The Influence of alpha7 and alpha4 beta2 Nicotinic Receptor Agonists on Feature Based Reversal Learning in Macaque Monkeys

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Abstract

Selective and non-selective nicotinic agonists are associated with improvements in higher order cognitive functions. However, the effects of selectively activating nicotinic sub-receptors on attention and learning are not well understood. In my project, I used two agonists selective for alpha7 (α7) and alpha4-beta2 (α4β2) nicotinic sub-receptors to test the effects of selective nicotinic activation on performance in a feature-based reversal learning task in non-human primate subjects. Overall, results showed that selective activation of nicotinic receptors led to improvements in different aspects of the task which were time and dose dependent: the optimal dose of α7 agonist improved performance accuracy and sped up learning of reversals in reward contingency, when drug plasma concentration was at its peak. In comparison, the best dose of α4β2 agonist reduced susceptibility to distraction. These findings are an important first step to identify the nicotinergic neuromodulatory mechanisms of attention and learning functions in the primate brain.

Acknowledgments

First, I wanted to thank my supervisor Dr. Thilo Womelsdorf for his continuous guidance and mentorship throughout my studies and research. I'm delighted to have made the decision to join your lab. I can't stress enough what a great learning experience the past two years was for me. I sincerely hope you remain a mentor to me after I complete my degree.

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Chapter 1-Introduction

1.1 Higher Executive Function

Higher executive functions are a collection of cognitive processes which enable the organism to respond and adapt to environmental changes. Executive functions include but are not limited to, working memory, attentional processes, and cognitive flexibility (Robbins, 1996). Each of these functions can be further differentiated into other processes. Working memory is the ability to temporarily store a restricted set of relevant information and process them to guide future behavior. Working memory is distinct but not independent from other forms of memory. According to the Baddeley and Hitch (1974) model, it consists of different components wherein different incoming visual, spatial and phonological information are integrated and transferred to long term memory. As such, working memory plays an essential role in thinking and decision making.

Attention describes the selective processing of information. Attention is often studied as a variety of processes which can include: 1) the ability to selectively attend to a set of incoming information at the expense of other subsets 2) the ability to sustain a selective attentive state over time and 3) the ability to shift the attentional allocation from one set of information to another (Bushnell and Strupp, 2009).

Cognitive flexibility involves adjustable changes in strategies i.e. a set of operations used to solve a problem to adapt to unexpected environmental changes (Izquierdo et al. 2016). Adjustment to novel situations requires recognition of event changes and directing attention to them, inhibition of previously appropriate responses that are not relevant to the new context, and devising new strategies that are applicable and goal-directed.

These higher order cognitive processes work in parallel and in coordination with each other to optimize decision making for reaching a goal, although the specifics of interaction between them is not well understood (Fougnie, 2008).

How neuromodulatory systems interact and modulate, i.e. increase or decrease the frequency and amplitude of neural activity in brain areas, is important for operation of executive functions. Neuromodulatory systems are composed of neurons that can modulate various populations of neurons in different brain areas via different groups of neurotransmitters. Dopaminergic, noradrenergic and cholinergic neuromodulatory systems, which employ dopamine, norepinephrine and acetylcholine neurotransmitters respectively, are suggested to interact and modulate visual working memory, selective attention as well as reversal learning (Izquierdo et al., 2016; Logue & Gould, 2014; Moore & Zirnsak, 2017).

For the remainder of this introduction, I will mainly focus on nicotinergic subsystem of the cholinergic modulation. I will discuss the putative physiological mechanisms underlying nicotinic receptor functions. I will then discuss task paradigms that are commonly implemented in rodents and non-human primates to study cognitive flexibility and reversal learning. I will then provide an overview of the current literature on specific nicotinic sub-receptors and of the gaps that this thesis aimed to fill.

1.2 Cholinergic Modulation of Cognition

1.2.1 Overview of Acetylcholine

Acetylcholine (ACh) is a widely-distributed neurotransmitter in both the peripheral nervous system (PNS) and central nervous system (CNS). The cholinergic system, which involves acetylcholine and the sites affected by it, underlies alertness, cerebral cortical development as well

as learning and memory processes in mammalian species (Schliebs & Arendt, 2006). The source of cholinergic projections, which are widely sent throughout the brain, originate mainly in the basal forebrain, but also from the pedunculopontine and laterodorsal tegmental areas (Picciotto, Higley, & Mineur, 2012). The basal forebrain is a collection of multiple subsections including the Nucleus basalis of Meynert (NBM) which provides wide projections to the cortex (Gratwicke et al., 2013) (**Fig. 1**). The NBM in rodents is primitive and not well differentiated from the surrounding structures while in non-human primates and humans, the NBM has reached its greatest development in size and is well differentiated from the surrounding cell types (Mesulam & Geula, 1988). This species specific difference is possibly due to the massive expansion of the cerebral cortex which is the main innervation target of the NBM in primates (Divac, 1975).

Post mortem studies have confirmed significant reductions of a particular cholinergic sub-type receptor (α7 nicotinic receptor) subunit protein in the frontal cortex of schizophrenia patients (Guan, Zhang, Blennow, & Nordberg, 1999) and significant loss of the NBM neurons in patients diagnosed with Alzheimer disease and Parkinson disease dementia (Etifnne et al., 1986). This significant neural loss has been associated with cortical cholinergic deficits and cognitive impairments in these patients (Gratwicke et al., 2013). Such evidence is indicative of notable contributions of the cholinergic system to learning and memory in humans. In non-human primates, local blockade of cholinergic receptors in the perihinal cortex lead to visual recognition impairment, possibly due to interference with storage processes of stimulus representation (Tang, Mishkin, & Aigner, 1997). Similarly, other local manipulations of the cholinergic system in the brain has shown deficits in specific aspects of memory formation in rodents (Hasselmo and Stern, 2006). The evidence from animal studies suggest that the cholinergic system has a preserved role in cognition and memory across different species (Gratwicke et al., 2013).

NSP: Nucleus subputaminalis
Ch4p: Posterior sector
Ch4i: intermediate sector
Ch4al: Anterolateral subsector
Ch4am: Anteromedial subsector

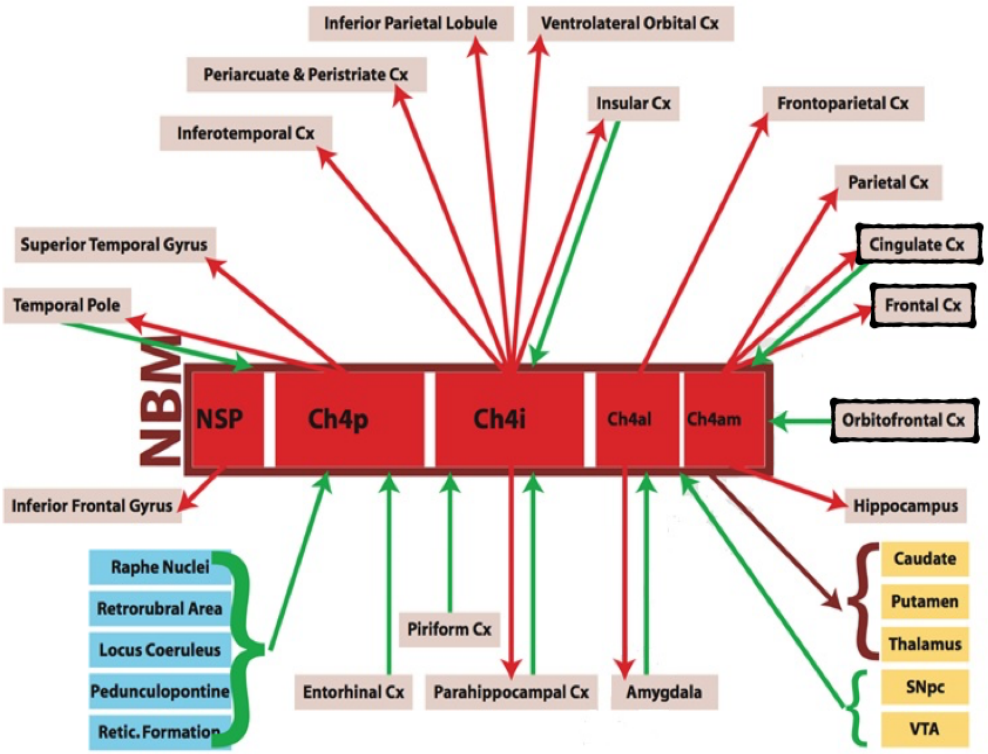

Figure 1 Proposed cortical and subcortical connections to the NBM for humans. These connections are suggested based on anatomical and histological experiments and observations in non-human primates, rodents and humans. The diagram shows different subcomponents of the NBM whose relative sizes are scaled with white line divisions. The green and red arrows represent afferent and efferent projections respectively. The main cortical areas identified to play role in attention set shifting and reversal learning are highlighted in black frames. (Modified from Gratwicke et al., 2013).

In the PNS, acetylcholine is involved as a neurotransmitter in neuromuscular junction and muscle activation. However, in the central nervous system (CNS) it acts as both neurotransmitter and neuromodulator, although its means of action are proposed to be predominantly through neuromodulation (Picciotto et al., 2012; Rowe & Hermens, 2006). Acetylcholine acts on two main

receptor groups (muscarinic and nicotinic) in both nervous systems. Each subsystem consists of various sub-receptors that differ from each other in terms of subunit component, local expression and pharmacokinetic properties (Dani, 2001).

Despite higher expression of muscarinic receptors compared to nicotinic receptors in the brain, both subsystems are suggested to be important for attention and learning (Gitelman & Prohovnik, 1992). Over the past few decades, clinical evidence consisting of genetic and molecular studies supported involvement of nicotinic receptors in some of the observed brain abnormalities and associated cognitive impairment in brain diseases such as schizophrenia and Alzheimer's disease. For instance, in-vivo studies have shown nicotinergic interaction with beta-amyloid pathologies in animal models of Alzheimer's disease (Schliebs & Arendt, 2006). Postmortem investigation of brain tissues in schizophrenia patients indicated reduction in a nicotinic sub-receptor expression (Leonard et al., 2000). Given these observations and development of pharmacological agents that can selectively agonize/antagonize different nicotinic sub-receptor, more neuro-pharmacological studies investigating cognitive modulation through nicotinic receptors have been conducted.

1.2.2 Specific Sub-Receptor Effects at the Physiological Level: α7 Versus α4β2

The nicotinic subsystem, which is composed of ligand-gated ion channels, is classified into a variety of receptors. In general, these receptors can be classified into three main functional classes based on their pharmacokinetic and physiological characteristics: muscle subunits; standard neuronal subunits (α2- α6 and β2- β4), which together form the nicotinic receptors in αβ combinations; and subunits (α7- α9) that form homomeric nACh receptors (Dani, 2001). The homomeric alpha7 (α7) and heteromeric alpha4 beta2 (α4β2) nicotinic receptors (**Fig. 2**) are the

dominant subtypes expressed in the mammalian brain (Dani, 2001) and of more interest for pharmacological interventions. The two sub-types differ from each other in molecular properties such as desensitization, affinity and permeability to Ca^{2+} ions. Receptor desensitization is a physiological process where the signaling of the receptor is attenuated in response to high concentration levels of the corresponding ligand or agonist. It can regulate receptor affinity (the ability of ligand binding to receptors), upregulation and synaptic plasticity (Mansvelder, Keath, & McGehee, 2002; Quick & Lester, 2002). Desensitization kinetics interact with activation and in-activation states of the receptors, although the exact dynamics are still not well-known (Levin, 2013; Quick & Lester, 2002; Suto & Zacharias, 2004). α7 receptors have comparatively low affinity for nicotine and rapid desensitization while α4β2 receptors possess higher affinity for nicotine and slower desensitization. Different desensitization kinetics of the nicotinic sub-receptors are suggested to underlie the rewarding and reinforcing properties of nicotine (Mansvelder et al., 2002; Quick & Lester, 2002). Previously it was shown that self-administration of nicotine by rats activates the ventral tegmental area (VTA) of the mesolimbic system, an important neural pathway for motivation and behavioral reinforcement. Mansvelder and colleagues (2002) showed that upon exposure to low nicotine concentrations, GABAergic inhibitory neurons expressing α4β2 nicotinic sub-receptors in the VTA become insensitive to the endogenous cholinergic transmission due to fast desensitization of α4β2 receptors. Thus, their inhibitory input to dopaminergic neurons is suppressed and consequently dopaminergic neurons are disinhibited. Meanwhile due to slow desensitization of α7 receptors, which are expressed in the glutamatergic excitatory neurons, the excitatory transmissions from glutamatergic neurons in the VTA is enhanced. If dopaminergic neurons are sufficiently depolarized, their overall excitability may be further increased as a result of this glutamatergic transmission. As such, it is

proposed that the desensitization kinetics of α4β2 and α7 receptors differentially contribute to reinforcing effects of nicotine and its addictive properties.

Permeability of receptors to Ca^{2+} is an important characteristic because this ion is involved in cascades of important signaling pathways involved in multiple synaptic plasticity forms such as long term potentiation (LTP), paired-pulse facilitation and depression and post-tetanic potentiation (PCP) (Citri & Malenka, 2008; Hunter, de Fiebre, Papke, Kem, & Meyer, 1994; Zucker, 1999). Nicotinic α7 receptors are highly permeable to Ca^{2+} ions while α4β2 receptors are much less permeable (Albuquerque, Pereira, Alkondon, & Rogers, 2009; Dani, 2001; Quick & Lester, 2002; Tanner, Chenoweth, & Tyndale, 2015). In addition, α7 receptors are reported to enhance activities of another highly Ca^{2+} permeable receptor known as N-methyl-D-aspartate (NMDA) receptor (Yang et al., 2013). NMDA receptors are glutamate receptors which are also permeable to Ca^{2+} ions and mediate their influx to post synaptic sites. As such, NMDA receptors are involved in mediating second messenger pathways important for neural plasticity and memory (Li & Tsien, 2009).

Chen and coleagues (2006) provided evidence for α7 receptor mediation of synaptic plasticity in CA1 region of the hippocampus. The authors reported that α7 receptor are necessary for LTP induction in the CA1 as blockade of α7 receptors in the Schaffer-collateral inhibited LTP induction in naïve rats. Moreover, application of an α7 receptor agonist (GTS-21) restored other neural plasticity processes such as PCP which were impaired because of treatment with beta-amyloid peptides in the same brain area.

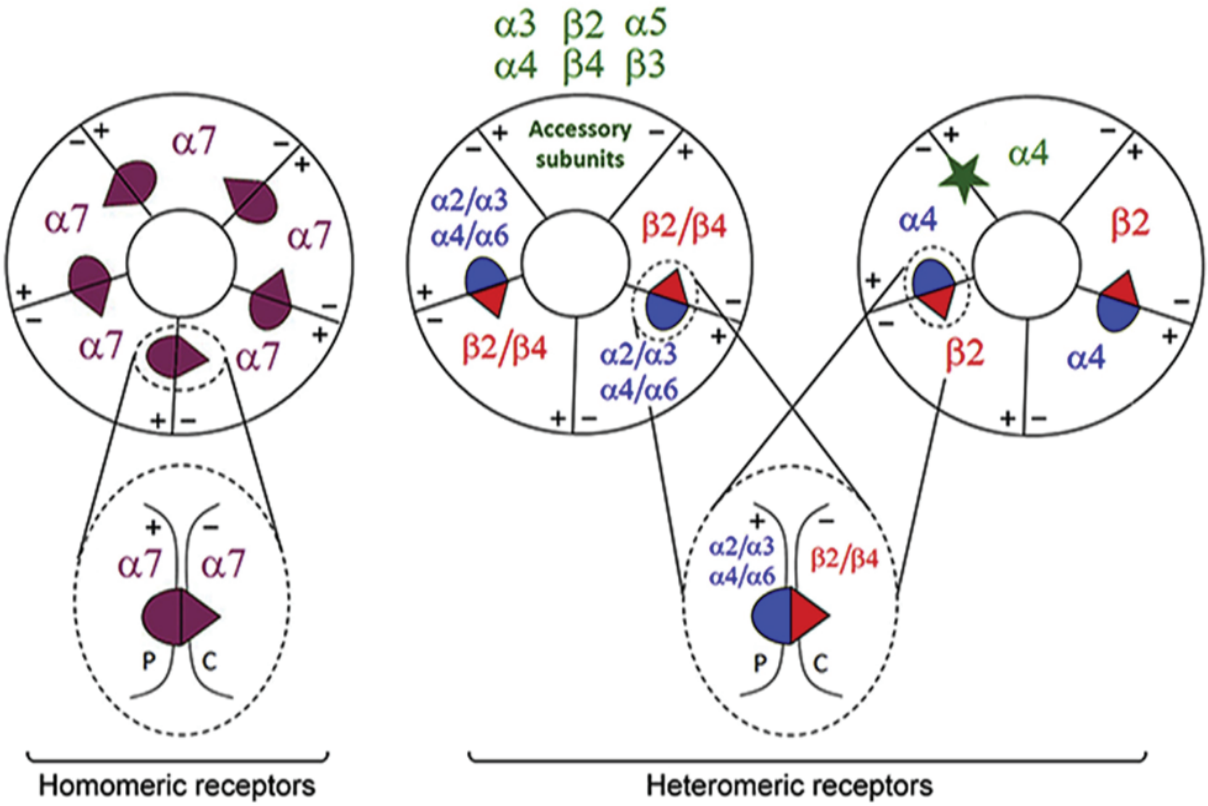

Figure 2 Two main homomeric and heteromeric nicotinic receptors and their subunit arrangements. The binding sites are color coded with different geometrical shapes. As seen in the diagram, heteromeric receptors possess different binding sites than homomeric (Zoli, Pistillo, & Gotti, 2015). This thesis will only focus on α7 homomeric and α4β2 hetermoeric subtype receptors.

In addition to subunit differences, expression patterns of sub-receptors across brain areas and cortical layers can affect modulation of neural activities by these receptors. In-vitro and in-vivo experiments have suggested across different cortical layers, pyramidal and interneurons

differentially contain α7 and α4β2 receptors in the mouse model. Moreover, neural activities of different cell types were also shown to be modulated by nicotinic sub-receptors in a layer dependent manner (Arroyo, Bennett, Aziz, Brown, & Hestrin, 2012; Bennett, Arroyo, Berns, & Hestrin, 2012; Poorthuis et al., 2013). For instance, in the deep layers of medial PFC (mPFC), pyramidal cells were activated by α7 receptors in layer V while in layer VI, the same cell types were activated by α4β2 receptors. In contrast, pyramidal cells in layers II/III were not identified to be positive for any of the nicotinic sub-receptors. Therefore, it was suggested that excitatory inputs to the superficial layers are not regulated by nicotinic sub-receptors (Poorthuis et al., 2013). In the studies mentioned above, nicotinic sub-receptor expression in cortical layers was inferred from the type of inward currents produced by cell types in response to local application of acetylcholine and pharmacological agents antagonizing α7 and α4β2 receptors. In other words, the receptors were not directly localized in identified cell types and as such, the reliability of the results is debatable.

Histological techniques for localization of nACh sub-receptors in both rodents and primates are available. However, the current techniques come with limits and hence the obtained data must be interpreted with caution (Gotti, Zoli, & Clementi, 2006). For instance, in situ hybridization technique is used in localizing the mRNA of receptor subunit, however, expression level of protein mRNAs does not necessarily correlate with the expression level of subunits in a region. Moreover, techniques such as immunocytochemistry that localize the subunit proteins face limitations such as lack of specificity of the antibodies implemented (Gotti, Zoli, & Clementi, 2006). However, current methods can still be informative of how comparable nACh receptor expressions are across different species (**Fig. 3**).

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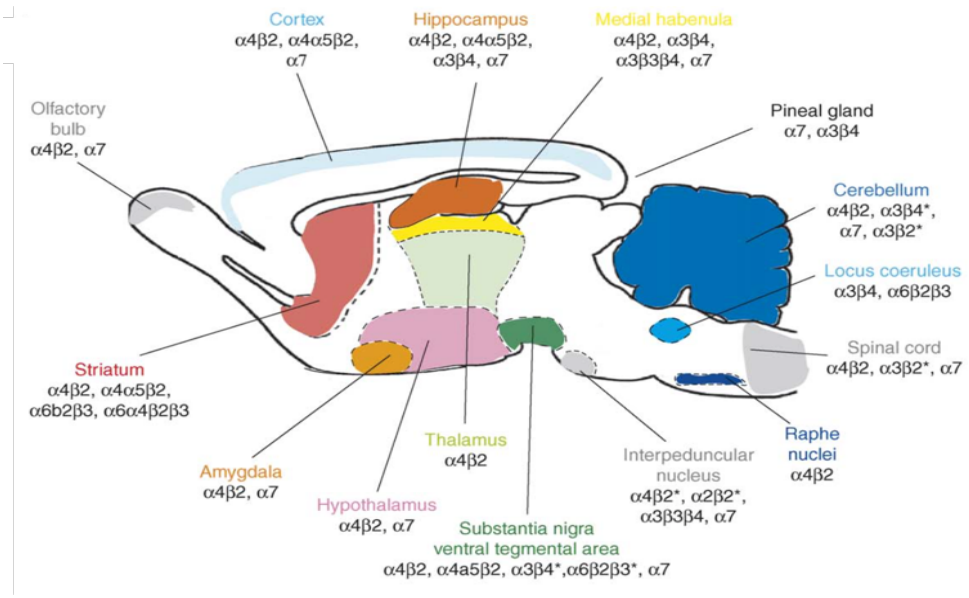

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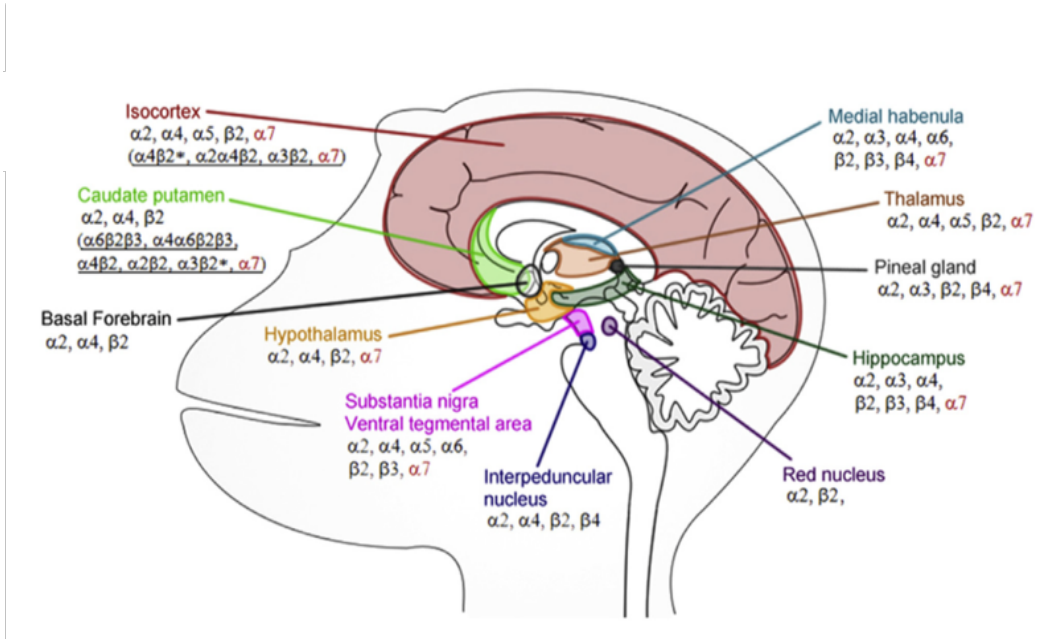

Figure 3 Expression of nACh sub-receptors in different brain areas of rodent (a) and non-human primate monkey (b). The distribution mapping of nACh sub-receptors was obtained by localization of subunit protein and mRNA techniques in rodent and monkey models respectively (Modified from Gotti et al., 2006; Zoli et al., 2015)

Using immunocytochemistry techniques in non-human primates, Disney and colleagues (2007) showed that receptors containing *β2 [1]subunits are mainly expressed in specific subclasses of interneurons in the visual cortex (V1). They also found that thalamocortical projections to recipient excitatory cells in layer IVc in V1 is mediated via pre-synaptic *β2 sub-receptors. Since no available antibodies passed the control experiments in the study, α7 receptors were not localized and consequently their contribution to neural responses and information flow could not be addressed. Based on these results, it is possible that *β2 sub-receptors modulate feedforward processing of information that from thalamus goes to layer IV and from there to cortical output layers in the visual cortex (Bloem, Poorthuis, & Mansvelder, 2014; Hasselmo & Sarter, 2011). Similarly, excitatory cells in layer V of rodent mPFC were shown to be modulated by *β2 sub-receptor mediated thalamic projections (Poorthuis et al., 2013). Additionally, the authors showed that these excitatory pyramidal cells contained α7 receptors, therefore suggesting response modulation of these cells by both sub-receptors.

Studies have shown that both systematic and local administration of nicotine is involved in bottom-up attentional orienting and can affect spatial attention in response to visual stimuli (Noudoost & Moore, 2011). However, how the cholinergic system modulates top-down attentional processing and neural activity in non-sensory cortical areas such as the mPFC remains obscure (Bloem, Poorthuis, & Mansvelder, 2014; Noudoost & Moore, 2011). To address this, in-vivo studies have started to emerge for assessing the effects of endogenously released ACh on task performance in behaving animals (Kuchibhotla et al., 2016; Letzkus et al., 2011). For instance, in an auditory fear-associated learning paradigm[2], Letzkus et al. (2011) identified a neural circuitry

[1] * β2 receptor refers to a heteromeric receptor with β2 as one of its composing subunits
[2] In fear-associated learning paradigms, animals learn to associate a neutral stimulus (e.g. auditory tone) with an unconditioned aversive stimulus (e.g. foot shock) and respond to the tone similar to how they do to the shock i.e. freeze

underlying performance in the task which was modulated by the cholinergic system. This circuitry involved the layer I interneuron mediated inhibition of particular interneuron sub-classes upon foot shocks in the superficial layers (II/III) and disinhibition of excitatory pyramidal cells in the same layers. The authors found that similar to foot shocks, photo-stimulation of basal forebrain axons alone led to excitation of layer I interneurons. Moreover, local injection of nicotinergic antagonists to auditory cortex (A1) during foot shock administration resulted in disinhibition blockade of pyramidal cells in layer II/III as well as reduction in freezing (i.e. impaired learning of fear association). The sub-receptor specificity involvement in cholinergic modulation of interneuron activity was not addressed by the experimenters. **Fig. 4** summarizes results about putative nACh sub-receptor localization in different cell types across sensory and prefrontal cortex based on the studies discussed above.

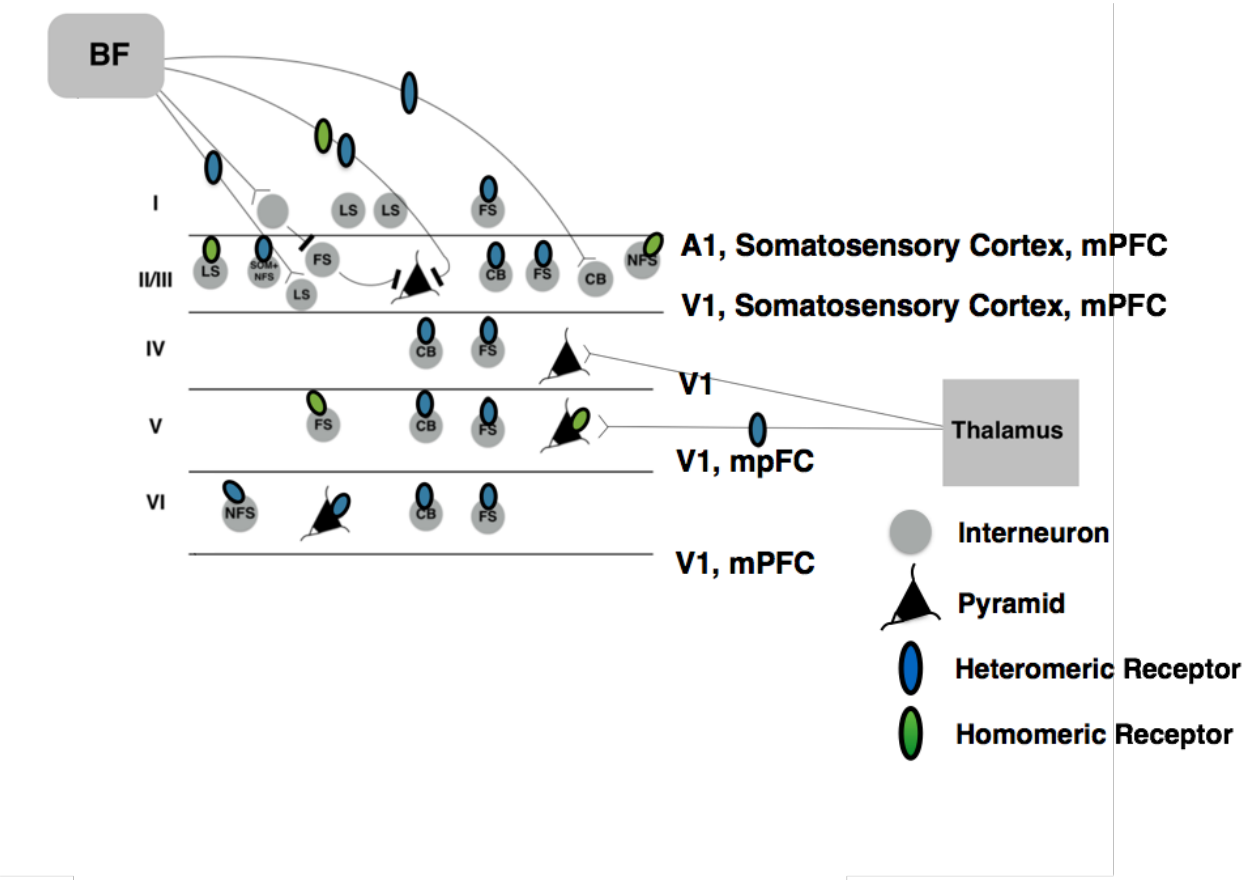

Figure 4 Summary of layer-specific nACh sub-receptor expression across sensory cortices and mPFC (Arroyo et al., 2012; Bennett et al., 2012; Disney, Aoki, & Hawken, 2007; Letzkus et al., 2011; Poorthuis et al., 2013). BF is an abbreviation for basal forebrain. Interneuron subclasses include fast and non-fast spiking (FS and NFS) as well late spiking (LS), calbindin and somatostatin positive (CB and SOM+). Homomeric receptor refers to receptors with α7 subunit while heteromeric to receptors with β2 subunit. Note that layer IV is missing in rodent mPFC and the cell types and nAChR expression shown in this diagram is based on a non-human primate study (Disney et al., 2007).

Neurochemical measurements have provided evidence that cholinergic transients in the mPFC during attention control are mediated differentially by α7 and α4β2 sub-receptors. In contrast to cholinergic modulation, cholinergic transients consist of fast sub-seconds to seconds

ACh release events (Parikh, Ji, Decker, & Sarter, 2010). These transients were released during a cue-detection period in a task which required subjects to detect cues predicting reward after a delay interval (Parikh, Kozak, Martinez, & Sarter, 2007). On the other hand, the transients were not released in trials where cues were missed by animals (rodents). In addition, these transients did not occur in another control brain region (motor cortex) (Parikh et al., 2007). The amplitude of cholinergic transients was specifically increased by presynaptic α4β2 but not α7 sub-receptors. Interestingly, performance of subjects in the task was positively correlated with the amplitude of these transients (Parikh et al., 2007). Thus, these results suggest distinct contributions of α4β2 and α7 sub-receptors to acetylcholine release in the PFC and attentional control.

Aside from regional- and layer-specific expression of sub-receptors, interaction of the cholinergic system with other neurotransmitter/neuromodulatory systems adds to the complexity of understanding the neural mechanisms underlying executive functions. The cholinergic system interacts with dopamine, norepinephrine and serotonergic circuitry (Logue & Gould, 2014). Presynaptic nACh sub-receptors are implicated in the release of neurotransmitters such as dopamine, norepinephrine, serotonin and glutamate (Duffy, Zhou, Milner, & Pickel, 2009). It is suggested that dopamine release mediated by heteromeric sub-receptors in the mPFC is important for attentional set-shifting while nicotinergic release of norepinephrine in the OFC is involved in cognitive flexibility such as reversal learning (Logue & Gould, 2014). It is also proposed that enhancement of NMDA receptor activity by homomeric nACh sub-receptors (α7)(**Fig. 5**) is critical for persistent network activity in the dlPFC in the absence of sensory stimuli (Yang et al., 2013). This finding suggests that α7 receptors play an important role in working memory.

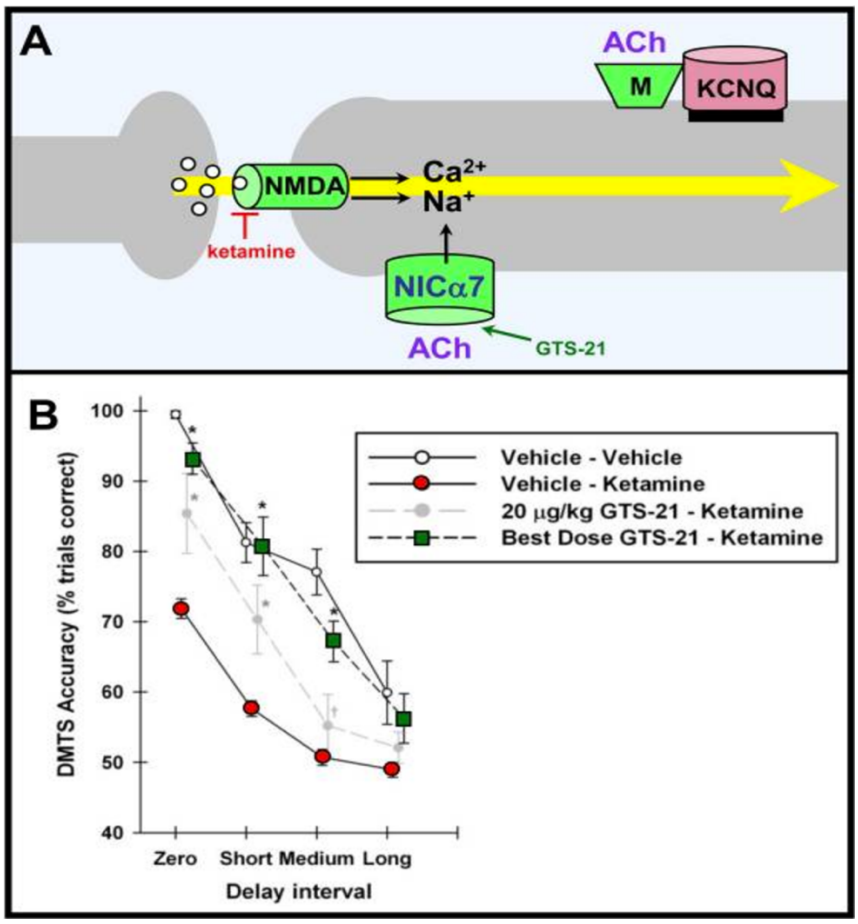

Figure 5 Interaction of α7 sub-receptor with NMDA receptors in PFC. (a) The coordination between these two receptors as well as muscarinic receptor blockade of potassium channel protein are proposed to be involved in rapid changes of cellular networks in the PFC (b) Reversal of ketamine –induced performance impairment in DMTS with an α7 sub-receptor agonist (GTS-21) in non-human primates. Ketamine is an agent which antagonizes NMDA receptors; Behavioral enhancement in these animal provides behavioral evidence for α7 actions on NMDA receptors (Modified from Arnsten, Paspalas, Gamo, Yang, & Wang, 2010).

Overall, little is known about the mechanisms underlying modulation of top-down attentional and higher order cognitive processes by cholinergic subsystems. How nACh sub-receptors specifically contribute to these processes depends on many factors such as differential expression in cortical and sub-cortical areas, laminar organization, cell type specificity and

interaction and coordination with other neurochemical networks. Additional research is required to shed light on neural mechanisms underlying cholinergic regulation of executive function.

1.3 Studying Nicotinergic Mediation of Higher Executive Functions

Previous studies trying to elucidate nicotinergic modulation used various task paradigms. These paradigms have specific strengths but also limitations. The most commonly used tasks in nicotinergic studies will be described and discussed in the following sections.

1.3.1 Delayed Match to Sample Task

This task is developed in different versions to study working memory and is mainly implemented in non-human primate studies. As shown in **Fig. 6**, a single stimulus (cue) is displayed on a screen. After a retention time interval (delay period), the subject is required to make a response by finding the match to the original cue. The stimuli and retention time intervals used in the task vary from trial to trial (Rodriguez and Paule, 2009). In some studies, distracting stimuli are sequentially displayed in the retention interval. Such manipulation allows for increasing the difficulty of the task. More importantly, it makes the task more comparable to real-world case scenarios where memory is retained after storing and processing new information (Miller, Li, & Desimone, 1993). Age-dependent cognitive deficits have been observed in monkeys performing DMTS. Thus, implementing this task with aged monkeys can be used to study Alzheimer's disease. Introduction of distractors during retention intervals also allows for evaluating sustained attention and making the task relevant for studying attention deficit disorders. DMTS tasks also demonstrate sensitivity to pharmacological testing. A summary of studies that investigated the effects of selective cholinergic pharmacological agents in DMTS tasks will be discussed in later sections.

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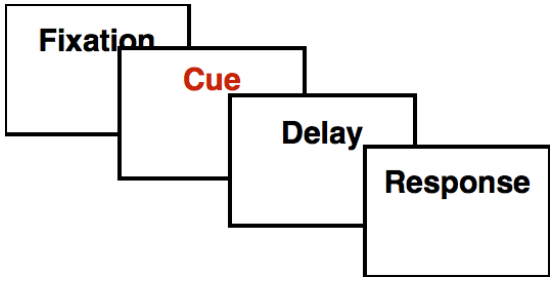

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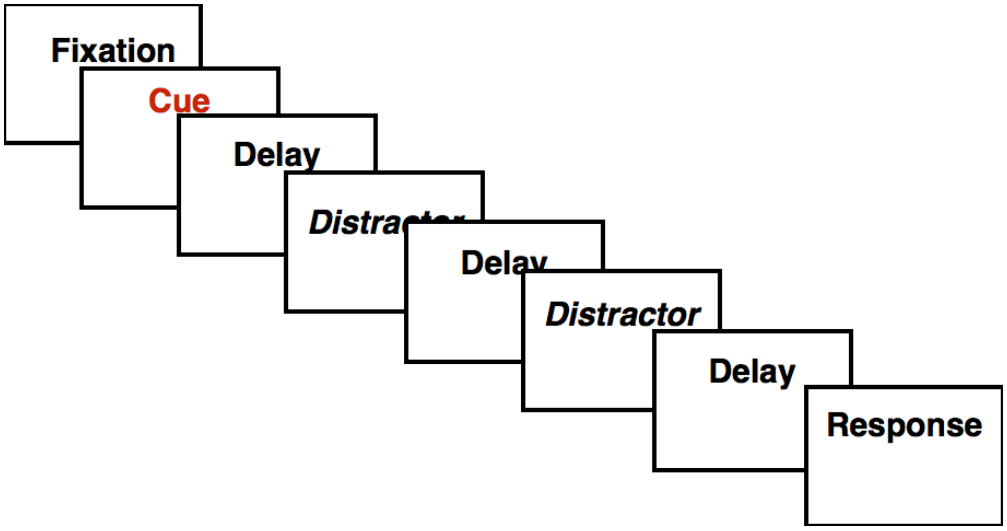

Figure 6 Delayed match to sample task (a) A schema of the task version without any intervening stimuli during retention intervals. Longer time intervals tend to cause greater impairment in performance. (b) A schema of the task version where variable number of distractors are introduced to increase the attention load and intricate the working memory-dependent performance.

1.3.2 5-Choice Serial Reaction Time Task

The 5- choice serial reaction time task (5-CSRTT) originally developed by Robbins et al. (1983) is used for studying sustained attention, mainly in rodents. This paradigm has been used in various studies investigating the nicotinergic effects on sustained attention (Hahn, Sharples, Wonnacott, Shoaib, & Stolerman, 2003; Hahn, Shoaib, & Stolerman, 2011; Hoyle, Genn, Fernandes, & Stolerman, 2006; Kolisnyk, Al-onaizi, Prado, & Prado, 2015; Young et al., 2004). **Table 1** summarizes the behavioral effects of a number of nicotinergic studies employing this task. Essentially, these tasks measure how well subjects can maintain their attentiveness to detecting a stimulus presented in different spatial locations. Typically, the animal is placed in an apparatus with 5 panels and a food magazine where reward can be obtained (**Fig. 7**). In each trial, brief flashes of light (cue) are presented in one of the panels in a pseudorandom sequence. The subject is required to detect the light in the right spatial location and respond to it by nose poking to the panel. The difficulty of the task is manipulated via duration and brightness of the light stimuli presented in a panel; shorter duration and less brightness increase the load on sustained attention and therefore lead to decreased performance. Another variant of this task is the 3-choice serial reaction time task (3-CSRTT) developed by Bunsey et al. (1995). Most nicotinergic studies use the 5-CSRTT in their experiments. To the best of my knowledge, only Tsutsui-Kimura et al. (2010) used this variant to assess the cognitive effects of two nicotinic sub-receptor drugs. This variant is very similar to the first, except for there are 3 panels in the apparatus and the food magazine is located differently, under the central point of the response port. The latter characteristic of 3-CSRTT is proposed to prevent the distracting effect of turning around and obtaining reward from the back of the apparatus. In both task variants, distracting stimuli (usually olfactory or auditory) can be introduced to assess selective attention. (Accornero et al., 2009; Bunsey & Strupp, 1995;

Moon et al., 2006). The distracting stimuli are usually presented after the trial onset and before cue presentation at the panels (Bushnell and Strupp, 2009). In general, five indices of behavioral measurement are reported with this task: 1) Correct response rate: the percentages of correct to total choices, 2) Omission rate: the percentage of non-initiated trials (omissions) by total trials in the task. 3) Pre-mature responses: number of responses made during the inter-trial periods, 4) Correct latency, defined as the time it took the subject to respond to the cue in the correct panel and 5) Magazine latency which is the time it took the subject to retrieve food at the magazine after nose poking at the correct panel (Robbins, 2002). Since its development, 5-CSRTT has been used in numerous experiments to study attentional dysfunction in disease models and effects of pharmacological interventions (Buschnell and Strupp, 2009). However, limitations imposed by behavioral measurements in this task can obscure the results of pharmacological studies. Correct and magazine latencies are sensitive to changes in locomotor activities and thus the effects observed in the task may not be due to cognitive-related aspects of a drug. In addition, pre-mature responses which are used as measures of impulsivity (i.e. actions without forethought) can be sensitive to pharmacological-induced variations in motor functions. Similarly, changes in omission rates may be due to non-cognitive effects of drugs and not increased distractibility per se. It has been shown that selective and non-selective nicotinic agonists can increase locomotor activities in rodents (Grottick et al., 2000; Reavill, CStolerman, 1990). Therefore, dissociation of cognitive from locomotive effects are of great importance when studying the effects of nicotinergic drugs in behavioral tasks.

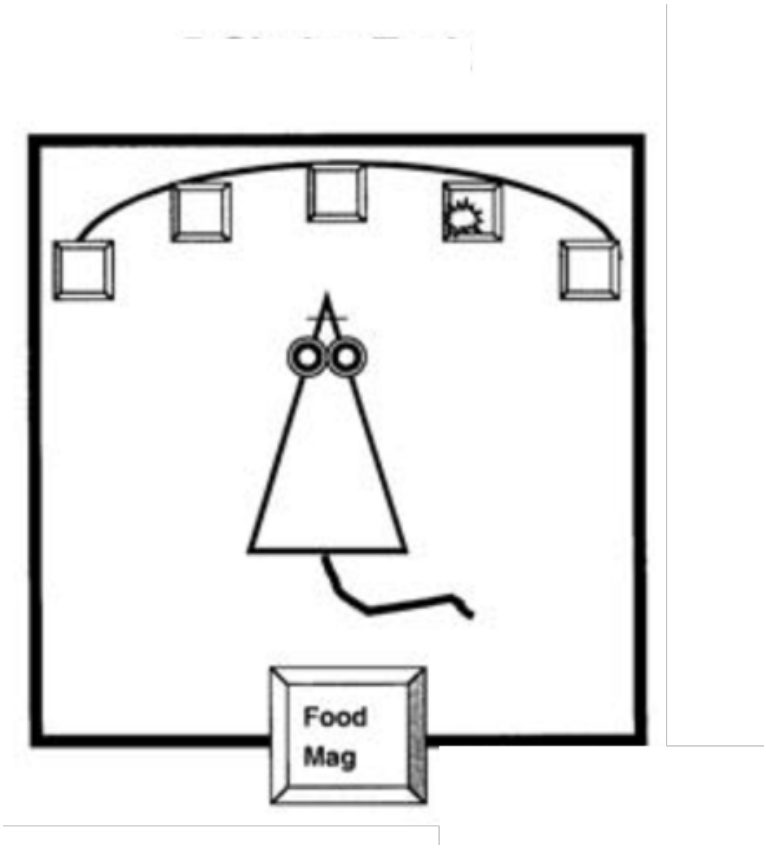

Figure 7 5 Choice serial reaction time paradigm for rodents. Common apparatus used for the task includes five spatial locations and a food magazine. In this example, light is illuminated in the second panel from right (Modified from Robbins, 2002).

1.3.3 Sustained Attention Task

As discussed above, it can be hard to disambiguate the attentional effects of independent variables such as pharmacological intervention from non-attentional processes in 5-CSRTT task. To provide better measurements for estimating response rates and false alarms (incorrect responses in the absence of stimuli/cue), newer versions of the sustained attention task were developed. These tasks first introduced by McGaughy and Sarter (1995) and collectively termed as sustained attention task (SAT) (**Fig. 8**), differ from 5-CSRTT tasks in few ways. First, the spatial aspect of the 5-CSRTT is removed as the cue (light stimulus) is presented only in one existing panel in the apparatus, however, the temporal aspect including timing of the trial onset and stimulus duration is maintained. Second and more importantly, discriminatory operations are involved. Once the cue is displayed, the subject is presented with two extendable levers to respond to the presence or absence of a light cue. As such, experimenters are enabled to dissociate between hits (correct responses to the presence of a stimulus), misses (lack of responses), false responses and correct rejections (correct response to the lack of a stimulus). Third, the levers are not available before cue presentation, thus, unlike 5-CSRTT, subjects cannot respond during inter-trial intervals.

To manipulate task difficulty, stimulus duration and inter-trial intervals can be varied. In addition, distracting stimuli can be introduced during inter-trial intervals. Presenting light flashes as distractors have shown to result in performance decrement over time (Howe et al., 2010).

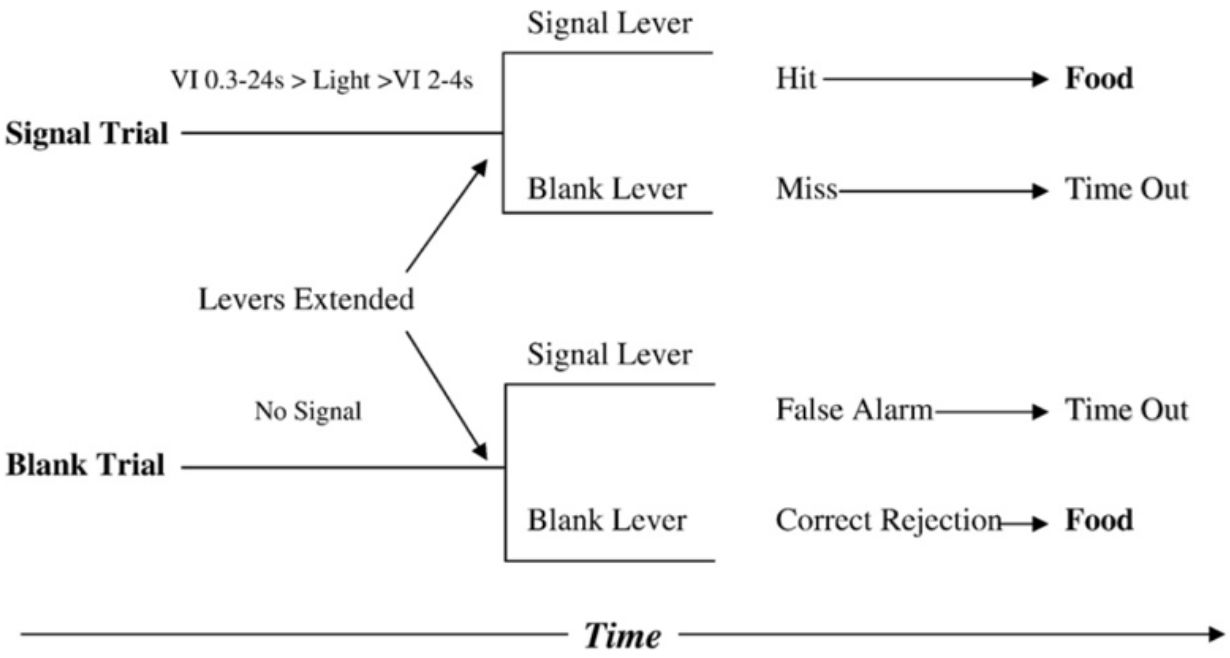

Figure 8 A visual signal detection task used to assess sustained attention in rodents. Signal levers are used by subjects to respond to signal trials, where light cue was illuminated and blank levers are used to respond to blank trials, where light cue was absent. Similar to 5-CSRTT, variable interval timings (VI) were considered prior to and post signal (light illumination) presentation. (Modified from Rezvani et al., 2009)**.**

1.3.4 Intra/Extra-Dimensional Set Shift Task

Intra/Extra-Dimensional Set Shift Task (ID/ED) is another task for evaluating executive functions which assesses and distinguishes between two different aspects of cognitive processes: 1) flexibility in regard to reversal of discrimination learning and 2) flexibility in regard to shifting attention from one perceptual dimension to another (Izquierdo et al., 2016). Essentially, the task consists of multiple stages which can be arranged in different numbers across task variants. The first stage starts with a simple discrimination wherein two exemplars (stimuli) differing in one feature (e.g. odor) are presented. The subject then needs to learn which exemplar is rewarded based on the dissimilar feature (i.e. odor). In the next stage, the subject learns a compound discrimination. The difference from the previous stage lies in the two exemplars differing from each other in both features (i.e. odor and texture). It should be kept in mind that the same feature that was rewarded in the first stage is still rewarded in this phase. This is followed by the first reversal event in the task i.e. reward contingencies switch such that the previously un-rewarded odor becomes rewarded for the first time. Subsequently, the subject is faced with a new set of exemplars. Both features are different but, similar to simple and compound discriminations, odor still determines which exemplar is rewarded. This is the intra-dimensional shift stage of the task which is followed by a second reversal. Next, the other feature which was not rewarded in the previous steps (i.e. texture) is rewarded and odor is not to be reinforced again. Finally, a third reversal in respect to texture takes place and the task ends.

ID/ED set shifting tasks have been implemented in both rodents and non-human primates. **Fig. 9** and **10** show a task example developed for each species. Conceptually, the tasks are similar between the animal models, however, there are few differences between them. The performance criterion for rodents is usually set to 6 consecutive trials and for non-human primates is set to 18/20

correct trials. Additionally, while rodents complete only one reversal at each stage, non-human primates perform serial reversals (Izquierdo et al., 2016). To the best of my knowledge, no non-human primate studies have used set shifting task to evaluate the effects of α4β2 and α7 nicotinic receptors. Therefore, the rest of this section will focus on ID/ED set shifting task in rodents.

For both species, the ED shift stage of the task is the most difficult of all phases and subjects require a higher number of trials to reach the criterion. Both species may complete all stages of the task within one session or across multiple sessions (Izquierdo et al., 2016). In rodents, the task variant that takes place all in one session is known as the "sand-digging" (**Fig. 9**) and originally developed by Birrell and Brown (2000). Given the time and technical efficiency, this variant is usually preferred to the "operant" task (Bushnell and Strupp, 2009) which is carried out across multiple sessions inside expensive operant chambers (Scheggia & Papaleo, 2016). However, there are limits with the sand-digging variant. First, unlike the operant-based task which is automated, sand-digging is carried out manually and the experimenter should present the trials and change exemplars by hand. This can also increase subjectivity-based biases in the measured parameters. Second, involving food related reinforcers in the exemplars can lead to possible choice biases by the subjects (Gilmour et al., 2013). Third, across multiple lesion and pharmacological studies (some examples provided in **Table 1**), only the ED shift stage was shown to be affected by the experimental manipulations (Bushnell and Strupp, 2009). The reason for this phenomenon may be related to the duration of this stage which varies between the two task variants. In the operant version, the ED shift stage is performed by subjects for a more prolonged time, and allows for evaluation of behavior during multiple phases: perseverative, chance and post-chance. These phases may allow studying perseverative responses to the previously rewarded exemplar, trial and error efforts, and a steadier learning of the new reward contingencies respectively. Therefore,

deficits in different attentional processes such as attention shift (i.e. problem of shifting from the previously rewarded exemplar, reflected in perseverative phase) and selective attention (i.e. problem of filtering out previously rewarded exemplar, reflected in post-chance phase) can be distinguished from each other (Scheggia & Papaleo, 2016). Despite these advantages, the operant-based task is not a very optimal design for pharmacological studies in which the temporal administration of drugs is an important variable. In other words, how performance in each stage can be affected by pharmacological manipulations can vary depending on when drugs are administered. For instance, drug administration before any reversal can affect acquisition (i.e. discrimination between stimuli) while after reversal can affect other learning processes (Gilmour et al., 2013).

Other paradigms have also been designed for evaluating reversal learning. Such tasks usually involve spatial discriminations (Redrobe et al., 2009; Terry, Plagenhoef, & Callahan, 2016; Thomsen, Christensen, Hansen, Redrobe, & Mikkelsen, 2009). The major limitation in these tasks is that reversals are done based on spatial locations and can involve employment of spatial strategies by subjects.

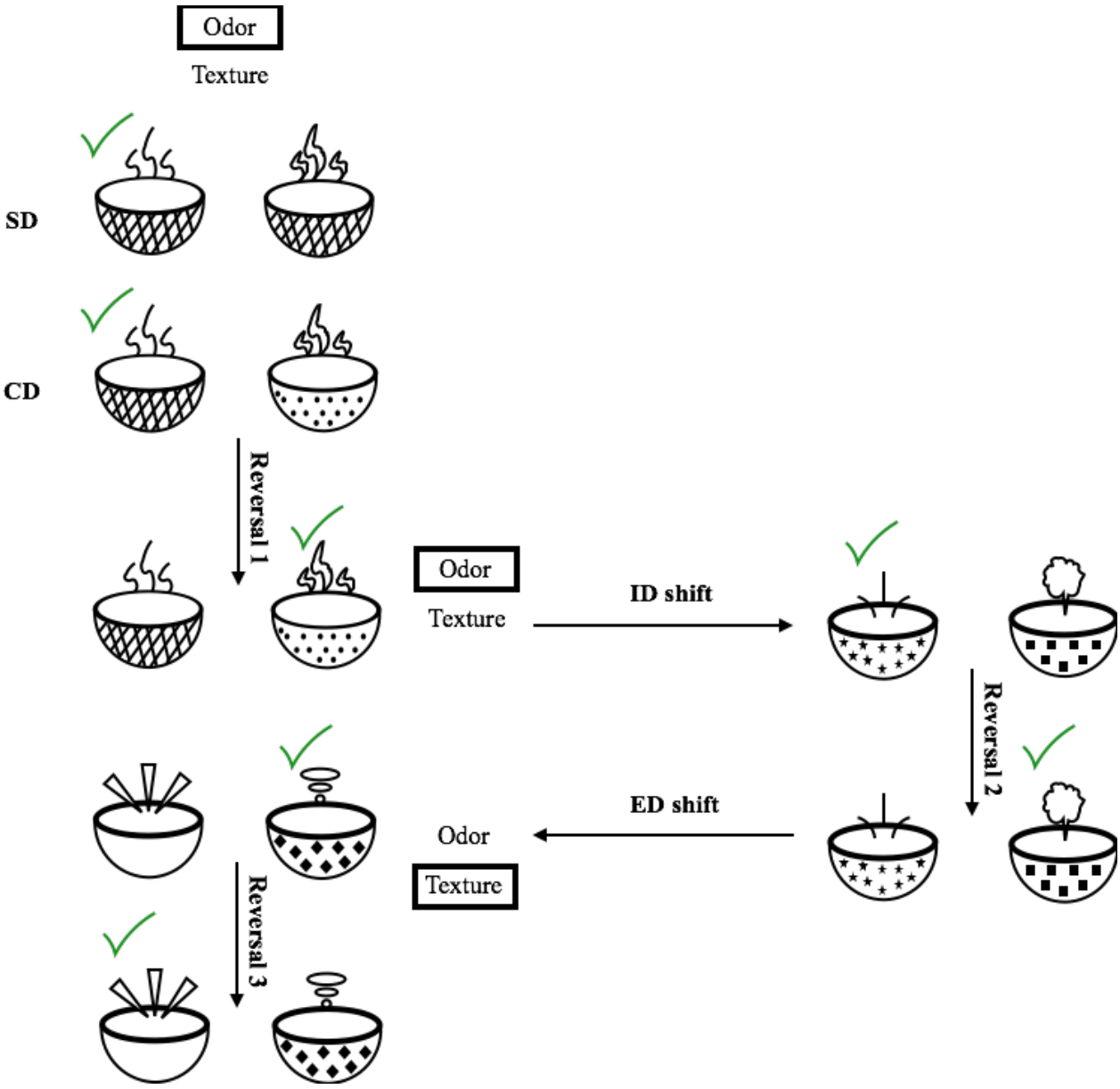

Figure 9 A sand-digging version of ID/ED shift task developed by Brown (2005). Prior to training for multiple stages of the task, subjects are trained to dig for the hidden food inside the bowls. The features enclosed by black rectangles are rewarded. Green checkmark indicates which compound stimulus is rewarded at each stage.

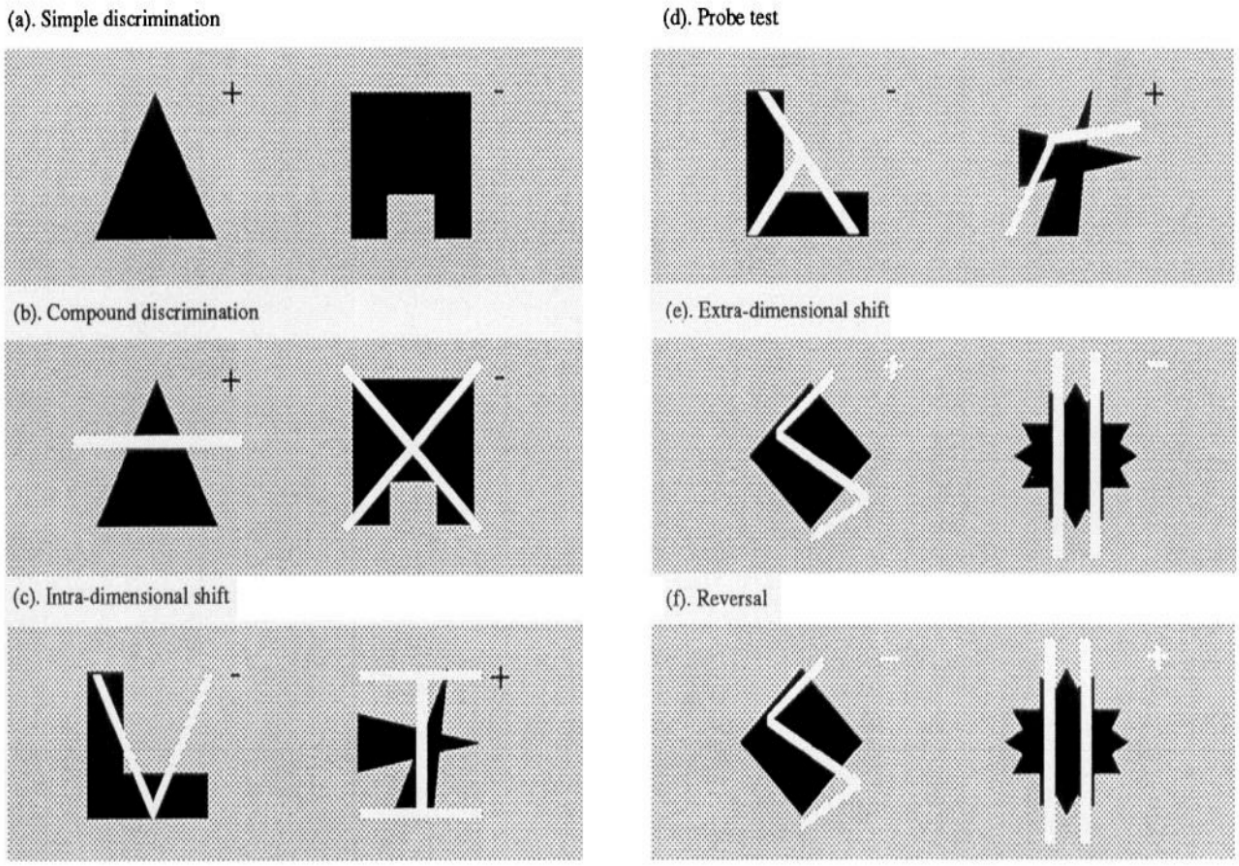

Figure 10 An ID/ED set shifting task implemented with marmoset monkeys. Although not shown, serial reversals follow the first reversal in this task (Roberts et al., 1994).

1.4 Nicotinic Sub-Receptor Effects at Behavioral Level: α7 Versus α4β2

Results from behavioral experiments investigating the specific contribution of nicotinic sub-receptors to learning and attention have not been very consistent. Depending on the agonist, selected doses and behavioral paradigms, different and sometimes seemingly contradicting effects of nAChRs on cognitive task have been reported. **Table 1** demonstrates a summary of rodent literature that has investigated the effects of α7 and α4β2 sub-receptor agonists on attention and ID/ED tasks.

Multiple studies showed cognitively enhancing effects of α7 sub-receptors in schizophrenia animal models measuring sensory gating (Hauser et al., 2009; O'Neill, Rieger, Kem, & Stevens, 2003; Wishka et al., 2006). Sensory gating is the ability to filter out irrelevant and unnecessary information in the environment and it is documented as one of the hallmarks of cognitive deficits in schizophrenia (Baker et al., 1987). However, auditory sensory gating per se is primarily not a measure of attention or learning. Other measurements such as response accuracy and omission errors in task constructs such as 5 CSRRT in rodents have shown potential involvement of both sub-receptors in mediating pro-cognitive effects. For instance, studies with both wild type and α7 knock-out (KO) models reported reduced level of omission errors by these sub-receptors (Hahn et al., 2011; Hoyle et al., 2006; Young et al., 2007; Young et al., 2004). Another pharmacological study also reported enhancing effects of α7 sub-receptor agonist in a SAT task where percentage of hits were increased (Rezvani et al., 2009). However, the literature is not void of inconsistencies: Kolisnyk (2015) and colleagues showed that while KO models of mice showed behavioral deficiency in 5-CSRTT task, they failed to show any significant improvement when systematically injected with two different α7 agonists. Interestingly, an α2β2 agonist ameliorated performance in the α7 KO subjects. Contrary to these results, the authors found improved performance in wild

type mice when injected with low doses of α7 agonists. In another study, α7 KO animals did not show any performance impairment compared to wild type while *β2 KO subjects experienced increase in omission errors. Additionally, re-expression of the receptors with *β2 subunits in the PFC reduced omission errors and resulted in a performance comparable to that of wild type mice (Guillem et al., 2011). Concordantly, Howe (2010) did not observe any significant behavioral changes as a result of α7 agonist administration in mice performing SAT and SAT-d tasks but they reported increases in percentage of hits with α4β2 agonist in SAT-d task only. Consistent with these findings, (Paolone, Angelakos, Meyer, Robinson, & Sarter, 2013) demonstrated differential ACh release in the PFC of two groups of rodents performing a SAT task. They found a higher level of ACh release in rodents employing optimal learning strategies compared to those utilizing poor strategies. Interestingly, the α4β2 agonist used in this study led to acetylcholine augmentation in the PFC of poor learners only. Literature on sub-receptor mediation of reversal learning is scarce. There are few studies on nicotinic sub-receptor effects on performance in attention set shifting tasks (Jones et al., 2014; McLean et al., 2012; Wallace & Porter, 2011). It was found that an α7 agonist restored performance in subjects experiencing phencyclidine-induced impairment in extra-dimensional switch such that subjects required fewer trials to reach the criterion (Wallace et al., 2011). Similarly, three different α7 agonists also resulted in enhanced improvement in ED shift (Jones et al., 2014), however, in comparison with Wallace et al. (2011) and McLean et al. (2012), there were two task-related differences: 1) the subjects were not tested in one session but over two days 2) reversal learning was not evaluated and only performance in ED shift was investigated. Both studies tested the effects of the agonists in animal models which were behaviorally impaired with NMDA receptor antagonizing agents. These results are consistent with the findings of Allison & Shoaib (2013) where the authors showed nicotinic–induced enhancement of performance in

healthy rodents during ED- shift stage of the task only. However, due to using nicotine (the general agonist), specific contributions of sub-receptors were not addressed in this study. To the knowledge of the author of this thesis, no studies have been conducted to test the effects of α4β2 sub-receptors on performance in set-shifting tasks.

There are different possibilities for the observed discrepancies across the literature: KO models can be limited in result interpretation given that these animals may develop different compensatory systems that otherwise would not exist in the wild type (Bloem et al., 2014). In fact, deletion of *β2 sub-receptors in mice can result in upregulation of muscarinic receptors in layer VI of the mPFC. This layer in healthy animals shows dominant expression of nicotinic sub-receptors wherein excitatory pyramidal cells are modulated by *β2 sub-receptors (Kassam, Herman, Goodfellow, Alves, & Lambe, 2008; Poorthuis et al., 2013; Tian, Bailey, De Biasi, Picciotto, & Lambe, 2011). Poor CNS penetration in agonists utilized in earlier studies and selection of doses higher than the optimal level were also suggested to be a possible reason for inconsistent results (Hahn et al., 2011; Tanner et al., 2015). Finally, task designs and measuring indices of behavior can also change results and interpretations.

Table 1 Effects of selective nicotinic sub-receptors on learning and attention in rodents across different studies. [1] Agonists selective for α7 nAChRs. [2] Agonists selective for α4β2 nAChRS. Doses that led to behavioral improvement are highlighted in bold font.

Pharmacological Agent	Task	Attention Control	Response Mapping	Doses Tested	References
SSR-180711[1]	ID/ED set shifting	Select newly rewarded exemplar over the previous one	Digging in the bowl containing the rewarded feature	1,3 and **10**[3] mg/kg	(Jones et al., 2014)
RG-3487[1]	ID/ED set shifting	Select newly rewarded exemplar over the previous one	Digging in the bowl containing the rewarded feature	**0.03**, **0.1**, **0.3** and **1** mg/kg	(Wallace et al., 2014)
	SAT	-	Press lever corresponding to presence or absence of a stimulus	**0.6 mg/kg**	(Rezvani et al., 2009)
GTS-21[1]	ID/ED set shifting	Select newly rewarded exemplar over the previous one	Digging in the bowl containing the rewarded feature	3,10 and **30** mg/kg	(Jones et al., 2014)
PNU-282,987	ID/ED set shifting	Select newly rewarded exemplar over the previous one	Digging in the bowl containing the rewarded feature	**3**, **10** and 30 mg/kg	(Jones et al., 2014)
PNU-120596	ID/ED set shifting	Select newly rewarded exemplar over the previous one	Digging in the bowl containing the rewarded feature	**10 mg/kg**	Mclean, 2012
AR-R17779	5-CSRTT	-	Nose-poke to the location of detected object	3,6,12 and 24 mg/kg	(Hahn et al., 2003)
PHA-543613[1]	5 CSRTT	-	Nose-poke to the location of detected object	0.33, **1** and 3 mg/kg	(Kolisnyk et al., 2015)
PNU-282,987[1]	5-CSRTT	-	Nose-poke to the location of detected object	**1**,3 and 5 mg/kg	(Kolisnyk et al., 2015)
ABT-418[2]	5 CSRTT	-	Nose-poke to the location of detected object	0.04, 0.13 and 0.39	(Kolisnyk et al., 2015)
				0.05, 0.1, 0.2 and **0.4** mg/kg	(Hahn et al., 2003)
ABT-089[2]	SAT	-	Press lever corresponding to presence or absence of a stimulus	**0.02** and 0.1 mg/kg	(Paolone et al., 2013)
S-38232[2]	SAT	-	Press lever corresponding to presence or absence of a light cue	0.03, 0.30, 1.00, and 3.00	(Howe et al., 2010)
	dSAT[4]	Respond to presence or absence of light cue while ignoring a flashing light distractor	Press lever corresponding to presence or absence of a light cue	0.03, **0.30**, 1.00, and 3.00	

[3] Effective doses are highlighted in bold font
[4] dSAT refers to a variant of SAT tasks that implement distractors.

In non-human primate models, multiple studies using the DMTS task have provided evidence for enhancing effects of both sub-receptors on working memory (**Table 2**). Evidence for selective attention modulation by nicotinergic sub-receptors comes from studies that implemented distractor in DMTS task (Buccafusco, Terry, Decker, & Gopalakrishnan, 2007; Prendergast et al., 1998). It should be noted that neither study has compared the effects of α7 to α4β2 sub-receptors and reported results for α4β2 agonists only. Insufficient number of studies on reversal learning are conducted in non-human primates. These studies were different in task design and measured indices of performance. Terry et al. (2016) carried out the reversal in ketamine-impaired subjects over 24-48 hours and only reported proportion of correct choices and error types such as perseverative tendencies but did not report trials to reach the criterion (i.e. learning rate). The main effects included higher proportion of correct responses under drug condition and attenuation of perseverative errors. Gould et al. (2013) conducted two reversals with different sets of exemplars (i.e. no serial reversals) and did not find effects on any behavioral components in either cocaine-impaired or healthy subjects. Neither study addressed possible differential sub-receptor modulation as they utilized agonists selective for both receptor types (Terry et al., 2016) or only agonists selective for α7 but not α4β2 sub-receptor. In summary, more experiments in non-human primates are needed to clarify the effects of the nicotinic sub-receptors on filtering distraction and reversal learning.

Table 2 Effects of selective nicotinic sub-receptors on learning and memory in non-human primates across different studies. [1] Agonists selective for α7 nAChRs. [2] Agonists selective for α4β2 nAChRS. [3] Agonists selective for both α7 and α4β2 nAChRs. Doses that led to behavioral improvement are highlighted in bold font.

Pharmacological Agent	Task	Attention Control	Doses Tested	References
GTS-21[1]	DMTS	-	3, 6.2, 12.4, **24.8** [5]and 49.6 μg/kg	(Briggs et al., 1997)
			2.5,5,10,**20**,**40** μg/kg	(Buccafusco & Terry, 2010)
PNU-282-987[1]	DMTS	-	**0.001**[6]**-0.56** μg/kg	(Gould et al., 2013)
	Reversal learning	Select newly rewarded object over the previous one	0.001-0.56 μg/kg	(Gould et al., 2013)
A-582941[1]	DMTS	-	**1.4**,**11,4** and 38 μg/kg	(Buccafusco et al., 2007)
PHA-534613[1]	Spatial working memory	-	0.01, **0.1**, 1, 10 μg/kg	(Yang et al., 2013)
ABT-418[2]	DMTS-D[7]	Visual distraction during delay (25% of trials)	0.41, 0.82, **1.64**, and 3.28 μg/kg	(Prendergast et al., 1998)
	DMTS	-	0.41, **0.82**, **1.64**, and 3.28 μg/kg	(Prendergast et al., 1998)
			0.41, 0.83, 1.64, **3.28**, 6.57 μg/kg	(Buccafusco et al., 1995)
ABT-089[2]	DMTS-D	Visual distraction during delay (25% of trials)	**1.1**, **2.2**, **4.4**, **8.8,** and **17.7** μg/kg	(Prendergast et al., 1998)
	DMTS	-	1.1, 2.2, 4.4, **8.8**, and 35.2 μg/kg	(Decker et al., 1997)
ABT-594[2]	DMTS-D	Visual distraction during delay (25% of trials)	**0.115**-3.7 μg/kg	(Buccafusco et al., 2007)
	DMTS	-	**0.115**-3.7 μg/kg	
Varenicline[3]	DMTS	-	**0.03, 1, 3.0** mg/kg	(Terry et al., 2016)
	DMTS	Visual distraction during delay (25% of trials)	**0.03**, **1** ,**3.0** mg/kg	(Terry et al., 2016)
	Reversal learning	Select newly rewarded object over the previous one	**0.03**, **1** ,**3.0** mg/kg	

[5] Effective doses are highlighted in bold font
[6] Individual best doses fell in the range of 0.01-0.1 mg/kg
[7] DMTS-D refers to a variant of DMTS task that implements distractors.

1.5 Scope of This Study

1.5.1 Alpha7 nAChR selective agonist (PHA-543613)

The first experiment discussed in this thesis employed PHA-543613 9*(N*-[(3*R*)-1-Azabicyclo [2.2.2] oct-3-yl] furo [2,3-*c*]pyridine-5-carboxamide); an agonist highly selective for α7 nAChRs. This agonist has been under development for cognitive impairments associated with schizophrenia. PHA543613 has a high affinity for α7 nicotinic receptors (nAChR) and acts as an antagonist on 5-HT3 receptors (Wishka et al., 2006). Because of negligible antagonist activity at both muscle and ganglion-like nicotinic receptors, rapid brain penetration and high oral bioavailability in rats (Wishka et al., 2006), PHA543613 was considered a good candidate for activating α7 nAChRs in this project. Only one non-human primate study exits in the literature which has used the same agonist for assessing changes in cognitive performance and its relevant neural activities (Yang et al., 2013). According to this study, PHA543613 showed a dose-dependent curve with optimal behavioral improvement occurring at low doses. Interestingly, the neural activity recorded by iontophoresis application of PHA543613 in the dlPFC also showed a dose-dependent effect on cell firing. The results of this study also indicated that α7 nACh receptors had a modulatory interaction with NMDA receptors and could enhance NMDA receptor-mediated glutamate transmission. Based on these results, the authors suggested that high dosages of agonists targeting cognitive deficits may result in non- specific effects on neuronal excitability and subsequent loss of beneficial effects and improvements.

1.5.2 Alpha4 beta2 nAChR selective agonist (ABT-089)

The second experiment utilized ABT-089 ([2-Methyl-3-(2-(S)-yrrolidinylmethoxy) pyridine dihydrochloride]), an agonist selective for α4β2 nACh receptors. This compound possesses good brain penetration and its pharmacokinetic properties have been studied in at least three animal

species including dogs, rats and monkeys (Rueter et al., 2004). This agonist has been under development for treatment of attention deficits associated with ADHD and Alzheimer's disease and has been tested on children aged 6-12 and adults 55-90 years old. ABT-089 has a high affinity for α4β2 nAChRs and its effects are weakened by non-competitive neuronal nicotinic receptor antagonist Mecamylamine (Rueter et al., 2004). ABT-089 has a low affinity for binding sites on cells that express muscle type nicotinic receptors. It also demonstrated a favorable safety profile, as shown by little or lack of cardiovascular and gastrointestinal irritations at plasma concentrations that are expected to be clinically beneficial (Rueter et al., 2004)**.** This pharmacological agent was previously used in few non-human primates to test for its effects on cognitive tasks. In DMTS-D task with monkey subjects, intramuscular (IM) administration of ABT-089 in a wide range of doses (1.1, 2.2, 4.4, 8.8, and 17.7 microgram/kg) was associated with reduction in distractibility and increase in accuracy (Prendergast et al., 1998). In another DMTS task with monkeys, the same agonist improved performance in the subsets of trials that contained the longest retention interval and therefore were most difficult for subjects. The performance improvement was more robust in aged subjects (Decker et al., 1997).

1.6 Hypotheses

I implemented a systematic injection of two different doses of PHA-543613 and ABT-089 agonists in one healthy rhesus monkey performing a feature based reversal learning task. The complexity of the task design allowed me to address the effects of agonists on multiple behavioral components. This increased the chance of evaluating specific contributions of nicotinergic sub-receptors to goal directed behavior and selective attention. Specifically, I looked at how the two agonists affected performance accuracy and learning rate, filtering distraction,

motivation, reward-history based performance and proportion of error types including impulsivity and perseverative rates. I hypothesized that ABT-089 and PHA-543613 would at least have some differential effects across behavioral measurements. Based on previous studies, I expected to see increased performance accuracy in reversal learning and enhancement of attentional processes by either agonist. Since previous studies suggested attenuation of distraction in DMTS task by α4β2 agonists (Buccafusco et al., 2007; Howe et al., 2010; Prendergast et al., 1998), I predicted that ABT-089 would improve distraction filtering in our task. I also hypothesized that either agonist can potentially reduce perseverative tendencies. Studies by Jones et al. (2014) and Terry et al. (2016) suggested that perseverative errors can be attenuated by agonists selective for α7 and agonists selective for both α4β2 and α7 sub-receptors respectively. I did not expect to see any effects on motivation as previous studies did not show any changes in this measurement for either α4β2 (Guillem et al., 2011) or α7 (Hahn et al., 2011; Young, Meves, Tarantino, Caldwell, & Geyer, 2011). Several studies have reported inverted U-shaped curve profile for nicotinergic agonists (Gould, Garg, Garg, & Nader, 2013; Kolisnyk et al., 2015; Redrobe et al., 2009; Wishka et al., 2006; Yang et al., 2013). This profile describes a non-linear relationship between dosage of pharmacological agents and their resulting behavioral changes. It states that the drug effects increase with dosage increase until they reach an optimal level at a particular point. Increasing the dose after this point leads to sub-optimal effects. Since I only used two doses, I could not make specific predictions in terms of an inverted U-shaped profile. However, I did expect to see changes in performance by at least one of the doses used for each agonist.

Chapter 2-Methods

2.1 Subjects and Apparatus

The data was collected from Monkey H, a 9-year-old adult male rhesus monkey (Macaca mulatta) subject who was previously trained on the task. Since March 2016, another 6-year-old male rhesus monkey known as Monkey K has been under training and still in progress. As part of training preparations, both subjects underwent surgeries to enable restraining head movements when performing the task. Following the surgery, the animals went through a recovery period during which they received necessary antibiotics and pain-management medications. Once recovered, animals were trained on the task with positive reinforcement such that they were rewarded with fluid (water or diluted juice) for each correct response to a trial. To maintain motivation throughout training and experiments, fluid intake was restricted during both experimental and control sessions. Unlimited access to primate biscuit chews was available to subjects and additionally, daily treats of nuts and dried fruits were provided. The subjects' fluid and food intake, weight, and behavior such as agitation and aggression were monitored closely by the experimenter and animal care personnel. All the surgical, training, experimental and drug protocols were approved by animal care committee in York university and in accordance with the guidelines of Canadian Council on Animal Care.

The training and experiments took place inside a dark booth were the monkey was seated inside a primate chair, head-fixed and 65 cm away from a 21-inch LCD monitor. The visual stimuli were generated using customized MATLAB (Mathworks, R2014a) code. The experimental task and reward administration were presented and controlled by MATLAB and MonkeyLogic toolbox. The fluid reward was delivered through a sipper tube which was placed in front of the primate's mouth and controlled by an air-pressured mechanical system. The eye positon was tracked by

Eye-link 1000 eye tracking system at 500 Hz sampling rate and the pupil dilatation and saccade movements were recorded by Cheetah software. The stimuli and how they were used were different across the two subjects as the second subject has not fully learned the task yet. The following information is regarding the task that Monkey H performed. The details on the stimuli and the version of the task that Monkey K has been under training with are disclosed in **Appendix A.1**.

All the stimuli were displayed against a black colored background. The fixation point was a 15-pixel size grey circle in the center of the display. The stimuli consisted of two circular apertures with moving red/black and green/black gratings of 2° radius both equally spaced within 5° away from the fixation points at right and left locations. The spatial frequency i.e. the motion speed of the movies was 1.20 cycles/degree. The location, movement direction of the stimuli and whether first the color or motion feature occurred prior to combination of both features were randomized throughout the sessions such that all possible 16 trial types were equally probable to occur during the task (**Fig.11**). The response target dots were both white and located 4° away up and downside of the fixation point.

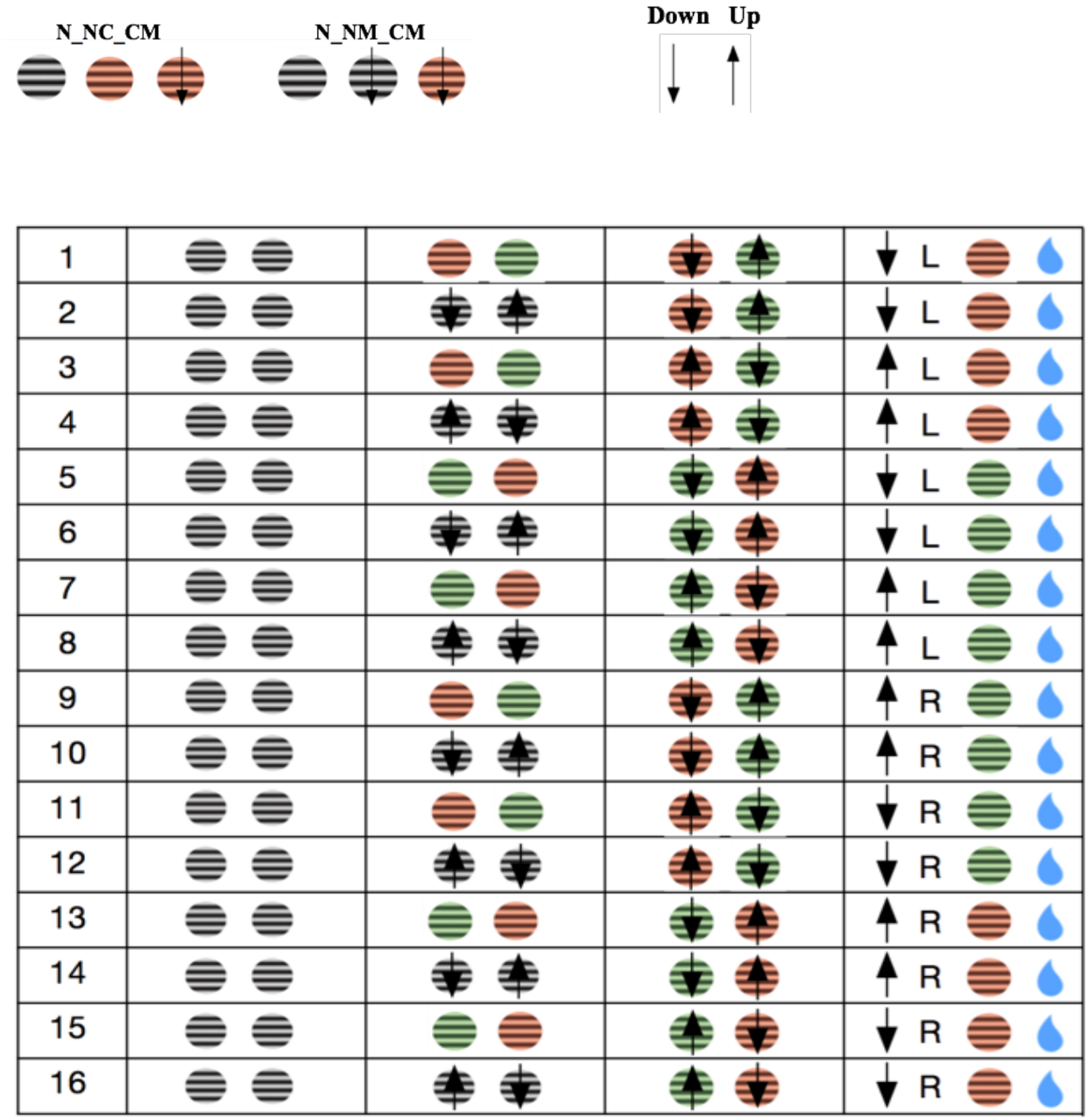

Figure 11 All possible trial conditions based on location, color and motion direction types as well as the precedence of the last two features. No color-No motion, No motion-Color, Color and motion (N_NC_CM) refers to a trial type where after a stationary phase, first color is presented and then motion comes on. No color-No motion, No color-Motion, Color and motion (N_NM_CM) refers to a trial type with a reverse order of feature presentation i.e. first motion and then color. L and R refer to left and right location respectively.

2.2 Behavioral Paradigm

The task aimed to evaluate reversal learning and attention in non-human primates with two differently colored visual stimuli (moving circles) on the screen. Multiple features including motion and color existed. The latter, would determine what stimulus would become rewarded in a block. Additionally, a dimming feature existed which served as a timing prompt for the subject to make a choice. Dimming of the stimuli cued the response to the motion direction of the rewarded or target stimulus. Three different timing conditions were possible in that regard: 1) the target stimulus dimmed first and then the non-rewarded stimulus would dim. 2) both stimuli dimmed simultaneously and 3) the non-rewarded stimulus dimmed first and then the target stimulus would dim. The response that the animal had to make was to saccade to the motion direction of the target (either up or down). If the subject responded to the motion direction of a target stimulus at the wrong dimming event, the response was considered a non-choice error and not considered in the analyses. The task involved reversal of the rewarded feature value, in other words, the rewarded color would switch multiple times throughout the session (**Fig.12-a**). This change would occur under two circumstances: 1) within a minimum of 30 trials, the subject reached 85% in a 12-trial window average. 2) 50 trials were completed; regardless of how performance was during that block. **Fig. 12-b** shows the timing frame of each event during the task in milliseconds. Each time frames is numbered in sequence.

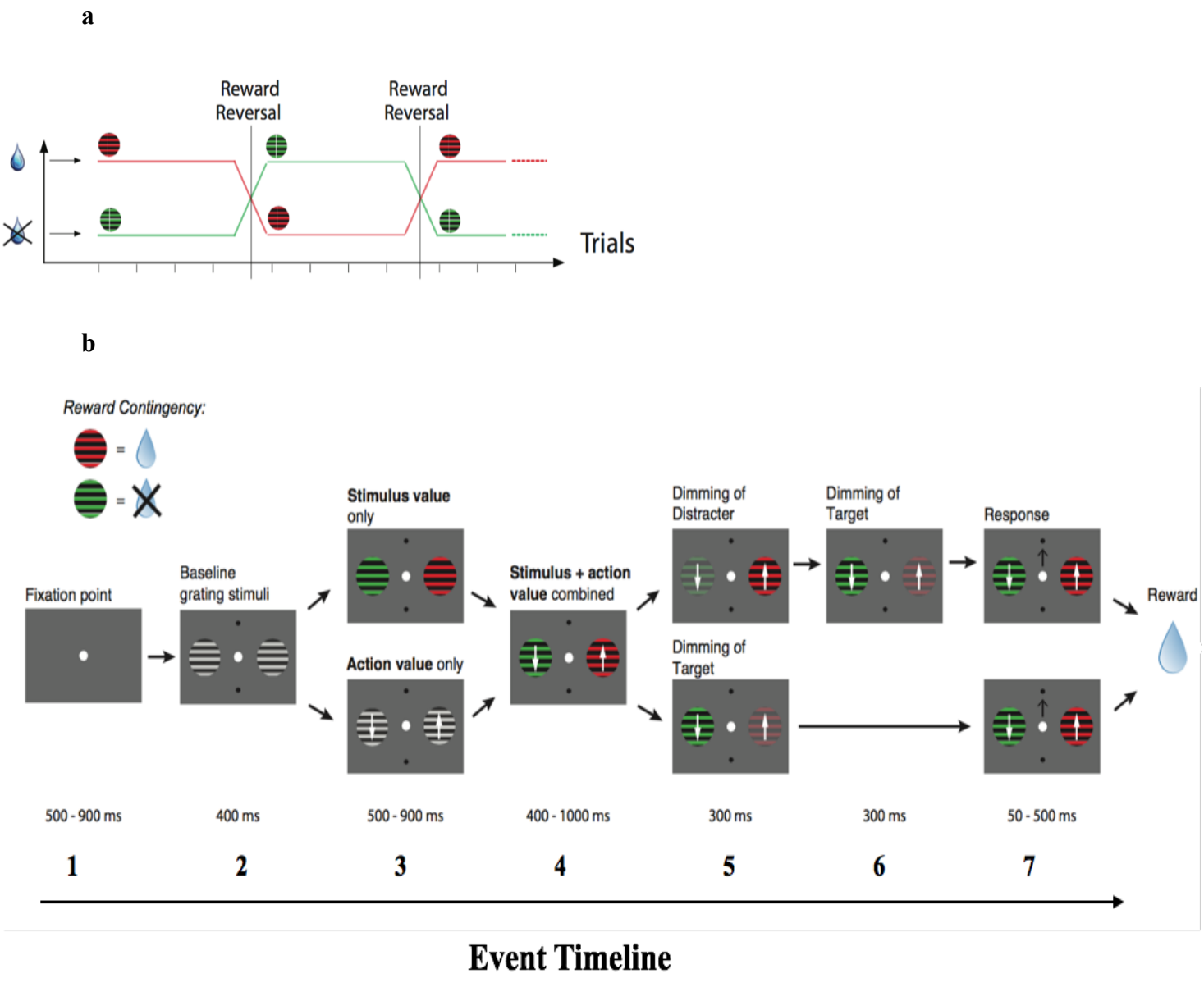

Figure 12 Feature-based reversal learning task: (a) depicts reversal of rewarded colors in a block-wise fashion. Each color was selected for the first block of the session every other day. (b) shows the timing frame of each event. The task starts with fixating on a central gray point for about 500-900 ms. Afterwards, a pair of grating stimuli appear on both sides of the central point and remain stationary for 400ms. After this period, there will be either a color onset (stimulus value) or a motion onset (action value). Either feature onset lasts about 500-900 ms and then both features will be combined. About 400-1000 ms later, either the target color or the distractor color dims for 300 ms. If the distractor is dimmed first, then after a period of 100-550 ms, the target starts to dim for 300 ms. The subject should make a choice only when the target color is dimmed. The subject has 50-500 ms to make a choice and must maintain his fixation on the target for at least 50 ms. If the choice is correct, they will be rewarded, if not, a 1000 ms time-out occurs before the start of the next trial.

2.3 Experimental Procedure

Monkey H did not receive either of the two drugs previously, however, he received Guanfacine (alpha2a-noradrenergic (a2a-NE) agonist) twice weekly about 14 months before the start of these experiments as part of a previous study conducted in the lab (Hassani et al., 2017). During this 4-month period, he was not part of any experiments and had unlimited access to fluid and food. Prior to the first experiment using PHA-543613 drug, Monkey H performed the task for three weeks to reach his baseline performance. The second experiment with ABT-089 started 3 months after the data was collected with PHA-543613 and the subject performed the task for a week prior to data collection to reach his baseline. No data was collected on Mondays as Monkey H would usually work unreliably on these days, probably because of being off work during the weekend. From Tuesday to Friday, total of two drug treatments of both doses were collected with at least one day between treatments. Assignment of the treatments was randomized such that each dose or vehicle could be administered at any day of the week to control for any biases that may have occurred because of variable performance throughout the week (**Fig. 13**). Both drugs and vehicle were administered via intramuscular (IM) injection. The vehicle injections occurring between drug treatments were also considered for analyses as control sessions. The injections were administered by a lab technician and the experimenter was blind to the treatment conditions. During both experiments, the subject was given at least 50 minutes to perform the task. When collecting data with PHA-543613, the criterion for stopping the subject was lack of any trial completion within the first 5 minutes after 50 minutes of working. To optimize this criterion when collecting data with ABT-089, the criterion was changed so that the animal would be stopped if he completed fewer than 20 trials within the first 5 minutes after 50 minutes of working. For PHA-543613 experiment, injection was administered 30±1 minute prior to start of the task. The time frame was

chosen as an estimate based on the rodent literature and the only non-human primate study in the literature (to the author's knowledge) where the same drug was orally administered in rhesus monkeys 60 minutes prior to the experiment (Bali et al., 2015; Kolisnyk et al., 2015; Sadigh-Eteghad, Talebi, Mahmoudi, Babri, & Shanehbandi, 2015; Wishka et al., 2006; Yang et al., 2013). For ABT-089 experiment, the IM injection occurred 10±1 minutes prior to the start of the task. This time frame was chosen based on previous non-human primate studies where the same drug was applied (Decker et al., 1997; Prendergast et al., 1998). All sessions were conducted at the same time of the day. The selected doses for PHA-543613 experiment were 0.125 and 0.250 mg/kg. These doses were chosen as an estimate from the rodent literature ((Bali et al., 2015; Kolisnyk et al., 2015; Sadigh-Eteghad, Talebi, Mahmoudi, Babri, & Shanehbandi, 2015; Wishka et al., 2006). The selected doses for ABT-089 experiment were 0.01and 0.02 mg/kg. These doses were chosen based on high and low values of the dose range previously tested in non-human primates. Initially, 0.04 mg/kg was selected as the higher dose for ABT-089; however, on the third collecting week, Monkey H experienced minor physiological side effects such as elevated heart rate and nausea which were attributed to the drug at the given high dose. Data collection with this dose was stopped immediately, and after a one-week recovery period, a lower dose (0.02 mg/kg) was selected to continue data collection. Following 8 and 12-week dose experimental protocols, the behavioral effects of PHA-543613 and ABT-089 doses respectively were tested. Data were excluded if they were collected from days when unexpected incidents such as staff intrusions, computer related problems etc. occurred. The first protocol provided 15 control sessions, 8 sessions with dose 0.250 mg/kg and 7 sessions with 0.125 mg/kg dose for PHA-543613. The second protocol provided 20 control sessions, 7 sessions with dose 0.02 mg/kg and 9 sessions with 0.01 mg/kg dose for ABT-089.

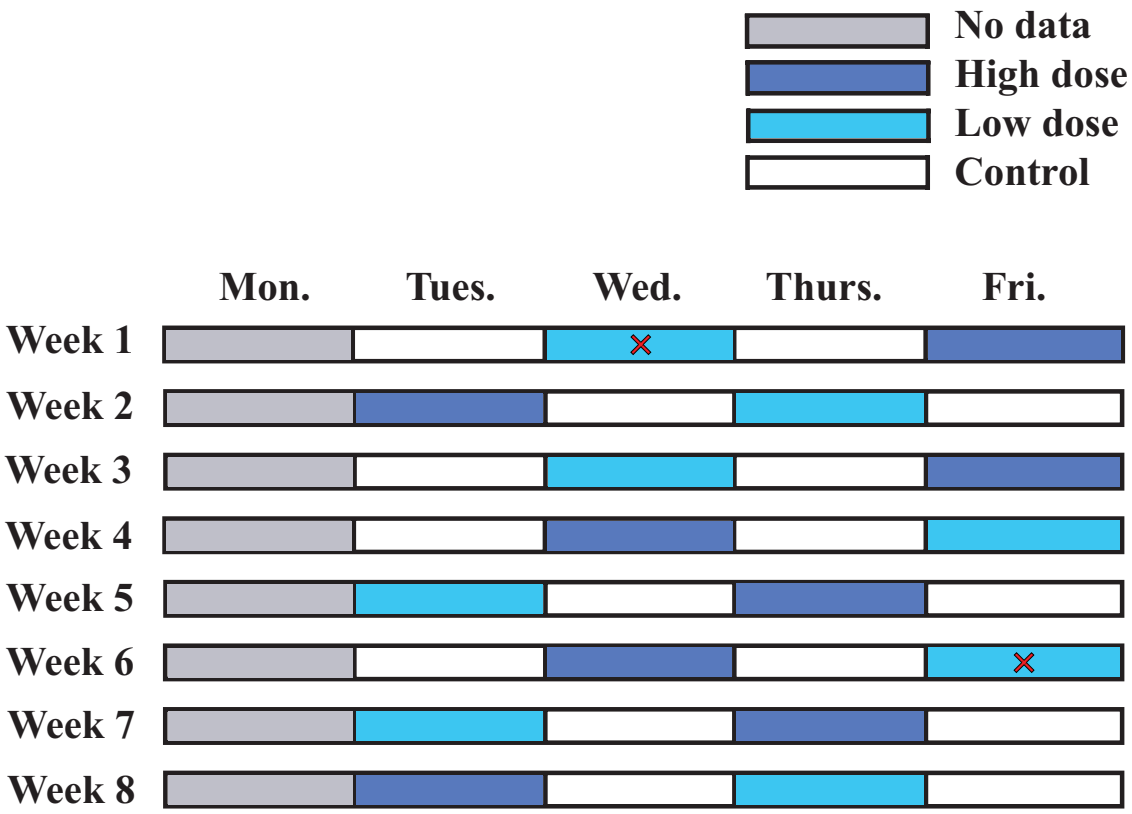

Figure 13 Random assignment of weekdays to drug doses and control sessions. No drug or vehicle injection happened on Mondays as Monkey H was not motivated enough to perform reliably. On control days, the subject was injected with sterile water**;** On drug treatments, the subject could be injected with either of the doses. The red cross marks the days from which data could not be used in the analysis for reasons explained previously.

2.4 HPLC Analysis of Blood Serum

To identify the peak concentration and the overall metabolism pattern of the drugs over time in blood, mass spectrometry HPLC analysis was conducted for both agonists. Nine blood samples of 300 μL in total (baseline and 8 samples after injection) were extracted from 10-year-old male rhesus macaque monkey S, weighing 10 kg on the day of sampling. The samples were taken at the following time points: 1 minute before drug injection (baseline), 9,15,25,40,70,100,160 & 220 minutes after injection. The higher drug dose (0.25 mg/kg) for PHA-534613 was considered for HPLC analysis while for ABT-089 the lower dose (0.01 mg/kg) was selected. Samples were centrifuged at 2000 rpm speed for about 40 minutes and after being spin filtered, stored for mass spectrometry HPLC processing.

The procedure was successful for PHA-543613, however, no ABT089 signal was observed in any of the blood samples and as a result no blood serum analysis could be obtained. It was possible that the injected drug dose was too low or the drug became completely protein bound in the blood and therefore would not have been able to pass through the spin filter.

In previous literature, the plasma exposure of ABT089 was measured in baboons under both bolus intravenous (IV) injection and slow infusion in doses ranging 0.04-1 mg/kg (Chin et al., 2011).

On average, the subject worked for 74 and 60 minutes during experimental sessions of PHA-543613 and ABT-089 respectively. Therefore, the results from Chin et al. (2011) and HPLC analysis of PHA-543613 (**Fig. 14** and **15**), suggested that the peak concentration and the subsequent drop in blood concentration were captured within the testing session time for both pharmacological agents.

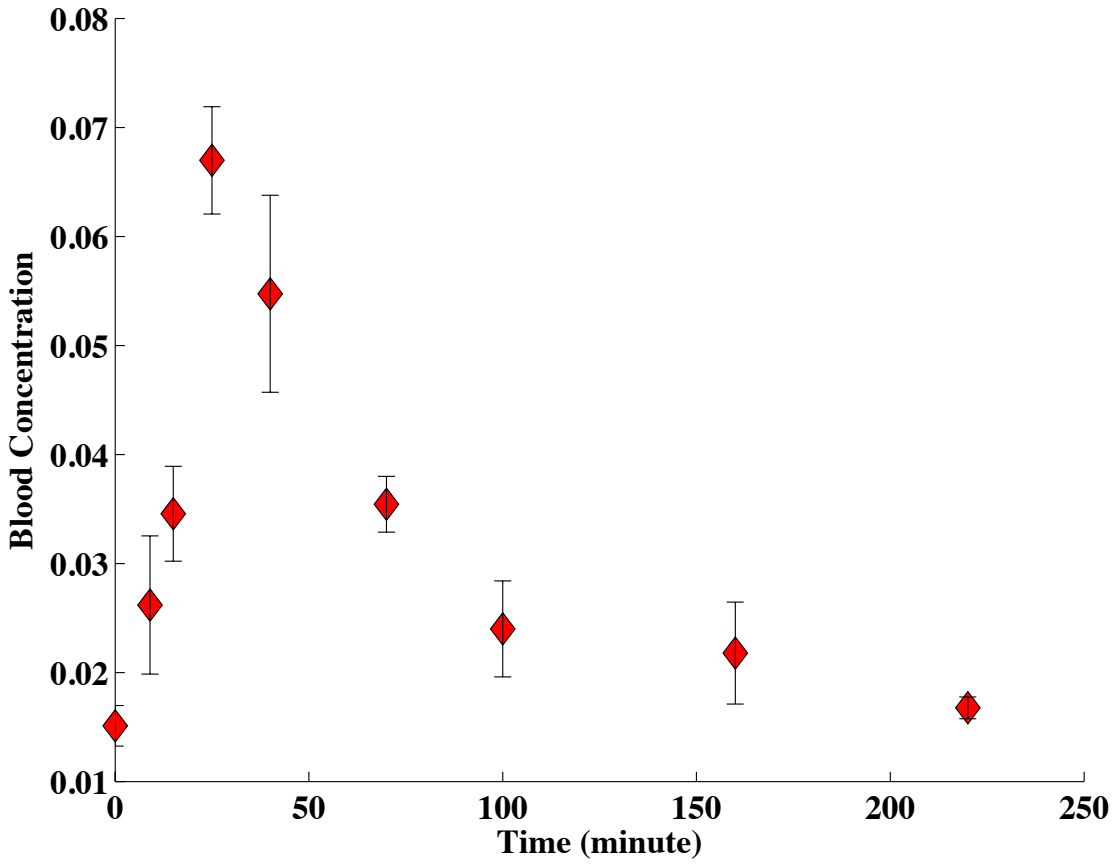

Figure 14 Measured PHA-543613 plasma exposure (mean ± standard deviation) in Monkey S. HPLC analysis of blood serum obtained over a period of 220 minutes after IM injection of 0.250 mg/kg dose.

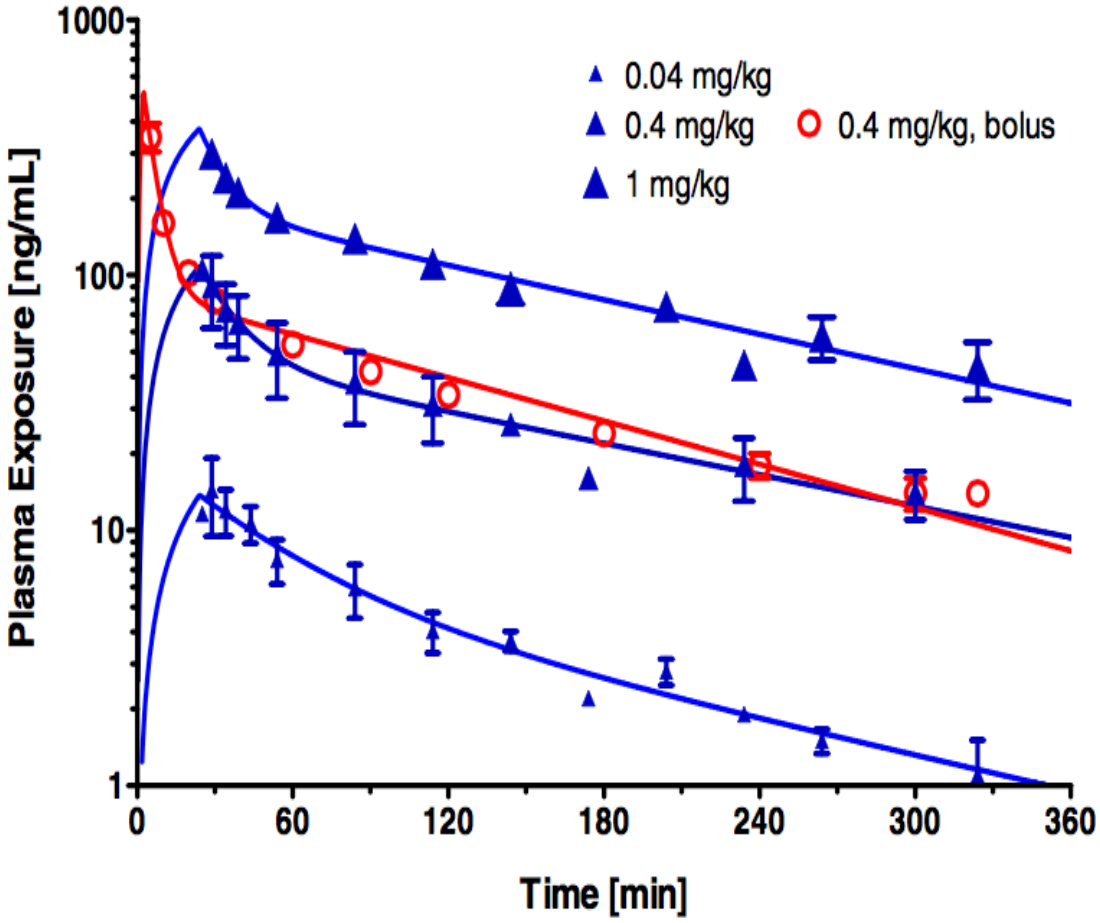

Figure 15 Measured ABT-089 plasma exposure (mean ± SEM) obtained under slow infusion vs bolus infusion in baboons. Red and blue data points refer to bolus and slow infusion injections respectively. The bolus infusion of IV injection is more comparable to the administration method used in this experiment. The lines represent the pharmacokinetic simulations conducted by the authors of the study

2.5 Data Analysis

All the experimental data were analyzed with Matlab. To assess the effects of systematic injection of PHA-543613 and ABT-089 on feature based reversal learning, different aspects of behavior were defined and quantified as described below. In some analyses, a moderately small number of data points existed in the drug condition which was considerably fewer than control. Therefore, data across all control and drug sessions were compared using non-parametric Wilcoxon rank sum test for performance accuracy in different time windows, trial type subsets based on dimming event and reward history and number of reversal blocks and rewarded trials. In addition, two-sample z-test for comparing proportions, and randomization test when correction for multiple comparison was needed were used.

Temporal variation of drug effects on performance. Trial by trial performance was estimated by using expectation maximization algorithm **(**See **Appendix B.1** for more details) developed by Smith (2004). Different overlapping time windows after the task started were selected to evaluate the temporal effects of drugs on behavior. The time windows included: 0-25, 12.5-37.5, 25-50, 37.5-62.5 minutes (**Fig. 16**). Performance accuracy was evaluated in I) throughout trials 1-25; II) During the learning period i.e. all trials up to the estimated learning trial by the algorithm; III) After the learning period i.e. all trials after the estimated learning trial up to trial 25. Different behavioral aspects such as speed of learning and asymptotic performance levels *after learning* were measured.

Dynamics of performance including learning speed and net increases in performance were measured via parameters of a hyperbolic ratio function fit to performance curves. The parameters of interest to make comparison between experimental and control sessions were C-50 (trial to reach half maximal of performance, exponent (slope) and R-max (maximum increase in performance

since baseline performance). A randomization test was used to evaluate the significance of the difference in performance between the experimental and control sessions (See **Appendix B.2** for more details).

Distraction filtering. Defined as the performance accuracy in trials were dimming of both stimuli occurred simultaneously. Here, performance was calculated as the proportion of correct choices within a backward trial window of 5 averaged across all blocks of a session (**Fig. 17**). In comparison to trials where dimming of the two stimuli did not happen simultaneously, performance in the same dimming trial type required a higher level of attentiveness to filter distraction. All dimming trial types were presented with the same ratio i.e. 1/3 each.

Motivation. Defined as total number of blocks performed and rewarded trials which would indicate the amount of fluid earned by the subject. Blocks were categorized as learned and not-learned; the latter defined as blocks not identified to be learned by the EM algorithm or their corresponding learning trial computed by the EM algorithm was higher than 24. The proportion of learned to total reversal blocks were computed as well and compared to each other with two sample proportions z-test (See **Appendix B.3**).

Effects of reward history in learning maintenance. Trials were grouped to 8 categories based on the number of consecutive rewarded trials after an unrewarded/error trial. The proportion of correct trials in each category was calculated to compare the effect of reward history on performance of a given trial. This analysis was conducted in a previous non-pharmacological study to investigate the mechanism underlying the role of anterior cingulate in reinforcement-guided behavior (Kennerley, Walton, Behrens, Buckley, & Rushworth, 2006).

Various Error Types. Error trials consisted of premature responses in time frames 2-4 and 4-6 as well choice-based errors (i.e. response to an incorrect stimulus). Proportion of different error

types to total errors in a session were computed. Pre-mature responses in time frame 2-4 were considered motivation-based errors. Motivation-based errors were defined as fixation breaks after stimulus onset (i.e. time frame 2) but before time frame 4 (i.e. the time frame when all the necessary information for making a response is available).

Pre-mature responses in time frame 4-6 were considered impulsivity-based errors. Impulsivity errors were defined as the number of trials in which erroneous saccades were made towards a stimulus after the onset of both motion and color feature (i.e. time frame 4) but before the dimming of that stimulus (i.e. time frame 5 or 6). The choice-based errors were analyzed within a successive pattern and termed as successive errors. These errors were defined as the number of successive choice errors ranging from 1-8 trial numbers following a correct trial.

For all analyses, performance was looked at within reversal blocks only i.e. the first block of the day was removed from analyses. Additionally, blocks with fewer than 16 trials (i.e. last block of the day where sometimes subject would not complete it) were excluded from the analysis.

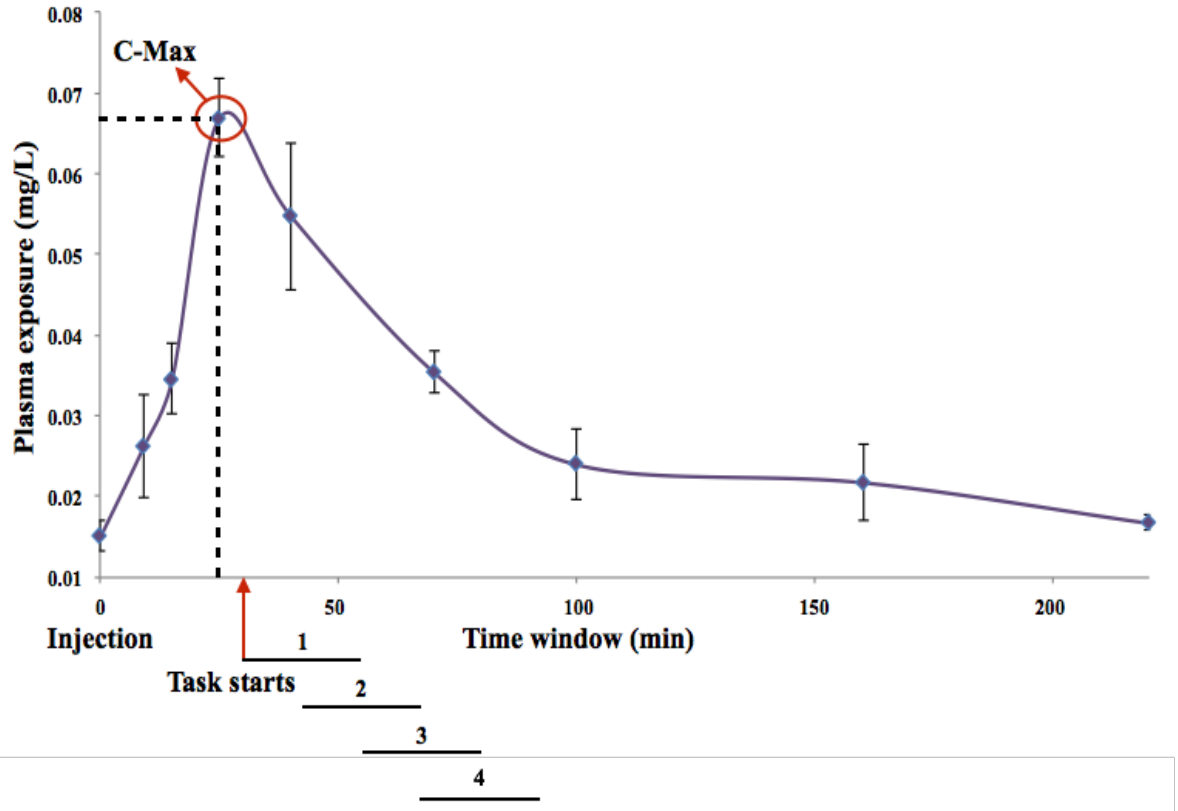

Figure 16 Arrangement of time windows after the task starts for investigating the temporal effects of drug treatments on behavior. Depicted are the main four overlapping periods selected for the analysis aligned against the drug concentration curve over time for PHA-543613, 250 mg/kg dose. Lines 1-4 refer to 0-25, 12.5-37.5, 25-50 and 37.5-62.5-minute time windows.

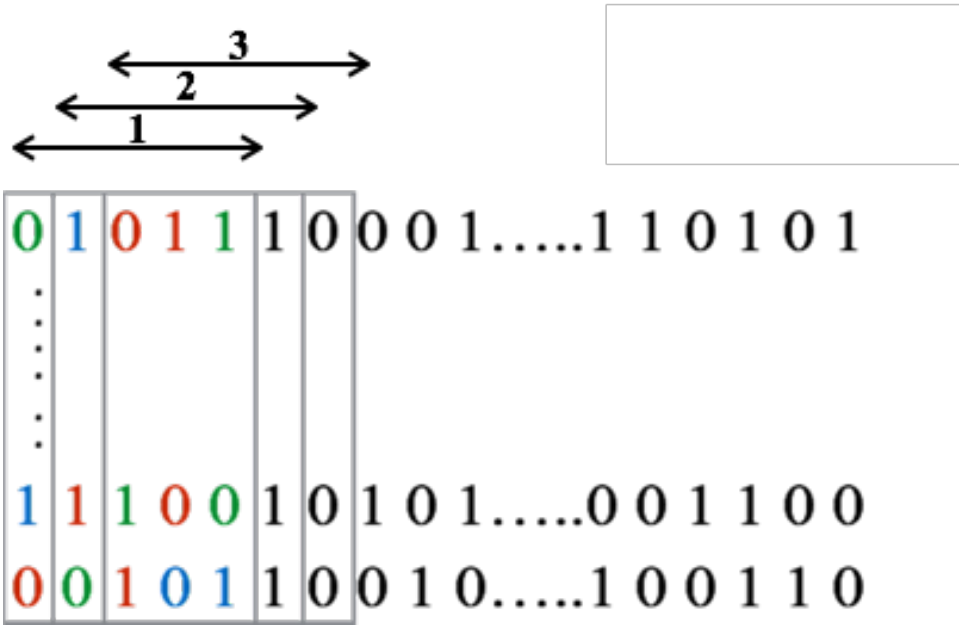

Figure 17 Proportion of correct choices calculated for each dimming type. Backward trial window of 5 were selected across all blocks of a session. Colors green, blue and red represent dim type first, same and second respectively. The proportion of correct to incorrect choices were extracted for each dimming trial type that existed within a given window. For instance, in window (1), the correct proportion at trial 1 for first dimming trial type was computed as: 2/5=0.4, for same dimming trial type as: 3/6= 0.5 and for second dimming trial type as: 3/4=0.75.

Chapter 3-Results

3.1 Temporal Effects of Selective nAChR Agonists on Performance

Performance in learned reversal blocks following administration of α7 and α4β2 agonists was compared with that of control sessions over all sessions (**Fig. 18** and **19**). Performance accuracy was analyzed in four different but overlapping time windows: 1) 0-25, 2) 12.5-37.5, 3) 25-50 and 4) 37.5-62.5 minutes after the task started. **Tables 3** and **4** show the number of blocks performed in all treatment and control days during time windows 1-4 and over the whole session. Results showed there was no difference in performance averaged across all trials between either drug doses and control when data was analyzed from blocks over the whole session (Wilcoxon ranksum, $p > 0.05$). However, within the four selected time windows, average performance was higher under PHA-543613 (0.250 mg/kg dose) than control conditions in 0-25-minute period only (Wilcoxon ranksum, $p=0.024$). The lower dose did not result in any significant increase or decrease in the performance accuracy compared to control sessions (Wilcoxon rankusm $p>0.05$). As for ABT-089 treatments, average performance was not significantly different than control sessions in either dose regimen over the whole session or selected time windows (Wilcoxon ranksum, $p>0.05$). As mentioned earlier, performance was also averaged across all trials *during* and *after learning*. The estimated learning trials from both control and drug sessions for a given time window were averaged to set the trial range for *during* and *after learning* periods. This resulted in the following average learning trials for performance over the whole session and within time window 1-4 respectively: PHA-543613 (dose 0.25 mg/kg) and control: 12, 7, 7, 8 and 8; PHA-543613 (dose 0.125 mg/kg) and control: 12, 7, 8, 8 and 8; ABT-089 (dose 0.02 mg/kg) and control: 9, 5, 6, 7 and 7; ABT-089 (dose 0.01 mg/kg) and control: 10, 6, 6, 7 and 6. Average performance *during learning* was not different between either PHA-543613 dose treatments and control sessions when

analyzing the data over the whole session or within time windows 1-4 (Wilcoxon ranksum, $p>0.05$). Similarly, *during learning* performance was not significantly different than control in ABT-089(0.02 mg/kg) treatment over the whole session or in either time windows (Wilcoxon ranksum, $p>0.05$). However, the lower dose treatment showed significantly lower average performance *during learning* within 12.5-37.5-minute time window (Wilcoxon rankusm, $p=0.01$). Next we analyzed *after learning* performance in all drug treatments and control sessions. In PHA-543613 (0.25 mg/kg) treatment, *after learning* performance was significantly higher than control within 0-25 and 12.5-37.5-minute time windows only (Wilcoxon ranksum, $p=0.031$ & $p=0.03$ respectively). The lower dose treatment was not associated with any significant difference in performance compared to control sessions over the whole session or within either time window. Similarly, neither ABT-089 dose regimen resulted in any significant difference in *after learning* performance compared to control sessions in any period (Wilcoxon ranksum, $p>0.05$).

Table 3 Number of blocks performed by Monkey H per each condition for all PHA-543613 treatments and control days during selected four time windows and over the whole session. Only reversal blocks that were identified as learned were quantified and used for the analysis. Note that consistent across all conditions, fewer blocks were performed later throughout the session i.e. the last time window.

Time interval	PHA-543613 (0.25 mg/kg)	PHA-543613 (0.125 mg/kg)	Control
0-25-minute	**30**	**25**	**50**
12.5-37.5-minute	**31**	**21**	**52**
25-50-minute	**29**	**19**	**39**
37.5-62.5-minute	**25**	**16**	**29**
Whole session period	**75**	**72**	**122**

Table 4 Number of blocks performed by Monkey H per each condition for all ABT-089 treatments and control days during the selected four time windows and over the whole session. Table descriptions are the same as Table 3.

Time interval	ABT-089 (0.02 mg/kg)	ABT-089 (0.01 mg/kg)	Control
0-25-minute	**26**	**36**	**83**
12.5-37.5-minute	**29**	**33**	**74**
25-50-minute	**21**	**30**	**61**
37.5-62.5-minute	**8**	**19**	**42**
Whole session period	**48**	**84**	**166**

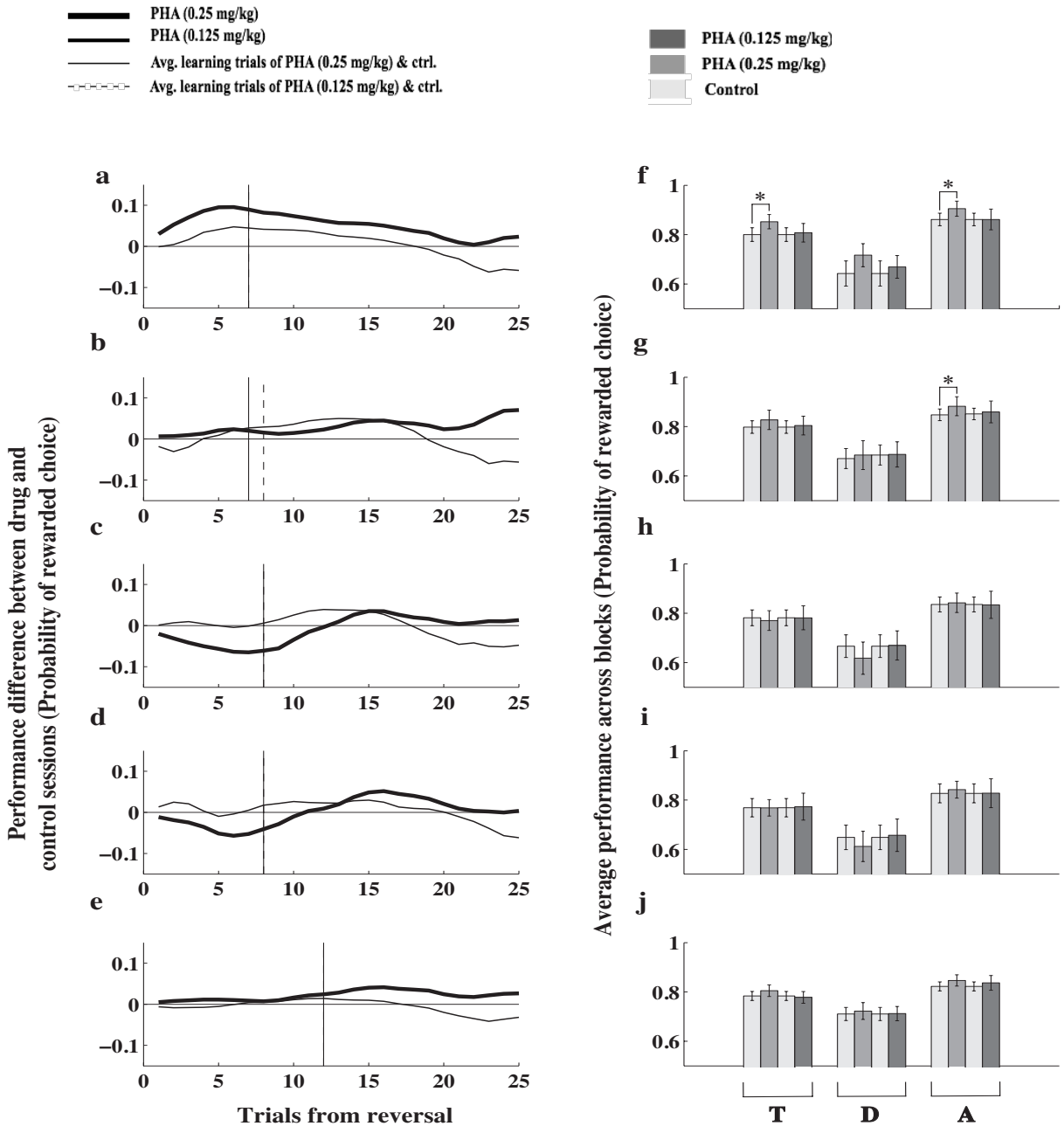

Figure 18 Performance across all blocks over the task session and within four selected time windows after the task started: PHA-543613 versus control. Panels (a-e) show the performance difference curves between PHA-543613 treatments and control sessions. All trials up until average learning trials are analyzed for "*during learning*" performance and the remaining trials after this period are analyzed for "*after learning*" performance. Panels (f-j) show average performance accuracy across all trials (T), *during* (D) and *after learning* (A). Panel "f" shows higher average performance compared to control accuracy across all trials within time window 1(Wilcoxon ranksum, p=0.024) and *after learning* within the time windows 1-2 (Wilcoxon ranksum, p=0.031 & p=0.030) under PHA-543613 (0.25 mg/kg). Error bars show 95% confidence intervals.

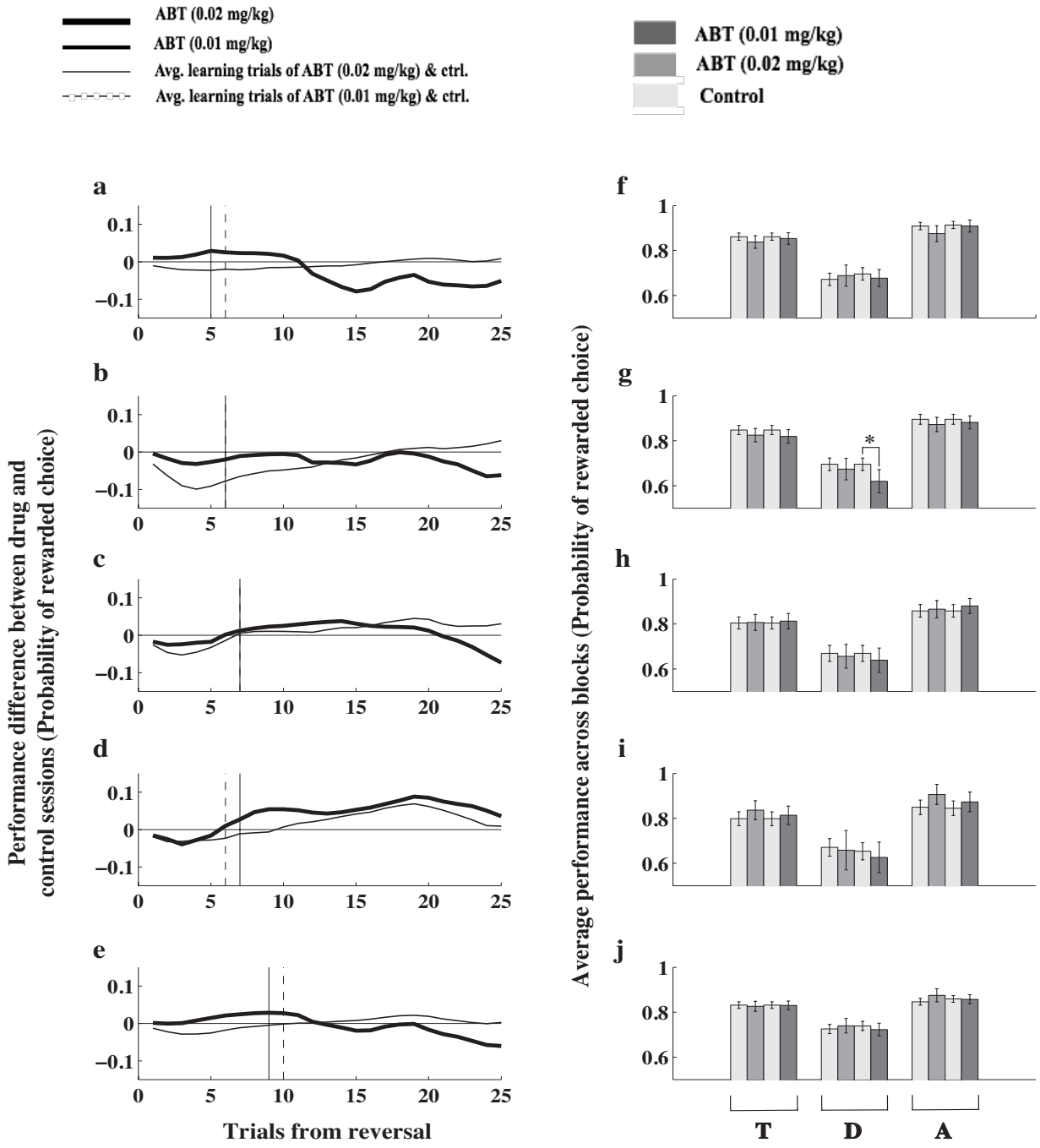

Figure 19 Performance across all blocks over the task session and within four selected time windows after the task started: ABT-089 versus control. Panel descriptions are the same as **Fig. 17**. Panel "g" shows lower average performance compared to control accuracy *during learning* within in time window 2 (Wilcoxon ranksum, p= 0.01) under ABT-089 (0.01mg/kg). Error bars show 95% confidence intervals.

To validate the previous finding regarding enhanced performance under the high dose of PHA-543613 with an additional metric, a function fitting approach was used to assess other dynamics of the performance. A hyperbolic ratio function (aka Naka-Rushton equation) was fit to the performance curves in all learned reversal blocks for each condition over the whole session and time windows 1-4. The following equation used in this analysis was reproduced from Williford and Maunsell (2006) with four parameters including Rmax, C50, n and m.

$$r = r_{\max} \cdot \left(\frac{c^n}{c^n + c_{50}^n} \right) + m$$

Rmax is the maximum attainable y value; In case of behavioral data analyzed in this thesis, Rmax is the difference between the maximum and minimum probability of being rewarded defined as parameter “m” in the function. The exponent “n” parameter represents how rapidly the y value (i.e. the probability of being rewarded) increases from at minimum level (i.e. chance level performance at the first trial in this case) to the saturating point (i.e. Rmax+m) and finally C50 represents the trial number at which the y value or probability of being rewarded is half maximal. This function was chosen to model the data as it included the parameters that defined the behavior of the data well i.e. it corresponds to the rising pattern of the performance which stabilizes or saturates at a certain level. Consequently, the parameters allow for meaningful interpretations of the data. For instance, a left shift of C50 would indicate learning a block in fewer number of trials. Acceptable ranges were chosen for all parameters from which randomly an initial value was assigned to estimate each parameter:

Rmax: the difference between minimum and maximum of probability of being rewarded were computed across all blocks. The initial “Rmax" value was randomly chosen from this range.

m: the minimum and maximum of the probability of being rewarded at the first trial across all

blocks were computed. The initial m value was randomly chosen from this range.

C50: the minimum and maximum of the trial number at which the probability of being rewarded is half maximal across all blocks were computed. The initial "c50" value was randomly chosen from this range.

n: the initial "n" value was chosen within 1-3 range.

Randomization tests to determine the significance difference for the parameters confirmed that there was no significant difference between ABT-089 (both doses) and PHA-543613 (0.125 mg/kg) and their corresponding control sessions ($p>0.05$). However, results showed that within the 0-25-time window, PHA-543613 (0.250 mg/kg) had a significantly lower value of C50 compared to control sessions ($p=0.0341$) (**Fig. 20**). No significant differences were observed for other parameters in either time windows for the same treatment ($p>0.05$)

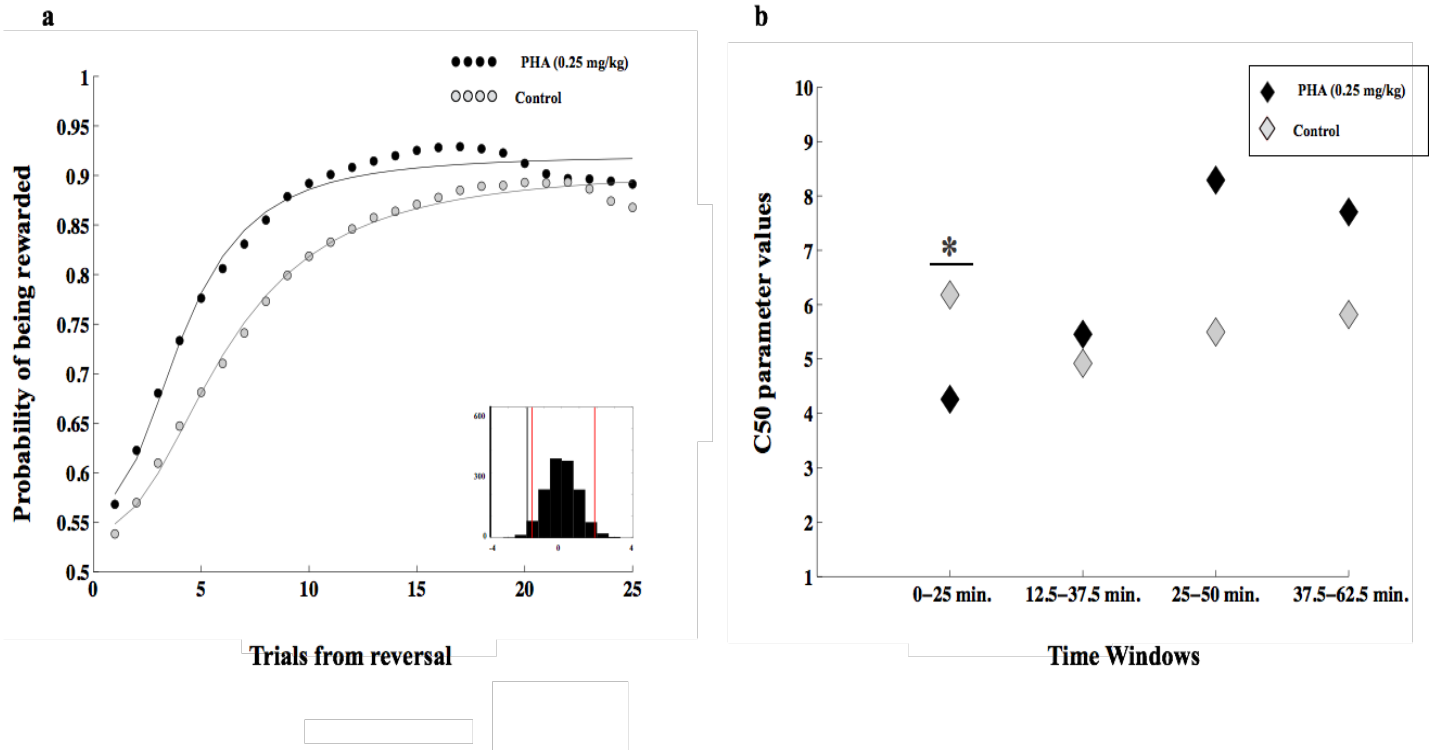

Figure 20 Hyperbolic-ratio function fit to performance curves within time window 1 under PHA-543613 (0.25 mg/kg) treatment and control. (a): depicts function fit to performance curve within 0-25-minute time window. The histogram shows the null distribution resulted from randomization procedure; Red lines represent 97.5 and 2.5 percentiles of the distribution and the black line the observed test statistics. (b): demonstrates the C50 parameters estimated by the function within all four time windows for the same drug treatment and control. This parameter was significantly lower under drug condition in time window 1 ($p=0.0341$).

3.2 Effects of Selective nAChR Agonists on Distraction Filtering

Performance accuracy was averaged across all 25 trials for all dimming type trials under drug and control conditions (**Fig. 21** and **22**). Under PHA-543613 treatments, the average performance accuracy was not significantly different than control in either dimming trial type (Wilcoxon ranksum, $p>0.05$). As for ABT-089 treatments, the higher dose yielded higher average performance accuracy than control only when both stimuli dimmed simultaneously (Wilcoxon ranksum, $p=0.033$). The lower dose of the same treatment was not associated with any significant difference in either dimming trial type (Wilcoxon ranksum, $p>0.05$).

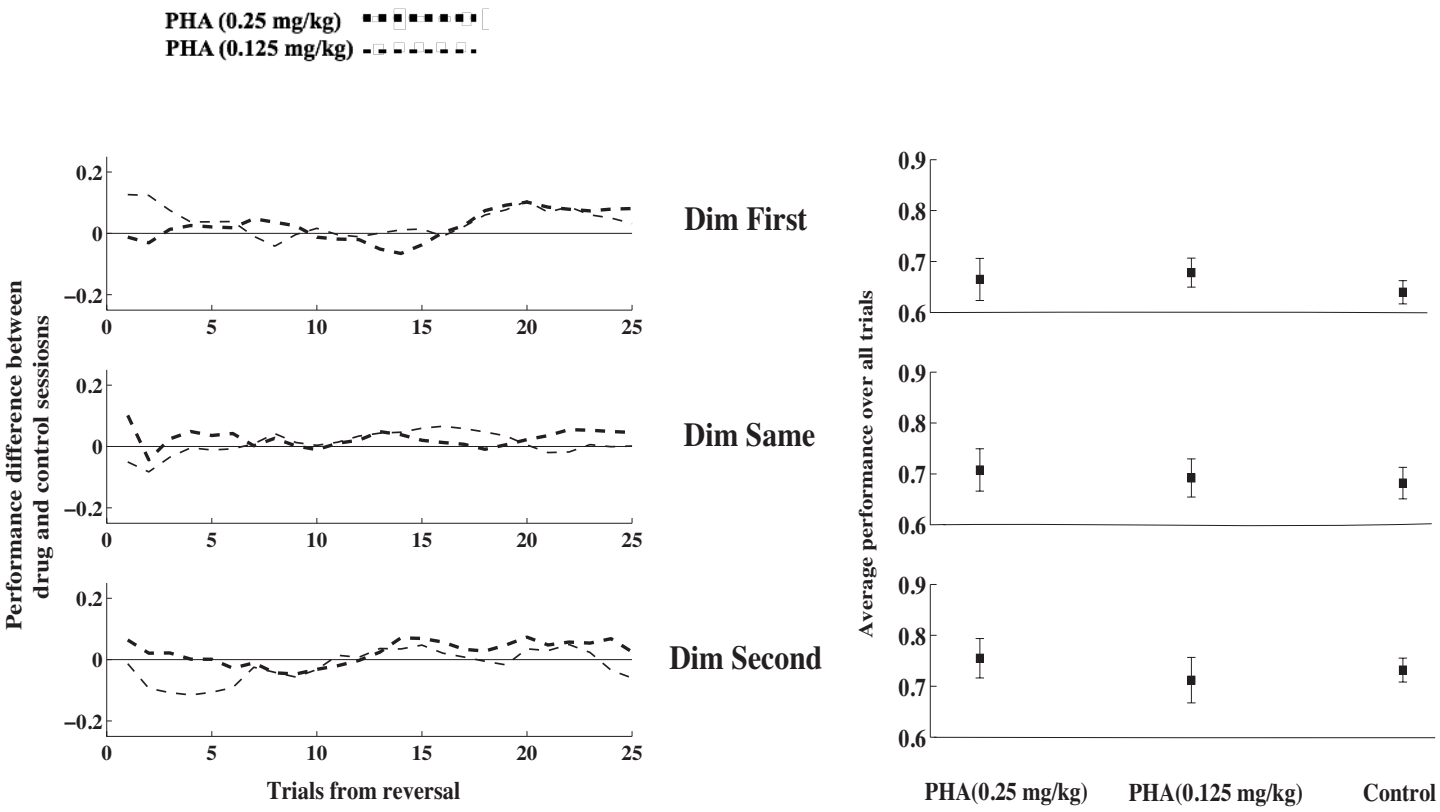

Figure 21 Average daily performance within each dimming trial type in PHA-543613 experiment. The panel on the left side shows performance difference curves between drug treatments and control sessions under first, same and second dimming trials. Trial numbers refer to the rank of that trial compared to all other trials from the same dimming subset; for example, third trial in dim first refers to the third "first dimming" trial that occurred in the block. The panel on the right side depicts the averaged performance across trials 1-25 in each corresponding performance difference curve. Neither drug doses affected performance in any of the dimming trial types.

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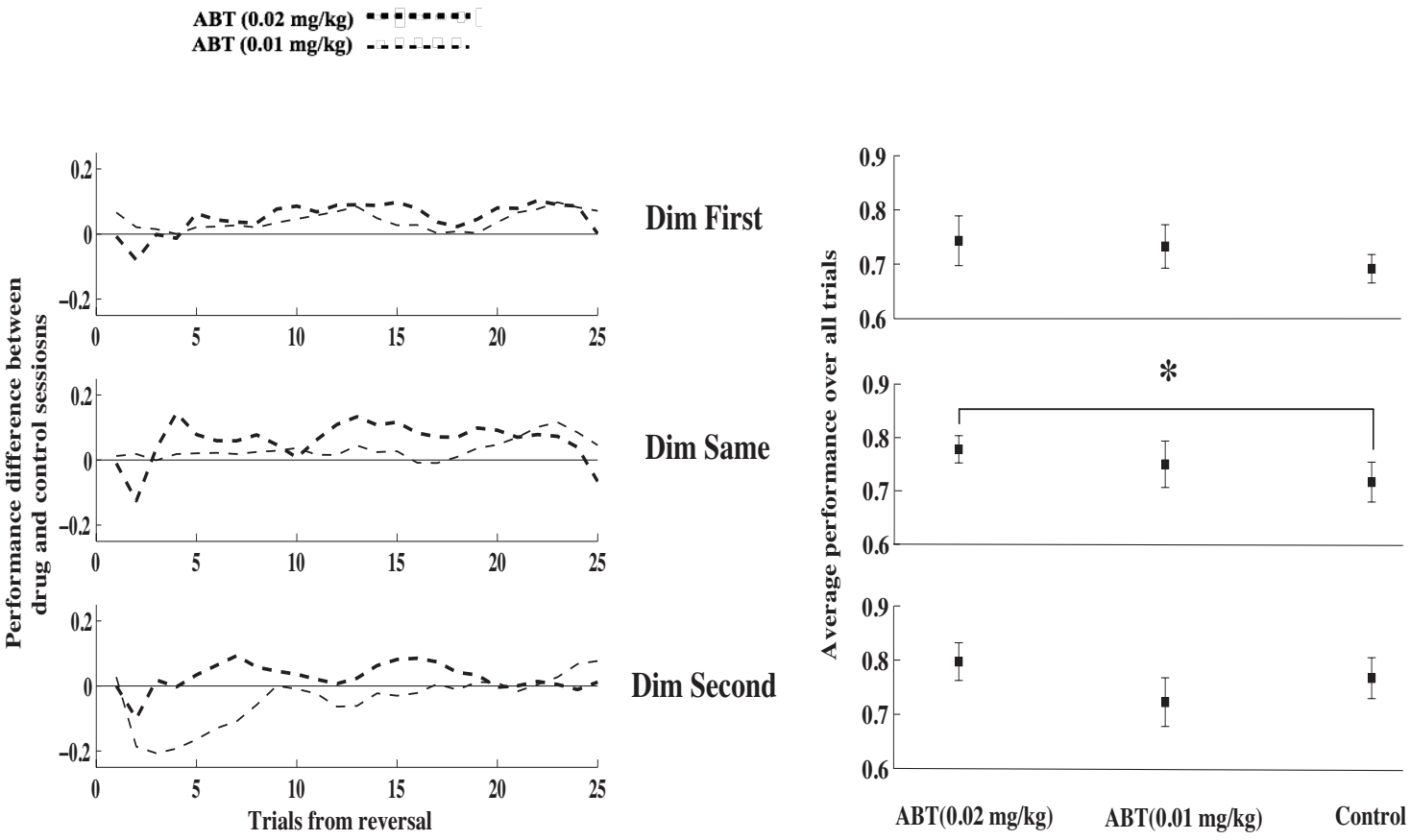

Figure 22 Average daily performance within each dimming trial type in ABT-089 experiment. Panel descriptions are the same as **Fig. 20**. Average performance in same dimming trial type only was significantly higher than control under ABT-089 (0.02 mg/kg) treatment (Wilcoxon ranksum, p=0.033)

3.3 Effects of Selective nAChR on Motivation

The average number of reversal blocks were 11.8, 12.7 and 11.3 for PHA-543613 (0.25 mg/kg), PHA-543613 (0.125 mg/kg) and control respectively and not significantly different from each other (Wilcoxon ranksum, $p>0.05$). Proportion of learned blocks in either PHA-543613 treatments were comparable to that of control (z test, $p>0.05$). In general, the subject tended to perform fewer number of blocks during the second protocol. The average daily number of total reversal blocks were 7, 10 and 9.7 For ABT-089 (0.02 mg/kg), ABT-089 (0.01 mg/kg) and control. Monkey H performed significantly fewer number of blocks than control when injected with higher dose of ABT-089 (Wilcoxon ranksum, $p=0.0255$). However, the proportion of learned blocks in ABT-089 (0.02 mg/kg) treatment was significantly higher than control (z test, $p=0.018$) (**Fig. 23**).

In addition to the total number of reversal blocks performed, the average earned reward was compared across drug treatment and control conditions using total number of rewarded choices throughout the whole session. Average number of total rewarded choices were 342, 401 and 354 for PHA-543613 (0.25 mg/kg), PHA-125 (0.125 mg/kg) and control respectively. There were no significant differences between either drug treatments and control (Wilcoxon ranksum, $p>0.05$). Likewise, average number of rewarded choices were similar across both drug treatments and control in ABT-089 experiment (Wilcoxon ranksum, $p>0.05$). Average number of total rewarded choices were 212, 304 and 264 for ABT-089 (0.02 mg/kg), ABT-089 (0.01 mg/kg) and control respectively.

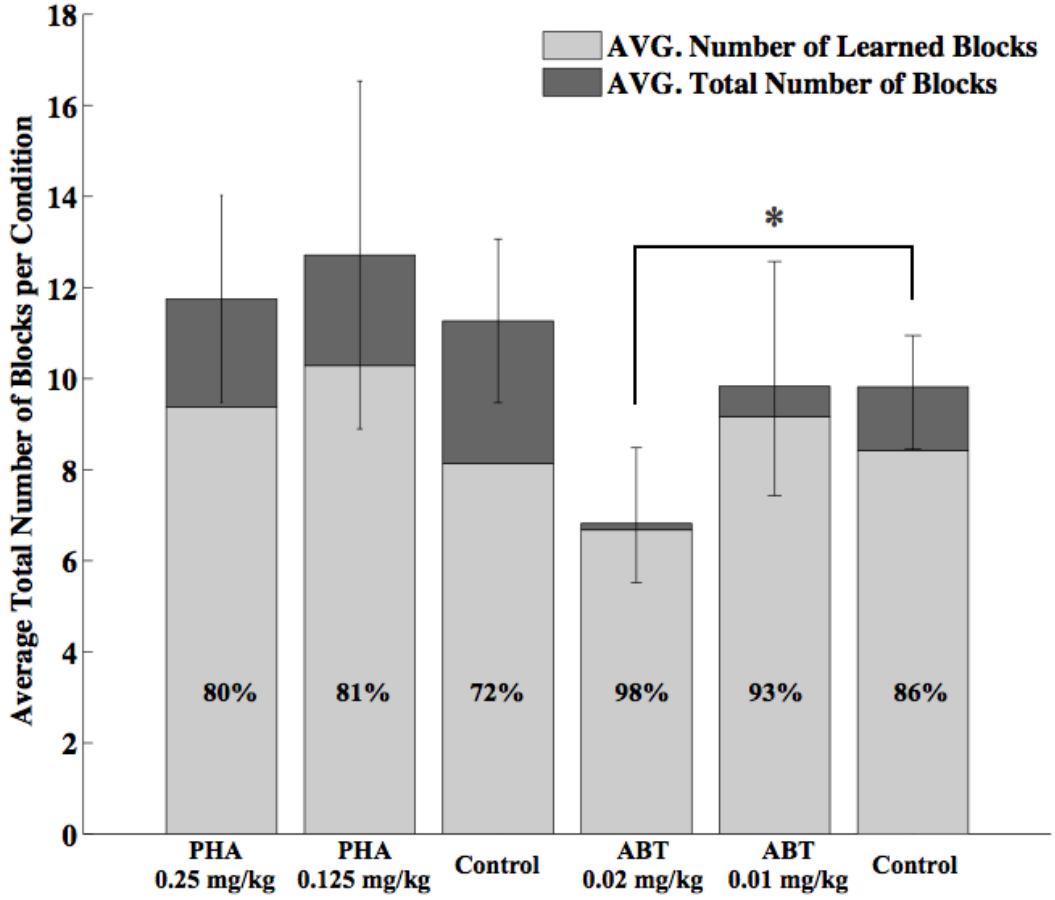

Figure 23 Average number of reversal blocks under all drug treatments and control sessions. Percentages depicted in each bar represents the average proportion of learned blocks (as identified by the EM algorithm). Total number of reversal blocks and proportion of learned blocks under both PHA-543613 treatments and control were comparable to each other. Total number of reversal blocks was significantly lower under ABT-089 (0.02 mg/kg) compared to control (Wilcoxon ranksum, p=0.0255). In contrast, the proportion of learned blocks was higher under the same treatment than control (z-test, p=0.018). The error bars represent 95% confidence intervals.

3.4 Effects of Selective nAChR on Reward History Based Performance

As seen in **Fig. 24**, The effect of reward history on performance of a given trial in each of the 8 categories was comparable across all drug treatments and control sessions and not significantly different from each other (Wilcoxon ranksum, $p>0.05$). It should be noted that by definition, the categories with higher number of consecutive correct trials would occur much less frequently compared to categories with low numbers of these trials. Looking at PHA-543613 (0.250 mg/kg) for instance, there were only an average of 15 trials in E+C8 category compared to 109 in E+C1.

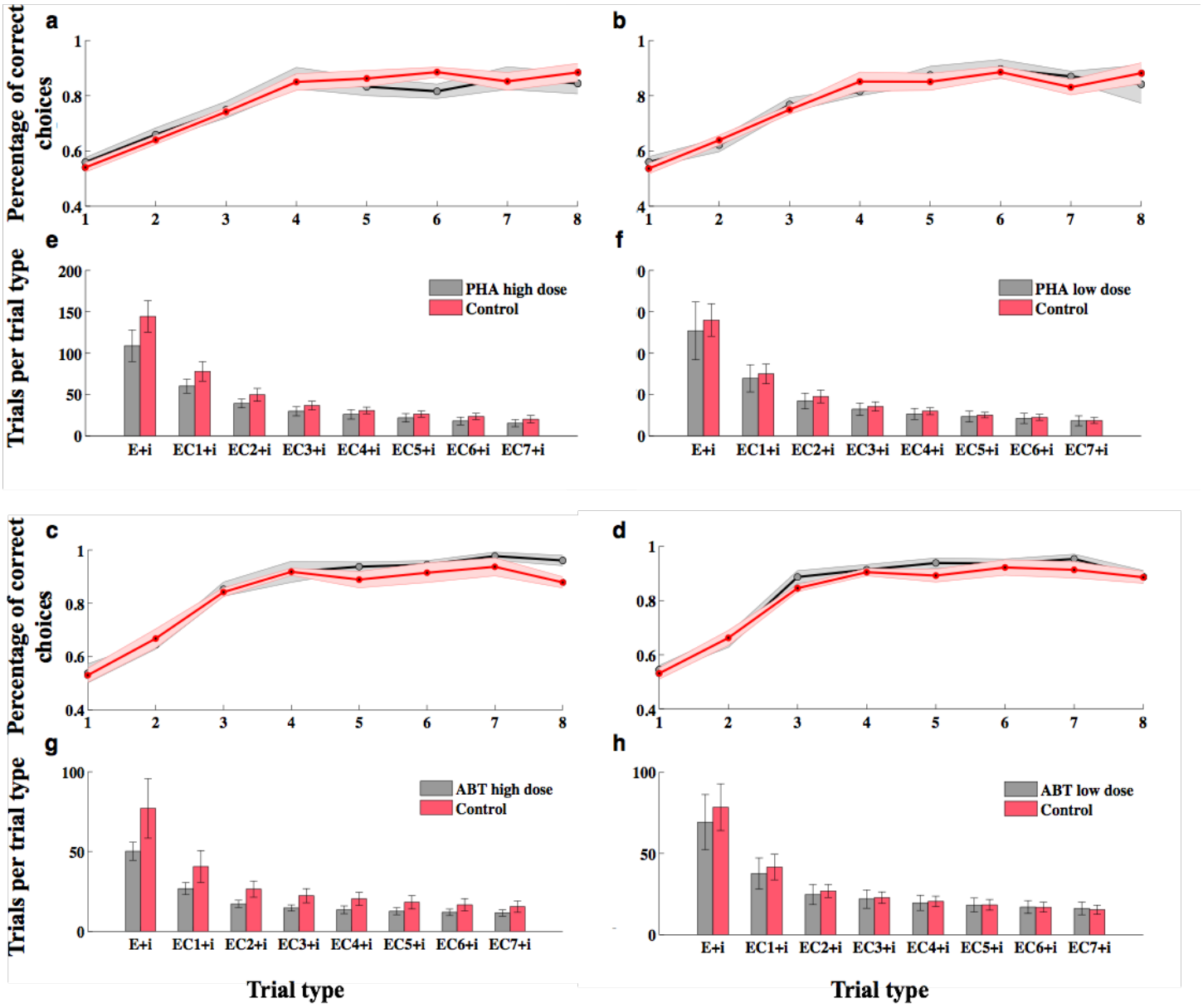

Figure 24 Reward history based performance under all drug treatments and control sessions. Panels a-d show performance curve defined as proportion of correct choices within each trial category. In all conditions, an enhancing trend in performance existed such that average proportion increased over the trial categories with higher number of correct choices after an error. However, this performance enhancement was consistent and not different across all drug treatment and control sessions (Wilcoxon ranksum, $p > 0.05$). The bar graphs e-g represent the average number of trials in each category. Error bars show 95% confidence intervals.

3.5 Effects of Selective nAChR on Error Types

Fig. 25 shows the proportion of each error type within a condition. The proportion of each error type i.e. successive-based errors, impulsivity-based errors and fixation break-based errors to total error types were computed. Generally, fixation break errors constitute the greatest proportion of all error types (on average 53.5 % across all drug and control conditions) while successive errors constitute the lowest proportion (on average 15 % across all drug and control condition). Results showed that the proportion of error types are very similar and consistent across all drug treatments and control sessions (Wilcoxon ranksum, $p>0.05$).

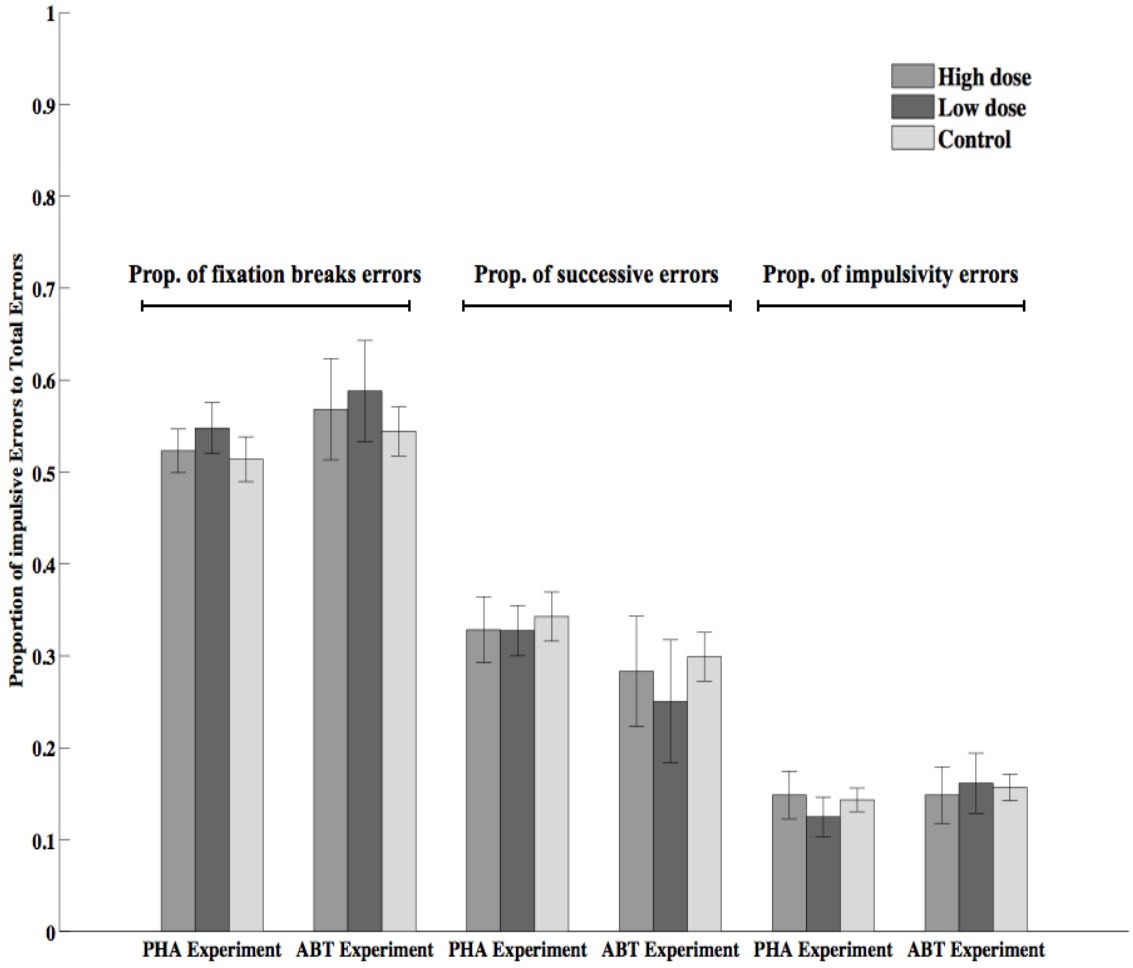

Figure 25 Proportion of three different error types in all drug treatment and control sessions. Fixation break errors constituted the highest error type across all conditions while impulsivity bases errors constituted the lowest. Neither drug treatment modulated any of the error types (Wilcoxon ranksum, $p>0.05$).

Chapter 4-Discussion

This thesis describes a project with qualities that distinguish it from the previous studies investigating the effects of nicotinic sub-receptors in animal models. First, the task used in this study allowed me to dissociate the effects on learning and attentional filtering from other motivational and behavioral measures of behavioral flexibility. Second, the dose selection protocol allowed a rapid identification of an optimal dose associated with performance improvement and a dose with no behavioral effects, thereby avoiding the complications that follow a fixed treatment schedule. Finally, multiple analysis approaches were used to evaluate behavioral indices and validate the main findings.

Overall, I found that the higher dose of each agonist emerged to be a dose that led to significant performance improvements. The behavioral enhancement induced by each agonist was unique and specific to different aspects of the task. The optimal dose of α7 receptor agonist increased overall performance accuracy and sped up learning of reversals in reward contingencies, but only within the first 25 minutes of the task when substantial concentration of the agonist was still present in the plasma. The optimal dose of α4β2 agonist was associated with improvement in trial types where filtering of distracting stimuli was most difficult. Neither agonist affected other behavioral measurements including motivation, impulsivity or perseverative rates. The lower dose of the α4β2 agonist was associated with lower performance accuracy during the learning period within a 12.5-37.5-minute time window after drug administration.

4.1.1 Selective Nicotinic Receptor Modulation of Distractor Filtering

In order to investigate the effects of selective nicotinergic agonists on control of interference from distractors, we averaged proportion of correct choices in each of the three existing subsets of trials and specifically, looked at trials in which both stimuli signaled the response simultaneously.

Proportion of correct choices in trials where both stimuli signaled the response at the same time was not significantly affected by either dose of PHA-543613. In contrast, proportion of correct choices was significantly increased in this subset of trials with the optimal dose of ABT-089 (See **Fig. 22**). In comparison with other trial types where the target stimulus signals the response prior to or after the distracting stimulus, trials in which both target and distracting stimuli signal the response simultaneously, had the highest attention demand to filter distraction. As such, this finding is consistent with my hypothesis that the α4β2 agonist would improve the ability to filter distracting stimuli. It is also consistent with previous rodent literature showing that α4β2 agonists enhanced performance in dSAT task (Howe et al., 2010); and with non-human primate studies where three different α4β2 agonists including ABT-089 improved performance accuracy in DMTS-D task (Buccafusco et al., 2007; Prendergast et al., 1998).

Interestingly, Prendergast and colleagues (1998) initially tested ABT-089 in a regular DMTS task and found no significant effects on performance. The authors then proceeded to test the drug in the more demanding DMTS-D task which includes distracting stimuli in delay periods. Consequently, they found that ABT-089 increased average number of correct choices in this variant of the task. Similarly, Howe et al. (2010) did not find any performance accuracy enhancement in regular version of SAT task but found increased hits in the variant with intervening distracting stimuli. In terms of effective doses, the optimal dose of ABT-089 in this thesis (0.02 mg/kg) was comparable to the highest effective dose of ABT-089 in Prendergast et al., (1998) study (i.e. 0.018 mg/kg) (for details on selected doses see **Table 2**).

The results of this thesis showed that α4β2 agonist can enhance control of interference from distractors and attenuate the influence of distractors on performance accuracy. This finding is consistent with those of other studies which suggest that α4β2 receptors may be more prominently

involved in mediating attentional processes when cognitive load is augmented (Buccafusco et al., 2007; Howe et al., 2010; Prendergast et al., 1998). Such enhancement effects on attention filtering by α4β2 agonists can be potentially mediated by evoked cholinergic transients in the PFC (Parikh et al., 2007) and facilitation of interactions between glutamatergic-cholinergic transients (Hasselmo & Sarter, 2011;Parikh et al., 2010).

4.1.2 Selective Nicotinic Receptor Modulation of Reversal Learning

Of the two agonists used in this study, the optimal dose of α7 agonist led to increase in probability of rewarded choice over all trials of a block within time windows that corresponded to the period when the drug was roughly at its peak concentration. The performance accuracy was enhanced most robustly in the *after learning* period in 0-25 and 12.5-37.5-minute time windows, suggesting a better maintenance of learning by PHA-543613 (See **Fig. 18 f** and **g**). Unlike 12.5-37.5-minute time window, performance accuracy in 0-25-minute time window was significantly enhanced throughout the whole block (See **Fig. 18 a** and **f**). Although the increased performance accuracy in the *during learning* period did not reach statistical significance, assessment of performance over all trials via hyperbolic-ratio function fitting showed that Monkey H reached half maximal of his performance in fewer trials compared to control sessions. Therefore, these results suggested that Monkey H learned block reversals faster during trials of the 0-25-minute time window. While one non-human primate study suggested improved performance accuracy in a spatial reversal learning task (Terry et al., 2016), neither rodent nor other non-human primate studies have reported more efficient reversal learning before. The difference in the findings of this thesis and previous experiments can be explained by how the reversal learning tasks were designed across the studies. In my thesis, I used a reversal learning task in which the subject performed serial reversals each daily session. This is in contrast with other studies in which subjects would perform one reversal

of an exemplar set per session. Rygula and colleagues (2010) proposed that exposure to learning reversals can result in utilizing strategies for optimal performance and showed that different brain regions of the PFC are recuited when marmosets reversed between a new set of stimuli than the same set several times. Another explanation could be temporal intervals within which the data was analyzed. In this project, both performance accuracy and faster learning were time-locked to early time windows. On the contrary, when analyzing the data over the whole session, these effects were non-existent. Previous selective nAChR agonist studies with reversal learning in both rodents and non-human primates analyzed the data within the overall session only and did not consider different time intervals. However, agonist-induced improvement in specific time blocks has been reported by previous studies using attentional tasks (5-CSRTT and SAT) and α7 pharmacological agents (Hahn et al., 2011; Rezvani et al., 2009). Similar to my results, the time windows that the behavioral effects were reported also paralleled the peak concentration of drugs.

Performance accuracy was not enhanced with ABT-089 over any time window. In fact, the lower dose led to decrease in performance accuracy in the *during learning* period within 12.5-37.5-minute time window. However, such a decrease in performance within this time window did not result in slower learning of the reversals.

In summary, we found that PHA-543613 broadly improves learning performance when the drug is at its peak concentration in the plasma. This is unlike the enhancing effects of ABT-089 which were specific to a subset of trials. Stimulation of α7 receptors has been proposed as a possible mechanism underlying ACh release in response to relevant events and subsequent strengthening of PFC network connectivity (Arnsten et al., 2010). Thus, an enhanced representation of relevant features in an environment (e.g. in this task color) can be a potential mechanism underlying the overall increased performance accuracy by α7 agonist PHA-543613.

4.1.3 No Net Influence on Motivation, Impulsivity or Perseveration

The agonists used in this study did not change all aspects of performance in a healthy subject. Motivation was not affected by either sub-receptor agonist. While Monkey H performed significantly fewer reversals blocks under the optimal dose of ABT-089, his earned reward i.e. fluid was comparable to control sessions. This is because with drug treatment, Monkey H performed the blocks more efficiently (as seen in higher proportion of learned blocks) and while doing fewer reversals, completed similar number of rewarded trials in both conditions. Therefore, consistent with previous studies, neither agonist impacted motivation (Guillem et al., 2011, Hahn et al., 2011; Young, Meves, Tarantino, Caldwell, & Geyer, 2011).

Neither agonists in this thesis affected impulsivity, motivation-based and successive errors (See **Fig. 23** and **25**). Hoyle and colleagues (2006) showed that depending on the variability of inter-trial intervals, lack of α7 subunit expression in mice can increase premature responses in 5-CSRTT task. When inter-trial intervals were fixed and no punishment delay period (time-out) followed these choices, the KO animals showed increased impulsivity. However, when inter-trial intervals were variable and time-outs included, KO subjects were not more impulsive than the wildtype. Therefore, other factors such as temporal prediction abilities may have been involved in the initial observed increased premature responses rather than impulsivity (Hoyle et al., 2006). The vast majority of 5-CSRTT studies limit themselves to reporting the percentage of accuracy and omission errors. Additionally, due to the nature of SAT and ID/ED set shifting tasks, it is not possible for animals to respond prematurely and as such, studies with these tasks do not report impulsivity. Consequently, effects of selective nicotinergic sub-receptors on impulsive behavior are not extensively investigated in either rodents or monkeys. For this project, I looked at pre-mature responses that occurred after the animal was exposed to all the necessary information for

making a response (i.e. color and motion) but before the go signal to respond (i.e. dimming of the stimulus). It should be noted that this is different from the time frame within which premature responses are calculated in 5-CSRTT which is the period before any stimulus (light cue) presentation.

I also did not find any drug-induced effects on fixation breaks in the time interval after stimuli presentation and before the display of all the necessary information for making a response. The lack of any significant effects on fixation breaks within this period implies that completing fewer trials with the optimal dose of ABT-089 was mainly due to lack of initiating a trial rather than breaking fixation when the stimuli were already displayed on the screen.

Finally, another error type that was looked into consisted of successive errors. These errors were counted after a correct response and could go as up as 8 consecutive choice-based error trials where Monkey H responded to the direction of the distracting stimulus. This measurement was independent of trial onset relative to the start of the block i.e. it was not limited to when the blocks just switched but included all errors of this nature throughout the whole block. Results did not show any drug-induced changes with either agonist and therefore, did not support my hypothesis that either agonist can potentially attenuate perseverative tendencies. This finding is in consistency with that of Gould et al. (2013) but at odds with those of Terry et al. (2016) and Jones et al. (2014). The discrepancy between results can be explained by how the errors were evaluated in each study i.e. whether the experimenters counted errors within a certain number of trials after the reversal (Jones et al., 2014; Terry et al., 2016) or counted them throughout all trials (Gould et al., 2013). Another explanation can be the use of animals whose performance were impaired by injection of pharmacological agents such as ketamine and phencyclidine (PCP) that are known to cause cognitive deficits (Jones et al., 2014; Terry et al., 2016). It is also possible that the selected drugs,

doses and timing of drug administration led to these different findings. For instance, Terry et al. (2016) used an α4β2 agonist which also strongly activated α7 receptors and administered the drug. While Terry et al. (2016) found attenuation of errors by all doses, Jones et al. (2014) found a dose-dependent effect with PNU-282987 (an α7 agonist with no interaction on α4β2 receptors) such that only the lowest dose was effective in reducing perseveration errors. However, the other effective dose of the study which similar to the lowest dose led to fewer trial to performance criterion in ED shift, failed to attenuate perseveration errors.

In summary, our findings show that nicotinic α4β2 and α7 sub-receptors do not improve performance through mediating motivation and different error types. Our findings suggest that the enhanced reversal learning mediated by α7 receptors occur in the absence of modulation of perseveration tendencies. Our findings also suggest that enhanced attentional control mediated by α4β2 receptors is independent of motivational factors. This finding is in consistency with a previous study which showed that increased response accuracy by non-selective nicotinic agonist was different from motivational-induced performance accuracy in rodents performing a 5-CSRTT task (Bizzaro and Stolerman, 2003).

4.2 Limitations

4.2.1 Lack of Washout Period in a Subset of Datasets

It is assumed that using a drug on one day still has a carryover effect on subsequent days and could influence behavior (Jerry J. Buccafusco, Letchworth, Bencherif, & Lippiello, 2005). Therefore, most pharmacological experiments consider washout periods in between treatment sessions. During these periods, no drug or control data are collected. However, no washout days were considered in between some of the drug and control sessions in the current study. This is unlike previous nicotinergic studies where usually a minimum of 1 washout day was considered (see for

example Jerry J. Buccafusco et al., 2007). However, it should be noted that in those studies, the control condition only takes place in the first day of the week and as such, drug treatments are also assigned to certain days of the week. This design does not control for behavioral variability of the subject which may rise as a result of performing in different days. Additionally, it does not allow for a blinded experiment and as such subjective biases may interfere with the data collection and result interpretation.

Based on the HPLC analysis for PHA-543613, the drug levels in blood serum should be back to baseline in less than 5 hours. Inferring from the results obtained with baboons (Chin et al, 2011) it can be expected that similar to PHA-543613, the plasma concentration of ABT-089 is most likely back to baseline in less than 24 hours. Decker and colleagues reported no behavioral-induced effects of ABT-089 in adult monkeys performing DMTS task which were assessed 24 hours after the IM drug administration. More importantly, aged monkeys in whom ABT-089 enhanced performance accuracy 10 minutes after drug injection, did not maintain the enhanced performance when evaluated 24 hours later. Thus, in-between treatment sessions in my study would be either comparable to control days that were scheduled at the beginning of the week or given the overall enhancing effects of both agonists, would be accompanied by a slight improvement in performance. Consequently, the results may have been even stronger if control days only happened at the beginning of the working week. Overall, while the scheduling of experimental and control conditions was not the most optimal in my thesis, it prevented problems common to fixed scheduling of treatments.

4.2.2. Nicotinergic Specificity of Behavioral Improvement

While the selected agonists in this study were mainly selective for a particular nicotinergic sub-receptor, they also interacted with other nicotinic or non-nicotinic receptors. Consequently, the

findings of this study should be interpreted with caution. PHA-543613 acts as an agonist on α7 nACh receptors but also acts as an antagonist on 5-HT3 serotonin receptors. Therefore, it could be possible that it was the antagonizing effects on 5-HT3 which led to increased performance accuracy and faster reversal learning. Previous studies with RG-3487 (a nicotinic agonist which similar to PHA-543613 is an agonist and antagonist for α7 and 5-HT3 receptors respectively) provided evidence that the cognitively enhancing effects of this agonist is most likely due to the drug acting on α7 nACh receptors (Boess et al., 2007; Wallace et al., 2011). These studies used MLA (an α7 antagonist) and observed that the performance improvement induced by RG-3487 was blocked by this α7 receptor antagonist. Therefore, while possible contribution of 5-HT3 receptors to the findings of my study about the effects of PHA-543613 cannot be completely ruled out, it is more likely that effects were mediated by activation of α7 receptors.

ABT-089 acts as an agonist on both α4β2 and α6β2 receptors, and as an antagonist on α3β4 receptors, which are expressed in the peripheral nervous system. In terms of interaction with α7 receptors, ABT-089 is shown to have a complex interaction with α7 receptors as it can both activate and desensitize them (Rueter et al., 2004), however, this interaction is reported to be insignificant (Lin et al., 1997; Marks, Wagemana, Gradya, Gopalakrishnanb, & Briggs, 2009; Rueter et al., 2004).

It could be possible that the effects found with ABT-089 in this thesis can be attributed to activation of α6β2 receptors. However, studies with other α4β2 agonists such as ABT-418 and ABT-594 which do not interact with α6β2 receptors, have also reported similar effects i.e. both agonists improved performance accuracy when distracting stimuli were included in the DMTS task (Buccafusco et al., 2007; Prendergast et al., 1998). Therefore, it is likely that the pro-cognitive effects found with ABT-089 in the current study are mediated by activating α4β2 sub-receptors.

4.2.3 Overly Trained Subject

Extensive exposure to discrimination between stimuli can facilitate reversal learning in animals. Often, subjects are tested on reversals shortly after reaching a certain performance criterion and therefore, over-training effects are not usually of concern in reversal learning tasks (Gilmour et al., 2013). In this thesis, data was collected from Monkey H which was over-trained on this task and used for 2 previous experiments in the lab prior to data collection for this project. The effects of over-training are evident in the observed high performance accuracy, in particular, in a subset of trials where the target stimulus dims after the distractor.

As a result of this over-training, it is possible that lack of significantly enhanced performance in the second-dim trial types could be due to the already ceiling performance in this subset of trial types. It is also possible that lack of effects on reversal learning was not observed with ABT-089 as the subject was already performing the blocks at a fast rate. Additionally, over-training can also explain the observed trends in the successive errors. When I started working with Monkey H, he would usually have more successive errors after a period of responding to the correct stimulus rather than at the beginning of the block when he was faced with the reversed reward contingencies. Such pattern indicates that he was likely predicting and expecting a change in the block and was already responding to the other stimulus which would be rewarded in the next block.

Longer periods of training can also reduce the frequency of premature responses in over-trained subjects (Hoyle et al., 2006). Therefore, lack of any observed effects on premature responses after color and motion features were presented can be due to higher proficiency of Monkey H in performing this task compared to his less experienced days.

4.2.4 A Single Case Study

The current project is a rigorous study of a single case of a young male adult monkey. This is in unlike other studies reviewed in this thesis which used multiple subjects. However, in some studies with multiple subjects, the between-subject variability in nicotinergic drug induced effects were not addressed clearly. For instance, Yang et al. (2013) presented individual PHA-543613 dose-dependent behavioral performance in DMTS task in two monkeys who had similar profiles. However, a total of 11 subjects including male, female, aged and young monkeys were included in the study. While the authors mention generally that performance was improved in both younger and older monkeys, they do not clearly state which specific aspect of performance was improved by other subjects. It is also not clear whether both genders benefited from the drug. A previous study with general nicotinic agonist by Buccafusco et al. (1999) suggested that drug-induced increase in performance accuracy is different across aged female and male monkeys. They also suggested that females may need a higher level of individualized dose selection and higher doses of nicotine to achieve the same behavioral effects as males. Between-subject variability in effective doses for inducing comparable behavioral improvement have been reported by a number of monkey studies who used selective nicotinergic agonists in subjects with comparable gender or/and ages (Decker et al., 1997; Gould et al., 2013). Since individualized best doses are selected from a series of options, there is a need for repeated testing of the assumed best dose in the same subject to ensure reliable behavioral enhancement with that particular dose (Buccafusco et al., 1995). However, dose repetitions are usually reported up to 2 or 3 times for each individual (see for example (Gould et al., 2013) and (Buccafusco et al., 1995)).

Each drug dose in this thesis has been repeated at least 6 times across different days of the week in a blinded experiment. Therefore, while current findings may be limited to one subject only, they provide reliable results in specific aspects of attention and learning.

4.3 Future Directions

Do differential behavioral effects also correspond to differential neural activities in brain areas important for cognitive flexibility and attention control when the monkey is administered with these two drugs? To address this question, another future step can be to conduct extracellular recording from multiple brain areas simultaneously as a subject is performing the task**.** As discussed in the introduction of this thesis, different brain areas are involved in reversal learning and attentional processes. Flexible goal-directed behavior is dependent on neuronal circuits in the prefrontal and anterior cingulate cortex (PFC/ACC) (Rothe, Quilodran, Sallet, & Procyk, 2011). Additionally, neural activities in cortico-striatal pathway have been shown to play important roles in memory and goal-directed learning (Asaad & Eskandar, 2011). The caudate nucleus, located in dorsal striatum, receives input from both ACC and PFC and disruptions in the connections between these areas are involved in neuropsychiatric disorders such as obsessive compulsive disorder (Tekin & Cummings, 2002).

Previous studies suggest that neuronal circuits in PFC/ACC implement rapid learning of feature relevance through dynamic neuromodulation of local circuit activities in the PFC (Arnsten AFT, Wang MJ, 2012; Noudoost & Moore, 2011). Neural recording can address how dynamic cholinergic neuromodulation facilitates rapid learning and flexible attentional feature selection and how this system affects PFC/ACC circuitry to support the mechanisms underlying such higher attentional processes.

Electrochemical in conjunction with electrophysiological recordings in behaving monkeys can enable us to measure the concentration of acetylcholine (or other neurotransmitters) in different brain areas during the task to understand where in the brain the functional effects on learning and distractibility reduction are brought about. It would also inform us of how cholinergic modulation induced by nicotinic sub-receptors affect the neural circuitry underlying cognitive flexibility and attentional processes (Vizi & Lendvai, 1999). Electrochemical methods are available in rodents and have been used to measure acetylcholine release in the PFC of behaving rodents (Parikh et al., 2007). However, advances towards building these methods are still in progress (Disney et al., 2015).

4.4 Conclusion

Overall, PHA-543613 and ABT-089 had differential effects on performance accuracy, learning rate and distraction filtering respectively. By implementing a complex feature-based reversal learning task which allowed for assessment of cognitive flexibility and attentional processes, these findings suggest that α7 and α4β2 nicotinergic sub-receptors play distinct roles in mediating higher order cognitive functions. The α7 agonist PHA-543613 broadly improved learning performance while the α4β2 agonist ABT-089 reduced distractibility and improved performance accuracy in trials with the highest attentional demand to filter distraction. The results of this thesis also suggested that the agonist-induced pro-cognitive effects were time and dose dependent. These findings add new insight about the distinct contributions of the two most prominent nicotinic sub-receptors in the mammalian brain to learning and attention in non-human primates**.**

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Appendix A Training of Monkey K

Monkey K has been under training with the objective of learning the feature based reversal learning task that Monkey H performed for this project. As of now, Monkey K can attend to a stimulus under all three different timings; albeit, in comparison to the final task version, there are two differences in this regard: 1) The color does not dim; it brightens as a signal of response timing. 2) The brightening is tied to motion onset; in other words, the go-cue signal is removed. Additionally, there are only eight trial types that Monkey K performs in the current task version (**Fig. 26**). These changes helped Monkey K to learn the concept of responding to a rewarded stimulus under different timings. Eventually, the goal is to add a go-cue signal such as a later step in training. Monkey K is also able to perform reversals with the guidance of a colored cue presented at the fixation point. Basically, the cue shows him which color is rewarded in each block. With this task version, he is capable of reaching a criterion of 80% within 10 trials in a block with a minimum of 30 and 50 trials. The goal is to phase out the cue so that Monkey K can do reversals with trial and error i.e. through receiving feedback from his choices. The stimuli and setup are comparable to ones that Monkey H was exposed to in terms of display background, motion speed, radius and distance from cue. However, the colors yellow and deep sky blue were chosen for Monkey K due to the animal's preferences. Moreover, as part of training the animal for the cued version of the task, a 20-pixel size rhombus (diamond) shaped cue was introduced for fixation point. To maintain consistency between the two subjects, the diamond cue will be changed to a 15-pixel size circle later in throughout the training.

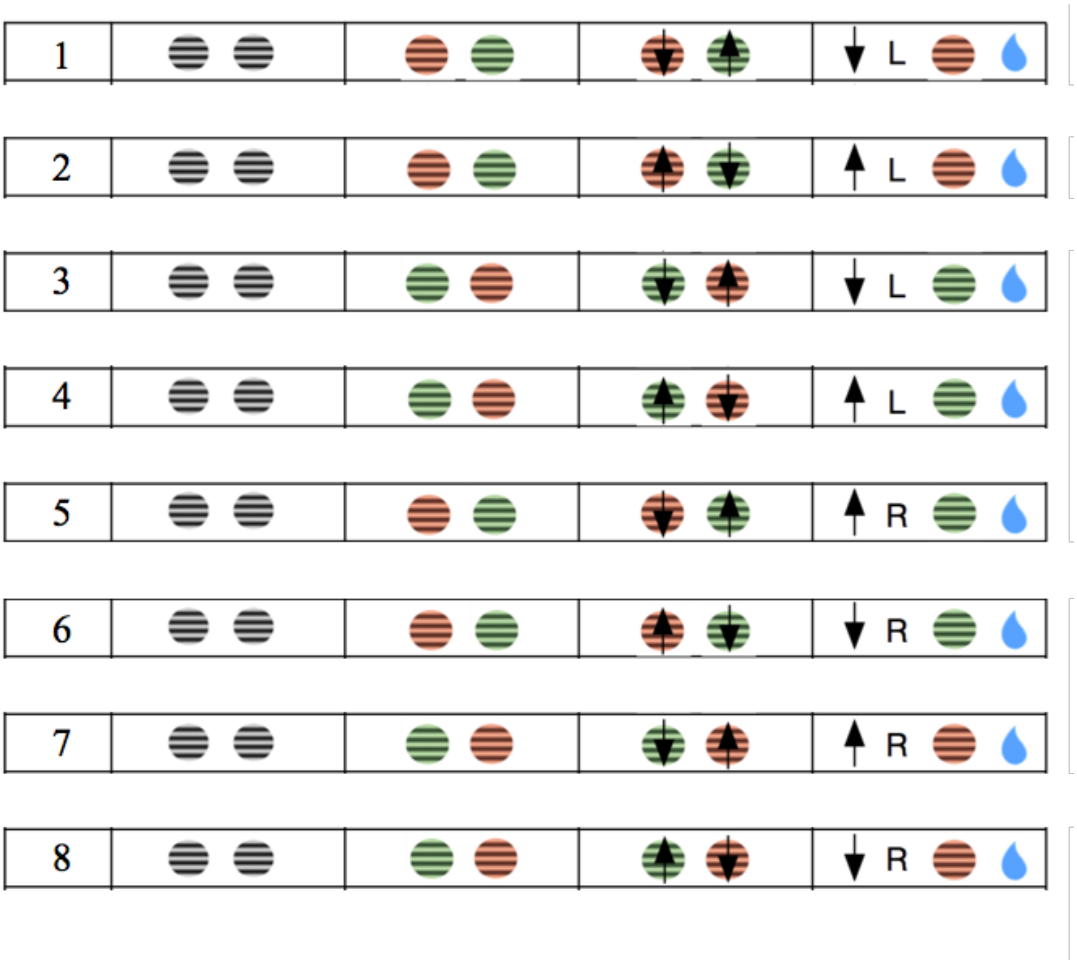

Figure 26 Eight possible conditions presented at the current task for Monkey K. Trials that started with motion onset first are non-existent in this version. It should be noted that the stimuli were colored as yellow and deep sky blue for Monkey K.

Appendix B.1 Expectation Maximization Algorithm

The model is a dynamic approach which estimates subject's learning as a probability of achieving a correct response as a function of each trial in a task with binary responses. Binary responses of the subject (correct or incorrect) are fed as input into the algorithm which in return provides a learning curve for each block. This state-space model paradigm consists of smoothing algorithm, which takes the perspective of an ideal observer, and filtering expectation maximization algorithms, which takes the perspective of the subject. The estimation of both gives a probability density for the correct response probability at a given trial. The mode values of this probability density were used to generate the learning curve in this thesis. The ideal observer also estimates a learning trial as the first trial on which the lower bound of the 95% confidence interval for the probability of a rewarded choice or correct answer is higher than obtaining the correct answer by chance (i.e. 50%) and remains above this chance level throughout the block (**Fig. 27**).

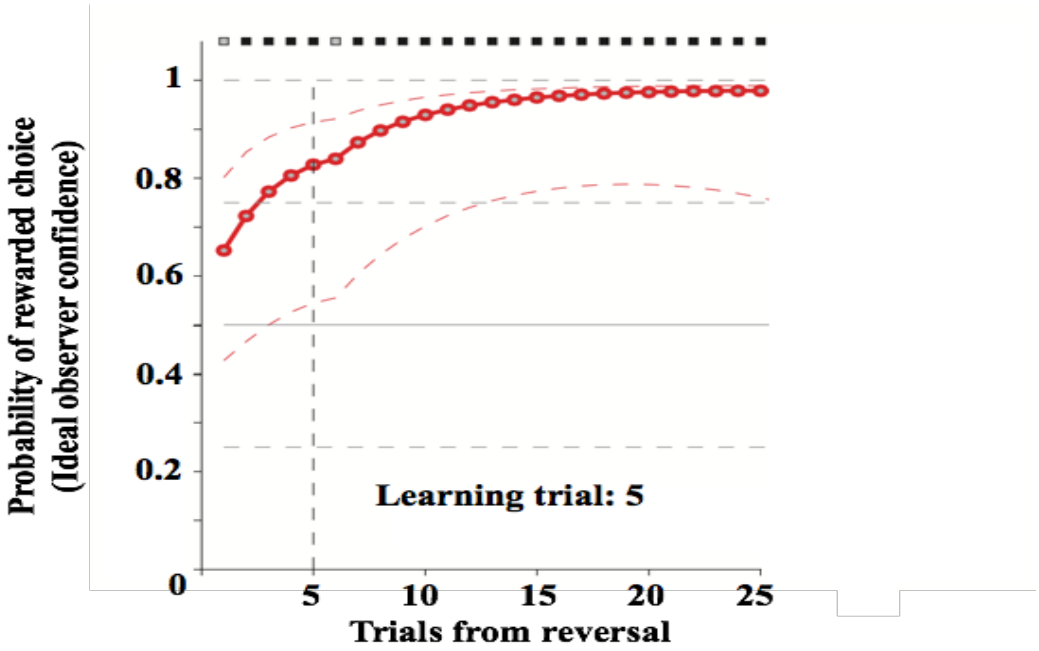

Figure 27 Probability of a rewarded choice per trial from reversal estimated by EM algorithm. The grey squares on top of the performance curve present non-choice errors while the black squares present correct choices. The ideal observer has estimated the learning occurring reliably above chance level at trial 5.

Appendix B.2 Randomization Test Implemented for Evaluation of Hyperbolic-Ration Function Fitting

A randomization procedure was carried out to test the significance of differences in the parameters of interest estimated by the hyperbolic-ratio function between drug and control conditions. The randomization procedure followed the steps described by Maris and Oostenveld (2007).

The difference between the estimated parameters of interest under each treatment were computed. Then, one dataset matrix was built out of the two datasets in which drug & control condition labels were randomized. A dataset as the same size of each condition was extracted. The function was fit to the averaged performance in all trials across all blocks of the newly constructed matrixes and afterward the parameters of interest were estimated in a similar way to datasets with the actual condition labels. This process was repeated 1500 times resulting in 1500 number of parameter values for each of the two matrix types whose experimental labels were shuffled. The distribution of root of mean square error (RSME) of all estimated parameters was constructed and any estimation that had RSME above 90% percentile of this distribution was excluded. The difference between the estimated parameter values were extracted as test statistics and the 97.5% and 2.5% percentiles of the null test statistics distribution was computed to conduct a two-tailed test at 0.05 alpha significance. These two values served as thresholds based on which the significance of the observed difference between the parameters of interest was determined. The proportion of values within the null distribution larger than the observed test statistics was calculated as the p value of this randomization procedure.

Appendix B.3 Two Sample Proportions Z-Test

In this thesis, the null hypothesis in a two sample proportions z-test stated that the difference between the proportions was zero. The z score was computed based on the equation below (Zar, 2010) where p1 is the first proportion value, p2 is the other proportion value. If null hypothesis was true, then p1-p2=0. P is the proportion of learned blocks calculated by pooling data from both control and drug conditions as shown by the following equation:

$$Z = \frac{(\hat{p}_1 - \hat{p}_2) - 0}{\sqrt{\hat{p}(1-\hat{p})\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

The null hypothesis was rejected if the computed z score at 0.05 alpha level in a two-tailed test was above 1.96. The *p* value was computed using "Table B.2: Proportions of the normal curve (one-tail)" from Zar (2010) by multiplying the value found in the table by 2 (since the test was two tailed).