OPTOGENETIC INHIBITION OF THE MPFC DURING DELAY DISCOUNTING

by

Shelby M. White

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STATEMENT OF COMMITTEE APPROVAL

Dr. Christopher Lapish, Chair
Department of Psychological Sciences
Dr. Cristine Czachowski
Department of Psychological Sciences
Dr. Marian Logrip
Department of Psychological Sciences

Approved by:
Dr. Nicholas J. Grahame
Head of the Graduate Program

To all my loved ones who bring me inspiration each day

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Author: White, Shelby, M. MS
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Committee Chair: Christopher C. Lapish

Impulsivity, or the tendency to act prematurely without foresight, has been linked to a diverse range of pathological conditions. Foresight refers to the ability to envision future rewards and events (i.e. prospectively sample) and has been associated with decreased impulsivity. One form of impulsivity is measured by the ability to delay gratification and is often studied in the framework of Delay Discounting (DD). DD provides the means to study impulsivity in a number of pathological conditions. However, whether impulsivity precedes the development of pathological states or results from the pathological state itself is not fully understood. This necessitates an understanding of neurobiological mechanisms contributing to decision making in both non-impulsive as well as impulsive populations of individuals.

Animal models allow invasive techniques to be used to dissect the neurocircuitry involved in decision making. Given that the decision-making process is an ongoing process rather than an isolated event, optogenetics provide the temporal and spatial specificity necessary for evaluating brain region specific contributions to decision making in DD. In the present study, optogenetics were used to assess the contribution of the medial Prefrontal Cortex (mPFC), a brain region involved in 'goal-directed' behavior, in the planning of future choices (i.e. prospective plans) and subsequent measures of impulsivity in an adjusting amount DD procedure. Optogenetic inhibition of mPFC was conducted in Wistar rats during different epochs of a DD task in order to assess how mPFC affects planning behavior in a population of rat not considered to be highly impulsive. Although no direct effects on planning behavior (e.g. consistency) were observed, inhibiting mPFC after a trial has been initiated and directly before a choice was made (Epoch 2) was observed to increase measures of impulsivity in comparison to days where no optogenetic manipulation occurred in a delay-specific manner. This suggests that mPFC differentially contributes to decision making at different delays. A pattern of associations between choice latency, impulsivity, and consistency began to emerge for inactivation occurring in Epoch 2, suggesting that mPFC

contributes to some aspect of planning choices during this epoch. Moreover, these results indicate that mPFC is involved in decision making in Wistar Rats. Understanding the direct role that mPFC plays in promoting choices of delayed rewards provides a neurobiological target for treatment aimed at reducing impulsivity in the clinical population.

INTRODUCTION

Impulsive Choice and Prospection

Impulsivity has been linked to many pathologies including substance use disorders (SUD), attention deficit hyperactivity disorder (ADHD), schizophrenia, pathological gambling, and obesity (Dalley, Everitt, & Robbins, 2011; Kobayashi & Schultz, 2008; Peterson, Hill, Marshall, Stuebing, & Kirkpatrick, 2015). Impulsivity is regarded as a multidimensional construct that refers to a set of heterogeneous traits and behavioral tendencies that likely reflect separate underlying processes (Cyders, 2015; De Wit, 2009; De Wit, Flory, Acheson, Mccloskey, & Manuck, 2007). Better understanding these separate processes requires impulsivity to be clearly defined by the behavioral tendencies being measured and the manner in which they are measured. Given that not all constructs of impulsivity are equally related to specific pathologies, clearly defining these processes and behavioral tendencies helps to improve reliability and translational approaches (Cyders, 2015). Understanding these processes and how they translate to pathologies is key to developing behavioral and/or pharmacological interventions focused on treatment and preventative measures.

Established behavioral measures of cognitive impulsivity, such as DD, are used in both clinical and preclinical research to assess impulsive choice (IC) behaviors, where IC refers to making impulsive decisions (Hamilton et al., 2015; Linsenbardt, Smoker, Janetsian-Fritz, & Lapish, 2016). Decision making refers to the process of choosing a particular action from a set of alternative options that are intended to result in an outcome beneficial to the agent (S. Kim & Lee, 2011). Decision making becomes maladaptive when choices are made without regard for future consequences (i.e. lack of prospection), and may be thought of as IC or non-planning impulsivity (Bevilacqua, Goldman, & Bevilacqua, 2013; Peterson et al., 2015). Alternatively, engaging in prospective thinking reduces IC, highlighting a potential target for behavioral intervention aimed at reducing IC (Liu, Feng, Chen, & Li, 2013; O'Donnell, Oluyomi Daniel, & Epstein, 2017; Peters & Büchel, 2010). IC is also associated with lack of planning (Hamilton et al., 2015). Although IC has been used synonymously with non-planning impulsivity, they are different yet related phenomena pertaining to future orientation (Steinberg et al., 2009). Planning refers to whether an individual makes a plan before acting, while prospection refers to mental time travel or a projection

of one's self into the future in order to pre-experience an event (Atance & Neill, 2001; Peters & Büchel, 2010; Steinberg et al., 2009). Lack of prospection and planning combined may disrupt the development of prospective strategies that benefit the decision maker, where strategies refer to an action-selection process (Powell & Redish, 2016). Therefore, it is hypothesized that a deficit in forming or maintaining a prospective behavioral plan contributes to IC by disrupting the development and/or maintenance of beneficial strategies.

Given that IC involves an inability to delay gratification of a larger later reward when presented with the option of a smaller sooner reward, DD provides a valuable tool for assessing how decision making changes over time (Hamilton et al., 2015). DD measures the rate at which an individual discounts the current value of a delayed reward, and is described by a temporal discounting function (S. Kim & Lee, 2011). Variations of the discount function exist, including but not limited to, the hyperbolic discounting function, exponential discounting function, exponential with a bonus for immediate rewards, and sum of multiple exponentials (Kurth-Nelson, Bickel, & Redish, 2012). The hyperbolic discounting function is widely used in most behavioral research, and the generation of this function appears to require the ability to use prospection (Kwan et al., 2012). This is demonstrated by patients with anterograde amnesia (i.e. lack of prospection) having linear discounting functions and controls (i.e. intact prospection) showing hyperbolic-shaped discounting (Kwan et al., 2012). IC is indicated by increased discounting, i.e., a steeper hyperbolic function (Bickel & Marsch, 2001; Bickel, Yi, Landes, Hill, & Baxter, 2011).

Clinical: Prefrontal Cortex Contribution to Impulsive Choice

It is not likely one brain region performs all the calculations needed to make a choice during DD procedures, but rather a complex network of neural systems interact to process events on a continuum that constantly requires past, current, and potential future events to be considered (Frost & McNaughton, 2017). One school of thought is that there are five distinct systems that likely contribute decision making in DD: (1) a sensory system taking in information, (2) a system for retrieving gain-related information (i.e. information about the immediate and delay rewards), (3) a system for creating representations of immediate vs. delayed rewards' subjective values, (4) a system capable of comparing the subjective values of immediate vs. delay gains that eventually *computes* a decision (5) the final system involved in sending the motor output needed to physically make the choice (Frost & McNaughton, 2017). A more simplified conceptualization resulting from

imaging studies is that there is a *beta* and *delta* system, where *beta* refers to brain regions that are more active to immediate gains and the *delta* system is active regardless of immediate or delayed gains (Frost & McNaughton, 2017).

One region of particular interest in clinical research on IC is the dorsolateral prefrontal cortex (dlPFC), as it is believed to be involved in monitoring information over delays, response control, flexibility, attention, and rule representation (Crews & Boettiger, 2009; Seamans, Lapish, & Durstewitz, 2008). The dlPFC has been included in what are considered the "goal control area" in the DD literature, likely contributing representations of events and would likely be a component of the *delta* system (Frost & McNaughton, 2017). Within the dlPFC, representation refers to high-level abstraction of general information about a concept, such as rules or strategies, which are formed or maintained in neuronal assemblies (Powell & Redish, 2016). Moreover, they can be stored either transiently or permanently in neuronal networks in order to aide in cognitive processes like decision making (Curtis & D'Esposito, 2003). Functional evidence supports the dlPFC's involvement in IC, including fMRI studies showing individuals with steeper discounting rates also show enhanced deactivation to delays of future rewards in the dlPFC, and that patients with damage to the dlPFC show increased IC (Ballard & Knutson, 2009; Cho et al., 2013). Animal models of IC are capable of further elucidating how these processes, or a disruption in these processes, affect IC behavior by using invasive techniques such as inactivation and lesioning.

Preclinical: The Medial Prefrontal Cortex and Prospection

The medial PFC (mPFC) of rodents, specifically the PL region, is often compared to the primate dlPFC (Wang, Yang, & Li, 2015). Further supporting this assertion, the PL has reciprocal connections to the mediodorsal nucleus of the thalamus (MD), which is considered by some to be a defining feature of the mammalian PFC (Delevich, Tucciaron, Huang, & Li, 2015). Conversely, the primate PFC contains a granular layer that developed at some point during evolutionary history, which the rodent PFC lacks (Passingham & Wise, 2012). It is argued that the MD projects to both granular and agranular areas of the primate cortex, and therefore using the MD projections as a defining feature of the PFC is not sufficient (Passingham & Wise, 2012). Moreover, given evidence from electrophysiological and lesioning studies, some contend that the rat mPFC is not homologous to the primate dlPFC, but rather encompasses similar functions as the dlPFC and anterior cingulate combined (Loos et al., 2010; Passingham & Wise, 2012; Seamans et al., 2008).

Understanding the similarities of functions that exist between the primate and rodent PFC requires an understanding of the information that the PL has can access. For instance, in addition to its connections to the MD, the PL also has connections to regions including the medial agranular cortex (AGm), VTA, striatum, and hippocampus (Hoover & Vertes, 2007; Vertes, 2004, 2006). These regions are proposed to be involved in processes such as integrating sensory information, reward processing, voluntary behavior, and memory, *respectively* (Hoover & Vertes, 2007; Vertes, 2004, 2006). There is also evidence that the AGm encodes preparation or planning of motor behaviors, implying the PL also has access to this information (Erlich, Bialek, & Brody, 2011). In considering the study of cognitive processes in rodent models of DD it is important to consider that the rodent mPFC, like the dlPFC, has been implicated in the processing of cognitive and emotional stimuli such as attention, inhibitory control, and executive control over aversive and appetitive stimuli that contribute to IC (Riga et al., 2014).

In sum, the PL neurocircuitry appears to be involved in integrating contextual (i.e. any set of cues which situate the animal in place and time, including task rules) and event-related (i.e. sensory cues and actions) information into behavior via sensory and limbic inputs to the PL, which enables activation of contextually appropriate representations of goals or task rules (Euston, Gruber, & Mcnaughton, 2013). This is important for DD, because it has been suggested that assigning value to the delayed reward requires it to be mapped onto the context in which it is received (Kurth-Nelson et al., 2012). Further, PL is proposed to be involved in a wide range of diverse processes that likely contribute to DD performance (Barrus & Winstanley, 2017; Gisquet-Verrier & Delatour, 2006; Hosking, Cocker, & Winstanley, 2016; Powell & Redish, 2016; Rich & Shapiro, 2009; Seamans et al., 2008). For example, attentional and flexible control over actions are important for prospective planning of delayed rewards, and subsequently, the development of prospective strategies (Gisquet-Verrier & Delatour, 2006; Linsenbardt et al., 2016). This is further supported by recent observations suggesting that the PL is necessary for recognizing the need for a change in strategy, and may be interpreted as contributing to *maintenance* of prospective strategies (Powell & Redish, 2016). Moreover, evidence suggests that maintenance of strategy representations occur after rules of a task have changed, but before the decision itself (Powell & Redish, 2016). The PL may also have a role in carrying out prospective strategies, given that PL lesions disrupt prospective foraging strategies in rats and performance on tasks that require switching between strategies or rules (Gisquet-Verrier & Delatour, 2006; Seamans et al., 2008).

Beyond the PL's possible role in prospective strategy, there are preclinical data to suggest that prospective strategies result in reduced IC behavior in Wistar rats. Wistar rats which appeared to use prospective strategies (i.e. they planned their future actions) to inform their decisions in a modified DD task also made less ICs when compared to alcohol preferring P rats that did not appear to be using any particular strategy (Linsenbardt et al., 2016). A modified adjusting amount DD task, which consisted of two retractable levers that were extended during both the initiation and choice epoch of the session, afforded the opportunity to assess decision intent (Linsenbardt et al., 2016). The idea that Wistars were using a prospective strategy stemmed from observations that Wistar rats that consistently used the same lever to initiate a trial and make their choice during that trial were less impulsive and took less time to make their choice (Linsenbardt et al., 2016). This was interpreted as decision intent, or proof that the animal had already decided which lever they were going to choose for a given trial (Linsenbardt et al., 2016). In sum, greater consistency between the lever selected to initiate a trial and the choice lever suggests that the animal planned its choices ahead of time (i.e. had a prospective plan). This ability to prospectively plan allowed for the development of a prospective strategy aimed at obtaining the most beneficial outcome for that session, which ultimately resulted in less IC. Additionally, this strategy was hypothesized to be formed at or close to the initiation of a trial (Linsenbardt et al., 2016). Given evidence that the PL plays an active role in rule representation and recognizing the need for a change in strategy, inactivating the PL in Wistar rats during critical time points in a modified adjusting amount DD task is hypothesized to result in IC by disrupting the ability to *develop* and/or update prospective strategies.

Rodent mPFC and Delay Discounting

In preclinical literature, mPFC has been less studied for its contributions to DD behavior, as it is not thought to be involved in valuation or choice during DD tasks (Fobbs & Mizumori, 2017). Nonetheless, this assertion should be taken with caution, as evidence from inactivation and lesioning studies remains inconclusive (Cardinal, Pennicott, Lakmali, Robbins, & Everitt, 2001; Feja & Koch, 2014). A study using muscimol to inactivate mPFC of rats claimed that DD performance was not affected (Feja & Koch, 2014). However, increases in omissions and lever response latencies at higher muscimol concentrations were observed, reportedly not due to motor

effects of the drug (Feja & Koch, 2014). This could be interpreted as a reduction in attentional or motivational processes that contribute to DD performance.

Conversely, lesioning mPFC in rats yields alternative results than those found in inactivation studies (Cardinal et al., 2001). Permanently lesioning mPFC results in a flattening of the discounting curve, i.e., choice of the large reward was reduced at short delays and increased at long delays (Cardinal et al., 2001). However, a closer look at the data reveals a critical flaw. Animals tested before lesions had very poor magnitude discrimination, i.e., they were not able to discriminate between large and small rewards at a zero-second delay. When tested with mPFC lesions, these same animals appeared to have failed magnitude discrimination almost completely. Still, this cannot be interpreted as a disruption in reward valuation, as poor magnitude discrimination in baseline measures make subsequent results uninterpretable. The reported interpretation of the observed performance alterations is that mPFC lesions disrupted tracking of time intervals (Cardinal et al., 2001). This interpretation seems unlikely given the animals' deficient performance at the zero-second delay. Alternatively, permanently lesioning mPFC may have allowed compensatory mechanisms to guide behavior (Feja & Koch, 2014). This alternative may also explain observations from the inactivation study described above. This explanation stems from evidence that multiple microinjections, at least in mice, have been shown to result in irreversible damage to brain tissue, essentially lesioning the area (Groblewski & Cunningham, 2013).

Optogenetic Techniques in Decision Making

Reducing the possibility of compensatory mechanisms necessitates use of inactivation techniques that provide high temporal resolution, such as optogenetics which occur on a millisecond time-scale (Janitzky, Lippert, Engelhorn, Tegtmeier, & Cope, 2015; Mattis et al., 2011). Decision making also occurs on a millisecond time-scale, supporting the utility of optogenetic inactivation in assessing PL functional contribution to IC (Martin & Potts, 2010). Further, both the aforementioned lesioning and inactivation studies used within-session adjusting delay DD with nose-poke holes to initiate the trial instead of levers (Feja & Koch, 2014; Cardinal et al., 2001). The use of nose-poke holes instead of levers to initiate the trial prevents the ability to detect decision intent (i.e. consistency between the initiation and choice lever for a given trial) during DD procedures. Without decision intent, it cannot be inferred that a prospective plan was

in place. This is important, considering that the PL involvement in rule representation and recognizing the need for change in strategy.

It may be that the PL plays a specific role in the *development* and *maintenance* of prospective plans that aide in decision making, and the lesioning and inactivation studies were not able to detect the PL's contribution to IC due to differences in methodology and/or compensatory mechanisms. In this regard, in order to understand how the PL contributes discretely to choices made during each trial to development and maintenance of prospective plans requires an understanding of the temporal dynamics. This can be accomplished by separating a trial into critical epochs, whereby optogenetics can be used to investigate the contribution of the PL to planning behavior in a temporally defined manner. Therefore Feja & Koch (2014) as well as Cardinal et al. (2001) were not able to detect development and/or maintenance of prospective plans due to an inability to detect decision intent, lack of temporal specificity, and possibility of compensatory mechanisms.

It is also important to consider these methodological differences, because contextual and event-related information contributing to the representation of goals or task rules are likely different in adjusting amount DD and adjusting delay DD. For instance, while an adjusting delay DD procedure allows animals to span all the delays (i.e. the delay lever either increases or decreases) within a single session and uses a fixed reward magnitude for both the immediate and delay lever, an adjusting amount procedure only exposes the animal to one delay per session and has one lever that 'adjusts' the reward magnitude on a trial to trial basis. An adjusting amount procedure requires multiple sessions at each delay to understand how subjective value of a reward changes with increasing delay to when it is received. This provides an opportunity to investigate the contribution of the PL to planning behavior in both an epoch-specific and delay-specific manner. At each delay, multiple sessions can be run to account for when either no manipulation occurred (baseline behavior) or when the PL is inactivated during a number of critical epochs. Assessing the role of the PL in prospective planning behavior and how it contributes to IC requires optogenetic inactivation of PL pyramidal cells, which comprise the majority of neurons in the cortex, in Wistar rats during critical epochs in a modified adjusting amount DD procedure in which decision intent can be assessed (Elston et al., 2011).

The green/yellow inhibitory archaerhodopsin (ArchT) is a valuable tool used in the investigation of functional significance of specific brain regions in behavior (Bernstein & Boyden,

2011). ArchT works as an outward proton pump on presynaptic cells in order to hyperpolarize specific cell types in a highly temporally precise manner (i.e., off kinetics of ~9ms), where a CamKII-α promoter can be used to target pyramidal glutamatergic projection neurons (El-Gaby et al., 2016; Yizhar, Fenno, Davidson, Mogri, & Deisseroth, 2011). ArchT expresses well on the cell membrane with excellent trafficking in long distances down axons (see Fig. 1B). In comparison to halorhodopsins, which have long inactivation states post-stimulation, ArchT recovers rapidly allowing for multiple stimulations over a given session (Han et al., 2011). In comparison to its predecessor, Arch, ArchT is also very light-sensitive, with irradiances in the optogenetic range of 1-10mW/mm^2 that are capable of covering large volumes of brain tissue with a single optic fiber (Han et al., 2011).

Lastly, the mechanism by which ArchT silences neurons is mediated by changes in pH restricted to the boutons of affected cells, which has been reported to differentially affected the rates of spontaneous vs evoked excitatory postsynaptic potentials (EPSP) (see Review by El-Gaby et al., 2016). Specifically, it was reported that ArchT activation-specific changes in bouton pH increased the rate of spontaneous EPSPs while reducing evoked EPSPs independent of action potential firing. This highlights a potential caveat to using this method of neural silencing. Although ArchT is reported to have excellent temporal specificity e.g., off kinetics of ~9ms, it is important to consider how changes in pH of sub-cellular compartments result in overall long-lasting changes in physiological processes that may extend beyond channel kinetics (El-Gaby et al., 2016; Yizhar et al., 2011). Nevertheless, multiple studies have demonstrated the utility of ArchT as an investigative tool in understanding how acutely and specifically silencing synaptic transmission affects trial-limited behavior during both working memory and decision-making tasks (Bercovici, 2017; El-Gaby et al., 2016).

ArchT can provide valuable information about cell-type specific contributions of the of the PL to IC behavior in a DD procedure during specific epochs. Provided that inhibition of pyramidal cell populations in the PL results in a deficit in this decision-making behavior, this population of cells can be further investigated to understand how pyramidal cells contribute to maladaptive computations that ultimately result in IC. Understanding how pyramidal neurons in the PL contribute to computations responsible for maladaptive decision-making can be used to develop computational models. These models can be used to investigate means by which maladaptive

computations can be altered to result in a decision process more beneficial for an individual, highlighting routes for prospective treatments.

Specific Hypotheses

It is hypothesized that optogenetic inactivation of PL mPFC pyramidal cells in an adjusted amount DD will result in increased IC by interfering with the *development* and/or *maintenance* of prospective strategies. Inactivation of mPFC from the start of the trial and terminating at the once an initiation lever was pressed (Epoch 1) is hypothesized to increase IC by disrupting *development* of the prospective planning process (i.e., decrease consistency). Increased IC as a consequence of PL inactivation will result in a reduction of the indifference point on days where Epoch 1 inactivation occurs in comparison to non-inactivation days. This is proposed to happen at the 4-second delay, given that this is the intermediate delay where floor or ceiling effects will not be encountered. It is also hypothesized that inactivation during this critical time period (Epoch 1) will result in increased initiation latencies and an increase in number of initiation omissions. Alternatively, inactivating after the trial has been initiated and ending once a choice has been made for a trial (Epoch 2) will result in a disruption of the *maintenance* of prospective planning. Inactivating during Epoch 2 is hypothesized to result in decreases in consistency between the initiation and choice levers accompanied by an increase in IC, reflected by a lower indifference point. Again, this is proposed to happen at the 4-sec delay. Further, it is hypothesized that there will be an increase in choice latencies as well as number of choice trial omissions.

METHODS

Animals

Twenty male Wistar rats were purchased from Envigo (Indianapolis, IN). Following arrival at the vivarium, animals were given 3 days to acclimate to new surroundings, including a 12-h reverse light/dark cycle with lights off at 7:00 AM. Following acclimation, animals were single housed and given at least a week prior to testing. All animals were at least 70 days of age prior to testing and had *ad lib* access to food and water prior to food restriction/habituation. Over the course of approximately two weeks, animals were food restricted to 85 % of their starting free-feeding weight and maintained under this condition throughout all experiments. However, animals were given a free feeding amount of food the day prior to surgery and during surgery recovery. Following recovery, animals were again food restricted under the same guidelines prior to resuming testing. All procedures were approved by the IUPUI School of Science Institutional Animal Care and Use Committee and were in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Operant Apparatus

Eight standard one-compartment operant boxes (20.3 cm × 15.9 cm × 21.3 cm; Med Associates, St Albans, VT) inside of sound attenuating chambers (ENV-018M; MED Associates, St. Albans, VT) were used for all behavioral procedures. Each box contained left and right retractable levers on one wall, left and right stimulus lights positioned immediately above each lever, and an easily accessible pellet hopper positioned between these left and right positioned devices. The opposite wall contained a house light and a tone generator (2900 Hz) on the topmost position.

Habituation/Shaping

Following single housing, animals were handled each day for a week to reduce the stress of attaching the optic patch cords to the implants later in the experiment. Ten 45mg sucrose pellets wrapped in a 1” square of Kimwipe were placed at the bottom of each animal’s home-cage on the

final day of food deprivation. Day 1 served as a habituation day and consisted of the animals becoming familiar with the layout and environment of the operant box. Animals were placed in the chamber for 30 minutes with 10 sucrose pellets in the hopper and no other stimuli present. On day 2 of shaping, illumination of the house light indicated the start of a trial and after 10 seconds, a single pellet was dispensed. Each of the 30 trials had an intertrial interval (ITI) of 15, 25, or 35 seconds, chosen at random for a total of 30 trials/pellets. Days 3 and 4 of shaping introduced the levers and cue lights associated with those levers. During this time, animals were manually trained to lever press using successive approximation. A single pellet was rewarded manually using a button located outside of the pellet hopper. The animal had to become increasingly closer to the lever to continue earning rewards. Eventually the animal learned that pressing the lever earned them a reward for every lever press and continued without help for the remainder of the session. One lever was trained per day. All rats started training on the right lever on day 3 and began training on the left lever on day 4. A minimum of 30 lever presses was required before moving on. Days 5 and 6 began with the illumination of the house light for 10 seconds followed by the extension of a single lever (i.e., left or right lever) and illumination of the corresponding cue light. A response on the lever resulted in the simultaneous sounding of a 100-ms tone (marking the response) and delivery of a single sucrose pellet. On day 5 the rats were shaped with the right lever, followed by the left lever on day 6. Days 5 and 6 shaping sessions terminated after either 30 trials/sucrose pellets earned or 30 minutes.

All of the stimuli were included on days 7 and 8. Illumination of the house light signaled the start of the trial and remained on for 10 seconds. Once extinguished, both levers extended and the animal was required to press either lever in order to initiate the start of the trial. No response for 10 seconds resulted in retraction of the levers followed by the illumination of the house light (10 seconds). Once a lever was pressed to initiate the trial, both levers retracted for 1 second and then were again presented with both cue lights illuminated above the respective levers. A response on either lever this time was marked with a 100ms tone and a single sucrose pellet delivered, simultaneously. Only the cue light above the chosen lever remained on for the remainder of the trial. The duration of the trials was always 35 seconds. These sessions were terminated either when 30 choices were made or when 35 minutes had elapsed. Over sessions 7 and 8, lever preference bias was determined for each animal.

Delay Discounting

The within-session adjusting amount DD procedure was a modified version of the procedure performed by Linsenbardt et al. (2016), which was adapted from Oberlin and Grahame (2009) and are illustrated in (see Fig. 1). Stimuli were presented in the exact manner detailed in days 7 and 8 of shaping except that the number of pellets delivered for a given trial was dependent on lever pressing contingencies detailed below. The "delay lever" was assigned to each animal as their non-preferred side. Choosing the delay lever always resulted in the delivery of 6 pellets following some delay (0, 1, 2, 4, 8, or 16-sec). The "immediate lever" was the opposite lever. Choosing the immediate lever resulted in 0-6 pellets delivered immediately (i.e. the adjusting amount lever). The number of pellets delivered following a response on the immediate lever (i.e. the 'value' of the immediate lever) always started at three on a given day. On "choice trials" each response on the immediate lever would decrease the number of pellets the immediate lever would dispense on the next trial by one (minimum 0 pellets) and a response on the delay lever would increase the number of pellets the immediate lever would dispense on the next trial by one (max 6 pellets). "Forced trials" were implemented for the immediate and delay levers, where two consecutive responses on the same lever would result in a forced trial for the non-chosen lever on the next trial (e.g. trial 1=immediate choice, trial 2=immediate choice, trial 3=delay forced). If an animal did not lever press for the forced trial, the forced trial would be presented again on subsequent trials until the lever was pressed. The animal had to eventually make a response on the forced trial in order to return to choice trials. There was no effect of forced trials on the value of the immediate lever.

The session terminated either after 30 choice trials or 35 minutes. The delays were completed in ascending order (0, 1, 2, 4, 8, 16-sec) with a day off in between the start of each new delay. Eight to twelve sessions were given at the 0-sec delay, four sessions at the 1 and 2-sec delay, and nine sessions at the 4, 8, and 16-sec delays (see Table 1). Magnitude discrimination was determined at the 0-sec delay with an exclusion criterion of 80% (4.8 pellets) of the maximum reward value (6 pellets). The average value of the immediate lever over the last ten choice trials was determined for the last three days of the 0, 1, and 2-sec delay and was used to determine the indifference point of each animal. Animals then either received surgery (group 1) or continued on and received surgery after completing the full DD curve (group 2) before re-experiencing each delay again (see Opsin Virus Delivery and Implantation of Optic Fibers below for detail).

For experimental delays (i.e., the 4, 8, and 16-sec delays where animals received optogenetic manipulation) the last 10 trials of each day for each condition (No inactivation, Epoch 1 inactivation, and Epoch 2 inactivation) were used to determine an indifference point for each condition at each experimental delay. Days 1 and 2 were excluded for the No inactivation condition, as animals were becoming familiar with the new delay and indifferences points were not yet stable. Therefore, the last 10 trials of days 4, 6, and 8 were used for calculating indifference points for the No inactivation condition. The last 10 trials of days where Epoch 1 as well as Epoch 2 inactivation occurred were taken for each animal for indifference points on optogenetic manipulation days (see table 1 for experimental design). The virus was allowed to express for at least three weeks before beginning experimental delays.

Surgical Preparation

Animals were placed inside a flow box and anaesthetized with isoflurane gas (2%) until sedated, at which point they were placed in a stereotaxic frame and maintained on 0.3-0.5% isoflurane for the duration of the surgery. Artificial tears were used to keep the animals' eyes from drying out. A local anesthetic (Marcaine; 5mg/kg), anti-inflammatory (Ketofen; 5mg/kg dose), and antibiotic (Cefazolin; 30mg/kg) were injected under the incision site (anesthetic) or via I.P. injection (anti-inflammatory and antibiotic) before beginning the incision.

Opsin Virus Delivery and Implantation of Optic Fibers

Surgery followed the completion of the last day of the 2-s delay for Group 1 (n=7). For Group 2 (non-implanted control animals from Group 1; n=10), animals received bilateral viral injections and fiber implantation following the completion of all delays and before re-exposure to delays in ascending order. Two syringe pumps (Pump 11 Elite; Harvard Apparatus, Holliston, MA) were attached to each arm of the stereotaxic frame and loaded with 2µL Hamilton syringes (7002KH, Hamilton Co., Reno, NV). Coordinates for PL mPFC viral injections occurred at a 20-degree angle and were as follows: +3.2mm AP, +2.0mm ML, -5.2mm DV from Bregma. Four anchoring screws were placed (two anterior and two posterior of the injection site) before beginning viral injections. Holes were drilled into the skull to allow the Hamilton syringes to penetrate the brain tissue. Animals then received bilateral injections of .65µL at a flow rate of .2µL/min of the inhibitory Adeno-associated virus (AAV-CaMKIIa-eArchT3.0-EYFP; K.

Deisseroth via UNC Vector Core) followed by 10 minutes of diffusion before retracting the Hamilton syringes. Subsequently, animals received fiber implantation of Dual Fiber-optic cannulas with guiding sockets (DFC_200/245-0.37_3.3mm_GS1.4_FLT; Doric Lenses Inc., Quebec, QC, Canada). Once the Dual Fiber-optic cannulas were in place, two-component dental cement was used to adhere the implants to the skull via anchoring screws.

Animals were given one-week post-surgery to recover before continuing in the operant boxes. Group 1 was given approximately three weeks of expression that included one week of surgery recovery and two weeks of re-food restriction necessary to return them to 85% free fed weight. Group 1 also had re-exposure to the 2-sec delay for two days before receiving a day off and starting the 4-sec delay. For Group 2, approximately four weeks of viral expression passed before starting experimental delays, consisting of one week of surgery recovery, and only one week of food restriction was needed to return animals to 85% free feeding weight. The remainder of the time virus was allowed to express occurred during re-exposure to the 0, 1, and 2-sec delays. Given that animals had previous experience with each delay (including the 0-sec delay), only four days/sessions of the 0-sec delay were needed to determine magnitude discrimination for Group 2 during their second exposure.

Optogenetic Stimulation

Animals were minimally restrained in the operant chambers. Two of the eight boxes were modified for optogenetic stimulation using a green (532nm) laser (MGL-FN-532-300mW; Ultralasers Inc., Toronto, Canada) operated through Med Associates Programming via a TTL (Med Associates, St Albans, VT). From the fiber coupler, a mono patch cord (MFP_200/240/900-0.22_1m_FC-FC; Doric Lenses Inc., Quebec, QC, Canada) was attached and traversed the sound attenuating chambers terminating at the rotary joint (FRJ_1x1_FC-FC; Doric Lenses) which attached a Branching Fiberoptic Patchcord (BFP(2)_200/240/ARMO-0.22_0.5m_FCM-GS1.4; Doric Lenses) that was the terminal connection to the animal via guiding socket at the top of the animal's skull. The terminal end of the mono patch cord was attached to a self-adjusting arm that would vertically lift the patch cord configuration when the animal reared in the operant chamber, while still allowing the rotary joint to swivel. Stimulation did not occur in pulses and remained on for the duration of the epoch to prevent rebound depolarization of cells. Stimulation at the tip of the fiber measured approximately 21mW resulting in predicted irradiance of ~60mW/mm^2 at the

fiber tip. Larger irradiance values were opted for in order to traverse the entire PL cortex with only one fiber per hemisphere.

Stimulation occurred at one of two different epochs during the task for a given session (Epoch 1 inactivation or Epoch 2 inactivation). Epoch 1 stimulation occurred from the start of a given trial and terminated once an animal initiated the trial. Stimulation remained on if the animal omitted initiating the trial until a response on an initiation lever was made. Epoch 2 stimulation occurred as soon as the animal initiated a trial and terminated once a choice was made. Stimulation remained if the choice was omitted until a choice was made on subsequent trials. Stimulation occurred on the third, fifth, seventh, and ninth session/day of the 4, 8, and 16 second delays in order to control for carry over effects of the stimulation as well as to obtain indifference points for the No Inactivation condition. All animals received stimulation at both Epoch 1 and Epoch 2 in a cross-over design (Table 1) so that half the animals received Epoch 1 on the third and seventh day and Epoch 2 on the fifth and ninth day and the other half of animals received the opposite configuration.

Immunohistochemistry

Animals were perfused within 14 days after behavioral testing with 4% PFA after receiving a Urethane anesthesia (1.5-2.0g/kg). Brains were then fixed in 4% PFA for 24 hours before being placed in a 30% sucrose solution (24-72 hours) and subsequently stored at -20 degrees Celsius until sliced 50 microns thick. A florescence imaging scope (Nikon Eclipse 80*i*; Melville, NY) was used to verify EYFP-tagged protein expression. In order to assess transduction of glutamatergic pyramidal cells within mPFC, slices were mounted on glass slides using an aqueous mounting medium (H-1000-10; Vectashield, Invitrogen). Photoshop (Adobe Systems, San Jose, CA, USA) was used to create a pictorial representation of placements and expression detail for all animals (Fig. 2A).

Behavioral Statistics

The data were analyzed with methods used by both Oberlin and Grahame (2009) and Linsenbardt et al. (2016). The Mazur hyperbolic discounting function is widely used in behavioral research both clinically and preclinically (Frost & McNaughton, 2017). The mean indifference point for all conditions (No inactivation, Epoch 1 inactivation, Epoch 2 inactivation) at delays 4,

8, and 16-sec were evaluated using a two-way ANOVA with both delay and condition as factors. Repeated measures were not used due to missing data points. A one-way ANOVA was used to calculate the differences across conditions for both the AUC as well as *k* value data analysis. The rate of discounting was determined using the hyperbolic fitting function (Mazur, 1987):

$$\boldsymbol{v = \frac{a}{1 + kd}}$$

In this function, *v* refers to the subjective value of the reward, *a* is the fixed value of the delay reward (6 pellets), *d* is the length of the delay (0, 1, 2, 4, 8, or 16-sec), and *k* is the value fitted by the hyperbolic function. This formula allows for the measure of impulsivity (*k*). *k* describes the steepness of the rate of discounting (i.e., steepness of the hyperbolic curve), where the larger the *k* value, the more impulsive an individual is. Statistics were performed on the 4, 8, and 16-sec delay specifically, given optogenetic manipulation did not occur at prior delays.

Given that omissions were minimal, the cumulative sum of initiation and choice omissions per animal for delays where an inactivation occurred were calculated. All non-normal data were natural log transformed except for omissions data, where non-parametric statistics were performed. Correlation and regression analyses were conducted on variables of interest in order to determine strength and significance of relationships between those variables. Tukey's multiple comparison or Dunnett's multiple comparison post-hoc tests were performed when appropriate. Outliers were considered data points for a given behavioral variable e.g., Latency, Omissions, or *k* value, had a distance from the median which exceeded 1.5 times the interquartile range and were determined on a variable by variable basis. No more than two outliers were excluded from a given analysis of a behavioral variable. All data were compiled using MatLab (Mathworks; Natick, MA). Data were analyzed and graphed using Graphpad Prism software (GraphPad Prism, v. 7.0b, La Jolla, CA). All significance α-values were set at 0.05.

RESULTS

Attrition/Placements

Three animals were excluded from the study after food-deprivation due to seizure-like activity. Two animals were excluded from Group 1 due to missed placements or lack of viral expression. Five animals were excluded from Group 2 due overgrown teeth (n=1) and to missed placements of either virus injection, optic fiber implant, or the combination (n=4). One animal was excluded from Group 2 due to not meeting magnitude discrimination criteria (Fig. 3). Of the animals injected with virus, those with placements had viral expression mostly in the PL mPFC with some animals having expression extending dorso-laterally into the anterior cingulate cortex and partially within the secondary motor cortex and some extending ventrally into the infralimbic mPFC (Fig. 2A). A representative image of eYPF-tagged protein expression in the PL mPFC can be seen in Figure 2B.

Two of the animals' data were not used for the Epoch 1 inactivation condition during the 8-sec delay due to patch cords breaking mid-session. One animal's data for one session was excluded for Epoch 2 inactivation condition during the 8s delay due to cage-leak on the previous night of testing resulting in poor behavior during testing. Three animals from Group 1 had fiber optic implants break during the 8-sec delay, which were repaired on the last day of the 8-sec delay using steel-reinforced epoxy before beginning the 16-sec delay.

Delay Discounting

At the 0-sec delay, it was apparent that a preference for the larger-later reward was present for all animals included in statistical analysis (Fig 3). Therefore, all animals included in the study passed stringent magnitude discrimination criteria. As the delay increased, preference for the delay lever decreased as preference for the immediate lever increased for all conditions (Fig. 4A, B). A two-way ANOVA was conducted to understand differences in the number of immediate or delayed choices across condition and delays. It was expected that the number of immediate choices would increase, and the number of delay choices would decrease as the session delay increased. A main effect of delay was observed for both number of delay [$F(2, 78) = 28.39$, $p<.0001$] and immediate

[F(2, 78) = 28.35, p<.0001] choices across the 4, 8, and 16-sec delays (e.g. delays with optogenetic manipulation). Post-hoc analysis indicated that the number of delay choices significantly differed for the 4 and 8-sec delays (p<.01), 4 and 16-sec delays (p<.0001), and the 8 and 16-sec delays (p < .01). There were also significant differences in number of immediate choices between the 4 and 8-sec delays (p<.001), 4 and 16-sec delays (p<.0001), and 8 and 16-sec delays (p <.01). This suggests that animals were discounting as a function of the delay. No significant differences were observed across conditions for number of delay [F(2, 78) = .99, p = .374] or immediate [F(2, 78) = .99, p = .373] choice trials (Fig. 4A, B). Therefore, the observed discounting was not influenced by the total number of trials completed at each delay.

The DD analysis can be seen in detail in Figures 5 through 7. A two-way ANOVA with condition and delays as factors was used to investigate the differences in indifference points across conditions for the 4, 8, and 16-sec delay. Significant main effects of delay [F(2, 78) = 26.98, p < .0001], and optogenetic manipulation [F(2, 78) = 3.33, p < .05] were observed showing that increasing delays resulted in a lower indifference point for all conditions, however, days where optogenetic manipulation occurred appeared to have lower indifference points than on days where No Inactivation occurred (Fig. 5A). Post-hoc testing with Dunnett's multiple comparisons test indicated that Inactivation and non-inactivation days differed significantly at the 8-sec (p < .05) delay. Epoch 2 Inactivation had a significantly lower indifference point than non-inactivation days during the 8-sec delay (p<.05) and effect size analysis (d=1.00) was found to exceed Cohen's (1988) convention for a large effect (d=.80; see Fig. 5A & B). Effect sizes were also evaluated in order to better understand the optogenetic manipulation in a small sample size. Although post-hoc analysis did not yield significant results for Epoch 1 Inactivation in comparison to No Inactivation condition (p=.08), a large effect size (d=1.00) was observed. Lastly, although neither Epoch 1 Inactivation (p=.35) nor Epoch 2 Inactivation (p=.09) significantly differed from the No Inactivation condition in post-hoc analysis at the 16-sec delay, effect size analysis for Epoch 1 (d=.52) and Epoch 2 inactivation (d=.88) exceeded Cohen's (1988) convention for medium (d=.50) and large (d=.80) effect sizes.

When the most impulsive animals were removed from analysis, the significant main effect of delay [F(2,60) = 31.71, p < .0001] and condition [F(2,60) = 6.47, p < .01] remained (Fig. 5B). Dunnett's multiple comparison indicated that at the 8-sec delay, No Inactivation significantly differed from Epoch 1 (p < .05) and Epoch 2 condition (p < .01). Large effect sizes were also

detected for Epoch 1 (d =1.94) and Epoch 2 (d = 1.74). This suggests that the most impulsive animals reduced indifference points for the No Inactivation condition which blunted the ability to detect an effect of Epoch 1 condition.

Alternative measures of impulsivity were also evaluated. Area under the curve (AUC) analysis, derived from experimental delays (4 through 16-sec) to include both (1) the indifference points for each condition across and (2) the best-fit lines from the hyperbolic fitting function (k). A one-way ANOVA was performed and no significant differences were observed for AUC between non-inactivation days and either inactivation epoch [$F(2, 24) = 2.52$, $p = .100$; Fig. 6A]. When the most impulsive animals were removed (Fig. 6B), there was a significant effect of inactivation on AUC [$F(2,18) = 6.58$, $p = .007$]. Dunnett's multiple comparison test indicated that No Inactivation was significantly different than Epoch 1 ($p < .05$) and Epoch 2 ($p < .01$). This suggests that the most impulsive animals reduced AUC in the No Inactivation condition, blunting the ability to detect a difference in AUC across conditions. A one-way ANOVA was also conducted to evaluate rates of discounting (k) across conditions (Fig. 6C). No significant differences were observed for k values on days where an inactivation occurred in comparison to non-inactivation days [$F(2, 24) = .99$, $p = .386$; Fig. 6C], even when the most impulsive animals were removed [$F(2, 18) = 3.35$, $p = .058$; Fig. 6D].

In order to further investigate the differences across conditions in the 8-sec delay, additional analyses were performed for this delay. At the 8-sec delay, the indifference point for Epoch 2 inactivation days is lower and significantly different than non-inactivation days ($p<.05$; Fig. 7A). When the most impulsive animals were removed from analysis, indifference points at the 8-sec delay for both Epoch 1 ($p < .05$) and Epoch 2 ($p < .01$) were significantly different than the No Inactivation condition (Fig. 7B). When looking across each day of the 8-sec delay (Fig. 7C), robust effects of inactivation (regardless of epoch) can be seen on days 5 and 7. Day 3 inactivation(s) were not significantly different from day 4 [$t(17) = .183$, $p = .857$, $d = .08$; Fig. 7C]. Day 5 inactivation(s) were significantly different from non-inactivation days 4 [$t(15) = 2.78$, $p = .01$, $d = 1.48$; Fig. 7C] and 6 [$t(15) = 3.24$, $p < .01$, $d = 1.72$; Fig. 7C]. Day 7 inactivation(s) were significantly different from non-inactivation day 6 [$t(15) = 2.78$, $p = .014$, $d = 1.49$; Fig. 7C] but not non-inactivation day 8 [$t(11) = 2.10$, $p = .059$, $d = 1.12$; Fig. 7C]. Lastly, day 9 inactivation(s) were not significantly different from day 8 [$t(11) = .10$, $p = .921$, $d = .05$; Fig. 7C].

When the most impulsive animals were removed from analysis of indifference points across days of the 8-sec delay, the effect of inactivation remained and was more robust for days 5 and 7. Day 3 inactivation(s) were not significantly different from day 4 [$t(14) = .594$, $p = .562$, $d = .29$; Fig. 7D]. Day 5 inactivation(s) were significantly different from non-inactivation days 4 [$t(11) = 3.59$, $p = .004$, $d = 2.23$; Fig. 7D] and 6 [$t(11) = 5.62$, $p < .001$, $d = 3.43$; Fig. 7D]. Day 7 inactivation(s) were significantly different from non-inactivation day 6 [$t(12) = 5.40$, $p < .001$, $d = 3.07$; Fig. 7D] and non-inactivation day 8 [$t(9) = 2.38$, $p = .041$, $d = 1.37$; Fig. 7D]. Lastly, day 9 inactivation(s) were not significantly different from day 8 [$t(9) = .076$, $p = .941$, $d = .05$; Fig. 7D]. The effects of inactivation were more robust when the most impulsive animals were removed.

Consistency

Given that decision intent (i.e. prospective strategy) was a main area of interest in this study, consistency between initiation and choice lever was quantified for choice trials. Observed consistency was calculated by dividing the total number of consistent choice trials (immediate and delay) by the total number of choice trials for a given session.

Observed consistency decreased across delays regardless of condition (Fig. 8). A significant main effect of delay on consistency was observed [$F(2, 79) = 4.02$, $p = .021$]. Post-hoc testing using Tukey's multiple comparison test indicated that consistency on the 4-sec delay significantly differed from the 16-sec delay ($p < .05$). No effect of inactivation was observed [$F(2, 79) = .180$, $p = .835$; Fig. 8].

Further, the probability of a consistent choice was dependent on the frequency of delay vs. immediate lever *initiations* as well as the delay vs. immediate lever *choices.* Chance probability of a consistent trial was calculated for each animal(*s)* at each delay(*d*):

$$P(\text{consistent choice n, d}) = \left[\left(\frac{D\ i,s,d}{T\ i,s,d}\right) * \left(\frac{D\ c,s,d}{T\ c,s,d}\right)\right] + \left[\left(\frac{I\ i,s,d}{T\ i,s,d}\right) * \left(\frac{I\ c,s,d}{T\ c,s,d}\right)\right]$$

D and *I* represent a response on the delay or immediate lever, respectively, and *T* is the total number of choices. The subscripts *i* and *c* correspond to a lever press in either the initiation or choice phase, respectively. There was a main effect of chance probability on delay [$F(2,79)=6.64$, $p<.01$; Fig. 8] and post-hoc analysis with Tukey's multiple comparison test indicated that chance probability at the 4-sec delay significantly differed from the 16-sec delay regardless of condition ($p<.01$).

Analysis of chance vs. observed consistency was also conducted to determine if consistent choices were made with greater than chance probability (Fig. 8). A main effect of delay [$F(2,158)=8.93$, $p<.001$] as well as a main effect of observation type (e.g. *chance* vs. *observed* values) [$F(5,158)=20.15$, $p<.0001$] was observed. Post-hoc analysis with Tukey's multiple comparison test indicated that the No inactivation *observed* consistency differed from No inactivation *chance* consistency at the 4 ($p<.05$), 8 ($p<.01$), and 16-sec delays ($p<.05$). Epoch 1 inactivation *observed* consistency also differed from the Epoch 1 inactivation *chance* consistency for the 4 ($p<.01$), 8 ($p<.05$), and 16-sec delays ($p<.05$). Epoch 2 inactivation *observed* consistency also differed from Epoch 2 inactivation *chance* consistency for the 4 ($p<.01$), 8 ($p<.01$), and 16-sec delays ($p<.05$). These differences between *observed* and *chance* consistency suggest that regardless of condition or delay, animals had a prospective plan.

Latency and Omissions

Response latencies were separated into *initiation* and *choice* latencies. The median response latencies were taken in the same manner as the way indifference points were calculated across delays and conditions. In order to understand how latencies differ across conditions and delays, a two-way ANOVA was conducted with delay and condition as factors. Initiation latency at the 4-sec delay was longer than either the 8 or 16-sec delays regardless of condition, where a significant main effect of delay [$F(2, 76) = 6.198$, $p < .01$; Fig. 9A] was observed. Post-hoc using Dunnett's multiple comparison test indicated that initiation latency differed for the 4 vs. 8-sec delay ($p > .01$) and the 4 vs.16-sec delay ($p = .039$; Fig. 9A). No effects of condition on initiation latency were observed [$F(2, 76) = .217$, $p = .81$; Fig. 9A].

For Choice Latency, significant effects were not observed for either delay [$F(2, 79) = 2.02$, $p = .83$; Fig. 9B] or optogenetic manipulation [$F(2, 79) = .068$, $p = .93$; Fig. 9B]. Lastly, there were no observed differences in the mean rank across conditions for cumulative number of initiation omissions (Kruskal-Wallis, $\chi 2 = 4.83$, $p=0.77$; Fig. 10A) or choice omissions (Kruskal-Wallis, $\chi 2 = 7.094$, $p=0.52$; Fig. 10B).

Relationship Between Behavioral Measures

In order to explore the relationship between behavioral measures of interest, correlation and regression analysis were conducted. Initiation latency was positively associated with natural

log-transformed *k* (impulsivity) values for Epoch 1 inactivation ($r(22) = .45$, $p < .05$) but not for No Inactivation ($r(28) = .35$, $p = .06$) or Epoch 2 Inactivation ($r(28) = .17$, $p = .37$; Fig.11A). A positive relationship between *initiation latency* and *impulsivity* was observed for Epoch 1 Inactivation ($b = .62$, $t(22) = 2.36$, $p = .027$), where longer latencies to initiate the trial was predictive of greater impulsivity scores (*k)*. Initiation latencies explained a significant portion of the variance in impulsivity scores for Epoch 1 [$R^2 = .20$, $F(1,22) = 5.58$, $p = .027$]. There were no differences between slopes across conditions for *initiation latency* vs. *k* [$F(2,78) = .928$, $p = .40$; Fig. 11A]. This suggests that condition does not change the degree to which *k* influences initiation latencies. However, there were differences in the Y-intercept [$F(2,80) = 3.15$, $p = .049$] which suggests that there were underlying differences in impulsivity (*k*) across conditions. This was confirmed by removing the most impulsive animals from analysis, as the association between *k* and initiation latency for Epoch 1 was no longer significant ($r(18) = .23$, $p = .28$; Fig. 11B).

Conversely, *choice latency* (Fig. 11C) during the Epoch 2 Inactivation condition was positively associated with natural log-transformed *k* values ($r(28) = .42$, $p < .05$) and this remained true when the most impulsive animals were removed from analysis ($r(24) = .42$, $p = .04$); Fig. 11D). Longer *choice latencies* were predictive of higher impulsivity scores (*k),* ($b = .990$, $t(28) = 2.46$, $p = .02$) for the Epoch 2 inactivation condition. For Epoch 2 inactivation condition, choice latency also explained a significant portion of the variance in impulsivity (*k)*, ($R^2 = .17$, $F(1,28) = 6.05$, $p = .02$). No significant associations were observed for Epoch 1 ($r(22) = .13$, $p = .55$) or No Inactivation Condition ($r(28) = .34$, $p = .07$; Fig. 11C, D). There were no differences in the slopes across conditions [$F(2,78) = 1.00$, $p = .37$], suggesting that condition does not change the degree to which *k* influences choice latencies.

Consistency was negatively associated with natural log-transformed *k* values (Fig. 12A) for all conditions including Epoch 1 inactivation ($r(26) = -.53$, $p < .01$), Epoch 2 inactivation ($r(26) = -.39$, $p < .05$) and the No Inactivation condition ($r(26) = -.61$, $p < .001$). Suggesting that lower consistency was predictive of greater impulsivity for all conditions ($b = -2.58$, $t(26) = 3.21$, $p = .004$; Epoch 1), ($b = -.201$, $t(26) = 3.27$, $p = .0409$; Epoch 2), and ($b = -3.41$, $t(26) = 3.89$, $p = .001$; No Inactivation). A significant amount of variance was accounted for by Epoch 1 condition ($R^2 = .28$, $F(1,26) = 1.10$, $p = .004$), Epoch 2 condition ($R^2 = .15$, $F(1,26) = 10.72$, $p = .041$), and No Inactivation condition ($R^2 = .37$, $F(1,26) = 15.17$, $p = .0006$). There were no differences between the slopes across conditions [$F(2,78) = 1.14$, $p = .33$], suggesting that the conditions do not differ on how consistency

contributes to impulsivity. Lastly, the most impulsive animals appeared to drive the associations between consistency and *k* for Epoch 1 and 2, as removal of these animals from analysis resulted in only the No Inactivation condition having a significant association between consistency and *k* (Fig. 12B).

Initiation latency was not associated with consistency (Fig. 12C) for any condition including No Inactivation (r(28)= .029, *p*=.88), Epoch 1 inactivation (r(26)= -.062, *p*=.20), and Epoch 2 Inactivation (r(28)= -.091, *p*=.63). This remained true when the most impulsive animals were removed ($r(24) = .09$, $p = .68$; No Inactivation, Fig. 12D), ($r(22) = -.25$, $p = .26$; Epoch 1, Fig. 12D), and ($r(24) = -.07$, $p = .74$; Epoch 2, Fig. 12D). Choice latency was negatively associated with consistency (Fig. 12E) for the No Inactivation (r(30)= -.53, *p*=.003) and the Epoch 2 inactivation (r(30)= -.46, *p*=.01) but not for the Epoch 1 inactivation condition (r (28)= -.28, *p*=.146). For both No Inactivation and Epoch 2 Inactivation condition, longer latencies to make a choice were predictive of lower impulsivity (*k*), (*b*= -.43, *t*(28)= 3.32, *p*= .003; No Inactivation) and (*b*= -.321, *t*(28)= 2.75, *p*= .01; Epoch 2). The longer the animal took to make a choice was predictive of lower consistency for the Epoch 2 inactivation and No inactivation conditions, and further, a significant amount of variance in consistency was accounted for by choice latency for No Inactivation (R^2= .28, F(1,28)= 11.01, *p*= .003) and Epoch 2 (R^2= .21, F(1,28)= 7.58, *p*= .01). There were no significant differences between slopes [F(2,82) = .391, *p*= .68], which suggests that there were no differences across conditions of choice latency contributing to consistency. Lastly, when the most impulsive animals were excluded from analysis, the only remaining significant association was in the No Inactivation condition for choice latency and consistency ($r(24) = -.43$, $p = .04$; Fig. 12F).

DISCUSSION

General Discussion

Given the general spread of virus (see Fig. 2A) covered more than just the PL region, effects of optogenetic manipulation will be addressed as mPFC-specific rather than PL mPFC-specific. Although mPFC of rodents in the past has been disregarded as not contributing to delay discounting behavior, current data suggest a role of mPFC in Wistar rats in IC behavior. It was hypothesized that inhibition of PL pyramidal cells during specific epochs of a modified delay discounting task would disrupt either the *development* (Epoch 1) of prospective plans or *maintenance* (Epoch 2) of prospective strategies derived from the prospective plans. Behavioral changes from inactivation were hypothesized to result in decreased consistency for both Epoch 1 and Epoch 2 Inactivation conditions that would include increased latencies, omissions, number of immediate trials, and a decrease in indifference point on days where optogenetic manipulation occurred. Epoch 1 was hypothesized to result in *initiation*-related changes and Epoch 2 contributing to *choice*-related changes in latencies and omissions. All of these changes were expected to occur at the 4-second delay, given that was the intermediate delay where ceiling or floor effects were not likely to encountered.

Consistency

Wistar rats performed above chance probability for all conditions with no effect of optogenetic manipulation (see Fig. 8), suggesting that a prospective plan was in place regardless of condition. However, the IRI was not manipulated due to length of the experiment. Previously, it has been seen that the greatest effects on consistency occur with increased latency between the initiation of a choice and the choice itself (Linsenbardt et al., 2016). Testing optogenetic manipulation at multiple delays provided the ability to obtain multiple measures of impulsivity e.g., *k,* AUC, and indifference points. Specifically, the AUC measurement has been regarded as a 'theoretically neutral' measure of DD, given the diversity of methodology of DD procedures e.g., adjusting amount and adjusting delay DD procedures (Kwan et al., 2012). Here, no differences in AUC or *k* were observed (Fig. 6A, C). However, if the most impulsive animals were removed,

AUC was significantly lower for both Epoch 1 and Epoch 2 compared to No Inactivation condition (Fig 6B). This would suggest optogenetic manipulation did not have an effect at all experimental delays. Here AUC and *k* are measures of impulsivity that reflect all experimental delays. Further, animals with a predisposition to high impulsivity (referred to here as the 'most impulsive animals') appeared to blunt the ability to detect effects of optogenetic manipulation.

Interpreting Change in Indifference Points

Optogenetic inhibition of mPFC of Wistar rats during the Epoch 2 (i.e. directly before a choice has been made) in an adjusting amount DD procedure affects the subjective value of the immediate reward (indifference point) in a delay-specific manner (Fig. 7A, B). Only at the 8-sec delay did optogenetic manipulation in Epoch 2 of the DD significantly decrease the indifference point relative to No Inactivation days. A possible explanation that could account for why more robust effects of optogenetic manipulation at the 8-sec delay were not observed pertains to variation in impulsiveness across animals. Although all animals were held to stringent magnitude discrimination criteria (80% of the maximum reward value), the degree to which each animal discounted was variable (Fig. 6 & 7). Two of the animals, although passing magnitude discrimination criteria, discounted to a greater degree than the remainder of the animals, but did not meet criteria as outliers. This is most apparent in the No Inactivation condition across the 8 and 16-sec delays where the two lowest data points are consistently the same animals (Fig. 7A, B).

Optogenetic inhibition of mPFC in animals that are highly impulsive may not disrupt IC. Evidence from clinical literature includes that continuous theta burst stimulation (TBS) using transcranial magnetic stimulation of the dlPFC reduces impulsivity (*k*) in healthy individuals compared to control, but not in pathological gamblers (Cho et al., 2010). Pathological gamblers are a population of individuals known for IC (for one review see Reynolds, 2006). It is possible that individuals (rodents or humans) that are highly impulsive rely less on prefrontal cortical regions for forming representations of delayed rewards during DD and instead rely on regions that are specific for immediate rewards i.e., the *beta* system. This may also explain why the effects of optogenetic manipulation at other delays was not apparent, given that the two highly impulsive animals reduced the average indifference points in the No Inactivation condition across experimental delays. Further, in a closer look at indifference points across delays (see Fig. 7A), if the most impulsive individuals were excluded (Fig. 7B), Epoch 1 Inactivation would have also

been significantly different than No Inactivation condition at the 8-sec delay. These relatively highly impulsive animals also contribute to why no differences in AUC (Fig. 6A, B) were observed across conditions. Differences across conditions for *k* values was trending once the most impulsive animals were removed, suggesting the need to increase sample size to increase power of analysis (Fig. 6C, D).

Relationship Between Indifference Points and Latency

Regarding the interpretations of the relationships between variables, it should be considered that there were no significant differences between the slopes of the lines across conditions for any regression analysis conducted, suggesting that a similar relationship between variables existed across conditions (i.e. Epoch 1, 2, and No Inactivation) for all relationships explored (see Fig. 11 & 12).

Effects of optogenetic manipulation on indifference points occur at the 8-sec delay with large effect sizes for the Epoch 2 inactivation condition vs. the No Inactivation condition. Although significant differences in indifference points were not observed across conditions at either the 8-sec for Epoch 1 or for either Epoch at the 16-sec delay, large and medium effect sizes were found for both Epochs at the 8 (large effect sizes for both Epochs) and 16-sec delays (medium and large effect sizes for Epoch 1 and 2, *respectively*; Fig. 7A). Considering the observed effect sizes, the consequences of optogenetic manipulation on indifference points may become more apparent if sample sizes were increased (n=10).

Shorter initiation latencies were also observed at the 8 and 16-sec delays compared to the 4-sec delay, although no effect of condition was observed (Fig. 9A). The effect of delay on initiation latency might be explained by greater attention to and/or motivation to complete a trial at the 8 and 16-sec delays compared to the 4-sec delay (Bizarro & Stolerman, 2003). More motivation to complete trials at the 8 and 16-sec delays are possible and may be due to fewer pellets/session being earned as delays increase. Motivation and attention track with goal directed behavior (H. Kim, Ährlund-Richter, Wang, Deisseroth, & Carlén, 2016), meaning it is possible that mPFC is more involved during decision making at the 8 and 16-sec delays. Consider that medium to large effect sizes of inactivation Epochs on indifference points occurred at the delays where initiation latencies were shown to decrease as further implementing mPFC of Wistar rats in

the '*delta-beta*' systems explanation of optogenetic manipulation contributing to decreased indifference points.

mPFC in this case would be a component of the *delta* system, responsible for contributing to the representation of the delay reward subjective value. Disruption in the development of the delayed reward representation would affect the downstream process of comparing the representations of the immediate and delayed rewards (see review Frost & McNaughton, 2017). In this case, the *beta* system has the potential to contribute the 'winning' signal, i.e., the comparator regions send the necessary information to the motor areas for the physical choice of the immediate reward to be executed.

For the Epoch 1 Inactivation condition, increased *initiation latency* predicted greater *impulsivity* (higher *k* values) (Fig. 11A). When the most impulsive animals were removed from analysis, this association was no longer significant, suggesting that the impulsive animals were influencing this effect (Fig. 11B). Only for the Epoch 2 Inactivation condition did longer *choice latencies* predict greater *impulsivity* (Fig. 11C). This association remained even after the most impulsive animals were removed (Fig. 11D). At least for choice latencies, it has been reported by Linsenbardt et al., (2016) that longer choice latencies are predictive of higher *k* values (impulsivity) in the more impulsive population of rats (Alcohol preferring P rats) but not Wistar rats (Linsenbardt et al., 2016). This would possibly explain why the significant associations of initiation latency and impulsivity were only significant when the two highly impulsive animals were included, as well as why Epoch 2 choice latencies were predictive of impulsivity. Latency seems to be involved in impulsivity regardless of whether impulsivity is artificially produced through optogenetics or whether the animals are naturally impulsive (i.e. the most impulsive animals from this study and the P-rat population from Linsenbardt et al., 2016).

Consider the converse of *shorter latencies* as an interpretation of *greater motivation/attention* and *longer latencies* translating to *less motivation/attention*. Given that there were no differences in the minimal number of initiation/choice omissions (see Fig. 10A, B), motivation was ruled out as the contributing factor. A more probable explanation is that inactivation of mPFC immediately after an initiation and directly before a choice (Epoch 2) disrupted top-down control over attention to the stimuli associated with delayed gain. Subsequently, the representation of the delay gain stimuli to be passed onto the comparator regions is affected, resulting in longer choice latencies predicting greater impulsivity. This explanation is

supported by a proposed framework of attention in the rodent mPFC by Sharpe & Killcross (2018) which states that a key role of the rodent mPFC is to direct control to contextual cues, attentional modulation, and task-setting cues in order to modulate stimulus-response pathways in a goal-directed manner. Further support stems from a study by Jo & Mizumori (2016) implicating mPFC in the regulation of dopaminergic responses in the ventral tegmental area to both predictive cues and rewards in a spatial DD task.

Relationship Between Consistency and Variables

It might be expected that the relationship between *choice latency* and *consistency* would also be significant if effects on attentional processes were responsible for the association between *choice latency* and *k* for the Epoch 2 Inactivation condition. For both the No Inactivation and Epoch 2 Inactivation condition, longer latencies to make a choice predicted lower consistency (Fig. 12E). However, if the two most impulsive animals were removed from analysis, this association was only significant for the No Inactivation condition (Fig. 12F). There was not a significant relationship between *initiation latency* and *consistency* for any condition (see Fig. 12C), suggesting that latency to initiate a choice was not related to decision intent (i.e. a prospective plan) regardless of condition.

The lack of significant associations between choice latency and consistency for the Epoch 2 condition does not necessarily mean that attentional processes were not interrupted via optogenetic inactivation of mPFC. The review by Sharpe & Killcross (2018) conceptualizes an attentional-response as, "one of a host of responses that can be elicited by a stimulus and modulated by task." In this view, it is possible that optogenetic inhibition of mPFC alters attentional responses to the necessary contextual and task-specific information to be updated or *maintained* discretely on each trial. It has been proposed that mPFC pyramidal neurons contribute to attentional processes (H. Kim et al., 2016). Specifically, synchronization of pyramidal neurons at a gamma frequency is suggested to contribute to preferential processing of task-relevant stimuli by increasing the probability of firing on appropriate downstream targets (Buzsáki & Wang, 2012; Salinas & Sejnowski, 2001). In light of these data, it is proposed that building an accurate representation of the delayed reward requires the subjective past, current, and future values of the immediate reward to be discretely at each trial, which requires *maintenance* of attentional responses to those values. Lastly, for all conditions *lower consistency* was predictive of *greater impulsivity* (Fig. 12A). Only

the No Inactivation condition had a significant association if the animals with the lowest impulsivity scores were removed, however (Fig. 12B). In other words, only in the least impulsive condition (No inactivation) did consistency predict impulsivity. This suggests that rats in the No Inactivation condition had a behavioral plan in place, and that optogenetic manipulation of mPFC disrupted that plan.

Establishing a Potential Role of mPFC in IC

In sum, Epoch 2 had significant associations where longer *choice* latencies predicted higher impulsivity (*k)* (Fig. 11C) and less *consistency* (Fig. 12E). This holds true when the most impulsive animals were removed from analysis (see Fig. 11D & 12F). Consider both the Epoch 2 Inactivation condition summary and that effects of initiation latency (Epoch 1 and No Inactivation) were apparent only with inclusion of the most impulsive animals from this study. This might again suggest differential influence of the '*beta-delta'* conceptualization of DD, where animals with induced IC via optogenetic inhibition of mPFC experience more influential power of mPFC during DD in comparison to animals impulsive by nature. Supporting this assumption, only the non-highly-impulsive animals experienced effects of optogenetic manipulation.

This study implicates mPFC of rodents as contributing to IC behavior in an adjusted amount DD procedure. The overall goal of this experiment was to understand mPFC's contribution to the *development* and *maintenance* of prospective strategies and subsequent effects on impulsivity during discrete epochs of a DD task. At a first glance, only a minor effect on indifference points at the 8-sec delay were apparent with no condition-specific effects on consistency or latency. Looking at the relationships between the variables, however, a pattern emerged between impulsivity (*k*), choice latency, and consistency for the Epoch 2 Inactivation condition. This suggests that mPFC is involved in the selection of delayed rewards during Epoch 2, specifically, for non-highly impulsive animals.

In light of these data it is proposed that the rodent mPFC is a component of the *delta* system in the '*beta-delta'* conceptualization of DD that is involved in IC behavior by selectively allocating attentional-resources to contextual and task-specific stimuli in order to contribute to well-informed representations of the immediate and delayed gains. For highly-impulsive rats, mPFC is likely less involved. Rather, the *beta* system exerts greater influence on choices that ultimately result in IC. Further, it is proposed that mPFC contributes discretely to choices made during each trial in order

to provide *maintenance* to stimulus-response relationships pertaining to the immediate and delayed rewards in order to modify goal directed behavior. Inhibiting mPFC pyramidal neurons directly before a choice appears to affect the allocation of attentional-resources that contribute to a relationship between greater impulsivity (*k)*, lower consistency, and increased choice latencies. One interpretation is that mPFC pyramidal neurons contribute to *maintenance* of prospective strategies to some degree moments before the decision is made.

Conclusions and Future Directions

Contrary to the hypothesis that effects of optogenetic manipulation of mPFC on behavioral measures would occur at the 4-second delay i.e., the intermediate delay, effects of were most apparent at the 8-second delay. A possible explanation is mPFC is involved to a greater degree at longer delays, perhaps through allocating appropriate attentional resources that are relevant to the task. Disruption in these attentional resources may contribute to the relationship that emerged between impulsivity, lower consistency, and choice latencies in the present study. In sum, these data suggest a role of the mPFC in DD.

Further studies implicating the rodent mPFC in DD should be conducted. More optogenetic manipulation studies with the procedure described here should be conducted to increase sample sizes and look for potential increases in effects of inactivation. To that notion, criteria for *k* (measure of impulsiveness) should be set for future studies involving inactivation of mPFC in order to fully assess how inactivation of mPFC in the non-impulsive population affects measures of impulsivity.

Moreover, future studies should investigate whether disruption in attentional processes involving mPFC are a contributing factor to the increased impulsivity observed in the current study. Considering involvement of mPFC pyramidal neurons synchronous activity in attentional processes, optogenetic stimulation procedures can be used to study pro-cognitive effects of mimicking naturally occurring gamma oscillatory phenomenon during this modified DD task (H. Kim et al., 2016)CITE). This would provide the framework for understanding how increasing the degree of influence from the component of the *delta* system involved in allocating appropriate attentional resources i.e., mPFC, has the potential to reduce IC in intrinsically impulsive animals.

Understanding the relationship between the *beta* and *delta* systems and how they differ between impulsive and non-impulsive animals will provide necessary information to investigate

treatments for the clinical population to address maladaptive decision making via cognitive (prospective thinking, mindfulness interventions), pharmaceutical (pro-cognitive drugs), or electrical-based therapy (e.g. TMS). Developing a treatment for IC has the potential to benefit a diverse population of individuals ranging from substance use disorders to obesity (Dalley et al., 2011).

TABLE

Table 1. Experimental Design for Optogenetic Manipulation Across the 4, 8, and 16-secon Delays.

Half of the animals in each group received configuration A and half of the animals received configuration B. The X indicates that No inactivation took place on that day. For days 3, 5, 7, and 9, either Epoch 1 inactivation occurred (indicated in both text as well as a magenta square) or Epoch 2 inactivation occurred (indicated in both text as well as a red triangle).

	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**
A	**X**	**X**	Epoch 1 ■	**X**	Epoch 2 ▲	**X**	Epoch 1 ■	**X**	Epoch 2 ▲
B	**X**	**X**	Epoch 2 ▲	**X**	Epoch 1 ■	**X**	Epoch 2 ▲	**X**	Epoch 1 ■

FIGURES

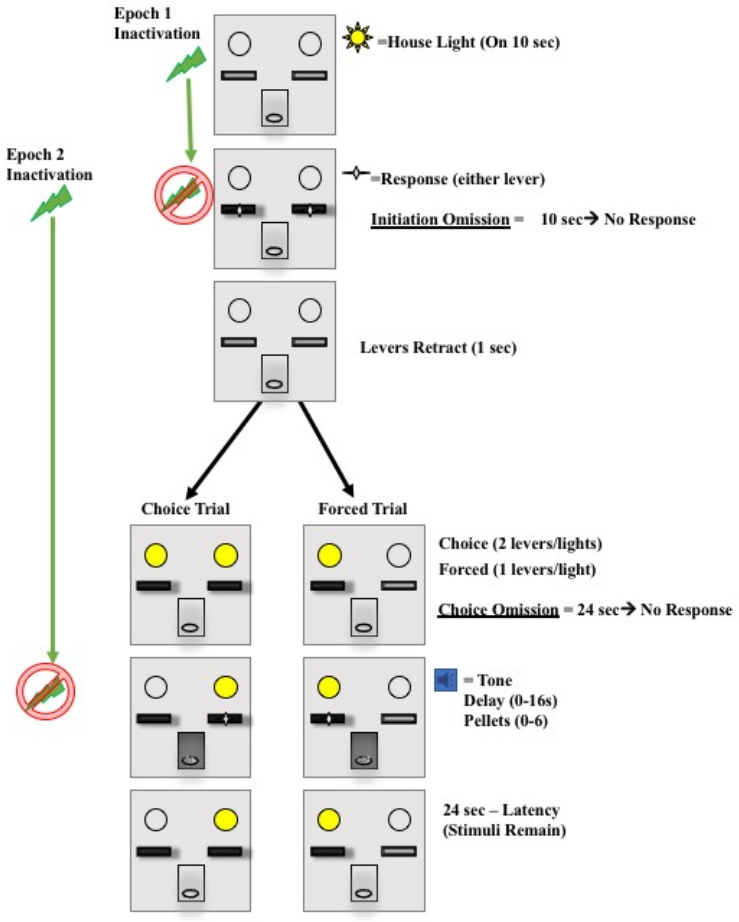


Figure 1. Schematic Representation of the Within-session Adjusting Amount Delay Discounting Procedure Modified from Linsenbardt et al. (2016).
Represented here is a full trial, which can either be choice or forced trial. The portion of the trial where Epoch 1 and Epoch 2 occur are indicated above.

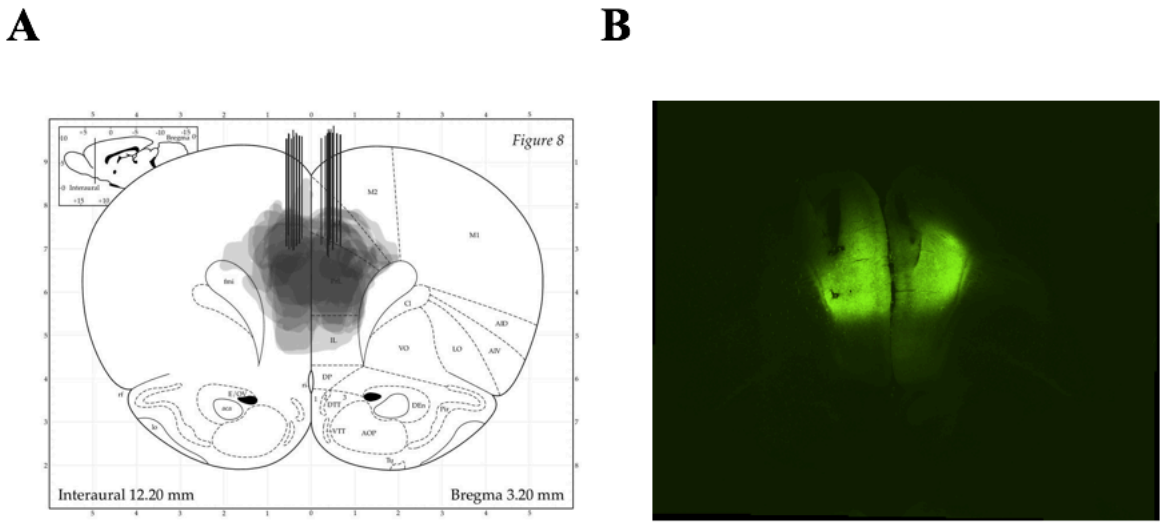


Figure 2. Placement Quantification and Representative Image for Group 1 and Group 2 Animals. (A) ArchT expression spread and optic fiber placements for all animals (n=10). (B) Representative image of viral spread and optic fiber placements.

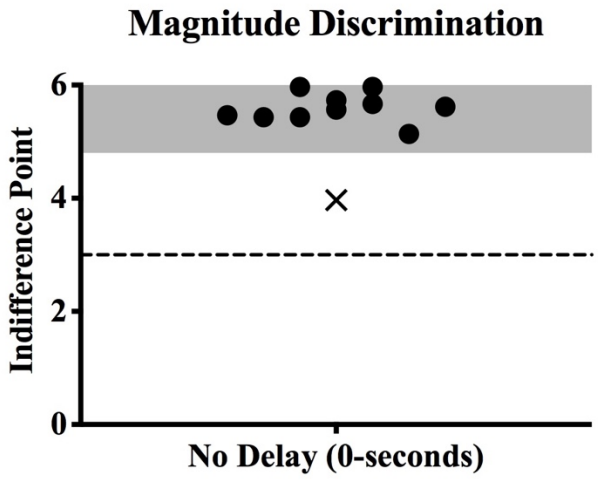


Figure 3. Magnitude Discrimination Test (0-sec delay).
One animal did not pass criteria for magnitude discrimination (indicated by the gray X). The shaded region indicates inclusion criteria of 80% of the maximum reward value (4.8 pellets). The dotted line indicates equal likelihood of choosing the delay and immediate lever. Each dot represents an individual animal.

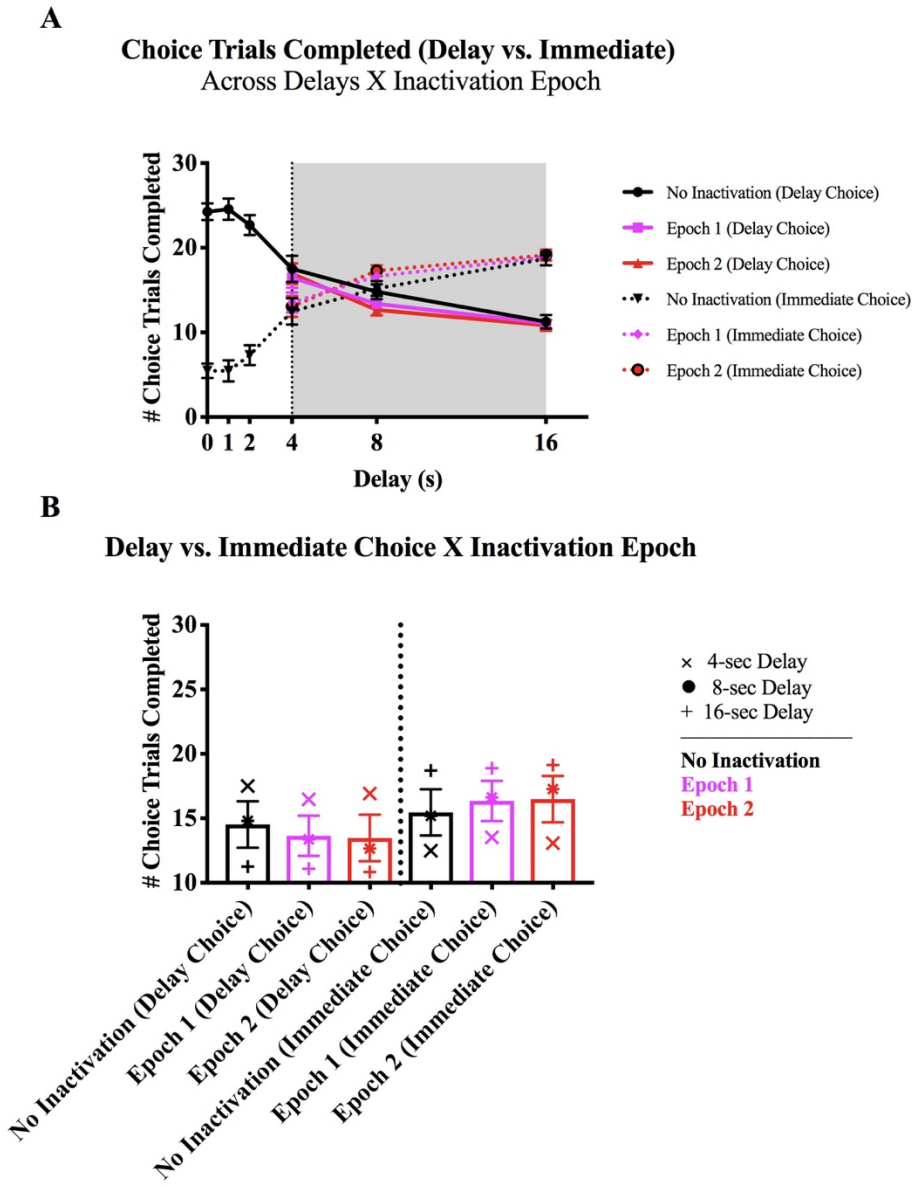


Figure 4. Number of Delay and Immediate Choice Trials Across Experimental Delays. Number of choice trials by Inactivation Epoch over delays (mean ±SEM) showing a decrease in number of delay choice trials and increase in number of immediate choice with increasing delay. The dotted line and shaded region indicate delays where optogenetic manipulation occurred. (B) Number of Immediate and Delay choices across delays and separated by condition (mean ±SEM). No significant differences were observed in number of delay/immediate choice trials by Inactivation Epoch. The dotted line indicates a separation between delay and immediate choice trials.

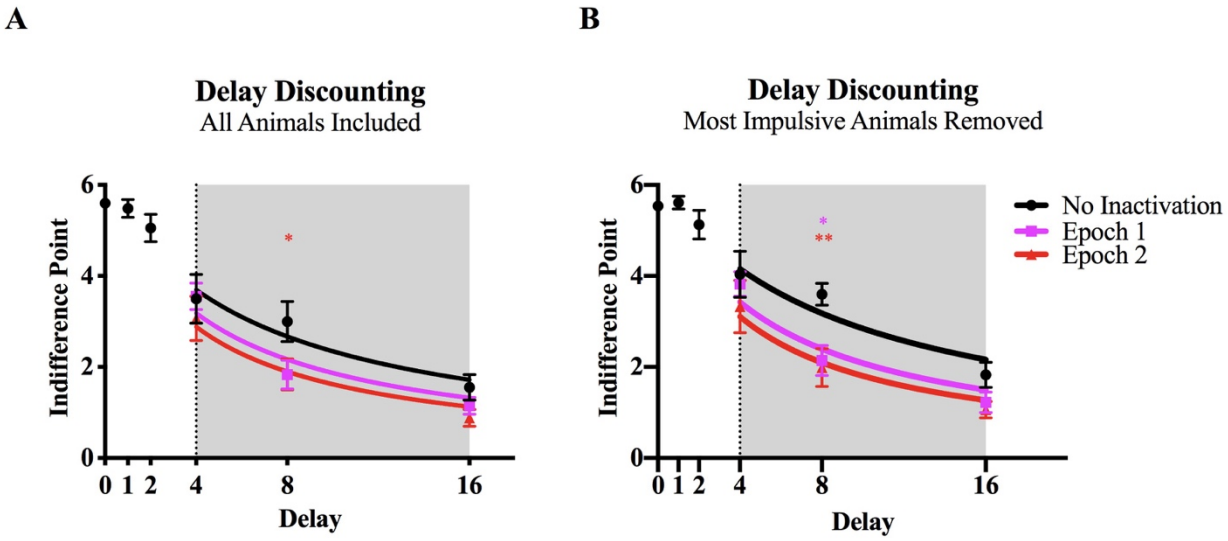


Figure 5. Delay Discounting Hyperbolic Curve.

All animals included (A) vs. when the most impulsive (n=2) animals were removed from analysis (B). (A & B) Hyperbolic curve fit to indifference points (mean ± SEM) for all animals across the 4, 8, and 16-sec delay for each condition. The asterisks indicate a significant difference in indifference points for Epoch 2 (red) or Epoch 1 (magenta) in comparison to No Inactivation. The shaded region indicates delays where optogenetic manipulations were conducted (* $p < .05$, ** $p < .01$).

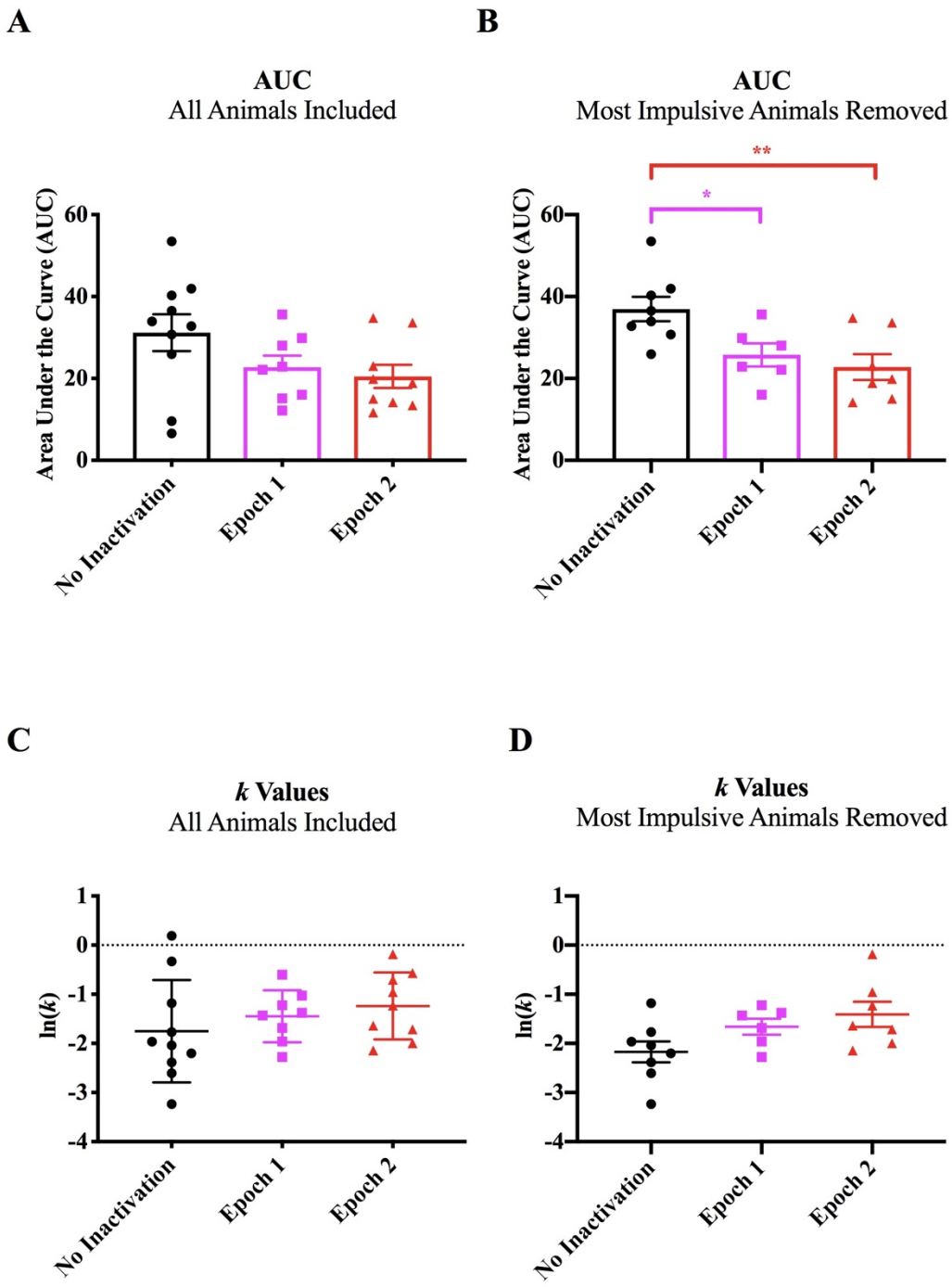


Figure 6. Additional Measures of Impulsivity (AUC and *k*).
All animals included (A & C) vs. when the most impulsive animals (n=2) are excluded from analysis (B & D). (A & C) AUC (mean ± SEM) as well as individual animals shown. (B & D) natural log transformed *k* values (mean± SEM) and individual animals. Asterisks indicate significant differences between Inactivation condition (Epoch 1, magenta; Epoch 2, red) and No Inactivation condition (black; * $p < .05$, ** $p < .01$).

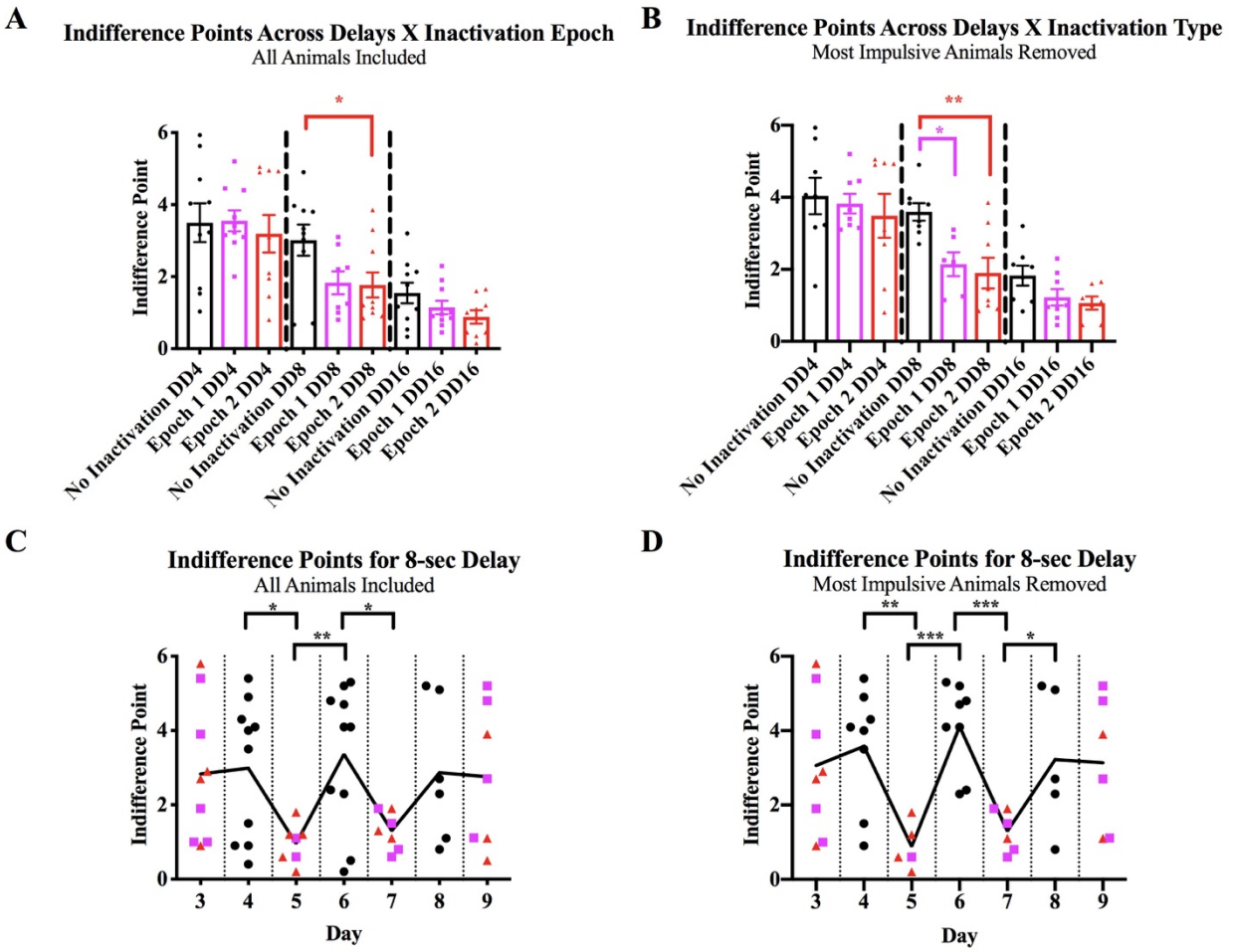


Figure 7. Indifference Points Separated by Condition Across Experimental Delays and Indifference Points Across Each Day of the 8-second Delay.

All animals included in analysis (A & C) vs. when the most impulsive animals (n=2) were removed (B & D). (A & B) Indifference points (mean ± SEM) as well as individual animals plotted across experimental delays. The asterisks (red, Epoch 2; magenta, Epoch 1) indicates indifference points at the 8-second delay were significantly different for No Inactivation condition compared to Inactivation conditions. Inactivation conditions resulted in lower indifference points compared to No Inactivation. The dotted lines indicate a separation between delays (DD4=4-sec, DD8=8-sec, DD16=16-sec delay; * $p < .05$, ** $p < .01$). (C & D) Indifference points across days of the 8-sec delay collapsed across Inactivation Epoch with mean plotted as well as individual animals. Asterisks indicate significant differences between a baseline day (days 4, 6, or 8) and an inactivation day (days 3, 5, 7, or 9). Black circles indicate No Inactivation, magenta squares indicate Epoch 1 Inactivation, and red triangles indicate Epoch 2 Inactivation (* $p < .05$, ** $p < .01$, and *** $p < .001$).

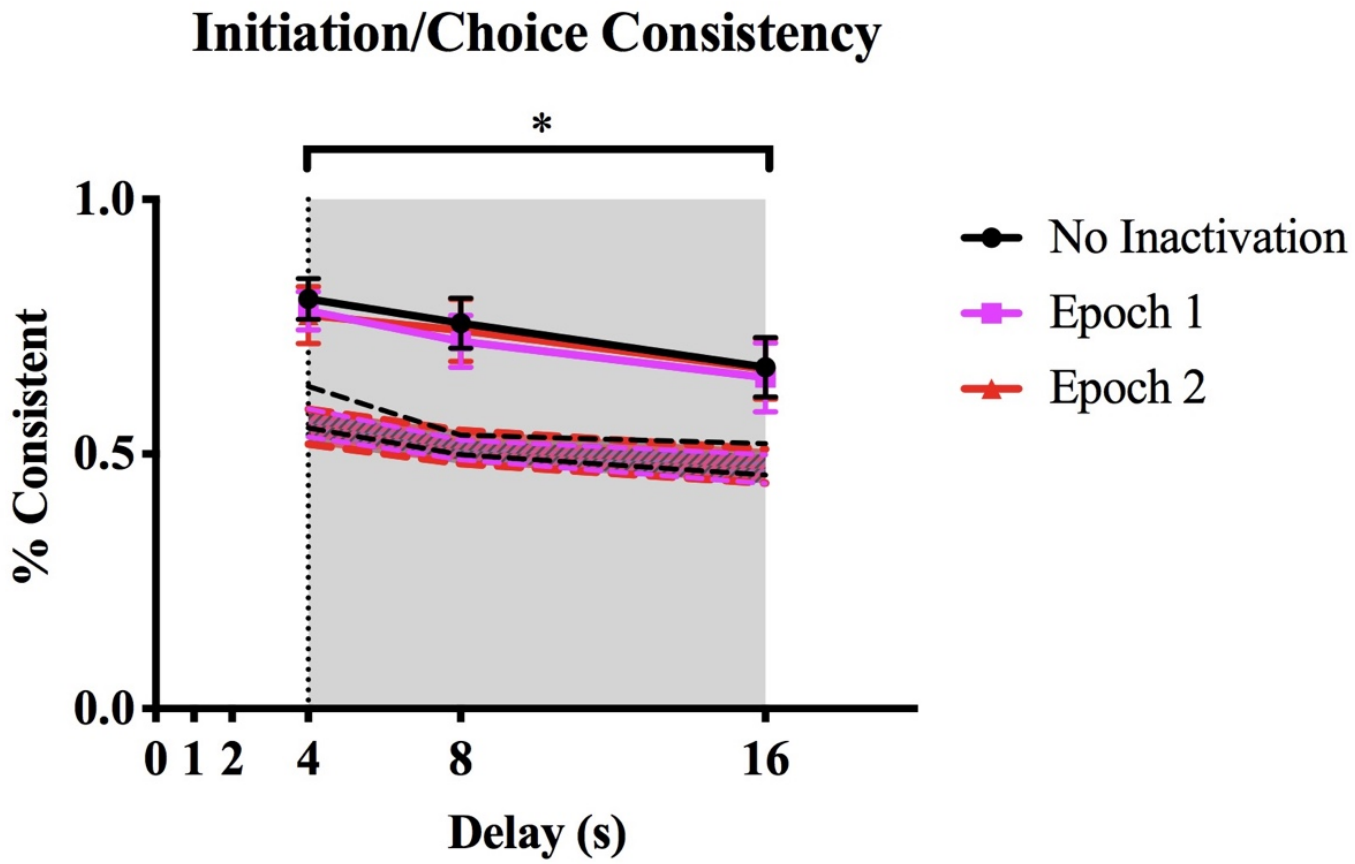


Figure 8. Observed and Chance Consistency for All Delays.

Consistency (mean ± SEM) over delays decreased regardless of condition. The dotted line at the 4-second delay .and shaded region indicate delays where optogenetic manipulation occurred. The asterisk indicates significant differences in consistency between the 4 and 16-second delays (* $p < .05$).

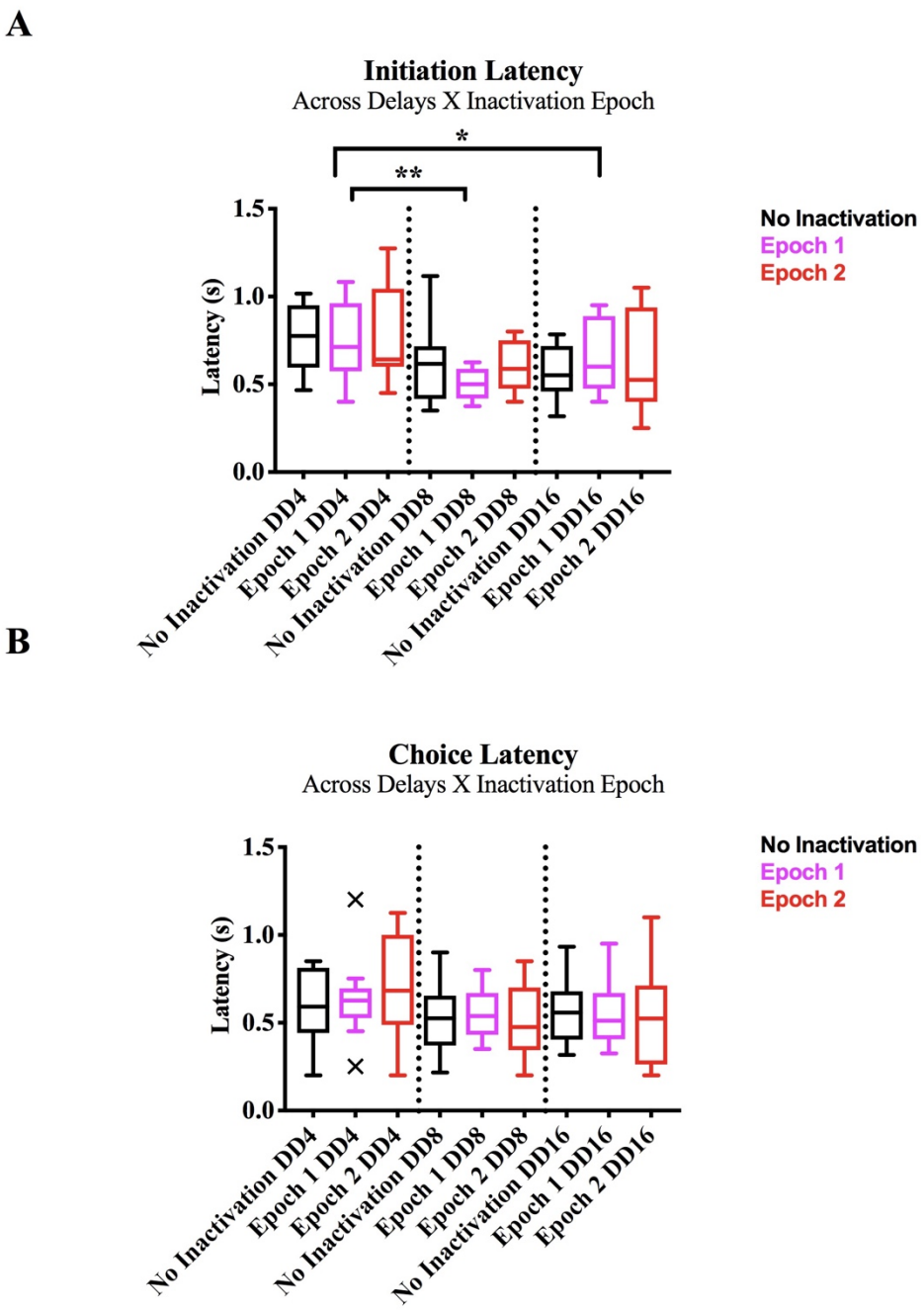


Figure 9. Initiation and Choice Latencies Across Experimental Delays.

Both Initiation as well as Choice Latencies are shown for each condition across each of the experimental delays as box plots. The dotted line indicates separations between delays. (A) Initiation Latency at the 4-sec delay was significantly longer for all conditions than both the 8 and 16-sec delays (* $p < .05$, ** $p < .01$) and (B) No differences in any condition across delays for Choice Latency. The X indicates outliers removed from analysis. The dotted lines indicate a separation between delays (DD4=4-sec, DD8=8-sec, and DD16=16-sec delay).

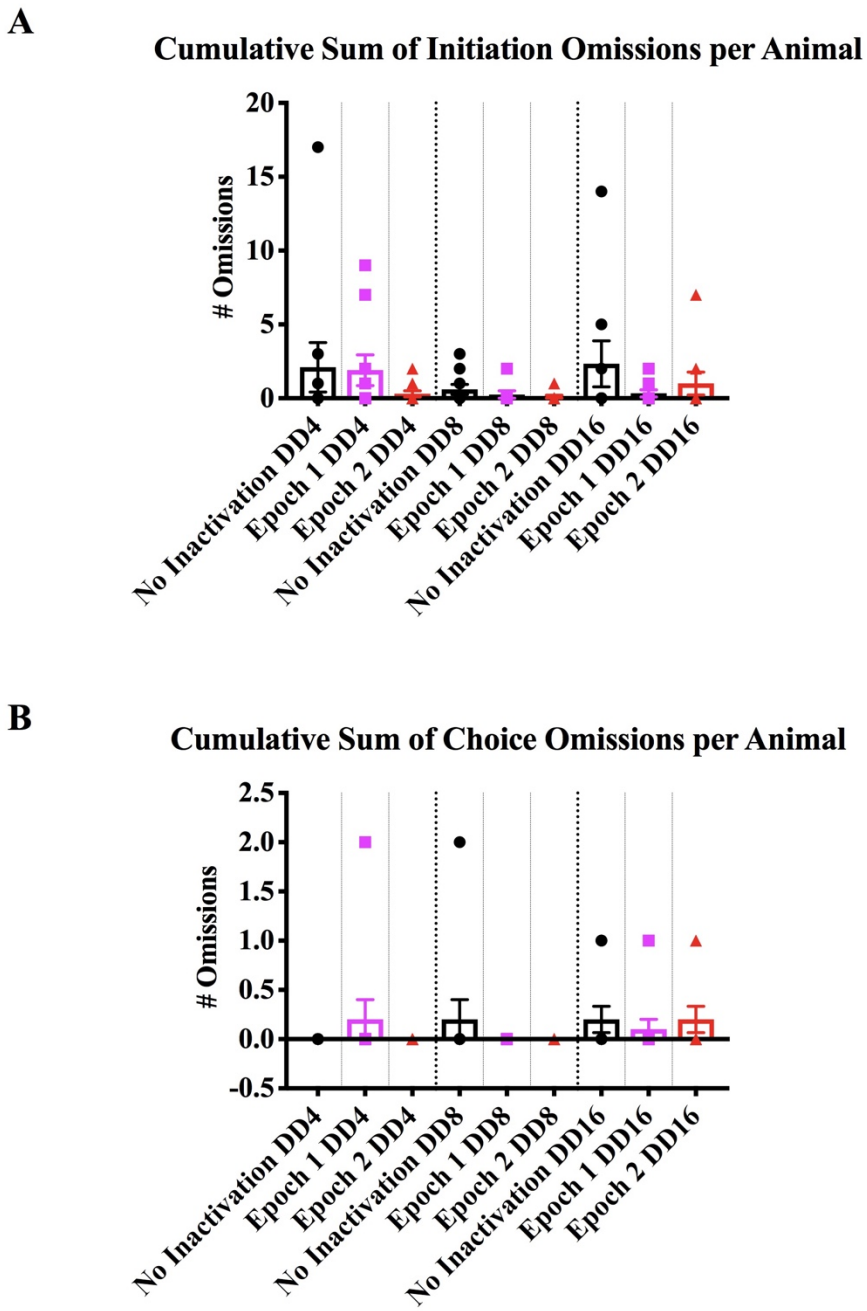


Figure 10. Initiation and Choice Omissions Across Experimental Delays.
Cumulative Initiation Omissions (A) and cumulative Choice Omissions (B) for each animal across delays and separated by condition. Black circles indicate No Inactivation, magenta squares indicate Epoch 1 Inactivation, and red triangles indicate Epoch 2 Inactivation. The black dotted lines indicate a separation between delays (DD4=4-sec, DD8=8-sec, and DD16=16-sec delays). The gray dotted lines indicate a separation between each condition.

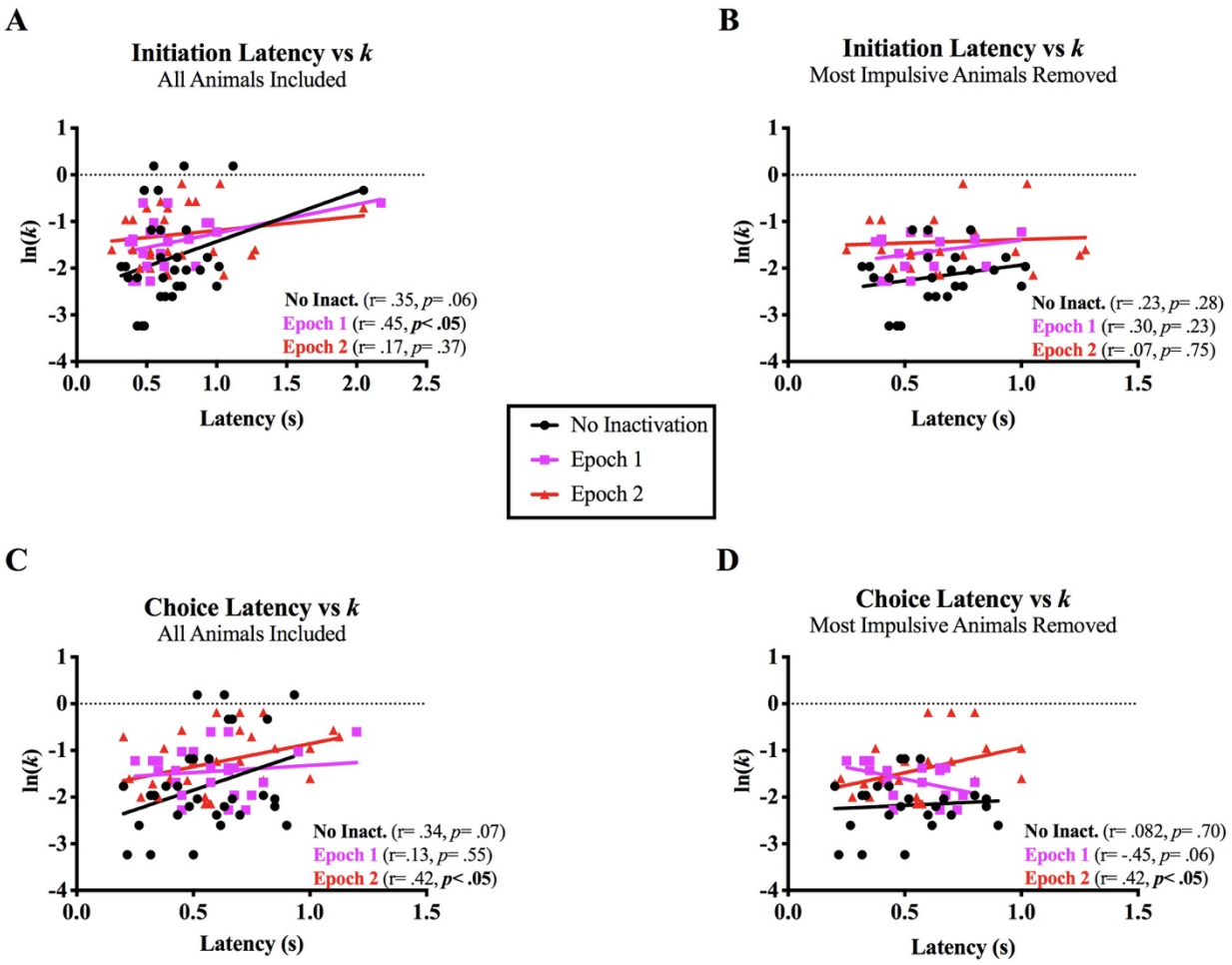


Figure 11. Relationship Between Latency and Natural Log Transformed *k* Values. All animals included (A & C) vs. when the most impulsive animals (n=2) were removed from analysis (B & D). (A, B, C, & D) Correlated impulsivity measures, *k,* with Latencies. (A & B) Impulsivity measure, *k*, positively associated with Initiation Latency for Epoch 1 Inactivation conditions (A) when impulsive animals are included vs. no significant association when impulsive animals are removed (B). (C & D) Impulsivity measure, *k*, positively associated with Choice Latency for Epoch 2 Inactivation condition both when impulsive animals are included (C) vs when removed from analysis (D). Significant associations between an Inactivation and No Inactivation condition are bolded and shown in the individual graphs.

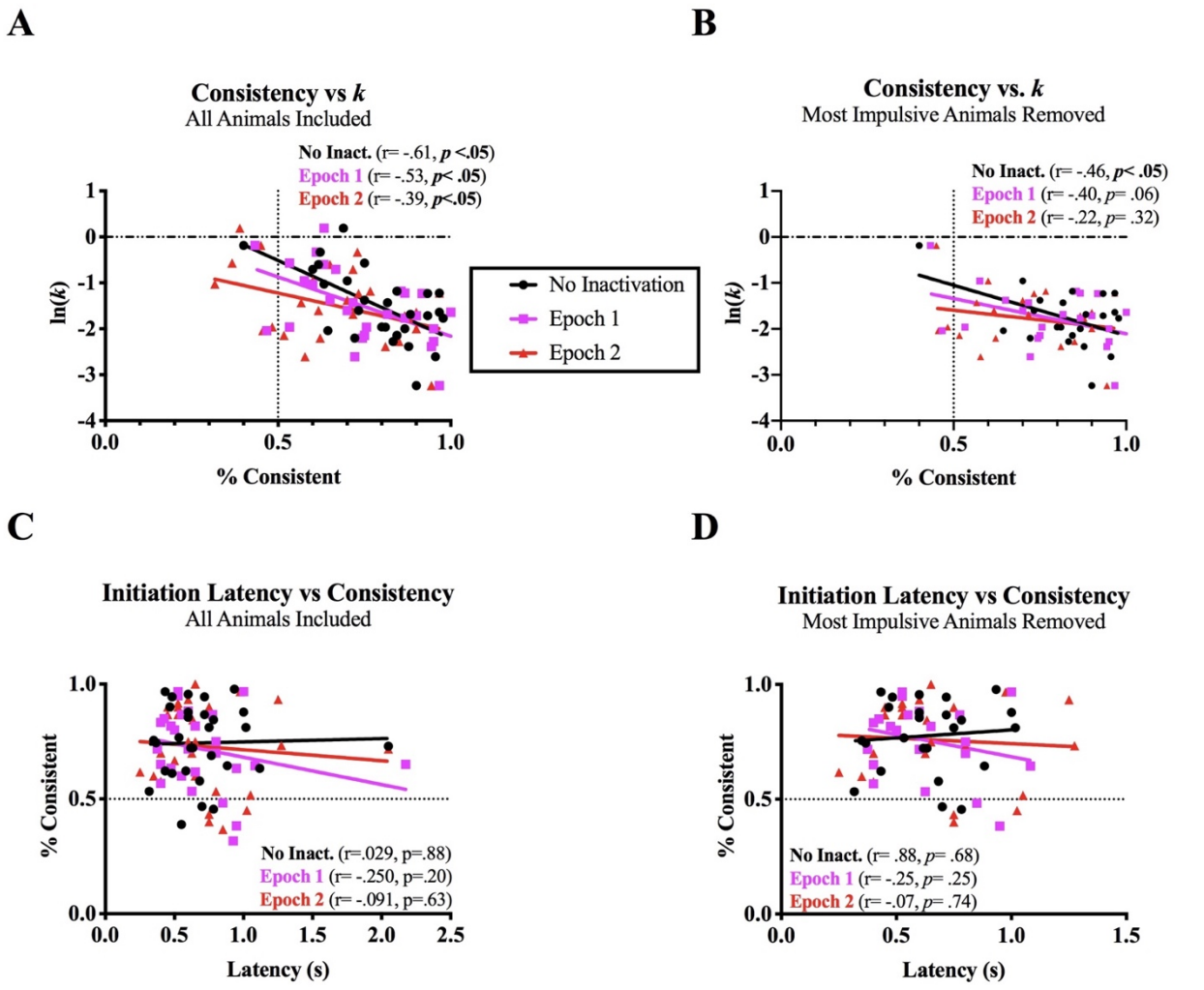
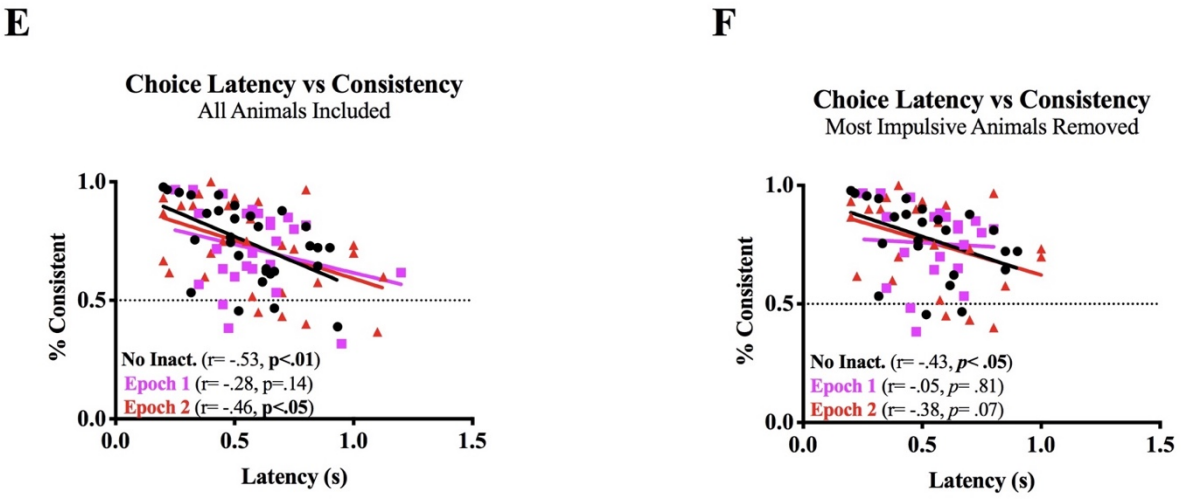


Figure 12. Relationship Between Consistency and Variables of Interest.
All animals included (A, C, & E) vs. when the most impulsive animals (n=2) were removed from analysis (B, D, & F). (A & B) Correlated impulsivity measure, *k*, with consistency. A significant association was detected for all conditions (A). Only the significant association in the No Inactivation condition remained when most impulsive animals were removed (B). (C, D, E & F) Correlated measures of consistency with Initiation Latency (C & D) and Choice Latency (E & F). Choice Latency was significantly associated with Consistency for both No Inactivation and Epoch 2 Inactivation conditions when all animals were included (E). Only the No Inactivation condition association between Choice Latency and Consistency remained significant when the most impulsive animals were removed (F). Significant associations between an Inactivation and No Inactivation condition are bolded and shown in the individual graphs.

Figure 12. continued



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