Interactions between prefrontal cholinergic and glutamatergic signaling support attentional function

by

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In loving memory of Doris J. Cutler

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Abstract

Attentional processes and capacities are central to cognitive performance. Cortically projecting cholinergic neurons of the basal forebrain are essential components of the neural systems underlying attentional function; however exactly how the cholinergic system supports attentional performance remains unknown. Our current model suggests that transient (scale of seconds) increases in acetylcholine (ACh) release in the prefrontal cortex foster the detection of predictive cues, and that the generation of such transients is dictated by glutamate released from mediodorsal thalamic afferents and the stimulation of ionotropic glutamate receptors. The studies that informed this model were based on data from anesthetized preparations and animals performing relatively simple instrumental tasks. Two of the primary aims of the work going in to my dissertation were to utilize a novel technique with a high degree of temporal precision to record glutamatergic and cholinergic activity in animals performing a sustained attention task (SAT) to characterize how these two major neurotransmitter systems interact to support attentional performance. The major results from these studies are that in contrast to signal detection per se, transient increases in ACh release mediate performance on signal trials requiring a shift from cue-independent to cue-dependent processing. The pattern of glutamate release in task performing animals suggests that in addition to recruiting

cholinergic mechanisms, glutamate plays an additional role in signaling choice. Finally our work revealing the interactions between cholinergic and glutamatergic mechanisms additionally predict and explain the observation that α4β2 nAChR agonists more robustly enhance attentional performance in the distractor version of the SAT than the non-selective nAChR agonist nicotine. The results from these studies suggest the limited beneficial effects of nicotine are due to its stimulation of long lasting release events that are unlikely to support specific cognitive operations. Collectively, the work included here has 1) redefined the role of cholinergic transient in attention 2) begun to explore the complex interplay between glutamatergic and cholinergic signaling in cognitive performance 3) demonstrated that by building upon our knowledge of the functions of and interactions between these two systems we can predict the pro-attentional efficacy of putative cognition enhancers

Chapter I

Introduction

Consider the case of a radar operator or air traffic controller whose job is to monitor a screen for the appearance of a small dot which signifies a plane or a submarine. So long as there is no signal, their thinking would be largely internally driven, perhaps focused upon remaining alert, thinking about how long it has been since the last time they saw a signal, or even when they might expect the next one to appear on screen. However, when this important signal does appear, their thinking would shift to recalling what the signal means in regards to the behaviors they now need to engage in order to successfully complete their job.

Most successful goal-directed behavior requires such shifts between cue-independent and cue-oriented attention (Burgess, Dumontheil, & Gilbert, 2007). There is some evidence from human imaging studies. For example, switching between internally and externally-focused processing increases activation in executive control areas, most notably the right rostrolateral prefrontal cortex (Brodmann's Area, BA, 10) (Burgess, et al., 2007; Henseler, Kruger, Dechent, & Gruber; McCaig, Dixon, Keramatian, Liu, & Christoff). Patients with lesions of this region perform well on executive tasks that rely exclusively on external cues, although they have difficulty in situations that require shifts between self-directed and cue-oriented behavior (Burgess, Veitch, de Lacy Costello, & Shallice, 2000;

Volle, Gonen-Yaacovi, Costello Ade, Gilbert, & Burgess). Furthermore, this region is dysfunctional in disorders associated with impairments in cue processing and organized behavior (Cools, Rogers, Barker, & Robbins, 2010; Laurens, Kiehl, Ngan, & Liddle, 2005). However on the whole, the neuronal mechanisms mediating such shifts are not well understood.

My work has been predominantly focused upon identifying neural mechanisms in the prefrontal cortex (PFC) that support attentional function. Over the years (and particularly the last month), we have redefined our thinking about how exactly these mechanisms support cognitive performance, culminating with the hypothesis that transient increases in prefrontal acetylcholine (ACh) release are critical for the shift from cue-independent to cue-dependent or cue-oriented processing. We have also begun to explore how prefrontal glutamatergic and cholinergic systems interact synergistically to support this cognitive operation.

The discussion to follow will begin with a background on the cholinergic system; its general organization and evidence from previous studies that support its role as a central component of the brain's attentional networks. Next I will discuss of the existing evidence regarding interactions between cholinergic and glutamatergic mechanisms in the prefrontal cortex. Finally, I will discuss the behavioral paradigm employed by all the studies included in this dissertation, the sustained attention task.

Cholinergic systems in cognition, focus on attention and prefrontal cortex

Ascending cholinergic projections originate from nuclei located within the brainstem and basal forebrain (Mesulam, Mufson, Wainer, & Levey, 1983). Brainstem cholinergic neurons are located in the pedunculopontine nucleus and laterodorsal tegmental nucleus, and go on to innervate several thalamic nuclei and as well as the basal forebrain (Rotter & Jacobwitz, 1981; Rye, Saper, Lee, & Wainer, 1987; Woolf & Butcher, 1986), and are hypothesized to play an important role in initiating REM sleep (Maloney et al., 1999). The term "basal forebrain" refers to cholinergic neurons that are present in the horizontal and vertical limbs of the diagonal band, the magnocellular neurons of the nucleus basalis (nBM), substantia innominata, the magnocellular preoptic nucleus, and the nucleus ansa lenticularis (Armstrong, Saper, Levey, Wainer, & Terry, 1983; Mesulam, et al., 1983; Woolf, Eckenstein, & Butcher, 1983). These neurons collectively provide the major source of the cholinergic innervation of the hippocampus, amygdala, and neocortex. Given the functional significance of these terminal fields, the cholinergic contribution to cognitive processes is overwhelmingly discussed in terms of these cortically projecting cholinergic neurons.

In primates, the primary source of cholinergic innervation of the PFC arises from the nBM. The corresponding source of neocortical cholinergic input in the rodent includes neurons within the nBM and substantia innominata (Armstrong, et al., 1983; Luiten, Gaykema, Traber, & Spencer, 1987; Mesulam, et al., 1983). The axons of cortically projecting basal forebrain cholinergic

neurons innervate all layers of the PFC, with particularly dense innervation of layers I, III, and V (Mrzljak & Goldman-Rakic, 1992, 1993; Mrzljak, Levey, & Goldman-Rakic, 1993; Mrzljak, Pappy, Leranth, & Goldman-Rakic, 1995). This pattern of innervation has been suggested to be indicative of a lamina-specific cholinergic modulation of cognitive function (Lysakowski, Wainer, Bruce, & Hersh, 1989).

ACh exerts its influence through actions on two major types of receptors in the central nervous system; ionotropic nicotinic receptors and metabotropic muscarinic receptors. Nicotinic acetylchoine receptors (nAChRs) are non-selective cation channels with a pentameric structure composed of α (2-10) and β (2-4) subunits. According to their binding affinity, nAChRs can be divided into two major classes: receptors with high affinity (nmol) for nicotine and receptors with high affinity for α-bungarotoxin. In the mammalian central nervous system, the most prevalent conformations of nAChRs are the α4β2 nAChR (high binding affinity for nicotine), and the homomeric α7 nAChR (high binding affinity for α-bungarotoxin) (Berg & Conroy, 2002; Dajas-Bailador & Wonnacott, 2004; Dickinson, Kew, & Wonnacott, 2008; Galzi & Changeux, 1995; Galzi, Edelstein, & Changeux, 1996; Wonnacott, 1997). The other class of receptor is the muscarinic, which is G-protein coupled, and 5 forms have been identified (M1-M5) with M1 and M2 being the most commonly discussed in terms of their function in the brain. Muscarinic receptors vary in which G-protein they are bound, and thus their effects on membrane potential. For example, M1 receptors are Gq coupled and typically described as being located post-synaptically with binding having a

net stimulatory effect. M2 receptors function primarily as presynaptic autoreceptors, are Gi coupled, and upon stimulation have a net inhibitory effect (Mrzljak, et al., 1993).

Early attempts at parsing out the functional relevance of basal forebrain cholinergic projections to the cortex relied primarily upon determining the effects of systemic administration of non-selective cholinomimetic compounds. modeling the effects of endogenously released ACh via the exogenous application of the neurotransmitter in discrete brain regions. For example, in the auditory cortex that the administration of ACh, when paired with a repeated, pure-tone stimulus, enhanced stimulus-specific neuron activity, or “signal”, while having no effect on spontaneous firing, or “noise” (Metherate & Weinberger, 1989). Furthermore, ACh can enhance responses to previously “weak” signals by decreasing the threshold needed to elicit an action potential (Metherate, Ashe, & Weinberger, 1990; Metherate & Weinberger, 1989, 1990). This phenomena is believed to be mediated through muscarinic, specifically M1, receptors, and reflects the ability of the cholinergic system to heighten the signal-to-noise ratio for relevant sensory stimuli (Hasselmo & Bower, 1992). As local infusions of ACh are unlikely to replicate concentrations of the transmitter resulting from endogenous release, the aforementioned studies failed to provide a physiological measure of cholinergic modulation. Therefore, the demonstration that basal forebrain stimulation can similarly enhance and suppress neural response in the auditory cortex elicited by thalamocortical activation (Metherate and Ashe, 1991) further emphasizes the role of ACh in modulating the signal-to-noise ratio. More recently, this preliminary

research has been reinterpreted as indicative of the ability of ACh to both enhance ascending input to the cortex from sensory systems and suppress spontaneous spiking (Hasselmo and McGaughy, 2004). Thus, the combined effects of ACh in cortical regions can shape the integration of stimuli into associative networks involved in attention (Hasselmo and McGaughy, 2004). Via stimulation of nAChRs and muscarinic receptors, ACh generally has been suggested to gate information flow between the cortical layers, allowing for greater attentional selectivity for salient stimuli (Munk, Roelfsema, Konig, Engel, & Singer, 1996; Xiang, Huguenard, & Prince, 1998).

Primary evidence in support of the importance of forebrain cholinergic systems in attention was derived from studies employing the immunotoxin 192-IgG saporin, which selectively targets the p75 neurotrophic receptor-expressing cholinergic neurons of the basal forebrain (Book, Wiley, & Schweitzer, 1992; Wiley, Oeltmann, & Lappi, 1991). Selective removal of cortical cholinergic inputs profoundly and permanently impairs performance in tasks designed to tax attentional processes and capacities (McGaughy, Dalley, Morrison, Everitt, & Robbins, 2002; McGaughy, Kaiser, & Sarter, 1996; Sarter, Hasselmo, Bruno, & Givens, 2005), specifically the ability to report the presentation of a predictive stimulus, while sparing the ability to indicate its absence (McGaughy, et al., 1996).

Further evidence for cholinergic modulation of attentional capacities was provided by studies utilizing *in vivo* microdialysis. These investigations have demonstrated that engagement in and performance of an attention task evokes

increases in fronto-cortical ACh release, while performance in tasks designed to control for motor behavior, reward presentation and reward retrieval do not (Arnold, Burk, Hodgson, Sarter, & Bruno, 2002). Further, ACh release is augmented in the face of increased demands on attentional control, such as those imposed by disruption of prefrontal cholinergic neurotransmission, attentional fatigue, or a distracting stimulus (Himmelheber, Sarter, & Bruno, 2001; Kozak, Bruno, & Sarter, 2006; Parikh, Kozak, Martinez, & Sarter, 2007; Sarter, Gehring, & Kozak, 2006; Sarter & Paolone, 2011; St Peters, Demeter, Lustig, Bruno, & Sarter, 2011)

Also important to note is that the function cortical cholinergic inputs in attentional performance is right lateralized; unilateral depletion of right hemispheric cholinergic inputs re-creates the profound impairments in attention observed in animals with bilateral cholinergic depletion (Martinez & Sarter, 2004) a finding concordant with the focus on right-hemispheric networks supporting attentional function in human imaging studies (Arrington, Carr, Mayer, & Rao, 2000; Corbetta, Kincade, Ollinger, McAvoy, & Shulman, 2000; Demeter, Hernandez-Garcia, Sarter, & Lustig, 2011). Additionally, studies have shown that in attention task performing, but not non-performing, animals, the capacity of the high affinity choline transporter, the rate limiting step of readily releasable ACh reserves, is augmented in the right prefrontal cortex (PFC) as a result of task performance, but not left PFC (Apparsundaram, Martinez, Parikh, Kozak, & Sarter, 2005).

The long standing notion of the cholinergic system as a modulator also carried with it ideas about its primary mode of neurotransmission; a slow, tonic state of release, as measured by microdialysis, that gradually and globally fluctuates over the course of task performance to modulate the state of cortical networks in response to demands on attentional/cognitive control (Paolone, 2010; Sarter, et al., 2006; Sarter & Paolone, 2011). With the optimization of enzyme selective biosensors capable of measuring ACh release on a sub-second time scale in task-performing animals, it has been revealed that in addition to this tonic mode, there is a fast, phasic mode of cholinergic neurotransmission that operates on a scale of seconds. These "transient" increases in cholinergic activity are evoked by attention capturing cues (Parikh, et al., 2007). Further, these cue-evoked release events are observed in the medial PFC (mPFC) and not in cortical control regions (e.g. primary motor cortex) (Parikh, et al., 2007). Together, these findings suggested a significant departure from the traditional conceptualization of cholinergic function; temporally and regionally specific signaling coincident with discrete cognitive and behavioral functions.

Interplay of cholinergic and glutamatergic mechanisms in the prefrontal cortex

Neuropharmacological studies provided the first level of insight into the local mPFC circuitry that underlies the generation of these transient cholinergic release events. For example, infusions of nicotinic receptor (nAChR) agonists into the mFPC can evoke the release of both glutamate and ACh (Gioanni, et al.,

1999; Lambe, Picciotto, & Aghajanian, 2003; Parikh, Ji, Decker, & Sarter, 2010; Parikh, Man, Decker, & Sarter, 2008). Further work in anesthetized animals has revealed that nAChR agonist-evoked increases in ACh release are attenuated by blockade of glutamatergic AMPA and NMDA receptors. Removal of mediodorsal thalamic input to the mPFC abolishes nAChR stimulated ACh release and suggests that this thalamic nuclei is a primary source of glutamatergic inputs to the mPFC. Cholinergic mechanisms also modulate glutamatergic afferents. For example, stimulation of α4β2 nAChRs evokes glutamate release. Furthermore, nAChR agonist evoked glutamate release is robustly attenuated in B2 -/- mutant mice (Parikh, et al., 2010; Parikh, et al., 2008). The combined evidence allows for the development of a model of prefrontal circuitry. Our working model suggests that cholinergic transients are the result of glutamate release from the mediodorsal thalamus stimulating ionotropic glutamate receptors located on the terminals of cholinergic inputs, resulting in the production of the cholinergic transient. In turn, glutamate release is also modulated by ACh through actions at α4β2 nAChR located on the axons of the thalamic inputs.

Measuring attention in rats: the Sustained Attention Task (SAT)

An illustration of the task that will be employed in all of the experiments included in my dissertation is provided in Figure 1.1. The sustained attention task (SAT) is comprised of two trial types, signal and non-signal. The presentation of signal and non-signal trials is randomized. Trials are separated

by a variable inter-trial interval (ITI) of 9±3 s to prevent animals from timing events. On a signal trial, a centrally located light on the intelligence panel is turned on for a variable amount of time (500,50,or 25 ms). Two seconds after the cue light is extinguished, the two response levers, located on either side of a reward port located beneath each the cue light, are extended into the operant chamber. A press on one of these levers, for example, the lever located to the left of the reward port, results in the presentation of a water reward, and is scored as a Hit. A press on the right lever is not rewarded, and scored as a Miss. In either case, after the response, the levers are retracted. If no response is made within 4 s, the levers are retracted and the trial is scored as an omission. On a non-signal trial, the levers are extended into the chamber after the ITI. A press on the right lever is rewarded and scored as a correct rejection (CR). A press on the left lever is not rewarded, and scored as a false alarm. Again, after a press on either lever, they are retracted, which triggers the ITI to begin. Sessions consist of ~200 trials, taking a total of 40 minutes. In addition, a houselight located at the back of the operant chamber remains illuminated throughout the task, preventing the animal from being able to monitor for changes in chamber luminescence and thereby necessitating maintained focus on the intelligence panel. This task has been validated as a measure of sustained attention in rats (McGaughy & Sarter, 1995), and has been adapted for use in human subjects (Demeter, Sarter, & Lustig, 2008). Further, the distractor version of the task (dSAT, houselight flashing on and off at a rate of 0.5 Hz) was recently selected by the Cognitive Neuroscience Treatment Research to Improve Cognition in

Schizophrenia (CNTRICS) group for research on the control of attention (Nuechterlein, Luck, Lustig, & Sarter, 2009).

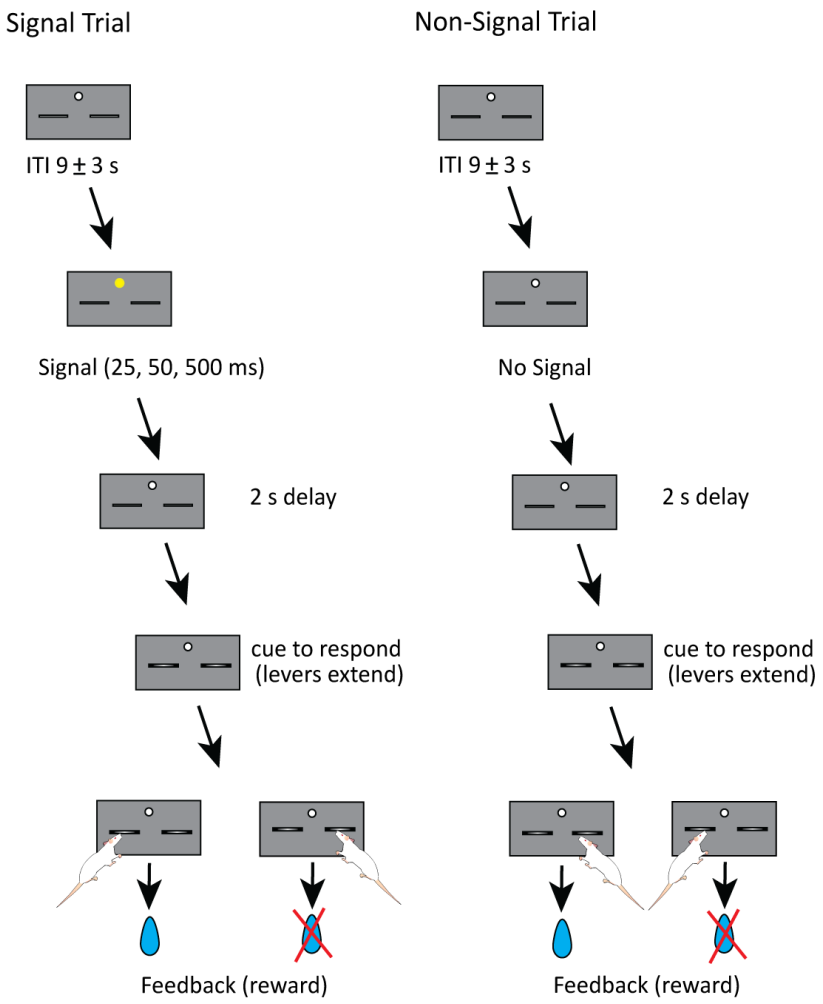

Figure 1.1

Main components of the sustained attention task (SAT). The task includes two types of trials, signal and non-signal. On a signal trial, after a variable inter-trial interval (9±3 s; ITI) a centrally located cue light on the intelligence panel is illuminated (500, 50, or 25 ms). After 2 s, 2 response levers are extended into the chamber. If the animal presses the correct lever (left in the above example) it is rewarded and the trial is scored as a hit (incorrect responses are scored as misses). On a non-signal trial, after the ITI the 2 levers are extended into the chamber and the animal must press the opposite lever (right), and then is presented with reward. Correct responses are scored as correct rejections (CR), incorrect as false alarms (FA)

Present Studies

The work that has gone in to my dissertation has been guided by two overlapping aims. The first is largely oriented towards basic science, and identifying the constituent neurobiological mechanism(s) that underlie fundamental aspects of cognition. Previous studies examining cholinergic-glutamatergic interactions in the mPFC were done in anesthetized animal preparations and in animals performing relatively simple tasks. By employing a novel method for measuring neurotransmission in near real-time, I have monitored fluctuations in both acetylcholine (ACh) and glutamate release in the medial prefrontal cortex (mPFC) of animals performing the SAT. By relating the changes in activity of these major chemical messaging systems to one another, I have added to our understanding of the neural substrates that support attentional performance and underlie shifts between cue-independent and cue-dependent processing. The second is to build upon this knowledge to explain and predict the pro-attentional efficacy of compounds with putative cognition enhancing abilities. Of the many compounds developed for the treatment of the cognitive impairments associated neuropsychiatric and neurodegenerative disorders, few have been met with great success in the clinical settings (Sarter, 1991, 2006; Sarter, Hagan, & Dudchenko, 1992a, 1992b; Sarter, Parikh, & Howe, 2009). Here, we have demonstrated that selective targeting of a subpopulation of

nicotinic acetylcholine receptors (nAChR) enhances attentional performance by facilitating the shift between cue-independent and cue-dependent processing.

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Chapter II

TRANSIENT INCREASES IN PREFRONTAL CHOLINERGIC NEUROTRANSMISSION MEDIATE THE SHIFT FROM CUE INDEPENDENT TO CUE DEPENDENT ATTENTION.

Summary

Cortical cholinergic inputs are necessary for performance in a variety of cognitive tasks, particularly those tapping attentional capacities (McGaughy, et al., 2002; McGaughy, Decker, & Sarter, 1999; McGaughy, et al., 1996). However precisely how the cholinergic system contributes to such performance has remained elusive. A recently developed method for *in vivo* measurement of acetylcholine (ACh) at a sub-second temporal resolution has led to new insights in our understanding of its function (Parikh, et al., 2007). The objective of this series of experiments was to test the hypothesis that 1) in animals performing a sustained attention task (SAT) involving signal and non-signal events, transient increases in medial prefrontal (mPFC) cholinergic activity mediate performance on trials requiring signal detection (Hits), and 2) removal of cholinergic inputs to the prefrontal cortex impairs signal detection performance. In agreement with our first hypothesis, we discovered that seconds-long increases in ACh are selective to signal trials resulting in Hits. Upon further analysis we discovered that not all detected cues evoke ACh release, but that the presence of such a release event depends upon the previous trial type. Specifically, ACh increases

were only observed on Hits if preceded by a non-signal response on the previous trial. In support of our second hypothesis, detection performance was only impaired in this subset of Hit trials in animals with prefrontal cholinergic lesions. These results suggest that rather than mediating detection *per se*, signal-evoked increases in ACh release contribute to the ability of prefrontal networks to shift from cue-independent attentional processing, to cue-dependent processing. It is further hypothesized that a complementary or even primary function of the event is to act as a reporter or learning signal, refining or strengthening the associative networks that afford such shifts.

Introduction

Cholinergic projections from the nucleus basalis of Meynert (medial wall of globus pallidus), substantia innominata (ventral to the globus pallidus) and the horizontal limb of the diagonal band (collectively termed basal forebrain, BF) to the neocortex have long been implicated as central to normal cognitive performance. Further, dysfunction within this ascending system has been linked to the cognitive impairments seen in schizophrenia, ADHD, and age-related cognitive decline (Bartus, Dean, Beer, & Lippa, 1982; Deutsch, 1971; Everitt & Robbins, 1997; Hasselmo & Sarter, 2010; McGaughy, et al., 2002; McGaughy, et al., 1999; McGaughy, et al., 1996; Sarter, et al., 2005; Sarter, Parikh, et al., 2009). A substantial literature places a special emphasis on this system as a mediator of attentional performance (Hasselmo & Sarter, 2010; McGaughy, et al., 2002; McGaughy, et al., 1999; McGaughy, et al., 1996; Sarter, et al., 2005; Sarter, Parikh, et al., 2009; Turchi & Sarter, 1997). In tasks that require reporting either the presence or absence of a cue, cortex-wide removal of cholinergic inputs selectively impairs the ability to utilize predictive cues to guide responding, while performance on non-cued trials remains intact (McGaughy, et al., 1996). Such evidence suggests that the role of the cortical cholinergic input system in attention is the optimization of signal detection and integration of stimuli into associative networks (Hasselmo & McGaughy, 2004; McGaughy, et al., 1999; McGaughy, et al., 1996; Parikh, et al., 2007; Sarter, et al., 2005). However, the

exact mechanisms through which cortical cholinergic inputs contribute to such performance remain poorly understood.

Attempts at clearly delineating the role of cortical cholinergic inputs have long been impeded by the lack of a technique capable of selectively measuring ACh release in real-time. Taking advantage of recent advances in enzyme-based biosensor technology, our lab has been involved in the development of a method for measuring ACh release at a high temporal resolution (<1s) in task-performing animals. This technique combines fixed-potential amperometry with *in vivo* electrochemistry to measure the changes in extracellular choline derived from the hydrolysis of ACh on a sub-second time scale. The ability of this method to measure choline generated by the hydrolysis of newly released ACh has been demonstrated previously (Parikh, et al., 2004). For example, removal of cholinergic inputs to the recording region or administration of neostigmine, an acetylcholinesterase (AChE) inhibitor, blocks KCl-evoked choline signals *in vivo*. Similarly, choline signals generated by pre-synaptic blockade of muscarinic receptors are blocked by tetrodotoxin (Parikh, et al., 2004). In these experiments, animals were anesthetized during the recording phase.

Initial attempts at employing this electrochemical method in task performing animals (Parikh, et al., 2007) utilized a relatively simple cued-appetitive response task that could be manually controlled and as such helped avoid many of the sources of electrostatic noise present in an typical operant chamber (motorized retractable levers, solenoids, etc.). In the task, a randomly occurring visual cue (every 90 ± 30 s) predicted the subsequent availability of

reward. Detected cues, as defined by an interruption of interoceptively guided behavior (such as grooming) and the initiation of reward seeking behavior (approach to the reward port location) evoked transient (seconds-long) increases in medial prefrontal (mPFC) cholinergic activity. On trials were cues did not evoke this behavioral response no change in cholinergic activity was observed even though animals were prompted to approach and retrieve reward with the sound of it being placed in the receptacle. These cue-evoked release events were selective to the mPFC, and were not observed in cortical control regions (e.g. primary motor cortex). Further, cue detection behavior in this task is dependent upon the generation of an ACh release event, as both selective lesions of prefrontal cholinergic inputs and pharmacological attenuation of transients impairs detection performance (Gietzen, 2010; Parikh, et al., 2007).

Taken together, the results of these experiments provided the first evidence that temporally and regionally specific changes in cholinergic activity are critical for a specific cognitive process (i.e., cue detection). It is important to note that in this context, and for the remainder of the discussion, cue detection refers to *"…the entry of information concerning the presence of a signal into a system that allows the subject to report the existence of the signal by an arbitrary response indicated by the experimenter"* (Posner, Snyder, & Davidson, 1980), and is therefore a distinctly cognitive as opposed to a sensory operation.

In the last 4 years, we have been able to further optimize our electrochemical recording techniques, and can now successfully record from animals performing fully automated operant tasks where fluctuations in current

evoked by task relevant stimuli (such as a detected cue) are rather small (2-5 pA). The cued appetitive response task is notably simple, and did not involve defined demands on attention which limited the interpretation of the results. The following experiments use a more stringent behavioral paradigm that requires higher cognitive demand to address this issue. Experiment 1 measured changes in mPFC cholinergic activity on a sub-second time scale in animals performing the sustained attention task (Figure 1; SAT). The task involves signal detection (in one out of four trial types) and, in contrast to the cued appetitive response task, was specifically designed to incorporate parameters that tax attentional capacities (McGaughy & Sarter, 1995; Parasuraman, 1987). Specifically, this task employs a relatively short ITI and randomly occurring signal and non-signal trials (each of which requires a response on discreet response manipulanda). These conditions necessitate a level of constant cognitive engagement. Incorrect responses to signal and non-signal events (misses and false alarms, respectively) require a lever press similar to correct responses (hits/correct rejections). Therefore, comparisons of changes in cholinergic activity between trial types are not confounded by different response topographies or levels of motivation (the failure to response within 4 s after the insertion of levers is counted as an omission; omissions are relatively rare and occur in less than 10% of trials). Finally, correct responses on both signal and non-signal trials are rewarded, providing an additional control for potential contributions of reward expectation and retrieval to increases in cholinergic activity. It should be noted that the use of the SAT in the present experiments is not to imply that cortical cholinergic activity

does not represent a critical component to other types of attention (divided, selective) as has been described previously (Turchi & Sarter, 1997). However, the specific hypotheses being tested in the proposed experiment address the involvement of cholinergic inputs in signal detection. As cue detection is integral for above chance performance in the SAT, it is an ideal paradigm for testing our specific hypothesis. To address the issue of the necessity of specifically prefrontal, as opposed to all cortical, cholinergic inputs for performance in this task an additional, preliminary, experiment assessed the affects of selective, bilateral prefrontal cholinergic deafferentation on detection performance. As both mPFC and orbitofrontal cortex have been implicated in supporting performance in this task (Lustig, 2011; Parikh, et al., 2007; Sarter, et al., 2005; Sarter, Parikh, et al., 2009; St Peters, et al., 2011), lesions were targeted to cover both regions of the frontal pole.

Materials and Methods

Subjects

Subjects were male Wistar rats approximately 60 days old (200-300g) at the onset of training. Animals were kept on a 14:10 light/dark cycle in a temperature and humidity-controlled vivarium. Water was only available as a reward during testing and for 30 min upon the completion of the training/testing session. Food was available *ad libitum*. All training and testing took place during the light cycle between 0800-1700 hours. Animals were maintained in accordance with the NIH guide for the Care and Use of Animals and experiments were conducted in

accordance with protocols approved by the University of Michigan Committee on Use and Care of Animals (UCUCA).

Apparatus

Rats were trained in one of twelve operant chambers (Med Associates, Georgia, Vt., USA) enclosed within a sound attenuating box and equipped with a fan to conceal any residual background noise. Each chamber was equipped with a water port located between two retractable levers. A central panel light was located at the front of each chamber above the water port. Additional panel lights were located above each lever. A house light was positioned at the back of the chamber. All training and testing programs were executed with a PC running Med-PC software (V. IV).

Electrochemical recordings were conducted in a 12” X 10” X 17” wooden operant chamber completely shielded by copper-wire mesh and equipped with two retractable levers constructed of fiberboard normally employed for electrical insulation. The receptacle used for water delivery was constructed from copper. The entire assembly was connected to an electrical ground. The relative location of the central panel light is the same as in the training chambers. The background illumination provided by the houselight, and the change in luminance following the presentation of the signal were kept constant between training and testing environments.

Pre-surgical training procedures

Animals were trained in the same chamber daily. In the first stage of training the two response levers were extended into the operant chamber and remained so throughout the session. A press on either lever led to access to 0.1 mL of water (FR-1 schedule), with the rule that if, for example, at any time the number of presses on the left lever was 5 greater than the number of presses on the right, the left lever becomes inactive until the right lever is pressed. This rule was designed to discourage the development of a lever or side bias. This phase of training continued until animals reached the criterion of 120 rewards per session (approximately 45 min) for two consecutive days.

In the second stage of training, animals had to discriminate between signal (illumination of central panel light) and non-signal (no illumination of central panel light) trials. It is important to note that the houselight prevents animals from being able to monitor for simple changes in chamber brightness, and must direct their focus to the intelligence panel. Each session included 160 trials equally divided between signal and non-signal. Correct responses were rewarded with water. On a signal trial, the signal light was turned for 1 second. Two seconds later the response levers were extended into the chamber. A press on the left lever was considered correct, and scored as a hit. A press on the right lever was scored as a miss. On non-signal trials, a press on the right lever was considered correct, and the trial scored as a correct rejection. A press of the left lever was considered incorrect, and scored as a false alarm (note: half of all

animals were trained with non-signal and signal response levers in the reverse position). The levers were retracted after being depressed regardless of whether the response was correct or incorrect. An omission was reported when animals failed to press either lever after four seconds, at which time both levers were retracted. Incorrect responses were followed by correction trials identical to the previous trial. After three consecutive errors on correction trials, animals were given a forced choice trial. If the error had occurred on a signal trial, the left lever alone was extended while the central panel light and the light directly above the correct lever remain illuminated. Only the right lever is extended into the chamber in the case of a non-signal trial. The inter trial interval (ITI) was 12±3 s during this stage of training. Performance criterion for this stage was >70% hits and correct rejections for three consecutive sessions.

In the third stage of training the correction and forced trials were dropped, and the ITI was decreased to 9±3 s. The signal duration remained fixed at 1 s and sessions consisted of a total of 162 trials. As with the previous stage, criterion for advancement was >70% hits and correct rejections for three consecutive sessions.

In the final version of the task, signal duration was shortened and varied (500, 50, or 25 ms). Training sessions were to a total of 162 trials, half signal and half non-signal. Performance was analyzed in 3 blocks of trials, each consisting of 54 trials. Signal and non-signal trials were presented in a pseudo-randomized order. Animals were trained on the final version of the task until performance

reached a plateau. Only animals with >70% hits to 500 ms signals and >70% correct rejections were employed in the present experiments.

Preparation and Calibration of Choline-Selective Microelectrodes

Multi-site microelectrodes were purchased from the Center for Microelectrode Technology at the University of Kentucky (Quanteon LLC, Nicholasville, KY; see Fig. 2.1 for a depiction of the measurement scheme). Each electrode array featured four 15x333 μm Platinum-recording sites arranged in side-by-side pairs. The pairs of electrodes were separated by 100 μm, and 30 μm separated sites in a pair. Connecting lines and recording sites were imprinted on a ceramic base ~ 125 μm thick. The connecting lines were coated with a layer of polyamide for insulation. The entire assembly was connected to circuit board. Microelectrodes were modified for recordings in freely moving animals by soldering four 2 cm pieces of enamel-coated magnet wire (30 ga) to gold terminals on the circuit board, each of which was connected to an individual recording site. The other end of the wire was connected to a female gold-pin. The gold-pins were inserted into a miniature 9-pin connector and glued to the microelectrode assembly using epoxy. The Ag/AgCl reference electrodes were constructed of 0.008" diameter silver wire (A-M Systems, Carlsberg, WA) soldered to a gold-pin, which was also inserted into the connector.

After assembly, electrodes were dip-coated with Nafion™ and then baked at 170°C for 4 minutes to repel anionic interferents from the platinum sites. The

bottom pair of recording sites were then coated with choline oxidase (CO) that had been cross-linked with a bovine serum-albumin (BSA)-glutaraldehyde mixture (1% CO, 1% BSA and 0.125% glutaraldehyde) using a 1µL syringe (Hamilton, Reno, NV, USA). The remaining two recording sites were coated with the BSA-glutaraldehyde solution alone and served to record background activity. Enzyme-coated microelectrodes were allowed to cure of 48-72 hrs in a desiccator prior to calibration.

Calibrations were performed using fixed potential amperometry with a voltage of 0.7 V versus Ag/AgCl reference electrode in a beaker containing 0.05M PBS solution which was constantly stirred and maintained at 37°C. Data was acquired at a rate of 5 Hz. After allowing 20 minutes for stabilization of background currents, aliquots of stock solutions of ascorbic acid (AA; 20 mM), choline (20 mM), and dopamine (DA; 2 mM) are added to the calibration beaker such that the final concentrations of the solutions are 250 µM AA, 20, 40, 60 and 80 µM choline and 2 µM DA. The slope (sensitivity), linearity (R^2) for choline, and selectivity ratio for AA and DA, were calculated for each individual recording site. The electrodes employed in the present experiments were characterized by a sensitivity for detecting choline: 7.34±1.84 pA/µM, a background current of <200 pA, selectivity for choline:AA: 275.30 ±101.74, and a highly linear response to increasing choline concentrations (20-80 µM): R^2: 0.997±0.001.

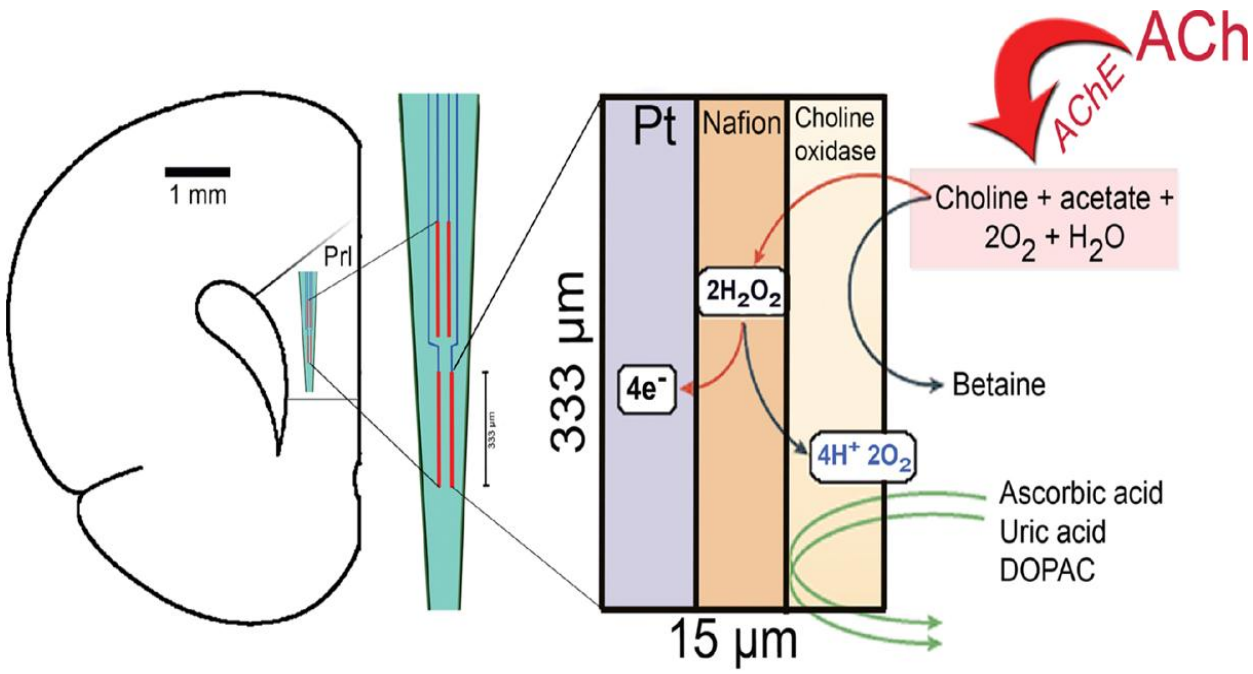

Figure 2.1

Measurement scheme for choline electrochemistry. Electrodes are implanted into the thalamic input layer of the prelimbic cortex. Newly released ACh is immediately hydrolyzed by acetylcholinesterase. The enzymatic reaction between choline and choline oxidase results in the production of hydrogen peroxide, which is then oxidized by a fixed potential of 700 mV. This process releases electrons which are measured as increases in current. The ability of this technique to measure changes in extracellular choline concentrations that are the result of newly released ACh has previously been validated (Parikh et al., 2004).

Surgery and in vivo recording

Upon demonstration of task proficiency, animals were acclimated to the test chamber. They were trained daily in the new environment until they re-established criterion level performance (approximately 2 weeks). After no less than three consecutive days of criterion level performance in the test chamber, animals were implanted with a CO-coated microelectrode. Surgeries were performed under aseptic conditions. Isoflurane (1-5%) was used to induce anesthesia (Anesco/Surgivet, Waukesha, WI). Anesthetized rats with shaved heads were placed in a stereotaxic frame (David Kopf, Model # 962, Tujunga, CA). Their body temperature was maintained at 37ºC using Deltaphase isothermal pad (Braintree Scientific, Braintree, MA).

Following cessation of the pedal reflex, the scalp was cleansed with betadine. A ~10 mm incision was made along the midline. Three stainless steel screws were threaded in the cranium. Additional holes were drilled above the right prelimbic cortex and above a remote spot in the left hemisphere to accommodate the Ag/AgCl reference electrode. The microelectrode assembly was slowly lowered into the prelimbic region of the right mPFC (AP: +3.0 mm; ML: -0.7 mm, measured from Bregma; DV: -3.5 mm, measured from dura) using a microdrive (MO-10; Narishige, International, East Meadow, NY) and anchored to the skull with dental cement. Topical antibiotic (bacitracin, polymixin and neomycin) was applied to the wound immediately after surgery. All animals given

antibiotic (amikacin; 25 mg/kg; s.c.), an analgesic (buprenorphine, 0.01 mg/kg; s.c.), and saline (1.0 mL; i.p.) for two days post-operatively while remaining in their home cages with food and water *ad libitum*. After a 48-hour recovery period, the water restriction schedule was resumed. On a test day, the animal was placed in the chamber and the microelectrode assembly connected to the FAST-16 system through a shielded cable, a low-impedance commutator, and a miniature headstage (mk-II RAT HAT; Quanteon, Lexington, KY). Recording sessions for the sustained attention task typically took place 3-5 days after surgery, allowing time for the animals to acclimate to the headstage. Amperometric recordings were collected every 500 ms (2 Hz) applying a fixed potential of 0.7 V to the microelectrode using the FAST-16 recording system. Data was captured using FAST-16 software. Amperometric recordings were time-locked by marking task events with TTL pulses.

Bilateral prefrontal cholinergic deafferentation

SAT training procedures for this experiment were identical to those for the recording experiments. The final version of the task varied slightly, only in that task length was set to a fixed amount of time (40 minutes, approximately 200 trials) as opposed to the number of trials. Following acquisition of criterion level performance, animals (n=5) received bilateral lesions of PFC cholinergic inputs. Two infusions, one in mPFC and one in orbitofrontal cortex, of 192 IgG-saporin were administered per hemisphere with a 1 µL Hamilton syringe (100 ng/0.5 µL;

coordinates from bregma: AP: +3.0, +3.7; ML: ±0.7, ±2.0 mm; DV: -3.0, -3.7 mm). To maximize the spread, the toxin was delivered with a bolus infusion. The needle was then left in place for 10 minutes to allow for diffusion. After recovering from surgery (1 week), animals were tested on the SAT for an additional 10 days.

Quantifying extent of cholinergic deafferentation

The extent of cholinergic dennervation in animals infused with the cholinotoxin was compared to non-performing animals (n=4) that were infused with a control toxin, saporin conjugated to the antibody for the mouse p75 receptor, and used for histological comparison. Animals were transcardially perfused with ice-cold 0.1 M phosphate buffered saline followed by a 4% paraformaldehyde solution. Brains were extracted, post-fixed for 24-hours, and then allowed to sit in 30% sucrose in phosphate buffered saline until they sunk. Brains were sliced in serial 40 µm sections on a freezing microtome (Leica Microsystems Inc, Bannockburn, IL).

Acetylcholinesterase (AChE) positive fibers were visualized using a modified version of the protocol outlined by Tago et al. (Tago, Kimura, & Maeda, 1986). Free-floating sections were rinsed in 0.1 M phosphate buffer, and then incubated in 0.1% hydrogen peroxide for 30 minutes. Sections were then rinsed in a 0.1M maleate buffer, and immersed in a 0.1 M sodium citrate, 5mM potassium ferracyanide, 30 mM cupric sulfate, and 30 mg of acetylthiocholine iodide in 0.1M maleate buffer. Sections were then rinsed in a 30mM Tris buffer

and incubated in 3,3' –diaminobenzidine with 0.75 mg of nickel ammonium sulfate per 250 ml of solution. Approximately 200 µL of 3% hydrogen peroxide per 20 mL of solution was added after ten minutes and sections were allowed to incubate until cortical layering could be detected. Sections were then rinsed in a 3mM Tris buffer and mounted on gel-coated slides.

Verification of the extent of the lesions was accomplished by photographing sections at a magnification of 40X, and overlaying a 40 X 25 µm grid in Photoshop. The number of AChE positive fibers making line crosses within a 4 X 4 square grid was quantified for two locations (one in mPFC and one from obitofrontal cortex) for each hemisphere from each animal.

Analysis of SAT Performance

In each session, the total number of Hits, Misses, correct rejections (CR), false alarms (FA), and omissions were recorded. Using these values, the relative number of Hits [$h/(h+m)$], correct rejections [$cr/(fa+cr)$], Misses (1-h), and FA (1-cr) were determined.

For the comparisons of the effect of bilateral prefrontal cholinergic deafferentation on SAT performance, data was lumped into four 3-day periods. The first period was the average performance over the 3 days immediately prior to surgery. This measure served as a pre-manipulation baseline. Because performance is uncharacteristically poor the first day on task after surgery, the data from this day was excluded from analyses of post-surgery performance.

Performance on days 2-4 post surgery was averaged together for the first post-surgery time period, days 5-7 the next, and finally days 8-10.

As detailed further below, the results of the electrochemistry experiments led us to hypothesize that the effects of prefrontal cholinergic deafferentation may manifest most robustly on performance on hit trials when the preceding trial was a CR or a Miss. To quantify performance on such trials, joint probabilities, which reflect the probability of two events co-occurring, were calculated for each of the four possible sequences of hits. An example: to calculate the joint probability of a Hit following a CR, first the probability of a hit is determined [$h/(h+m)$]. Next the probability that a CR precedes a signal trial is calculated [(#CR preceding signal trials/total #CR)]. Employing the multiplicative law, the joint probability was determined by calculating the product of these two terms (Howell, 1989).

Electrochemical signal processing

Current recordings from each platinum site were normalized by dividing the raw current value at each time point by the change in current following the addition of DA observed on that site during calibration. The normalized currents recorded from the non-CO coated sites were then subtracted from the normalized currents recorded at the CO-coated sites ("self-referencing"). These subtracted values were then converted to approximations of extracellular choline by dividing by the sensitivity of the CO-coated electrode determined in calibration.

Trial by trial analysis of electrochemical data

Data was organized in 0.5 s time bins, and normalized to the data point 5 seconds prior to the signal or non signal event (e.g. 7 s prior to lever extension) by setting this point equal to 0 and every point after the difference from this value. The choline concentrations for each data point over the 2 s prior to the onset of the signal (or the analogous time period on a non-signal trial) were averaged together and served as the pre-trial baseline. Because we were interested in changes in cholinergic activity proximal to cue and lever extension only the 4 s after the signal were used for statistical analysis. To reiterate, there is a two second delay between cue and lever extension on a signal trial, thus this time window includes a 2 s time window following signal and lever extension. Absolute changes in extracellular choline levels reported in the results are the difference between peak choline concentration value and the pre-cue baseline period. A total of 191 Hits, 236 CR, and 102 Misses were included in the final analyses. False alarms occurred only rarely (9.88% of all trials recorded). Because of the low number of such trials they were not included in the analyses.

Statistical analysis

Hits during SAT performance were analyzed with repeated-measures ANOVAs with the factors of signal duration and block of performance. CR and

the percent of omitted trials were analyzed with one-way repeated measures ANOVA with a factor of Block. The electrochemical data were analyzed repeated measures ANOVAs with a factor of time (Baseline, followed by the 4 seconds post cue). A significant change in extracellular choline levels relative to baseline would be indicated by a main effect of time.

The consequences of removal of prefrontal cholinergic inputs on performance in the SAT were analyzed with repeated measures ANOVA with an additional factor of day (baseline, Day 2-4, Day 5-7, Day 8-10). Joint probabilities were calculated for each hit sequence (across signal duration). Any significant alterations in performance, relative to baseline, are indicated by a main effect of Day.

Results of all statistical tests are reported with Huyhn-Feldt corrected values. All post hoc analyses employed the least significant difference test (LSD). When warranted, main effects, interactions, and exploratory analyses were carried out with paired samples t-tests ($\alpha=0.05$).

Results

SAT Performance during electrochemistry

Animals' performance in the SAT during recording sessions is summarized in Figure 2.2. The percentage of signal trials resulting in hits was signal duration dependent ($F(2,8)=27.32$; $p<0.001$) with the percentage of detected trials declining with shorter signal durations. Animals correctly rejected

79.06±5.09% of non-signal events. Animals also omitted very few trials (4.44±2.96% of trials/session). Finally, performance did not vary across blocks of task performance for any measure (all p's >0.20). Response latencies (time between lever extension and press) followed a fairly predictable pattern with the fastest responses on hits (640.51±34.57 ms, recall the animal has detected the cue and can begin preparing to respond prior to lever extension) and consistent across all other trial types, always taking less than one second (overall mean: 844.43 ± 13.76 ms).

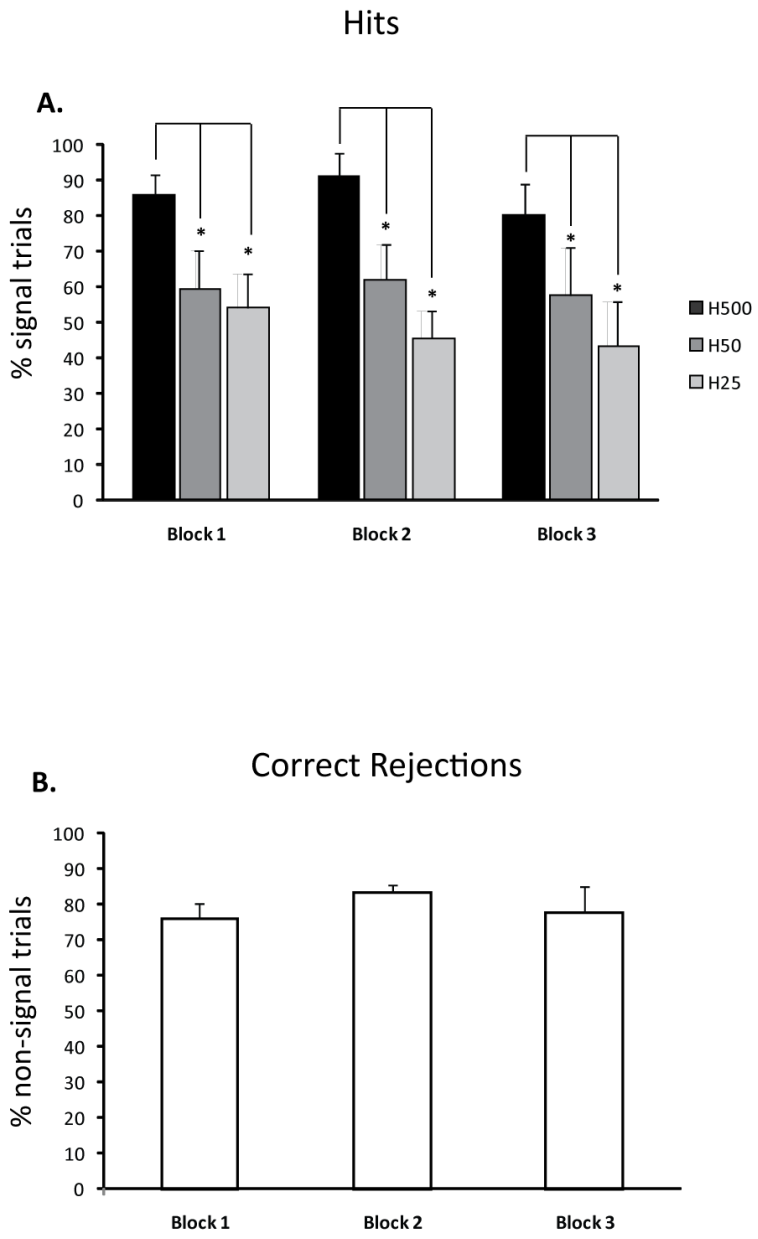

Figure 2.2

Performance during choline electrochemistry experiments (Y axis; % of correct trials, X axis: Block of trials; M;SEM). Performance on signal trials (A) was signal duration dependent, and animals correctly rejected (B) the majority on non-signal trials. Performance on both trial types did not vary across the time spent on task. The number of omitted trials was low (4.44±2.96%) and response latencies were less than one second for all trial types (overall mean 844.43 ± 13.76 ms).

Cholinergic activity during SAT performance

As predicted detected cues, or hits, were associated with an increase in extracellular choline levels that began following the presentation of the cue and prior the extension of the lever (main effect of time; ($F(9, 1710) = 7.21$, $p<0.001$). Levels of extracellular choline were significantly elevated relative to baseline by the time of lever extension (2 s post cue; $p=0.002$), and continued to rise to 130.43 ± 30.57 nM above baseline at 4 s post cue (Figure 2.3a). As is clear from Figure 2.3a, extracellular choline levels continued to rise even beyond the 4 s time point, eventually peaking 163.31 ± 31.19 nM above baseline 6.5 s post-signal. On signal trials that resulted in a Miss, there was no significant change in extracellular choline levels following the presentation of the cue or across the 4 s time window relative to baseline ($F(9,909)=1.70$, $p=0.137$). On trials resulting in a correct rejection (Figure 2.3b), there was no increase in extracellular choline concentrations ($F(9,2106)=2.51$, $p=0.012$). Levels actually declined and reached a low point 1 s after lever extension (-79.10 ± 28.05 nM, $p=0.005$).

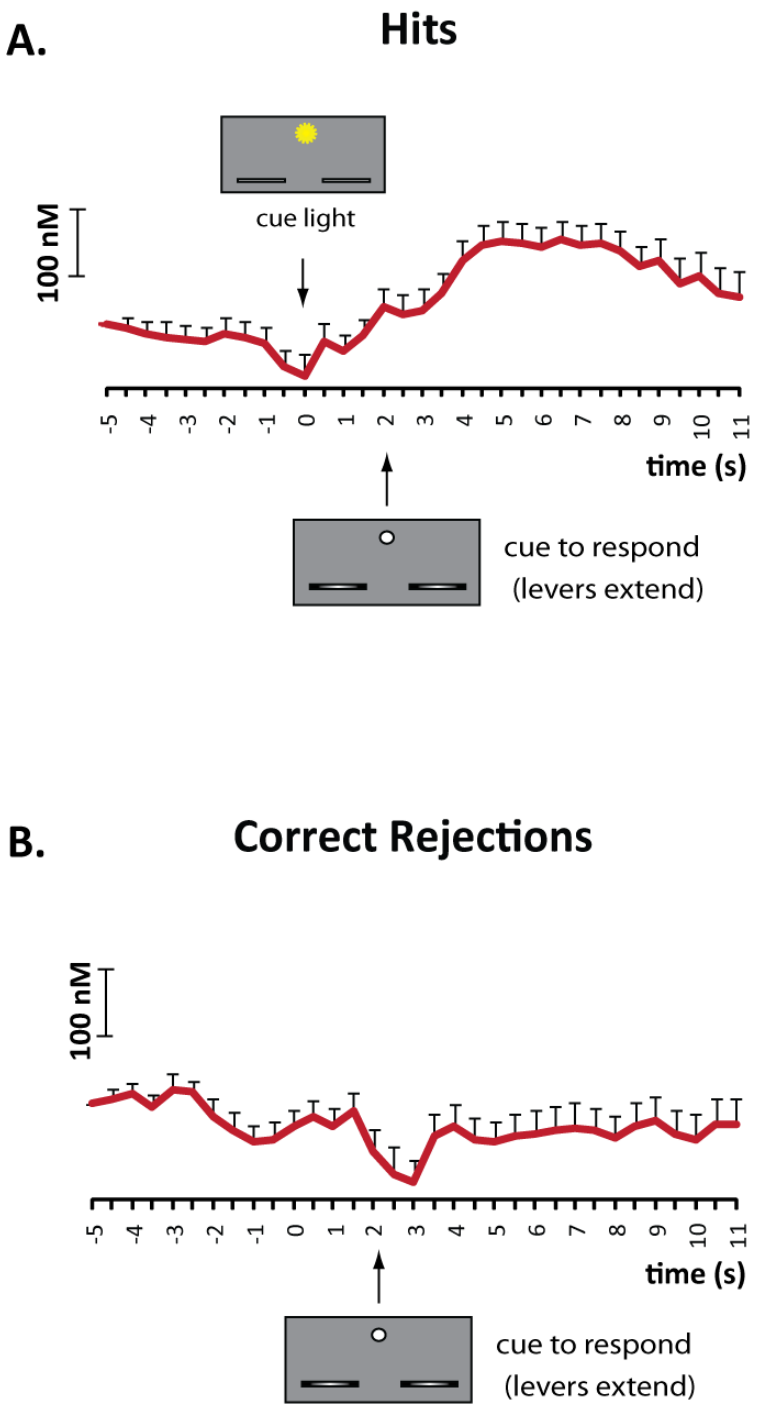

Figure 2.3

In agreement with previous studies suggesting that increases in cholinergic activity mediate cue detection, levels of extracellular choline began to increase following the cue on hit trials (A). Correct rejections, which also include a lever press, reward presentation, and retrieval, were not associated with such increases and actually tended to decrease (B).

Variability in cholinergic activity on hits

An interesting caveat of the data described above is that increases in cholinergic activity were only seen ~3/5 (60.69%) of hits analyzed. Two exploratory analyses were carried out to attempt to account for this variability. The first examined the change in choline concentrations on hits as a function of signal duration. As expected, the number of Hit trials available for analysis differed by signal duration, so a random subset of Hits to 500ms and 50ms signals was selected for comparison with hits to 25 ms signal. There was no evidence that extracellular choline concentrations varied as a function of signal duration (Main effect of signal duration: $F(2,86)=0.44$, $p=0.636$). Next, Hit trials were classified and separated by the response on the previous trial. When the data was parsed in this way a pattern began to emerge. As illustrated in Figure 2.4 if the hit was preceded by a CR (2.4b) or Miss (2.4c), there was an increase in cholinergic activity following the presentation of the cue. In contrast, if the hit was preceded by another hit trial, cue detection did not evoke a change in extracellular choline levels on the second Hit trial of the sequence. The number of hits of each trial sequence type differed, the fewest number being 32 for Miss→Hit sequences. 32 CR→Hit and 32 Hit→Hit sequences were randomly selected, and compared with 2-way repeated measures ANOVA with the factors of time and sequence. Extracellular choline levels varied across the 4 s time window as a function of the previous trial (time X sequence interaction; $F(18,558) = 3.06$, $p=0.002$). There was no difference in the pre-trial baseline levels of extracellular choline between trial sequences ($F(2,62)=.291$, $p=0.737$). Further

analyses of changes in choline levels were done within individual trial sequences. For CR→Hit sequences, there was a significant increase in cholinergic activity ($F(9,801)=5.91$, $p<0.001$). Extracellular levels began increasing after the termination of the cue, reaching levels above baseline by the time the lever was extended ($p=0.022$), and continued to rise throughout the next 2 s (Figure 2.4b, peak of increase at 4 s: 151.00 ± 41.45 nM). The rise in current levels continued past the 4 s time point, peaking at 212.13 ± 42.29 nM above baseline at 6.5 s post signal. There was also a significant increase in cholinergic activity on Miss→Hit trials ($F(9,279)=4.18$, $p=0.002$). Like CR→Hit trials, extracellular choline levels began to rise following the presentation of the cue. The pattern of change did differ slightly, with the increase in extracellular choline reaching significance by 1.5 s post cue (LSD $p=0.004$), however the overall trend was the same with levels increasing throughout the remainder of the 4 s period (peak change from baseline at 4 s: 282.43 ± 88.1 nM) and beyond. By 6.5 s post signal, levels had reached 368.70 ± 95.31 nM above baseline. In contrast, on a 2 hit sequence (Hit→Hit), extracellular choline levels did not change over the 4 s window ($F(9,612)=1.599$, $p=0.181$). The overall increase in extracellular choline levels appeared greater on Miss→Hit sequences than on CR→Hit, however the two sequences did not differ in a comparison using the same randomly selected trials above, even if the analysis included the entire 11 s post cue (main effect of sequence and sequence X time interaction, both p's >0.23).

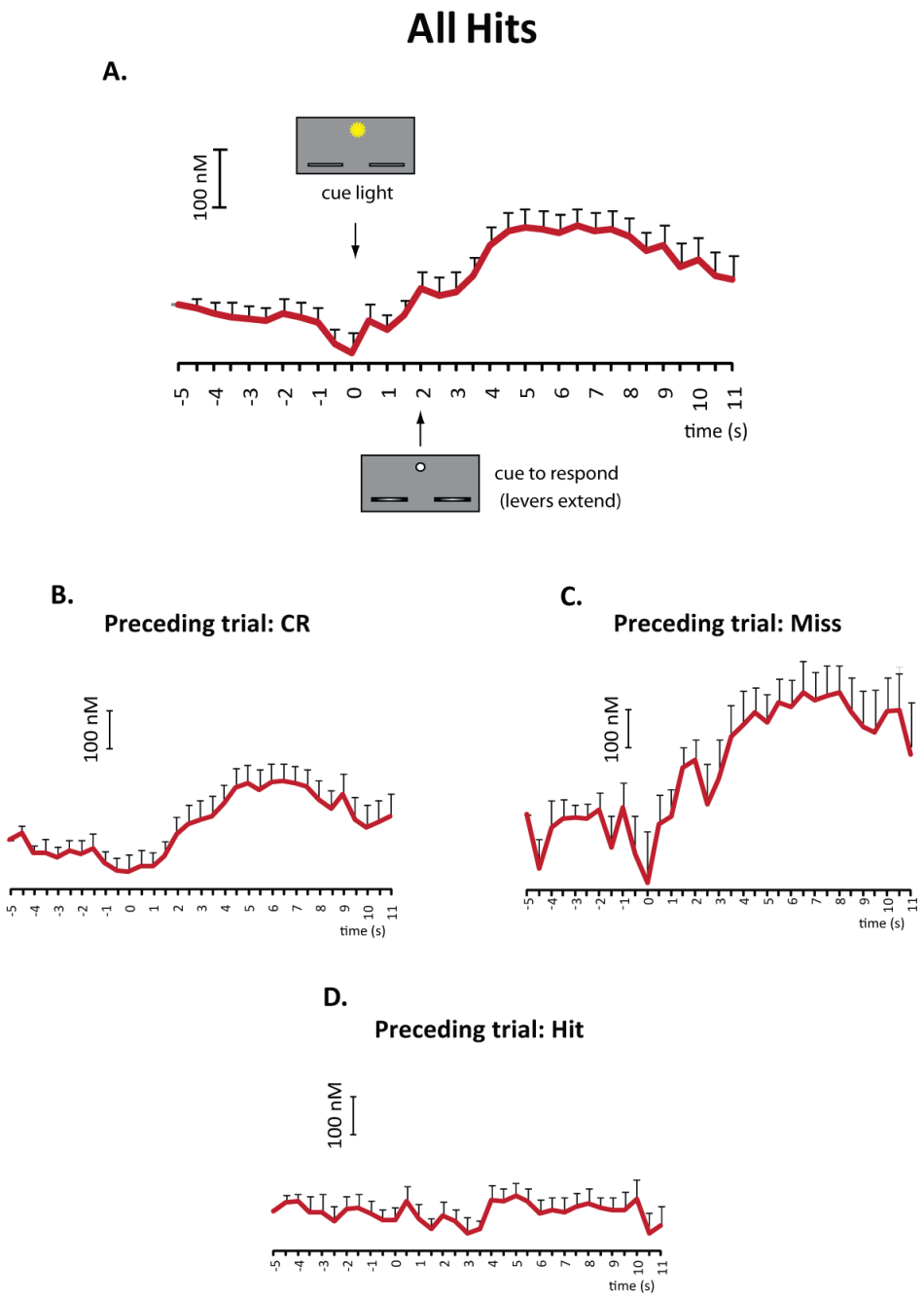

Figure 2.4

In contrast to our preliminary hypothesis, not all detected cues evoked increases in cholinergic activity. Whether a Hit was associated with significant increase in extracellular choline levels was dependent upon the previous trial type. Hit trials preceded by a CR or Miss evoked an increase (A and B), while on Hit→Hit sequences signal presentation and detection did not evoke an increase.

Effect of bilateral prefrontal cholinergic deafferentation

The extent of cholinergic deafferentation is presented in Figure 2.5. All animals received infusions of the cholinotoxin in both the mPFC and the orbitofrontal cortex. Compared to controls, there was a modest reduction (41.4 ±7.2%) of AChE positive fibers in the mPFC (2.5a, 2.5b). The extent of fiber reduction was more robust (80.50 ± 3.62%) in the oribitofrontal portion of the PFC (2.5c, 2.5d). The reason for the discrepancy in the effectiveness of the toxin in the two different regions is unknown, but as will be described below, the limited effects on performance following the lesion may be attributable to the lack of deafferentation in the mPFC. Preliminary behavioral results are illustrated in Figure 2.6. Bilateral prefrontal cholinergic deafferentation had limited impacts performance on the SAT, although the pattern of results is consistent with the electrochemical data. Performance on non-signal trials was not impaired (Figure 2.6b, effect of Day; $F(3,12)=1.32$, $p=0.32$), and the proportion of omitted trials did not significantly increase (Day; $F(3,12)= 2.78$, $p=0.15$). The number of hits (irrespective of sequence) did not differ significantly from baseline (effect of Day; $F(3,12)=1.84$, $p=0.199$). Although the statistical test did not reach significance, as illustrated in Figure 2.6a there was a trend towards reduced numbers of detected trials, most evident at the middle, 50 ms signal duration, reaching its lowest point between days 5-7 post surgery (paired-t test comparing 50 ms hits at baseline and days 5-7; ($t(4)=2.81$, $p=0.049$)). Thus it seems

possible that the lack of effects in the omnibus test was due to the relatively small n, lesion, and a lack of statistical power.

Subsequent analyses explored the effect of prefrontal cholinergic deafferentation on performance on hit sequences. Joint probabilities were calculated as described above for each of the 3 possible hit contingencies (CR→Hit, Miss→Hit, Hit→Hit). Relative to baseline, the probability for a Hit following a Miss was significantly reduced (Figure 2.6c, Main effect of Day $F(3,12)=5.13$, $p=0.030$). This decrease was most robust over Days 2-4 (LSD, $p=0.048$), and although performance did not fully recover, additional pairwise comparisons were not significant for Days 5-7 or 8-10. The probability for a hit on all other sequences was not significantly affected by cholinergic deafferentation (all p's >0.180).

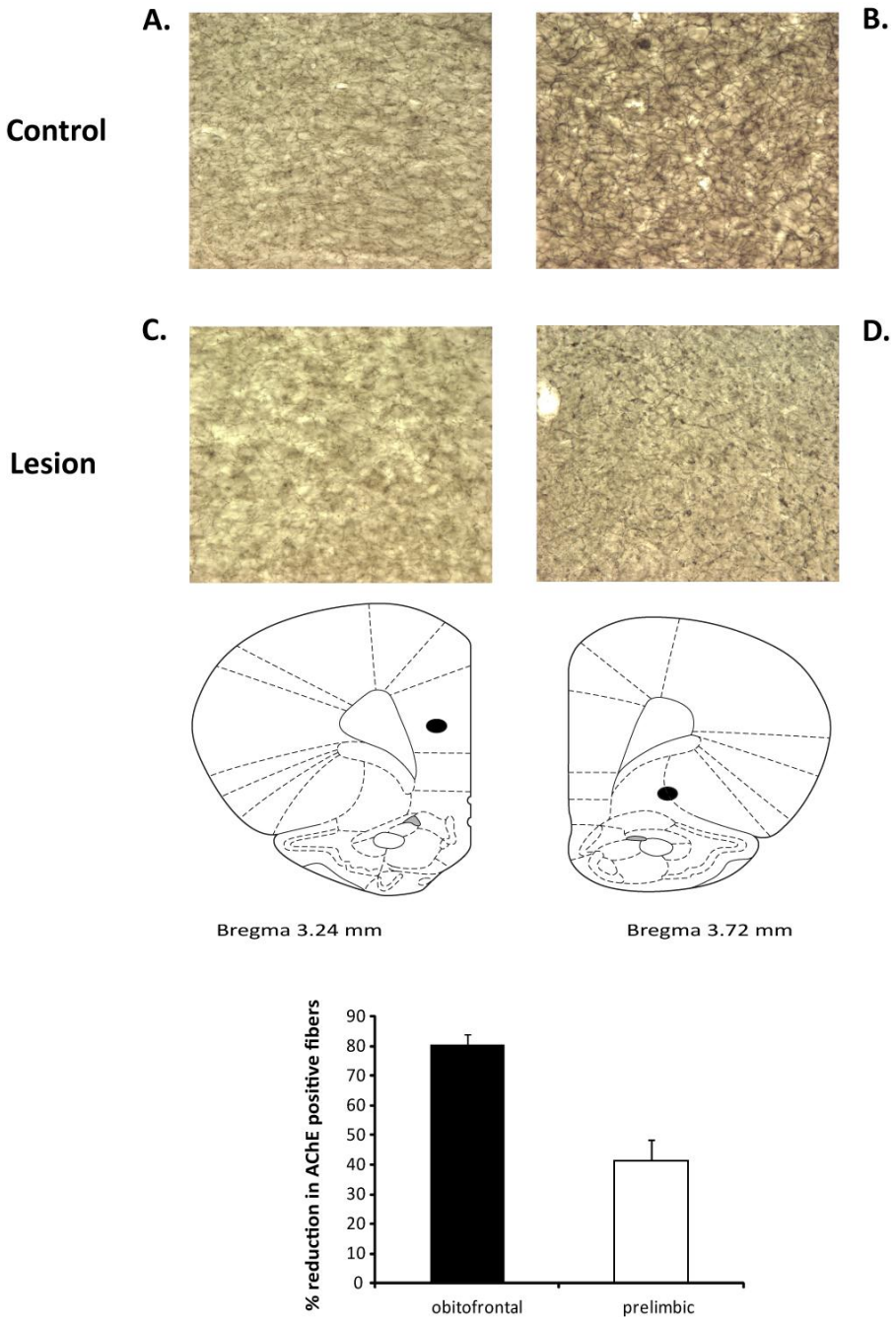

Figure 2.5

Representative images of the extent of cholinergic deafferentation in the mPFC and orbitofrontal cortex. The black dots on the coronal sections indicate the location the image was taken from. The toxin modestly reduced the number of AChE positive fibers in the more anterior, prelimbic regions (A control,C lesion). The toxin had a more profound effect on fiber density in orbitofrontal areas (B control, D lesion).

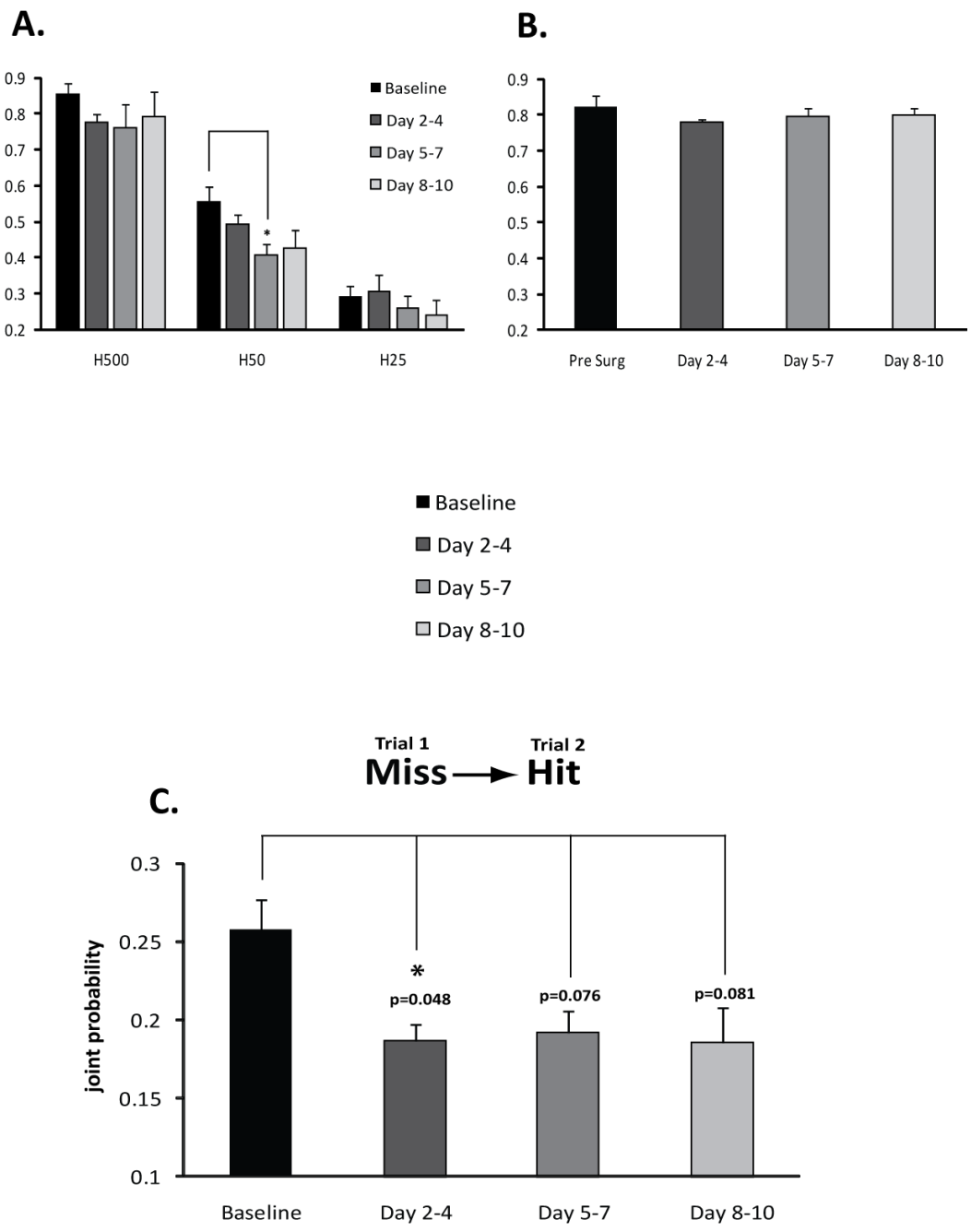

Figure 2.6

SAT performance following bilateral, prefrontal cholinergic deafferentation. Performance on signal trials was moderately impaired (A). Performance on non signal trials did not differ, relative to pre-surgery levels, following removal of prefrontal cholinergic inputs (B). Joint probability analyses support the proposition that cue detection was particularly impaired on trials involving a non-signal-to-signal shift, as reflected by a reduced probability for a Hit following a Miss (C)

Discussion

Prefrontal cholinergic transients in SAT-performing rats

Based upon the effects of selective removal of cholinergic inputs to the cortex, and preliminary experiments employing choline sensitive microelectrodes in animals performing a cued appetitive response task (McGaughy, et al., 1996; Parikh, et al., 2007), we hypothesized that detected cues on Hits evoke increases in cholinergic neurotransmission in prefrontal regions, and removal of prefrontal cholinergic inputs would impair Hit performance. In the present study, only Hits were associated with increases in cholinergic activity. However in contrast to our initial hypothesis, such increases were only seen when the Hit was preceded by a CR or Miss. In addition, bilateral removal of prefrontal cholinergic inputs selectively impaired performance on Miss→Hit trial sequences, while performance on other Hit sequences was spared. Because of the trial sequence-dependent occurrence of increases in cholinergic activity in hit trials, and the selective performance impairment, our relatively straightforward hypothesis about the role of these increases in cue detection must be revisited. At present, we have focused on two different possible explanations of the selectivity of cholinergic activity in the SAT, each of which places emphasis upon different parts of the cholinergic release event. The first focuses solely upon the trial sequence dependence of an evoked cholinergic signal, and particular attention is paid to the fact that levels of cholinergic activity are significantly elevated at the time the animals are prompted to make their response. The

functional relevance of the remainder of the signal is not considered. The second explanation attempts to account for the sequence dependency, as well as the full time course of the release, the major assumption being that as choline levels continue to increase, ACh release and hydrolyzation must be ongoing. Future experiments designed to test both of these possibilities will be addressed in the General Discussion.

Cue-evoked increases in cholinergic activity mediate the shift between cue independent to cue dependent attention

Cholinergic activity begins to rise following the offset of the cue during hit trials if preceded by a non signal trial response (CR or Miss), reaching levels significantly above baseline at the time of lever extension when the animal must then indicate the presence or absence of the signal. It has been proposed that in order for a cue to be detected (as defined by Posner, see above), the afferent (presumably mediodorsal thalamic, discussed further in Chapter III) representation of the cue must be allowed to be integrated into ongoing processing so that it can be used to guide responding (Hasselmo & Sarter, 2010). In the cortex, stimulation of muscarinic receptors can suppress intracortical inputs, while afferent input is unaffected (Hasselmo & Bower, 1992). To extend this neurophysiological finding to performance in the SAT, non-signal trial responding does not require cue detection and therefore responses are based primarily upon intracortical or associational processing. By suppressing synaptic communication between cortical structures, a cholinergic release event could

function to effectively suppress interfering or competing activity and allow the representation of the cue to inform action selection.

An extension of this theory is that the transition from non-signal to signal based attentional processing requires that the state of PFC circuitry be shifted from one dominated by intracortical inputs (cue-independent processing) to one that facilitates the utilization of external, predictive cues (cue-dependent processing). A cholinergic transient need not be generated by every detected cue, as if the previous trial was a detected signal trial, then the state of the network would have already been shifted.

Shifts from cue-independent to cue-dependent processing are associated with significant right PFC (Broadman's area 10) activation in human imaging studies (Burgess, et al., 2007). Interestingly, a recent study in humans performing a reverse-engineered version of the SAT demonstrated that Hits on such non-signal→signal sequences evoke greater right PFC activation in Broadman's area 10 than on signal→signal Hit sequences (Lustig, 2011). Thus, the data described here may be indicative of a transient increase in cholinergic activity underlying the shift from cue-independent to cue-dependent processing modes.

Increases in cholinergic activity as a learning signal

Although the increase cholinergic activity began early in the trial, it continued to increase well beyond this time point, peaking at around 6.5 s post-cue. Thus, cue-evoked cholinergic transients during hits in incongruent trial

sequences may have another function beyond attention mode shifts and cue detection, perhaps acting as a reporter or learning signal. ACh can increase the strength of the relationship between cue and response by augmenting long-term potentiation in both the hippocampus and cortex, effectively lowering the stimulation threshold for inducing an increase in synaptic strength (Hasselmo & Barkai, 1995; Huerta & Lisman, 1993). Stimulation of basal forebrain cholinergic projections have been shown to enhance the cortical coding of a sensory stimulus (Goard & Dan, 2009; Metherate & Ashe, 1991, 1993), which has been suggested to both aid in the formation and later support association between a conditioned and unconditioned stimulus (Weinberger, 2003).

In the context of animals performing the sustained attention task, the learning signal may serve to stabilize or increase the readiness for shifting from cue-independent to cue-oriented attention and refresh the processing of the response rule for cues (e.g., “if signal press left lever”). During successive hits, no second or third learning signals are generated. Thus, a prior correct rejection or a miss, or prior cue-independent attention, and the shift to cue-oriented attention are conditions for generating this learning signal.

Reconciling past results

Whether cholinergic transients selectively mediate the transition from cue dependent to cue independent processing, or also reflect a reporter or learning signal that strengthens cue→response→reward associations, both afford alternative interpretations of the previous study with the cued-appetitive response

task (Parikh, et al., 2007). The long ITI (90±30 s) cued-appetitive task fostered engagement in cue-independent behavior (e.g. grooming). Therefore, if the first speculation is correct, every detected cue evoked a cholinergic transient because the animal shifted from cue-dependent to cue-independent behavior during the ITI.

If cholinergic transients act as a learning signal, they would not be predicted to be evoked by reward delivery, even though they act as a cue for reward port approach in miss trials. This behavior is unrelated to strengthening the association between a predictive cue and reward. It's also intriguing to consider the results of additional experiments conducted by Parikh and colleagues. In the first series, the interval between cue and reward delivery was varied (either 6 or 2 s post cue). In either case, the cholinergic transient peaked at the time of reward delivery. This observation is consistent with the hypothesis that cholinergic activity "confirms" or reinforces an incongruent detection requiring reward delivery as the final step. Further, in trials in which the cue was detected but reward was not delivered, the amplitude of cholinergic transients were attenuated and peaked within 3 s after the cue (cue-reward period was 6±2 s). This data could be interpreted as indicating that in the absence of reward, detection confirmation collapsed, and as a consequence blocked the learning signal. Together, both seem consistent with the second hypothesis that delivery strengthens the association between cue and reward. It is important to reiterate that reward *per se* does not elicit cholinergic activity, as indicated by the absence

of cholinergic transients during rewarded misses in the former study or during successive hits and CR in SAT-performing animals.

Effects of prefrontal cholinergic lesions

We chose to target the prelimbic and orbitofrontal cortices for cholinergic deafferentation because of where we observe cholinergic transients (prelimbic), and based upon the recent replication of the sequence dependent increases in activation on hits in humans performing the SAT (an anatomical analogue of Broadmann's area 10 does not exist in rats, although it was our speculation that the rodent orbitofrontal cortex would be the closest functional homologue). Cholinergic deafferentation of these two areas was not sufficient to impair performance on all Hit sequences associated with increases in cholinergic activity. The lack of robust effects could be attributed to the extent of the lesion in prelimbic areas being limited (~40%). However, it could also be the case that there exists a distributed network of cortical areas where cholinergic input is an important contributor that were not targeted. For example, the posterior parietal cortex is a prominent feature in many models of attentional networks (Corbetta, et al., 2000; Shulman, d'Avossa, Tansy, & Corbetta, 2002). Cholinergic inputs to this area modulate cue evoked neural activity on Hits in animals performing the SAT (Broussard, Karelina, Sarter, & Givens, 2009) and are necessary for the performance enhancing effects of nucleus accumbens shell stimulation in animals performing a version of the task that includes a distracting stimulus (St Peters, et al., 2011). Thus a more extensive cholinergic lesion is likely

necessary to create a robust impairment in performance on CR→Hit and Miss→Hit trials.

The effects of the lesion in this study did however follow an interesting pattern. The difference in the amplitude of cholinergic activity on CR→Hit and Miss→Hit sequences was not statistically significant. Inspection of Figure 2.4 however, indicates a trend towards a greater increase in extracellular choline on a Miss→Hit sequence. Further analyses and perhaps the addition of just 1-2 animals to the data set may bare this difference out, and indicate larger amplitudes of cholinergic transients for Hits after a Miss when compared with hits after a correct rejection. Such a finding could be interpreted as indicating that cue-independent attention in the SAT is enforced by detection failures (misses), and a larger transient is needed to drive a shift or, a larger learning signal is generated to reinforce the response rules. If such transitions require a greater increase in prefrontal cholinergic neurotransmission, this could explain why even a limited lesion such as the one in this study could impair performance on these trials.

Implications for pre-clinical research

Here we report that cue evoked, transient increases in ACh release mediate not signal detection as previously hypothesized, but specifically when a transition from cue-independent to cue-dependent processing is required. Given the hypothesized role of cholinergic dysregulation in the cognitive impairments associated with psychopathology it is not surprising that many cholinomimetic

drugs have developed as potential treatments. However, with the exception of selective agonists of α4β2 nicotinic receptors which have shown promise, few have been clinically effective (Bartus, et al., 1982; Deutsch, 1971; Everitt & Robbins, 1997; Freedman, et al., 2008; Sarter, 2006; Sarter, Parikh, et al., 2009). This lack of efficacy is likely due to misconceptions regarding the role of the cholinergic system in cognitive performance. By helping to define the precise cognitive operations under cholinergic control and more specifically, how cholinergic systems orchestrate these functions, the present research will hopefully help to guide the development of pharmacotherapies towards a more productive future.

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Chapter III

Prefrontal glutamatergic signaling during attentional performance: relationship to cholinergic activity

Summary

Mounting evidence suggests prefrontal cholinergic-glutamatergic interactions mediate attentional processes and capacities. Transient increases in medial prefrontal (mPFC) cholinergic activity (scale of seconds) mediate the shift between cue-independent and cue-dependent attentional processing. Previous work in anesthetized preparations suggests that the generation of cholinergic transients depends in part on the stimulation of ionotropic glutamate receptors by glutamate released from mediodorsal thalamic afferents (Parikh, et al., 2010; Parikh, et al., 2007; Parikh, et al., 2008). The present experiments measured real-time glutamate release in the thalamic input layer of the mPFC in animals performing a sustained attention task (SAT). We hypothesized that on signal trials resulting in a Hit, the cue would evoked glutamate release. The rise and peak of this glutamate release will, on average, precede that observed on cholinergic transients. As Hit rates are signal duration dependent, the amplitude of cue-evoked glutamate release would vary as a function of signal duration. Tonic cholinergic activity (scale of minutes) functions to support the control of attention. Mediodorsal thalamic projections to the mPFC express α4β2 nicotinic

receptors presynaptically, and thus can themselves be modulated by such tonic cholinergic activity. We hypothesized that the amplitude of glutamatergic transients would be larger during periods of good performance (when tonic levels would be elevated) than in periods of poor performance. Correct rejections and Misses will not be associated with increases in glutamate release. In agreement with our hypothesis, the cue on signal trials resulting in Hits evoked an increase in glutamate release. The amplitude of this cue-evoked release was signal duration dependent. Further, the glutamatergic transients on Hits during periods of good performance were characterized by larger amplitudes than Hits during poor performance. In contrast to our hypotheses, lever extension on both CR and Misses also evoked glutamate release. The presence of glutamatergic transients at lever extension on CR and Misses suggests that in addition to supporting the generation of cholinergic transients, gluatmatergic transients may also represent the detection of any stimulus that prompts a decision or choice and the initiation of a response.

Introduction

Attentional functions and capacities are central to cognitive performance. The cortical cholinergic input system is a necessary component of the neural circuitry of attention (Everitt & Robbins, 1997; McGaughy, et al., 2002; McGaughy, et al., 1999; McGaughy, et al., 1996; Sarter, et al., 2005). We have recently shown that transient increases in cholinergic activity in the medial prefrontal cortex (mPFC) mediate the ability to shift from cue independent to cue dependent attentional processing, and may also serve as a learning or reporter signal to reinforce the ability to engage in such shifts (Hasselmo & Sarter, 2010; Parikh, et al., 2007; Sarter, Parikh, et al., 2009). Mechanistic studies of the mPFC circuitry underlying the generation of these cholinergic transients have revealed that they are a product of local cholinergic-glutamatergic interactions. They require glutamate release and the stimulation of ionotropic glutamate receptors located on cholinergic terminals (Parikh, et al., 2008). Such glutamate release originates from projections from the mediodorsal thalamic nucleus (Parikh, et al., 2010). These thalamic inputs are hypothesized to act as an extension of the thalamic reticular nucleus "attentional searchlight" (Crick, 1984), and help recruit attentional mechanisms by importing information about a predictive cue to prefrontal networks, in turn engaging mPFC cholinergic mechanisms, and ultimately generating cholinergic transients.

We have developed a model of a prefrontal network illustrating such glutamatergic-cholinergic interactions in attentional performance (see Figure 3.1).

An important aspect of the model concerns the presence of two modes of cholinergic neurotransmission (Hasselmo & Sarter, 2010; Parikh, et al., 2007). In addition to transient or brief increases in ACh release, there is a tonically active mode of cholinergic activity that changes on the scale of tens of seconds to minutes. Current evidence suggests that levels of tonic cholinergic activity are mediated by prefrontal-nucleus accumbens-basal forebrain interactions, and function to support the control of attention (Sarter & Paolone, 2011; St Peters, et al., 2011). For example, engagement in and performance of tasks taxing attentional capacities, but not tasks designed to control for locomotor activity and reward presentation, evoke tonic increases in ACh release (Arnold, et al., 2002). Further, tonic cholinergic activity is increased in the face of enhanced demands on attentional control, such as those imposed by disruption of prefrontal cholinergic neurotransmission, attentional fatigue, or a distracting stimulus (Kozak, et al., 2006; Sarter, et al., 2006; Sarter & Paolone, 2011; St Peters, et al., 2011). Thalamic afferents to the mPFC express α4β2 nicotinic acetylcholine receptors (nAChRs) (Dickinson, et al., 2008; Lambe, et al., 2003). The current model speculates that tonic cholinergic activity modulates glutamate release from mediodorsal thalamic afferents via $\alpha 4\beta 2$* nicotinic acetylcholine receptors (nAChRs). These receptors are situated on the terminals of the thalamic input (Purple projection from BF in figure 3.1 (Lambe, et al., 2003; Parikh, et al., 2010; Parikh, et al., 2008), and thus support attentional performance by dictating the likelihood of a cholinergic transient.

Although the current evidence largely supports the model depicted in Figure 3.1, the initial experiments that informed the function and circuitry of this mPFC attention network utilized either relatively simple instrumental tasks or anesthetized preparations, prohibiting efforts to directly demonstrate how cholinergic and glutamatergic mechanisms might interact to mediate attentional performance. In the present experiments glutamate release was measured on a sub-second time scale in the thalamic input layer of the mPFC of animals performing a sustained attention task (SAT). We tested hypotheses that 1) in an operant test of sustained attention which includes the complexity of both signal and non-signal trials, detected signals evoke glutamatergic transients 2) as glutamate and stimulation of ionotropic receptors stimulate cholinergic transients, the onset and peak of glutamatergic transients will precede cholinergic transients. 3) Performance on signal trials in the SAT is signal duration dependent, and glutamate release appears to determine the likelihood of generating a cholinergic transient, therefore the amplitude of cue evoked glutamate release will also vary as a function of signal duration 4) trials resulting in a Miss will be associated with a smaller or undetectable glutamate release event than those measured on Hits 5) responses on non-signal trials will not be associated with any significant change in glutamatergic activity. A final exploratory analysis was conducted to assess the potential role of tonic modulation of prefrontal glutamatergic signaling. The characteristics of cue-evoked glutamatergic activity on Hits during periods of good performance (when tonic levels of cholinergic activity would be hypothesized to augmented) was compared to periods of poor performance

(when performance hovers around chance levels, reflecting a lack of attentional control and thus tonic release would be relatively lower).

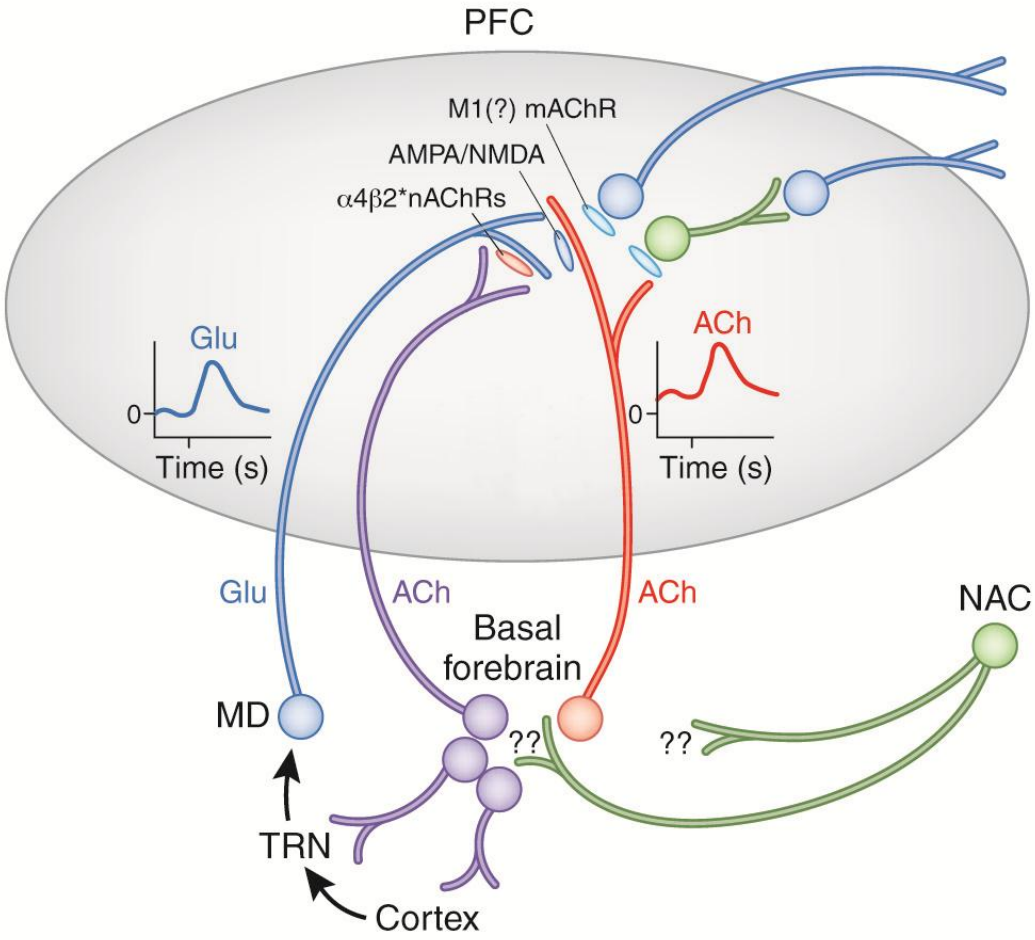

Figure 3.1

Hypothesized model of mPFC circuitry underlying cholinergic transients and associated attentional performance. Cholinergic transients are the product of glutamate release from mediodorsal thalamic afferents (blue input) stimulating ionotropic (AMPA/NMDA) receptors situated on the terminals of cholinergic inputs (red input). A second, tonic, mode of cholinergic activity (purple input) modulates the amplitude of cue-evoked glutamate release by actions at α4β2 nAChRs, thereby controlling the probability of evoking a cholinergic transient and a successful shift between cue independent and cue dependent attention. ***Figure taken from Hasselmo & Sarter, 2010***

MATERIALS AND METHODS

Subjects

Subjects (n=3) were male Wistar rats approximately 60 days old (200-300g) at the onset of training. Animals were kept on a 14:10 light/dark cycle in a temperature and humidity-controlled vivarium. Water was only available as a reward during testing and for 30 min upon the completion of the training/testing session. Food was available *ad libitum*. All training and testing took place during the light cycle between 0800-1700 hours. Animals were maintained in accordance with the NIH guide for the Care and Use of Animals and experiments were conducted in accordance with protocols approved by the University of Michigan Committee on Use and Care of Animals (UCUCA).

Apparatus

Rats were trained in one of twelve operant chambers (Med Associates, Georgia, Vt., USA) enclosed within a sound attenuating box and equipped with a fan to conceal any residual background noise. Each chamber was equipped with a water port located between two retractable levers. A central panel light was located at the front of each chamber above the water port. Additional panel lights were located above each lever. A house light was positioned at the back of the chamber. All training and testing programs were executed with a PC running Med-PC software (V. IV).

Electrochemical recordings were conducted in a 12” X 10” X 17” wooden operant chamber completely shielded by copper-wire mesh and equipped with two retractable levers constructed of fiberboard normally employed for electrical insulation. The receptacle used for water delivery was constructed from copper. The entire assembly was connected to an electrical ground. The relative location of the central panel light is the same as in the training chambers. The background illumination provided by the houselight, and the change in luminance following the presentation of the signal were kept constant between training and testing environments.

Pre-surgical training procedures

Animals were trained in the same chamber daily, and a houselight located at the back of the chamber is illuminated throughout training/test sessions. In the first stage of training the two response levers were extended into the operant chamber and remained so throughout the session. A press on either lever led to access to 0.1 mL of water (FR-1 schedule), with the rule that if, for example, at any time the number of presses on the left lever was 5 greater than the number of presses on the right, the left lever becomes inactive until the right lever is pressed. This rule was designed to discourage the development of a lever or side bias. This phase of training continued until animals reached the criterion of 120 rewards per session (approximately 45 min) for two consecutive days.

In the second stage of training, animals had to discriminate between signal (illumination of central panel light) and non-signal (no illumination of central panel light) trials. It is important to note that the houselight prevents animals from being able to monitor for simple changes in chamber brightness, and must direct their focus to the intelligence panel for above chance performance. Each session included 160 trials equally divided between signal and non-signal. Correct responses were rewarded with water. On a signal trial, the signal light was turned for 1 second. Two seconds later the response levers were extended into the chamber. A press on the left lever was considered correct, and scored as a hit. A press on the right lever was scored as a miss. On non-signal trials, a press on the right lever was considered correct, and the trial scored as a correct rejection. A press of the left lever was considered incorrect, and scored as a false alarm (note: half of all animals were trained with non-signal and signal response levers in the reverse position). The levers were retracted after being depressed regardless of whether the response was correct or incorrect. An omission was reported when animals failed to press either lever after four seconds, at which time both levers were retracted. Incorrect responses were followed by correction trials identical to the previous trial. After three consecutive errors on correction trials, animals were given a forced choice trial. If the error had occurred on a signal trial, the left lever alone was extended while the central panel light and the light directly above the correct lever remain illuminated. Only the right lever is extended into the chamber in the case of a non-signal trial. The inter trial interval (ITI) was 12±3 s during this stage of

training. Performance criterion for this stage was >70% hits and correct rejections for three consecutive sessions.

In the third stage of training the correction and forced trials were dropped, and the ITI was decreased to 9±3 s. The signal duration remained fixed at 1 s and sessions consisted of a total of 162 trials. As with the previous stage, criterion for advancement was >70% hits and correct rejections for three consecutive sessions.

In the final version of the task, signal duration was shortened and varied (500, 50, or 25 ms). Training sessions were timed to last 40 minutes with a total of ~200 trials, half signal and half non-signal. Performance was analyzed in 5 blocks of trials, each 8 minutes in duration. Signal and non-signal trials were presented in a pseudo-randomized order. Animals were trained on the final version of the task until performance reached a plateau. Only animals with >70% hits to 500 ms signals and >70% correct rejections were employed in the present experiments.

Preparation and Calibration of Enzyme-Selective Microelectrodes

Multi-site microelectrodes were purchased from the Center for Microelectrode Technology at the University of Kentucky (Quanteon LLC, Nicholasville, KY; see Fig. 1A). Each electrode array featured four 15x333 μm Platinum-recording sites arranged in side-by-side pairs. The pairs of electrodes were separated by 100 μm, and 30 μm separated sites in a pair. Connecting

lines and recording sites were imprinted on a ceramic base ~ 125 µm thick. The connecting lines were coated with a layer of polyamide for insulation. The entire assembly was connected to circuit board. Microelectrodes were modified for recordings in freely moving animals by soldering four 2 cm pieces of enamel-coated magnet wire (30 ga) to gold terminals on the circuit board, each of which was connected to an individual recording site. The other end of the wire was connected to a female gold-pin. The gold-pins were inserted into a miniature 9-pin connector and glued to the microelectrode assembly using epoxy. The Ag/AgCl reference electrodes were constructed of 0.008" diameter silver wire (A-M Systems, Carlsberg, WA) soldered to a gold-pin, which was also inserted into the connector.

After assembly, electrodes were dip-coated with Nafion™ and then baked at 170°C for 4 minutes to repel anionic interferents from the platinum sites. The bottom pair of recording sites were then coated with glutamate oxidase (GO) that had been cross-linked with a bovine serum-albumin (BSA)-glutaraldehyde mixture (1% GO, 1% BSA and 0.125% glutaraldehyde) using a 1µL syringe (Hamilton, Reno, NV, USA). The remaining two recording sites were coated with the BSA-glutaraldehyde solution alone and served to record background activity. Enzyme-coated microelectrodes were allowed to cure of 48-72 hrs in a desiccator prior to calibration (See Figure 3.2 for illustration of measurement scheme.

Calibrations were performed using fixed potential amperometry with a voltage of 0.7 V versus Ag/AgCl reference electrode in a beaker containing

0.05M PBS solution which was constantly stirred and maintained at 37°C. Data was acquired at a rate of 5 Hz. After allowing 20 minutes for stabilization of background currents, aliquots of stock solutions of ascorbic acid (AA; 20 mM), glutamate (20 mM), and dopamine (DA; 2 mM) are added to the calibration beaker such that the final concentrations of the solutions are 250 µM AA, 20, 40, 60 and 80 µM glutamate and 2 µM DA. The slope (sensitivity), linearity (R^2) for glutamate, as well as selectivity ratio for AA and DA, are calculated for each individual recording site and recordings from GO-coated sites were self-referenced. The electrodes employed in these experiments were characterized by a sensitivity for detecting glutamate of 7.57±0.81 pA/µM, a background current of <200 pA, a selectivity for glutamate:AA: of 379.74±285.41, and a highly linear response to increasing glutamate concentrations (20-80 µM): R^2=0.95± 0.04.

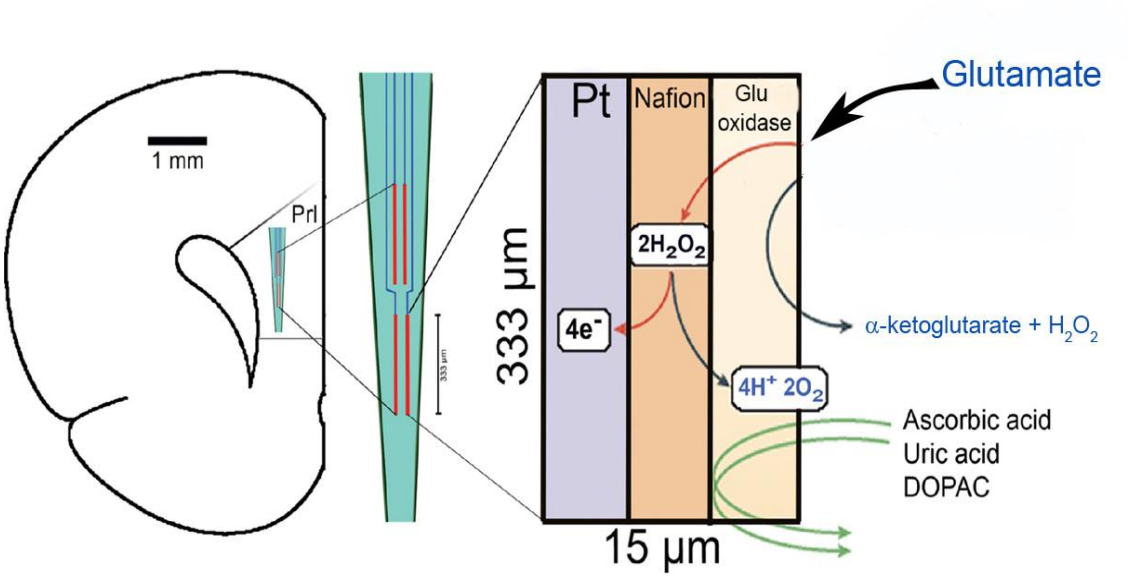

Figure 3.2

Measurement scheme for glutamate electrochemistry. Platinum recording electrodes are dip coated with nafion to repel anionic interferents (such as ascorbic acid). Glutamate oxidase is immobilized on the platinum surface with a BSA-glutaraldehyde protein matrix. Extra-synaptic glutamate comes in to contact with glutamate oxidase, resulting in the production of α-ketoglutarate and hydrogen peroxide. This hydrogen peroxide is oxidized by the fixed potential of 700 mv, releasing electrons that are measured as changes in current. Only 2 of the 4 recording sites are coated with glutamate oxidase, the other two are coated with the protein matrix alone to serve as sentinels for self-referencing. In off-line data analysis, the current recorded on the sentinels is subtracted from that recorded on the coated sites. The remaining current changes are attributed to glutamate release.

Surgery and in vivo recording

Upon demonstration of task proficiency, animals were acclimated to the test chamber. They were trained daily in the new environment until they re-established criterion level performance (approximately 2 weeks). After no less than three consecutive days of criterion level performance in the test chamber, animals were implanted with a GO-coated microelectrode. Surgeries were performed under aseptic conditions. Isoflurane (1-5%) was used to induce anesthesia (Anesco/Surgivet, Waukesha, WI). Anesthetized rats with shaved heads were placed in a stereotaxic frame (David Kopf, Model # 962, Tujunga, CA). Their body temperature was maintained at 37ºC using Deltaphase isothermal pad (Braintree Scientific, Braintree, MA).

Following cessation of the pedal reflex, the scalp was cleansed with betadine. A ~10 mm incision was made along the midline. Three stainless steel screws were threaded in the cranium. Additional holes were drilled above the right prelimbic cortex and above a remote spot in the left hemisphere to accommodate the Ag/AgCl reference electrode. The microelectrode assembly was slowly lowered into the prelimbic region of the right mPFC (AP: +3.0 mm; ML: -0.7 mm, measured from Bregma; DV: -3.5 mm, measured from dura) using a microdrive (MO-10; Narishige, International, East Meadow, NY) and anchored to the skull with dental cement. Topical antibiotic (bacitracin, polymixin and neomycin) was applied around the incision immediately after surgery. All animals given antibiotic (amikacin; 25 mg/kg; s.c.), an analgesic (buprenorphine, 0.01

mg/kg; s.c.), and saline (1.0 mL; i.p.) for two days post-operatively while remaining in their home cages with food and water *ad libitum*. After a 48-hour recovery period, the water restriction schedule was resumed. On a test day, the animal was placed in the chamber and the microelectrode assembly connected to the FAST-16 system through a shielded cable, a low-impedance commutator, and a miniature headstage (mk-II RAT HAT; Quanteon, Lexington, KY). Recording sessions for the sustained attention task typically took place 3-5 days after surgery, allowing time for the animals to acclimate to the headstage. Amperometric recordings were collected every 500 ms (2 Hz sampling rate) applying a fixed potential of 0.7 V to the microelectrode using the FAST-16 recording system. Data was captured using FAST-16 software. Amperometric recordings were time-locked by marking task events with TTL pulses.

Analysis of Performance

In each session, the total number of Hits, Misses, correct rejections (CR), false alarms (FA), and omissions were recorded. Using these values, the relative number of Hits [$h/(h+m)$], correct rejections [$cr/(fa+cr)$], Misses (1-h), and false alarms (1-cr) were determined. An additional measure, the SAT score, was also calculated. SAT scores take in to account performance on both signal and non-signal trials and serve as an index of overall attentional performance. Scores can range from -1 to 1, with 1 indicating all Hits and CR, 0 chance performance, and -1 all Misses and FA.

Electrochemical signal processing

Current recordings from each platinum site were normalized by dividing the raw current value at each time point by the change in current following the addition of DA observed on that site during calibration. The normalized currents recorded from the non-GO coated sites were then subtracted from the normalized currents recorded at the GO-coated sites ("self-referencing"). These subtracted values were then converted to approximations of extracellular choline by dividing by the sensitivity of the GO-coated electrode determined in calibration.

Trial by trial analysis of electrochemical data

Data was organized in 500 ms time bins, and normalized to the data point 5 seconds prior to the signal or non-signal event (e.g. 7 s prior to lever extension on a non-signal trial) by setting this point equal to 0 and every point after the difference from this value. The glutamate concentrations for each data point over the 2 s prior to the onset of the signal (or the analogous time period on a non-signal trial) were averaged together and served as the pre-trial baseline. Because we were interested in changes in glutamatergic activity proximal to cue and lever extension only the subsequent 4 s post-baseline were used for statistical analysis. As noted in the description of the SAT above, there is a two second delay between cue and lever extension on a signal trial, thus this time

window includes an analogous 2 s time window following signal and lever extension. Absolute changes in extracellular glutamate levels reported in the results are the difference between peak glutamate concentration value and the pre-cue baseline period. A total of 154 Hits, 185 CR, and 95 Misses were included in the final analyses. As in Chapter II, FA occurred only rarely (12.5% of electrochemical data available) and therefore were not included in the analyses.

Statistical analysis

Performance during the SAT was analyzed with repeated-measures ANOVAs with block of performance (and stimulus duration for signal trials) as factors. The electrochemical data were analyzed with repeated measures ANOVAs with a factor of time (Baseline, followed by the 4 seconds post cue). A significant change in extracellular glutamate levels relative to baseline would be indicated by a main effect of time.

Results of all statistical tests are reported with Huyhn-Feldt corrected values. All post hoc analyses examining the change from baseline levels employed the least significant difference test (LSD). When warranted, main effects, interactions, and exploratory analyses were carried out with paired samples t-tests (α=0.05).

Results

Performance

The percentage of detected trials declined with shorter signal durations (M±SEM; 500 ms Hits: 70.00±6.66%, 50 ms Hits: 55.00±6.72%, 25 ms Hits 52.56±7.95%). Animals correctly rejected 73.74±9.54% of non-signal events. Animals also omitted very few trials (4.85±2.11% of trials/session). Response latencies (time from extension of the response lever into the operant chamber to lever press) were fastest for hits (710.51±41.69 ms), most likely due to the fact that on detected trials, the animal can begin to initiate a response prior to lever extension. The latency to respond was similar across all other trial types always taking around 1 s (901.19 ± 24.98 ms).

Glutamatergic activity during SAT performance

Hits

As illustrated in figure 3.3a, the concentration of extracellular glutamate increased following the signal on a Hit (main effect of time ($F(9,1377)=12.00$, $p<0.001$)). Glutamate was significantly increased by 500 ms following cue offset, peaked after lever extension (2.5 s post cue, 72.53 ± 16.31 nM above baseline), and declined following response and reward. Additionally, determination of the onset and peak of glutamatergic transients (aim 2) is based upon the averaged population trace and not data from individual trials.

Influence of trial sequence

A trial by trial analysis indicated a significant amount of variability in the amplitude of cue evoked glutamate release on Hits. Given the trial sequence dependency of cholinergic transients, we next examined glutamate release on hits based upon the preceding trial type. In contrast to cholinergic transients, glutamate release events on hit trials are not modulated by trial sequence. On Hits that were preceded by a CR (Figure 3.3b), glutamate levels began to rise following the offset of the cue, peaking after the extension of the response levers (time point 2.5 s post cue, 85.38±23.31 nM above baseline), and then rapidly declined (main effect of time; $F(9,540)=5.442$, $p<0.001$, time point 0.5 s - 2.5 s LSDs all p's <0.05). There were relatively few Hit trials preceded by Misses that were available for analysis (total of 18). As a result, statistical tests did not indicate a robust increase in extracellular glutamate levels on such hits, although as is evident in Figure 3.3c, there was a similar pattern in glutamatergic activity following the cue on a detected trial (peak at time point 1.5 sec post cue, 85.50±60.36 nM above baseline). Finally, on Hits preceded by another Hit (3.6d), glutamate began to rise after the cue, peaking around the time of the extension of the response lever (main effect of time; $F(9,423)=5.480$, $p<0.001$. Time points 0.5-3 s, all p's <0.03).

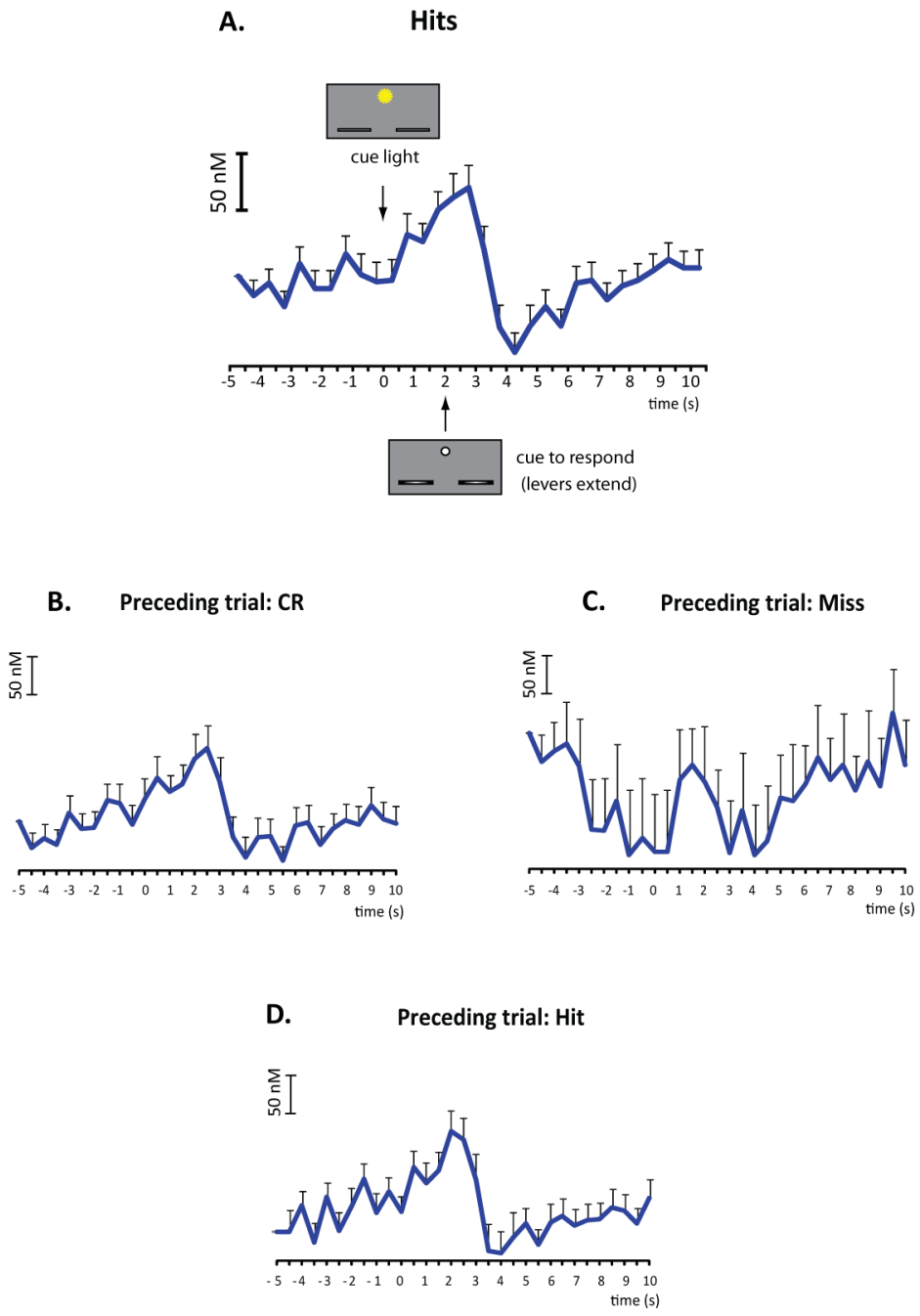

Figure 3.3

On signal trials resulting in a hit, extracellular glutamate levels began rising with the onset of the cue, peaking at the time of lever extension and response (A). Unlike cholinergic transients on hits which are only seen on hits preceded by CR or Misses, all hit sequences are associated with transient increases in glutamate release (B-D).

Influence of signal duration on the amplitude of evoked glutamate release on Hits

In the SAT, the duration that the cue light is illuminated is varied (either 500, 50, or 25 ms) In terms of performance, the proportion of Hits varied by signal duration, with more Hits on trials with the longest signals. As shown in Figure 3.4, the amplitude of the glutamate release event also varied as a function of signal duration. Following a 500 ms signal (Figure 3.4b), glutamate began to rise immediately and peaked 2.5 s later, 100.65±26.78 nM above baseline (Main effect of time: $F(9,522)=6.768$, $p<0.001$, LSDs: time point 0.5 sec $p=0.025$, time points 1.5 -3 s, all p's <0.044). For 50 ms Hits (Figure 3.4c), the evoked glutamate response was on average smaller, peaking 2 s post cue, 89.20 ± 30.35 nM above baseline (Main effect of time; $F(9,414)=3.567$, $p=0.001$. Time points 2 s and 2.5 s post cue, both p's <0.011). The magnitude of the increase was even smaller on 25 ms Hits (3.4d). Although levels of glutamate trend towards an increase following the cue no individual point actually reached statistically significant levels above baseline. The closest time point was the peak at 2 s post cue ($p=0.105$), 42.86±47.81 nM above baseline concentrations.

As a reminder, as of now we only have data from 3 animals in 3 recording sessions. It is our belief that the trend in the amplitude of the release event is strong enough that the differences between Hits following different signal durations will be become more obvious with the addition of more data.

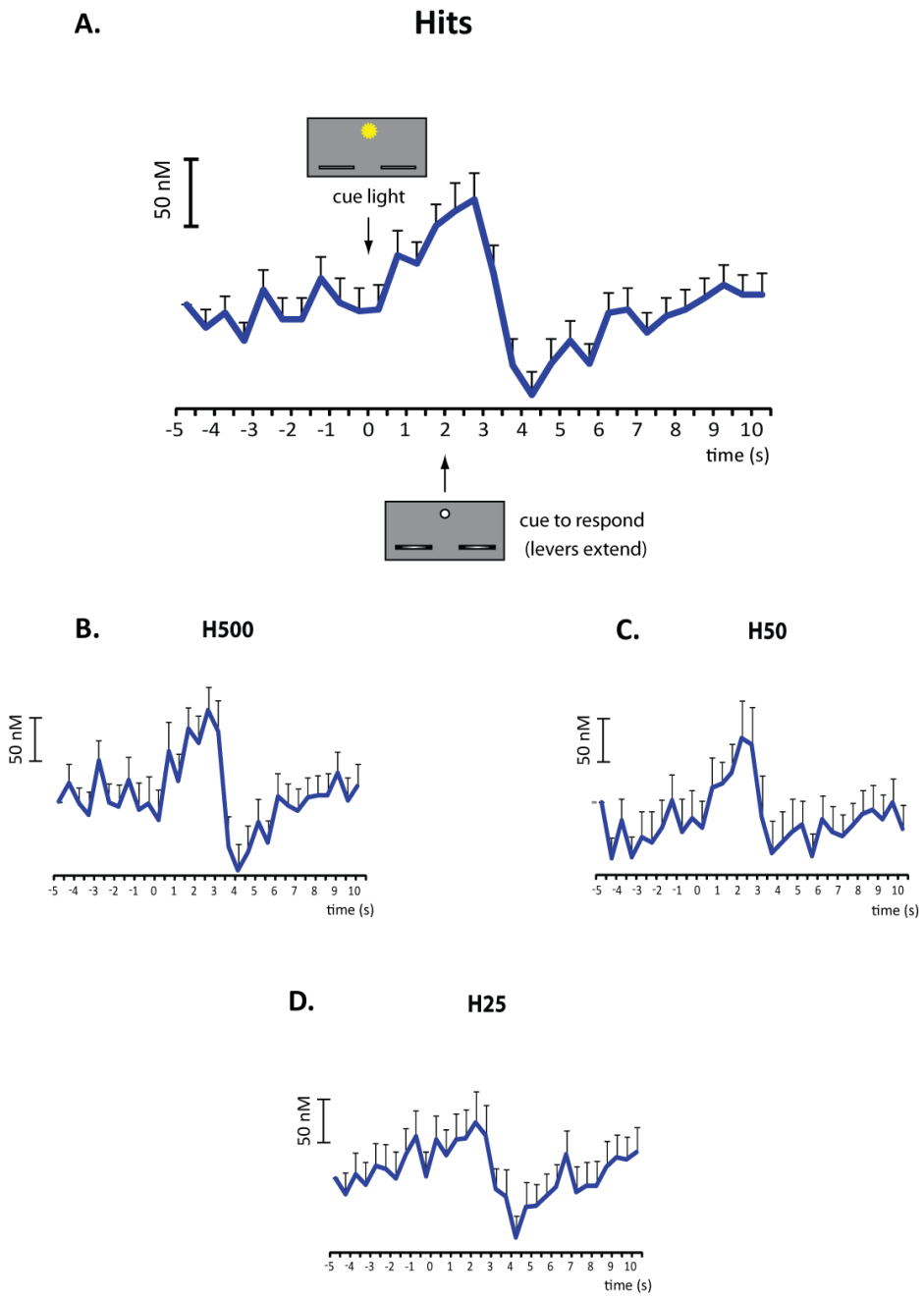

Figure 3.4

Detected cues evoked transient increases in glutamate release (A). Additional analyses suggest that the amplitude of cue-evoked glutamate release varies as a function of signal duration, with the amplitudes following 500 ms signals (B) being larger than those following 50 ms signals (C). For 25 ms signals (D), concentrations of extracellular glutamate did not exceed those at baseline. At this point, it is speculated that this is due to the relatively few number of trials included in the overall analysis, and addition of more data will show that these brief signals evoke small, but robust, increases in glutamate.

Glutamatergic activity on Correct Rejections and Misses

We were surprised to find in contrast to our preliminary hypothesis, and to the pattern of cholinergic activity, there was also a change in glutamate concentration on CR trials (Figure 3.5a). This elevation was coincident with extension of the response lever into the operant chamber (main effect of time ($F(9,1656)=39.86$, $p<0.001$; time of lever extension→2 s post, all LSDs $p<0.001$). This increase peaked at 1 s after lever extension, 302.48±34.17 nM above baseline.

The results from Misses were also unexpected (Figure 3.5b). We had predicted that on a Miss, the cue would evoke a small amount of glutamate release, however much less than on a hit, and presumably not enough to engage transient cholinergic mechanisms. However, glutamate release on Miss trials was remarkably similar to that observed on CR at the time of lever extension (main effect of time ($F(9,846)=9.57$, $p<0.001$; time of lever extension→2 s post, all LSDs $p<0.001$).. There was no change following cue presentation. This increase peaked at 1.5 s after the lever had been extended into the chamber, at 291.11 ± 83.61 nM above baseline.

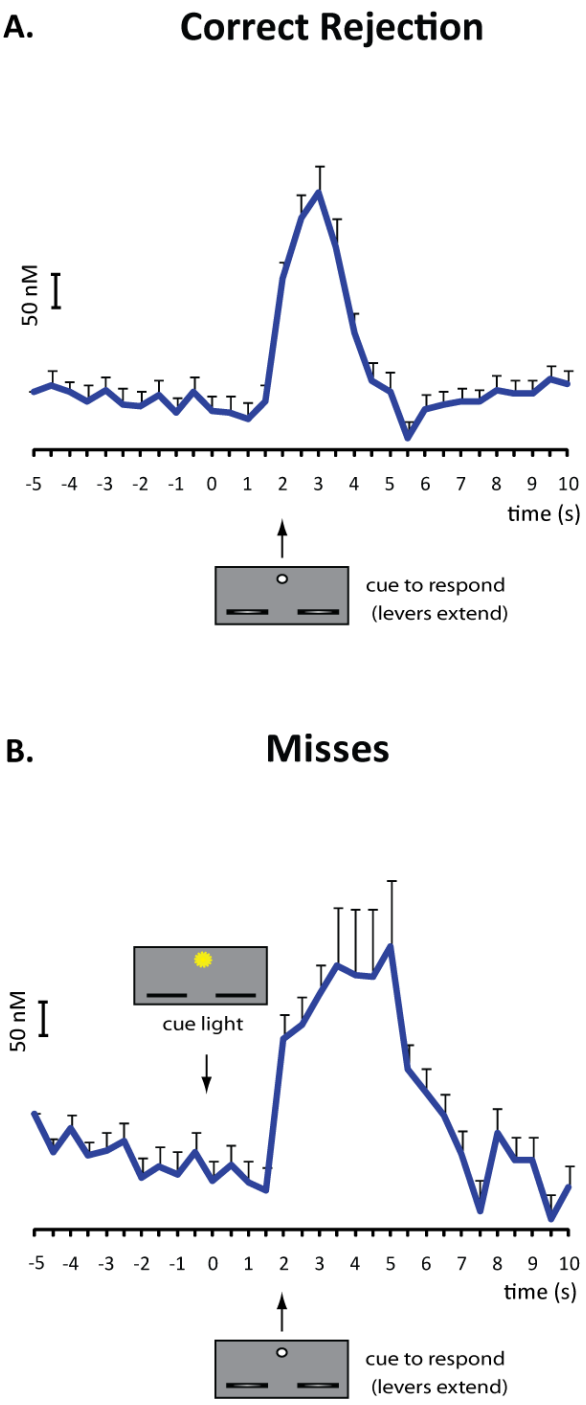

Figure 3.5
Contrary to our hypothesis, both CR (A) and Misses (B) were associated with glutamatergic transients during the period the levers were extended in to the chambers. Interestingly, for both CR and Misses, this time point coincides with the first indication to make a response.

Cue-evoked glutamate release during periods of good vs. poor performance

According to our model (Figure 3.1), the amplitude of glutamatergic transients should be modulated by tonic cholinergic activity. To address this issue, we conducted a preliminary analysis comparing glutamatergic transients recorded during periods of "good" vs "poor" performance. "Good" versus "poor" performance was defined using the SAT score. Again, this measure collapses performance on both signal and non-signal trials into a score ranging from -1 to 1, with 1 indicating perfect response accuracy, 0 random lever selection, and -1 perfectly wrong performance. The behavioral data from each animal was broken down in to 3 min blocks. SAT scores lower than 0.35 were defined as periods of poor performance. Animals typically exhibited 2-3 such periods per session. Overall, periods of good performance were characterized by SAT scores of 0.57±0.02, while periods of poor performance had a mean SAT score of 0.19±0.03 (Figure 3.6a $F(1,2)=476.67$, $p=0.002$).

Electrochemical data was then separated based on trial type from periods of good or poor performance. We next wanted to directly compare the amplitude of cue evoked glutamate on Hits from each. However, during periods of poor performance, animals had fewer Hits overall, most notably on 500 ms and 25 ms signal duration trials. Because the amplitude of glutamate on Hits appears to vary by signal duration, equivalent numbers were randomly selected and used for statistical comparison. The peak amplitude of glutamate release on Hits during

periods of good performance (Figure 3.6b) was 98.08 ± 23.87 nM above baseline, compared to 54.57±21.99 nM during periods of poor performance (Figure 3.6c; performance X interaction; $F(9,639)=2.068$, $p=0.039$).

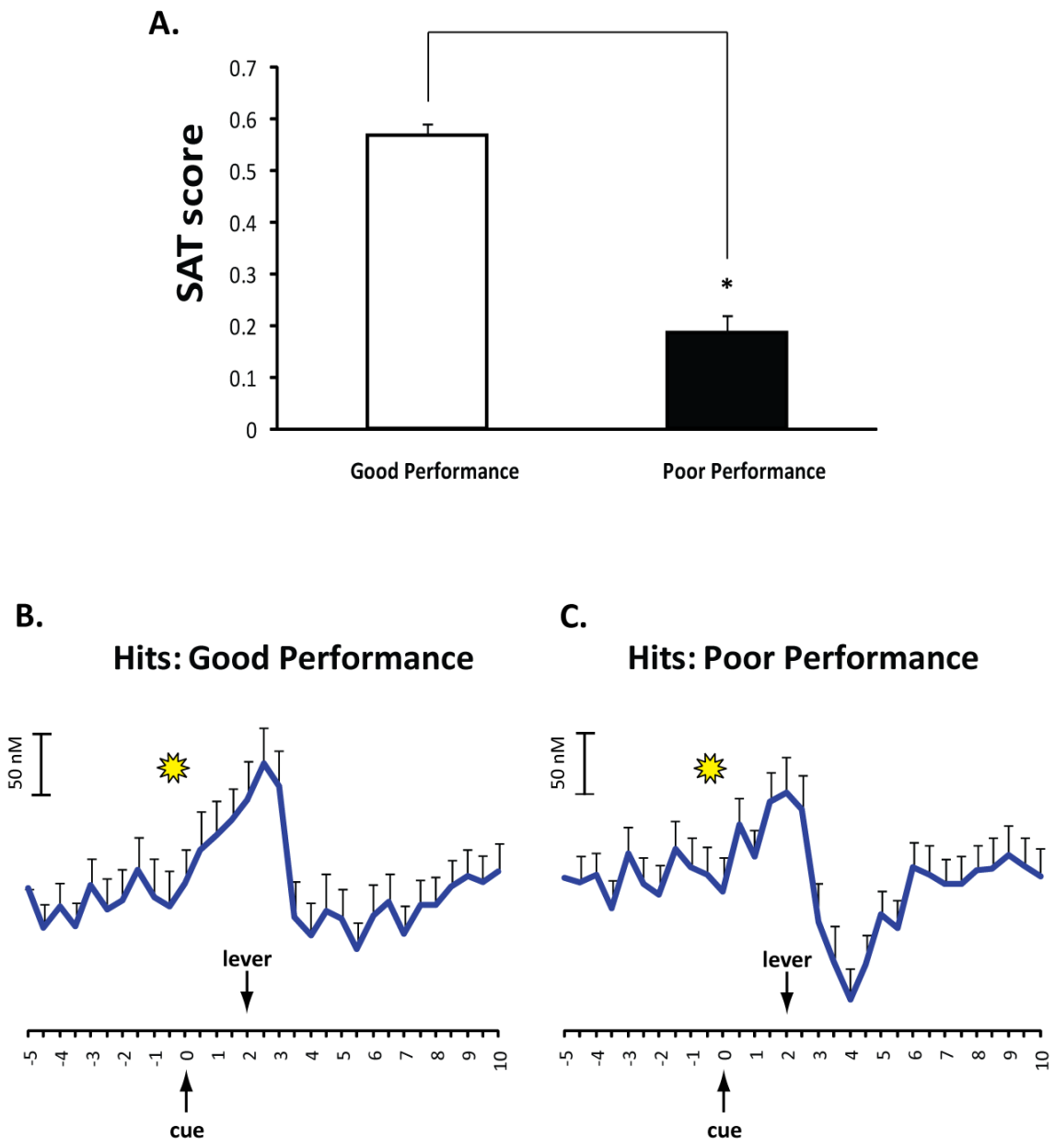

Figure 3.6

Performance varied across the behavioral test session. Good performance and poor performance periods were defined based upon SAT scores. SAT scores range from -1 or 1, with 1 representing all Hits and CRs, 0 chance performance, and -1 all Misses and FA. Good periods of performance were characterized by a SAT score of 0.57±0.021, while the SAT score during periods of poor performance hovered around chance (0.19±0.03) (A). During periods of poor performance, the amplitude of evoked glutamate release on hits was attenuated (C) in comparison to periods of good performance (B).

Discussion

Summary

Here we report that cues evoke glutamatergic transients in all detected signal trials, irrespective of sequence. The amplitudes of glutamatergic transients in trials that ended with a Hit were larger for cues with longer durations (e.g. 500>50>25 ms). On signal trials resulting in a Miss, or non-signal trials resulting in a CR, lever extension, representing the first indication that the animal can respond, also evoked a robust increase in glutamate release. Furthermore, we compared the amplitudes of glutamatergic transients from trials yielding hits during periods of high levels of performance (Figure 3.6b; SAT scores >0.35 averaged over all durations) with periods of near random performance (SAT scores: <0.35). The amplitudes of glutamatergic transients were markedly lower during poor performance periods (Fig. 3.6c). The discussion to follow will compare the characteristics of cholinergic and glutamatergic activity and address their potential relationship to one another. It will begin with the patterns that were predicted by our initial hypotheses, and follow with observations that deserve further explanation

Direct comparison of Cholinergic and Glutamatergic activity

Time course and tonic modulation

Based upon previous studies in anesthetized animals we hypothesized that cue evoked glutamate release stimulates a cholinergic transient (recall figure 3.1, cue evoked glutamate release (blue projection) drives cholinergic signaling through stimulation of ionotropic glutamate receptors situated on cholinergic inputs (red projection)). Examination of the timing of the onset of the two release events (glutamate→ACh) supports this hypothesis. Cue evoked glutamate release on Hit begins immediately following the offset of the detected stimulus, reaching levels significantly above baseline by 500 ms post-cue. Cholinergic transients begin to rise approximately 1 s after cue offset and reach levels above baseline by ~2 s. Thus in terms of time course, it seems possible that detected cue-evoked glutamate release in turn recruits local cholinergic mechanisms to produce a cholinergic transient.

We predicted that amplitudes of cue-evoked glutamate release on Hits would be larger during periods of good performance compared to periods of poor performance due to tonic cholinergic modulation of glutamate release (Figure 3.1, purple projection). During periods of good performance, the amplitude of cue evoked glutamate release was almost twice as large as during periods of poor performance (change from baseline; good performance: 98.08 ± 23.87 nM, poor performance: 54.57 ± 21.99 nM). Based upon these data, it does seem plausible that tonic cholinergic activity modulates cue-evoked glutamate release during SAT performance.

Effect of signal duration

We had predicted that the amplitude of glutamate release would be signal duration dependent, given the signal duration dependent Hit rates that characterize task performance. Indeed, the amplitude of cue-evoked glutamate on Hits is largest following 500 ms signals, and smallest for 25 ms signals. Cholinergic transients (based upon the current data) are not modulated by signal duration. This pattern suggests that the amplitude of glutamate release dictates the probability of generating a cholinergic transient.

Glutamate release events are not sequence dependent

Cholinergic transients are observed only on Hit trials that are preceded by CR and Misses. Glutamatergic transients are observed on all hits, regardless of sequence. This introduces a complication and suggests additional modulation at prefrontal synapses acting to selectively suppress cholinergic activity on Hit→Hit sequences. Possible mechanisms accounting for this are explored in the General Discussion.

A separate role for glutamate?

One of the most striking differences between the patterns of glutamate and ACh release events in the SAT is the presence of a large glutamatergic transient at lever extension on CR and Misses, where we see no measurable

change in cholinergic activity. This suggests that in addition to any potential mediation of cholinergic release events, glutamatergic signaling must also play a separate role in supporting task performance. To examine the glutamate data on its own: On Hits, a glutamatergic transient is generated by the detected cue. This transient peaks at the time of lever extension, and then sharply drops off following lever press and reward. On a CR, a large transient is evoked by the lever which peaks during the response period and then begins to decline following lever press and reward. On a Miss, again a large transient is evoked by the lever. .

The cue-light on a Hit and the lever on a CR represent the first signal to initiate a choice or a decision and are coincident with the initiation of a response (note; the lever does not evoke a glutamate release event on Hits). This signal is maintained until the choice is made and declines after receiving feedback that the choice was correct. On a Miss, the lever provides the same information that it is time to make a choice. However, after the response is made, the reward is not presented, and suggests that glutamate release does not necessarily need to be concurrent with reward.

Concluding Remarks

We began with a fairly straightforward hypothesis based upon earlier work. Results from anesthetized studies determined that mediodorsal thalamic input is required for the generation of cholinergic transients (Parikh, et al., 2010). This

glutamatergic input was hypothesized to recruit local prefrontal cholinergic mechanisms, subsequently generating a cholinergic transient. Mediodorsal thalamic inputs express α4β2 nicotinic receptors, thus tonic cholinergic activity can positively modulate the glutamatergic representation of the cue and vary the probability of signal detection (or an attention mode shift). Some of our hypotheses were supported. Glutamate release precedes cholinergic release and thus it is feasible that cue-evoked glutamatergic transients stimulate cholinergic transients under some conditions (i.e. Hit trial following a CR or a Miss). The amplitude of glutamate release is also tightly correlated with signal duration dependent hit rates; larger glutamate release events are more likely to generate a cholinergic transient and a Hit on 500 ms trials, while the smaller release event on 25 ms makes a cholinergic transient and a Hit less likely. This speculation is of course restricted to trials requiring a shift from cue-independent to cue-dependent processing given the trial sequence dependency of cholinergic transients.

Based upon the results from anesthetized studies showing the mediodorsal thalamic input is necessary for the generation of cholinergic transients (Parikh, et al., 2010), such glutamate release is hypothesized to be necessary for generating cholinergic transients in SAT performing animals. The presence of large glutamate release events at lever extension on CR and Misses indicates that it is not sufficient, and further suggests a separate or additional role for glutamate in task performance other than cholinergic activation. Future experiments, including how thalamic lesions impair task performance as well as

glutamatergic and cholinergic transients in task performing animals, will be necessary to resolve the complex relationship between cholinergic and glutamatergic signaling.

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Chapter IV: Enhancement of attentional performance by selective stimulation of α4β2 nAChRs: underlying cholinergic mechansims

Summary

Impairments in attention are a major component of the cognitive symptoms of neuropsychiatric and neurodegenerative disorders. The non-selective nicotinic acetylcholine receptor (nAChR) agonist nicotine has frequently been demonstrated to facilitate aspects of attention in humans and animals; however, these effects often were difficult to demonstrate and remained relatively small. Using an operant sustained attention task (SAT), including a distractor condition (dSAT), that was previously cross-validated for research in animals and humans, administration of the selective α4β2* nAChR agonist S 38232, but not nicotine, was found to robustly benefit the attentional performance of intact rats, specifically during the recovery of performance following distractor presentation. Consistent with evidence indicating the role of transient increases in cholinergic activity in attentional performance, and the cholinergic mediation of effects of nAChR agonists, S 38232 enhanced the detection of signals specifically in trials that involved shifts from cue-independent to cue-dependent attention. Electrochemical recordings of prefrontal cholinergic activity evoked by S 38232 and nicotine indicated that the α4β2* nAChR agonist evoked profoundly "sharper" increases in release, mirroring those that mediate shifts in attention. By co-administering the α7 nAChR antagonist MLA, the release events evoked by

nicotine are “sharpened”. Accordingly, co-administration of nicotine and MLA also enhanced the recovery of attentional performance following distracter presentation. These results indicate that compared with nicotine, α4β2* nAChR agonists produce significant enhancement of attentional performance and that the dSAT represents a useful behavioral screening tool. Finally, the electrochemical evidence supports the hypothesis that nAChR agonist-evoked increases in cholinergic activity that closely mimic those seen in performing animals predict greater pro-attentional efficacy than agonists evoking longer lasting increases in cholinergic neurotransmission.

INTRODUCTION

Efforts to discover and develop treatments for the cognitive symptoms of neuropsychiatric and neurodegenerative disorders have been hampered by the absence of effective "benchmark" drugs and the unavailability of preclinical screening and characterization procedures that reliably predict clinical efficacy of putative cognition enhancers. Furthermore, comprehensive theories that would define promising target mechanisms for the development of cognition enhancers have remained rare (Hagan & Jones, 2005; Sarter, 2006).

Attentional functions and capacities are key variables of cognitive performance (Sarter, 2006; Sarter, et al., 2005), and as such have been extensively targeted for drug induced cognition enhancement. Cholinergic activity is necessary for the performance of attention tasks (McGaughy, et al., 1996; Sarter, 2006; Sarter, et al., 2005). The development of enzyme-coated microelectrodes for monitoring real-time acetylcholine (ACh) release revealed that brief (on the scale of seconds) increases in cholinergic activity (henceforth called ‘transients’) mediate the detection of cues in attentional contexts (Parikh, et al., 2007). This finding helped explain the exclusive impairment in the ability to report the presence of a signal observed after cholinergic lesions, whereas non-signal trial-response accuracy remains completely spared (McGaughy, et al., 1996). More recent evidence recording transients from animals performing the SAT has further refined this theory (see below).

The demonstration of the beneficial attentional effects of nicotine, the most extensively studied nicotinic acetylcholine receptor (nAChR) agonist, in healthy humans, patient groups, as well as in intact animals and animal models, has been less than straight forward. The magnitude of the effects of nicotine often remained relatively small and depended on individual task parameters, treatment regimen and the subjects' prior exposure to nicotine (Mirza & Stolerman, 1998; Sarter, Parikh, et al., 2009). In contrast, accumulating evidence indicates that selective agonists at α4β2* nAChRs more robustly and reliably enhance attentional performance in patients and laboratory animals (Grottick & Higgins, 2000; Lippiello, Letchworth, Gatto, Traina, & Bencherif, 2006; McGaughy, et al., 1999; Potter, et al., 1999; Prendergast, et al., 1998; Wilens, et al., 1999; Wilens & Decker, 2007; Wilens, Verlinden, Adler, Wozniak, & West, 2006).

Local administration of α4β2 nAChR agonists evoke abrupt increases in cholinergic activity within the cortex that mirror, specifically in terms of rise time, amplitude, and decay rate, the 'sharp' transients observed in task performance (Parikh, et al., 2008). In contrast, nicotine generates long-lasting release events (up to and over 1 min) that are partly mediated through stimulation of the α7 nAChR (Parikh, et al., 2010; Parikh, et al., 2008). On the basis of these and additional results we hypothesized that α4β2 agonists more robustly enhance attentional performance than nicotine, as the long release events supported by nicotine may limit its ability to enhance trial-based performance in tasking involving cholinergically mediated cognitive operations occurring on much shorter time scales (Sarter, Parikh, et al., 2009).

The first aim of the present study was to compare the effects of a full agonist at α4β2 nAChRs, S 38232, with the effects of the non-selective agonist nicotine on the performance of rats in the distractor version of the sustained attention task (dSAT). This task was originally developed for research in animals, has been cross-validated for research in humans (Demeter, et al., 2008), and recently was suggested by the CNTRICS Initiative (Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia) for research concerning 'control of attention' (for details see (Nuechterlein, et al., 2009). The stabilization and recovery of attentional performance following distractor presentation is thought to be mediated, top-down, via activation of prefrontal networks (Demeter, et al., 2011; Johnston, Levin, Koval, & Everling, 2007; Weissman, Warner, & Woldorff, 2004). As high demands on cognitive performance are a major determinant of showing attentional benefits of nicotine in healthy subjects (Newhouse, Potter, & Singh, 2004), dSAT performance was expected to reveal such effects. The second aim of this study was to determine the properties of cholinergic release events evoked by the nicotine and S 38232. Third, we tested the hypothesis that co-administration of nicotine and the α7 nAChR antagonist MLA results in greater pro-attentional effects due to the honing nicotine-evoked ACh release by α7 nAChR blockade.

Finally, in animals performing the SAT, transient increases in prefrontal cholinergic activity were observed specifically in signal trials that yielded a hit (or a detection) if such trials were preceded by non-signal trials yielding correct rejections, or by signal trials yielding misses (see Chapter 2). This finding

suggests that prefrontal cholinergic transients shift prefrontal circuitry into a mode that allows cues to control attention (cue-dependent attention), and away from a mode that controls attention based on task rules not involving cue detection (cue-independent attention). Therefore, a final analysis explored the possibility that that the beneficial effect of the α4β2 nAChR agonist is due to the facilitation of performance on trials requiring such shifts.

METHODS

Subjects

For the behavioral experiments, subjects were male Wistar rats (Harlan, IN) approximately 200-300 g at the onset of training. Animals were kept on a 14:10 light/dark cycle in a temperature and humidity-controlled vivarium. Water was available only as a reward during testing and for 30 min upon the completion of a training/testing session. Food was available *ad libitum*. All training and testing took place during the light cycle. Subjects used for electrochemical studies were adult male Fisher/Brown Norway hybrid rats (FBNF1; Harlan, IN; n=21) approximately 200-250 g at the beginning of the experiments. Food and water was available *ad libitum.* All animals were maintained in accordance with the NIH guide for the Care and Use of Animals and experiments were conducted in accordance with protocols approved by the University of Michigan Committee on Use and Care of Animals (UCUCA).

Sustained attention task (SAT) acquisition and performance criteria

Animals were trained in the same operant chamber daily. In the first stage of training animals were shaped to press each of the two response levers to gain access to a water reward. A press on either lever led to access to 0.1 mL of water (FR-1 schedule), with the rule that if at any time there were 5 more presses on one lever relative to the other, the lever becomes inactive until the other is pressed. This rule was designed to discourage the development of a lever or side bias. This phase of training continued until animals reached the criterion of 120 rewards per session (approximately 45 min) for two consecutive days.

In the second stage of training, animals had to discriminate between signal (illumination of central panel light) and non-signal (no illumination of central panel light) trials. Each session included 160 trials equally divided between signal and non-signal. Correct responses were rewarded with water. On a signal trial, the signal light was turned for 1 second, followed two seconds later by the extension of the response levers into the chamber. A press on the left lever was considered correct, and scored as a Hit. A press on the right lever was scored as a Miss. On non-signal trials, a press on the right lever was considered correct, and the trial scored as a correct rejection (CR). A press of the left lever was considered incorrect, and scored as a false alarm (FA) (note: half of all animals were trained with non-signal and signal response levers in the reverse position). The levers were retracted after being depressed regardless of whether the response was correct or incorrect. If no press occurred after four seconds,

both levers were retracted and the trial was scored as an omission. Incorrect responses were followed by correction trials identical to the previous trial. After three consecutive errors on correction trials, animals were given a forced choice trial. The inter trial interval (lTI) was 12±3 s during this stage of training. Performance criterion for this stage was >70% hits and correct rejections for three consecutive sessions.

In the third stage of training the ITI was decreased to 9±3 s and there were no correction or forced trials. The signal duration remained 1 s and sessions consisted of a total of 162 trials. As with the previous stage, criterion for advancement was >70% hits and correct rejections for three consecutive sessions.

In the final version of the task, signal duration was shortened and varied (500, 50, or 25 ms). Training sessions were timed to 40 minutes, equivalent to ~200 trials, half signal and half non-signal. Animals were trained on the final version of the task until performance reached a plateau. Only animals with >70% hits to 500 ms signals and >70% correct rejections were employed in the present experiments. After reaching criterion animals continued daily practice sessions and were habituated for one week to systemic injections (i.p.) of saline.

Assessment of performance during the distractor condition (dSAT)

In the dSAT, the first 8-min block of trials (block 1) was identical to the SAT described above. This block was followed by a 16-min block (block 2) with

the distractor (chamber houselight flashing on/off at 0.5 Hz) turned on. Following distractor termination, performance recovery was determined during a final 16-min block of the regular SAT (block 3). Animals practiced the dSAT a minimum of two times before the effects of S 38232 and nicotine on performance were tested. Individual distractor test sessions were separated by a minimum of 2 days/SAT practice sessions, with performance at or above criterion level. Importantly, our evidence suggests that repeated exposure to the distractor does not significantly alter the efficacy of the distractor or the rate of post-distractor performance recovery.

Measures of SAT and dSAT performance

Data from both SAT and dSAT test sessions were grouped into three blocks of trials for analysis; the first 8 minutes of task performance (block 1), the second 16 minutes of task performance (block 2), and the final 16 minutes of task performance (block 3). For each session, the total number Hits and Misses by signal duration, CR, FA, and omissions, were recorded. Using these values, the relative number of Hits (h=H/(H+M)), correct rejections (CR=CR/(FA+CR)), misses (1-Hits), and false alarms (1-CR) were determined. To generate an index of the animals' overall performance on both signal and non-signal trials, a sustained attention task score (SAT, dSAT for distracter sessions) was calculated (SAT or dSAT= (Hits-FA)/(2(Hits+FA)-(Hits+FA)^2)). SAT/dSAT scores range from -1 to 1, with 1 indicating perfect accuracy in signal and non-signal

trials, 0 chance performance, and -1 depicting that all responses were incorrect (misses and false alarms). The SAT/dSAT score is a variation of the sensitivity index (Frey, 1973) and is based upon the relative number of hits and false alarms, as opposed to the probability of such responses, and thus is not confounded by errors of omission. Scores were calculated for each block of trials, signal duration, as well as averaged across signal durations.

Analysis of the probability of a hit depending on prior trial outcome

We hypothesized that nAChR agonist-induced enhancement in performance manifests primarily in terms of increases in hits (event 1), and specifically if such hits were preceded by non-signal trials resulting in a correct rejection (event 2). Employing the multiplicative law of probability (see Chapter 2 for further explanation), the joint probability for two such events, as well as for several control events, was calculated (Howell, 1989).

Drugs, drug administration, and doses

S 38232, a pyridinylamino-cyclopropanamine derivative, was obtained from Institut deRecherches Internationales Servier (Courbevoie, France). The compound is a full agonist at α4β2 nAChRs (Lagostena, et al., 2010) with an EC_{50} of $3.4x10^{-6}$ M to rat α4β2 nAChRs expressed in Xenopus Laevis oocytes. Furthermore, the compound has a low partial agonist activity at rat α7 nAChR

(EC_{50} of $1.3x10^{-4}$ M). Nicotine (nicotine hydrogene tartrate; Sigma-Aldrich, St. Louis, MO) and S 38232 were administered to two groups of animals. SAT performance was determined after administration of S 38232 (n=11; 0.03, 0.30, 1.00, and 3.00 mg/kg) or nicotine (n=14; 0.02, 0.10, 0.40 mg/kg; base weights). Both compounds were dissolved in sterile saline. Animals were given a systemic injection (i.p.) and placed into the operant chambers for the duration of a pre-task wait period (15 min for nicotine, 30 min for S 38232). The administration of doses and vehicle was randomized for each animal. Successive administrations of drug doses were separated by a minimum of 2 days/sessions where the animal's SAT performance was at or above criterion level. Vehicle was administered prior to all other test sessions. The selection of doses for dSAT testing was based on effects on SAT performance and therefore is explained in Results. Finally, we tested the effects of nicotine (0.1 mg/kg) administered together with the α7 nAChR antagonist MLA citrate hydrate (MLA; Sigma-Aldrich; n=9; 1.0 or 5.0 mg/kg; 1ml/kg for all injections; 15 min before task onset). All compounds were dissolved in sterile saline. The pH of the solutions (7.4-7.6) was adjeusted using sodium hydroxide solution. These effects were determined in animals that also received nicotine alone before a dSAT performance test to allow for within-subjects comparisons.

Electrochemical recordings of nAChR agonist evoked cholinergic transients *in vivo*

Cholinergic transients were measured using ceramic based choline selective microelectrodes and fixed-potential amperometry (Quanteon, Nicholasville, KY). Each electrode was equipped with four platinum recording sites arranged in side by side pairs. Choline oxidase (CO) was cross-linked with a bovine serum albumin (BSA)/glutaradehyde solution and immobilzed on the bottom pair of recording sites. The other pair was coated with the BSA/glutaraldehyde solution alone and served to record fluctuations in background current. *Meta*-phenylene diamine (*m*PD) was electropolymerized onto the surface of the recording sites to block electroactive interferents. Electrodes were calibrated *in vitro* and characterized by a sensitivity for detecting choline of 8.57±1.40 pA/µM, a selectivity of choline:AA of 512.32±276.93, and a highly linear response to increasing choline concentrations (20-80 µM): R^2: 0.957±0.012. Animals were anaesthetized with urethane (1.25-1.5 g/kg; i.p.) and placed in a stereotaxic frame. Body temperature was maintained at 37 °C with an isothermal pad. Single barrel glass capillaries (1.0 mm X 0.58 mm, 6 in; A-M systems, Inc., Carlsborg, WA) were pulled using a micropipette puller (Model # 51210, Stoelting, Wood Dale, IL). Micropipettes (inner tip diameter ~15 ìm) were attached to the microelectrode with the tip centered between the two pairs of recording sites, ~70 ìm from the surface of the electrode. The assembly was positioned in either the right or left medial prefrontal cortex (AP: +3.0 mm, ML ± 0.7 mm, DV: -3.0 mm from bregma). An Ag/AgCl reference wire was implanted in the opposite hemisphere, and a fixed potential of +0.7 V was held between the reference and recording electrodes. Amperometric recordings were made at 1Hz,

and data was digitized using a FAST-16 recording system (Quanteon LLC, Nicholasville, KY). Experiments began following stabilization of baseline current (45-60 min). Drug solutions were pressure-ejected through the micropipettes and ejection volumes were monitored via a stereoscope equipped with a reticule. S 38232 was delivered via intracranial pressure ejections of 200 nL of drug solution (40 pmol, n=5; 200 pmol, n=6, and 2 nmol, n=5). Cholinergic transients evoked by S 38232 were compared to those evoked by identical amounts of nicotine (data taken from Parikh *et al,* 2008). In addition, the effects of dihydro-â-erythoidine (DHβE), a relatively selective β2 nAChR antagonist, on signals evoked by S 38232 was assessed. DHβE (Tocris Bioscience; Ellisville, MO) was infused (1.6 nmol in 800 nL over 5 s), and was followed 3 min later by a series of 3 pressure ejections of S 38232 (2 nmol, n=5). Current recordings on CO-coated channels were self-referenced by subtracting the current recordings from the sites coated with the protein matrix alone. Transients evoked by S 38232 or nicotine were compared with respect of peak amplitudes and signal decay rate (t50; time required for the signal to decline by 50% of peak amplitude). Data from 3 cholinergic signals per drug manipulation and per animal were averaged and used for statistical analysis.

Statistical methods

Performance was analyzed using repeated measures ANOVAs with dose of drug, performance block, and signal duration as within-subjects factors. Mixed

model ANOVAs with the between-subjects factor of group were used to demonstrate that baseline performance did not differ between animals treated with nicotine or S 38232. One-way ANOVAs with dose as a between subjects variable were used to determine dose-response relationships and to compare evoked cholinergic transients evoked by the two nAChR agonists. All post hoc analyses employed the least significant differenced test (LSD). When necessary, main effects and interactions were further investigated with independent and paired samples t-tests (α=0.05). Exact p-values were reported.

RESULTS

Baseline SAT performance

Following the administration of vehicle, SAT performance did not differ between animals being treated with nicotine and S 38232, with respect to the overall SAT score or the individual measures of accuracy in signal (hits) and non-signal trials (correct rejections; all effects of group $p>0.13$). Likewise, the effects of group did not interact with any of the task parameters (signal duration or block; all $p>0.21$). SAT and Hit scores declined significantly with decreasing signal duration (SAT: $F(2,46)=112.67$; $p<0.001$; hits: $F(2,46)=184.09$; $p<0.001$; see Figure 4.1a). Animals correctly rejected 88.37±1.21% of non-signal events (Figure 4.1b). SAT performance did not vary across the 3 blocks of trials, and the effects of block did not interact with group and/or signal duration (all $p>0.45$). Finally, animals omitted few trials (0.88±0.26% of ~200 trials/session) and the

number of omissions did not differ between the groups (p=0.44).

Effects of nicotine on SAT performance

Administration of the non-selective nAChR agonist nicotine (0.02, 0.1, 0.4 mg/kg) did not enhance SAT performance (dose: $F(3,39)=1.31$; $p=0.28$). Further, as illustrated in Figure 4.1c, the highest dose of nicotine produced a small, but robust decrease in the Hit rate to longest signals (dose X signal: $F(6,78)=5.50$; $p<0.001$; post hoc comparisons indicated on figure). Furthermore, all three doses of nicotine resulted in a decrease in the relative number of CR during block 1. These effects were not seen during subsequent blocks of trials (dose: $F(3,39)=5.63$; $p=0.013$; dose X block: $F(6,78)=6.42$; $p<0.001$; Figure 4e). Finally, the highest dose of nicotine also increased the number of omitted trials ($F(3,39)=8.05$; $p=0.014$), from 0.66±0.29% after vehicle to 12.29±4.07% after administration of 0.4 mg/kg).

Effects of S 38232 on SAT performance

Administration of S 38232 (0.03, 0.3, 1.0 and 3.0 mg/kg) did not affect Hits (Figure 4.1d; all main effects and interactions including dose: $p>0.10$). However, the highest dose of S 38232 resulted in a small yet significant decrease in the relative number of CR during the second block of trials (Figure 4.1f; dose: $F(4,40)=0.84$; $p=0.49$; dose X block: $(F8,80)=2.31$; $p=0.03$). The number of

omitted trials increased over blocks of trials ($F(2,20)=5.30$; $p=0.02$), from 1.46±0.66% in block 1 to 2.26±0.65% in block 3; however, this increase was not affected by the administration of S 38232 ($F(8,80)=1.90$; $p=0.15$).

Taken together, neither the administration of the selective α4β2* nAChR agonist nor the non-selective agonist nicotine benefited SAT performance. Following the administration of the highest dose of either compound, relatively small-sized impairments in SAT performance were observed and remained limited to a particular block of trials.

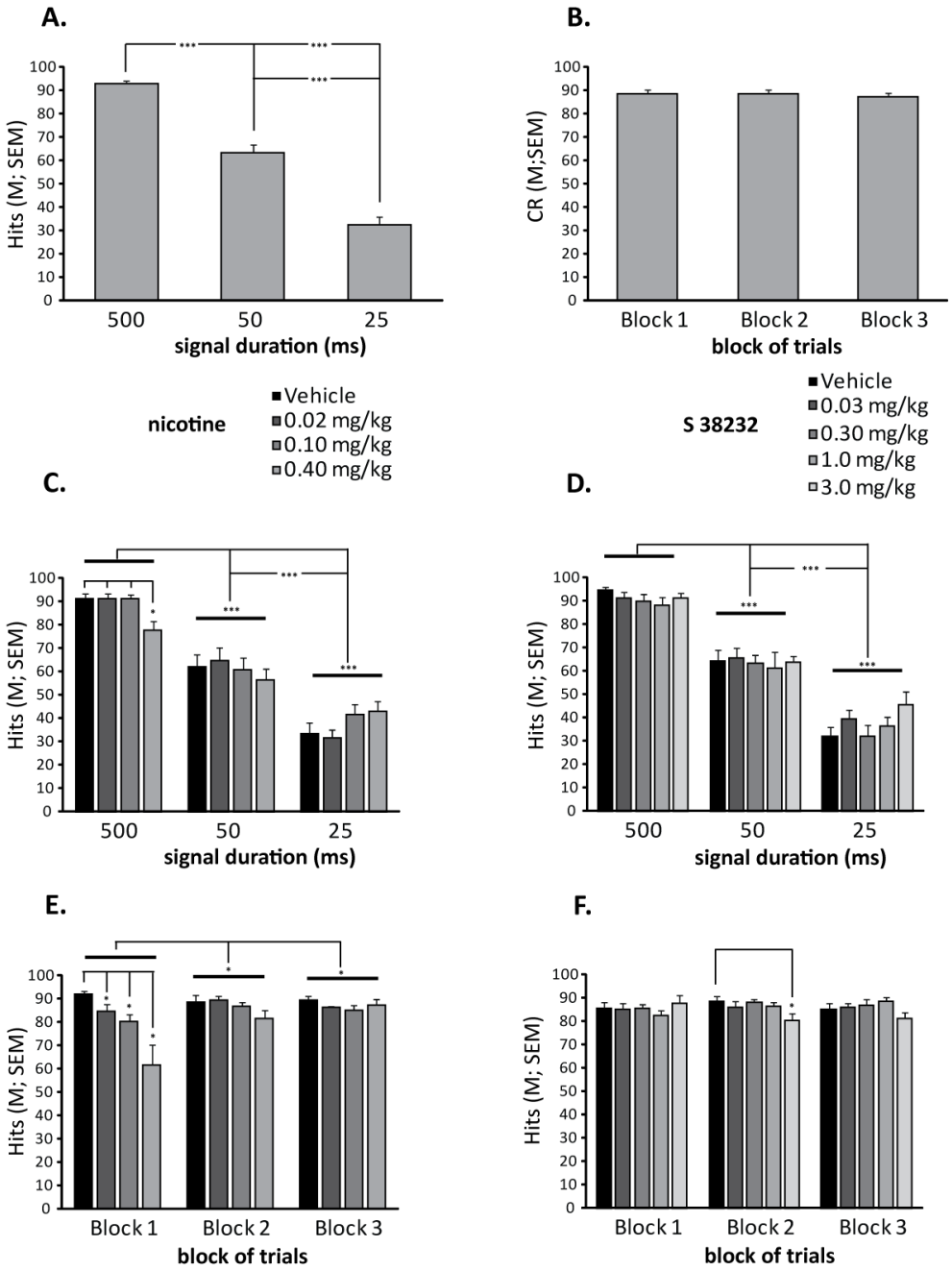

Figure 4.1

Baseline SAT performance and effects of S 38232 and nicotine. (A) Depicts baseline Hit rate as a function of signal duration. (B) CR rate over the three blocks of trials. Baseline performance did not differ between the groups treated with S 38232 and nicotine. (C) Administration of the high dose of nicotine decreased the number of Hits on 500 ms signals. (D) Administration of S 38232 did not affect Hits. (E) All doses of nicotine decreased the CR in the first block. (F) There was a small decrease in the number of CR in block 2 after the highest dose of S 38232. (*p<0.05, **p<0.01, ***p<0.001).

Distractor-induced impairment and performance recovery

The dSAT baseline (vehicle) performance did not differ between animals scheduled to be treated with S 38232 or nicotine (all main effects of group and interactions involving group: $p>0.20$). As illustrated in Figure 4.2a and b, presentation of the distractor resulted in robust decreases in both the relative number of Hits and CR. Concerning Hits, the effect of block interacted significantly with signal duration (block: ($F(2.46)=23.66$; $p<0.001$; block X signal: $F(4,92)=16.21$; $p<0.001$). Post hoc comparisons (illustrated in 4.2a) indicated an acute decrease in Hits during the distractor block and a partial but incomplete recovery of the Hit rate to longest signals during the post-distractor block. In contrast, Hits to medium signals did not recover and Hits to the shortest signals were lower during the post-distractor block than during both preceding blocks of trials. In contrast, the distractor-induced decrease in CR recovered completely during block 3 (main effect of block: ($F(2.46)=73.26$; $p<0.001$); Figure 4.2b).

Facilitation of dSAT performance by S 38232

Based on the detrimental effect of the highest dose of S 38233 on SAT performance (see above), and inspection of (insignificant) effects of dose on all measures of SAT performance, a dose of 0.3 mg/kg was selected for dSAT testing. In the analysis of effects on dSAT performance, putative drug effects were expected to interact with the factor 'block', reflecting that drug effects would

manifest in the presence of, or subsequent to, distractor presentation. Such an interaction was found with respect to Hits ($F(2,20)=6.68$; $p=0.006$).

As would be expected, based on the effects of this dose of S 38232 on SAT performance (above), post hoc comparisons did not indicate an effect of S 38232 on Hits in the pre-distractor period (block 1). Likewise, S 38232 did not affect the low number of Hits during the presence of the distractor in block 2. However, S 38232 enhanced the Hit rate during the post-distractor block 3 ($F(1,10)=7.09$; $p=0.024$; Figure 4.2d).

Effects of nicotine on dSAT performance

As illustrated in Figure 4.1c, SAT performance was robustly impaired by administration of the highest dose of nicotine (0.4 mg/kg). Therefore the next to the highest dose was selected for dSAT testing (0.1 mg/kg). Furthermore, this dose was previously shown to enhance attentional performance in the presence of bursts of white noise. (Hahn, Shoaib, & Stolerman, 2002). However, we did not find any main effects of dose (all $p>0.59$) and no interactions between the effects of nicotine and block of trials on any measure of performance (4.2c; all $p>0.11$).

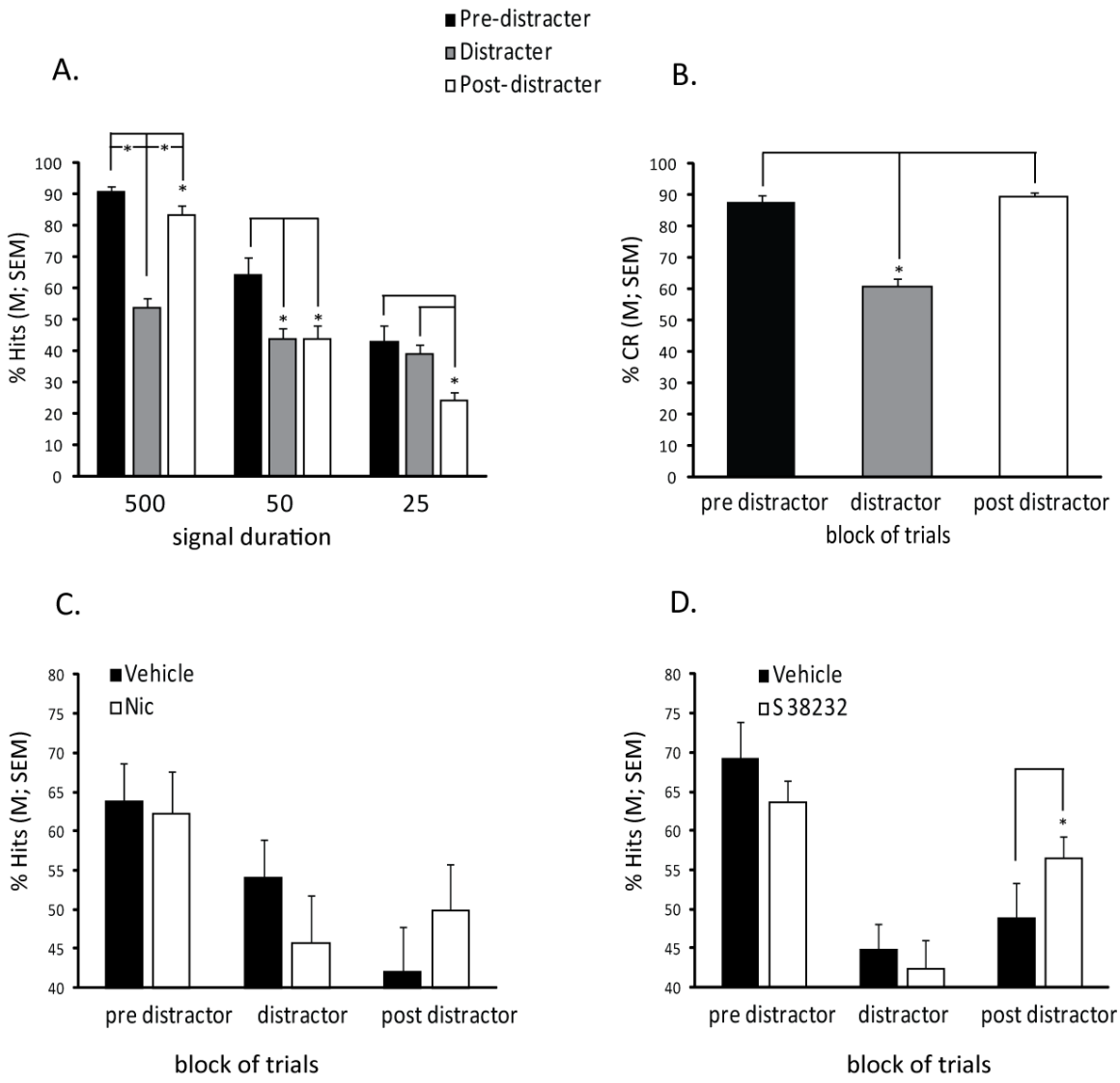

Figure 4.2

Characterization of the effects of distractor presentation on performance: (A) depicts the relative number of Hits for all for all three signal durations and across the three blocks of trials. The Hit rate to longest, but not shortest, signals recovered partly during the post-distractor block of trials. In contrast, CR recovered completely (B). Shown in (C), the nicotine did not affect dSAT performance. (D) Administration of S 38232 benefited the Hit rate during the post-distractor block of trials.

nAChR agonist-evoked cholinergic transients

We previously demonstrated that nAChR agonists evoke transient increases in acetylcholine (ACh) release in the prefrontal cortex and identified major aspects of the underlying neuronal mechanisms. This evidence supports the hypothesis that the greater pro-attentional efficacy of selective α4β2 nAChR agonists is related to their ability to evoke large and "sharp" cholinergic transients in contrast to nicotine (Parikh, et al., 2008; Sarter, Parikh, et al., 2009). Thus, we determined the cholinergic transients evoked by S 38232 and compared their amplitudes and decay rates with those evoked by nicotine.

The choline-sensitive microelectrodes used in these experiments were characterized by a sensitivity of 8.57±1.40 pA/μM, and a selectivity ratio for choline of relative to ascorbic acid. Administration of S 38232 (40, 200, 2000 pmol) evoked cholinergic transients that were characterized by dose-dependent increases in amplitude ($F(2,13)=29.05$; $p<0.001$; post hoc LSDs indicated that the amplitude after the two higher doses was larger than those evoked by 40 pmol; Figure 4.3a and d). Consistent with the classification of S 38232 as a selective α4β2* nAChR agonist, the amplitude of the cholinergic transient evoked by S 38232 (2 nmol) was almost completely attenuated by co-administration of the relatively β2-selective antagonist DHβE (1.6 nmol; residual amplitude: 1.43±0.19 μM; $t(8)=192.81$; $p<0.001$).

Compared with the amplitudes of cholinergic transients evoked by nicotine (40 and 200 pmol, 4 and 20 nmol; Parikh *et al,* 2008), S 38232 was significantly more potent than nicotine in evoking cholinergic transients (Figure 4.3a,d).

Comparisons between the amplitudes evoked by the two lower doses of S 38232 indicated larger cholinergic signal amplitudes compared with the amplitudes evoked by identical doses of nicotine (40 pmol: $t(8)=3.94$; $p=0.004$; 200 pmol: $t(8)=3.11$; $p=0.01$). The amplitude produced by the highest dose of S 38232 corresponded with that evoked by the highest dose of nicotine (20 nmol; $p=0.59$). As Illustrated in Figure 4.3d, amplitudes generally reached a plateau at 5.5-6 μM, indicating that the greater potency of the selective agonist was not associated with greater efficacy.

Cholinergic transients evoked by S 38232 were robustly 'sharper' than those evoked by nicotine. First, as illustrated in Figure 4.3c, dose of S 38232 did not affect the relatively short rise time of transients evoked by this compound (time from administration of the compound to peak amplitude; $F(2,15)=0.15$; $p=0.858$; 5.73±0.28 s). In contrast, nicotine-evoked signals required robustly more time to reach peak amplitude, up to almost 30 s for the highest dose (note again that peak amplitudes did not differ between the highest dose of S 38232 and nicotine; above). Even at the second dose, 200 pmol, nicotine-evoked signals were slower to reach peak amplitude (nicotine: 21.75 ± 7.60 s; S 38232: 5.94±1.04 s; $t(8)=2.62$, $p=0.03$).

Second, as illustrated in Figure 4.3e, t_{50} values of cholinergic signals evoked by S 38232 remained below 10 s even after the highest dose, contrasting with nicotine-evoked signals that required to almost 70 s to decline by 50% from peak values after the highest dose. Dose of S 38232 affected the time required for the amplitude of cholinergic transients to decrease by 50% from peak levels

(t_{50}) ($F(2,15)=4.14$; $p=0.041$). However, post hoc LSDs revealed that this effect was due to a relatively small increase in t_{50} (about 2 s) after the administration of 200 pmol when compared with the effects of the lower and higher dose (40 pmol and 2 nmol; Figure 4.3e). Even at the second dose, 200 pmol, nicotine-evoked signals were significantly slower to decay (t_{50}; nicotine: 39.75±12.43 s; S 38232: 9.95±0.72 s; $t(8)=2.82$; $p=0.02$). Collectively, these results indicate that S 38232 evokes cholinergic transients with greater potency when compared with nicotine, and that cholinergic signals evoked by S 38232 differ from those resulting from nicotine with regard to rise times and decay rates, yielding robustly 'sharper' cholinergic transients evoked by S 38232.

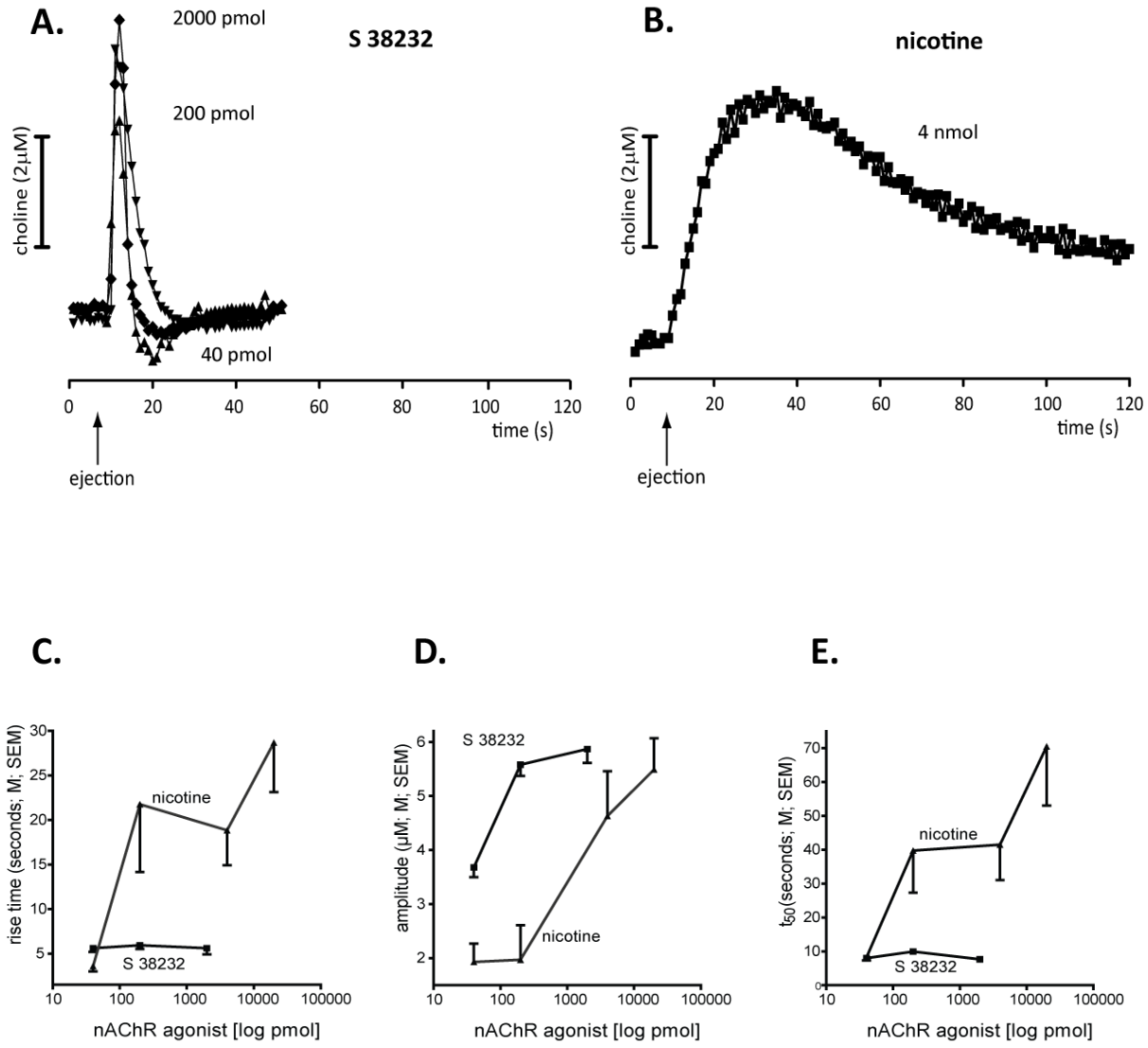

Figure 4.3

Transient increases in prefrontal acetylcholine release evoked by S 38232 and nicotine (the nicotine data are used for comparison and were adopted from (Parikh, et al., 2008)). (A,B) provides examples of individual traces evoked by S 38232 (A) and nicotine (B).. (C) The time required for transients to reach peak amplitude ('rise time'). Note the relatively fast and stable rise times for S 38232 compared to nicotine. (D) Peak amplitudes. S 38232 was more potent, but not efficacious, than nicotine. (E) Transients evoked by nicotine were relatively slow to return to baseline taking ~70 s to decrease by 50% of peak (t_{50}). In contrast, t_{50} values for S 38232 were flat, also taking less than 10 s. Taken together, S 38232 evokes 'sharper' transients than nicotine.

Blockade of α7 nAChR 'sharpens' nicotine-evoked transients

As illustrated in figure 4.4 b-e, nicotine-evoked cholinergic transients were characterized by slower rise times and decay rates when compared with those evoked by the selective α4β2 nAChR agonist. We earlier observed that blockade of the α7 nAChR with MLA did not affect the amplitude of nicotine-evoked cholinergic transients, but partly attenuated the slow rise time and slow decay rate (Figure 4.4 b-e; these data were taken from Parikh et al., 2008 and integrated into figure 4.3 to provide a justification for the test of the effects of the co-administration of nicotine and MLA on dSAT performance). More recently, we determined the effects of nicotine in mice lacking the α7 nAChR and again found that the slow rise time and decay rate of nicotine-evoked cholinergic signals are partly mediated through the α7 nAChR (Parikh, et al., 2010). Thus, with respect to cholinergic transients, blocking the α7 nAChR converts nicotine into a more selective α4β2 nAChR agonist.

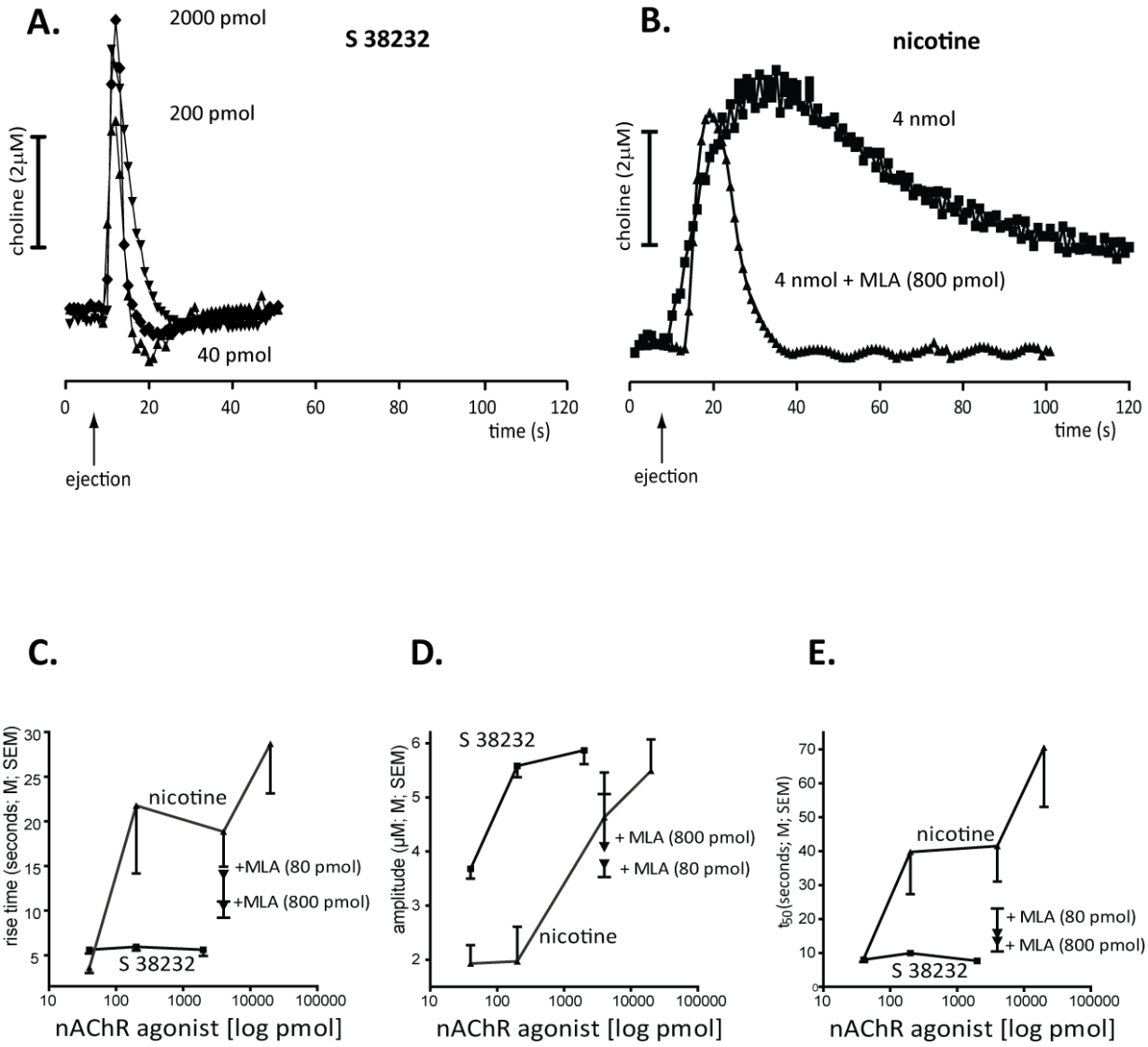

Figure 4.4

Effect of MLA on nicotine evoked cholinergic transients. (B) Nicotine-evoked cholinergic transients are 'sharpened' by MLA. (C) Blocking the α7 nAChR, by co-administration of MLA, shortened the rise time of nicotine-evoked cholinergic transients. (D) Blocking the α7 nAChR did not reduce the amplitudes of nicotine-evoked transients. (E) Co-administration of MLA accelerated the decay rate of nicotine-evoked cholinergic signals, reducing t_{50} values to close to those for the α4β2 selective agonist.

Co-administration of nicotine and MLA enhances dSAT performance

Compared with the administration of saline or nicotine (0.1mg/kg) alone, co-administration of this dose of nicotine and MLA (1.0 mg/kg) enhanced the relative number of hits during dSAT performance. As indicated in the Methods, we also tested a higher dose of MLA (5.0 mg/kg); however, co-administration of nicotine and the higher dose of MLA impaired performance, likely reflecting non-selective antagonist effects of MLA (Lopez-Hernandez, et al., 2009). Therefore, these data are not described.

Compared with vehicle and nicotine administered alone, the co-administration of nicotine and MLA enhance the Hit rate across all three blocks of trials of dSAT performance (Figure 4.5b; main effect of treatment: $F(2,16)=5.46$; $p=0.016$). Figure 4.5a shows the effects over blocks. This figure suggests that the co-treatment increased Hits particularly robustly during the post-distractor block, as with S 38232, although the effect of treatment did not interact with the effect of block ($p=0.18$).

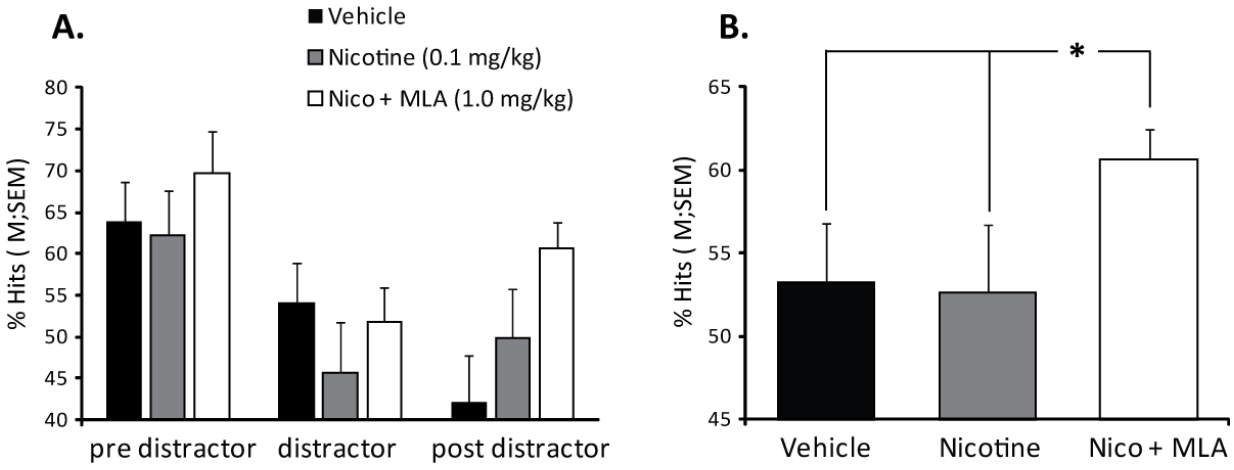

Figure 4.5

Effect of nicotine and MLA co-treatment. Co-administration of nicotine (0.1mg/kg) and MLA (1.0 mg/kg) enhanced the Hit rate across all blocks of the dSAT test session (B). Although (A) suggests that the beneficial effects of the co-treatment were most robust in the post-distractor recovery period, there was no treatment X block interaction. Here we again demonstrate that nicotine administered alone does not significantly benefit dSAT performance.

Effects of S 38232 on the performance in signal trials that followed correct rejections or misses

This analysis was based on the following rationale. We previously demonstrated that the detection of signals requires a transient, second-based increase in prefrontal cholinergic transmission (Parikh, et al., 2007). More recent evidence indicates that such transients are evoked in Hits specifically if they follow non-signal trials that yielded a correct rejection or signal trials that yielded a Miss (and thus may be considered perceived non-signal trials). Analyses of the joint probability of a Hit on signal trials and for these signal trials to follow CR or Misses were conducted to test the prediction that the increase in hits produced by S 38232 during the post-distractor block 3 was due primarily to hits in signal trials that involved cue independent and cue dependent processing. Administration of S 38232 significantly increased the joint probability for a Hit in signal trials if preceded by CR or Misses (see bar graph in Figure 4.6; $t(9)=2.72$; $p=0.023$). In contrast, treatment with S 38232 did not affect the joint probability for hits that were preceded by hits or false alarms ($t(9)=1.03$; $p=0.33$).

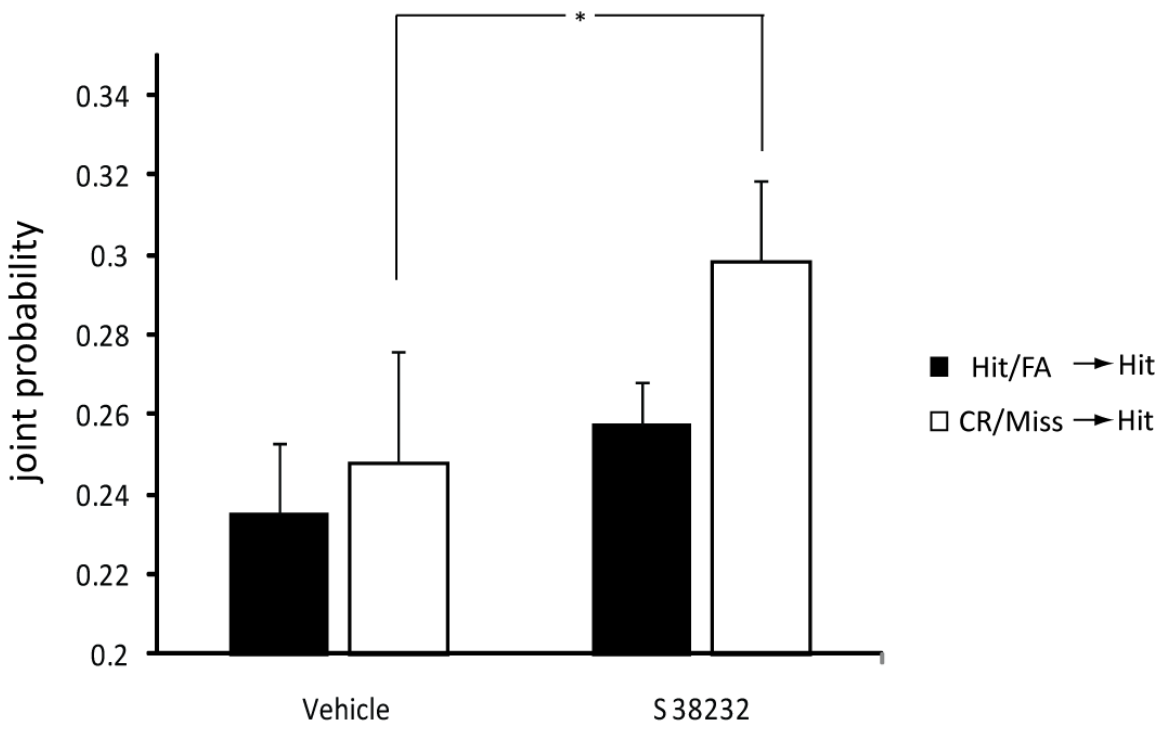

Figure 4.6

Analysis of the joint probabilities for Hit trials during the post-distractor period where the beneficial effects of S 38232 were observed. Compared to performance under vehicle, S 38232 increased the joint probability for a Hit following either a CR or a Miss. The joint probability for a Hit following another Hit or a False Alarm was not augmented. This evidence forms the prediction that nAChR agonist-evoked enhancement of attentional performance manifests primarily in terms of Hits in trials that require a shift from cue-independent to cue-dependent processing.

DISCUSSION

The main results of the behavioral and electrochemical experiments described above indicate that administration of a selective α4β2 nAChR agonist, but not nicotine, facilitates attentional performance under taxing conditions. Furthermore, compared with nicotine, the α4β2 nAChR agonist was more potent in evoking cholinergic transients which were characterized by very rapid termination of release, yielding "sharp" spikes in cholinergic activity. The more slowly rising and decaying transients evoked by nicotine are ‘sharpened’ by blocking the a7 nAChR; accordingly, we found that co-administration of nicotine with MLA robustly increased the Hit rate of dSAT-performing animals. Results from trial-sequence analyses indicated that the beneficial performance effects were due to an increase in Hits in (signal) trials that followed either CR or Misses. Combined, these findings are consistent with the hypothesis that such selective agonists are able to amplify without broadening such transients, and thereby benefit the detection of signals, or more specifically performance on signal trials requiring a shift from cue-independent processing, in attention tasks. The discussion below will focus on a) the lack of beneficial effects of nicotine, b) the cognitive and neuronal mechanisms underlying the attentional effects of the selective α4β2* nAChR agonist, c) the prediction of attentional enhancement based on the characteristics of nAChR agonist-evoked cholinergic transients, and d) the implication of these findings for strategies focusing on the treatment of the cognitive symptoms of a range of disorders.

As noted in the Introduction, the demonstration of beneficial attentional effects of nicotine in healthy, non-smoking humans and intact animals has been less than straight forward and effect sizes have remained generally small. In healthy, non-smoking humans, nicotine most consistently enhanced measures of response speed but rarely produced robust increases in measures of cue detection; rather, faster responding was observed occasionally to be associated with fewer detections (Foulds, et al., 1996; Giessing, Thiel, Rosler, & Fink, 2006; Hahn, et al., 2007; Kleykamp, Jennings, Blank, & Eissenberg, 2005; Newhouse, et al., 2004; Sahakian, Jones, Levy, Gray, & Warburton, 1989; Thiel & Fink, 2008).

In intact animals, acute administration of nicotine likewise decreased response times but less consistently affected detection rate (Blondel, Sanger, & Moser, 2000; Bushnell, Oshiro, & Padnos, 1997; Stewart, Burke, & Marrocco, 2001; Turchi, Holley, & Sarter, 1995), and depended on specific task parameters (Mirza & Stolerman, 1998) or strain of animals (Mirza & Bright, 2001; Stolerman, Mirza, Hahn, & Shoaib, 2000). Thus, the present evidence, indicating the absence of beneficial effects on performance accuracy is largely consistent with the literature (note that response latency measures generated by the current task are uninformative because of the 2-s delay between event and lever presentation). Previous experiments showed that administration of nicotine (at the same dose used to assess dSAT performance) attenuated the detrimental effects of bursts of white noise on five-choice serial reaction time task performance (Hahn, et al., 2002). The distracter condition used in our task is

more efficacious, as indicated by near random lever selection during block 2, thereby possibly limiting the efficacy of any treatment to attenuate the acute effects of the distractor. Thus it was expected that block 2 performance remained unaffected by nicotine. However, nicotine also did not benefit post-distractor performance recovery. We will come back to discussing this negative finding further below in the context of nicotine-evoked cholinergic transients.

In contrast to nicotine, the selective α4β2* nAChR agonist robustly enhanced post-distractor dSAT performance. Although the evidence from studies in humans, including patients, and animal experiments remains limited, such ligands appear to produce more robust attentional effects than nicotine (Dunbar, et al., 2007; Grottick & Higgins, 2000; McGaughy, et al., 1999; Wilens, et al., 1999; Wilens & Decker, 2007; Wilens, et al., 2006). Our evidence further indicates that the performance-enhancing effects of the α4β2 nAChR agonist were due to an increase in detection rate (or Hits) and that the increase in Hits was found to occur in trial sequences that involved a shift from cue independent (no signal, response selection guided by propositional rules) to cue dependent attention (signal controls response selection in accordance with task rules). A neurobiological explanation of the superior efficacy of α4β2 nAChR agonists over nicotine will be offered further below, and this explanation predicts that the beneficial effects of α4β2* nAChR agonists are due to enhancing such shifts. The detection of signals, specifically if involving a shift from cue-independent to cue-dependent attention, requires transient increases in cholinergic activity in prefrontal cortex. Ideally, the effects of nicotine and α4β2* selective nAChR

agonists on such transients, recorded in performing animals, would be expected to reveal the basis for their differential efficacy. Unfortunately, such studies remain extremely technically challenging. However, the limited evidence available from such studies indicates that the properties of cholinergic transients evoked by local administration of nAChR ligands in anesthetized animals generalize to the augmentation of transients recorded in performing animals (Gietzen, 2010). Our electrochemical studies indicated that such transients are a product of local prefrontal glutamatergic-cholinergic interactions (Parikh, et al., 2008) mirroring the conclusions from prior psychopharmacological studies (Quarta, et al., 2007). Furthermore, α4β2* nAChRs control the amplitude of these transients; largely based on stimulation of such receptors situated on the glutamatergic terminals of thalamic afferents (Gioanni, et al., 1999). Resulting in stimulation of ionotropic glutamate receptors that may be directly located on the terminals of cholinergic afferents (Sarter, Parikh, et al., 2009). This model predicts, as indicated by the current data, that α4β2* nAChR agonists potently evoke, in terms of amplitude, cholinergic transients. Our prior data also suggest that the slow decay rate of nicotine-evoked cholinergic transients is indicative of ongoing and slowly diminishing acetylcholine release, is unrelated to effects mediated via α4β2* nAChRs, but are due, in part, to stimulation of α7 nAChR. Notably, the decay rate of atttentional performance mediating cholinergic transients corresponds with those evoked by α4β2* nAChR agonists, but not nicotine (Parikh, et al., 2007; Parikh, et al., 2008).

Taken together, this evidence suggests the hypothesis that α4β2* nAChR agonists potently evoke "sharp" cholinergic transients and are more efficacious in enhancing cue detection and associated shifts between attention modes than agonists that evoke more lasting increases in release. Consistent with this hypothesis, we recently found that stimulation of α7 nAChRs produced extremely long-lasting increases in ACh release (Paolone, 2010) but, similar to experiments on the attentional effects of other α7 nAChR agonists (Grottick, Haman, Wyler, & Higgins, 2003; Grottick & Higgins, 2000; Hahn, Sharples, Wonnacott, Shoaib, & Stolerman, 2003) failed to benefit attentional performance. Thus, nicotine binding at receptors other than α4β2 nAChRs may interfere with the enhancing effects that result from stimulation of α4β2 nAChR. The attentional enhancement produced by selective α4β2 nAChR agonists is closely related to the "sharpness" of the cholinergic transients evoked by such compounds, combined with their greater potency in terms of the amplitudes of the cholinergic transients. The detrimental significance of lasting increases in ACh release can also be illustrated on the basis of the time scale at which shifts from cue-independent to cue-dependent attention occur in this task. Such a mode shift is triggered by the insertion of a preattentionally processed cue into prefrontal circuitry, fostering effective cue detection by this circuit (Sarter, Parikh, et al., 2009). The occurrence of such a mode shift is evidenced by subsequent detection, or a hit, and it occurs within ~3 s after cue presentation. Thus, if nAChR agonists benefit performance by augmenting detection- and mode shift mediating cholinergic transients (Gietzen, 2010), it would be extremely difficult to conceptualize the

benefits of increases in ACh release that last for 30-70 s in the case of nicotine or tens of minutes in the case of α7 nAChR agonists (Paolone, 2010). The usefulness of the present behavioral and electrochemical approaches for basic research and drug development research deserve comment.

Consistent with the neurobiological conceptualization, the finding that the beneficial effects of the α4β2* nAChR agonist are due to the facilitation of attention mode shifts confirms the importance of the randomized sequencing of signal and explicit non-signal trials that differentiates the present task from other attention tasks. Second, the focus on dSAT performance over performance in the SAT is of clinical relevance as, for example, the attentional deficits of schizophrenic patients manifest primarily in the context of challenges to performance (Nuechterlein, et al., 2009; Sarter, Martinez, & Kozak, 2009). Such challenges further activate prefrontal circuitry, in part due to greater cholinergic activity as indicated by our prior experiments using microdialysis (Kozak, et al., 2006). Treatment effects are expected to occur primarily during the recovery period, in part due to the tremendous efficacy of the distractor, but also while mechanisms are activated in order to recover attentional performance (Sarter, et al., 2006). Such effects may allow more defined interpretation than effects against an acute distractor, particularly in terms of predicting the potential usefulness of a drug for treating attentional impairments which, in most groups of patients with neuropsychiatric and neurodegenerative disorders or brain injury, are a function of the demands on effort and top-down control (Stulemeijer, Andriessen, Brauer, Vos, & Van Der Werf, 2007).

Finally, the current evidence, combined with our prior work (Parikh, et al., 2008), further suggests that characterizing the properties of cholinergic transients may serve as a useful neuropharmacological screening procedure for nAChR agonists. Although it will be necessary to further demonstrate that the amplitudes and decay rates of such transients predict the characteristics of the augmentation of such transients in performing animals (Gietzen, 2010), the present results substantiate the hypothesis that compounds which potently evoke second-based increases in ACh release benefit attentional performance more robustly than compounds that less potently increase ACh release and produce much longer lasting (minutes) release events. The present evidence indicates that nAChR mediated cholinergic activity in the cortex controls shifts in attention modes (Greenwood, Lin, Sundararajan, Fryxell, & Parasuraman, 2009) and that α4β2* nAChR agonists benefit attentional performance by enhancing the efficacy of shifts from cue-independent to cue-dependent attention. Impairments in the ability to disengage from endogenously-guided attention and allowing attention to be controlled by behaviorally significant stimuli, often termed "inattention", are essential elements of the cognitive symptoms of major disorders, including ADHD, age-related cognitive disorders, and schizophrenia. The evidence described above suggests therapeutic efficacy of α4β2* nAChR agonists for the cognitive symptoms of such disorders.

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Chapter V

General discussion

Synopsis

The main goals of the experiments included in my dissertation were to 1) employ a novel technique capable of monitoring changes in cholinergic activity at a on a sub-second resolution in task performing animals to better understand the function of cholinergic signaling during attentional performance 2) to apply this high temporal resolution electrochemical technique to glutamate release in animals performing the same attention task to provide insight into the interaction between these two major neurotransmitter systems in the context of attentional performance 3) to demonstrate that by building upon our knowledge of the functions of and interactions between these two systems, we can predict and explain why selective α4β2 nAChR agonists more robustly enhance attentional performance than the non-selective nAChR agonist nicotine.

Disregulation of attentional processes plays a role in a host of psychological disorders from ADHD to schizophrenia. Attention is essential to learning and memory and gates all learning events. Understanding the mechanisms underlying attentional function is paramount to understanding their disorder in disease states and to creating cognitive enhancers.

The studies described here give robust support for the cholingeric system's involvement in attentional processes. Acetylcholine is widely transmitted and therefore was not thought to be involved in discrete cognitive processes. The studies described here advance the understanding of the psychological function of the cholinergic system, affording it a role in specific attentional processes. By demonstrating the interaction of choline and glutamate in cue detection, these studies also describe a neurobiological mechanism for this specificity of function. This proposed mechanism can be tested by further hypothesis driven experiments and allow creation of compounds with putative cognition enhancing abilities.

The previously described work demonstrates for the first time subsecond data on both acetylcholine and glutamate transmission. New techniques with faster rates of data acquisition that allow detection of the actual chemical signal advance the field and our understanding of neural function. They force new hypotheses to be created and tested. What follows is a summary of the major findings from each chapter as well a potential directions for future research to further clarify the results of the present studies.

General Limitations

There are a few limitations to the studies described. The lesion studies described had a major caveat. Lesion of the cholinergic inputs to the prefrontal cortex destroyed both modes of cholinergic transmission, both tonic and phasic. Therefore we are unable to make strong conclusions about whether the deficits

seen in those animals that received lesions were due to the lack of phasic or tonic acetylcholine. The electrochemical studies described also need expansion. First, all recording reported were made from layer V of the prefrontal cortex, the thalamic input layer. Transient cholinergic increases that underlie cue detection need sensory information from the thalamus, therefore these same transients would not be expected in other cortical layers. Recordings were not made from I and VI, which predominately receive information from other cortical areas. The currents findings would be strengthened if laminar specificity could be demonstrated. Second, based on previous data that demonstrates depletion of right hemispheric cholinergic inputs impairs performance on Hit trials and SAT performance leads to an up regulation of choline transporter in the right hemisphere, I recorded choline release from the right hemisphere only. To more completely understand the function of the cholingeric system in attentional processes, both hemispheres need to be tested. Finally, only the prefrontal cortex was recorded from. Therefore, it is unknown if these transients are unique to the prefrontal cortex.

Chapter 2 Summary

Previous experiments utilizing a selective cholinergic toxin to remove cholinergic inputs to the cortex, and preliminary experiments employing choline-sensitive microelectrodes in animals performing a cued appetitive response task led us to hypothesize that in the SAT, detected cues on Hits would evoke increases in cholinergic neurotransmission in prefrontal regions, and that removal

of prefrontal cholinergic inputs would impair Hit performance (McGaughy, et al., 1996; Parikh, et al., 2007). Here, we demonstrated that increases in cholinergic activity are only seen only on Hits, but only if that Hit is preceded by a CR or Miss. In accordance with the electrochemical data, removal of cholinergic input to the prefrontal cortex impaired performance on only on Miss→Hit trial sequences. Because of the trial sequence-dependent occurrence of increases in cholinergic activity on hit trials, and the selective performance impairment following the lesion, our initial hypothesis concerning the role of cholinergic transients in mediating cue detection were revisited. We have focused on two different possible explanations for the selectivity of cholinergic activity in the SAT, each of which places emphasis upon different parts of the cholinergic release event and neither is mutually exclusive of the other. The first is that cue-evoked increases in cholinergic activity mediate the shift between cue-independent to cue-dependent attention. This hypothesis focuses solely upon the trial sequence dependence of an evoked cholinergic signal, and particular attention is paid to the fact that levels of cholinergic activity are significantly elevated at the time the animals are prompted to make their response. The functional relevance of the remainder of the signal is not considered. The second is that increases in cholinergic activity as a reporter or learning signal, and attempts to account for the sequence dependency, as well as the full time course of the release. The major assumption is that as choline levels continue to increase, ACh release and hydrolyzation must be ongoing, and this on-going release is functionally relevant to current or future performance.

Future Experiments, Chapter 2

We have begun to plan additional experiments to help clarify the function of these cholinergic transients. The first addresses the selectivity of cholinergic transients for non-signal→signal trial sequences. I have designed a "tracking" task where a cue light situated above each response lever indicates which must be pressed in order to receive reward and thus only signal trials exist. Non-signal trials have been engineered out of the task. For continuity, I have kept as many of the parameters of this task consistent with the SAT as possible; the houselight remains on throughout the task, it includes a variable inter-trial interval of 9±3 s, variable stimulus durations (500, 50, 25 ms), and task length is set at 40 minutes. The hypothesis is that in this task, which involves no shifts from cue-independent to cue-dependent processing, detected cues will not evoke cholinergic transients. Further, prefrontal cholinergic deafferentation will not impair performance.

Clarifying whether cholinergic transients exclusively mediate the shift between cue-independent and cue-dependent processing, or also act as a learning signal, we have discussed two possible experiments. The first is to lengthen the interval between the cue and the extension of the response levers. The prediction of the results requires some speculation. If cholinergic transients act as a learning signal, it could be expected that cholinergic activity helps reinforce the association between signal-response-and reward. Under this hypothesis acetylcholine release begins to increase following the offset of the cue on, for example, a CR→Hit sequence. Release would continue through the interval between cue and response lever, and as with the cholinergic transients in

the present study, continue to rise for following response and reward retrieval. In sum, the shape of the cholinergic transients described here would be broadened extending across the added time of the longer ITI. Note that in this instance the onset of the cholinergic transient begins with the cue, the first event in a chain of events (cue→response→reward) that are being associated. A second possibility is that in a version of the SAT with a longer interval between the visual cue, the onset and peak of the transient might be right shifted to coincide with the timing of lever extension, response, and retrieval. If the peak can be broadened or right-shifted, the transient could be interpreted as a learning signal as reward delivery acts as the final confirmation. If cholinergic transients simply mediate the shift from cue independent to cue dependent processing, the transients in the version of the task with the lengthened interval would have the same characteristics as those in the current experiments as in either case it is the visual cue and the visual cue alone that is responsible for the cue-independent to cue-dependent attention shift.

A second approach could take advantage of ***(Chat)::Cre*** rats (Witten, et al.) and the ability to silence cholinergic release using the chloride pump Halorhodopsin during incongruent trial sequences. If the cholinergic signal is mediating the shift between cue-independent and cue-dependent processing alone, then silencing cholinergic signaling should acutely impair performance and trials involving shifts. Alternatively, if the cholinergic transient is a learning signal, performance would remain intact during the first incongruent sequences where

ACh release has been blocked, and decline slowly over time as associations are weakened due to the lack of the reinforcing cholinergic signal.

Chapter 3 Summary

Detected cues evoked glutamatergic transients in all hit trials, irrespective of sequence. The amplitudes of glutamatergic transients in trials that ended with a hit were larger for longer cues. On signal trials resulting in a Miss, or CR, lever extension, representing the first indication that it is time to make a response also evoked a robust increase in glutamate release. Furthermore, we compared the amplitudes of glutamatergic transients from trials yielding hits during periods of high levels of performance (SAT scores >0.35 averaged over all durations) with periods of near random performance (SAT scores: <0.35). The amplitudes of glutamatergic transients were markedly lower during poor performance periods (Fig. 3.7b). Furthermore, and in contrast to our hypothesis, lever extension evoked a glutamate release event on CR and Misses.

Glutamate release events are not sequence dependent

Cholinergic transients are observed only on Hit trials that are preceded by CR and Misses. Glutamatergic transients are observed on all hits, regardless of sequence. One possible means to selectively suppress cholinergic activity on

Hit→Hit sequences is inhibition presumably by GABAergic interneurons which are known to express both nicotinic and muscarinic acetylcholine receptors can act primarily to inhibit subsequent acitivation (Bandyopadhyay, Sutor, & Hablitz, 2006; Disney & Aoki, 2008). Thus ACh release events suppress future release events via a GABAergic mechanism. A second possibility is that synaptic spillover from the ACh release event on Hit trials activates presynaptic muscarinic M2 receptors attenuating subsequent release events. As M2 receptors are G_i coupled, signaling would decrease the probability of a second release event. A third possibility is that there is a cholinergic release event on every Hit, but the release event on a Hit→Hit sequence is more attenuated through mechanisms of habituation. However, why this release event would be smaller, even though the glumatergic transient is not, is not supportive of this theory.

A final consideration concerns a mechanism that explains the carryover of the network shift between successive Hit trials. In combination with calcium influx presumably caused by glutamate release and activation of NMDA receptors, ACh release leads to sustained, persistent spiking of cortical neurons through activation of non-specific cation currents. Such sustained spiking has been hypothesized to help effectively maintain stimulus-response contingencies and to serve as a preparatory mechanism for the next trial. This mechanism may also be capable maintaining prefrontal networks in a state that fosters cue detection (Fransen, Alonso, & Hasselmo, 2002; Hasselmo & Sarter, 2010; Hasselmo &

Stern, 2006). Future studies will be needed to determine which, if any, of these mechanisms may be contributing to these differences.

Future experiments, Chapter 3

The results from this experiment are very preliminary, therefore, the primary goal is to collect more data and verify that the present results are robust and reproducible. One study that is necessary and is planned assesses the effects of mediodorsal thalamic lesions on SAT performance as well as cholinergic and glutamatergic transients in task-performing animals.

Chapter 4 Summary

The main results of the behavioral and electrochemical experiments described above indicate that administration of a selective α4β2 nAChR agonist, but not nicotine, facilitates attentional performance under taxing conditions. Furthermore, compared with nicotine, the α4β2 nAChR agonist was more potent in evoking cholinergic transients which were characterized by very rapid termination of release, yielding "sharp" spikes in cholinergic activity. The more slowly rising and decaying transients evoked by nicotine are ‘sharpened’ by blocking the a7 nAChR; accordingly, we found that co-administration of nicotine with MLA robustly increased the Hit rate of dSAT-performing animals. Results from trial-sequence analyses indicated that the beneficial performance effects of

the α4β2 nAChR were due to an increase in Hits in (signal) trials that followed either CR or Misses.

Combined, these findings are consistent with the hypothesis that such selective agonists enhance cue evoked glutamate release from mediodorsal thalamic afferents and in turn, enhance the probability of generating a cholinergic transient (in at least the subset of Hit trials requiring a shift from cue-independent to cue-dependent processing). Nicotine should also enhance glutamate release through the same mechanism. However, though its actions at the α7 nAChR it produces a long lasting release event. The exact mechanism underlying the α7 nAChR's ability to create extended release events is not clear. Previous studies have explored the possibility that it is through an interaction with prefrontal dopaminergic inputs. Removal of dopaminergic input to the PFC does attenuate the amplitude of α mediated ACh release, but not the long duration of the release event (Parikh, et al., 2010). Further studies have demonstrated that α7 nAChR stimulation leads to calcium induced calcium release (CICR), which could promote extended release (Dickinson, et al., 2008). Regardless of the mechanism, as we have shown here, such long duration release events do not facilitate a precise cognitive operation like the shift from cue-independent to cue-dependent processing.

Future analyses, Chapter 4

I plan to go back to my existing data set and conduct further analyses to determine if, like administration of the α4β2 nAChR, co-administration of nicotine

and MLA increase the possibility of a Hit in trials requiring a shift from cue-independent to cue-dependent processing. Furthermore, a more detailed analysis of SAT performance (including joint probabilities for Hits preceded by CR and Misses, as well as Hits preceded by Hits) following administration of nicotine will be undertaken. One further possibility is that if cholinergic transients are indeed a learning signal, than perhaps by augmenting them there may be some carryover effects into the next day's test session (i.e. perhaps performance on CR→Hit, Miss→Hit trials the day after receiving the α4β2 agonist remains elevated). Future analyses will examine this possibility as well.

Concluding Remarks

While much of the data in the current thesis remains preliminary, I have been able to provide new insight into the function of prefrontal cholinergic and glutamatergic mechanisms in the support of attentional performance. Based upon the results of the study presented in Chapter 4, the current evidence also suggests that our combined electrochemical/behavioral approach may serve as a useful pre-clinical screening tool for the testing of compounds with putative cognition enhancing properties.

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