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Cholinergic modulation of auditory and prefrontal cortical interactions

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Dissertation

CHOLINERGIC MODULATION OF AUDITORY AND PREFRONTAL CORTICAL INTERACTIONS

by

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DEDICATION

In loving memory of my Father, D. Richard James. His spark will live on.
ACKNOWLEDGMENTS

I would like to thank my dissertation advisory committee for guiding me through this process and providing me with essential and rich feedback along the way. Their insights have been a crucial component of the production of this work. I would also like to express gratitude for all of the members of my laboratory who offered advice and technical knowledge over the past five years. Specifically, I would like to offer special thanks to Dr. Howard Gritton for his invaluable guidance and scientific insight during the production of these works.

I would like to sincerely thank my co-advisers Dr. Nancy Kopell and Dr. Xue Han. Working with them both has been a tremendous opportunity. Dr. Nancy Kopell has given me a truly unique perspective on the cortical dynamics of attention that is rich in complexity and scope. Dr. Xue Han has taught me a dynamic and multi-faceted approach to addressing scientific inquiry, and shown me how to confront the depth and subtlety of experimentation.

Finally, I would like to thank my Mother and Father for supporting me throughout the years, and my long-time friend and mentor Dr. Les Schaffer for his invaluable advice and scholarly conference. I could not have made it this far without their guidance.
ABSTRACT

Much of the previous work investigating the influence of cholinergic tone on cortical circuits has emphasized global states of arousal and local circuit dynamics; however the cholinergic system is well-suited to coordinate large-scale cortical interactions due to its diffuse cortically projecting arborization and diverse influence on the various cell types within the cortical microcircuit. In this thesis I examined the function of cortical cholinergic tone in supporting long-range cortical interactions, feed-forward sensory signaling, and active processing of behaviorally relevant stimuli. I utilized optogenetic stimulation and silencing of the cholinergic nucleus basalis while recording from the auditory-prefrontal cortical circuit as well as performing local drug infusions in awake mice. I demonstrate that prefrontal cortex actively responds to cortico-cortical sensory input in animals passively presented with acoustic stimuli and that muscarinic receptor binding within auditory cortex is essential for feedforward pathways from auditory cortex to transmit sensory related neural signals. Specifically, muscarinic antagonists applied to the auditory cortex disrupt sensory signaling within auditory cortex as well as bottom up signaling to prefrontal cortex. Furthermore, muscarinic antagonists attenuated the influence of cortical cholinergic release on
recording channels closest to drug infusion, confirming the efficacy of muscarinic antagonism, and demonstrating that aspects of cholinergic modulation are locally generated within cortical circuits, while others are globally generated in large networks. In task performing animals, I observed that optogenetic silencing of cholinergic nucleus basalis neurons attenuates the magnitude of PFC alpha power following correct behavioral choice and that alpha in prefrontal and auditory cortical LFPs are actively involved in behavioral learning during extinction, suggesting that cholinergic tone is involved in maintaining and updating the value of stimuli across behavioral trials. In summary, my thesis supports a model where endogenous cholinergic signaling is an essential component of normal auditory processing during low attentive states, contributes to circuit activation through local and large network mechanisms, and supports essential cortical dynamics that contribute to active behavioral processing of stimuli.
TABLE OF CONTENTS

DEDICATION ......................................................................................................................... iv

ACKNOWLEDGMENTS ............................................................................................................ v

ABSTRACT ............................................................................................................................ vi

TABLE OF CONTENTS ........................................................................................................... viii

LIST OF TABLES .................................................................................................................. xi

LIST OF FIGURES ................................................................................................................ xii

LIST OF ABBREVIATIONS ..................................................................................................... xiii

CHAPTER ONE: INTRODUCTION ............................................................................................. 1

The Role of Prefrontal Cortex in Passive Sensory Processing ............................................. 1

Cholinergic Modulation of Cortical Circuits ........................................................................ 3

Potential Mechanisms of Extinction Learning in Cortex ..................................................... 5

CHAPTER TWO: DISTINCT CHOLINERGIC RECEPTOR SUBTYPES REGULATE AUDITORY-PREFRONTAL CORTEX COMMUNICATION DURING PASSIVE AUDITORY PROCESSING ......................................................................................................................... 9

Introduction .......................................................................................................................... 9

Summary of Findings ............................................................................................................ 11

Methods for Chapters Two & Three .................................................................................... 12

Results ................................................................................................................................... 20

Prefrontal cortex is responsive to auditory stimuli during passive listening ............ 21
Cholinergic signaling modulates response properties in auditory cortex ........... 25
Cholinergic signaling in auditory cortex modulates sensory response properties in
prefrontal cortex........................................................................................................ 33
Conclusions.................................................................................................................... 33
Discussion..................................................................................................................... 35

CHAPTER THREE: DISRUPTION OF OPTOGENETICALLY INDUCED
CHOLINERGIC CORTICAL DESYNCHRONY WITH LOCAL APPLICATION OF
CHOLINERGIC BLOCKADE...................................................................................... 40
Introduction.................................................................................................................... 40
Summary of findings...................................................................................................... 42
Methods........................................................................................................................ 43
Results.......................................................................................................................... 44
Conclusions.................................................................................................................... 51
Discussion..................................................................................................................... 52

CHAPTER FOUR: CORTICAL CHOLINERGIC RELEASE MODULATES
NEUROPHYSIOLOGICAL SIGNATURES OF EXTINCTION LEARNING IN
PREFRONTAL AND AUDITORY CORTEX ................................................................. 54
Introduction.................................................................................................................... 54
Summary of findings...................................................................................................... 56
Methods........................................................................................................................ 57
Results.......................................................................................................................... 62
LFP Dynamics During Extinction Learning.................................................................... 66
LIST OF TABLES

Table 2.1 Statistical comparison of CSD RMS values between drug and baseline

conditions ....................................................................................................................... 30

Table 2.2 Statistical comparisons of granular layer sink duration between drug and
baseline conditions ....................................................................................................... 30

Table 2.3 Statistical comparisons of granular layer source latency between drug and
baseline conditions ....................................................................................................... 30

Table 2.4: Statistical comparison of granular layer current sink latency between drug and
baseline conditions ....................................................................................................... 31

Table 3.1 Statistical comparison of LFP power in low and high frequency ranges between
drug and baseline conditions .................................................................................... 46

Table 3.2 Statistical comparison of cholinergically induced LFP power in high and low
frequency ranges during drug and baseline conditions .............................................. 51
# LIST OF FIGURES

Figure 2.1: Experimental paradigm and placement histology. ........................................ 19

Figure 2.2: White noise stimuli in elicit strong activation in PFC and AC. ....................... 22

Figure 2.3: ERP responses and latencies to white noise stimuli in auditory and prefrontal
cortex.......................................................................................................................... 24

Figure 2.4: Scopolamine attenuates CSD responses in auditory cortex. ......................... 28

Figure 2.5: Mecamylamine delays CSD current sink onset latency in auditory cortex.... 32

Figure 2.6: Scopolamine infusion in the auditory cortex reduced amplitude of sound
evoked LFP responses in medial frontal cortex.......................................................... 34

Figure 3.1: Optogenetically induced cortical desynchrony in auditory and medial frontal
cortex.......................................................................................................................... 47

Figure 3.2: Scopolamine attenuates decrease in low power induced by cholinergic
activation.................................................................................................................... 49

Figure 4.1: Behavioral training paradigm................................................................. 60

Figure 4.2: Reward-related lick rate during extinction learning................................. 63

Figure 4.3: Neuronal firing rates in PFC during extinction learning............................. 64

Figure 4.4: PFC LFP signatures of behavioral responses............................................. 65

Figure 4.5: PFC LFP activity changes during extinction learning............................... 68

Figure 4.6 AC LFP activity changes during extinction learning.................................. 71

Figure 4.8: PFC LFP activity during cholinergic silencing......................................... 75

Figure 4.9: Cholinergic nucleus basalis neuron silenced by optogenetics..................... 79
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC</td>
<td>Auditory Cortex</td>
</tr>
<tr>
<td>PFC</td>
<td>Prefrontal Cortex</td>
</tr>
<tr>
<td>NB</td>
<td>Nucleus Basalis</td>
</tr>
<tr>
<td>LFP</td>
<td>Local Field Potential</td>
</tr>
<tr>
<td>MUA</td>
<td>Multi-Unit Activity</td>
</tr>
<tr>
<td>WSR Test</td>
<td>Wilcoxon Signed-Rank Test</td>
</tr>
<tr>
<td>ACSF</td>
<td>Artificial Cerebrospinal Fluid</td>
</tr>
<tr>
<td>SOM</td>
<td>Somatostatin</td>
</tr>
<tr>
<td>PV</td>
<td>Parvalbumin</td>
</tr>
<tr>
<td>LTS</td>
<td>Low Threshold-Spiking</td>
</tr>
<tr>
<td>FS</td>
<td>Fast-Spiking</td>
</tr>
</tbody>
</table>
CHAPTER ONE: INTRODUCTION

In this thesis I propose that cholinergic modulation of cortical circuits is a potential substrate of neuronal interaction across large brain networks during stimulus processing, and that it promotes transient states of cortical activation that are supportive of attentional processing. In neocortex, cholinergic input originates in the basal forebrain nuclei, projecting to all of cortex, most heavily in frontal areas (Zaborszky 2012). Previous work has shown that cortical cholinergic release modulates cortical circuits in response to ascending sensory input; induces local circuit synchrony in primary sensory cortices; and in the prefrontal cortex correlates highly with attentional orienting (Goard et al. 2009; Roopun et al. 2010). Additionally, it has been proposed that cholinergic modulation facilitates cortical maintenance of sensory information during executive functioning tasks (Hasselmo and Sarter 2011). Furthermore pharmacological studies in the monkey visual system have demonstrated that increasing acetylcholine in cortical circuits improves attentional enhancement of V1 neuronal firing responses (Herrero et. al 2008; Thiele et. al 2012), providing a direct link between cholinergic tone and attentional processing in bottom up sensory cortical circuits. These studies have expanded our understanding of cholinergic involvement in aspects of attention, however, the interaction between executive functioning and bottom up sensory regions of cortex during states of cholinergic activation is not well understood.

The Role of Prefrontal Cortex in Passive Sensory Processing

I examined auditory processing in the auditory (AC) and prefrontal cortex (PFC) as a model of top down and bottom up functioning in cortical circuits. Studies have
demonstrated in primates that auditory information is represented in prefrontal regions, and that there are cortico-cortical pathways projecting from auditory to prefrontal cortex (Romanski et al. 1992; Romanski et al. 2002; Romanski et al. 2005; Barbas et al. 1985; Barbas 1995). These pathways potentially relay sensory information from auditory cortex to decision making areas of the cortex. AC itself undergoes alterations in response properties during learning and attention that shapes the cortical response to behaviorally relevant stimuli (Rutkowski et al. 2005; Bieszczad et al. 2010; Rodgers et al. 2014; Yin et al. 2014, Fritz et al. 2007). Similarly, stimulation of the cholinergic nucleus basalis (NB) of the basal forebrain induces plasticity that biases cortical responses toward temporally paired acoustic tones (Kilgard and Merzenich 1998; Kilgard and Merzenich 1998; Polley, Steinberg et al. 2006; Fritz, Elhilali et al. 2007; Froemke et al. 2007). And it has been shown that this plasticity can take place over long periods of time, expanding the representation of a paired tone within the auditory cortical tonotopy (Kilgard & Merzenich 1998) or very short timescales; altering the balance of excitation and inhibition at the cortico-cortical synapse (Froemke et. al 2007). In this way, auditory cortex likely also plays an active role in transforming sensory related information in order to impact executive functioning related to sensory stimuli. These studies have extensively characterized the processes by which attention can transform auditory response properties, however, we do not yet understand how cholinergic influences modulate cortical dynamics between brain structures.
Cholinergic Modulation of Cortical Circuits

In previous modeling works cholinergic receptor types have been proposed to modulate cortical dynamics, shifting neural populations into modes of activity that are conducive to attentional processing (Soto et al. 2006, Ainsworth et al. 2011, Borgers et al. 2005, Borgers et al. 2008, Lee et al. 2013). At a circuit level, the influence of muscarinic receptors—a class of cholinergic receptors—are extremely varied; potentially modulating cell types that play a role in restricting or permitting feed-forward signaling and top down control of sensory circuits. Specifically, they reduce hyperpolarizing M-current in pyramidal neurons, increasing excitability; reduce spike-frequency adaptation; hyperpolarize fast spiking interneurons (FS cells); and reduce m-currents in superficial layer low threshold spiking interneurons (LTS cells) (Krnjevic et al. 1971, Schwindt, et al. 1988; Nunez et al. 2012; Kruglikov et al. 2008; Xiang et al. 1998, Chen et al. 2015). Additionally, it has been demonstrated that muscarinic receptors types have a diverse functionality in modulating cortical dynamics during sensory perception and attentional processing, reducing the attentional modulation of V1 neurons (Herrero et al. 2008) and cholinergically induced decorrelation of cortical neurons (Goard et al. 2009, Pinto et al. 2013).

With such varied actions on cortical neurons, determining the precise role of muscarinic receptor activation in modulating cortical circuits during cholinergic activation is a daunting task. Numerous studies have investigated the role of muscarinic receptors by stimulating the cholinergic nucleus basalis while taking electrophysiological recordings in cortex and performing local or systemic drug infusions (Berg et al. 2005, Metherate et
Cholinergic stimulation induces a phenomenon known as cortical desynchrony, which is a disruption in the baseline spectral properties of the cortical LFP. In these studies, muscarinic blockade was demonstrated to alter the spectral properties of cortical desynchrony, reducing or abolishing the cholinergically induced disruption of the cortical baseline LFP. However many of these studies have utilized electrical stimulation of the basal forebrain, stimulating all cell types of the basal forebrain. More recently, studies have begun to utilize optogenetic techniques to investigate specific receptor antagonism during cholinergic activation of cortical circuits (Chen et al. 2015, Kalmbach et al. 2012, Pinto et al. 2013, Kalmbach and Waters 2014). The cholinergic nucleus basalis is a perfect candidate for optogenetics since the cholinergic neurons are interspersed with a variety of other cell types (Zaborszky et al. 2012). By expressing opsins specifically in neurons that produce choline acetyltransferase—an enzyme involved in the production of acetylcholine—researchers have been able to target cholinergic neurons in the nucleus basalis, allowing them to induce or suppress cholinergic release without stimulating or activating unrelated neural pathways or neuromodulators.

However, even with the use of optogenetics to capture the effect of distinct cholinergic cell types, many studies make use of an anesthetized preparation, which can introduce unintended experimental variables. This can be problematic since, cortical cholinergic release has been shown to be critically involved in the sleep/wake cycle, pacing large scale EEG patterns associated with activation and arousal (for a review read Jones 2004). Additionally anesthetics such as urethane, or ketamine have been shown to have
influences over the field potential activity of the cortical resting state (Sharma et al. 2010, Rivolta et al. 2015).

In this investigation an awake preparation is used in order to eliminate these confounding elements in cortical cholinergic release under blockade of nicotinic and muscarinic receptor types. Additionally, I am able to perform local drug infusions while probing the prefrontal and auditory cortical circuits with multichannel recording arrays. In this way it is possible to determine the locality of receptor antagonist disruption of cholinergically induced cortical desynchrony with respect to receptor blockade. This will build upon previous research, allowing for discernment of aspects of cortical desynchrony that are either locally or globally generated.

**Potential Mechanisms of Extinction Learning in Cortex**

Several studies have characterized the time course of cholinergic release in rodent prefrontal cortex (PFC) during successful detection of behavioral cues (Parikh et al 2007, Howe et al 2013). These studies demonstrate not only that cholinergic release in PFC is strongly associated with the detection of cues, but that it is released when animals must shift from processing irrelevant to relevant cues on consecutive trials, suggesting that cholinergic signaling in PFC is highly sensitive to transitions between external and internal attentive processing. This process was thoroughly investigated by utilizing in vivo electrophysiological recordings in rodent PFC during extinction learning. During extinction learning, animals had to learn to suppress responses to stimuli previously associated with reward. In this way, extinction learning is a transition from externally, to internally driven response to sensory stimuli. By examining the network dynamics of
extinction learning *in vivo* it can be determined whether or not aspects of the neuronal response correlate with extinction learning.

Alpha band oscillations (typically 8-13 Hz) in cortex are traditionally thought of as being suppressive of distracting or irrelevant stimuli, appearing in areas that process sensory stimuli that are not behaviorally relevant, and decreasing in magnitude upon presentation of sensory stimuli (Nikouline et al. 2000, for a review read Payne & Sekuler 2014), making them a likely candidate for involvement in the process of extinction learning. Cortical alpha has proven to have a variety of roles in cortical circuits during attention, provoking much debate over their function. For instance, alpha band oscillations have also been shown to be involved in internal working memory processing (Jensen et al. 2002). And they have been observed to coincide with increased attentional processing in relevant brain areas (Mo et al. 2011). In this way, alpha oscillation seem to have a multi-functional role, suppressing irrelevant external stimuli, while enhancing the internal processing of others.

Beta oscillations have similarly been linked to attentional processing and processing of behavioral cues in cortical structures (for a review, read Engel and Fries 2010). Furthermore, beta band synchrony has been shown to be involved in the selection of task rules during cognitive flexibility tasks in primates (Buschman et al. 2012) and beta oscillations in basal ganglia have been proposed to reflect an internal association between sensory cues and selection of motor responses (Leventhal et al. 2012). These findings suggest that beta oscillations may be involved in the process of extinction learning, since
subjects must gradually learn to override previously-learned stimulus response associations.

Interestingly, alpha oscillations (8-13 Hz) are conspicuously close in frequency range to the range of frequencies reduced by activation of cortical acetylcholine (typically 1-10 Hz). Furthermore, cortical alpha oscillations can have different underlying neurophysiologies in distinct brain areas (Bollimunta et al. 2008). In light of this, cholinergic mechanisms in cortical circuits may be involved in the generation or suppression of types of cortical alpha under attentional task demands. Similarly, beta oscillations have been shown to be induced by acetylcholine in specific layers of bottom-up sensory cortex (Roopun et al. 2010), and modeling predicts that generation of beta oscillations in sensory cortex involves cholinergic modulation of specific cell types (Lee et al. 2013). While the mechanisms of beta in PFC are not completely understood, involvement of acetylcholine in bottom-up sensory cortex suggests that cholinergic modulation may similarly be involved in the generation of beta in PFC.

In this thesis I will address several the predictions and research directives put forward above: I will determine if cholinergic receptor binding is an essential influence in promoting feedforward interaction between auditory and prefrontal cortex, if muscarinic receptor activation contributes to cholinergically induced cortical desynchrony in the waking state either globally or locally, and ascertain the influences of cholinergic signaling that contribute to the neurophysiological phenomena characterized during extinction learning. By addressing these questions I can contribute to our understanding
of the role of cortical cholinergic modulation in attentional processing, and further evaluate previous research describing auditory and prefrontal dynamics during attention.
CHAPTER TWO: DISTINCT CHOLINERGIC RECEPTOR SUBTYPES REGULATE AUDITORY-PREFRONTAL CORTEX COMMUNICATION DURING PASSIVE AUDITORY PROCESSING

Introduction

The prefrontal cortex (PFC) in mammals has been associated with various cognitive functions, such as stimulus categorization and integration, decision making, and executive control (Miller and Cohen 2001; Dalley et al. 2004; Roy et al. 2014). PFC neurons exhibit a high degree of selectivity for conditioned sensory stimuli and can be differentially regulated by context or task demands (Maren and Quirk 2004; Chang et al. 2010; Euston et al. 2012; Hyman et al. 2012; Moorman and Aston-Jones 2015). While much is known about how PFC is modulated within the context of well learned behavioral tasks, the intrinsic response properties of PFC to non-behaviorally relevant sensory stimuli is less well studied. In particular, how much sensory information is conveyed to prefrontal neurons intrinsically and is there an underlying mechanism that gates sensory signals into executive control regions under these conditions?

Cortical synchrony is thought to enhance communication across cortical areas, such as between sensory cortices and PFC, in ways that promote sensory processing (Fries et al. 2001, Buschman and Miller 2007, Siegel et al. 2012). Since the basal forebrain cholinergic system provides diffuse and global innervation of the cortex, it is well suited to modulate coordination of neural activity across large brain networks. Additionally, recent studies have demonstrated that activation of the cholinergic nucleus basalis produced profound influences of neural processing within cortical regions (Golmayo et
al. 2003, Goard and Dan 2009, Letzkus et al. 2011, Pinto et al. 2013, Chen et al. 2015). Much of our understanding of cholinergic influences on stimulus processing comes from task performing animals, or cortical plasticity induced through temporal pairing of nucleus basalis stimulation with sensory stimuli (Kilgard and Merzenich 2002, Froemke et al. 2007, Goard and Dan 2009, Letzkus et al. 2011, Pinto et al. 2013). While this intriguing evidence suggests that the cholinergic system is important in modulating neural signals during active behavioral states, little is known about how the cholinergic system contributes to feed-forward sensory processing in the absence of well-defined behavioral associations.

Cholinergic modulation of cortical processing is mediated by nicotinic and muscarinic receptors in complementary ways. In AC, nicotinic receptors located on thalamic projection terminals have been shown to augment presynaptic input (Gil et al. 1997, Lambe et al. 2003, Kawai et al. 2007). In addition, nicotinic receptors are expressed on excitatory and inhibitory AC neurons, which upon activation can increase cortical excitability (Roerig et al. 1997, Chu et al. 2000, Levy and Aoki 2002). Furthermore, muscarinic receptors have been shown to decrease recurrent peri-somatic inhibition of layer V pyramidal neurons in the AC, enhancing excitability (Kruglikov and Rudy 2008, Nunez et al. 2012). Given their diverse functionality, it is theorized that nicotinic and muscarinic receptors could promote cortical representation of sensory inputs through their coordinated activation, likely by increasing signal to noise ratio of sensory information (Hasselmo and Sarter 2011).
To investigate how PFC and AC coordinate sensory processing in passive states, and how the cholinergic system modulates their interaction through distinct receptor types, we combined electrophysiological, pharmacological and optogenetic (discussed in chapter three) techniques in awake head fixed mice, during presentation of a neutral auditory stimulus. We discovered that PFC receives sensory input during passive listening, and cholinergic signaling in the auditory cortex plays an important role in modulating responses in PFC through distinct receptor pathways during passive or low attentive states.

**Summary of Findings**

We found that PFC responds robustly to auditory stimuli during passive conditions, providing novel evidence that PFC is involved in processing auditory stimuli in the absence of behavioral context. Furthermore, response latencies in PFC were slightly longer than those observed in AC, which is consistent with a mono or poly-synaptic delay between structures. Nicotinic receptor blockade in AC increases the onset latency of AC sound responses in the granular layers, suggesting that nicotinic receptors are involved in the timing of thalamocortical input in AC. On the other hand, muscarinic receptor blockade in AC both attenuated and abolished phases of sound evoked CSDs in AC. Similar reductions of sensory related CSDs induced by application of a mixture of GABA antagonists has been shown to reflect blockade of intracortical input in auditory cortex (Hap pel et al., 2010). Our results, demonstrating similar effects with muscarinic antagonism provide new evidence that muscarinic receptor binding is necessary for intracortical interaction within AC. Further evidence from our study directly links
muscarinic receptor blockade within AC to reductions in the magnitude of sound responses in PFC, providing evidence both that AC relays auditory information to PFC, and that muscarinic receptors are involved in sensory relay from AC to PFC.

Methods for Chapters Two & Three

The methods below describe experimental and data analysis techniques that were used to produce results shown in chapters two and three. At baseline, there were two types of experimental manipulations: acoustic white noise stimuli, and optogenetic stimulation of the cholinergic nucleus basalis. Both of these manipulations were introduced before, and after infusion of cholinergic antagonists into the auditory cortex. The effects of cholinergic antagonists on the cortical responses to sound are discussed in chapter two, while the influence of cholinergic antagonists on the cortical response to optogenetically induced cholinergic stimulation are discussed in chapter three.

All procedures involving animals were approved by the Boston University Institutional Animal Care and Use Committee (IACUC). A total of 34 mice were used in this study (n=28 Chat-ChR2 and n=6 Ai32) transgenic mice (3-6 months-old, on the day of recording). Chat-ChR2 mice were obtained by crossing Chat-Cre line (B6;129S6-Chat^{tm1(cre)Lowl}/J) with Ai32 line (B6;129S-Gt(ROSA)26Sor^{tm32(CAG-COP4*H134R/EYFP)Hze}/J (both were obtained from Jackson Laboratory, Maine). Ai32 animals served as controls for all optogenetic manipulations.

Surgical Procedures: Mice were surgically implanted with a headplate as described in a previous publication (McCarthy et al. 2011). Briefly, under isoflurane aesthesia a custom
head-plate designed to allow access to the prefrontal cortex and the auditory cortex was anchored to the skull through 3 anchor screws. A fourth screw was placed over the cerebellum and connected to a metal pin to serve as the ground electrode on the recording day.

**In Vivo Electrophysiology:** Upon complete recovery, typically one week after surgery, mice were habituated to the recording environment and the head fix apparatus, and they were exposed to a sham recording session. Recording occurred on the subsequent day following habituation. During recording, mice were head fixed through a custom holder that was anchored to the recording table and loosely wrapped in a breathable mesh (Butler Schein, Dublin, OH; Vet-Flex EZTear) to increase comfort. Recording electrodes were slowly lowered through two small craniotomies into PFC (AP -2.0, ML +0.4, DV -2.75) and the auditory cortex (AP +2.3 to +3.6, ML +4.0 to +4.5, DV -1.0) using motorized micromanipulators (Siskiyou, Grants Pass, OR) at a very slow speed. A 200 um optical fiber was lowered through a third craniotomy on a Microdrive (Siskiyou, Grants Pass, OR) into the nucleus basalis (NB; AP +0.5, ML +1.5, DV -4.5) for optical stimulation.

*Extracellular* recordings were made with a multichannel RZ2 recording system (Tucker Davis Technologies, Alachua, FL) in an electrically shielded sound attenuation chamber. A 16 contact linear probe (Neuronexus, Ann Arbor, MI; model: A1x16-10mm-100-177-A16) with 100 um spacing was inserted dorsal-ventrally into the PFC. A 32 channel probe (4 shanks, 8 sites per shank with 100 um spacing between contacts and 400 um spacing between shanks (Neuronexus, Ann Arbor, MI; model: A4x8-5mm-100-400-177-
A32) was positioned into AC, perpendicular to the cortical surface. Because of the curvature of the cortical surface, not all four of the shanks could be placed at precisely the same depth during each experiment. Probes were advanced until all probe contacts were within the cortical tissue. Shanks were spaced 1.2 mm along the rostro-caudal axis of the auditory cortex.

To record spikes, signals were digitized at 24,414 Hz and bandpass filtered between 300-5000 Hz. Spikes were identified by threshold crossings and were manually set at the beginning of each recording session. Timestamps and waveform snippets (1.3 ms long) were stored for later offline processing. Local field potentials (LFPs) were digitized at 3051.8 Hz, and low pass filtered at 1 kHz.

**Pharmacology:** The 32 channel recording electrode in AC was also coupled to an infusion pipette under control of a picospritzer that allowed for local drug infusions (Figure 2.1B). The infusion pipette was a pulled glass pipet with an opening of approximately 5 um in diameter, and was placed so that the tip was halfway between the two innermost electrode shanks and terminated halfway between the deepest and most superficial recording site. After a baseline recording session, the picospritzer was used to deliver 500 nL of drug into the auditory cortex over approximately 5-10 minutes. An additional 20-30 minutes was allowed to elapse before beginning post-drug recording sessions. We infused cholinergic drugs at two different dosages in order to assess the drug effects at different levels of receptor blockade. We infused the muscarinic antagonist scopolamine at a low dose of 10ug/uL and a high dose of 100ug/uL, and the nicotinic antagonist mecamylamine at a low dose of 1ug/uL and a high dose of 10ug/uL.
dissolved in artificial cerebro-spinal fluid (ACSF, Toscris Bioscience, Bristol, UK). ACSF alone was used as vehicle alone control for infusions.

**Generation of Auditory Stimulus:** White noise auditory stimuli were generated with a RZ2 Bioamp processor and RP2.1 real time processor (Tucker Davis Technologies), and digitized at a frequency of 48828 kHz. Stimuli were played with a multi-field magnetic speaker (Tucker Davis Technologies, model: MF1), positioned at 20 cm from the animals head, and calibrated to be 70 dB SPL with a conditioning amplifier and microphone (Bruel and Kjaer, Naerum, Denmark; amplifier: model: type 2690—0S2; microphone: type 4939-A-011). The stimuli consisted of 500 milliseconds white noise bursts (5ms linear ramp to 90% of peak amplitude).

**Optogenetic Stimulation (discussed in chapter three):** Laser light for optogenetic stimulation of nucleus basalis was delivered through a multimode optically shielded 200um fiber (Thorlabs, Newton NJ; model: BFH48-200), coupled to a 473nmDPSS laser (Shangai Laser Ltd., Shanghai, China; model: BL473T5-200FC). The tip of the fiber was stripped bare to minimize tissue damage, and the laser power was calibrated to 15mW at the fiber tip prior to insertion. 40 Hz square light pulses (20% duty cycle) lasting 750 ms was delivered via TTL trigger from the RZ2 recording system.

**Experimental Protocol:** The recording protocol consisted of several combinations of optogenetic and acoustical sound stimuli. Two trial types were analyzed, one consisted of a white noise burst alone, and the other consisted a 750 ms optogenetic stimulation alone. Both trial types were presented to mice 100 times, with their order randomized. The inter-trial interval (ITI) was 10 seconds (*Figure 2.1, D*).
**Histology:** At the end of the experiments, mice were anesthetized and transcardially perfused. Brains were removed and histologically processed for verification of electrode placement using cresyl (reagent catalog number) staining. To validate the expression of ChR2 reporter was isolated to cholinergic neurons we also processed tissue with antibodies directed against ChAT. Sections designated to undergo antibody staining were rinsed with 0.05 M Tris-HCL-buffer (Tris, pH=7.6) followed by 60 min rinse in blocking buffer (5% serum; Jackson ImmunoResearch, 017-000-121) with 0.2% triton. Tissue was then incubated for 24 hours with goat anti-ChAT antibody (Millipore; Temecula, CA) diluted at 1:500. The following day, sections were rinsed 3X at 10 min each in TrisHCL and then incubated with a secondary antibody (Alexa Fluor 594 donkey-anti-goat; Life Technologies A11058; Grand Island, NY) at 1:200 for 2 hours. Sections were rinsed again and then mounted on gelatin-coated slides, allowed to dry, and coverslipped using an anti-fade mounting media (Vectashield; H-1400, Burlingame, CA).

**Data Analysis:** All data analysis was performed with custom Matlab functions (MathWorks, Natick, MA). Statistical tests were either Friedman’s test, for comparisons of drug and proximity to infusion pipette or Wilcoxon signed-rank tests for comparisons between baseline and post-infusion measurements. In some instances the logarithm of the data values were used for statistical analysis.

LFPs were first bandpass filtered between 2 Hz and 150 Hz and down-sampled by a factor of 8 (381 Hz) prior to further analysis. In awake head fixed conditions, we occasionally found motion induced artifact. To remove motion artifact, we took the root mean square (RMS) in 100 ms bins for all recorded trials. We rejected trials if the
channel-averaged RMS curve exceeded 5 standard deviations at any point during the response window.

Current source density (CSD) analysis estimates the second spatial derivative of LFP signals to determine the relative current flows across the cortical laminar depth profile. CSDs were calculated using the LFPs recorded from the laminar depth probes in the auditory cortex as described previously (Nicholson and Freeman 1975; Mitzdorf 1985). We averaged LFPs on each channel across all trials prior to CSD calculation. First, we applied smoothing across channels as described in (Sakata and Harris 2009).

\[ \Phi(z) = \frac{\Phi(z+\Delta z) + 2\Phi(z) + \Phi(z-\Delta z)}{4} \]

Where \( z \) is the depth perpendicular to the cortical surface, \( \Delta z \) is the electrode spacing, and \( \Phi \) is the potential. Then we estimated the CSD (Nicholson and Freeman 1975; Mitzdorf 1985).

\[ -CSD(z) \approx \frac{\Phi(z+\Delta z) - 2\Phi(z) + \Phi(z-\Delta z)}{\Delta z^2} \]

To quantify the absolute changes in current flow across cortical laminar depth profile, we calculated root mean square (RMS) values across depths within analysis windows of interest. To quantify effects on CSD across populations upon drug infusion we averaged the two inner or two outer shanks in order to reflect the symmetrical orientation with respect to the infusion pipette. For display purposes, CSDs were interpolated linearly and plotted as pseudocolor images.

**ERP quantification:** Event related potentials (ERPs) were calculated for all electrode sites in PFC. To present the overall effect on LFP in PFC upon sound presentation, we
averaged LFP recorded across all trials, and all electrodes. ERP magnitude was calculated by subtracting the minimum from the maximum value of the ERP within 100 ms following stimulus onset.

*Spike analysis:* Spike snippets were analyzed using Offline Sorter (Plexon Inc., Dallas, TX), and manually clustered in PCA space. Spike peri-stimulus time histograms (PSTHs) were calculated using 20 ms time bins for population and statistical analysis. For AC multi-units, signals were considered responsive if the mean z-score in either an early (5-40 ms) or late (40-100 ms) window exceeded an absolute value of 2. Similarly, in PFC multi-unit signals were considered responsive if their mean z-score exceeded an absolute value of 2 during the 25-155 ms window.

*Spectral analysis:* We used the open source software package Chronux (www.chronux.org) to compute multi-taper spectrograms. Three tapers and a 500 ms sliding window with 5 ms step were set as parameters for the analysis. Single trial spectrograms were first calculated, and then averaged across trials. We then calculated trial averaged spectrograms of the LFP for each channel, and then further averaged across channels before normalizing them to their baseline. Baseline was calculated by averaging the LFP signals during the 5 second window immediately before stimulus onset.

*Latency Analysis:* CSD magnitude was used to estimate latency from sound onset within auditory cortex. The channel with the largest current sink was chosen to estimate latency and we further limited this analysis to those electrode shanks in AC with sink magnitudes
Figure 2.1: Experimental paradigm and placement histology.

A: Head fixed mouse preparation with electrode and optical fiber placements during passive listening recording. Recording configuration consists of 2 multi-channel electrodes, an infusion pipette that is integrated into the auditory cortex recording electrode, and a shielded optical fiber. B: Anatomical placement of recording electrodes and infusion pipette represented to scale in a frontal (prefrontal) and horizontal section (auditory cortex) (see methods). C: Histological sections of electrode and fiber placement. (i): Section from auditory cortex representative of electrode placement for this recording area. (ii): Nissl stain showing optical fiber track and placement in nuc. basalis (iii): Section adjacent to (ii) showing ChR2 GFP reporter expression in tissue co-labeled with antibodies directed against choline acetyl transferase (Chat). Note expression is limited to cells also expressing Chat (red). (iv): Section from frontal cortex demonstrating electrode placement from a representative animal. D: Trial configuration schematic. Trials consisted of 100 presentations of white noise and 100 optical stimulation trials across two separate blocks. Trials were randomized over the recording session within each block. Block 1 consisted of a baseline recording session that was followed by a drug or vehicle infusion. The block 2 recording session began 30 min after infusion and was identical in trial number to block 1.
that exceeded three standard deviations from the baseline within the 50 ms post sound onset. Latency was chosen as the exact point at which the CSD crossed 3 s.d. from baseline, while the endpoint of the sink was chosen as the first zero-crossing following the initial dip. Peak source latencies were chosen as latency of the local maximum following the initial dip, within 500 ms from sound onset. Values less than 10ms (1 occurrence) were considered to be erroneous measurements and excluded.

For latency estimation in PFC and comparisons between PFC and AC, we calculated latencies from ERP magnitudes. In PFC channels were either averaged together, or analyzed separately to examine depth-wise latency. In auditory cortex, ERPs were taken from the channel with the largest magnitude current sink. Similar to CSD latency analysis, we limited our analysis to ERPs that exceed 3 standard deviations of the baseline activity within 100 ms post sound onset for PFC, or 50 ms post sound onset for AC. Latencies were chosen as the point at which the ERP exceeded 3 s.d. from baseline.

**Results**

In order to better understand how the prefrontal cortex (PFC) processes auditory stimuli, and how PFC responses are coordinated with those seen in the auditory cortex (AC) during passive listening, we simultaneously recorded from AC and PFC using multi-contact electrodes (Figure 2.1A-C). In addition, to examine how the cholinergic system might contribute to this process, we used local pharmacology to block cholinergic receptor subtypes by infusing selective antagonists into AC through an infusion pipette (Figure 2.1B, right) as well as optogenetically stimulating the cholinergic nucleus basalis
in Chat-ChR2 transgenic mice that express ChR2 selectively in cholinergic neurons. We employed white noise auditory stimulus at a relatively high sound intensity (70dB) based on previous findings that PFC neurons tend to code more strongly for complex auditory stimuli (Romanski and Goldman-Rakic 2002, Romanski et al. 2005, Plakke and Romanski 2014). Awake, head-fixed animals were presented with 100 trials of white noise stimulus, with a 10 second ITI (Figure 2.1A, D), intermixed with another 100 trials of optogenetic stimulation (Figure 2.1D). At the conclusion of each experiment, histology was performed to verify electrode and optical fiber placement, and to confirm ChR2-GFP expression was selectively expressed in cholinergic cells (Figure 2.1, C).

Prefrontal cortex is responsive to auditory stimuli during passive listening
While it has been well established that neurons in PFC respond robustly to auditory stimuli that have been associated with task-relevant stimuli (Maren and Quirk 2004; Chang et al. 2010; Euston et al. 2012; Hyman et al. 2012, Moorman and Aston-Jones 2015), it remains unclear to what extent PFC neurons respond to auditory input during passive listening. In order to address this, we quantified multi-unit activity (MUAs) in passive listening conditions (Figure 2.2A, C). We found that a total 48% of PFC MUAs were significantly modulated upon white noise presentation, with 46% positively modulated and 2% negatively modulated (n=89 MUAs recorded in 28 mice) (Figure 2.2C, right). Thus, as a population, responsive MUAs exhibited an overall increase in firing rate upon sound presentation. The increase in firing rate is followed by a reduction below the baseline immediately after sound offset, which last for ~500 ms before returning to baseline (Figure 2.2C, left). In AC, a similar diversity of neuronal responses
were observed, but with a much larger fraction, 75% of MUAs, being responsive to white noise presentation (total n=151 neurons recorded in 28 mice). Of which, 74% are positively modulated (Fig. 2B,D), and 1% are negatively modulated (Fig. 2D, right). The majority of the responsive MUAs in AC exhibited a large transient increase immediately

**Figure 2.2: White noise stimuli in elicit strong activation in PFC and AC.**

A: (left) Example MUA from PFC increasing firing rate during white noise stimulus. (right) Example PFC MUA decreasing firing rate. B: Same as A, for AC. C: (left) Population spiking average of sound responsive PFC MUAs during white noise presentation. (right) Proportion of responsive MUAs recorded in PFC. D: Same as C, for AC.
following sound onset, however, some neurons also exhibited low levels of sustained and tonic activation throughout the duration of the stimulus (Fig. 2B, left).

We further examined the auditory stimulus evoked voltage deflections in the recorded local field potentials (LFPs) in both AC and PFC. We found that auditory stimuli effectively produced large event related potentials (ERPs) on all 16 sites in PFC (Figure 2.3Ai). Because of the anatomy of mouse PFC (Fig. 2.1B, Left), our laminar recording could not access the cortical layers perpendicularly. Since we inserted our electrode approximately 0.4 mm from the midline, our recording configuration likely sampled the middle layers, around layers 3-4, across multiple depths that are roughly defined in mice as cingulate cortex, prelimbic cortex, and infralimbic cortex (Figure 2.1B). PFC ERPs had an average onset latency of 28.34±7.25, (mean±S.D., Figure 2.3Di). Interestingly, we detected a significant correlation between ERP amplitude and dorsal-ventral depth ($R =-0.196, p= 4.09e-5$), with the strongest LFP deflections observed in the most superficial sites in the cingulate cortex. In addition, we detected a systematic increase in latencies with depth, with the deepest recording sites lagging ~5ms of that observed in the most superficial recording sites (Fig 3Ci). The changes in ERP amplitude and latency along the dorsal-ventral axis of PFC suggest that mouse PFC may receive varying levels of auditory inputs.

In AC, white noise evoked robust ERPs in most of the recording sites. Some shanks exhibited larger ERPs than others, likely because sections of the auditory cortex are outside the frequency range of the band-limited white noise presented. The largest ERPs were in the middle layers, layers 3-5, ~300-500 um from the surface of the cortex (Figure
Figure 2.3: ERP responses and latencies to white noise stimuli in auditory and prefrontal cortex.

Ai: Example of depth-wise LFP response to white noise stimulus in the PFC. (left) Recording shank inserted along the dorsal-ventral axis of the medial frontal cortex. (right) ERP responses to white noise stimuli taken from corresponding channels. Aii: Same as Ai, for four shank recording electrode in AC. Bi: Population ERP from PFC, in response to white noise. Bii: Same as Bi, for granular layer AC channels. Ci: Mean +/- S.E.M response latencies for PFC ERPs across all recording depths. Cii: Mean +/- S.E.M. response latencies for granular layer ERPs across the rostro-caudal axis of AC. Di: Histogram of ERP latencies from PFC. Dii: Same as Di, for AC granular layer ERPs.
3Aii). The changes in the amplitude of AC ERPs closely paralleled the firing rate changes of MUAs, with the most robust changes observed immediately after tone onset (Figure 2.3Aii), consistent with previous results from AI, AAF, or AII (Stiebler, Neulist et al. 1997; Joachimsthaler, Uhlmann et al. 2014). The mean onset latency of granular layer ERPs was 18.5±3.03 ms (mean±S.D., n=28 mice, Figure 2.3Cii, Dii), about 10ms shorter than that observed in PFC (28.34±7.25, mean±S.D.). Taken together, these results demonstrate that mouse PFC, as well as AC, is robustly modulated by auditory stimuli during passive listening. Nearly half of recorded PFC neurons showed significant responses upon tone presentation, and the auditory evoked responses seen in PFC lag that in AC by about 10 ms, consistent with a relay of auditory information in AC before reaching PFC.

**Cholinergic signaling modulates response properties in auditory cortex**

In order to determine how different cholinergic receptors might influence sound processing in AC and PFC, we infused cholinergic antagonists into AC through an infusion pipette placed between shanks two and three of the four shank electrode (Figure 2.1B and Figure 2.4C). Infusion volumes were limited to 500nL for all drugs and vehicle. We infused the muscarinic receptor antagonist scopolamine at a higher dose (100ug/ul) and a lower dose (10ug/ul), as well as the nicotinic antagonist mecamylamine at a higher dose (10ug/ul) and a lower dose (1ug/ul) that have been shown to influence behavioral performance in active and passive experimental paradigms (Ingles et al. 1993, Maruki et al. 2003, Santucci and Shaw 2003, Rogers and Kesner 2004, Boix-Trelis et al. 2007).
To evaluate the effects of cholinergic receptor blockade on the time course of the AC stimulus response across cortical layers we characterized sound evoked current source density (CSDs). CSD analysis estimates the second spatial derivative of laminar LFP signals to calculate the current flows across the cortical depth over time. It has previously been shown that different inputs to AC may be dissociated in the temporal domains using CSD analysis. For example, an initial, short latency sink observed in middle layers of AC has been attributed to strong thalamocortical feedforward activation, although local intra-columnar activity due to recurrent feedback within layer IV may also contribute to this sink (Happel et al. 2010). The subsequent current source that emerged after the initial sink reflects primarily intracortical inputs from horizontal afferents in the supra-granular, or infra-granular layers, leading to a latent current source in the granular/middle layers. We found that white-noise stimulus produced a large current sink in the granular layers at a depth of ~300-500 um from cortical surface (Fig. 4A, blue color), followed by a strong source that emerged ~70 ms after tone onset (Fig. 4A, red color). To quantify the CSD profile across animals and across drug treatment conditions, we computed the population CSD profiles by taking the CSD trace with the largest magnitude current sink on each recording shank, presumably reflecting the granular layer, and then averaged across animals and shanks (Fig. 4B). The strong sink occurred at a latency of 18.5±3.03 ms from stimulus onset and lasted 55.93±4.24ms (mean±S.D., based on return to zero crossing). The subsequent current source lasted 58.26±40.19 (mean±S.D., Figure 2.5B). These response profiles are consistent with the general findings in AC (Szymanski et al. 2009,

After administering high dose scopolamine, the CSDs profiles on the two inner shanks were largely attenuated, while the responses on the two outer shanks remained robust and un-altered (Fig 4D). To quantify the effects of the infusion across mice, we grouped responses from the middlemost (inner) shanks, closer to the infusion pipette, and the two outer shanks, further away from the infusion pipette, so that any drug concentration differences due to diffusion would be constant within the populations. We calculated the latency and magnitude of CSD changes during the early sink (Early) and late source (Late) periods (figure 2.4B and figure 2.4C). We defined the early sink period as the period in which the mean granular layer current flow was negative in magnitude (within 72ms after sound onset), during which the gross current flow is likely dominated by thalamocortical/intracortical inputs. The late source period was defined as the period in which the mean granular layer current flow was positive (72-190ms after sound onset, Figure 2.4B), during which the current flow is primarily influenced by cortico-cortical inputs.

We first examined the magnitude of the changes in granular layer CSD profile upon drug infusion. We found that on the inner shank, high dose scopolamine infusion drastically attenuated both early sink, (Wilcoxon signed-rank test, p=0.047), and completely abolished the late source (Wilcoxon signed-rank test, p=0.047) on the inner shanks (Figure 2.4F, top). High dose scopolamine infusion however produced no effect on the outer shanks (Wilcoxon signed-rank test, p=1 for early sink, and, p=0.297 for late source)
The lack of effects on the outer shank is consistent with a lower concentration of scopolamine due to diffusion. This is consistent with our observation.

Figure 2.4: Scopolamine attenuates CSD responses in auditory cortex.

A: Example laminar ERPs in AC overlaid on trial averaged CSD calculated from ERPs. B: Population mean +/- s.e.m. granular layer CSD. Red dotted line represents 0 mV/mm. Zero crossings define our analysis windows, capturing the initial granular layer current sink 0-72 ms, and the post-synaptic current source 72-186 ms. C: Diagram illustrating recording/infusion configuration in AC. Inner shanks (2&3) are closer to infusion pipette, and will have a higher concentration of infused drug nearby than the outer shanks (3&4) due to diffusion. D: CSD across four probe shanks prior to scopolamine infusion and E: after scopolamine infusion. Bar graphs represent the RMS across CSDs in the early and late windows for each CSD plot. F: Population mean +/- s.e.m. granular layer CSD on the inner and outer shanks (black) before scopolamine infusion (red) after scopolamine infusion. G: (top) RMS of across CSDs for ACSF infusion population during early (left) and late (right) analysis windows. Bar height represents population mean, error bars are S.E.M. (middle) same as top for scopolamine 100ug/ul infusion. (bottom) same as top for 10 ug/ul mecamylamine.
that low dose scopolamine infusion failed to produce any effects on inner sites.

Interestingly, while high dose scopolamine infusion failed to influence the magnitude of CSDs on the outer shanks, we noticed that it significantly increased the duration of the current sink from about 72ms to ~142ms (N=7 mice, Wilcoxon signed-rank test, p=0.03) (Fig. 4F, bottom). Additionally, it delayed the peak of the latent granular layer source (N=7 mice, Wilcoxon signed-rank test, p=0.002). These temporal changes observed on the outer shanks are consistent with the idea that intracortical inputs originating from adjacent inner shank cortical regions are delayed or attenuated upon muscarinic blockade.

Neither ACSF, nor any other drug conditions, produced effect on early sink or late source CSD amplitude on the inner or outer shanks in either of the two windows analyzed (Table 2.1 and Figure 2.4G). Together, these results suggest that endogenous level of muscarinic receptor activation is necessary for cortico-cortical signaling within auditory cortex.

<table>
<thead>
<tr>
<th>Drug</th>
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<th>WSR test</th>
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</thead>
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</tr>
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</tr>
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<td>72-190 ms</td>
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<td>WSR test</td>
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Table 2.1 Statistical comparison of CSD RMS values between drug and baseline conditions

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Table 2.2 Statistical comparisons of granular layer sink duration between drug and baseline conditions

We then compared the onset latency before and after drug infusion for each of the antagonists used, as well as ACSF control. Mecamylamine at both high and low doses significantly delayed the sound response on the inner shanks (lose dose (1µg/µL): Wilcoxon signed-rank test, p=0.004; high dose (10µg/µL): Wilcoxon signed-rank test,
p=0.037). Onset latencies did not differ in controls or low dose scopolamine groups on the inner shanks (Table 2.4 and Figure 2.5B and 2.5D). Given the attenuation of the evoked response upon high dose scopolamine infusion on the inner shank, it was difficult to accurately measure onset latency without considering the changes in amplitude. However, when we examined the latency to peak, we found that high dose scopolamine did not alter latency to peak (Figure 2.4F), suggesting that thalamocortical input was likely conserved. No difference in onset latency was detected in any of the drug or vehicle conditions on the outer shanks (Wilcoxon signed-rank test, p> 0.05). Together, these findings suggest that the timing of the initial sound response depends on intrinsic nicotinic receptor activation. Although nicotinic blockade with mecamylamine did not alter the auditory evoked response amplitude, it delayed the responses by several milliseconds (Figure 2.5A, 2.5D).

<table>
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Table 2.4: Statistical comparison of granular layer current sink latency between drug and baseline conditions.

Interestingly, in all drug or vehicle infusion conditions, we did not observe changes in individual AC MUAs responses, suggesting that neurons in auditory cortex remained responsive to white noise stimuli even though CSDs had been drastically altered with cholinergic receptor antagonism. Together, these results suggest that muscarinic receptors
play a significant role in both the early and late phase of auditory evoked responses. Blocking muscarinic receptors reduced both early stimulus response mediated by

Figure 2.5: Mecamylamine delays CSD current sink onset latency in auditory cortex.

A: Representative example of CSD profile from an animal prior to drug infusion (top) and following infusion of low-dose mecamylamine (bottom). Onset latency of granular layer sink (blue) is demarcated by the dashed line in the pre and post infusion periods. B: Population granular layer CSD mean CSD+/-SEM for vehicle infused animals (top) before (black) and after (blue) infusion for the inner shank sites. Population response following mecamylamine (bottom) infusion are shown for comparison (red) to the pre-infusion condition (black). C and D: CSD mean population latency onsets for all drug conditions and vehicle compared to baseline are shown for outer shanks (C) and inner shanks (D) +/- SEM. Note for inner shanks the attenuation of the current sink amplitude prevented us from including it in the latency analysis (* = p<0.05).
thalamocortical and intracolumnar excitation and the late phase cortico-cortically mediated response. In contrast, blocking nicotinic receptors delayed the onset of responses in the granular layers, but did not affect CSD amplitude.

**Cholinergic signaling in auditory cortex modulates sensory response properties in prefrontal cortex**

Next we sought to determine how different cholinergic receptor systems are involved in the modulation of sound evoked PFC responses. We discovered that high dose scopolamine infusion in AC significantly reduced ERP magnitude measured in PFC (Wilcoxon signed-rank test, $p=0.031$; Figure 2.6Aiii, B), and reduced the number of responsive MUAs by 70%. Low dose scopolamine infusion produced a moderate decrease in PFC ERP magnitude, but failed to reach significance (Wilcoxon signed-rank test, $p=0.125$, Figure 2.6B). The reduction in ERP magnitude upon scopolamine infusion was observed throughout the recording depth, and there was no correlation between ERP amplitude changes and depth ($R=-0.021$, $p=0.826$). No changes in ERP or MUAs were observed upon infusion of ACSF or mecamylamine at either dose (Wilcoxon signed-rank test, $p>0.05$, Fig. 6Ai, ii and B). Together, these results further demonstrate that PFC auditory responses are relayed in AC, and that endogenous muscarinic activation in AC modulates the information relay from AC to PFC.

**Conclusions**

In summary, our results demonstrate that ERPs and firing rate increases accompany white noise presentation in PFC, as well as auditory cortex, providing novel evidence that
PFC is involved in passive processing of sound. Since attenuation of early phase, and abolishment of late phase CSD responses induced by muscarinic blockade is consistent with previous findings utilizing cocktails of GABAergic blockers designed to target

Figure 2.6: Scopolamine infusion in the auditory cortex reduced amplitude of sound evoked LFP responses in medial frontal cortex.

A: Example ERP demonstrating ERP amplitude following local AC infusion of Ai: ACSF, Aii Mecamylamine, and Aiii: Scopolamine. (black) pre infusion ERP, (red) post infusion ERP. B: Median post-infusion ERP amplitude for ACSF and scopolamine 100ug/ul infusion conditions, expressed as a percentage of pre-infusion amplitude. Error bars are S.E.M. C: Example of an individual neuron in prefrontal cortex that lost sound responsivity following infusion of scopolamine into auditory cortex.
cortico-cortical activity, we also conclude that muscarinic activity is involved in cortico-cortical processing within auditory cortex during waking, passive processing of sensory stimuli (Happel et al. 2010). Furthermore, the reduction in ERP amplitude in PFC upon infusion of scopolamine into AC, strongly supports a model where AC relays auditory neural pathways to PFC, and these pathways are dependent on basal levels of muscarinic binding to function.

**Discussion**

This study aimed to better understand how auditory signals presented during low attentive states are processed in PFC, as well as how cholinergic influences modulate or gate sensory processing. We performed simultaneous recordings in AC and PFC in awake head-fixed mice while they were subjected to repeated presentations of a white noise stimulus. White noise was chosen to better recruit as much of AC tonotopy as possible, so that comparisons between recording penetration sites could be made to assess drug effects, and possibly to measure PFC responses (Romanski and LeDoux 1992; Campeau and Davis 1995; LeDoux 2000; Romanski and Goldman-Rakic 2002).

We found that PFC receives sensory input during passive listening, with strong ERPs and about half of the recorded neurons significantly modulated, indicating that PFC activation occurs even though the animals were not engaged in a behavioral task. Additionally, we found robust changes across all auditory shanks, exhibiting CSD patterns similar to those previously reported in primary auditory cortex with a response latency of 11-18ms after tone onset (Szymanski et al. 2009, Happel et al. 2010, Szymanski, et al. 2011, Intskirveli
and Metherate 2012, Metherate et al. 2012), suggesting that the majority of our recordings were made from primary auditory areas.

Because synaptic integration in auditory cortex depends on interactions between afferent thalamocortical input, local intracortical, and long-range corticocortical interactions (Happel et al. 2010), we closely examined CSD amplitude and latency and compared them to responses in the presence of cholinergic antagonists. We found that local muscarinic blockade in AC reduced sensory-related activation in PFC, suggesting that sensory information is relayed from AC to PFC, and that basal levels of muscarinic activation are important to convey information about auditory stimuli. Responses seen in PFC were delayed by ~10 ms from activation in AC, suggesting one or two synaptic delays between them. In addition, the results that scopolamine attenuated both the initial current sink and the later current source supports the conclusion that muscarinic receptors play a complimentary role in modulating response properties in auditory cortex independently from thalamocortical influences which primarily influence the early current sink. It is likely that muscarinic receptor activation can modulate intra-columnar propagation and/or influence feedforward cortico-cortical signaling. This, together with the results demonstrating attenuation of PFC ERPs, support the conclusion that muscarinic receptors play an active role in gating cortico-cortical synaptic activation during low attentional demands.

One possibility for this gating to occur is that acetylcholine, at low levels, effectively lowers the threshold for excitation and promotes feedforward excitation by increasing the
m-currents in layer II/III low threshold spiking interneurons, which have been proposed to gate intercolumnar signaling by inhibiting fast spiking interneurons (personnel correspondence – Jung Lee; Chen, Sugihara et al. 2015). Our \textit{in vivo} finding is somewhat contradictory to previous in vitro studies demonstrating intracortical activity can be attenuated by muscarinic receptor activation presumably through presynaptic inhibition (Hasselmo and Bower 1992; Kimura and Baughman 1997). A potential explanation for this difference is that under conditions of enhanced cholinergic release, as during states of hypervigilance or sustained attention, augmented acetylcholine release promotes activation of presynaptic muscarinic receptors that contribute to a decrease in cortico-cortical signaling and favoring thalamocortical inputs. This effect, for example, has been demonstrated during visual attention tasks, where acetylcholine has been shown to enhance response properties in the attended visual field and this augmentation can be selectively blocked by scopolamine (Herrero et al. 2008). Future experiments will be necessary to fully understand this relationship; however our data suggests that the traditional views of muscarinic receptors mechanisms in cortico-cortical signaling may differ during periods of low cholinergic tone from those that support high attentive states.

We also found that signaling through nicotinic receptors during passive listening enhanced the relative timing of thalamic inputs into AC. The nicotinic antagonist mecamylamine significantly delayed the sound evoked CSD response at both doses for the sites nearest the drug infusion but did not alter timing at distal sites. Perhaps unexpectedly, we found that mecamylamine had no effect on LFP amplitude or CSD amplitude at either dose used in our study. Based on previous studies (Kawai et al. 2007;
Intskirveli and Metherate 2012), we expected that mecamylamine may attenuate the auditory evoked amplitudes at short time scales in response to white noise but we found no evidence of this. In evaluating this result, it is important to consider mechanisms of cholinergic influences on nicotinic receptors may vary by experimental condition and the attentional demands. Furthermore, an explanation for the lack of effect of mecamylamine on the magnitude of AC CSDs and PFC ERPs, is that in this paradigm mice were passively presented with auditory stimuli, and as mentioned previously, nicotinic receptors have been shown to modulate cortical dynamics during active behavioral tasks. In other studies where nicotinic agonists have demonstrated enhanced probability of firing, particularly at best frequencies, the stimulus is generally presented at lower intensities (<60dB) where the probability of a response is presumably lower. In addition, the effects of nicotine on stimulus processing are complicated by the fact that much of our knowledge of nicotinic influences comes from task performing animals where cholinergic tone is presumably much higher. Under these conditions acetylcholine release is thought to improve sensory encoding by decreasing the correlated activity of cortical neurons (Cohen and Maunsell 2009) and enhancing responses to relevant stimuli during sensory discrimination (Liang et al. 2008). Although we found that nicotinic antagonists delayed response onset for our sound stimulus, this delay had no effect on the amplitude or timing of the response we measured in frontal cortex and suggests that at least during passive sound presentation, nicotinic receptors do not contribute to gating of auditory stimuli to association cortices.
Taken together, our data provide direct evidence that cholinergic mechanisms play a role in ascending auditory stimulus processing and are complimentary to the roles ascribed for cholinergics in facilitating top-down mediated attentional performance (Sarter et al. 2005, Guillem et al. 2011, Hasselmo and Sarter 2011). We found no evidence that either antagonist by itself impaired the ability of the neurons to respond to a particular stimulus within auditory cortex, suggesting that endogenous cholinergic signaling is not necessary for thalamocortical input. Instead, it appears that muscarinic receptor activation contributes to circuit dynamics that are permissive to cortical gating. Acetylcholine may promote long-range synaptic communication by enhancing intrinsic oscillatory patterns in order to bind networks of neurons together in stimulus processing (Roopun et al. 2010). In this regard, cortical projecting cholinergic systems may contribute to signal processing by gating information flow between cortices, as well as between thalamus and the cortex. Future experiments will be necessary to determine how this process is accomplished, however, new research suggests that superficial SOM cells may play an active role in cholinergic modulating neuronal decorrelation that could be associated with a shift in corticocortical connectivity (Chen et al. 2015). It will be important to better understand how cholinergic receptor activity contributes to signal processing across a variety of attentive states and how that contributes to our framework of how synchrony can facilitate intracortical communication.
CHAPTER THREE: DISRUPTION OF OPTOGENETICALLY INDUCED CHOLINERGIC CORTICAL DESYNCHRONY WITH LOCAL APPLICATION OF CHOLINERGIC BLOCKADE

Introduction

Much of the understanding about the dynamics of cholinergic modulation in cortex comes from studies using electrical stimulation of the basal forebrain (Berg et al. 2005, Metherate et al. 1992, Bakin et al. 1996, Goard & Dan 2009). Gross electrical stimulation depolarizes all neurons near the stimulation electrode non-specifically (Tehovnick, 1996). This is problematic when examining the function of the cholinergic nucleus basalis, because it is comprised of a variety of cell types that produce different neurotransmitters. Optogenetics successfully averts this experimental obstacle because cholinergic neurons can be genetically targeted allowing for testing of specific hypotheses regarding their functions. A widely observed effect of cholinergic efflux in cortical circuits is cortical desynchrony; a phenomenon characterized by a simultaneous decrease in cortical LFP power at low frequencies (>10 Hz) and increase in power at higher frequencies (10-100 Hz). It has previously been observed in visual and auditory cortices coincident with optogenetic or electrical activation of the nucleus basalis (Metherate and Ashe 1993; Goard and Dan 2009, Kalmbach et al. 2012; Pinto et al. 2013, Kalmbach and Waters 2014). Within the last ten years, many of the early results from electrical stimulation studies of cholinergic modulation of cortical dynamics have been confirmed with the use of optogenetics (Chen et al. 2015, Kalmbach et al. 2012, Pinto et al. 2013, Kalmbach and Waters 2014), however most of these studies have taken place in
reduced preparations, including the use of anesthetized animals and the systemic application of cholinergic antagonists. We know that cortical dynamics during sleep are fundamentally different from waking, and that basal forebrain activity is intricately related to the sleep/wake cycle (Anaclet et al. 2015). Additionally, systemic injections can influence an animal’s entire central nervous system, confounding results. In awake animals, how do we confirm that our optogenetic, and pharmacological manipulations are effective, without introducing unintended experimental confounds?

We sought to overcome these research obstacles by developing a method of optogenetically stimulating the cholinergic nucleus basalis (NB) of awake mice, while infusing cholinergic drugs locally into the auditory cortex and taking multi-channel electrophysiological recordings. We inserted a four-shank, 32 channel laminar probe into the auditory cortex coupled to an infusion pipette and centered the pipette tip on the four probe shanks in order to capture regions of cortical tissue both within, and outside the volume tissue reached by diffusion of drugs. We infused the muscarinic antagonist scopolamine, and the nicotinic antagonist, mecamylamine in order to determine the local contribution of nicotinic and muscarinic receptors to local changes in cortical activity in auditory cortex. Additionally, we inserted a probe in the prefrontal cortex—a region heavily innervated by the nucleus basalis cholinergic projections, but far away from our site of infusion (Zaborszky et al. 2012). Using these methods we were effectively able to access the cholinergic nuclei within NB and its projection system and capture their interaction within the auditory cortex.
Several studies have demonstrated that muscarinic antagonists block aspects of cortical desynchrony as well as altering baseline LFP and EEG patterns (Berg et al. 2005, Metherate et al. 1992, Bakin et al. 1996, Kalmbach and Waters 2014). We found that muscarinic receptor blockade reduced low frequency power (1-10 Hz) during cholinergic stimulation, and reduced high frequency power (10-100 Hz) during periods of baseline. We are able to show that alterations of the baseline cortical LFP following scopolamine infusion take place on the inner recording shanks only, closest to the infusion pipette, while alterations in power during cortical desynchrony take place across the auditory cortex despite the confined nature of the infusion.

Summary of findings

We demonstrate that blockade of muscarinic receptors via scopolamine infusion in AC, reduces LFP power at high frequencies (10-100 Hz), only on the inner electrode shanks, closest to the site of scopolamine infusion. We can further conclude that the inner electrode shanks are within the region reached by diffusion of the drug, while the outer shanks remain outside the area of infusion. We also provide novel evidence that cortical desynchrony induced by optogenetic stimulation of the cholinergic nucleus basalis occurs in PFC. Furthermore, while it has been demonstrated previously that muscarinic blockade can disrupt cholinergically induced cortical desynchrony (Berg et al. 2005, Metherate et al. 1992, Bakin et al. 1996) we demonstrate novel findings that the specific disruption of the low frequency (<10 Hz) decrease in LFP power via muscarinic blockade occurs within the region of infusion (inner shanks), as well as neighboring regions, outside the volume of tissue reached by the drug upon administration (outer shanks).
This finding demonstrates that the low frequency decrease in power associated with
cortical desynchrony is supported by mutual activity among neighboring cortical areas,
and that it relies on activity that is relayed along lateral cortical connections. Essentially,
by disrupting activity at muscarinic synapses within a small portion of auditory cortex,
we were also able to disrupt the pattern of cortical desynchrony across a large expanse of
auditory cortical tissue, traversed by our four shank recording array.

**Methods**

All data analyzed in this chapter was collected during the same recording sessions
described in the methods section of chapter two. To summarize the methods from
chapter two, we performed acute multi-channel recordings and optical stimulation in
Chat-ChR2 transgenic mice in the awake, head-fixed condition. We took laminar
recordings across the rostro-caudal axis of auditory cortex and depth-wise recordings
across the dorsal-ventral axis of PFC, while optogenetically stimulating the cholinergic
nucleus basalis (Figure 2.1). Light stimulation consisted of 750 ms of 40 Hz (473 nm)
laser stimulation emitted at the tip of a 200 um optical fiber inserted acutely into the
nucleus basalis. Light stimulation trials were interleaved with trials presenting acoustical
white noise stimuli (discussed in chapter 2). The inter-trial interval between trials was 10
seconds, and each trial type occurred 100 times during a recording block. Pharmacology
consisted of local drug infusions, through an infusion pipette affixed to the multi-channel
electrode array inserted in the auditory cortex. The tip of the infusion pipette was place
so that it was halfway between the two inner shanks of the four shank electrode (Figure
2.1B, 2.4C). Cholinergic antagonists (scopolamine and mecamylamine), as well as ACSF
for control, were infused after an initial recording block, and a second block was recorded following infusion.

**Results**

In order to better understand how endogenous cholinergic signaling contributes to AC and PFC interactions, we utilized optogenetic stimulation to elicit cortical cholinergic release in the presence of each antagonist during passive listening. A widely observed effect of cholinergic activation on sensory cortices is cortical desynchrony, a phenomenon characterized by a simultaneous decrease in cortical LFP power at low frequencies (<10 Hz) and increase in power at high frequencies (10-100 Hz), and is often calculated as the ratio of high frequency power (10-100Hz) to low frequency power (1-10Hz). While optogenetic or electrical activation of nucleus basalis has been shown to produce cortical desynchrony in sensory cortices and motor cortex (Metherate and Ashe 1993, Berg et al. 2005; Goard and Dan 2009; Kalmbach et al. 2012, Pinto et al. 2013, Kalmbach and Waters 2014), it is unclear whether it occurs in PFC. In addition, it is unclear how different receptors mediate distinct frequency patterns relevant for cholinergically induced cortical desynchrony effects. We positioned an optical fiber in the nucleus basalis to optogenetically stimulate cortically projecting cholinergic neurons in Chat-Ch2 transgenic mice, while simultaneously recorded in AC and PFC in the presence or absence of cholinergic receptor blockers (Fig. 1D, condition 2). Control experiments were conducted in Ai32 transgenic littermates.

Before examining the effects of optogenetic stimulation of the cholinergic nucleus basalis, we first compared changes in LFP power before and after drug infusion in the
absence of optogenetic stimulation. Systemic application of cholinergic antagonists have been shown to modulate cortical LFP power (Kalmbach and Waters 2014). We found that local infusion of high dose scopolamine in AC significantly reduced AC LFP power at high frequencies of 10-100Hz (Wilcoxon signed-rank test, p=0.031), but not lower frequencies (1-10 Hz) (Wilcoxon signed-rank test, p=0.156; Figure 3.1A). This was observed only on the inner electrode shanks (Wilcoxon signed-rank test, p=0.031), but not the outer shanks (Wilcoxon signed-rank test, p=0.156; Figure 3.1A and 3.1B), consistent with drug diffusion induced concentration differences. Other drug conditions, including low dose scopolamine, high and low dose mecamylamine, and ACSF failed to alter LFP powers (Wilcoxon signed-rank test, p>0.05). Together, these results demonstrate that the endogenous muscarinic receptor activation is important in coordinating neural synchrony within AC.

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Table 3.1 Statistical comparison of LFP power in low and high frequency ranges between drug and baseline conditions

We next quantified optogenetically evoked LFP power changes in both AC and PFC in Chat-ChR2 mice in the absence of any auditory stimuli and before infusing drugs. We found that optogenetic stimulation of cholinergic nucleus basalis evoked a significant increase in cortical desynchrony in both AC (Figure 3.1B (right); Wilcoxon signed rank test, p=3.79e-6) and PFC (Figure 3.1C (right); Wilcoxon signed rank test, p=1.318e-5). Cortical desynchrony was not observed upon light stimulation in control Ai32 only transgenic mice (Figure 3.1B (left) and 3.1C (left), Wilcoxon signed rank test, p=0.219),
Figure 3.1: Optogenetically induced cortical desynchrony in auditory and medial frontal cortex.

A: Population spectrum of baseline LFP in auditory cortex. (Left) Population mean spectra of LFPs +/- SEM before (black) and after (red) infusion of scopolamine. (Right) Population mean +/- SEM power within the 1-10 Hz and 10-100 Hz power bands on the inner and outer shanks for (left) ACSF and (right) scopolamine 100 ug/uL. B: Trial averaged, baseline normalized spectrograms of auditory cortex LFP centered on 750ms optogenetic activation of cholinergic nucleus basalis neurons. (left, top) population average of all trials from control ChR2+/Chat-, (right, top) from ChR2+/Chat+. (bottom) ratio of low power (1-10 Hz) to high power (10-100 Hz) in spectrograms. C: Same as B, for medial frontal cortex.
confirming that light mediated cholinergic activation is responsible for the observed cortical desynchrony. While it has not been reported to our knowledge that cortical desynchrony occurs in PFC, the observed cortical desynchrony in PFC here, is consistent with the anatomical projection patterns of cholinergic nucleus basalis, which sends strong afferents to the frontal regions, in addition to sensory cortices.

We then examined the influence of selective cholinergic receptor blockade on cortical desynchrony induced by optogenetic stimulation of cholinergic nucleus basalis, by comparing the cortical desynchrony induced by optogenetic stimulation before versus after drug infusion. Interestingly, upon infusion of high dose scopolamine, optogenetically induced decrease in 1-10 Hz LFP power within AC was abolished (chi-square=15.8, p= 7.025 e-5), on both the inner (Figure 3.2B top; Wilcoxon signed-rank test, p=0.016) and outer shanks (Figure 3.2B top; Wilcoxon signed-rank test, p=0.016). Since scopolamine infusion directly influences baseline LFPs only on the inner shanks, this findings suggests that the effect observed in the low frequency range on the outer shanks during cholinergic activation is caused by disruptions of cortical desynchrony in the adjacent cortical tissue, recorded on the inner shanks. This further suggests that within a cortical region, lateral cortical interactions as well as local cortical dynamics contribute to the generation cortical desynchrony upon cholinergic activation.

Additionally, high dose scopolamine failed alter optogenetically induced high frequency power on both the inner and outer shanks (Wilcoxon signed-rank test, p>0.05).

Mecamylamine and ACSF control did not produce any change at high or low frequencies
at either dose on inner or outer shanks (all Wilcoxon signed-rank test, p>0.05). In PFC, none of the drugs or vehicle altered the LFP power.

Figure 3.2: Scopolamine attenuates decrease in low power induced by cholinergic activation.

A: (top, left) Example LFP spectrogram demonstrating cortical desynchrony before infusion of scopolamine in auditory cortex, (bottom, left) same as top for medial frontal cortex. (right) Cortical desynchrony in auditory (top) and medial frontal cortex (bottom) following infusion of scopolamine 100ug/ul into auditory cortex. B: Mean post-infusion power +/- SEM, expressed as percentage of pre-infusion power, in low (1-10 Hz) and high (10-100 Hz) frequency bands during the 0-750ms following onset of cholinergic activation in AC (left) and PFC (right). AC measurements are split into inner and outer shanks, as they are in cortical regions with differing concentrations of infused...
induced optogenetically at either high or low frequencies (Figure 3.2C bottom; all
Wilcoxon signed-rank test, p>0.05). While high dose scopolamine altered cholinergically
induced cortical desynchrony in AC, it failed to alter it in the PFC, suggesting that
cortical desynchrony is locally generated within PFC, and that the effects produced by
local scopolamine infusion in AC were confined to AC and not relayed to PFC.

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<td>10-100 Hz</td>
<td>N/A</td>
<td>p=0.625</td>
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<td>10-100 Hz</td>
<td>Chi-square=1.07, p=0.301</td>
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<td>1-10 Hz</td>
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<td>10-100 Hz</td>
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<td>outer</td>
<td>10-100 Hz</td>
<td>N/A</td>
<td>p=0.438</td>
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Table 3.2 Statistical comparison of cholinergically induced LFP power in high and low frequency ranges during drug and baseline conditions.

We demonstrate that selective activation of cholinergic nucleus basalis using optogenetics techniques can reliably induce cortical desynchrony in both AC and PFC, consistent with the broadly projection patterns of these cholinergic neurons. While in AC both an increase in high frequency LFP power and a decrease in low frequency LFP power contribute to the observed cortical desynchrony, in PFC, cortical desynchrony is primarily expressed as an increase in high frequency oscillations. Upon local muscarinic blockade in AC, we detected local changes during cholinergic activation, specifically at low frequencies, but not high frequencies, suggesting that only the low frequency power decrease in AC depends on muscarinic activation. Furthermore, the disruption of low power decrease occurred on the outer, as well as the inner shanks, indicating that local disruption of cortical desynchrony similarly disrupts cholinergic dynamics in neighboring regions of cortex. However, the lack of differences in PFC cortical desynchrony between drug conditions applied in AC demonstrates that the low frequency dynamic observed in AC is not relayed to PFC.

**Conclusions**

These results provide novel evidence that muscarinic blockade attenuates high frequency power in the cortical local field potential. Furthermore, we demonstrated that muscarinic blockade of the low frequency component of cholinergically induced cortical
desynchrony extends beyond the regions of tissue directly in contact with the administered drug. This strongly supports a model, whereby cortical desynchrony relies on the mutual activity of neighboring regions of cortex, and that activity patterns that support low frequency decrease in power are supported by lateral connections within cortical tissue.

**Discussion**

We wanted to investigate how optogenetically evoked cholinergic release modulates cortical circuits in auditory cortex and how antagonists at the doses we employed modulate those circuit dynamics. Furthermore, we wanted to expand the existing research in cholinergic modulation of cortical dynamics by utilizing local cortical infusion in order to isolate the cortical terminals of the cholinergic projection system and observe the influence of local blockade in the waking state.

We found that optogenetic stimulation of NB cholinergic neurons alters the spectral properties of cortical LFPs recorded with a laminar probe in both auditory cortex and prefrontal cortex (Figure 3.1) and is consistent with previous findings of enhanced neocortical desynchrony with electrical or optogenetic stimulation (Metherate and Ashe 1993, Goard and Dan 2009, Kalmbach et al. 2012, Pinto et al. 2013, Kalmbach and Waters 2014). In addition the evoked desynchrony in auditory cortex and prefrontal cortex was short lasting, as has been shown in visual cortex previously (Pinto et al. 2013). Cholinergic antagonists have been shown to attenuate optogenetically evoked cortical desynchrony (Kalmbach and Waters 2014) which is consistent with our findings in auditory cortex that scopolamine robustly attenuated the low frequency component (1-10
Hz). Interestingly, the low frequency component of cortical desynchrony was reduced on all recording shanks, despite—as demonstrated in chapter 2—that the infusion itself does not diffuse across cortical tissue to the outer shanks. This indicates that a local disruption in cortical desynchrony similarly influences adjacent cortical areas, suggesting that neuronal desynchronization relies on the interactions between cell populations within circuits that are distributed within cortical areas.

In comparison, mecamylamine had no significant effect on cortical desynchrony at either of the doses tested here. It is interesting that scopolamine was effective in blocking the reduction in low frequency power associated with cortical desynchrony. New research suggests that superficial SOM cells may play an active role in cholinergic modulation of cortical desynchrony and neuronal decorrelation (Chen et al. 2015). Interestingly superficial layer SOM interneurons in visual cortex have been shown to be activated by input from surrounding cortex, indicating that they receive lateral connections (Adesnik et al. 2012). This provides a potential mechanism for our results, demonstrating that disruption of cortical desynchrony induced by muscarinic blockade extends beyond the region of drug infusion. Perhaps SOM interneurons rely on the summation of surrounding inputs as well as local cholinergic mechanisms to support circuit dynamics that produce cortical desynchrony.
CHAPTER FOUR: CORTICAL CHOLINERGIC RELEASE MODULATES NEUROPHYSIOLOGICAL SIGNATURES OF EXTINCTION LEARNING IN PREFRONTAL AND AUDITORY CORTEX

Introduction

Selective attention and cognitive flexibility—the ability to update task rules when conditions change—are two notable deficits often characterized in individuals with impaired executive function. The behavioral consequences of cholinergic lesions and acetylcholine depletion in cortex are subtle, specifically impairing animals in reversal learning tasks and attentional orienting (for a review, read Robbins & Roberts 2007, McGaughy et al. 1996). These impairments suggest that cholinergic modulation in cortex is strongly involved in detection of stimuli, and adapting to changing relevance of cues. Additionally, cholinergic modulation of cortical circuits has been proposed to play a role in the maintenance of sensory input in cortical circuits (Zhou et al. 2011), as well as internally driven attentional processing (Howe et al. 2013).

Before examining the role of cholinergic modulation of cortical circuits during stimulus discrimination, it was necessary to identify underlying neurophysiological signatures of learning-related task performance. Since mice have an inherently limited capacity for executive functioning, we chose to utilize an extinction-learning paradigm in order to examine the process by which task rules update during learning. In a simple reinforcement paradigm extinction occurs when an appetitive or aversive association that has been paired with a conditioned stimulus ceases to be conditionally reinforced and subjects then learn that an extinguished stimulus has no behavioral consequence. The
mechanisms by which cortical circuits engage with stimulus-related input during learning are not completely understood, however extinction learning and auditory discrimination are closely linked, since they both require subjects to suppress responding to stimuli which don’t carry any associative meaning.

Since the prefrontal cortex has demonstrated a causal role in the extinction of associative memories in rodents (Van Den Oever et al. 2013), and gene expression in regions of rodent PFC are correlated with phases of extinction learning (Cruz et. al 2015) it was necessary to determine which aspects ongoing neurophysiology within PFC were most closely related to extinction learning. We chose to utilize simultaneous electrophysiological recordings in both the auditory and prefrontal cortex during an auditory extinction-learning task. During the task, two previously reinforced stimuli were presented, while reward was withdrawn from one and maintained for the other.

We found that alpha and beta frequency LFPs occur in PFC following presentation of an extinguished tone once the association with reward is unpaired. Furthermore, we found that at the same time alpha, but not beta rhythms occur in AC. Alpha and beta rhythms have both been shown to be related to attentional processing. Alpha rhythms have been shown to be involved in suppressing neural responses to distracting stimuli and suppressing motor commands, as well as contributing to internal working memory processes (Mirpour & Bisley 2013, Pani et. al 2014, Palva & Palva 2007). Furthermore, beta rhythms have similarly been linked to internal attentional processes as well as self-guided decision making (Iversen et al. 2009, Pesaran et al. 2008). Their co-occurrence during this task suggests that neural dynamics in PFC are actively involved in adapting to
changing rules, as well as self-guided choice during behavioral uncertainty. It is important to note that the alpha and beta LFP increases were observed after mice had already completed conditioned responses (CRs), indicating that the observed synchrony was involved in the process of post response self-evaluation or updating of task rules once each trial was complete.

Having first identified alpha and beta band synchrony in PFC as crucial components of discrimination learning, we ran a follow up recording session after the sensory discrimination was well-learned. In order to investigate how cortical cholinergic signaling is involved with the underlying dynamics that we observed during extinction learning, we employed optogenetic silencing of cholinergic nucleus basalis neurons. Interestingly, we found that suppression of nucleus basalis cholinergic neurons attenuated the magnitude of alpha and beta power in PFC following correct rejections of extinguished stimuli and misses of rewarded stimuli. Our results suggest that cholinergic modulation supports neurophysiological dynamics of endogenous attention, working memory, and sensory discrimination processing.

Summary of findings

Here we demonstrate that alpha and beta power LFPs are associated with the process of behavioral extinction learning in PFC, and that alpha power is involved with learning in AC. Specifically, alpha power LFPs occur following presentations of extinguished and rewarded tones during the early phases of learning in AC, while alpha and beta LFPs only occur following the extinguished tone in PFC. This suggests that PFC engages in the process of updating task rules associated with extinction earlier than AC. During best
behavioral performance, alpha occurs selectively for the extinguished tone in both AC and PFC, suggesting that working memory processes in PFC are transferred to bottom up sensory processing regions of cortex, and that this process is related to successful extinction learning. Furthermore, cholinergic silencing on subsequent recording sessions effectively attenuated the alpha and beta power in PFC following correct rejections, suggesting that the cholinergic system is intricately involved in the generation of learning-associated synchrony in cortical circuits.

**Methods**

All procedures involving animals were approved by the Boston University Institutional Animal Care and Use Committee (IACUC). A total of 11 mice were used in this study (n=11 ChAT-Arch) transgenic mice (3-6 months-old, on the day of recording). Chat-Arch mice were obtained by crossing Chat-Cre line with Ai35 (Archaerhodopsin) line (both were obtained from Jackson Laboratory, Maine).

*Surgical Procedures:* Mice were surgically implanted with head plates in a manner identical to that described in chapter 2. Head plates were implanted under isoflurane anesthesia and mice were allowed to recover completely before beginning behavioral training.

*In Vivo Electrophysiology:* Electrophysiology was performed in a manner similar to that described in chapter 2. On the first recording day, recording electrodes were slowly lowered through two small craniotomies into the right hemisphere PFC (AP -2.0, ML +0.4, DV -2.75) and the auditory cortex (AP +2.3 to +3.6, ML +4.0 to +4.5, DV -1.0)
using motorized micromanipulators (Siskiyou, Grants Pass, OR) at a very slow speed. On
the second recording day a recording electrode was lowered into the left hemisphere PFC,
and bilateral 200 um optical fibers were lowered through two small craniotomies using a
Microdrive (Siskiyou, Grants Pass, OR) into the nucleus basalis in both hemispheres
(NB; AP +0.5, ML +1.5, DV -4.5) for optical silencing.

**Extracellular** recordings were made in the same manner as described in chapter 2.
Spikes were detected with a manual threshold set at the beginning of each experiment and
stored for further analysis with offline sorter (Plexon Inc.).

**Generation of Auditory Stimulus:** Tone stimuli were generated with the same apparatus
described in chapter 2. The stimuli consisted of 500 milliseconds pure tones (5ms cosine
ramp to 90% of peak amplitude).

**Optogenetic Stimulation:** Laser light for optogenetic stimulation of nucleus basalis was
delivered through a multimode optically shielded 200um fiber (Thorlabs, Newton NJ;
model: BFH48-200), coupled to a 532nmDPSS laser (Shangai Laser Ltd., Shanghai,
China; model: BL473T5-200FC). The tip of the fiber was stripped bare to minimize
tissue damage, and the laser power was calibrated to 15mW at the fiber tip prior to
insertion. 750 ms square waves were delivered via TTL trigger from the RZ2 recording
system.

**Behavioral Training:** Mice were water deprived and trained on a two tone operant
behavioral paradigm in which they learned to lick a water spout—coupled to an IR beam
sensor—to receive a small water reward following presentation of either of two
conditioned tonal stimuli. Mice were trained over the course of approximately 1 month
until they reached a criteria of performance of 80% correct trials each day. Water reward consisted of a small (5 uL) droplet of 0.02% saccharine water mixed so that mice would complete several hundred trials before becoming sated each day. On the day of recording, the reward associated with one of the tones was removed, un-pairing the tone from the reward and promoting suppression of conditioned responding for the tone. Mice were then trained over several more days, until the tone discrimination was well learned

*Experimental Protocol:* Conditioned stimuli were presented every 9 +/- 3 second and were followed by a 2 second reward window during which the mice could lick for a water reward. On the day of tone extinction, mice were run until they received 50 rewards before beginning the extinction training. For extinction learning and subsequent behavioral sessions, the reward window was consistently withdrawn from one of the tones. On the second recording day, following several more days of tone discrimination training, mice were allowed to receive 25 rewards before optogentic trials began. Optogenetic silencing consisted of a 750 ms square pulse of laser light beginning 250 ms before onset of rewarded or extinguished tones and co-terminating with tones.

*Data Analysis:* All data analysis was performed with custom Matlab functions (MathWorks, Natick, MA). Statistical tests were either a repeated measures ANOVA, for comparisons of normalized power between behavioral and optogenetic silencing conditions, paired ttests, or Wilcoxon signed rank tests for comparisons of firing rate and lick rate data. All data that was analyzed with ANOVAs or ttests was first tested for normality using a Lillie test. All data that did not fit a normal distribution was analyzed
Mice were water deprived for 5-7 days before being trained to lick a water spout for reward delivery. The first phase of training consisted of pavlovian conditioning, during which mice received an automatic water reward following presentation of either of two conditioned stimuli (CS). During the next phase of training, water rewards were triggered by mouse licks within the 2 second interval following presentations of either CS. (operant conditioning). In the first experimental session, lick-triggered water release was denied for one of the two CS tones, and acute recordings were taken in auditory and prefrontal cortex. The removal of reward caused mice to undergo extinction of the association between reward and CS for one of the tones. After 1-2 more days of behavioral training mice typically completed the process of extinction learning and were acutely implanted with a prefrontal recording depth electrode, and bilateral optical fibers in the nucleus basalis.

using a non-parametric Wilcoxon signed-rank test. In some instances the logarithm of the data values were used for statistical analysis.

LFPs were down-sampled to 381 Hz prior to further analysis. In awake head fixed conditions, we occasionally found motion-induced artifact. To remove motion artifact, we took the root mean square (RMS) in 100 ms bins for all recorded trials. We rejected trials if the channel-averaged RMS curve exceeded 5 standard deviations at any point during the response window.
For analysis of auditory cortex LFPs, current source density was calculated in the manner described in chapter 2, in order to determine the magnitude of stimulus induced granular layer current sinks. Of the four shanks, the shank with the largest sink was chosen for further spectral analysis relative to each tonal stimulus.

**Spike analysis:** Spike snippets were analyzed using Offline Sorter (Plexon Inc., Dallas, TX), and manually clustered in PCA space. Spike peri-stimulus time histograms (PSTHs) were calculated using 20 ms time bins for population and statistical analysis. As a test for cell responsiveness, PFC cells were deemed responsive if their mean firing rate exceeded a z score of 2 during the 500 ms following cue onset. Since AC neurons were more responsive to tone onset, they were deemed responsive if their mean z score exceeded 2 in the first 50 ms following cue onset.

**Lick analysis:** Lick traces were thresholded so that lick onset was determined as the time point at which the lick trace reached ~50% of its peak value. Lick timestamps were then binned into 50 ms bins and peri-stimulus time histograms were constructed around sound onset for each tone/trial type.

**Spectral analysis:** We used the open source software package Chronux (www.chronux.org) to compute multi-taper spectrograms. Three tapers and a 500 ms sliding window with 5 ms step were set as parameters for the analysis. Single trial spectrograms were first calculated, and then averaged across trials. We then calculated trial averaged spectrograms of the LFP for each channel, and then further averaged across channels before normalizing them to their baseline. Baseline was calculated by
averaging the LFP signals during the 3 second window immediately before stimulus onset.

**Results**

To determine the level of activation in AC and PFC during behavioral performance we examined MUAs in both regions during baseline, and during the first fifty trials after extinction learning commenced. For analysis of these intervals, we were able to include data from all mice, regardless of whether or not they learned the task in subsequent intervals of behavioral performance. During baseline trials mice increased their lick rate by a factor of ~8 during the first second of the reward window for both tones (Figure 4.2A, black traces). During the first interval of extinction learning mice licked within the reward interval for both tones. However, the lick rate dropped to roughly 50% of its previous rate upon onset of the extinguished tone (n=11 mice, Wilcoxon signed rank test, P=0.0005, figure 4.2A, red traces), indicating that the withdrawal of reward caused them to reduce their rate of licking.

In PFC, during baseline trials, neurons fired robustly in response to both conditioned stimuli throughout the duration of the tones, increasing on average by a factor of 2.5-3 of the baseline firing rate (9/58 MUAs responsive for both tones, Figure 4.3A, black trace). This confirms previous studies demonstrating that PFC neurons are engaged by behaviorally relevant stimuli (Maren and Quirk 2004; Chang, Berke et al. 2010; Euston, Gruber et al. 2012; Hyman, Ma et al. 2012; Moorman and Aston-Jones 2015). Once extinction training commenced, PFC firing rates significantly attenuated in magnitude during presentation of the extinguished tone, while remaining unchanged following the
rewarded tone (n=58 neurons, Wilcoxon signed rank test, P=0.0005, Figure 4.3A, red trace) although 9/58 MUAs remained responsive. Since PFC firing rates decrease in response to the extinguished tone, but still deviate from baseline during this interval, they are likely involved in the expectation of reward, as well as the retrieval of reward. In auditory cortex, 20/106 neurons were responsive to the rewarded tone and 14/106 were responsive to the to-be-extinguished tone at baseline. The number of responsive MUAs in AC did not change for the extinguished tone in the first 50 trials following removal of reward, and MUAs did not alter their firing rates (n=106 neurons, Wilcoxon signed rank test, P=0.139), suggesting that AC neurons were responsive to the tonal stimuli themselves rather than their behavioral associations.

In order to investigate this dynamic further, we analyzed the spectral properties of the LFP in mice during behavioral performance. We first wanted to determine which aspects

![Figure 4.2: Reward-related lick rate during extinction learning.](image)

A: Normalized lick rate during presentation of the rewarded tone (REW) during baseline trials (black), and during the first 50 trials after extinction training begins (red). (Right) Same as left, for the extinguished tone. B: Average ratio of normalized lick rate during the reward window (0-2 seconds) in CONF and BL. Error bars are S.E.M.
of the observed neurophysiology were related merely to the motor action of licking. Mice periodically licked the reward port throughout the training sessions during the inter-trial interval, perhaps constantly monitoring for reward, or for excess water droplets on the reward port. Figure 4.4, C shows an average PFC LFP spectrogram of 50 non-rewarded licks during the inter-trial interval from 5 good performing animals. During lick motor execution there is clearly a large decrease in the beta and alpha bands of the LFP. This is consistent with decreases in beta band power that have been widely reported.
during motor execution in rodents, monkeys, and humans (Pfurtscheller et al. 2003; Park et al. 2013, Tzagarakis et al. 2010).

Beta and alpha band reductions are also seen immediately following the cue on hit trials in which the animals licked and received reward following the rewarded tone, and on false alarms trials in which the animal licked following the extinguished tone (figure 4.4). Interestingly, this reduction in LFP power also occurs following correct rejections; when the animal successfully suppressed his lick response following presentation of the extinguished tone (Figure 4.4, E), suggesting that the reduction in beta band power may have a complex role supporting both behaviorally relevant cue processing as well as suppression of motor activity. Interestingly, following tone extinction, there is a
relatively strong gamma component centered at 50-70 Hz that appears approximately one second after cue presentation on hit trials that does not appear following pre-extinction hits (figure 4.4 A, B), although direct comparisons are difficult because the baseline period contains limited trials. Since gamma becomes prominent following tone extinction it may reflect that gamma is necessary during cued attention tasks, but only if the animal is required to discriminate between stimuli.

**LFP Dynamics During Extinction Learning**

In order to investigate learning-related changes in LFP power during behavioral performance we limited further analysis to animals which reached criterion (see methods) during the recording session. These animals had a window of 50 trials during of their behavioral performance in which their behavioral d prime score was 2 or greater. We chose the window of highest behavioral performance as the best performance period (BP). Similarly we chose the first 50 trials following the initial removal of reward as the confusion interval (CONF) and the trials before devaluation consisted of the baseline period (BL). In this way we could observe LFP signals in the three intervals most relevant to the animal’s behavioral learning. Additionally, there were three time intervals of interest within each trial: the cue presentation interval (0-500 ms post sound onset) during which the rewarded and extinguished tones were played, the lick interval (0-2 seconds post sound onset) during which the mouse could lick for reward on rewarded trials (note that I refer to this as the lick interval even on trials in which the mouse could not receive reward), and the post-lick window (2-5 seconds post sound onset) during which the animal had completed stimulus-associated licking and waited for the next trial.
Figure 4.5: PFC LFP activity changes during extinction learning.

A: (top, left) Normalized PFC spectrogram triggered on onset of rewarded tones during the BL interval. (top, right) Same as left, for the tone that will be extinguished. (center) Normalized alpha power in the post reward window (2-5 seconds) for rewarded (blue) and to-be extinguished tones (red) during the baseline period (BL). (bottom) same as center, for beta power. Bars plotted in pairs for trial type, arranged according to depth along the recording electrode. B: Same as A, for the first fifty trials after extinction training begins (CONF). C: Same as A, for the fifty trials of best performance during extinction learning (BP). D: Channel averaged PFC alpha in the post-reward window for BL, CONF, and BP periods. Data is averaged from A, B, and C (bottom plots). In all histograms, error bars are S.E.M. Black arrows highlight periods of relevant alpha and beta power in the LFP spectrograms.
A strong period of alpha (8-13 Hz) and beta (13-18 Hz) band power in the PFC LFP is visible in the post-lick interval of CONF extinguished tones that is not visible prior to extinction (Figure 4.5 B, A). Furthermore the alpha and beta is maintained during BP for the extinguished tone (Figure 4.5C). In both intervals the alpha and beta occur consistently across all depths of the PFC recording sites (Figure 4.5 B,C). ANOVA reveals a significant main effect for alpha power and learning interval (i.e. BL, CONF, and BP windows, F(2,8)= 31.28, p=0.0002) and a significant interaction between interval and trial type (i.e. extinguished and rewarded tone presentations, F(2,8)=4.51, p=0.049). Similarly, there was a main effect for beta power and learning interval (F(2,8)=19.86, p=0.0008), and a significant interaction between interval and trial type (F(2,8)=4.86, p=0.041). These results demonstrate that alpha and beta LFPs are associated with the process of stimulus selection during extinction learning.

In AC, increases in alpha frequency LFP power occur following both rewarded and extinguished tones in CONF, but persist only for the extinguished tone in BP (Figure 4.6 B, C). ANOVA revealed a significant main effect for alpha and learning interval in AC (F(2,8)=5.2, p=0.038), but no significant interaction between learning interval and trial type (F(2,8)=0.27, p=0.77)). It is likely that there was no interaction between trial type and learning interval for alpha power because alpha did not occur selectively for the extinguished tone until BP; remaining for both trial types during CONF (Figure 4.6 B). This result suggests that cortical alpha power occurs selectively for rewarded/non-rewarded trial type later in AC than in PFC, perhaps reflecting delayed top down input from PFC during behavioral learning. Additionally, there was no main effect for learning
Figure 4.6 AC LFP activity changes during extinction learning.

A: (top, left) Normalized AC spectrogram triggered on onset of rewarded tones during the BL interval. (top, right) Same as left, for the tone that will be extinguished. (middle) Normalized alpha power in the post-reward window (2-5 seconds) for rewarded (blue) and to-be extinguished tones (red) during the baseline period (BL). (bottom) Same as middle for beta power. Bars plotted in pairs for trial type, arranged according to depth along the AC recording electrode. B: Same as A, for the first fifty trials after extinction training begins (CONF). C: Same as A, for the fifty trials of best performance during extinction learning (BP). D: Channel averaged PFC alpha in the post-reward window for BL, CONF, and BP periods. Data is averaged from A, B, and C (bottom plots). Black arrows highlight periods of relevant alpha power in the LFP spectrograms.
interval and no interaction between learning interval and trial type for beta (P>0.05). The absence of beta in AC suggests that the beta observed in PFC is specific to top-down cortical processing. Interestingly, in BP, AC alpha is weaker in superficial layers, and strongest in the deep layers, suggesting either that top down input projects to the deep layers of auditory cortex, or that it influences cortical interneurons that play a role in alpha generation and project to the deep layers (figure 4.6 B, C).

*Cholinergic Silencing During Tone Discrimination*

On the second recording day, after animals reached criterion for tone discrimination, optogenetic silencing of the cholinergic nucleus basalis was performed on 50% of trials. Silencing trials were divided evenly between rewarded and devalued tones and interleaved throughout the behavioral session. Additionally, the electrophysiological data was grouped into response outcomes: hits, misses, correct rejections, and false alarms. Lick rates did not differ between silencing and non-silencing conditions during any trial type (Wilcoxon signed rank test, p>0.05). However, there was a small increase in lick rate toward the end of the licking response (Figure 4.7 A, right) that may be induced by cholinergic silencing. Interestingly, MUAs in PFC also had a moderate increase in firing rate over the course of the reward window during false alarm silencing trials (figure 4.7, C), suggesting that increased PFC activation during false alarms may have prolonged the duration of licking (Wilcoxon signed rank test, p=0.021). However cholinergic silencing elicited no change in firing rates during hits, misses and correct rejections (Wilcoxon signed-rank test p>0.05).
Interestingly, alpha and beta power were still prominent in the PFC in the post-lick window, following presentations of the extinguished stimulus (figure 4.8 B, D), indicating that alpha and beta in PFC are not only involved in the process of extinction learning, but also involved in the maintenance of extinction over time. On correct rejection trials, NB silencing largely attenuated the magnitude of PFC alpha, however this

Figure 4.7: Licking behavior and PFC firing rates during cholinergic silencing. A Normalized, stimulus-triggered lick rates post extinction learning, for non-optogenetic (black) and optogenetic (red) trials. Licking traces are sorted by trial type: hits, false alarms, misses, and correct rejections. B: Ratio of lick rates during silencing and non-silencing trials, for hits (blue) and false alarms (red). C: Average normalized firing rate for PFC neurons during non-silencing (blue) and silencing (red) trials, grouped by trial outcome. Error bars are S.E.M.
Figure 4.8: PFC LFP activity during cholinergic silencing.

A: (top,left) Normalized PFC spectrogram triggered on onset hit trials (n=11 mice). (top,right) Same as left, for optogenetic silencing trials. (middle) Normalized alpha power in the post reward window (2-5 seconds) for non-silencing (red) and cholinergic silencing trials (blue). (bottom) Same as middle, for beta power. Bars are plotted in pairs for optogenetic treatment, arranged according to depth along the PFC recording electrode. B, C, and D: same as A, for false alarms, misses, and correct rejections, respectively. Error bars are S.E.M. Black arrows highlight relevant periods of the silencing condition in which alpha and beta power were reduced.
was only a trend (paired t test, \( p=0.074 \), Figure 4.8, D) and cholinergic silencing significantly reduced the magnitude of PFC beta following correct rejections (paired t test, \( p=0.016 \)). Furthermore, cholinergic silencing reduced power in the alpha (paired t test \( p=0.005 \), figure 4.8 C) and beta (paired t test, \( p=0.0499 \)) bands during the post reward window following misses. But, alpha and beta power were not significantly different in post lick intervals for false alarms (paired t test, \( p>0.05 \), figure 4.8 B), or hits (paired t test, Figure 4.8, A). These results suggest that cholinergic silencing during cue presentation facilitates working memory processes that occur in the post-lick interval of trial types in which the mouse did not elicit a behavioral response—misses and false alarms. A potential interpretation of these findings is that cholinergic signaling is involved in maintaining the value of the stimulus during the reward window, and allowing post-trial endogenous working memory processes to occur after behavioral responses.

Conclusions

In this chapter, we show that alpha frequency LFPs are strongly associated with extinction learning in both auditory and prefrontal cortex. While beta frequency LFPs co-occur with alpha in PFC, they do not occur in AC, suggesting that the beta we observe is specific to executive functioning regions. With respect to alpha in AC and alpha/beta in PFC, increased LFP power occurs following extinguished tone presentations in both AC and PFC during learning. Furthermore, increases in alpha and beta power occur selectively for the extinguished tone in PFC earlier during the course of learning than
alpha in AC, suggesting that cognitive processing associated with stimulus selection occurs earlier in PFC than in AC. After completion of extinction learning, we show that cholinergic silencing attenuates alpha and beta in PFC following trial types in which mice did not elicit a motor response (correct rejections and misses), indicating that cholinergic tone during stimulus processing is crucial to the production of post-response LFP dynamics in PFC.

**Discussion**

Using simultaneous recording in auditory and prefrontal cortex during extinction learning we were able to demonstrate that specific bands of the LFP are modulated by task demands. Beta and alpha power are largely reduced during licking behavior and tone presentation, consistent with studies demonstrating reduced beta during motor activity and reduced alpha power during sensory perception (Pfurtscheller et al. 2003; Park et al. 2013, Tzagarakis et al. 2010, Nikouline et al. 2000) however there are also substantial beta reductions during correct rejections, suggesting that beta reductions may also have an additional role in cue processing. Interestingly, there was strong oscillatory LFP activity that emerged in the post-lick window, following alpha and beta reductions. These occurred specifically following initiation of extinction learning in both auditory and prefrontal cortex. During CONF, alpha power LFPs in AC followed presentation of both the rewarded and extinguished tones, while in PFC alpha and beta power emerged following the extinguished tone alone. By the BP interval, alpha began to occur selectively for the extinguished tone in AC, while alpha and beta maintained selectivity in PFC. These results suggest that both PFC and AC are engaged by extinction learning and
that engagement in PFC precedes AC. As mentioned previously, alpha band activity has been linked to internal working memory processing (Jensen et al. 2002, Bollimunta et al. 2008) and beta power has been shown to be involved in working memory and self-directed choice (Iversen et al. 2009, Pesaran et al. 2008). One explanation for the increase in alpha LFP power during extinction learning is that it represents working memory processes related to updating the animals internal representation of task rules as he learns the extinction task. In this way alpha and beta may represent an internal register of the stimulus association as the mouse encounters new information about it.

Optogenetic silencing of the cholinergic nucleus basalis, during the second phase of behavioral sessions—tone discrimination performance—altered the spectral properties of the cue evoked LFP profile, altering the increases in alpha and beta activity in the post-licking time interval observed previously during learning. Specifically, cholinergic silencing attenuated the magnitude of alpha and beta LFP power on correct rejection trials and elicited a large reduction of alpha and beta on miss trials. These results are interesting because cholinergic silencing only extended through the duration of tonal cues. Reductions in the alpha and beta bands occurred later, during the post lick interval (2-5 second post cue). Therefore the reductions in power were not due to temporal overlap with cholinergic silencing, but still occurred as a consequence of it.

A potential explanation of these findings is that cholinergic tone is necessary for the maintenance of the identity of the tonal stimulus in PFC throughout the licking interval, and that when cholinergic tone is inhibited, the mouse was not able to process the
stimulus in working memory. This interpretation is in line with previously established studies demonstrating that acetylcholine is involved in the maintenance of information in cortical circuits (Zhou et al. 2011). Similarly, cholinergic efflux is related to cognitive switching from externally driven to internally driven responding (Howe et al. 2013). In light of this, reductions in alpha and beta may reflect a prolonged period of externally driven cue monitoring, suggesting that cholinergic tone may also be necessary for disengagement from external stimuli, as well as engagement in internal processing.

Figure 4.9: Cholinergic nucleus basalis neuron silenced by optogenetics.

(top) Raster plot of a nucleus basalis neuron silenced with 1 second 473 nm laser light across 100 trials. (bottom) Firing rate histogram of (top).
Another interpretation of the reduction in power induced by silencing is that it is due to rebound spiking of cholinergic neurons of the nucleus basalis following the silencing period. As demonstrated in chapter 3, a decrease in cortical low frequency power is often observed to coincide with cholinergic activation. However, this is unlikely, since the reductions in power continue for several seconds after silencing ends and, as shown in figure 4.9, we recorded the firing rate time course of cholinergic nucleus basalis neurons and did not observe prolonged rebound spiking, although there may be classes of cholinergic interneurons that do exhibit this behavior. Additionally, in chapter 3, we observed the time course of cortical desynchrony in the cortex during cholinergic stimulation and demonstrate that it does not extend for a prolonged duration after cholinergic stimulation ends, suggesting that acetylcholine is rapidly metabolized after it is released.
CHAPTER FIVE: CONCLUSIONS

I have addressed three main research directives outlined in chapter 1: Firstly, I have demonstrated that cholinergic receptor binding in cortical circuits contributes to feedforward sensory pathways between bottom up sensory and top down decision making regions of cortex. Secondly, I have addressed prior research on cholinergically induced cortical desynchrony, confirming prior findings through the use of optogenetic manipulations in an awake preparation, and observed that aspects of cortical desynchrony are generated within distributed circuitry within cortical regions, as shown across AC. I have determined that alpha and beta frequency LFP power in the prefrontal and auditory cortex are involved with the process of extinction learning and that the production of alpha and beta power in PFC depends on cholinergic signaling in PFC. These findings suggest that cholinergic release in cortical circuits is intricately involved in cortical interactions within large brain networks and supports cortical dynamics involved in attentional processing.

Much of the work in auditory neuroscience has focused on the cochlea, cochlear nerve, brainstem and thalamic nuclei, inferior colliculus, and the auditory cortical tonotopy. However, it has become increasingly evident that auditory cortex constitutes only a link in a large network of brain areas involved in the processing of acoustic stimuli. For example, we know that PFC is involved in processing auditory stimuli during behavioral tasks (Romanski et al. 1992, Romanski et al. 2002, Romanski et al. 2005) and results from chapter 2 demonstrated that PFC processes auditory stimuli during passive stimulus presentation, establishing a role for PFC in the auditory processing network independent
of behavioral context. Similarly, primary auditory cortex has been shown to process sensory information from other modalities, receiving and sending cortico-cortical processing streams during sensory perception (Lakatos et al. 2008, Lakatos et al. 2007). Corticocortical interactions are a crucial component of auditory cortical processing, shaping its response dynamically during behavior. In the attentive state, regions of the auditory cortical tonotopy interact corticocortically to enhance attended, and suppress unattended auditory stimuli (O’Connell et al. 2014). As shown in chapter 2, muscarinic blockade has a profound influence over the ability for corticocortical interactions within AC to occur, suggesting that cholinergic signaling is integrally involved in the transformation of auditory information as it flows along sensory pathways. Furthermore, in chapter 3, I demonstrated that cortical desynchrony is generated in distributed circuits, indicating that states of cholinergic activation rely on complex corticocortical interactions across AC. Finally, since muscarinic receptor activation was shown to be necessary for sensory pathways to carry stimulus information from AC to PFC, it is evident that cholinergic mechanisms are involved in conveying sensory signals along long-range cortico-cortical pathways. Together, these results have important implications for large-brain networks during attention, establishing cholinergic receptor types as a potential gateway for neuronal interaction within cortical areas and between distal regions of cortex.

In chapter 4, I demonstrated that alpha and beta LFP power coincides with extinction learning. As mentioned previously, cortical alpha rhythms have been shown to have several roles in attentional processing, and to have a variety of mechanisms of generation
(for a review read Palva & Palva 2007, Mo et al. 2011, Haegens et al. 2015). Similarly, beta has been shown to have a diversity of roles in cognitive function that depend on brain region and task demands (for a review read, Engel and Fries 2010). Interestingly beta frequency LFPs have been shown to coincide with stimulus-reward associations in PFC, and in basal ganglia—a region closely linked to PFC function—they have been shown to be involved in behavioral associations between cues and motor responses (HajiHosseini & Holroyd 2015, Leventhal et al. 2012). I observed that beta and alpha synchrony in PFC and alpha synchrony alone in AC occur following behavioral reward windows—after animals have elicited or withheld motor responses. This suggests that in this context alpha and beta reflect a state of post-performance evaluation or internal mapping between cue and motor response.

My results demonstrating a cholinergic silencing induced decrease in alpha and beta LFP power in PFC following presentation of extinguished tones has interesting implications related to the function of oscillations in cortical circuits during sensory discrimination tasks. Cholinergic modulation of cortical circuits decreases cortical LFP power at lower range frequencies (<10 Hz), which is also a phenomenon that occurs during sensory processing (Nikouline et. al, 2000). This suggests that the reduction in low frequencies during cholinergic activation may be permissive to cortical sensory processing. Furthermore, in vitro electrophysiological studies and modeling works have shown that the effects of neocortical cholinergic modulation are specific to cortical region, layer, and cell type in many cases allowing complex, dynamic interactions to occur (Xiang et al. 1998, Kawaguchi et al. 1997, Roopun et al. 2010, Lee et al. 2013). These local dynamics
suggest that acetylcholine can have profound effects on neural signaling, particularly during selective attention, which involves complex states of large scale neural synchrony (Siegel et al. 2012, Fries et al. 2001, Buschman et al. 2007).

An explanation for the generation of alpha and beta following correct rejections of extinguished stimuli and it’s modulation by cholinergic silencing is that it requires large scale interactions within multiple sensory and executive functioning regions of cortex to occur during sensory processing. Since cholinergic silencing potentially reduces corticocortical interactions across distributed cortical circuitry, perhaps internal working memory processing is unable to continue following response windows. Specifically, immediately following the reward window of the extinguished tones, animals may hold the tone in working memory in order to evaluate their internal representation of its contextual meaning. If this process is interrupted by reduced cholinergic signaling, and subsequent loss of sensory information stored in cortical circuits, then post-response evaluation of self-guided behavior may be similarly disrupted. Furthermore, much of the extinction and reward circuitry is thought to be subcortical (Saddoris et al. 2015, Bassareo et al. 2015, Chen et al. 2015), providing a potential explanation for the lack of behavioral effect associated with cholinergic silencing, and suggesting that interactions between sensory and executive functioning areas of the cortex are more important for internal working memory processing that occurs after animals have already either responded, or successfully withheld a response in this task.
ABBREVIATIONS FOR BIBLIOGRAPHY

Ann N Y Acad Sci........................................ Annals of the New York Academy of Sciences
Annu Rev Neurosci................................. Annual Review of Neuroscience
Behav Brain Res..................................... Behavioural Brain Research
Behav Neurosci....................................... Behavioral Neuroscience
Brain Res Bull....................................... Brain Research Bulletin
Brain Res Brain Res Rev........................ Brain Research. Brain Research Reviews
Cereb Cortex......................................... Cerebral Cortex
Clin Neurophysiol.................................. Clinical Neurophysiology
Curr Biol............................................. Current Biology
Curr Dir Psychol Sci............................... Current Directions in Psychological Sciences
Curr Opin Neurobiol................................ Current Opinion in Neurobiology
Eur J Neurosci....................................... European Journal of Neuroscience
Front Behav Neurosci............................ Frontiers in Behavioral Neuroscience
Front Neural Circuits............................. Frontiers in Neural Circuits
Front Neurosci....................................... Frontiers in Neuroscience
Hear Res............................................... Hearing Research
J Comp Physiol A.................................. Journal of Comparative Physiology. A, Sensory, Neural, and Behavioral Physiology
J Neurophysiol...................................... Journal of Neurophysiology
J Neurosci............................................ Journal of Neuroscience
J Neurosci Methods............................... Journal of Neuroscience Methods
J Physiol.................................................................Journal of Physiology
Learn Mem..............................................................Learning & Memory
Nat Commun..............................................................Nature Communications
Nat Neurosci..............................................................Nature Neuroscience
Nat Rev Neurosci......................................................Nature Reviews. Neuroscience
Neurobiol Learn Mem..............................................Neurobiology of Learning and Memory
Neurosci Biobehav Rev..............................................Neuroscience & Biobehavioral Reviews
Neurosci Lett.................................................................Neuroscience Letters
Philos Trans R Soc Lond B Biol Sci.................................Philosophical Transactions of the Royal Society B: Biological Sciences
Physiol Rev.................................................................Physiological Reviews
PLoS Comp Biol.............................................................PLoS Computational Biology
Proc Natl Acad Sci USA.....................................................Proceedings of the National Academy of Sciences of the United States of America
Prog Brain Res.............................................................Progress in Brain Research
Schizophr Bull...............................................................Schizophrenia Bulletin
Sci Rep.................................................................Scientific Reports
Trends Neurosci...........................................................Trends in Neuroscience
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