Activity level (restlessness, pacing, reluctance to move)		daily
☒ Facial expression (eyes dull, pupils dilated, pinning of ears)		daily
☐ Sweating or salivation (stressed rodents salivate excessively)		
☐ Oculonasal discharge (rats shed porphyrin pigment)		
☐ Teeth grinding (rabbits, livestock)		
☐ Other, Specify:		
☐ Other, Specify:		

For each monitoring parameter checked above, indicate the action you will take if observations suggest pain, distress, or a decline in animal well-being.

K12. SPECIMEN COLLECTION FROM LIVE ANIMALS

Will specimens be collected from living animals during or after the procedure(s)?

◉ No. Specimens will not be collected from living animals.

○ Yes. Define the specimen type and collection details below.

☐ Fluids (e.g., blood, lymph, ascites, CSF, GI fluids, etc.

Fluid type (specify): Collection method:

Volume (mls) per collection: Frequency of collection:

Method of disposal:

☐ Solid Tissues

Tissue type (specify): Collection method:

Volume (mm3) per collection: Frequency of collection:

Method of disposal:

K13. HUMANE ENDPOINTS THAT WILL PROMPT INTERVENTION

Select those humane endpoints from the list below that are appropriate within the context of the proposed study for determining when intervention for humane reasons will occur. For each endpoint selected, indicate the action that will be taken should the endpoint be reached.

	Humane Endpoint that will prompt: >>>	Veterinary Consultation	Euthanasia	Other Action *(define)*
☐	Infection unrelated to the protocol.	☒	☒	Or put on antibiotics an monitor
☐	Signs of moderate to severe pain or distress that was not anticipated by the study plan.	☒	☒	
☐	Body weight loss exceeding 15% of free-feeding body weight relative to an age-matched reference.	☒	☒	Put wet food in cage, give antibiotics and monitor closely.
☐	Mutilation of operative site or other self mutilation.	☒	☒	Give break from task and put on free water. Monitor closely.
☐	Neurological disorders (e.g., seizures, blindness, ataxia) that were not anticipated by the study plan.	☐	☒	
☐	Cardiopulmonary disorders (e.g. sudden weakness, vascular collapse, coma) that were not anticipated by the study plan.	☐	☒	
☐	Abnormal feeding or defecation for 48 hours (e.g., decreased feed or water intake and/or decreased fecal production that is unrelated to the study plan).	☒	☒	
☐	Non-weight bearing for 72 hours (e.g., difficulty walking, inability to maintain upright posture)	☐	☒	
☐	Other (specify):	☐	☐	
☐	Other (specify):	☐	☐	

Add Sections K1 through K13 for another species.

PART II: PROTOCOL SPECIFIC INFORMATION (Append to Part I, if applicable)
SECTION M: BREEDING COLONY (INCLUDING TRANSGENIC/KO BREEDING AND USE)

M1. JUSTIFICATION

Could the animals that will be bred be purchased from commercial sources in the required number?

- ○ No. The animals are not available commercially in sufficient number.
- ◉ Yes. The animals are available commercially.

Please describe the rationale for breeding these animals at the University of Maryland:

Please identify the source of breeders:

- ☒ Colony managed by a UMCP Investigator. Specify source protocol #:
- ☐ Obtained from another institution. Identify source:
- ☒ Purchased from a vendor (e.g., Harlan, Charles River, etc.) Identify source: Charles River

M2. BREEDING COLONY SPECIES AND NUMBERS

List species to be bred, indicate the number of breeders required, and provide **estimates** for the numbers of offspring expected and their ultimate disposition. Protocols have a life span of 3 years, list numbers anticipated over a 3 year period.

	Species	Strain	# of Male Breeders	# of Female Breeders	Expected # of Offspring	Estimated # of Offspring used for this Protocol (over 3 year)	Estimated # Of Offspring transferred to another Protocol	Estimated # Of Offspring euthanized without use
Add Species	Rattus Norvegicus	Long Evans	10	20	200	100	0	100

M3. DISPOSITION OF BREEDERS AND UNNEEDED OFFSPRING

Indicate the final disposition of retired breeders:

☒ Euthanasia according to protocol ☐ Used in experiments ☐ Other

Please explain any planned experimental use and/or other disposition:

If euthanasia without use is indicate in section Q3 above, please explain why the surplus offspring cannot be used for this protocol or by another investigator.

Females are not needed because they do not show the intended effects, but the males are viable. We can use male breeders in another protocol.

M4. BREEDING PLAN

The breeding method will be:

- ○ Monogamous (single male and female per cage)
- ◉ Harem (single male and multiple females). Please indicate which of the following will apply:
  - ☒ Males will be removed once females are confirmed pregnant.
  - ☒ Females will not bred again until the offspring are weaned.
  - ☒ Individual pregnant females will be moved to new cages prior to delivery of offspring.
  - ☒ Females and their litters will be moved to larger cages to provide required floor space.

Special care (feed, water, temp, humidity, air flow) required for this breeding colony:

- ◉ No special care is required.
- ○ Special care is necessary to keep these animals healthy. The required special care is as follows *(describe)*:

Weaning of rodents will occur at:

- ☒ 21 days of age or earlier
- ☐ 22 days of age or later (specify strains affected and justification of extending weaning beyond 21 days)

- (•) Special care beyond routine animal care IS NOT necessary.
- ( ) Special care IS necessary to keep these animals healthy. The required special care is as follows *(describe)*:

Genotyping:

- (•) Genotyping is not necessary for this protocol.
- ( ) Genotyping will be performed on tissue obtained by the method(s) defined below.
  - [ ] Tail snipping (mice).
    - ( ) The tail snip will be taken after 21 days of age.
    - ( ) The tail snip will be taken prior to 21 days of age.

      Justify the delayed genotyping and describe the anesthetic regimen to be used for the procedure:
  - [ ] Oral swabs.
  - [ ] Blood collection.
  - [ ] Other *(describe)*:

M5. SPECIES AND GENOTYPE

(Complete this section only if breeding genetically engineered animals. Otherwise, go to section M6.)

List species to be used, identify genetic lines, and indicate any induction method that is necessary.

	Species	Strain / Genotype
Add Species		

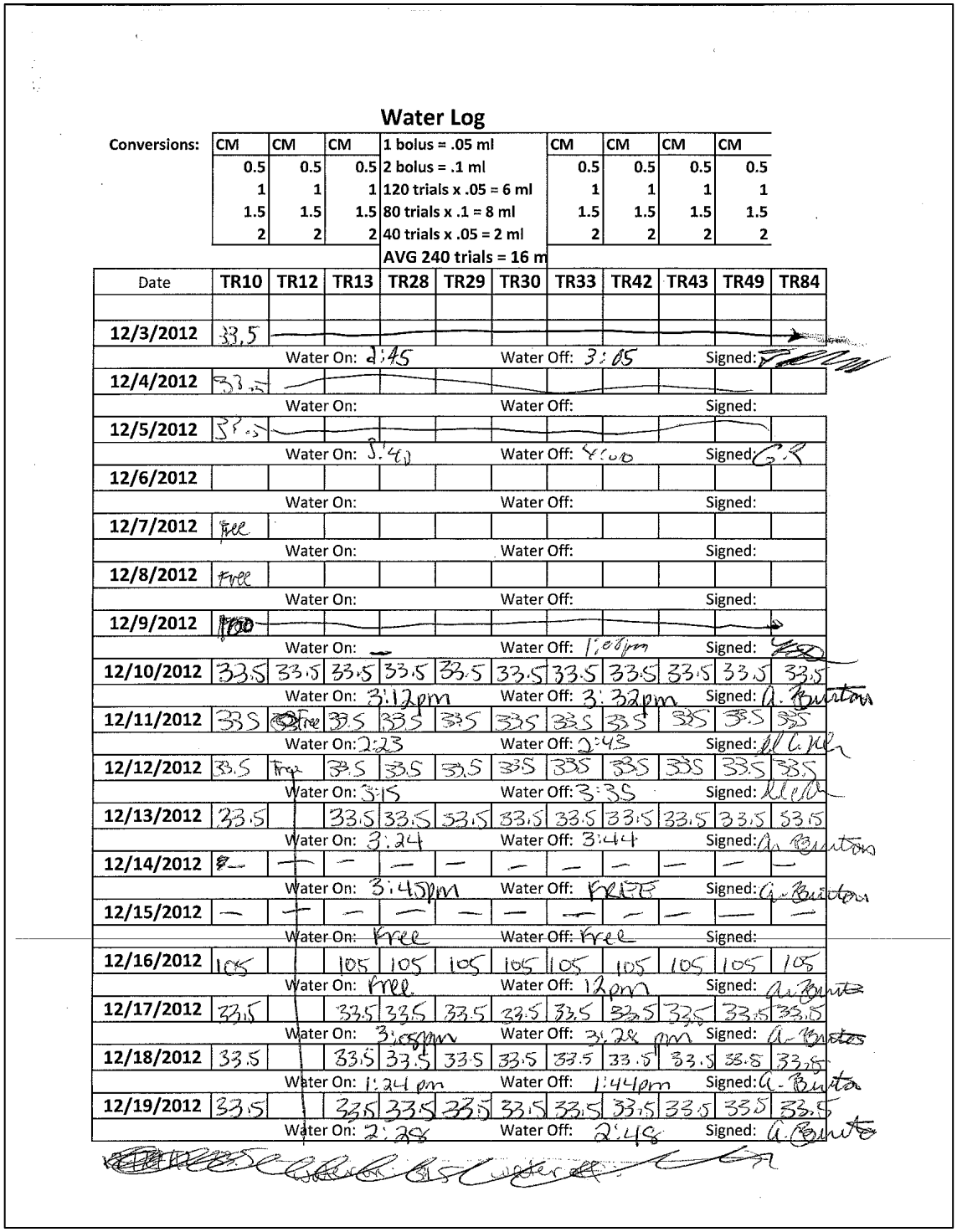
M6. RECORD KEEPING *(Applies to all breeding colonies of conventional and genetically engineered animals.)*

Indicate the record-keeping system that will be used to document health surveillance and the maintenance of well-being for the conventional and/or genetically-engineered animals.

- [x] Special care is not required and documentation will be provided by standard observation records.
- [ ] Special care outlined in section M4 will be documented by the record-keeping sheet attached to this application.
- [ ] Special care outlined in section M4 will be documented as follows:

Appendix B: Sample Data Collection Sheets

Appendix B1: Water log for mothers.



Water Log

Conversions:	CM	CM	CM	1 bolus = .05 ml	CM	CM	CM	CM
	0.5	0.5	0.5	2 bolus = .1 ml	0.5	0.5	0.5	0.5
	1	1	1	120 trials x .05 = 6 ml	1	1	1	1
	1.5	1.5	1.5	80 trials x .1 = 8 ml	1.5	1.5	1.5	1.5
	2	2	2	40 trials x .05 = 2 ml	2	2	2	2
				AVG 240 trials = 16 m				

Date	TR10	TR12	TR13	TR28	TR29	TR30	TR33	TR42	TR43	TR49	TR84
12/3/2012	33.5										
	Water On: 2:45					Water Off: 3:05				Signed:	
12/4/2012	33.5										
	Water On:					Water Off:				Signed:	
12/5/2012	33.5										
	Water On: 3:40					Water Off: 4:00				Signed:	
12/6/2012											
	Water On:					Water Off:				Signed:	
12/7/2012	free										
	Water On:					Water Off:				Signed:	
12/8/2012	free										
	Water On:					Water Off:				Signed:	
12/9/2012	free										
	Water On: —					Water Off: 1:08pm				Signed:	
12/10/2012	33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5
	Water On: 3:12pm					Water Off: 3:32pm				Signed: A. Burton	
12/11/2012	33.5	free	33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5
	Water On: 2:23					Water Off: 2:43				Signed:	
12/12/2012	33.5	free	33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5
	Water On: 3:15					Water Off: 3:35				Signed:	
12/13/2012	33.5		33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5
	Water On: 3:24					Water Off: 3:44				Signed: A. Burton	
12/14/2012	—	—	—	—	—	—	—	—	—	—	—
	Water On: 3:45pm					Water Off: FREE				Signed: A. Burton	
12/15/2012	—	—	—	—	—	—	—	—	—	—	—
	Water On: free					Water Off: free				Signed:	
12/16/2012	105		105	105	105	105	105	105	105	105	105
	Water On: free					Water Off: 12pm				Signed: A. Burton	
12/17/2012	33.5		33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5
	Water On: 3:08pm					Water Off: 3:28 pm				Signed: A. Burton	
12/18/2012	33.5		33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5
	Water On: 1:24 pm					Water Off: 1:44pm				Signed: A. Burton	
12/19/2012	33.5		33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5	33.5
	Water On: 2:28					Water Off: 2:48				Signed: A. Burton	

Appendix B2. Weight log for pups.

TR Rats Weight Log								
	TR65	TR71	TR35	TR42	TR43	TR49	TR73	TR84
8/23/2012	227	258	308	299	294	265	249	257
8/30/2012	265	296	335	324	325	283	283	290
9/6/2012	307	335	[illegible]	370	360	318	329	343
9/13/2012	368	332	391	384	398	350	353	358
9/20/2012	390	362	410	404	380	408	389	390
9/27/2012	425	379	446	416	402	443	416	425
10/4/2012	443	415	467	443	433	475	450	449
10/11/2012	485	433	495	455	445	503	479	485
10/18/2012	521	522	553	527	481	524	538	534
10/25/2012	—	500	—	522	505	545	570	573
11/1/2012	—	435	—	476	495	540	555	525
11/8/2012	—	393	—	445	485	532	—	510
11/15/2012	—	350	—	464	495	535	—	535
11/22/2012	—	380	—	452	514	520	—	530
11/29/2012	—	—	—	505	510	569	—	580
12/6/2012	—	—	—	500	523	588	—	600
12/13/2012	—	—	—	541	537	590	—	613
12/20/2012	—	—	—	810	540	570	—	606
12/27/2012	—	—	—	593	563	607	—	625
1/3/2013	—	—	—	515	576	602	—	626
1/10/2013	—	—	—	595	555	610	—	621
1/17/2013	—	—	—	575	540	620	—	625
1/24/2013	—	—	—				—	
1/31/2013	—	—	—				—	
2/7/2013	—	—	—				—	
2/14/2013	—	—	—				—	
2/21/2013	—	—	—				—	

Appendix B3. Behavioral task recording log.

Rat AB01

Time In	Date	Comments
10:16	2/26/13	Multibox. 100ms. Shaping=Y. Phase 1.
9:30	2/27/13	Rack 4. All choice. Blockers. Link Reward to Side Lights. preO=150. odor=100 PreF=0 2 Boli Max Respons=60 Delay same length=N 97 trials
8:20	2/28/13	Rack 4. 18/20 Choice. Blockers. Link Reward to Side Lights. PreO=150 Odor=100. PreF=0. 2 Boli. Max Resp=30. Delay same length=N. 93 trials. No problem switching.
7:30	3/1/13	Rack 4 16/20 choice Blockers. Link reward to side lights. preO=200 odor=100 pref=0 2 boli max resp=60 delay same length=N 87 trials No problem switching
7:30	3/4/13	Rack 1 14/20 choice Reward size 90 Link Reward to Side lights preO=250 odor=100 pref=0 2 Boli delay same length=N 194 trials No problem switching
11:15	3/5/13	Rack 4. stop sig. No link. odor=2,3. Prob=50. No stop trials. Use stop Lights=Y. preO=300. odor=100. PreF=0. 144 trials 10/20
7:45	3/7/13	Rack 4. Stop Sig. Odor=2,3 Stop=0/20. Prob=50. Use Stop Lights. PreO=350 Odor=100 Pref=0. Error ITI=4000! ~160 trials
9:45	3/8/13	Rack 1 Stop Sig Odor=2,3 Stop=0/20 prob=50 use stop lights pref=400 odor=100 pref=0 error ITI=4000 168 trials.
7:00	3/11/13	Rack 1 stop sig odor=2,3 stop=0/20 prob=50 use stop lights preO=650 odor=100 pref=0 error ITI=5000. 66 trials. percent correct: 49%.
9:40	3/12/13	Rack 1. stop sig. odors=2,3. stop=0/20. prob=250. use stop lights. preO=~~500~~ 5000. odor=100. pref=0 error ITI=~~5000~~ 5000. 147 trials.
10:00	3/13/13	Rack 4. stop sig. Odor=2,3. stop=0/20 Prob=50 use stop lights preO=550 odor=100 pref=0 Error ITI=5000. 157 trials. 66%
	3/14/13	Rack 2. Stop sig. odor=2,3. Stop=0/20. Prob=50. use stop lights. preO=600. odor=100. PrefF=0. Error ITI=5000. 173 trials.
10a	3/15/13	Rack 3. Stopsig. Odor=2,3 Stop=0/20. Prob=50. Stoplights. PreO=650 Odor=100. Pref=0. Error ITI=5000. 137 trials.
11:40a	3/22/13	Rack 1, stopsig., odor=2,3, stop=0/20, prob=50, use stoplights, preO=700, odor=100, pref=0, error ITI=5000, 135 trials, 47%.
11:20a	3/25/13	Rack 4, stopsig, odors 2&3, stop=0/20, prob=50, use stoplights, preO=750, odor=100, pref=0, error ITI=5000, 116 trials 53%
10:03	3/26/13	Rack 4. stop sig. odors=2,3. stop=0/20. prob=50. use stop lights. preO=800. odor=100. pref=0 error ITI=5000. E 140 trials 53%
8:43	3/27/13	Rack 4. stop sig. odors 2,3. stop=0/20. prob=50. use stop lights. preO=850 odor=100 PrefF=0 error ITI=5000 113 trials 57%
1:25	3/28/13	Rack 1 stop sig. odors=2,3. stop=0/20. Prob=50 use stop lights. preO=~~4000~~ 4000. odor=100. pref=0. error ITI=5000. 125 trials 10/20
8:25	3/29/13	Rack 4 stop sig odor=2,3 stop=0/20 prob=50 use stop lights preO=950 odor=100 pref=0 error ITI=5000 64 trials. 9/30. 49%
10:45	4/1/13	Rack 1 stop sig odor=2,3 stop=0/20 prob=50 use stop lights preO=1000 Odor=100 Pref=0 error ITI=5000 81 trials. 11/20.
11:30	4/2/13	Rack 4. stop sig. odor=2,3 stop=0/20. Prob=50. use stop lights. preO=1050. odor=100. pref=0. error ITI=5000. 86 trials 12/20
8:41	4/3/13	Rack 1. stop sig. odor 2,3 stop=0/20 prob=50. use stop lights. preO=~~1000~~ 1000 odor=100 pref=0 Error ITI=5000 73 trials 13/20 51%
10:15	4/4/13	Rack 3. stop sig. odor=2,3. stop=0/20. prob=50. use stop lights. preO=1000. odor=100. pref=0. error ITI=5000. 109 trials. 10/20. 51%
8:30	4/5/13	Rack 4. Odor=2,3. Stop=0/20. End=W. Delay=0. Prob=50. Use Stoplights. PreO=1000. Odor=100. Pref=0. Error ITI=5000. 155 trials. 10/20. 55%

Rat AB01

Time In	Date	Comments
8:30	4/8/13	Rack1 stop sig odor=2,3 stop=3/20 Prob=50 end=w delay=0 use stop lights pre0=1000 pref=0 odor=100 error ITI = 5000 57% 117 trials
11:25	4/9/13	Rack 3. stop sig. odor=2,3. stop=4/20. prob=50. end=w delay=0 use stop lights. pre0=1000. pref=0. odor=100. error ITI=5000. 116 13/20
6:25	4/10/13	Rack 4. stop sig. odor=2,3. stop=4/20. prob=50. end=w/delay=0 use stop lights pre0=1000 pref=0 odor=100 error ITI=5000 118 trials. 14/20.
10:11	4/11/13	Rack1 - stop sig. odor=2,3, stop=4/20. prob=50. end=w delay=0 use stop lights. pre0=1000. pref=0. odor=100. error ITI=5000. 146 trials. 11/20. 56%
9:40	4/12/13	Rack2. Odor=2,3. Stop=4/20. Prob=50 End=W. Delay=0. Pre0=1000. Odor=100. PreF=0. Error ITI=5000. 146 trials. 14/20. 61%
10:09	4/16/13	Rack2. Odor=2,3. Stop=4/20. Prob=50. End=W. Delay=0. Pre0=1000 Odor=100. PreF=200. Error ITI=4000. 160 trials. 11/20.
9:47	4/17/13	Rack1. odor=2,3 stop=4/20 Prob=50 End=w. delay=0. pre0=1000 Odor=100 preF=300 Error ITI=350. 202 trials 50%.
1:30	4/18/13	Rack1 odor=2,3. stop=4/20. prob=50. end=w delay=0. pre0=1000 odor=100 preF=400. error ITI=3000. 177 trials 11/20
8:15	4/19/13	Rack4 odor=2,3 stop=2,3 prob=50 Delay=0 End=W pre0=1000 odor=100 pref=500 error ITI=2500. 156 trials. 11/20 59%
9:05	4/22/13	Rack1 Odor=2,3 Stop=2,3 prob=50 delay=0 end=W pre0=1000 odor=100 pref=600 error ITI=2000 171 trials 5b%
10:08	4/23/13	Rack 4. odor=2,3. stop=2,3. prob=50. delay=0. end=w pre0=1000. odor=100. pref=700. error ITI=1000. 183 trials. 13/20. 56%
11:40	4/24/13	Rack1. odor 2,3 stop=2,3 prob=50 delay=0 end=W pre0=1000 odor=100. pref=800. 188 trials, 57%
9:30	4/25/13	Rack4. odor=2,3. stop=2,3. prob=50. delay=0. end=w. pre0=1000. odor=100. pref=900. 200 trials. 18/20.
7:30	4/26/13	Rack4 odor=2,3 Stop=2,3 prob=50 delay=0 end=w pre0=1000 odor=100 pref=800-1000 187 trials
10:48	4/29/13	Rack odor=2,3 stop=4/20 prob=50 End=w delay=0 pre0=1000 odor=100 pref=800-1000 189 trials. 11/20. 46%

Sucrose

Electrophysiology Surgery Sheet – mPFC

Investigator Roesch **Protocol #:** _____ **Surgeon:** Bryden

Animal ID: AB01 **Weight:** 406 (grams) **Sex:** M **Date:** 4/30/13

Start time: 9:15 am/pm **End time:** 11:30 am/pm

Anesthetic gas type: Iso **Nose Cone?** ☑ yes ☐ no

Returned to cage: 11:45 am/pm

Post-Op Analgesics and Medications

Drug	Dose*	Route	Time
Bup	.10mL	SQ	5/1/13 8am

*Total mg/ml.

RIGHT HEMI

Measurements for implant:

From Bregma: Anterior: 48.26
Lateral: 7.25 7.85
Ventral: _____

	LEFT		RIGHT		
Bregma AP	58.06	61.36	____	____	+3.3 mm anterior to bregma
Midline Lateral	15.42	16.02	____	____	+0.6 mm lateral to bregma
From Dura Ventral	19.1 Start	17.1 Intended Stop	17.0 Actual Stop	____ ____	2 mm ventral to brain

* advance at rate of 100 um/minute*

Description of Operative Procedure and Day of Surgery Notes:

Appendix B4. Surgery sheet.

Post-Op Recovery

Record information every 15-30 minutes until fully recovered (ANIMAL SHOULD BE STERNAL OR STANDING, HOLDING HEAD UP, AND RESPONSIVE TO STIMULATION)

Time	Respiration	Color	Sedation*	Comments/Additional Observations	Initials
11:30a	Normal	Pink	4		DWB
11:40a	Normal	Pink	3		DWB
11:50	Normal	Pink	2		DWB

***Sedation Level:**

1 = Alert, Responsive, Moving around
2 = Alert, Responsive, Not active
3 = Eyes open, Responsive, Groggy
4= Eyes closed, Nonresponsive, Heavily sedated

Euthanasia

Surgeon: _____ **Date:** _____ **Time:** _____

Perfusion: yes / no **Anesthetic Gas Type:** _____ **Decapitated:** yes / no

Comments:

_____

_____

_____

_____

_____

To Carcass Freezer: _____ **am/pm**

Appendix B5. Single-unit recording log.

NO Cells

tafile: AB01052120 13.plx

Session Length: 2:01

Animal #: AB01

Date: 5/21/13

Estimated electrode depth: 2.18

Stop-Signal Delay: 0

Rack #: 3
stopsignal_070111.exe Odors: 2,3

A/D Channels --> Electrode:

#1	
#2	Maybe Cell
#3	
#4	
#5	
#6	Maybe Cell
#7	
#8	

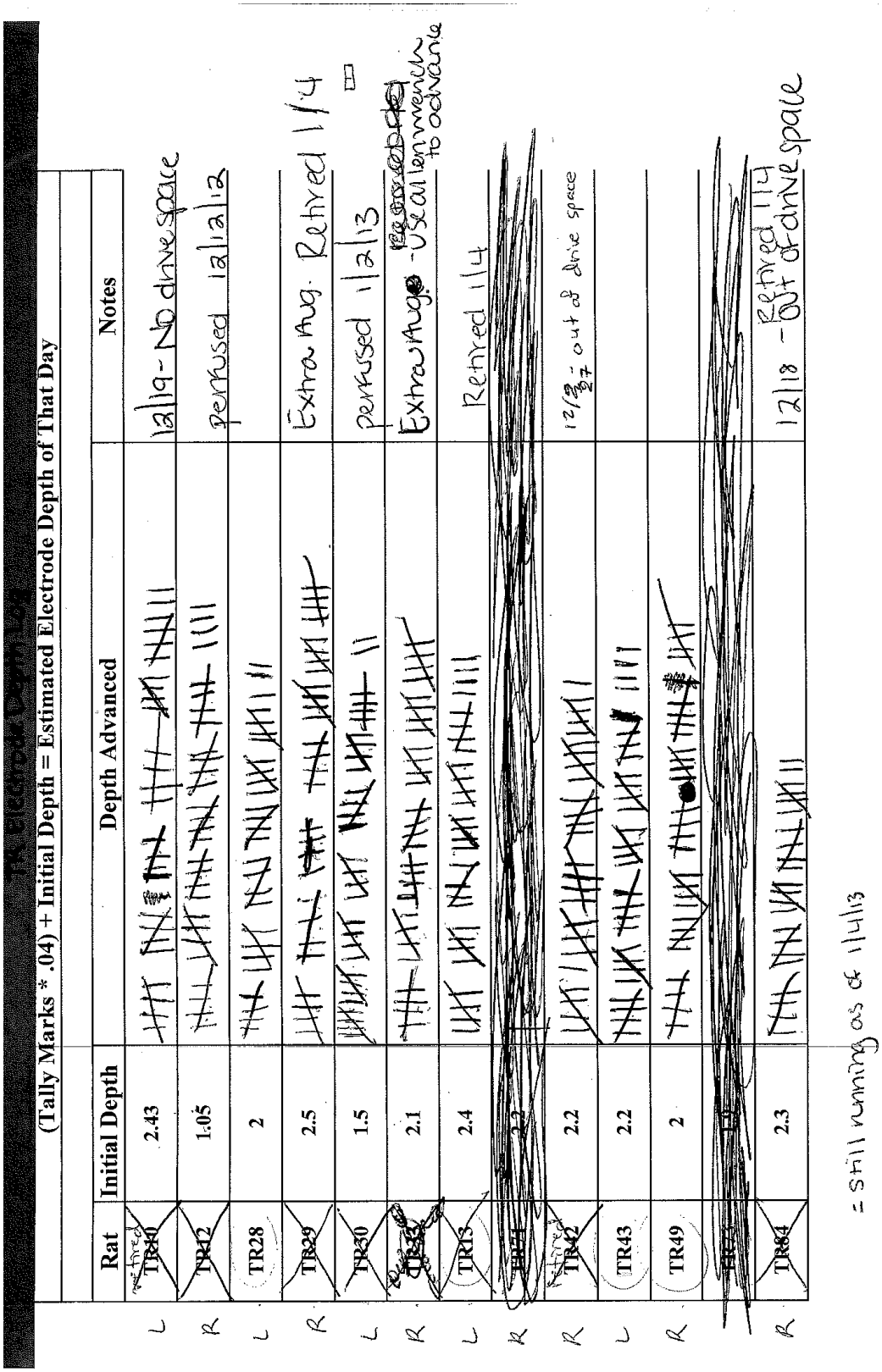
Reward = 100; Stop trials = 4/20; End Stop Signal = W; Probability = 50
SS Relays as Odors; PreOdor = 1000; Odor = 100; PreFluid Delay = 800-1000

Percent Correct	Number of Trials
67	255

Additional Comments:

Adv. 1/8

Appendix B6. Electrode advancements log.



Appendix C: Experimental Protocols

Electrode making 7/10/07

1. Cut down an augit's pins to half length, leaving the middle one full length. Use the dremel tool to shave each down.
2. Make cannula: Snip it with the large scissors to about 20 mm. Then shave it down until it is no longer flattened at the end. Then clear out the end of shavings with an insect pin. Then work on the opposite end. Shave it down little by little until it is exactly the right length, measuring each time using the calipers. For ABL it should be 17.50 mm. For OFC, 15.00 mm. For mPFC = 13.00 mm. When it is just a tiny hair above the correct length, bevel it using the dremel, by tilting the cannula. Make sure the reamer goes all the way through the cannula.
3. Solder the cannula to a pin. The pin should be ground down so that the closed end is short. Hold the pin on another pin that is held in a vice. The end of the cannula should be between the two grooves (towards the back groove), with the beveled side up. Make sure the cannula is straight. Use the little clamps to hold the cannula in place. Put a little flux and then solder it by tapping with the solder iron with a little bit of solder on it.
4. Cut 11 wires for the electrode, with the small scissors. Each should be about 3 inches. Cut them onto a pair of forceps lying on the table, so that they are easier to grab once cut. Grab them with your fingers and even out the ends by snipping with the scissors. Then get a little ~~spit~~ and twist them together, running your fingers all the way to the end. Then put the bundle down on the forceps, and let them dry. After 1-2 minutes, grab the bundle with the blunt forceps, 2-3 inches from the end, and align it with the cannula (being held on the pin-holder). Push the end into the beveled side and work them through until they come out the other end. Then pull them very gently out. Then trim the wires so they are all even, and about 1 -2 cm out.
5. Push the other end of the wires with the side of a forceps so that they are curved up into a right angle from the cannula, and splay (fan) them out. Then put a drop of glue on the beveled end of the cannula to glue the wires.
6. Slide the pin that the cannula is soldered to onto the center pin on the augit you are using. Make sure the splayed wires point towards the white dot on the other side of the augit.
7. Wrap each wire, using the microscope, around one shortened pin. Wrap them counterclockwise for the right-most 5 pins, and clockwise for the leftmost 5 pins. Start with the pins furthest away from the white dot. Wrap them at least 6-10 times.
8. Take a fine forceps and run it along the end of the wrapped wire. It will eventually break, and in the process will strip off some of the insulation. Push that end so that it is very close to the pin (i.e. not sticking out). Then paint the whole pin with the silver conductor paint. As you do each pin, go back to the last pin and paint a second coat of the silver paint. Often you have to hold the end of the wire against the pin as the paint dries. Make sure it is inside of the paint when it is completely dry.

9. Test all of the wires, by inserting the end of the bundle into a small beaker of saline, and attach one battery lead to the saline and the other to hold in the augit in turn. The end of the wire should bubble if the connection is good. Make sure the positive/negative is the right alignment (I'm not sure which is correct to make it bubble).
10. Cover with another coat of silver paint, and then test again when dry. To fix a wire that doesn't work, strip off the paint with the forceps and find the end and restrip it, or reattach it to the pin with the paint. There will be one extra wire when you are done. Use that wire if one of the other wires does not work after several tries. Otherwise cut the 11th wire off.
11. Put a donut of green putty (one drop of liquid mixed with the putty from the tube) around the pins to cover them so that it covers from the cannula all the way down to the augit surface. Leave a lip around the edge of the augit.
12. Add a covering of pink dental cement over the green putty. Use equal amounts of powder and liquid to make it. Apply it with a spatula onto the green putty. Use the lip of the augit to catch the cement flowing down so that it doesn't run over the side of the augit. Wipe it off the side if it does run over. Make a sealed covering up to the cannula.
13. Solder a round nut onto each screw that you will need for the electrode (3 in total for each electrode). Screw the nut down until it is 2 mm from the head of the screw. Set the screw onto an allen wrench, so that it is pointing up. Put a little flux on the nut. Drop a tiny bit of solder onto the top of the nut (facing up). Test it by screwing a second nut down all the way to the soldered one. The soldered one should not move at all, and the second one should freely screw all the way up to it. (i.e. there should not be solder in the threads).
14. Pot electrode:
    a. Grease the mold using high-vac grease.
    b. Screws into the tripod. Screw them all the way down and back up, then insert into a beaker of WD-40 and screw down and back up again. End up with each screw 15 mm, using the calipers.
    c. Score the side of the augit, using the dremel tool, with three x's. (They will look white).
    d. Put grease in the space between the nut and the screw-head – just a tiny bit on all sides.
    e. Fill the top of the screw-head with grease (the cavity where the allen wrench goes).
    f. Grease the top of the augit (which will be facing down in the mold)
    g. As you put the augit into the mold, center the cannula against one side of the triangle.
    h. Carefully put the tripod, screw-heads down, over the electrode and lower it into the mold so that each screw-head sits in one corner of the mold.
    i. Make the pink cement again, (1 to 1) and use a big syringe and 19 ga. needle to suck it up. Drop it into the mold along each side of the triangle (away from the screws). It will flow down over the augit and the screw-heads. Fill it up to the level of the mold, and then try to put a little bit of semi-hard cement onto the rounded cement (from before) that covers the

5 scoops
1½ droppers

pins (in order to make sure the augit is held firmly). If some of the cement gets onto the flat part of the mold, clear it off when it is partially dry. You will have to cut it away from the rest of the cement and then peel it off.

j. When it is mostly hard, after 10 minutes, remove from the mold by unscrewing the sides, splitting the mold in half. Immediately turn each screw on the electrode a half turn back and forth, to break it free from the cement. Do this again every few minutes until it is completely dry.

5 min

5ml syringe

19G needle

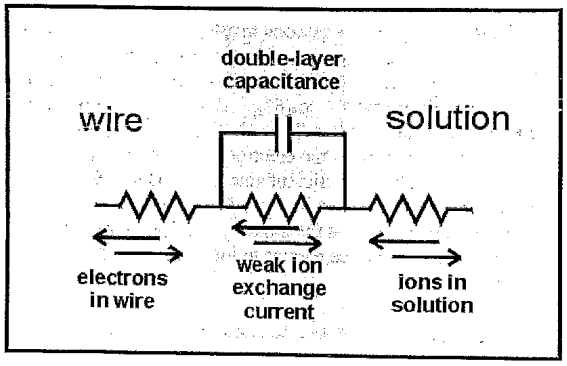
Metal Microelectrodes for Recording in Behaving Animals

Electrode impedance is a primary factor in the performance of any electrophysiological recording system. Electrode impedance describes the electrical characteristics of the complex interface between the metal wire microelectrode and the extracellular recording medium.

Modeling Electrode Impedance

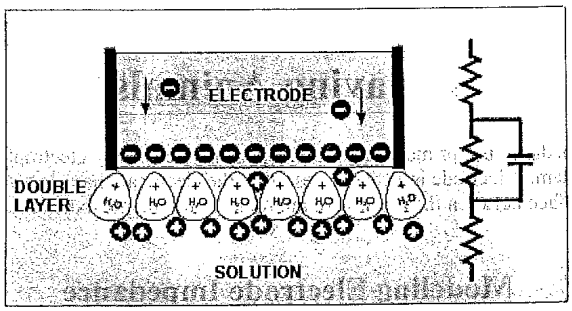
The equivalent electrical circuit of a metal microelectrode immersed in an electrolyte solution is shown in Figure 1. It consists of both resistive and capacitive elements. The resistive portion represents the mobility of charge carriers on each side of the solution/metal interface plus the weak exchange of ions across the so-called "double-layer".

Figure 1



The double-layer is created by polarized water molecules at the metal/brain interface. These water molecules form a thin dielectric (Figure 2). Thus, the double-layer is modelled by a capacitor. The exchange of ions across the double layer is weak and the interface is considered polarizable. That is, ions do not physically pass into and out of the electrode. Instead, changes in ionic concentration in the extracellular space attract (or repel) electrons to (or from) the interface.

Figure 2



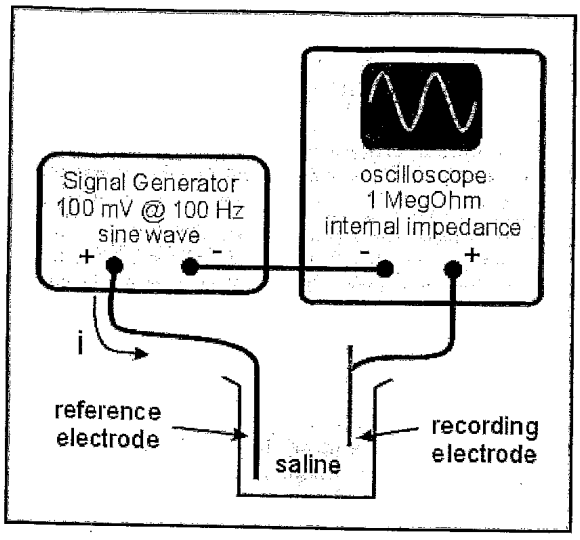
Both the capacitance and the resistance of this interface (Figure 2) are dependent on the size of the surface area of the metal in contact with the solution. In general, more surface area results in lower contact resistance and higher double-layer capacitance. Recall that higher capacitance values have smaller capacitive reactance at any given frequency. Thus, the overall effect of increasing surface area is to reduce electrode impedance.

Electrode impedance is related to Johnson (or thermal) noise. Johnson (or thermal) noise is generated by random movement of electrons in all resistive impedance elements. In general, at any given temperature, Johnson noise is proportional to resistive impedance. If the resistive impedance of the electrode is too high, these random fluctuations will interfere with the electrophysiological recording. Because electrode impedance is largely determined by surface area of the electrode tip, increasing tip diameter is one way to reduce the Johnson noise inherent to the electrode. Unfortunately, many applications require small tip diameters to obtain sufficient single-unit isolation. An alternative approach to reducing Johnson noise is to increase the surface area at the electrode tip without increasing tip diameter. This is the strategy employed by both "bubbling" and electroplating, two methods that reduce electrode impedance without sacrificing the selectivity of the microelectrode.

Measuring Electrode Impedance

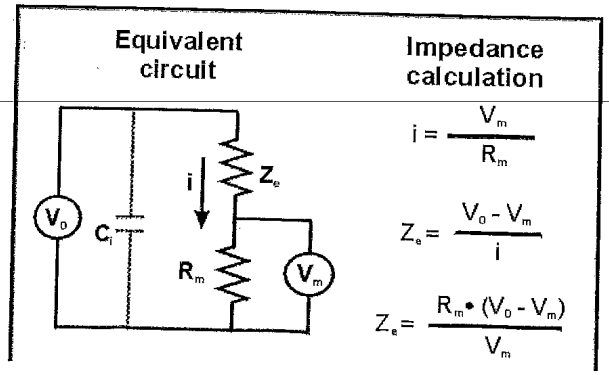
The impedance of an electrode is measured by passing a small AC current through the electrode, measuring the voltage drop across a known resistance placed in series with the electrode and using this information to calculate the impedance. In our lab we use a battery-powered sine-wave generator, an oscilloscope and a beaker of saline arranged as shown in Figure 3. The sine wave generator is set to 100 mV and 100 Hz. The oscilloscope sweep rate and sensitivity is adjusted to display several cycles of the test waveform. The reference electrode should have a large surface area.

Figure 3



Electrode impedance may be calculated directly from the oscilloscope reading using the equation in Figure 4 below. In our lab, we use a table to make quick determinations of electrode impedance from measured voltages. In this analysis, R_m is the internal impedance of the oscilloscope (1 megohm in our example), V_0 is the output of the sine-wave generator (100 millivolts in our example) and V_m is the value displayed on the oscilloscope. The electrode impedance measured will be dependent on the frequency of the signal generator. Electrode impedance will decrease as frequency is increased consistent with the model shown in Figure 1.

Figure 4



See Also: Table for Computation of Impedance

Practical Considerations

Immerse the electrode to a consistent depth. When a metal microelectrode is immersed in a conductive electrolyte, the wire and the solution form the plates of a capacitor (shown as C_i, above). The insulation coating the electrode wire is the dielectric and the capacitance depends on the depth of immersion. Thus, it is important to immerse the electrode to a consistent depth for each test so as to keep C_i invariant between tests. I typically immerse the tips to a depth of 2 mm.

Check the voltage output of the signal generator. Make sure the sine wave is devoid of DC offset (zero mean) by checking it with an oscilloscope or DC voltmeter.

Check the frequency of the sine wave. Impedance is dependent on the frequency of the signal used to measure it. For impedance measurements to be comparable and consistent, they must be made using the same frequency signal each time.

Procedures to Reduce Electrode Impedance

A small diameter tip is necessary to detect the local ionic changes that are generated extracellularly by an action potential. Wire diameter is chosen based on the cellular properties of the particular brain area being recorded. The surface area of the tip of the electrode is a strong determinant of the impedance of the electrode. Both capacitive reactance and contact resistance are reduced by increasing the surface area of the exposed electrode tip. Thus, procedures that increase surface area will result in electrodes with lower impedance. The impedance reduction procedures described below change the profile of the exposed tip without changing the diameter of the electrode tip. Thus, reducing impedance without sacrificing unit selectivity.

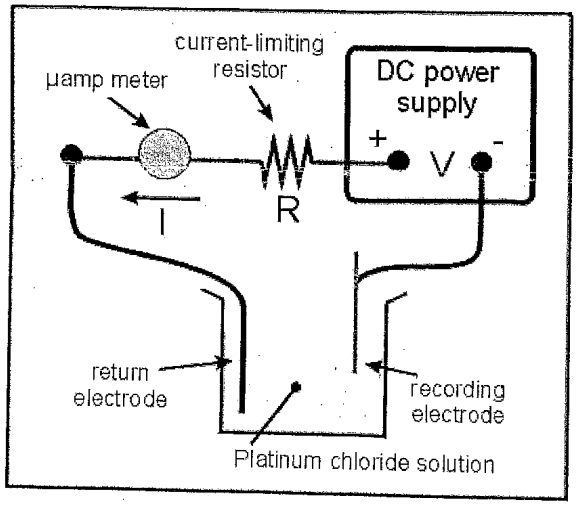
Method 1: the "bubbling" technique. One simple and inelegant method that has worked for us empirically is the byproduct of a procedure known as "bubbling" used to test the continuity of each wire. This procedure involves passing anodal current through each wire from a DC source, such as a 9 V or 12 V battery, and back through a saline solution. The current causes bubbles to form in the saline solution at the tip of the wire. This "bubbling" process confirms the integrity of the connections inside the electrode assembly and also results in a lowering of the impedance measured at the electrode tip. Impedance is reduced because the strong current that produces the bubbles in solution also causes etching of the electrode tip. This etching process increases the exposed surface area of the tip without changing the diameter, which determines the recording characteristics of the electrode. Damage to the insulation may also occur however, so the goal of this method would be to minimize insulation damage, which can be viewed under a stereomicroscope, while etching the tips sufficiently to achieve a useable impedance on each wire. Too much damage to the insulation will result in a non-

selective electrode that is unable to isolate single cells from their neighbors.

Method 2: plating. Another more conventional method of reducing electrode impedance is through electrolytic deposition of an inert metal onto the electrode tip (electroplating). Electroplating also reduces impedance by increasing the effective surface area without increasing the tip diameter. Platinum electroplating is accomplished by placing the electrode tips into a solution of platinum chloride and applying a small current such that the platinum in solution is reduced, causing platinum deposition at the tip of the metal electrode. I plate our electrodes using a solution of hydrogen hexachloroplatinate (8% PtCl4 by weight; Sigma Chemicals) with a multi-channel, constant-current plating device available from Eclectic Engineering Studio.

A simple constant-current plating circuit is formed using a large resistance (R) and a DC voltage source (V) as in Figure 5, below. The resistor used in this circuit must be at least ten times larger than the largest anticipated DC resistance of the electrode. The plating current is computed using Ohm's law.

Figure 5



In constructing such a circuit, the current (I) should be kept relatively small (1 to 10 microamps), and the return electrode should be either graphite or platinum to prevent contamination of the platinum chloride solution.

Immersion procedure for platinum electrodeposition. Both the plating current and the concentration of the plating solution will affect the immersion time required for sufficient plating. Typically we plate our electrodes at 5 microamps for 4-6 seconds in two separate immersions. Prior to each immersion, the electrodes are dipped into a 90% EtOH solution to reduce small air bubbles and remove dirt particles that can have a negative impact on electrolytic deposition. Wetting the tips with EtOH and repeating the

process several times serves to both reduce and distribute these factors and results in a more uniform impedance across the different wires. It is important to visually examine the tips of the wires when determining the optimal parameters for time, current, and platinum concentration. Prepare the electrodes for plating by cutting the tips using fine surgical scissors. Prior to plating, the exposed electrode tip should be "shiny" and the insulation should be intact. After plating to the desired impedance, the tips will have a rough "matte" appearance and impedance is typically reduced by 50-75%. If the insulation appears ragged or degraded, then the wires have been damaged, indicating that the current, duration or concentration of the plating solution should be reduced.

Variability of results. Finally it is worth noting that the exact parameters change slightly between days and on different electrodes, so we typically practice plating each electrode several times before making a final determination of the correct values. This approach is possible because extra wire is fed through the guide cannula to be cut off prior to surgery, thus several plating attempts are made before the wires are cut to their final length. Some variation in the final impedance between wires is normal and likely results from local variables in the plating environment or the mating of each wire through the electrode assembly.

Web Content from: Schoenbaum, G. Olfactory Learning and the Neurophysiological Study of Rat Prefrontal Function. In: CRC Series: Methods and Frontiers in Neuroscience. Edited by S.A. Simon and M.A.L. Nicolelis, CRC Press, NY, 2000.

This web page coauthored by Kevin B. Austin, Ph.D., Eclectic Engineering Studio, www.EclecticStudio.com

Calculating Electrode Impedance Using a Table

V_m (mV)	Z_e (kΩ)
50	1000
52	923
54	852
56	786
58	724
60	667
62	613
64	563
66	515
68	471
70	429
72	389
74	351
76	316
78	282
80	250
82	220
84	190
86	163
88	136
90	111
92	87
94	64
96	42

In our lab, we use this table (left) to compute electrode impedance (Z_e) from a measured voltage (V_m). The table applies to measurements made using the circuit in Metal Microelectrodes for Recording in Behaving Animals and these parameters:

Source voltage: V_0 = 100 mV pk-pk, sinusoid (100 Hz)

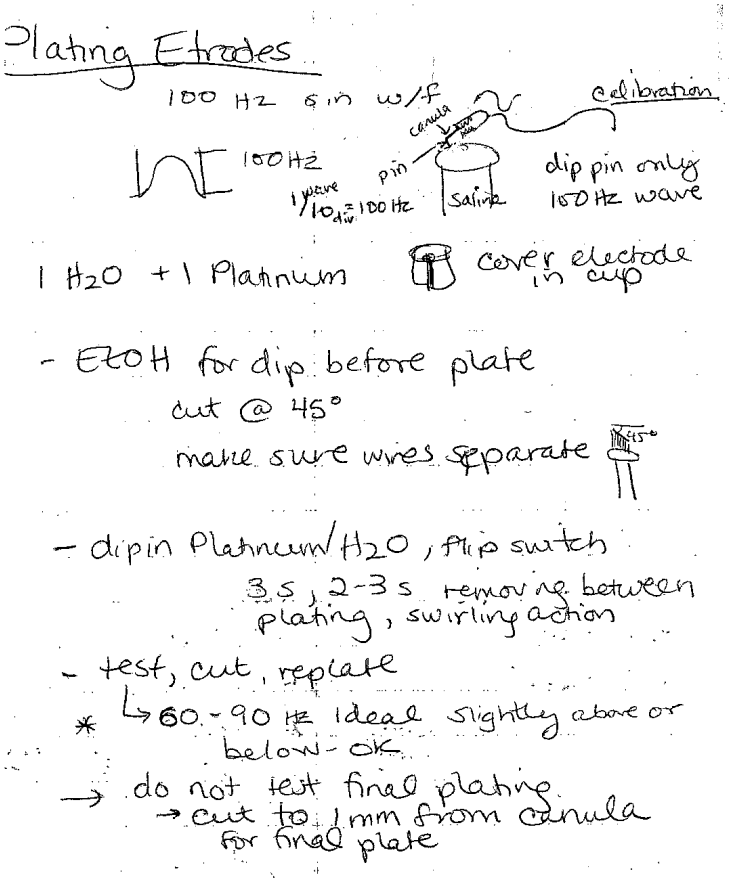
Oscilloscope internal impedance: R_m = 1 megohm

You may wish to adapt this technique for your particular situation. To do this, create a spreadsheet using an equation to compute impedance from your setup-specific parameters. One possible variation from our setup would use a digital voltmeter instead of an oscilloscope. This substitution would most likely require you to change R_m from 1 megohm to 10 megohms as most digital voltmeters have 10 megohms internal impedance. If you decide to use a digital voltmeter, make sure you are measuring "AC volts".

Web Content from: Schoenbaum, G. Olfactory Learning and the Neurophysiological Study of Rat Prefrontal Function. In: CRC Series: Methods and Frontiers in Neuroscience. Edited by S.A. Simon and M.A.L. Nicolelis, CRC Press, NY, 2000.

This web page coauthored by Kevin B. Austin, PhD., Eclectic Engineering Studio,

Appendix C1. Electrode protocol. Courtesy of the Schoenbaum lab.



•Surgery•

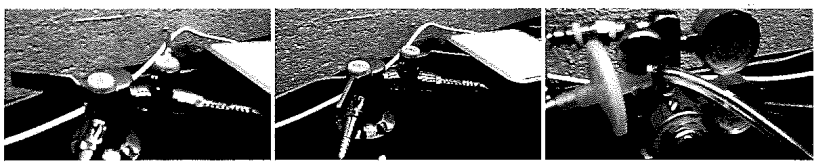
Weigh rat
Fill iso
Turn on o2
Alcohol dremel bit
Bead sterilize all tips and screwdriver
Box with o2
Rat in o2 box for 5 mins
Turn on iso
Rat in for 5 mins
Shave rat head and ears
Place back in box
Turn iso to nosecone
Ear bars
Bite bar
Push away tongue
Turn down iso
Heatpad for 3 mins
Heatpad with paper towel
Alcohol wipe
Betadine wipe
.1 lidocane injection
Reglove
Make incision
Scrape away tissue and muscle
Clamp hemostats onto muscle and over eyes
Clean with saline and gauze throughout
Mark lamda
Mark bregma
Attach pin to stereotax arm
Drop pin onto bregma
Measure height
Drop pin on lambda
Measure height
Two heights should be within 100 ums
Adjust head bar to account for difference
Remeasure
Write down measurements

Measure and drop pin onto drive spot
Mark drive spot
Remove stereotax arm
Drill spot
Clean out drill hole
Drop tripod onto hole
Mark spots for screws
Remove tripod
Drill screw holes
Screw screws
Dip electrode in alcohol
Attach electrode and rotate to wanted position
Straighten canula with background edges
Measure coordinates of the wires just above bregma
Remeasure
Redrill if necessary
Clean out hole of dura with microscope
Align electrode
Watch wires go into brain
Mark brain height
Advance 100 ums per minute
May come up short,
Cement mix. 4-5 scoops dental cement powder.
Dropper or two of grip liquid
Get around screws and around tripod. Get up and over the center of tripod and connect edges
Wait to dry
Break off jagged pieces
Fix a good squeeze of neosporin with .5 lidocane mixture
Disconnect fur and skin from cement
Neosporin cemented areas and inside
Unattach stereotax arm
Disconnect Popsicle stick
Slowly turn down lidocane
Take out from bite bar
Take out of ear bars
Heat heatpad for 3 mins
Place under recovery cage

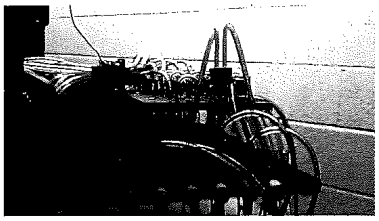
Appendix C2. Behavioral task training protocol.

Protocol for training TR rats

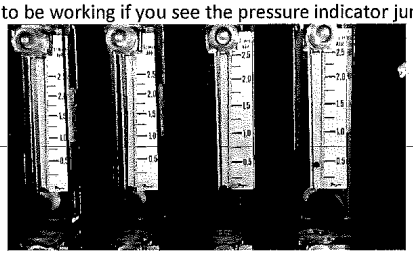
-Turn on the vacuum and air valves for boxes 5-8 in the nook. The air valve is the circular silver knob and must be turned all the way to the left. Do not touch the knob with red tape that says "Do not touch".



-Get 4 sucrose flasks from the fridge in 2205 and plug them into the green fluid lines of each rack.

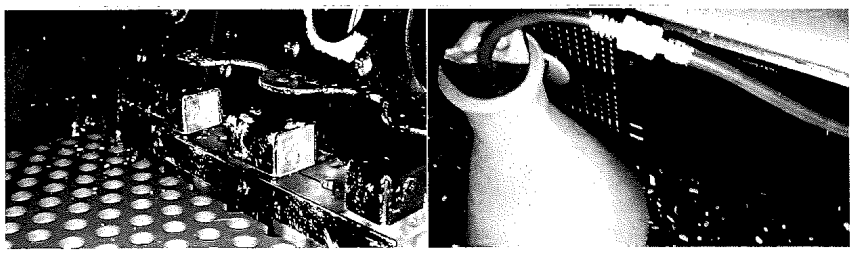


-Turn on the racks by flipping the big red switch at the bottom of both racks.
-Turn on all four computers and the video monitor.
-You will be prompted to login to a computer. After logging in, you can switch to the other four computers by using the button combo of "Scroll lock", "Scroll lock", Number, "Enter" (where Number is the number of the computer you want to switch to. 1 is Atlas, 2 is Aura, 3 is Eurybia, and 4 is Ares).
-Log in all four computers with the password "annasohn"
-Open the folder labeled: "Shortcut to BEHAVPRGM" or just "BEHAVPRGRM"
-Open the file named: "stopsignal_070111.exe"
-Input 0 when prompted to test processors.
-Input 1 when prompted for box number.
-When prompted to test odors, input y (meaning yes). Then, press enter repeatedly to test all 16 odor lines, which are considered to be working if you see the pressure indicator jump when a line is selected.



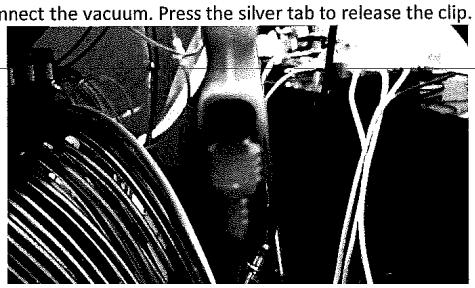
-When prompted to test fluids, input y. Make sure that the sucrose flasks are connected to the green fluid lines, the green fluid lines are completely connected, the blue and red lines are clamped with hemostats, and the vacuum is connected.

-You will see a fluids menu where you can select 1-6, 7, 8, 9, or 10. 1 through 6 denotes the 6 thin, colored fluid lines. 1 is the far right green line, 2 is the blue line next to it, 3 is the red line next to the blue line, and so forth. 7 will flush the lines and this is what you need to do first. When you press 7 and then enter, it will prompt you to press enter again. When you press enter, the vacuum will attempt to flush sucrose from the flask all the way down to the trap at the bottom of the rack. If you do not hear the fluid streaming down or see any fluid flowing through the tube leading directly to the trap, then you need to press your finger completely over both of the reward wells inside the box and alternate tapping both wells. Doing so will create suction and help to draw the fluid down.

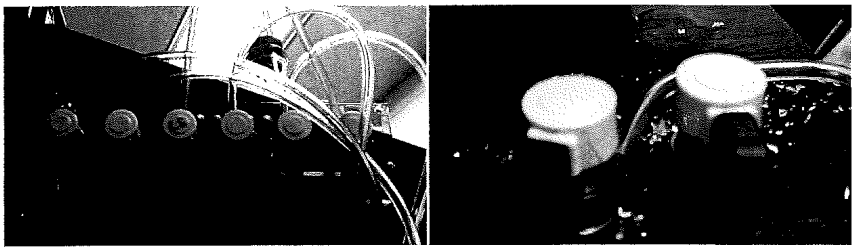


-The first fluid to be flushed down is called Right Fluid A by the program and refers to the green sucrose line 4 from the right. When you establish that the fluid is in fact flowing into the trap, press enter to move onto Left Fluid A. This is the green sucrose line on the far right. Again, establish that fluid makes it all the way to the trap. You can ignore Fluids B and C, so press enter repeatedly until the program asks if this is acceptable. Press y.

-Now you need to disconnect the vacuum. Press the silver tab to release the clip.



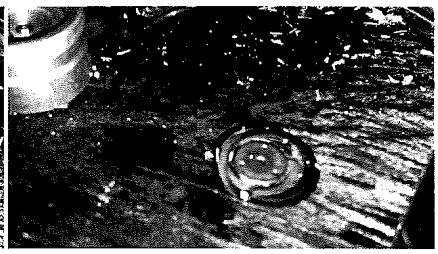
-Now that the vacuum is disconnected, you can look at the fluids menu. You will see that you can select 1 through 10 again. Press 1 and enter. You will then be prompted to input a value. Input 100 and enter, and then when asked if this is acceptable, press y. Then, press 4 and enter. Again, input 100 and enter, and then y. You have set the fluid levels for the green sucrose lines (1 corresponds to the left sucrose line and 4 corresponds to the right sucrose line). You now need to take a flashlight (image not included) and make sure that the fluid levels are equal in both wells. You should see about half of the well filled with fluid in both cases. If they are not equal, then take a paper towel and wipe out both wells, then repeat the process 3 or 4 times. If they are not equal at this point, then the problem is likely to be with the solenoids.



-There are two solenoids that "gate" the flask to the rest of the green sucrose lines and two solenoids at the back of the rack that "gate" the exit of fluid in the well down to the trap.

-If the wells are constantly filling with fluid, then the first two solenoids could be clogged with mold or rust. Unscrew the metal ring under the yellow cap to disassemble the solenoid. Make sure that the sucrose is flushed out of the green lines completely (repeat the flush with 7 on the fluid menu while the green tubes are unplugged) so that no fluid leaks out during this troubleshooting. When the solenoid is open, it looks like this.

-There will be the top of the solenoid (held in this picture) and the base of the solenoid. There will also be a circular spider valve attached by magnetism to one of these two components and a silver washer on top of the spider valve.

-Remove the spider valve from the base with a screwdriver (just pry it up) or remove the spider valve from the top by sliding it off. Clean any rust or black mold off of the components with a wet paper towel or a metal tool good for scraping. Then, take the spider valve and orient it so that the little rubber part in the middle faces into the hole of the base of the solenoid (or look at it as the rubber part is facing away from the yellow cap of the top of the solenoid). Then, place the silver washer on top of the spider valve as seen in the second picture. Then, screw the top of the solenoid back onto the base.

-If the wells are not constantly filling with fluid, but rather are not filling with fluid at all (after multiple attempts of filling the wells normally), then the second set of solenoids could be the issue. Repeat the exact same process, but look for rat hairs that could be clogging the hole in the base of the solenoid.

-Once you have set the two green sucrose line fluid amounts to 100, you may press enter when you are on the fluids menu. This will take you to the next step of the setup process.
-Once the behavioral program is set up, put the appropriate rat into the box and let him run.

-When his session is done, press "Ctrl" + "n" to end the program. Write down the appropriate information, including number of trials and correct trials out of 20.

-If you are the last person to run rats in a day, then you must also shut down the racks.

-When you hit Ctrl + n, you will be given the option to calibrate fluids again. Press "y" to return to the fluids menu. Remove the green sucrose tubes from the flask on top of the rack and place them into another flask of hot, nearly steaming water. Then, press "7", "enter", and "enter" again to begin flushing through Right Fluid A, or the fourth fluid line from the right. Flush half of the contents of the hot water flask through this line. Then, press "enter" again to flush the last half of the flask through Left Fluid A. Once this is done, unclamp the red and blue line hemostats. Get a plastic squirtbottle and fill it with hot, nearly steaming water (make sure to put the cap on afterwards). Then, press "enter" to activate Right Fluid B and squirt water through all four red and blue lines for ten seconds each. Once this is done, you will be prompted "Is this acceptable?". Press "n" and go back through all 6 lines while there are no flasks connected to them. Flush through all 6 lines so that all water is cleared out. Then, reclamp the red and blue lines.

-Remove the trap at the bottom of the rack by unclipping the white clasp on the left and removing the rubber stopper on top. Dump all of the waste water down the drain and rinse the trap with hot water.

-Take a paper towel wetted with hot water and clean out the wells, the odor port, and the floor of the box. Then, remove the poop tray and use a paper towel to brush off all debris into the trashcan outside in the hall. Rinse the poop tray with hot water in the sink after this and return it to its slot under the box.

- Shut Down Computers
- Turn Off Rack
- Turn off Vac and Air

Appendix D: MATLAB Data Analysis Script

```
align = 16;
pre = -5;
post = 5;

e1start = 22;
add_to_e1start = -2;
e1stop = 22;
add_to_e1stop = -1;

e2start = 16;
add_to_e2start = 0;
e2stop = 17;
add_to_e2stop = 0;

for cell_count = 1:24;
%control below baseline
if cell_count ==1 load tr12112020;  cell=sig001a_1;hem=1; end
if cell_count ==2 load tr84110620;  cell=sig008a_1;hem=1; end
if cell_count ==3 load tr84110720;  cell=sig004a_1;hem=1; end
if cell_count ==4 load tr84122820;  cell=sig006a_1;hem=1; end
if cell_count ==5 load tr84122720;  cell=sig004a_1;hem=1; end
if cell_count ==6 load tr84122020;  cell=sig004a_1;hem=1; end
if cell_count ==7 load tr84122020;  cell=sig004b_1;hem=1; end
if cell_count ==8 load tr84121920;  cell=sig001a_1;hem=1; end
if cell_count ==9 load tr84121920;  cell=sig003a_1;hem=1; end
if cell_count ==10 load tr84121920;  cell=sig003b_1;hem=1; end
if cell_count ==11 load tr84121820;  cell=sig002a_1;hem=1; end
if cell_count ==12 load tr84121820;  cell=sig008a_1;hem=1; end
if cell_count ==13 load tr84121720;  cell=sig001a_1;hem=1; end
if cell_count ==14 load tr84121420;  cell=sig004a_1;hem=1; end
if cell_count ==15 load tr84121320;  cell=sig001a_1;hem=1; end
if cell_count ==16 load tr84121320;  cell=sig008a_1;hem=1; end
if cell_count ==17 load tr84121220;  cell=sig004a_1;hem=1; end
if cell_count ==18 load tr84121120;  cell=sig001a_1;hem=1; end
if cell_count ==19 load tr84121020;  cell=sig006a_1;hem=1; end
if cell_count ==20 load tr84120720;  cell=sig003a_1;hem=1; end
if cell_count ==21 load tr84120620;  cell=sig006a_1;hem=1; end
if cell_count ==22 load tr84120620;  cell=sig006b_1;hem=1; end
if cell_count ==23 load tr84120320;  cell=sig003a_1;hem=1; end
if cell_count ==24 load tr84113020;  cell=sig007a_1;hem=1; end

%Use lights on (LO) and light off (LF) for start and
%stop of each trials.

%Use correct ITI (CI) to split into correct trials only
light_on=[];

all_trials = cat(2,LO,LF);
all_trials(:,3:40) = -999;
```

`

```
%column 3 = reward delivered on left
b=1;
for a = 1:length(all_trials(:,1)),
if b > length(StrobedDIO00252(:,1))
    break
end
if StrobedDIO00252(b,1) < all_trials(a,2) &&
StrobedDIO00252(b,1) > all_trials(a,1)
all_trials(a,3) = StrobedDIO00252(b,1);
b=b+1;
else
all_trials(a,3) = -999;
end
end

%column 4 = reward delivered on right
b=1;

for a = 1:length(all_trials(:,1)),
if b > length(StrobedDIO00253(:,1))
    break
end
if StrobedDIO00253(b,1) < all_trials(a,2) &&
StrobedDIO00253(b,1) > all_trials(a,1)
all_trials(a,4) = StrobedDIO00253(b,1);
b=b+1;
else
all_trials(a,4) = -999;
end
end

%column 5 = 1st odor used
b=1;
for a = 1:length(all_trials(:,1)),
if b > length(StrobedDIO00002(:,1))
    break
end
if StrobedDIO00002(b,1) < all_trials(a,2) &&
StrobedDIO00002(b,1) > all_trials(a,1)
all_trials(a,5) =  StrobedDIO00002(b,1);
b=b+1;
else
all_trials(a,5) = -999;
end
end

%column 6 = 2nd odor used
b=1;
for a = 1:length(all_trials(:,1)),
if b > length(StrobedDIO00003(:,1))
    break
```

`

```
end
if StrobedDIO00003(b,1) < all_trials(a,2) &&
StrobedDIO00003(b,1) > all_trials(a,1)
all_trials(a,6) = StrobedDIO00003(b,1);
b=b+1;
else
all_trials(a,6) = -999;
end
end

%column 7 = stop signal on
b=1;
for a = 1:length(all_trials(:,1)),
if b > length(StrobedDIO00302(:,1))
    break
end
if StrobedDIO00302(b,1) < all_trials(a,2) &&
StrobedDIO00302(b,1) > all_trials(a,1)
all_trials(a,7) = StrobedDIO00302(b,1);
b=b+1;
else
all_trials(a,7) = -999;
end
end

%column 8 = stop signal off
b=1;
for a = 1:length(all_trials(:,1)),
if b > length(StrobedDIO00303(:,1))
    break
end
if StrobedDIO00303(b,1) < all_trials(a,2) &&
StrobedDIO00303(b,1) > all_trials(a,1)
all_trials(a,8) = StrobedDIO00303(b,1);
b=b+1;
else
all_trials(a,8) = -999;
end
end

%FIND TIME OF WHEN BROKE BEAM IN LEFT WELL
b=1;
for a = 1:length(all_trials(:,1))
if b > length(WPL(:,1))
    break
end
if WPL(b,1) < all_trials(a,2) &&  WPL(b,1) > all_trials(a,1)
all_trials(a,12) = WPL(b,1);
b=b+1;
else
all_trials(a,12) = -999;
end
```

`

```
end

%FIND TIME OF WHEN BROKE BEAM IN RIGHT WELL
b=1;
for a = 1:length(all_trials(:,1)),
if b > length(WPR(:,1))
    break
end
if WPR(b,1) < all_trials(a,2) &&  WPR(b,1) > all_trials(a,1)
all_trials(a,13) = WPR(b,1);
b=b+1;
else
all_trials(a,13) = -999;
end
end

%FIND TIME OF WHEN LEFT THE LEFT WELL
b=1;
for a = 1:length(all_trials(:,1)),
if b > length(WUL(:,1))
    break
end
if WUL(b,1) < all_trials(a,2) &&  WUL(b,1) > all_trials(a,1)
all_trials(a,14) = WUL(b,1);
b=b+1;
else
all_trials(a,14) = -999;
end
end

%FIND TIME OF WHEN LEFT THE RIGHT WELL
b=1;
for a = 1:length(all_trials(:,1)),
if b > length(WUR(:,1))
    break
end
if WUR(b,1) < all_trials(a,2) &&  WUR(b,1) > all_trials(a,1)
all_trials(a,15) = WUR(b,1);
b=b+1;
else
all_trials(a,15) = -999;
end
end

%FIND TIME OF WHEN LEFT odor port
b=1;
for a = 1:length(all_trials(:,1)),
if b > length(OU(:,1))
    break
end
if OU(b,1) < all_trials(a,2) &&  OU(b,1) > all_trials(a,1)
all_trials(a,16) = OU(b,1);
```

`

```
b=b+1;
else
all_trials(a,16) = -999;
end
end

%well entry time for left and right
for a = 1:length(all_trials(:,1)),
if all_trials(a,12) > -999
    all_trials(a,17) = all_trials(a,12);
end
if all_trials(a,13) > -999
    all_trials(a,17) = all_trials(a,13);
end
end

%well exit time for left and right
for a = 1:length(all_trials(:,1)),
if all_trials(a,14) > -999
    all_trials(a,18) = all_trials(a,14);
end
if all_trials(a,15) > -999
    all_trials(a,18) = all_trials(a,15);
end
end

%reward delivered right and left
for a = 1:length(all_trials(:,1)),
if all_trials(a,3) > -999
    all_trials(a,20) = all_trials(a,3);
end
if all_trials(a,4) > -999
    all_trials(a,20) = all_trials(a,4);
end
end

%time odor on for all odors
for a = 1:length(all_trials(:,1)),
if all_trials(a,5) > -999
    all_trials(a,22) = all_trials(a,5);
end
% if all_trials(a,6) > -999
%     all_trials(a,22) = all_trials(a,6);
% end
if all_trials(a,6) > -999
    all_trials(a,22) = all_trials(a,6);
end
end

%Was the last trial rewarded?
for a = 2:length(all_trials(:,1))
    junk = -999;
```

`

```
    if all_trials(a-1,20)>-999
        junk = all_trials(a-1,20);
    end
    all_trials(a,24) = junk;
end

%last trial went left
for a = 2:length(all_trials(:,1))
       junk = -999;
        if all_trials(a-1,12)>-999
            junk = all_trials(a-1,12);
        end
        all_trials(a,29) = junk;
end

%last trial went right
for a = 2:length(all_trials(:,1))
        junk = -999;
        if all_trials(a-1,13)>-999
            junk = all_trials(a-1,13);
        end
        all_trials(a,30) = junk;
end

%last trial was left odor
for a = 2:length(all_trials(:,1))
        junk = -999;
        if all_trials(a-1,5)>-999
            junk = all_trials(a-1,5);
        end
        all_trials(a,31) = junk;
end

%last trial was right odor
for a = 2:length(all_trials(:,1))
        junk = -999;
        if all_trials(a-1,6)>-999
            junk = all_trials(a-1,6);
        end
        all_trials(a,32) = junk;
end

% 2 trials before was left odor     % and correct
for a = 3:length(all_trials(:,1))
        junk = -999;
        if all_trials(a-2,5)>-999 % & all_trials(a-2,20)>-999
            junk = all_trials(a-1,5);
        end
        all_trials(a,34) = junk;
end

% 2 trials before was right odor      % and correct
```

```
for a = 3:length(all_trials(:,1))
        junk = -999;
        if all_trials(a-2,6)>-999  %& all_trials(a-2,20)>-999
            junk = all_trials(a-1,6);
        end
        all_trials(a,35) = junk;
end

% 3 trials before was left odor            % and correct
for a = 4:length(all_trials(:,1))
        junk = -999;
        if all_trials(a-3,5)>-999 %& all_trials(a-3,20)>-999
            junk = all_trials(a-1,5);
        end
        all_trials(a,36) = junk;
end

% 3 trials before was right odor               %and correct
for a = 4:length(all_trials(:,1))
        junk = -999;
        if all_trials(a-3,6)>-999 % & all_trials(a-3,20)>-999
            junk = all_trials(a-1,6);
        end
        all_trials(a,37) = junk;
end

%FIND TIME OF WHEN BROKE BEAM IN ODOR PORT
b=1;
for a = 1:length(all_trials(:,1)),
if b > length(OP(:,1))
    break
end
if OP(b,1) < all_trials(a,2) &&  OP(b,1) > all_trials(a,1)
all_trials(a,35) = OP(b,1);
b=b+1;
else
all_trials(a,35) = -999;
end
end

% REACTION TIME FOR ALL
all_trials(:,25) = (all_trials(:,16)-all_trials(:,22)) - .5;

% MOVEMENT TIME FOR ALL
all_trials(:,26) = (all_trials(:,17)-all_trials(:,16));

% LIGHT ON LATENCY
all_trials(:,27) = (all_trials(:,22)-all_trials(:,1)) - .5;

% EPOCH OF INTEREST 2
for a=1:length(all_trials(:,1))
```

`

```
all_trials(a,32) = (length(find(cell(:,1) <
(all_trials(a,e1stop)+add_to_e1stop)  & cell(:,1) >=
(all_trials(a,e1start)+add_to_e1start))) /
((all_trials(a,e1stop)+add_to_e1stop)-
(all_trials(a,e1start)+add_to_e1start)));
end
% EPOCH OF INTEREST 2
for a=1:length(all_trials(:,1))
all_trials(a,33) = (length(find(cell(:,1) <
(all_trials(a,e2stop)+add_to_e2stop)  ...
& cell(:,1) >= (all_trials(a,e2start)+add_to_e2start)))...
/ ((all_trials(a,e2stop)+add_to_e2stop)-
(all_trials(a,e2start)+add_to_e2start)));
end

%
%AVERAGE FIRING RATE FOR POPULATION HISTOGRAM ALIGNED TO SOME
EPOCH
bin_centers=pre:0.1:post;
  bin_centers = bin_centers - 0.05;
for a = 1:length(all_trials(:,1)),
        spike_times_idx = find(cell(:,1) <=
(all_trials(a,align)+post)  & cell(:,1) >=
(all_trials(a,align)+pre));
        spike_times = cell(spike_times_idx);
        spike_times_normalized = spike_times -
all_trials(a,align);
         hist_trial = hist(spike_times_normalized,bin_centers);
         all_trials(a,40:(40+length(hist_trial(1,:)))-1) =
hist_trial;
end
%%%%%%


% %Times of when these events happened
% 1. House Light on
% 2. House Light off
% 3. Reward delivered on left
% 4. Reward delivered on right
% 5. Odor 1 onset [left]
% 6. Odor 2 onset [right]
% 7. Stop signal on
% 8. Stop signal off
% 12. Broke beam in left fluid well
% 13. Broke beam in right fluid well
% 14. Exited left fluid well
% 15. Exited right fluid well
% 16. Exited odor port
% 17. Well entry time for either left or right
% 18. Well exit time for left or right
% 20. Reward delivery for left or right
% 22. Time of odor onset for all odors
```

`

```
% 25. Reaction time
% 26. Movement time
% 27. Latency to nosepoke after houselights come on.
% 32. baseline epoch
% 33. analysis epoch
% 40 through end of matrix = spiking over time

% These idx variables will find all trials in a session that fit
a
% specific description. The important ones in this code are
lf_idx and
% rt_idx, which find all left trials (go and stop) and all right
trials (go
% and stop)

go_lf_idx = find(all_trials(:,20)>-999 & all_trials(:,5)>-999 &
all_trials(:,7)==-999);
go_rt_idx = find(all_trials(:,20)>-999 & all_trials(:,6)>-999 &
all_trials(:,7)==-999);
st_lf_idx = find(all_trials(:,20)>-999 & all_trials(:,6)>-999 &
all_trials(:,7)>-999);
st_rt_idx = find(all_trials(:,20)>-999 & all_trials(:,5)>-999 &
all_trials(:,7)>-999);

lf_idx = find(all_trials(:,20)>-999 & all_trials(:,14)>-999);
rt_idx = find(all_trials(:,20)>-999 & all_trials(:,15)>-999);

% Firing rate during epoch of interest, odor port exit to well
entry, for
% all trial types as well as each type individually

go_lf_epoch = (all_trials(go_lf_idx,33));
go_rt_epoch = (all_trials(go_rt_idx,33));
st_lf_epoch = (all_trials(st_lf_idx,33));
st_rt_epoch = (all_trials(st_rt_idx,33));

lf_epoch = (all_trials(lf_idx,33));
rt_epoch = (all_trials(rt_idx,33));

%Histogram for firing during the trial, 5 seconds before well
entry to 5
%seconds after well entry
go_lf_hist = all_trials(go_lf_idx,40:length(all_trials(1,:)));
go_rt_hist = all_trials(go_rt_idx,40:length(all_trials(1,:)));
st_lf_hist = all_trials(st_lf_idx,40:length(all_trials(1,:)));
st_rt_hist = all_trials(st_rt_idx,40:length(all_trials(1,:)));

lf_hist = all_trials(lf_idx,40:length(all_trials(1,:)));
rt_hist = all_trials(rt_idx,40:length(all_trials(1,:)));

%Take the means of each trial epoch firing rate
lf_epoch_m = mean(lf_epoch);
```

`

```
rt_epoch_m = mean(rt_epoch);

go_lf_epoch_m = mean(go_lf_epoch);
go_rt_epoch_m = mean(go_rt_epoch);
st_lf_epoch_m = mean(st_lf_epoch);
st_rt_epoch_m = mean(st_rt_epoch);

% This defines the direction that the cell prefers.
if lf_epoch_m > rt_epoch_m
   go_preferred = go_lf_epoch_m;
   st_preferred = st_lf_epoch_m;
   go_preferred_hist = go_lf_hist;
   st_preferred_hist = st_lf_hist;
   go_nonpreferred = go_rt_epoch_m;
   st_nonpreferred = st_rt_epoch_m;
   go_nonpreferred_hist = go_rt_hist;
   st_nonpreferred_hist = st_rt_hist;
end

if rt_epoch_m > lf_epoch_m
   go_preferred = go_rt_epoch_m;
   st_preferred = st_rt_epoch_m;
   go_preferred_hist = go_rt_hist;
   st_preferred_hist = st_rt_hist;
   go_nonpreferred = go_lf_epoch_m;
   st_nonpreferred = st_lf_epoch_m;
   go_nonpreferred_hist = go_lf_hist;
   st_nonpreferred_hist = st_lf_hist;
end

% This defines the direction of the trial relative to the
electrode placement
go_ipsi = go_rt_epoch_m;
go_ipsi_hist = go_rt_hist;
go_contra = go_lf_epoch_m;
go_contra_hist = go_lf_hist;

st_ipsi = st_rt_epoch_m;
st_ipsi_hist = st_rt_hist;
st_contra = st_lf_epoch_m;
st_contra_hist = st_lf_hist;

if contraipsi == 0
      go_ipsi = go_lf_epoch_m;
      go_ispi_hist = go_lf_hist;
      go_contra = go_rt_epoch_m;
      go_contra_hist = go_rt_hist;

      st_ipsi = st_lf_epoch_m;
      st_ipsi_hist = st_lf_hist;
      st_contra = st_rt_epoch_m;
      st_contra_hist = st_rt_hist;
```

`

```
end

% These eight lines create the histogram means
go_preferred_hist_m(cell_count,:) = mean(go_preferred_hist);
st_preferred_hist_m(cell_count,:) = mean(st_preferred_hist);
go_nonpreferred_hist_m(cell_count,:) =
mean(go_nonpreferred_hist);
st_nonpreferred_hist_m(cell_count,:) =
mean(st_nonpreferred_hist);

go_ipsi_hist_m(cell_count,:) = mean(go_ipsi_hist);
go_contra_hist_m(cell_count,:) = mean(go_contra_hist);
st_ipsi_hist_m(cell_count,:) = mean(st_ipsi_hist);
st_contra_hist_m(cell_count,:) = mean(st_contra_hist);

% These lines define a variety of distributions
go_pnp_dist = (go_preferred-
go_nonpreferred)/(go_preferred+go_nonpreferred);
stop_pnp_dist = (st_preferred-
st_nonpreferred)/(st_preferred+st_nonpreferred);
preferred_dist = (((st_preferred)-
(go_preferred))/((st_preferred)+(go_preferred)));
nonpreferred_dist = (((st_nonpreferred)-
(go_nonpreferred))/((st_nonpreferred)+(go_nonpreferred)));

go_ci_dist = (((go_ipsi)-(go_contra))/((go_ipsi)+(go_contra)));
stop_ci_dist = (((st_ipsi)-(st_contra))/((st_ipsi)+(st_contra)));
contra_dist = (((st_contra)-
(go_contra))/((st_contra)+(go_contra)));
ipsi_dist = (((st_ipsi)-(go_ipsi))/((st_ipsi)+(go_ipsi)));

% Keep the numbers you want before the end of the loop.
numbers(cell_count,:) = [go_pnp_dist stop_pnp_dist preferred_dist
nonpreferred_dist go_ci_dist stop_ci_dist contra_dist ipsi_dist];

%Clear data
all_trials = [];
OU = [];
cell=[];

end %this 'end' is the end after it cycles through all cells in
the very first
%loop.

go_preferred_pop_m = mean(go_preferred_hist_m,1);
st_preferred_pop_m = mean(st_preferred_hist_m,1);
go_nonpreferred_pop_m = mean(go_nonpreferred_hist_m,1);
st_nonpreferred_pop_m = mean(st_nonpreferred_hist_m,1);

% The following four lines smooth the population histograms.
go_preferred_pop_m_smooth = smooth(bin_centers,
go_preferred_pop_m, .1, 'rloess');
```

`

```
st_preferred_pop_m_smooth = smooth(bin_centers,
st_preferred_pop_m, .1, 'rloess');
go_nonpreferred_pop_m_smooth = smooth(bin_centers,
go_nonpreferred_pop_m, .1, 'rloess');
st_nonpreferred_pop_m_smooth = smooth(bin_centers,
st_nonpreferred_pop_m, .1, 'rloess');

figure
plot(bin_centers, go_preferred_pop_m_smooth, 'b'); hold on;
plot(bin_centers, st_preferred_pop_m_smooth, 'r'); hold on;
plot(bin_centers, go_nonpreferred_pop_m_smooth, 'g'); hold on;
plot(bin_centers, st_nonpreferred_pop_m_smooth, 'y'); hold off;
legend('Go Preferred', 'Stop Preferred', 'Go Nonpreferred', 'Stop
Nonpreferred');
xlabel('Time (s)');
ylabel('spikes/s');
title('Control Below Baseline, Stop vs Go, Preferred vs
Nonpreferred');

bin = -1:.1:1; bin=bin-0.05;

figure
idx_numbers = 1;
hist(numbers(:,idx_numbers),bin); hold on;
axis square
xlabel('(go_p-go_np)/(go_p+go_np)');
ylabel('spikes/s');
n = length(numbers(:,idx_numbers));
sr = signrank(numbers(:,idx_numbers));
title(['Control Below Baseline Preferred/Nonpreferred
Distribution, Go Trials', ' ', num2str(n), ' ', num2str(sr)]);
axis tight;

figure
idx_numbers = 2;
hist(numbers(:,idx_numbers),bin); hold on;
axis square
xlabel('(st_p-st_np)/(st_p+st_np)');
ylabel('spikes/s');
n = length(numbers(:,idx_numbers));
sr = signrank(numbers(:,idx_numbers));
title(['Control Below Baseline Preferred/Nonpreferred
Distribution, Stop Trials', ' ', num2str(n), ' ', num2str(sr)]);
axis tight;

figure
idx_numbers = 3;
hist(numbers(:,idx_numbers),bin); hold on;
axis square
xlabel('(stop-go)/(stop+go)');
ylabel('spikes/s');
n = length(numbers(:,idx_numbers));
```

`

```
sr = signrank(numbers(:,idx_numbers));
title(['Nicotine Above Baseline Stop/Go Distribution, Preferred
Direction', ' ', num2str(n), ' ', num2str(sr)]);
axis tight;

figure
idx_numbers = 4;
hist(numbers(:,idx_numbers),bin); hold on;
axis square
xlabel('(stop-go)/(stop+go)');
ylabel('spikes/s');
n = length(numbers(:,idx_numbers));
sr = signrank(numbers(:,idx_numbers));
title(['Nicotine Above Baseline Stop/Go Distribution,
Nonpreferred Direction', ' ', num2str(n), ' ', num2str(sr)]);
axis tight;
```

Glossary

5-choice serial reaction time task (5-CSRTT) - A method of measuring impulsivity and visual process in rats. Rats are given a brief visual stimulus, then make a choice from 5 wells in response to the stimulus. Lower accuracy and higher reaction times are correlated with ADHD symptoms.

Acetylcholine - A neurotransmitter which is involved in decision-making and attention, among other things. It also guides dopaminergic neurons during fetal brain development. Acetylcholine binds the nicotinic acetylcholinergic receptor (nAchR), which nicotine can also bind as an agonist. The neurons that produce acetylcholine are part of the cholinergic system.

Agonist - A molecule that mimics a neurotransmitter and binds its receptor, causing the same effect as the neurotransmitter itself.

Animal model - A non-human animal that represents a disease through structurally and functionally homologous physiology.

Antagonist - A molecule that blocks a neurotransmitter from binding its receptor, preventing the effect of the neurotransmitter.

Anterior cingulate cortex (ACC) - The front of the cingulate cortex; this region is responsible for decision-making, reward anticipation, and impulse control, among other things.

Attention - In this paper, attention refers to demonstrating responses relevant to a behavioral task.

Blood oxygen level-dependent functional MRI (BOLD fMRI) - An imaging technique which detects changes in brain activity by measuring the amount of oxygen delivered by the blood to a certain brain area at a given time during a task.

Cerebrum - The brain. Regions of the cerebrum are divided into several anatomical regions: dorsal (upper), ventral (lower), lateral (left and right edges), medial (center), anterior (front), and posterior (back).

Conditioned stimulus - A stimulus that would not normally elicit a response which is paired with one that would (the unconditioned stimulus) to train a subject to respond to the conditioned stimulus the same as they would to the unconditioned stimulus. For instance, when a puff of air to the eye is preceded by a tone, subjects learn to close their eyes at the tone.

Cortex - The outermost structure of neural tissue in the human cerebrum

Directional index - The difference between preferred and non-preferred direction trial activity ((preferred - non-preferred)/(preferred + non-preferred)) for each neuron.

Dopamine - A neurotransmitter which is involved in reward, cognition, and motor control. Dopamine binds its receptors D2 and D3 and it taken from the synapse by its transporter DAT. The neurons that produce dopaminergic are part of the dopaminergic system.

Executive functioning - The theorized cognitive system responsible for managing other cognitive processes, including working memory, planning and execution of tasks, and problem solving.

Frontal lobe - An area of the cerebrum responsible for executive functioning, among other things.

GO trial - A SST trial during which only the go-signal is presented.

Hippocampus - A brain region involved in memory formation, emotion, navigation, and spatial orientation.

Homology - When some part of the physiology of two different species are conserved from the same ancestral origin. Species can show structural homology, in which they have structures that are anatomically similar, and functional homology, in which they have structures which serve the same function in both species.

Hyperactivity - In this paper, hyperactivity refers to increased locomotion.

Impulsivity - In this paper, impulsivity refers to failing to inhibit an already-initiated response to a stimulus. Impulsivity is here measured by increased SSRT and decreased stop accuracy.

Inhibition - In this paper, inhibition refers to stopping an already-initiated response to a stimulus. This should not be confused with inhibitory neurons, which cause downstream neurons to fire less often and are not necessarily involved in response inhibition.

Limbic system - A collection of structures involved in emotion, long-term memory, behavior, and motivation.

Magnetic resonance imaging (MRI) - An imaging technique which shows soft tissue structures of the body and can be used to measure cortical volumes.

Monaminergic - Neurons that produce the monamine neurotransmitters, such as dopamine and noradrenaline, are called monaminergic.

Neurotransmitter - A signaling molecule which binds a receptor and causes a neuron to fire or not fire. Neurotransmitters are produced by upstream, afferent neurons, released

into the synaptic space, then bind the receptor of the downstream, efferent neuron, after which they are taken back into the upstream neuron via transporters.

Noradrenaline - A neurotransmitter which is involved in attention, among other things. Noradrenaline binds alpha and beta receptors. The neurons that produce noradrenaline are part of the adrenergic system.

Nucleus accumbens (NAcc) - A brain region involved in pleasure, fear, impulsivity, reward, and learning.

Parietal lobe - An area of the cerebrum responsible for integrating sensory information.

Positron Emission Tomography (PET) - An imaging technique which senses gamma rays emitted by a radioactive tracer bound to a biologically active molecule to trace the activity of the molecule, such as dopamine.

Prefrontal Cortex (PFC) - The anterior portion of the frontal lobes; this region is involved in complex cognitive and social behavior such as decision-making, personality expression, and problem solving as it directs thoughts and actions in accordance with internal goals.

Projections - Neural pathways to and from a brain area which can be divided into afferents (input pathways) and efferents (output pathways). Neural activity in a brain region is influenced by its afferents, and a brain region in turn affects its own efferents. A brain region which sends projections to another region is said to innervate that area.

Race model - The theory that a behavior is the result of two competing responses to stimuli. Here, it is used to describe competition between responding to the go-signal and responding to the stop-signal.

Receptor - In this paper, a receptor refers to a molecule on the surface of a neuron which binds a neurotransmitter, affecting whether that neuron does or does not fire.

Regulation - In this paper, regulation refers to when the expression of a protein, in particular, receptors, is changed. A receptor can be upregulated when more of the molecule is found on the surface of neurons or downregulated when less of the molecule is found.

Serotonin - A neurotransmitter involved in anxiety and depression, among other things. It binds the 5-HT receptor.

Single unit/neuron recording - A recording of action potentials of a single neuron from the outside of the cell.

Stop signal reaction time (SSRT) - The time needed by the subjects to inhibit the initiated response to the go-signal and change their behavior to the conditioned response of the stop-signal

Stop index - The difference between STOP and GO trial activity (STOP - GO/STOP + GO) for each neuron.

STOP trial - A SST trial during which a stop-signal is presented following the go-signal.

Stop-signal delay - The time between the GO signal and the STOP signal.

Stop-signal task (SST) - A behavioral tasks that measures impulsivity by gauging how quickly an already-initiated response to a stimulus is inhibited. In the task, the subject is trained to respond to a conditioned stimulus, known as the go-signal. After this initial training, the subjects practice restraining their response to the go-signal and responding to a second conditioned stimulus, known as the stop-signal. All of the trials begin with the go-signal; however, on a minority of trials (~20%), the stop-signal appears after the go-signal. This is so that the subject becomes accustomed to quickly reacting habitually to the go-signal, and thus making it more difficult to inhibit the response on stop-signal trials.

Striatum - A cerebrum structure which helps coordinate motivation with body movement, such as by inhibiting a rat turning left after a right directional STOP signal has been shown.

Transporter - A molecule on the surface of an upstream neuron which brings neurotransmitters back from the synaptic space into the neuron to stop it from binding receptors on the downstream neuron.

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