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Auditory working memory: contributions of lateral prefrontal cortex and acetylcholine in non-human primates

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AUDITORY WORKING MEMORY: CONTRIBUTIONS OF LATERAL PREFRONTAL CORTEX AND ACETYLCHOLINE IN NON-HUMAN PRIMATES

by

Bethany Joy Plakke Anderson

An Abstract

Of a thesis submitted in partial fulfillment
of the requirements for the Doctor of
Philosophy degree in Psychology
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May 2010

Thesis Supervisor: Associate Professor Amy Poremba

ABSTRACT

Traditionally, working memory and its neural underpinnings have been studied in the visual domain. A rich and diverse amount of research has investigated the lateral prefrontal cortex (lPFC) as a primary area for visual working memory, while another line of research has found the neurotransmitter acetylcholine (ACh) to be involved. This dissertation used auditory cues and found similar patterns of activity for processing auditory working memory information within a task compared to visual working memory processes. The first two experimental chapters demonstrated that the cholinergic system is involved in auditory working memory in a comparable fashion to its role in visual working memory. In chapter 2, blocking ACh impaired performance on an auditory working memory task in a dose dependent manner. Chapter 3 investigated the specificity of the effect of blocking ACh by administering an ACh agonist (physostigmine) at the same time as an ACh antagonist (scopolamine). When both drugs were administered together performance on the delayed matching-to-sample task (DMTS) task improved compared to performance on scopolamine alone. These results support the hypothesis that ACh is involved in auditory working memory.

Chapter 4 investigated the neural correlates of auditory working memory in area 46 and found that this region of the lPFC contains neurons that are responsive to auditory working memory components in a very similar way to how it this region encodes information during visual working memory tasks. Neurons in the lPFC are responsive to visual or auditory cues, the delay portion of tasks, the wait time (i.e. decision making period), response, and reward times. This type of coding provides support for the

theories that position the lPFC as a key player in recognition and working memory regardless of modality.

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Iowa City, Iowa

CERTIFICATE OF APPROVAL

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PH.D. THESIS

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This is to certify that the Ph.D. thesis of

Bethany Joy Plakke Anderson

has been approved by the Examining Committee
for the thesis requirement for the Doctor of Philosophy
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Steven Anderson

Dedicated to my loving husband, parents, and family.

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Traditionally, working memory and its neural underpinnings have been studied in the visual domain. A rich and diverse amount of research has investigated the lateral prefrontal cortex (lPFC) as a primary area for visual working memory, while another line of research has found the neurotransmitter acetylcholine (ACh) to be involved. This dissertation used auditory cues and found similar patterns of activity for processing auditory working memory information within a task compared to visual working memory processes. The first two experimental chapters demonstrated that the cholinergic system is involved in auditory working memory in a comparable fashion to its role in visual working memory. In chapter 2, blocking ACh impaired performance on an auditory working memory task in a dose dependent manner. Chapter 3 investigated the specificity of the effect of blocking ACh by administering an ACh agonist (physostigmine) at the same time as an ACh antagonist (scopolamine). When both drugs were administered together performance on the delayed matching-to-sample task (DMTS) task improved compared to performance on scopolamine alone. These results support the hypothesis that ACh is involved in auditory working memory.

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LIST OF ABBREVIATIONS

ACh:	Acetylcholine
AS:	Arcuate sulcus
CM:	Corrrect Match
CNM:	Correct Nonmatch
DA:	Dopamine
dB:	Decibels
dlPFC:	Dorsolateral prefrontal cortex
DMTS:	Delayed matching-to-sample
DNMTS:	Delayed nonmatching-to-sample
FEF:	Anterior arcuate cortex
fMRI	Functional magnetic resonance imaging
FR:	Firing rate
HNR:	Harmonics-to-noise ratio
IM:	Incorrect match
INM:	Incorrect nonmatch
ISI:	Interstimulus interval
IT:	Inferior temporal cortex
ITI:	Intertrial interval
MD:	Medial dorsal nucleus of thalamus
lPFC:	Lateral prefrontal cortex
mPFC:	Medial prefrontal cortex
PET:	Positron emission tomography
PFC:	Prefrontal cortex
PPC:	Posterior parietal cortex
PS:	Principal sulcus
rSTG:	Rostral superior temporal gyrus
ScHCl:	Scopolamine hydrochloride
STG:	Superior temporal gyrus
STS:	Superior temporal sulcus
vlPFC:	Ventrolateral prefrontal cortex

CHAPTER 1. INTRODUCTION

Daily life requires the use of working and recognition memory. Performing tasks such as holding a phone number in mind while dialing, doing "mental math," and tracking incoming environmental cues to solve everyday problems all utilize working and/or recognition memory.

The common definition of working memory is storing and updating 'on-line' information. Baddeley and Hitch classically defined working memory as a main attention controller known as the "central executive" which is supported by two subsystems; the visuospatial sketchpad and the phonological loop (Baddeley & Hitch, 1974; Baddeley, 2000). This model has been recently updated by Baddeley to include an 'episodic buffer' that deals with multi-dimensional information and temporally stores it for the visuospatioal sketchpad or phonological loop to use (Baddeley, 2000). The buffer can be used to help recall a piece of episodic memory or deal with language. It can act as a temporary storage place to hold items from long-term memory that are being used during working memory demand (Baddeley, 2000). Working memory is also often defined as keeping track of and representing stimuli that are no longer present in the current situation (Goldman-Rakic, 1996). Thus, the processes of manipulating or maintaining information for a few seconds to minutes are frequently referred to as working memory. Our delayed matching-to-sample (DMTS) task requires maintenance of an auditory stimulus over a delay period and it is considered a test of working memory.

Recognition memory can be short- or long-term and requires the subject to realize that an object previously seen is the same or different from the object currently being

presented. Both DMTS and delayed nonmatch-to-sample (DNMTS) tasks can utilize working and recognition memory. Traditionally the DMTS or DNMTS tasks have involved visual cues, however there have been some experiments in which tactile, auditory, audio/visual, or olfactory exemplars were used as cues (Colombo & Graziano, 1994; Fritz et al., 2005; Kikuchi-Yorioka & Sawaguchi, 2000; Kowalska et al., 2001; Meunier et al., 1996; Mishkin & Delacour, 1975; Murray & Mishkin, 1983; Otto & Eichenbaum, 1992; Sugihara et al., 2006; Watanabe, 1992). There are other tasks that can test recognition memory but the current studies use a DMTS task.

In the DMTS task used in the proposed studies, an auditory stimulus is presented for 500 msec and is followed by a delay period (2-10 seconds). After that a second sound stimulus is presented for 500 msec. If the second sound is the same as the first sound a GO response consisting of pressing the button, results in delivery of a reward. If the second sound is different from the first then a NO-GO response or not pressing the button, is required to be correct. This task tests working memory because the stimulus is no longer present over the delay gap and recognition memory because the animal must recognize that it is the same sound to perform correctly.

Visual Recognition Memory

Neural Correlates of Visual Recognition Memory

Visual recognition memory has been studied across multiple species including: rats, pigeons, cats, primates and humans (Kraemer & Roberts, 1985; Mumby & Pinel, 1994; Murray & Mishkin, 1986; Okudzhava et al., 2008; Prusky et al., 2004; Suzuki et al., 1993; Yassa & Stark, 2008). Both lesion and imaging work has indicated the perirhinal, entorhinal, and parahippocampal cortices to be the essential structures

underlying visual recognition memory function (Buffalo et al., 2000; Davachi & Goldman-Rakic, 2001; Málková et al., 2001; Meunier et al., 1993; Murray & Mishkin, 1986; Suzuki et al., 1993; Suzuki et al., 1997; Yassa & Stark, 2008; Zola-Morgan et al., 1989). Other structures that are also implicated in visual recognition memory are hippocampus, parietal lobe, inferior temporal cortex, and medial thalamus (Aggleton & Mishkin, 1983; Miller & Desimone, 1991; Prusky et al., 2004; Xu, 2007).

Recording and imaging studies using visual stimuli have implicated major roles for the inferior temporal cortex (IT) and the parietal lobe in recognition memory (Miller & Desimone, 1993; Miller & Desimone 1991; Nee & Jonides, 2008). For example, during a recording study, cells in the IT suppressed their firing rate to visual stimuli that were repeatedly presented (Miller & Desimone, 1993). It was suggested that the IT assists with sensory and memory processes by suppressing activity to recently seen items, allowing novel visual stimuli to be identified (Miller & Desimone, 1993). The parietal lobe has been suggested to be important for maintaining features of objects such as color or shape (Kawasaki et al., 2008). Other researchers have suggested that the parietal lobe may assist in recognition memory by interacting with frontal circuits to maintain attention on a specific visual object (Nee & Jonides, 2008). While some of the neural substrates for visual recognition memory have been identified it is unknown whether these same substrates underlie auditory recognition memory.

Recognition memory and Acetylcholine

Another finding from the visual short-term memory literature is that the cholinergic system is involved in visual memory processes. It has been demonstrated that blocking the cholinergic system with drugs like scopolamine (a muscarinic receptor

antagonist) impairs performance on a wide array of visual working memory tasks (e.g., Bachevalier and Mishkin, 1994; Goldman-Rakic, 1987; Málková et al., 2001). While many brain areas have been implicated in short-term or working memory for both visual and auditory cues, the underlying neurotransmitter systems for auditory memory have not been examined in depth.

Recently, receptors for dopamine and serotonin were found to be involved in recognition memory; however this was a task that examined the ratio of time mice looked at or inspected familiar versus novel objects, and did not require an overt choice behavior (Nagai et al., 2008). Since recognition memory requires multiple cognitive processes (encoding, retrieval) it is likely that more than one neurotransmitter system is involved. Nonetheless, the cholinergic system has been implicated in tasks requiring visual short-term memory. In addition, multiple studies have shown that blocking muscarinic receptors impairs visual memory performance on a variety of tasks including, DMTS, DNMTS, self-ordered spatial search, and serial-probe recognition (Aigner & Mishkin, 1986; Hironaka & Ando, 1996; Myers et al., 2002; Penetar & McDonough, 1983; Ogura & Aigner, 1993; Taffe et al., 1999). These data demonstrate that the cholinergic system has a clear role in visual recognition memory; however, its precise role in auditory recognition memory has yet to be determined.

Other acetylcholine receptor antagonists, such as atropine, that affect the cholinergic system by blocking receptor binding, also impair short-term memory (Penetar & McDonough, 1983). To test this hypothesis, Penetar & McDonough (1983) trained monkeys (*Macaca mulatta*) in a DMTS task with variable delays of 0, 4, 8 or 16 seconds. Performance was significantly decreased at the 4, 8, and 16 second delays when

administered the highest atropine dose (440 µg/kg). While at the second highest dose of atropine (140 µg/kg) only the 8 and 16 second delays were affected (Penetar & McDonough, 1983). This demonstrates that blocking muscarinic receptors impairs visual memory performance and supports the hypothesis that the cholinergic system underlies some visual memory functions.

The use of acetylcholine (ACh) for memory processing may be conserved across species as blocking it also impairs mainly visual memory performance in rats, pigeons, monkeys and humans (Elsmore et al., 1989; Flicker et al., 1990; Hudzik & Wenger, 1993; Pontecorvo & Evans, 1985; Pontecorvo, et al., 1991; Spinelli et al., 2006). Pontecorvo et al., (1991) used mixed sensory stimuli using a light and tone as stimuli, and levers for responding in a DMTS task; wherein rats had to remember which stimuli had been most recently administered and then choose the appropriate lever for matching and nonmatching trials. Rats injected with scopolamine showed dose dependent effects and were impaired in their choice accuracy.

In addition, when the main inputs of the cholinergic system were damaged in monkeys (*Macaca fasicularis*) with ibotenic-acid, visual recognition memory was impaired (Aigner et al., 1991). When only part of the system was damaged, such as the nucleus basalis of Meynert, or the medial septal and diagonal band nuclei, there was no deficit in performance on a DNMTS task. Nevertheless, when both areas were damaged a decline in performance was observed (Aigner et al., 1991). This supports the notion that the acetylcholine system is engaged in processes that mediate visual recognition memory. Perhaps the acetylcholine system is important for auditory recognition memory as well. The first group of experiments contained in this dissertation (Chapters 2, 3) will

examine acetylcholine muscarinic receptors and their effects on auditory recognition memory.

Acetylcholine has also been implicated as a neural modulator for plasticity within the auditory cortex (Weinberger, 2007). Through several studies Weinberger and colleagues have demonstrated that individual neurons within the auditory cortex change their best frequency response, the frequency for which a neuron has the strongest firing rate. Thus, when ACh was administered to the cortex in cats their receptive field changed (Ashe et al., 1989; McKenna et al., 1989; Metherate & Weinberger, 1990). In addition, the effects of administration of ACh are attenuated by administration of atropine, a muscarinic receptor blocker (McKenna et al., 1989), signifying there may be an endogenous role for ACh in auditory cortex plasticity. Weinberger and colleagues have also investigated the role of ACh in auditory learning paradigms, and found that stimulation of the nucleus basalis (a main source of ACh for the cerebral cortex) can change receptive fields and tonotopic maps in auditory cortex (Weinberger, 1995; 1998). More recently, their lab has found that as rats acquired an auditory conditioned stimulus ACh was released in primary auditory cortex (Butt et al., 2009). This supports the hypothesis that ACh is necessary for auditory cortical plasticity during learning.

Acetylcholine and Attention

While the focus of the pharmacological experiments in this dissertation is to examine memory, the role of attention will also be considered. It has been suggested that the cholinergic system is important for attentional processes, which includes encoding of visual stimuli and cue detection (Bentley et al., 2004; Hasselmo & McGaughy, 2004; Parikh & Sarter, 2008; Parikh et al., 2007). However, when the cholinergic system is

compromised, impairments in performance on memory tasks are not due to a deficit in attention alone, indicating a role for acetylcholine in memory as well as attention (Chudasama et al., 2004).

Auditory Short-term Memory

Auditory short-term memory has not been examined nearly to the extent that visual short-term memory has. There have been a few studies in birds, dogs, dolphins, and primates, as well as some studies done in humans that examine auditory memory (Colombo & D'Amato, 1986; Downing et al., 1988; Fritz et al., 2005; Herman & Gordon, 1974; Kowalska et al., 2001; McFarland & Cacace, 1995; McFarland & Cacace, 1992; Zokoll et al., 2008; Zokoll et al., 2007). In animal work it has been found that auditory short-term memory is less durable than visual short-term memory and that extensive training must be undergone to obtain a reasonable learning criterion (Colombo & D'Amato, 1986; Fritz et al., 2005; Wright, 2007).

Humans

For humans most short-term auditory memory studies examine pitch, verbal memory, or examine list memory effects (Connine, 2004; Deutsch, 1972; Deutsch, 1970; Gaab et al., 2003; Jusczyk et al., 1995; McFarland & Cacace, 1995; Peterson & Peterson, 1959). Studies that use verbal cues to study short-term auditory memory may be confounded by using language because the sounds themselves could be encoded or rehearsed within the phonological loop or a subvocal rehearsal system, which themselves are associated with different anatomical areas, the supramarginal gyrus and Broca's area respectively (Paulesu et al., 1993). The verbal memory being stored in these cases is not a 'pure' auditory memory, but may be engaging a language code to be remembered. Some

research suggests that the use of non-verbal auditory stimuli is beneficial to understanding short-term auditory memory in humans, but there has been little work to examine the capacity of humans within the short-term domain (McFarland & Cacace, 1992; Pollack, 1972). What has been examined includes a wide array of concepts such as rhythmicity or pitch memory. For example it has been found that auditory rhythms are easier to learn than visual rhythms or when a single pitch is repeated memory performance improves (Deutsch, 1972; Glenberg et al., 1989). Other studies have investigated auditory list memory, rehearsal, and compared serial position curves for audition and vision. Results show that humans can demonstrate recency effects, that rehearsal can improve performance, and that serial position curves are similar for auditory and visual short-term memory (McFarland & Cacace, 1992; Keller et al., 1995; Ward et al., 2005). Some work in humans has also found that children have a smaller auditory capacity measured by a shorter span length, but that even infants (1-2 months old) can encode and recognize an auditory sequence, in this case a nursery rhyme after 3 days (Cacace & McFarland, 1992; Spence, 1996). While all of these studies expand our knowledge base about audition and memory, there is less known about specific brain areas or underlying neurotransmitter systems that could be involved in auditory recognition memory.

Animals

Early animal work demonstrated that monkeys could perform in an auditory recognition memory task (Stepien et al., 1960). Stimuli used in this study were click trains with frequencies of 5 per second or 20 per second, however the rhythmic nature of the stimuli could have tested a different memory system than if more straightforward

auditory stimuli were used. Kojima (1985) trained monkeys in an auditory DMTS task, and found that performance dropped to almost chance at 16 seconds. These early studies established that auditory recognition memory could be explored but used simple auditory stimulus types (buzzers, tones, clicks) and only a few number of example stimuli such as two or three tones.

In a study with starlings (*Sturnus vulgaris*), utilizing a DNMTS task, the average short-term auditory recognition memory capacity was found to be between 4 and 6 seconds (Zokoll et al., 2007). Between individuals there was large behavioral variation, with some birds performing very well at delays up to 19.7 seconds (Zokoll et al., 2007). In a follow up study, delays were lengthened to 26.8 seconds, but birds' performance at these longer delays dropped to 65.2 % correct on average (Zokoll et al., 2008). These studies suggest that even in species that rely on audition for complex song learning and mate selection, short-term auditory recognition memory tasks are difficult to perform at longer delays (Eens et al., 1991; Mountjoy & Lemon, 1996).

Other studies with primates (*Macaca mulatta*) have examined auditory list memory and serial position effects (Wright, 2002). It was found that for audition the longer the delay interval up to 30 seconds, the stronger the recency effect (Wright, 1999). This was explained through a series of experiments as a result of a decrease in proactive interference of items at the end of the list, which resulted in a recency effect (Wright, 1999; Wright & Roediger, 2003).

The above studies by Wright and colleagues demonstrate that monkeys can perform auditory recognition memory, however these studies did not examine the brain areas that underlie auditory memory function. Some lesions addresed this question;

African green monkeys (*Ceropithecus cethiœs sabœus*) with lateral medial temporal lobe damage were impaired on performance with no delay (Cordeau & Mahut, 1964). This was argued as more of impairment in discrimination ability rather than a memory impairment (Cordeau & Mahut, 1964). Later work showed the auditory association cortex was critical to performance in an auditory short-term memory DMTS task in monkeys (*Cebus apella*) (Colombo et al., 1990). In this task there was no impairment in sound discrimination, but there were clear deficits in memory processes (Colombo et al., 1990). More recently, it was found that the superior temporal gyrus is critical for auditory recognition memory in primates (*Macaca mulatta*) using a DMTS task (Fritz et al., 2005). Monkeys with rhinal cortex lesions were not impaired in performance on the same DMTS task. This was a surprising finding given that the rhinal cortices are critical for visual recognition memory (Buffalo et al., 2000; Fritz et al., 2005; Málková et al., 2001; Meunier et al., 1993; Murray & Mishkin, 1986; Suzuki et al., 1993; Zola-Morgan et al., 1989). The auditory memory performance in control animals fell to less than 75% correct at a delay of about 37 seconds. This 75% correct performance level is similar to visual performance of animals with lesions of rhinal cortices (Fritz et al., 2005). Thus, normal animals in the auditory task behave as if they are not utilizing the rhinal cortices and when that region is lesioned, there is no further decrement observed in their behavioral performance. This could mean that the monkeys are relying more on a working or short-term memory system to perform this version of the auditory DMTS task (Fritz et al, 2005).

A lesion study in dogs provides support for this working memory assertion. Lesions of the perirhinal and entorhinal cortices or the hippocampus did not disrupt performance

in an auditory short-term recognition DMTS task (Kowalska et al., 2001). The hippocampus and surrounding cortices are known for their importance to long-term memory. Since these areas are not crucial for short-term auditory memory performance another brain system is most likely involved.

Although difficult to train, animals can learn auditory recognition memory tasks. High performance accuracy varies across species, but on average occurs at delays of 60 seconds or less (Fritz et al., 2005; Kowalska et al., 2001; Zokoll et al., 2008). Lesion work has found that the superior temporal gyrus is critical for short-term auditory recognition memory in monkeys, while other lesion work has shown that the hippocampus and rhinal brain areas are not. This could indicate that regions important for short-term or working memory, such as lateral prefrontal cortex (lPFC), could be important for short-term auditory memory demands.

The lateral PFC

Anatomy of the PFC

Various brain regions have been hypothesized to play a role in short-term recognition or working memory. While the specific brain regions may depend on task requirements, one brain region that is consistently important for short-term or working memory demands is the lateral prefrontal cortex (Goldman-Rakic, 1987; Habeck et al., 2005). The next section of this chapter will provide important background about the lateral PFC and its role in working memory.

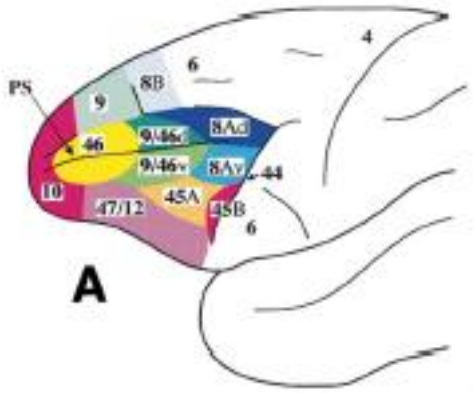

Figure 1. Lateral view of PFC showing area 46 which surrounds the principal sulcus. Adapted from Fuster, 2001.

The PFC is comprised of three major divisions: the lateral PFC, the medial PFC (mPFC), and the orbital frontal area. LPFC is further divided into the dorsolateral PFC (dlPFC) consisting of areas 8 (A &B), 9, 9/46 and 46, and ventrolateral PFC (vlPFC) comprised of areas 12/47, and 45 (Figure 1, lateral view). LPFC is generally important for ordering information such that behavior and speech is organized in a timely manner. In non-human primates, area 46 is defined as the cortical banks that surround the principal sulcus (PS).

In humans, the analogous region surrounds the middle frontal sulcus and extends up to the superior frontal sulcus, (Figure 2A).

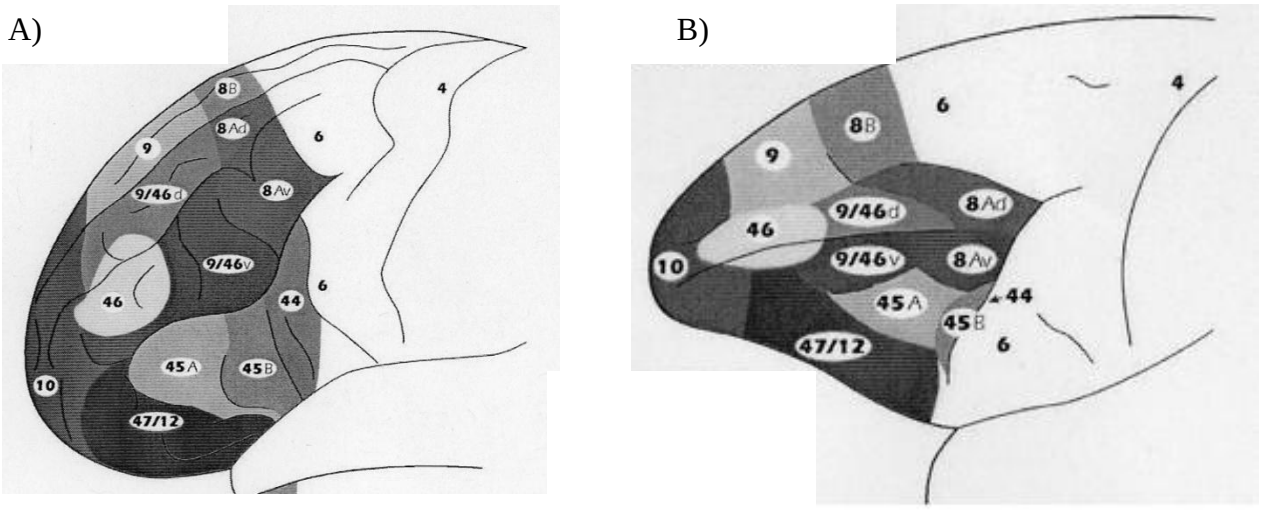

Figure 2. Schematics of the lateral view of a human and a monkey brain. A) Human prefrontal regions, adapted from Petrides & Pandya, 1999. B) Monkey prefrontal regions, adapted from Petrides & Pandya, 1999.

In humans, area 45 is part of the triangular area of the inferior frontal gyrus, (Figure 2A). In non-human primates, area 45 begins at the rostral most end of the arcuate sulcus and extends upward to the infraprinciple dimple, which sits below the principal sulcus see (Figure 2B).

Preliminary results from these rhesus macaques in a positron emission tomography (PET) study found activity in area 46 during passive listening (Poremba et al., 2000), and this region has frequently been implicated in visual working memory tasks (Fuster, 2001), thus it may be important in facilitating auditory recognition memory. Area 45 has also been indicated as an important region for working memory processes (Fuster, 2001).

Important Afferents to the Region

In order to understand why the lPFC may play a role in auditory short-term memory, the important afferents need to be appreciated. The PFC as a whole receives connections from almost all areas of the brain including the three other cerebral lobes, the cerebellum, the brainstem, and multiple subcortical structures (Porrino & Goldman-Rakic, 1982). One of the major inputs to the dlPFC is the medial dorsal nucleus of the thalamus (MD) (Giguere & Goldman-Rakic, 1988; Goldman-Rakic & Porrino, 1985; Negyessy & Goldman-Rakic, 2005). The MD thalamus collects information from the temporal lobe, amygdala, inferior temporal cortex, and the mesencephalic reticular formation and forwards this to the dlPFC (Fuster, 1997). The MD thalamus has been shown to be fundamental to visual recognition memory (Aggelton & Mishkin, 1983), which means it could also be important to auditory recognition memory. Connections from the MD thalamus to the dlPFC could also influence performance on the auditory DMTS task. A myriad of sensory information also makes its way to the PFC. Jones & Powell (1970) summarized previous tracing work and found that the lPFC receives auditory, visual, and somatic input. This incoming sensory information is important for processes that are necessary in working memory tasks such as detecting stimuli.

Since vision is the most commonly used modality to study working memory within lPFC, a brief description of the visual pathways that project to the lPFC will be reviewed. Visual input from the primary visual cortex follows two main routes. One is ventral, and follows from V1 through regions of prestriate cortex to area TEO and TE, which then sends projections to the vlPFC (Macko et al., 1982; Mishkin, et al., 1983; Mishkin & Ungerleider, 1982). A second dorsal pathway begins in V1 and travels

through prestriate cortex to parietal cortex, which then projects to prearcuate cortex which sends direct projections to the dlPFC (Mishkin & Ungerleider, 1982; Macko & Mishkin, 1985). These routes have been implicated in processing object identification (ventral route) and object location (dorsal route), (Figure 3A) (Macko & Mishkin, 1985). These two main routes project different types of information to distinct regions within the lateral PFC and may play a role in the functional organization of the lPFC.

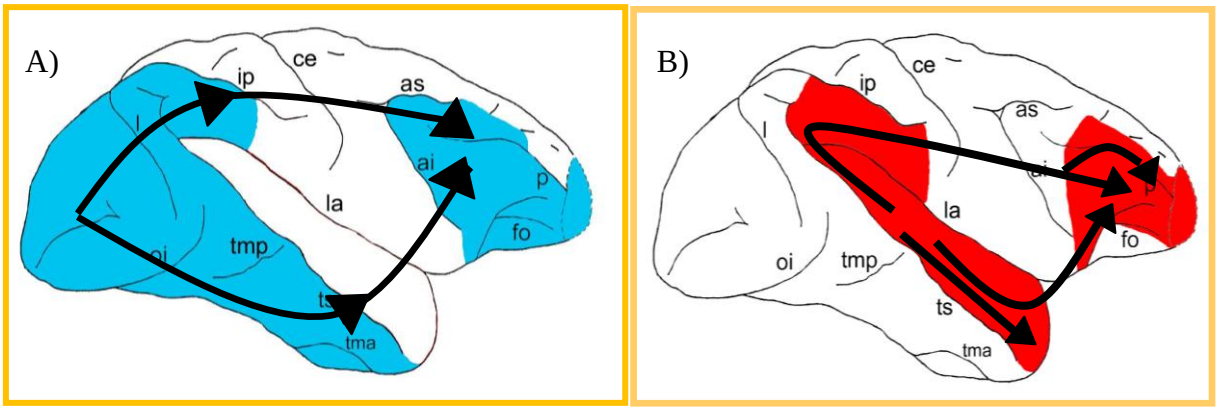

Figure 3. Schematics depicting modality pathways. A) Pathways showing convergence of visual information in lateral PFC. (Adapted from Macko & Mishkin, 1985) B) Pathways showing convergence of auditory information in lateral PFC. (Adapted from Poremba et al., 2003; Kaas & Hacket, 1999).

Multiple auditory areas project to the lPFC. Some work has suggested that auditory processing is similar to visual processing in that it may project to the PFC via "what" and "where" pathways, (Figure 3B). The superior temporal cortex is one area that sends multiple projections to the lPFC (Romanski et al., 1999a). Auditory association cortex areas of the lateral belt are responsive to complex auditory stimuli and are arranged by cochleotopic fields. The anterolateral area is associated with object identification

(specifically monkey calls), and the caudolateral area is associated with spatial location (Rauschecker & Tian, 2000). A tracing study revealed that the anterolateral area projected to dorsolateral areas of the lPFC and the caudolateral area projected to more ventrolateral areas of the lPFC (Romanski et al., 1999b). These anatomical findings support the spatial and non-spatial segregation of auditory information in the lPFC.

More recently, this has been explored further in humans. Findings from multiple studies that used functional magnetic resonance imaging (fMRI) or PET have found support for dorsal and ventral streams of auditory information in humans projecting to the lPFC (Alain et al., 2001; Arnott et al., 2005; Arnott et. al., 2004; Parker et al., 2005; Rämä et al., 2004). The imaging work and anatomical projections suggest that the lPFC receives auditory "what" and "where" information. Further scrutiny examining if this segregation is maintained, within the lateral PFC, seems warranted but is not the focus of the proposed studies. However, it is possible 'auditory object' cells [that are cells that fire more to a specific sound (coo) or specific sound categories (monkey vocalization versus human vocalization)] could be found within the lPFC. A general definition of an auditory object can be defined as particular sound stimulus (Griffiths & Warren, 2004). This has been found for an area of the ventral lateral PFC (Romanski et al., 2005) but has not yet been examined in the dlPFC.

In both humans and monkeys lPFC is a region that is important for processing complex calls associated with communication (Romanski & Averbeck, 2009). Primary auditory cortex, the lateral belt regions surrounding auditory cortex, the temporal area of the superior temporal sulcus (STS), and the superior temporal gyrus (STG) have all been found to process species-specific vocalizations (Ghazanfar et al., 2008; Poremba et al.,

2004; Romanski & Averbeck, 2009; Wang, 2000). Primary auditory cortex projects to the anterolateral belt an area that specifically processes monkey vocalizations. The anterolateral belt and the rostral STG project to the lPFC (Munoz, 2009; Romanski, 1999b). Neurons in the lPFC are responsive to species-specific vocalizations as well as other complex sound stimuli (Averbeck & Romanski, 2006; Gifford et al., 2005; Romanski et al., 2005; Russ et al. 2008). Thus, lPFC is a region of interest for studying how the brain processes complex communication sounds, however, the majority of the studies examining this question have focused on vlPFC, specifically area 45.

Important Efferents from the Region

The dlPFC may influence behavior in the auditory DMTS task by its projections to other brain regions that are important for auditory memory. The lPFC projects to the MD thalamus, pons, amygdala, anterior cingulate cortex and the hippocampus (Fuster, 1997). Another important output from the regions surrounding the PS, is to the temporal and parietal areas which serve as associative areas for sensory information. Area 46 also sends projections to the upper bank of the superior temporal sulcus as part of the uncinate fascicles. The superior temporal gyrus has already been shown to be crucial for auditory recognition memory, thus this projection could be an important one for our DMTS task performance (Fritz et al., 2005). These outputs could serve as loops where the dlPFC sends out information and then receives feedback. One set of these connection loops projects through the caudate and the anterior putamen, which projects to the substantia nigra, which then can project back to the dlPFC directly or indirectly via the MD thalamus (Fuster, 1997). These connections could assist with sending signals for motor

control and may also receive feedback that could be used for working memory or task performance.

Another set of connections that could play an important role in controlling behavior are known as the frontal-parietal and parietal-frontal network. Selemon & Goldman-Rakic (1988) injected two tracers into the PS and part of the posterior parietal cortex (PPC) and found many joint projections into multiple cortical and subcortical regions. These included ipsilateral cortical areas such as: the supplementary motor cortex, the dorsal premotor cortex, the ventral premotor cortex, the anterior arcuate cortex (FEF), orbitofrontal cortex, the anterior and posterior cingulate cortex, the medial parietal cortex, the insular cortex, and the superior temporal cortex (Selemon & Goldman-Rakic, 1988). Some of the cortical structures that shared input from the PS and PPC had alternating columns of input where one column received input from the PS and the column next to it received input from the PPC. Sometimes the input from the PFC and PPC alternated by layer such as in the superior temporal gyrus: where layers I, III and V were innervated by the PFC and layers IV and VI were innervated by the PPC. These authors suggest that these common pathways may play a role in coordinating important functions for goal-directed behavior including, attention, spatial perception, memory and spatially guided movement (Selemon & Goldman-Rakic, 1988). Therefore, the lPFC could be relaying important information about auditory cues to secondary auditory areas within the superior temporal gyrus or motor areas that could be utilized during the DMTS task.

Neurotransmitters and the PFC

Many neurotransmitters exist within the PFC including norepinephrine, dopamine, serotonin, and acetylcholine. Amino acids such as GABA, (γ–aminobutyric

acid) the main inhibitory transmitter, and glutamate, the main excitatory transmitter are present as well. Norepinephrine is thought to play a role in processing somatosensory information in PFC. Dopamine (DA) is seen as being important for learning and memory within the PFC, as injections of D1 antagonists can aid in working memory. Increasing dopamine in PFC of rats produced a deficit in a working memory task (Murphy et al., 1996). However, too much suppression can cause impairment, suggesting there is an ideal level of DA for the PFC to operate smoothly. DA receptors within the PFC are involved in neurological diseases such as Parkinson's disease and schizophrenia, and are associated with decline in cognitive function as the disease progresses (Grace et al., 2007; Moustafa et al., 2008). How these neurotransmitters contribute to working memory is currently being studied. Serotonin has also been associated with schizophrenia and PFC (Remington, 2008). Lastly, ACh is associated with assisting in excitatory and inhibitory responses of cells within the dlPFC (Fuster, 1997; Goldman-Rakic, 1996; Rao et al., 1999). It is possible that DA, serotonin, and ACh all play a role in working memory processes, depending on task demands.

Lateral PFC and Working Memory

Evidence for the role of the lPFC in working memory comes from lesion and electrophysiological studies. Lesions in both humans and animals cause impairments in delay tasks when the lPFC is damaged. The electrophysiological evidence indicates that particular cells in the lPFC respond to certain parts of a delay task; including the delay time itself, the cues presented, and the response of the animal (Funahashi et al., 1989; Funahashi et al, 1990; Funahashi et al., 1991; Fuster & Alexander, 1971; Fuster, 1973). All of these neuronal types (those that encode cues, delay etc.) could correspond to the

information being held in working memory. Many researchers in the field support the hypothesis that one undertaking of the lateral PFC is to play a role in working memory (Fuster, 2001; Fuster, 2000; Goldman-Rakic, 1987; Levy & Goldman-Rakic, 2000; Miller & Cohen, 2001; Owen, 1997; Petrides, 2000).

Other brain regions that contribute to working memory include parietal cortex, temporal cortex, thalamus, and hippocampus (Courtney et al., 1996; Davachi & Goldman-Rakic, 2001; Awh et al., 1996; Miller & Desimone, 1991). These different brain regions support working memory demand based on what the task requires. For example, spatial working memory interacts with areas of the parietal lobe and object memory interacts with areas of the temporal lobe including the perirhinal cortices (Courtney et al., 1996; Davachi & Goldman-Rakic, 2001; Miller & Desimone, 1991). While these other brain areas provide support for working memory, the PFC is argued to be the key component for organizing working memory, in that it facilitates sending information to these regions and may also play a role in guiding behavior (Fuster, 2001; Miller, 2000).

Another key area in the lPFC supporting working memory is area 46 **(**Goldman-Rakic, 1987). Thus, area 46 contributes to the role of the PFC in guiding goal-directed behavior. The next sections of this paper will discuss some of the main evidence from lesion, imaging, and electrophysiological studies that help support the position of the lPFC in working memory and why it might be involved in our DMTS task.

Lesion studies

The importance of the lPFC's involvement in working memory and other cognitive tasks is demonstrated by lesion work. Early work by Hitzig and Ferrier found that natural

lesions of the region affected attention and intelligence more so than sensory or motor components (Goldman-Rakic, 1987). In 1936, Jacobsen used a delay-response task to illustrate impaired behavior of primates that had prefrontal cortex damage. He further demonstrated that the main problem with these monkeys was related to the demands of the delay aspect of the task and was not related to a lack of visual or motor skills (Curtis & D'Esposito, 2004). Starting in the 1950's, Pribram and Mishkin completed a series of studies with large lesions of the prefrontal lobes in monkeys and baboons. These lesions created deficits in delay response, delay spatial alternation, and delay object alternation tasks (Mishkin et al., 1969; Mishkin & Pribram 1956; Mishkin & Pribram, 1955; Pribram et al., 1952; Pribram & Mishkin, 1956). In an attempt to understand better the precise deficit of a smaller area of cortex, more specific lesions of area 46 were completed and tested with a spatial delay alternation task. These smaller lesions of the middle section of area 46 resulted in complete failure to relearn the task postoperatively even after 1000 trials (Butters & Pandya, 1969). This type of delayed alternation task emphasized the importance of the delay or working memory component that the lPFC plays a part in, especially area 46. Multiple studies demonstrate that lesions of the lPFC lead to spatial and delay deficits (Malmo, 1942; Mishkin, 1957; Mishkin & Manning, 1978; Passingham, 1975). Lesions of other regions of the PFC such as the mPFC did not cause as severe deficits in delayed response performance (Curtis & D'Esposito, 2004).

Further work by Funahashi et al., (1993) provided more evidence for the implication of area 46 in working memory. Unilateral lesions of area 46 in a spatial occulomotor delay response task resulted in memory impairments for the opposite visual field (Funahashi, et al., 1993). Work by Petrides (1995) examined the role of dlPFC in visual

object-delay tasks. When the dlPFC (areas 46 and 9) was lesioned in two non-spatial object-delay tasks a clear impairment was found, with monkeys performing at chance levels (Petrides, 1995). Clearly, area 46 is applied more for working memory demands than for spatial or non-spatial domains.

Humans with frontal lobe damage are frequently described as unmotivated, apathetic, disinterested and lacking drive (Shallice & Burgess, 1991). If the damage is mostly in the lPFC then planning becomes a problem. Patients may be able to follow directions to perform a task, such as making a salad, but may be impaired on planning a series of events such as a complete meal (salad, main course, dessert).

Frontal lobe patients have also been examined on tasks such as the tower of London task or self-ordered tasks, which require planning of solutions and then execution of the moves in a sequence, placing a heavy load on working memory (Owen et al., 1990). Patients with lPFC damage were less efficient at planning and completing the tower of London task and had impaired performance on the self-ordered task as well (Owen et al., 1990; Petrides & Milner, 1982). More recent work has found that humans with frontal lesions are still impaired on working memory tasks (Barcelo & Knight, 2007). It has been found however, that damage unilaterally does not impair performance for rehearsal or maintenance processes in working memory tasks (D'Esposito et al., 2006; Owen et al., 1995). The authors suggest that for humans bi-lateral damage is necessary to impair working memory performance, because other brain networks may compensate if there is only unilateral damage (D'Esposito et al., 2006).

One study that used a DMTS task, examined auditory working memory in patients with dlPFC damage, found impairment only when there was a distracter (a series of tone

pips) presented during the delay period (Chao & Knight, 1998). Although the patients were not impaired compared to control participants in the no distracter condition, this could be because the patients only had unilateral damage or because the delay time was only 5 seconds. Thus, the lPFC could still be an important site of plasticity for auditory working memory processes.

PFC and Attention

Another function of the PFC is to modulate attentional demand. This is frequently described as assisting in selecting relevant stimuli or interacting with other brain networks to help select a behavior (Fuster, 2001; Miller, 2000). It is suggested that the PFC can exert control by sending excitatory signals to other brain regions to help maintain an informational set or select a behavioral output sequence (Miller, 2000). This interaction between attending to a particular stimulus and maintaining that stimulus in memory is argued as being one purpose of the PFC. The PFC assists with working memory by guiding attention and by interacting with other regions such as the inferior temporal cortex to complete task demands (Nee & Jonides, 2008).

Imaging Studies

Neural imaging serves as another important technique for probing working memory in humans and non-human primates. Using 2-DG (2de-oxyglucose) Friedman & Goldman-Rakic (1994) found that monkeys that underwent training in three working memory tasks had a 19% increase in local cerebral glucose utilization in the lPFC during metabolic mapping, compared to controls involved in associative learning tasks. This suggests that increased activity in lPFC is important for working memory tasks.

Human imaging studies using PET or fMRI also support the role of the lPFC in working memory. Many visual working memory tasks that use faces, visual objects, visuospatial components, or verbal cues have activation in lPFC (Awh et al., 1996; Bor et al., 2004; Bunge et al., 2003; Courtney et al., 1997; Courtney et al., 1996; McCarthy et al., 1996; McCarthy et al., 1994; Owen et al., 1998; Owen et al., 1996; Petrides et al., 1993a; Petrides et al., 1993b; Postle et al., 2000; Postle et al., 1999; Rypma & D'Esposito, 1999; Stern et al., 2000). A meta-analysis of over 20 studies found, that even across multiple working memory tasks using various procedures and stimuli, there was significant activation of the lPFC in all of the studies (Owen et al., 2005). More current work has begun to examine separate processes within working memory such as encoding of stimuli, attending to task rules, manipulation of information, or response selection, and all of these studies find activation within the lPFC (D'Esposito et al., 2000; D'Esposito et al., 1999; D'Esposito et al., 1998; Petrides et al., 2002; Postle et al., 2003; Postle et al., 2000; Postle et al., 1999; Rowe et al., 2008; Rypma & D'Esposito, 2003). While the exact role of the lPFC is still being determined, it is a key component in the processes of working memory.

Electrophysiological studies

Recording studies also support the role of the lPFC in working memory. Multiple studies report that dlPFC neurons responded during various working memory delay tasks. Fuster and Alexander (1971) used a delay response task and found sustained activation of 65% of the cells in area 46 during delays of up to 60 seconds. Fuster et al., (1982) later reported that half the cells recorded from in area 46 responded during the delay period of a DMTS task. Other groups have found similar results, for example Miller et al., (1996)

found cells that were selective for particular stimuli in the lPFC during a visual DMTS task.

In addition, Azuma & Suzuki (1984) found neurons that responded to a direction of a sound source, and hinted that these cells may play a role in attention. Further electrophysiological work using various paradigms has provided evidence for the dlPFC in working memory (Bodner et al, 1996; Carlson et al., 1997; Funahashi, et al., 1989; Fuster et al., 2000; Kojima & Goldman-Rakic, 1982; Miller et al., 1996; Quintana & Fuster, 1999; Watanabe, 1992; Wilson et al., 1993). All of these recording studies have found neurons in or around the PS which selectively responded to stimuli in the delay task as well as to the delay itself, some of which maintained or increased firing rates over the delay period. However, only a few of these studies used auditory cues to examine this region's role in memory.

Bodner et al., (1996) used pairs of cues such that after a high tone was played, a 10 sec delay would follow, and the monkey had to select a red light in order to earn a reward. If the low tone was played the monkey had to select a green light to be rewarded. While the use of the auditory cue was one of the first used in a recording study, the animal could have immediately switched to remembering the trained color associated with the tone; and may not have been actively been remembering the auditory stimulus.

Another study that recorded from the lPFC used a conditioning procedure where a cue (visual or auditory) signaled the forthcoming presentation of the unconditioned stimulus (juice) (Watanabe, 1992), i.e., high tone = juice, low tone = no juice. In this case some cells in the prefrontal region fired more to the cue that predicted juice reward, while some cells fired more to the cue type (high or low tone) regardless of whether the

tone predicted a coming reward (Watanabe, 1992). This was an important contribution showing that cells in the lPFC are responsive to auditory cues, but it was not a test of auditory recognition memory.

The main gap in this field is that most of the research conducted has used visual stimuli. This proposal seeks to expand the current knowledge of the lPFC by using a different modality, audition. The lPFC receives auditory input from auditory cortex such as the anterior belt cortex and the caudal belt (Romanski et al., 1999a). The lPFC has also exhibited neural responses to a range of auditory stimuli from tones and clicks to monkey vocalizations and was shown to be active during a passive listening task as well as to sounds over a memory gap (Azuma & Suzuki, 1984; Bodner et al., 1996; Newman & Lindsley, 1976; Poremba et al., 2003). Others have suggested an auditory domain could lie within the vlPFC as cells here fired to auditory stimuli but not to visual stimuli or eye movements (Romanski & Goldman-Rakic, 2002). Neurons in the lPFC also fire to auditory location cues in a delay task (Kikuchi-Yorioka & Sawaguchi, 2000). Consequently, it is expected that because the lPFC has auditory memory location cells, (cells that fire more to sounds coming from a particular location) and is responsive to sounds during passive listening, it could also have auditory cells that are responsive to a particular stimulus (coo) or stimulus type (monkey vocalization vs. human vocalization) during the DMTS task. Cells which encode single sound stimuli, as well as ones that are more generally responsive to multiple sound stimuli could elucidate how the lPFC is involved in auditory short-term recognition memory.

Summary

Working and recognition memory have been widely studied within the visual domain. One region that has been identified as essential to working memory is the lPFC, while the cholinergic system has been identified to assist with visual recognition memory. Does another modality, utilize the same neurotransmitter system and the same neural region (lPFC) as visual recognition memory?

Experiments in Chapters 2 and 3 will test the cholinergic system in an auditory recognition memory task. A DMTS task will be used with auditory cues and differing doses of scopolamine, a muscarinic receptor antagonist, as well another acetylcholine agent, physostigmine (acetylcholineasterase inhibitor). Improved performance when both scopolamine and physostigmine are administered would support the hypothesis that the cholinergic system is involved in auditory working memory.

Chapter 4 will utilize electrophysiology within the lPFC to measure neuronal activation during the same DMTS task and during passive listening. Comparing the same cell's responsiveness to sound stimuli within both the DMTS task and during passive listening will elucidate the organization of the lPFC and how it contributes to complex behaviors. In addition, cells within the lPFC could be attuned to particular rules of the DMTS task, such that more cells fire to match trials versus nonmatch trials. This study provides an opportunity to expand our knowledge of how the lPFC operates in a working/recognition memory task in a new modality.

This dissertation examines auditory working and recognition memory in two ways. First examining a possible role for the cholinergic system and second by recording from a

region (lPFC) associated with visual working memory. It is anticipated that these two approaches will begin to determine if auditory working memory relies on the same neurotransmitter (acetylcholine) and if it employs a similar brain region (lPFC) utilized by visual working memory demand.

CHAPTER 2. A ROLE FOR ACETLYCHOLINE IN SHORT-TERM AUDITORY MEMORY

In our daily lives, decisions that require short-term memory including judgments of stimulus recency (working memory) or stimulus familiarity (recognition memory) are critical for a multitude of basic tasks such as conversation, reading this sentence, and finding your way home. One behavioral task that assesses short-term memory and that utilizes both working and recognition memory is delay matching-to-sample (DMTS). Typically during DMTS a sample visual object is presented and then a delay memory period comes next and is followed by the previously presented sample object and a novel choice object. The animal is rewarded for choosing the previously presented sample stimulus. There are several pieces of evidence that point to the brain areas involved with working and recognition memory using visual cues (e.g., Bachevalier and Mishkin, 1994; Fuster, 1982; Goldman-Rakic, 1987; Málková et al., 2001) and a few using auditory/visual or auditory cues (Fritz et al., 2005; Kikuchi-Yorioka & Sawaguchi, 2000; Sugihara et al., 2006; Watanabe, 1992). While many brain areas have been implicated in short-term memory for both visual and auditory cues, the underlying neurotransmitter systems for auditory memories have not been examined.

One possible neurotransmitter system for auditory memory, important for tasks requiring visual short-term memory, is the cholinergic system. Several studies have shown that blocking muscarinic receptors impairs memory performance on a variety of tasks including, DMTS, delay nonmatching-to-sample (DNMTS), self-ordered spatial search, and serial-probe recognition (Aigner & Mishkin, 1986; Hironaka & Ando, 1996; Ogura & Aigner, 1993; Myers et al., 2002; Penetar & McDonough, 1983; Taffe et al.,

1999). Other acetylcholine receptor antagonists, such as atropine, that affect the cholinergic system by blocking receptor binding, also impair short-term memory (Penetar & McDonough, 1983). Here, monkeys were trained in a DMTS task with variable delays of 0, 4, 8 or 16 seconds. Performance was significantly decreased at the 4, 8, and 16 second delays when administered the highest atropine dose (440 µg/kg). While at the second highest dose of atropine (140 µg/kg) only the 8 and 16 second delays were affected (Penetar & McDonough, 1983).

The use of acetylcholine for memory processing may be conserved across species as blocking it also impairs mainly visual memory performance in rats, pigeons, monkeys and humans (Elsmore et al., 1989; Flicker et al., 1990; Hudzik & Wenger, 1993; Pontecorvo, et al., 1991; Pontecorvo & Evans, 1985; Spinelli et al., 2006). For example, Pontecorvo et al., 1991 used mixed sensory stimuli using a light and tone as stimuli, and levers for responding in a DMTS task; wherein rats had to remember which stimuli had been most recently administered and then choose the appropriate lever for matching and nonmatching trials. Animals injected with scopolamine showed dose dependent effects and were impaired in their choice accuracy.

Although we know some of the brain areas that are involved, we do not know what neurotransmitters might be important for auditory recognition memory. Thus, the proposed studies are designed to assess the effects of ACh on auditory recognition memory.

Methods

Subjects

Five rhesus monkeys (*Macaca mulatta*), 3 females and 2 males (11 to 12 years old; 5-10 kg), were born and raised in captivity, and housed in Spence Laboratories at the University of Iowa (12-hr light/ dark cycle). Monkeys were fed standard monkey chow (Harlan Teklad Global Diet, Madison, WI, USA) with fresh fruit and vegetables. The majority of food was given after training each day. Water was provided ad libitum in the home cage with all animals given environmental enrichment. Each animal's weight was maintained above 85% of starting weight and adjusted upwards based on age. The Institutional Animal Care and Use Committee at the University of Iowa approved all procedures.

Sound Stimulus Selection

Sound stimuli (900) included: tones, music, human voices (speech and non-speech sounds), monkey calls, bird calls, other animal calls, and manmade sounds (such as cars, train whistles or airplanes), were eventually repeated throughout training so none were unfamiliar. However, because they are pseudorandomly presented in a trial unique fashion prediction based on familiarity is not possible. Sound stimulus duration was truncated at 500 ms, and all sounds played from a single speaker positioned just above the response button.

Conditioning Apparatus

Monkeys sat comfortably in restraint chairs placed inside a sound attenuation chamber. There was a response button (7.5 cm square) in front (height 45.7 cm; 12.7 cm

from monkey's chest), a speaker (height 55.9 cm), and a copper tube connected to a dish (2.5 cm from monkey's fingertips) from which to collect a reward. A house light provided illumination throughout the training session. A stimulus light remained on during the intertrial interval (ITI). LabView software (National Instruments, Austin, TX) controlled lights, sound stimuli, and treat dispenser. To the upper left of the monkey a small video camera allowed observation by the experimenter.

Basic Training Procedure

The DMTS task used approximately 75 stimulus set sounds/day. Training sessions were held 5 days a week, 50 trials/session. The task was designed as a go/no-go task (Figure 4). For match trials the monkey was to respond by pressing the response button releasing a small chocolate candy reward. For nonmatch trials the monkey was not to respond. If the monkey pressed the button after a nonmatch trial they received a 500 ms air puff reminder not to respond (a mild punishment). This mild air puff is applied semi-randomly during normal training after nonmatch errors to discourage incorrect responding. During sessions with saline or drug injections, animals only received air puff after the first incorrect nonmatch trial.

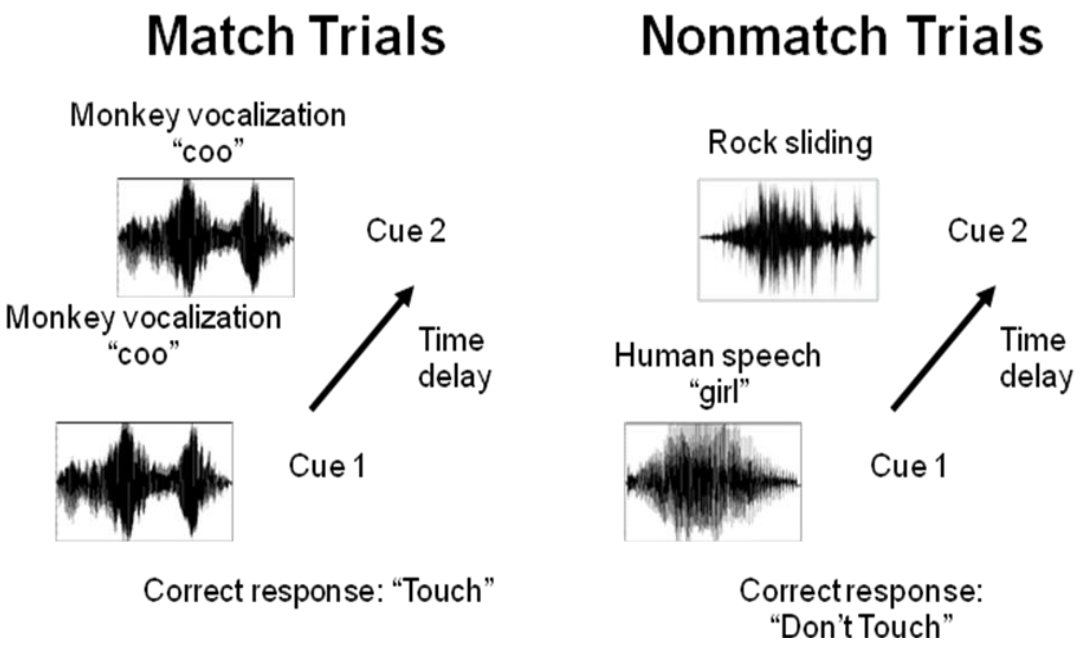

Figure 4. This diagram illustrates the design of the go/no-go task. The left panel illustrates match trials that consist of a first sound presentation, in this case a "coo" followed by a variable time delay of 500 ms, 2500 ms, or 5000 ms. The second sound presentation was also the same "coo," thus the animal should respond. The right panel illustrates nonmatch trials that consist of a first sound presentation, in this case "girl" followed by the delay, and then the second sound nonmatching presentation, "rock sliding," thus the animal should not respond. Each daily session consisted of 25 match and 25 nonmatch trials.

Variable DMTS

Match and nonmatch trials consisted of a 500 ms sound followed by a pseudo-randomly selected inter stimulus interval (ISI) of 500 ms, 2500 ms, or 5000 ms. Then a second 500 ms sound was played, and the response button lit up for 1000 ms. This happened on both match and nonmatch trials as a cue that signaled the possible response time, and did not in any way signal which were match versus nonmatch trials. If the

animal did not respond during this time interval, it lost the chance for a reward on that trial, and the ITI of 12000 ms began.

Monkeys trained to a criterion of 80% or better on this variable ISI schedule before the saline and drug doses were administered. The three time delays were chosen because they were well within the auditory short-term memory capacity for all five monkeys. With the standard training at 5000 ms, they could perform the task at shorter delays. We wanted to ascertain performance with these relatively short delays, which a larger number of our trained monkeys can consistently perform. Other work has shown that when delays are lengthened past 37.5 seconds on a similar auditory task performance starts to drop below 70% correct (Fritz et al., 2005).

Drug Protocol for Variable DMTS

All monkeys served as within subject controls. After meeting behavioral criterion, they were injected with saline, 3 µg, 5 µg, 10 µg of scopolamine hydrochloride (salt), (Sigma-Aldrich, St. Louis), per 1 kilogram of weight, intramuscular injection (i.m.). Drug doses were selected based on similar ranges in other scopolamine studies with rhesus macaques (Aigner & Mishkin, 1986; Ogura & Aigner, 1993). All animals received two sessions with each drug dose, and five sessions with saline. All means reported are the average of those sessions. Drug or saline was administered 30 minutes before the behavioral session. Drug dose sessions were assigned in a semi-random order and counter balanced so that some monkeys received 3 µg, 5 µg, then 10 µg, while others received 10 µg, 3 µg, 5 µg etc. Saline was administered on the first weekday of training, followed by a drug dose day, then a training day.

Food Reward Control Test

To examine the effects of scopolamine on the animals' response to food rewards, i.e., motivation, without a memory demand, we compared sessions with saline and a 5 μg/kg dose of scopolamine per 1 kilogram of weight during a food test. During the regular DMTS training the session lasts about 20 minutes and animals work to receive 20-25 rewards. In these food reward test sessions, monkeys were placed in the sound booth (20 minutes after injection) and given one small treat through the pellet dispenser as during regular DMTS, per minute for 20 minutes. The pellet dispenser emits the sound of the solenoid turning on and off to drive the delivery device. There is also the sound of the pellet falling through the copper delivery tube. Monkeys only had to reach for the reward upon hearing the pellet dispenser release the treat. The control saline injection session with the food reward test occurred the day before the scopolamine injection session.

Same Sound DMTS Control Test

To investigate whether the monkeys could pay attention to a simple task that did not require memory within a trial we designed a task that presented sound trials with a repeated white noise stimulus (25) and no sound trials (25). On every sound and no sound trial the lighted response button was briefly lit just as in the variable memory delay DMTS task. Button presses on the sound trials resulted in food reward and button presses during the no sound trials were scored as errors. For the sound trials the delay was set at 500 ms but the same white noise sample was used for every stimulus on every trial. There was a variable ITI of 8000, 10000, 12000 ms so that the animal could not predict when

the next sound trial would start. We compared sessions with saline and a 5 μg/kg dose of scopolamine per 1 kilogram of weight (30 minute wait time).

Low Memory DMTS Control Test

To investigate whether the monkeys were attending to the cues and performing the basic task we shortened the ISI delay to 50 ms. This is an extremely short ISI but still allows for the detection of two separate sounds. Both match (N = 25) and nonmatch (N = 25) trials were presented with the short ISI. The very short delay was so slight virtually no memory demand is present. This concept is similar to some visual paradigms, which present the sample and then leave the sample up while presenting the choice stimulus (Robbins et al., 1997; Taffe et al., 1999). The trial unique sound stimulus set and ITIs were the same as those used in the variable DMTS task. We compared sessions with saline and a 5 μg/kg dose of scopolamine per 1 kilogram of weight (30 minute wait time). This design reduces the memory component but still tests whether the monkeys are attending and able to process sound quality beyond the white noise presented in the same sound DMTS.

Description of Analyses

Performance of the animals, measured by percent error (the number of incorrect trials/by the total number of trials; per session), was analyzed. The variable DMTS task was analyzed with a repeated measures analysis of variance (ANOVA) using SPSS 13 software (SPSS, Chicago, IL, USA), one within factor was dosage (saline, or 3 μg, 5 μg, 10 μg of scopolamine) and the other within factor was ISI delay (500 ms, 2500 ms, 5000 ms). Two separate ANOVAs were used for match and nonmatch trials as response

requirements differed. In order to balance the statistical design, we selected two of the saline sessions that were closest to the mean across all saline sessions. The match latency to respond was also analyzed with repeated measures ANOVAs using the same within factors as above. The *p*-value was set at 0.05. The food reward test, same sound DMTS task, and low memory DMTS task were analyzed with planned independent *t*-statistics with the *p*-value set at 0.05.

For the nonmatch latency data we used the Bonferroni procedure, with Keppel's modification, to correct for the "family-wise" error rate among comparisons of *t*-statistics (Keppel, 1982). Under each delay, the latency to respond under the 4 drug conditions was examined. Thus, 18 pairwise comparisons were conducted to reveal differences between saline and scopolamine conditions. The 4 drug conditions were then entered as the experimental treatment, the number of degrees of freedom for the treatment source of variance (4 – 1 = 3) was multiplied by the standard critical probability level (0.05), and the product was divided by the number of *t*-test comparisons (i.e., 18), yielding the corrected, critical probability level of 0.008.

Results

Performance Results of Variable DMTS

Animals met a criterion of 80% or better on average for match and nonmatch trials before beginning drug sessions. Performance for match and nonmatch trials was calculated using the number of incorrect responses divided by the total number of responses of that type and converted to percent error. For performance on match trials, there was a significant effect of dose ($F_{3, 27} = 16.03$, $p \leq 0.05$; Figure 5) showing an

increase in percent error at all three delays. For performance on match trials, there was no significant effect of delay and no significant interaction.

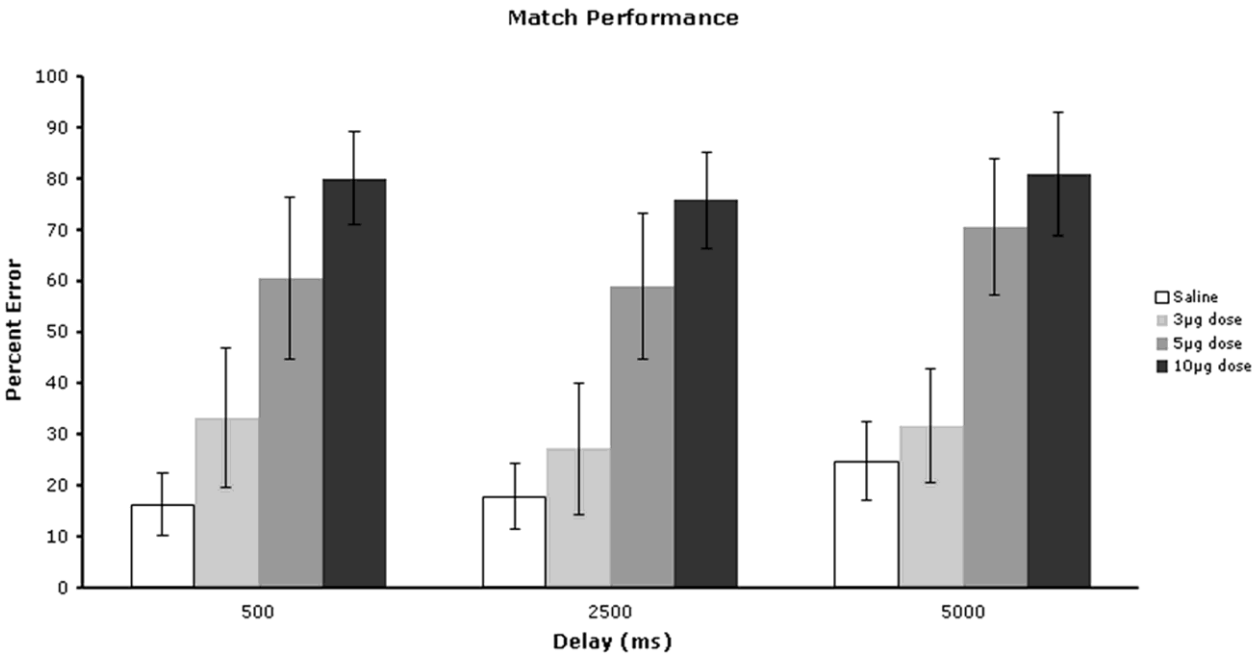

Figure 5. Match performance as measured by percent error, significant effect of dose. Performance on 5 μg and 10 μg doses was significantly impaired compared to saline ($p \leq 0.05$). Performance on 5 μg and 10 μg doses was also significantly impaired compared to the 3 μg dose ($p \leq 0.05$). (Plakke et al., 2008).

For performance on nonmatch trials, there were no significant main effects of dose or delay and no significant interaction effect.

In addition to overall behavioral performance, we also examined the latency to respond. On match trials responding is a correct response, however on nonmatch trials responding is an incorrect response and considered an error. On the highest dose (10µg dose) animals rarely responded to nonmatch trials. For this reason, some animals were missing latencies for response errors on nonmatch and an ANOVA was not viable. Instead we used *t*-tests, with a corrected *p*-value (0.008) for multiple comparisons to examine differences between dosages at each delay. There were no significant response latency differences for match or nonmatch trials.

Food Reward Control Test Outcome

During saline sessions for the food reward test monkeys reached for and obtained all 20 treats. During the scopolamine sessions all but one of the monkeys reached for and obtained all 20 treats made obtainable throughout the food reward test. There was no significant difference between the number of rewards taken on saline versus scopolamine during the food reward test (*t*-test: $p = 0.35$).

Same Sound DMTS Control Test Outcome

Animals performed well on the same sound DMTS task during both saline and scopolamine conditions. There were no significant differences in performance between the saline and scopolamine session for sound match trials (*t*-test: $p = 0.90$) nor on the light only trials (*t*-test: $p = 0.37$).

Low Memory DMTS Control Test Outcome

There were no significant differences on low memory demand DMTS task performance for match or nonmatch trials between saline and scopolamine sessions (*t*-test: match trials $p = 0.51$; nonmatch trials $p = 0.18$).

Discussion

Blocking muscarinic transmission with scopolamine impairs performance of auditory DMTS. The two higher dosages of ScHCl impaired performance on the auditory variable DMTS at all three delays. Additional tests indicate that this deficit is more likely due to a deficit in auditory memory than in attention as the intermediate dose of ScHCl did not impair performance with very short delays of 50 ms.

A decrease in responding could be interpreted as a general lack of motivation and/or a motor deficit caused by impaired muscarinic transmission. However, in this study neither of those explanations can account for the decrement in responding on match trials. Performance on nonmatch trials during which a motor response should be withheld for a correct response was not impaired, i.e., no significant changes in over or under-responding. If the basic deficit on match trials was a decrement in overall responsiveness we would expect to see a significant decrease in errors made on the nonmatch trials as well. Response latency on match and nonmatch trials was not affected either. This does not support the argument that decreases in performance are due to motor impairment. Results of the food reward test demonstrate that even while under the influence of the same dose of scopolamine that led to deficits on the memory task, the animals were still motivated to reach for and consume treats. The simple food reward task in which monkeys routinely responded under the influence of scopolamine to retrieve and eat food

rewards, and the decrease in responding on match trials indicates a DMTS specific problem that is not attributable to a lack of motivation or motor impairment.

Given that the animals were impaired at all three original delays of 500 ms, 2500 ms, and 5000 ms one could argue that it was attentional processing that was affected. Acetylcholine is important for cue detection, which is affiliated with attentional control (Parikh et al., 2007). In opposition to this finding however, the food reward test demonstrates that the animals are attending in some capacity in that they hear the sound of the pellet dispenser, orient, and obtain the food reward while under the influence of scopolamine. Furthermore, while on the intermediate dose of scopolamine, the monkeys performed well on the same sound DMTS task but with a very simple, repeated, white noise stimulus thus with a lower memory demand at a delay of 500 ms. The monkeys responded well to the sound presentations and ignored the light only trials confirming their ability to detect sound. Although the monkeys were impaired at the shortest 500 ms delay during variable DMTS with scopolamine, performance at the briefest delay of 50 ms was not impaired in the low memory DMTS suggesting that encoding of auditory stimuli was intact. A half second delay may seem incredibly short in a visual task, but in an auditory task this may be a substantial memory delay as auditory memory performance starts to fall below 70% at only 37.5 seconds as compared to the visual system that may have a capacity measured in minutes to hours (Fritz et al., 2005). Good performance on these control conditions demonstrates that the monkeys were attending and responding to sound, food, and matching sound stimuli. Thus the observed deficit induced by scopolamine in the sound unique DMTS task, with the three delays (500, 2500, 5000 ms), cannot be solely due to attentional problems.

Our current findings show that performance on an auditory memory task is impaired when the cholinergic system is temporarily disabled with a receptor antagonist. These findings are similar to work done in the visual field where short-term recognition memory was examined by Aigner and Mishkin using a DNMTS wherein the delay between sample and test object was 15 seconds (1986). Several other non-human primate and other animal studies (Hironaka & Ando, 1996; Myers et al., 2002; Ogura & Aigner, 1993; Penetar & McDonough, 1983; Taffe et al., 1999), as well as a human study (Robbins et al., 1997), suggest a role for acetylcholine in visual short-term memory. Although task requirements differed across the experiments all tested some form of short-term memory and consistently found across multiple species that blocking acetylcholine impaired visual memory performance. Taken together with our current results concerning the important role of acetylcholine in auditory memory, we suggest that a similar mechanism utilizing the cholinergic system may be conserved for short-term memory across multiple modalities.

Visual short-term memory relies on several brain areas including areas of the frontal lobe, rhinal cortex, parietal lobe, and other visual cortical areas (Champod & Petrides, 2007; Fritz et al., 2005; Hironaka & Ando, 1996; Turchi et al., 2005; Xu & Chun, 2007). Auditory short-term memory relies on areas within the medial temporal lobe such as the superior temporal gyrus (Fritz et al., 2005), but other areas such as the prefrontal cortex may also be involved (Bodner et al., 1996). Working memory is thought to rely heavily on the prefrontal cortex and its involvement has been demonstrated in neurophysiological and imaging work (Artchakov et al., 2007; DePisapia et al., 2007; Miller et al., 1996). The prefrontal cortex receives cholinergic input and blocking

cholinergic input directly via prefrontal infusions of scopolamine has been shown to impair visual working memory (Chudasama et al., 2004). A possible link between visual and auditory memory could be the neurotransmitter system involved, as well as shared brain areas like the prefrontal cortex (Kikuchi-Yorioka & Sawaguchi, 2000; Romanski & Goldman-Rakic, 2002; Watanabe, 1992).

Future studies could address if cholinergic agonists improve auditory memory as some have shown using visual cues (Aigner & Mishkin, 1986; Bentley et al., 2004; Ogura & Aigner, 1993; Penetar & McDonough, 1983), determine the exact process that is impaired, e.g., encoding of stimuli, storage, or retrieval (Aigner et al., 1991; Robbins et al., 1997; Weinberger et al., 2006), or determine if acetylcholine is important across all modalities for other similar types of tasks. The current findings lend support to the idea that the cholinergic system plays a role in short-term memory performance regardless of the modality of the given cues and suggests that diseases and medications affecting the cholinergic system may generally influence short-term memory performance in at least two modalities.

CHAPTER 3. PHYSOSTIGMINE ALLEVIATES BEHAVIORAL DEFICIT CAUSED BY A CHOLINERGIC ANTAGONIST DURING DMTS PERFORMANCE

Although a few neural structures have been suggested to be involved in auditory working memory, information concerning the neurotransmitters underlying auditory memory is sparse. In Chapter 2, we demonstrated that scopolamine, a cholinergic receptor antagonist, impaired performance on an auditory go/no-go delay matching-to-sample task (DMTS). In this experiment in order to verify the role of the cholinergic system as a possible modulator for auditory short-term memory, physostigmine, a cholinergic agonist, was used to assess if the scopolamine induced deficit could be diminished. Physostigmine is an acetylcholinesterase inhibitor and so indirectly stimulates nicotinic and muscarinic receptors. Additionally, longer variable delays were used to systematically assess the memory deficit. One behavioral task that assesses short-term memory and that uses both working and recognition memory is delayed matching-to-sample (DMTS). Typically during DMTS, a sample visual object is presented followed by a delay memory period after which the previously presented sample object and a novel choice object are presented. The animal is rewarded for choosing the previously presented sample stimulus. There are several pieces of evidence that point to the brain areas involved with working and recognition memory using visual cues (e.g., Bachevalier and Mishkin, 1994; Fuster, 1997; Goldman-Rakic, 1987; Málková et al., 2001), and a few using auditory/visual or auditory cues (Fritz et al., 2005; Kikuchi-Yorioka & Sawaguchi, 2000; Sugihara et al., 2006; Watanabe, 1992;).

One possible neurotransmitter system for auditory memory, important for tasks requiring visual short-term memory, is the cholinergic system. Several studies have shown that blocking muscarinic receptors impairs memory performance on a variety of tasks including, DMTS, delay non-matching-to-sample (DNMTS), self-ordered spatial search, and serial-probe recognition (Aigner & Mishkin, 1986; Aigner, Walker & Mishkin, 1991; Hironaka & Ando, 1996; Myers et al., 2002; Ogura & Aigner, 1993; Penetar & McDonough, 1983; Taffe et al., 1999;). Other acetylcholine receptor antagonists, such as atropine, that affect the cholinergic system by blocking receptor binding, also impair short-term memory (Penetar & McDonough, 1983). Penetar & McDonough (1983) found that monkeys were trained in a delayed matching-to-sample task with variable delays of 0, 4, 8 or 16 seconds. Performance was significantly decreased at the 4, 8, and 16 second delays when administered the highest atropine dose (440 µg/kg). While at the second highest dose of atropine (140 µg/kg) only the 8 and 16 second delays were affected (Penetar & McDonough, 1983). This suggests that a particular level of neurotransmitter is needed to perform a memory task, and when the cholinergic system is partly impaired (via a lower dose of drug) only the more difficult longer memory demands are impaired.

The use of acetylcholine for memory processing may be conserved across species as blocking it also impairs mainly visual memory performance in rats, pigeons, monkeys and humans (Elsmore et al., 1989; Flicker et al., 1990; Pontecorvo & Evans, 1985; Pontecorvo, et al., 1991; Hudzik & Wenger, 1993; Spinelli et al., 2006). For example, Pontecorvo et al., 1991 used mixed sensory stimuli using a light and tone as stimuli, and levers for responding in a delayed matching-to-sample task (DMTS); wherein rats had to

remember which stimuli had been most recently administered and then choose the appropriate lever for matching and nonmatching trials. Animals injected with scopolamine showed dose dependent effects and were impaired in their choice accuracy. When the same neurotransmitter system is involved across species for a similar demand, in this case short-term memory, it suggests it is a good candidate to also be conserved across modality.

Previously, our lab demonstrated a performance deficit for the auditory DMTS task with delays lasting up to five seconds (Plakke et al., 2008). Here, we want to expand our understanding of cholinergic system involvement during auditory recognition memory by using the same DMTS task with longer memory delays and verify the specificity of acetylcholine involvement. We utilize the acetylcholine agonist (physostigmine) in combination with the previously used antagonist (scopolamine) to see if the behavioral deficit can be reduced, compared to performance when scopolamine is used alone. The DMTS task utilizes a go/no-go response paradigm during which two sound presentations are separated by variable time delays ranging from 2 to 10 seconds in length. The two sound presentations either match, for which a button press response is required to achieve a small food reward, or the two sound presentations do not match, for which there should be no button press and no reward is given.

In order to examine the effects of physostigmine and scopolamine together, both were first administered individually to assess how they affected behavior. For this experiment the neural system was again challenged by scopolamine hydrochloride (ScHCl), but longer memory delays were used compared to Plakke et al., 2008. Physostigmine was also administered at the same time as scopolamine to determine if this

agonist alleviated the antagonist induced deficit, thus testing the specificity of acetylcholine involvement. Based on the evidence that blocking the cholinergic system induced a short-term memory deficit at delays up to five seconds we hypothesize that blocking the cholinergic system with scopolamine hydrochloride (ScHCl), a muscarinic cholinergic receptor antagonist, will also impair auditory short-term memory at longer delays (up to ten seconds) and that performance reversal will be observed with the addition of a specific cholinergic agonist. Before administering the acetylcholine agonist physostigmine, an acetylcholineasterase inhibitior, in combination with scopolamine, we administered it alone to test its effects on performance.

Methods

Subjects

Four rhesus monkeys (*Macaca mulatta*), 2 females and 2 males (12 to 13 years old; 5-10 kg), were born and raised in captivity, and housed in Spence Laboratories at the University of Iowa (12-hr light/ dark cycle). Monkeys were fed standard monkey chow (Harlan Teklad Global Diet, Madison, WI, USA), fresh fruit, and vegetables. The majority of food was given after training each day. Water was provided ad libitum in the home cage with all animals given environmental enrichment. Each animal's weight was maintained above 85% of starting weight and adjusted upwards based on age. The Institutional Animal Care and Use Committee at the University of Iowa approved all procedures.

Sound Stimulus Selection

Sound stimuli (~900), tones, music, human voices (speech and non-speech sounds), monkey vocalizations, bird calls, other animal calls, and manmade sounds (e.g. cars, train whistles or airplanes), were eventually repeated throughout training so none are unfamiliar. However, because they are pseudorandomly presented in a trial unique fashion prediction based on familiarity is not possible. Sound stimulus duration was truncated at 500 ms, and all sounds played from a single speaker positioned just above the response button.

Conditioning Apparatus

Monkeys sat comfortably in restraint chairs placed inside a sound attenuation chamber. There was a response button in front (height 18 inches; 5 inches from monkey's chest), a speaker (height 22 inches), and a copper tube connected to a dish (1 inch from monkey's fingertips) from which to collect reward. A house light provided illumination throughout the training session. A stimulus light remained on during the ITI. LabView software (National Instruments, Austin, TX) controlled lights, sound stimuli, and treat dispenser. To the upper left of the monkey a small video camera allowed observation by the experimenter.

Basic Training Procedure

The DMTS task used approximately 90 stimulus set sounds/day. Training sessions were held 5 days a week, 60 trials/session. The task was designed as a go/no-go task. For match trials the monkey was to respond by pressing the response button releasing a small chocolate candy reward. For nonmatch trials the monkey was not to respond. If the monkey pressed the button after a nonmatch trial they received a 500 ms

air puff reminder not to respond. This mild air puff is applied semi-randomly during normal training after nonmatch errors to discourage incorrect responding.

Variable DMTS

Match and nonmatch trials consisted of a 500 ms sound followed by a pseudo-randomly selected inter stimulus interval (ISI) of 2, 4, 6, 8 or 10 seconds. Then a second 500 ms sound was played, and the response button lit up for 1000 ms. This happened on both match and nonmatch trials as a cue that signaled the possible response time, and did not in any way signal which were match versus nonmatch trials. If the animal did not respond during this time interval, it lost the chance for a reward on that trial, and the ITI of 12 seconds began. Monkeys were trained to a criterion of 80% or better on this variable ISI schedule before the saline and drug doses were administered. These five time delays were chosen because we were expanding the delay period from our previous work and a similar delay length has been used for a cross modal auditory-visual task (Fuster, Bodner, & Kroger, 2000). Other work has shown that when delays are lengthened past 37.5 seconds on a similar auditory task performance starts to rapidly decline (Fritz et al., 2005).

Drug Protocol for Variable DMTS

All monkeys served as within subject controls. After meeting behavioral criterion, they were injected with saline, or 10 µg of scopolamine hydrochloride (salt), (Sigma-Aldrich, St. Louis), per 1 kilogram of weight. The drug dose was selected based on our previous study as well as other studies that have used scopolamine with macaques (Aigner & Mishkin, 1986; Ogura & Aigner, 1993; Plakke et al., 2008). All animals received two sessions with saline and two sessions with 10 µg/ kg scopolamine. All

means reported are the average of those sessions. Drug or saline was administered 30 minutes before the behavioral session. Drug dose sessions were assigned in a semi-random order and counter balanced.

Following the scopolamine and saline drug sessions, the animals were injected with 3.2 µg, 10 µg or 32 µg of physostigmine, eserine salt (Sigma-Aldrich, St. Louis) per 0.5 kilogram of weight, and 15 minute wait time. These 3 doses were selected based on work done in the visual domain (Aigner & Mishkin, 1986). To control for possible order effects these drug sessions were counterbalanced.

Finally, animals were injected with 10 µg scopolamine and at the same time 20 µg physostigmine. After a 30 minute wait time the 60 DMTS trials began. The procedure of administering the drugs at the same time was adopted from Rupniak et al., (1991).

Food Reward Control Test

To examine the effects of scopolamine on the animals' response to food rewards, i.e., motivation, without a memory demand, we compared sessions with saline and a 10 µg/kg dose of scopolamine per 1 kilogram of weight during a food test. During the regular DMTS training the session lasts about 20 minutes and animals work to receive 20-25 rewards. In these food reward test sessions, monkeys were placed in the sound booth (30 minutes after injection) and given one small treat through the pellet dispenser as during regular DMTS, per minute for 20 minutes. The pellet dispenser emits the sound of the solenoid turning on and off to drive the delivery device. There is also the sound of the pellet falling through the copper delivery tube. Monkeys only had to reach for the

reward upon hearing the pellet dispenser release the treat. The control saline injection session with food reward test occurred the day before the scopolamine injection session.

Same Sound DMTS Control Test

To investigate whether the monkeys could pay attention to a simple task that did not require memory within a trial we designed a task that presented sound trials with a repeated white noise stimulus (30) and no sound trials (30). On every sound and no sound trial the lighted response button was briefly lit just as in the variable memory delay DMTS task. Button presses on the sound trials resulted in food reward and button presses during the no sound trials were scored as errors. For sound trials the delay was set at 500 ms but the same white noise sample was used for every stimulus on every trial. Variable ITIs (8000, 10000, 12000 ms) prevented animals from predicting when each trial would start. We compared sessions with saline and a 10 μg/kg dose of scopolamine per 1 kilogram of weight (30 minute wait time).

Low Memory DMTS Control Test

To investigate whether the monkeys were attending to the cues and performing the basic task we shortened the ISI delay to 50 ms. This is an extremely short ISI but still allows for the detection of two separate sounds. Both match (N = 30) and nonmatch (N = 30) trials were presented with the short ISI. The very short delay was so slight virtually no memory demand is present. This concept is similar to some visual paradigms, which present the sample and then leave the sample up while presenting the choice stimulus (Taffe et al., 1999). The trial unique sound stimulus set and ITIs were the same as those used in the variable DMTS task. We compared sessions with saline and a 10 μg/kg dose

of scopolamine per 1 kilogram of weight (30 minute wait time). This design reduces the memory component but still tests whether the monkeys are attending and able to process sound quality beyond the white noise presented in the same sound DMTS.

Description of Analyses

Performance of the animals, measured by percent error (the number of incorrect trials/by the total number of trials; per session), was analyzed. The variable DMTS task was analyzed with a repeated measures analysis of variance (ANOVA) using SPSS 13 software (SPSS, Chicago, IL, USA), one within factor was treatment (saline, 10 µg of scopolamine alone, or 10 µg scopolamine and 20 µg physostigmine administered together) and the other within factor was ISI delay (2, 4, 6, 8, 10 sec). Two separate ANOVAs were used for match and nonmatch trials as response requirements differed. Another ANOVA was run to compare performance on the DMTS task under saline and the three doses of physostigmine. Using within factors, dosage (saline, 3.2 µg , 10 µg , 32 µg physostigmine) and ISI delay (2, 4, 6, 8, 10 sec). Least significant difference (LSD) post-hocs were used if there was a significant main effect. The match latency to respond was also analyzed with repeated measures ANOVAs using the same within factors as above. The *p*-value was set at 0.05. The food reward test, same sound DMTS task, and low memory DMTS task were analyzed with paired *t*-statistics with the *p*-value set at 0.05.

For the some nonmatch latency data we used the Bonferroni procedure, with Keppel's modification, to correct for the "family-wise" error rate among comparisons of *t*-statistics (Keppel, 1982). For the days when scopolamine and both scopolamine and physostigmine were administered, under each delay, the latency to respond under the 3

drug conditions was examined. Thus, 10 pair wise comparisons were conducted to reveal differences between saline and drug conditions. The 3 drug conditions were then entered as the experimental treatment, the number of degrees of freedom for the treatment source of variance (3-1= 2) was multiplied by the standard critical probability level (0.05), and the product was divided by the number of *t*-test comparisons (i.e., 10), yielding the corrected, critical probability level of 0.01.

Results

Variable DMTS Results

Animals met a criterion of 75% or better on average for match and nonmatch trials before beginning drug sessions. Performance for match and nonmatch trials was calculated using the number of incorrect responses divided by the total number of responses of that type and converted to percent error.

Under the influence of saline or physostigmine alone for performance on match and nonmatch trials, there was no significant interaction or effects of dose or delay in percent error, see Figure 6 and 7.

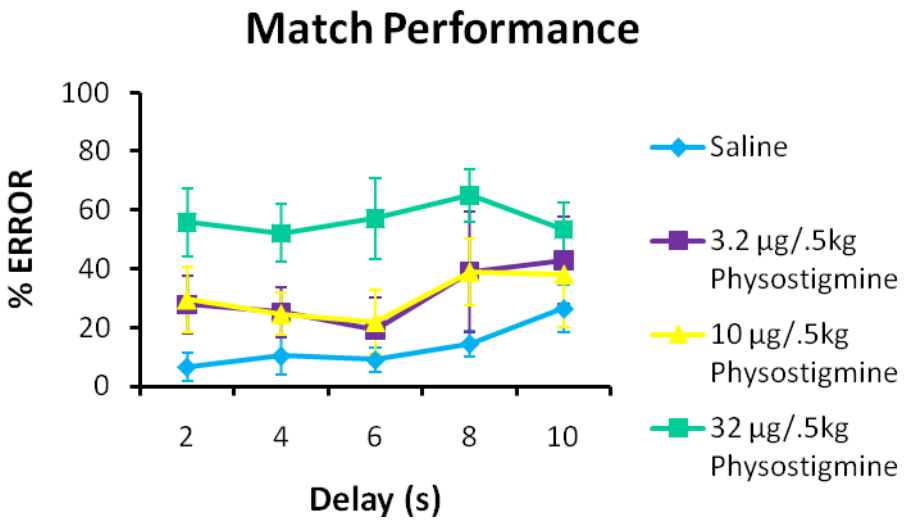

Figure 6. Performance on match trials, under the influence of physostigmine, there were no significant differences compared to saline.

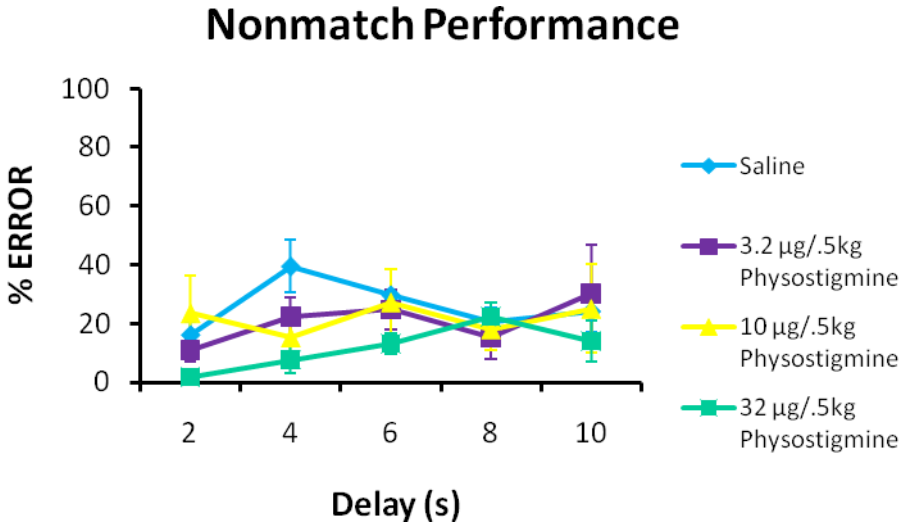

Figure 7. Performance on nonmatch trials, under the influence of physostigmine, there were no significant differences compared to saline.

In addition to overall behavioral performance, we also examined the latency to respond. On match trials responding is a correct response, however on nonmatch trials responding is an incorrect response and considered an error. There were no significant main effects or interactions for latency to respond on match or nonmatch trials see Figure 8 and 9.

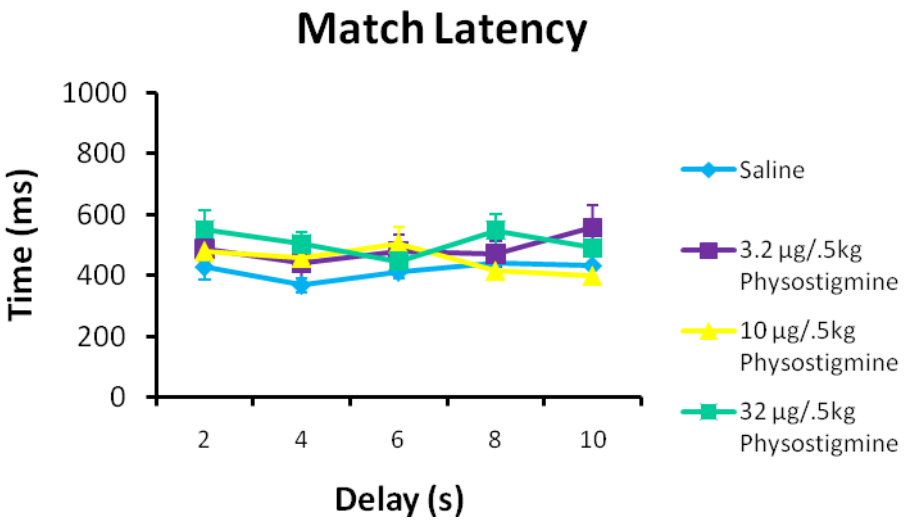

Figure 8. Response latency on match trials, under the influence of physostigmine, there were no significant differences compared to saline.

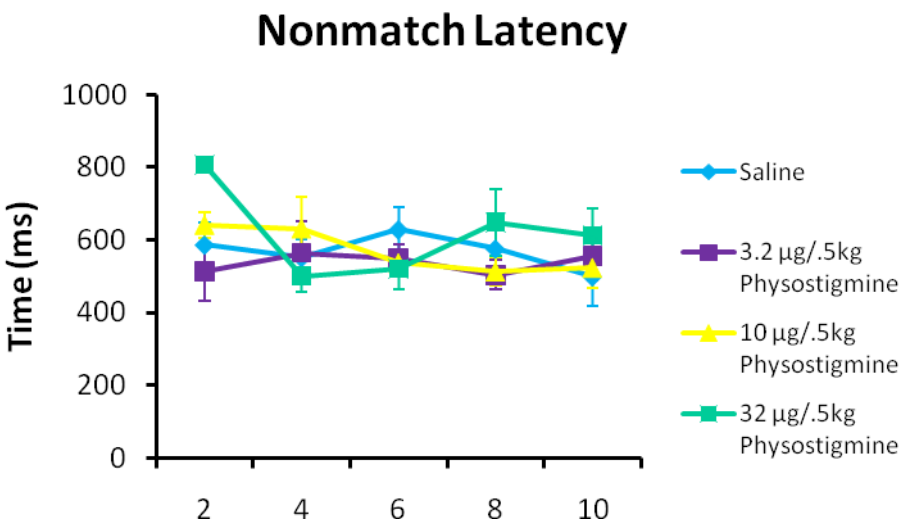

Figure 9. Response latency on nonmatch trials, under the influence of physostigmine, there were no significant differences compared to saline.

Comparing administration of saline, scopolamine alone, or the combination day for performance on match trials there was a significant effect of treatment ($F_{2,3.2} = 20.12$, $p = 0.018$). The LSD post-hoc tests showed that performance on the 10 µg scopolamine alone was impaired compared to performance on saline and when both scopolamine and physostigmine were administered ($p \leq 0.05; \leq 0.05$), see Figure 10. The LSD post-hoc tests also showed that performance on the day when both drugs were administered was impaired compared to performance on saline alone ($p \leq 0.05$). For latency to respond there was no significant differences between saline and drug conditions see Figure 12.

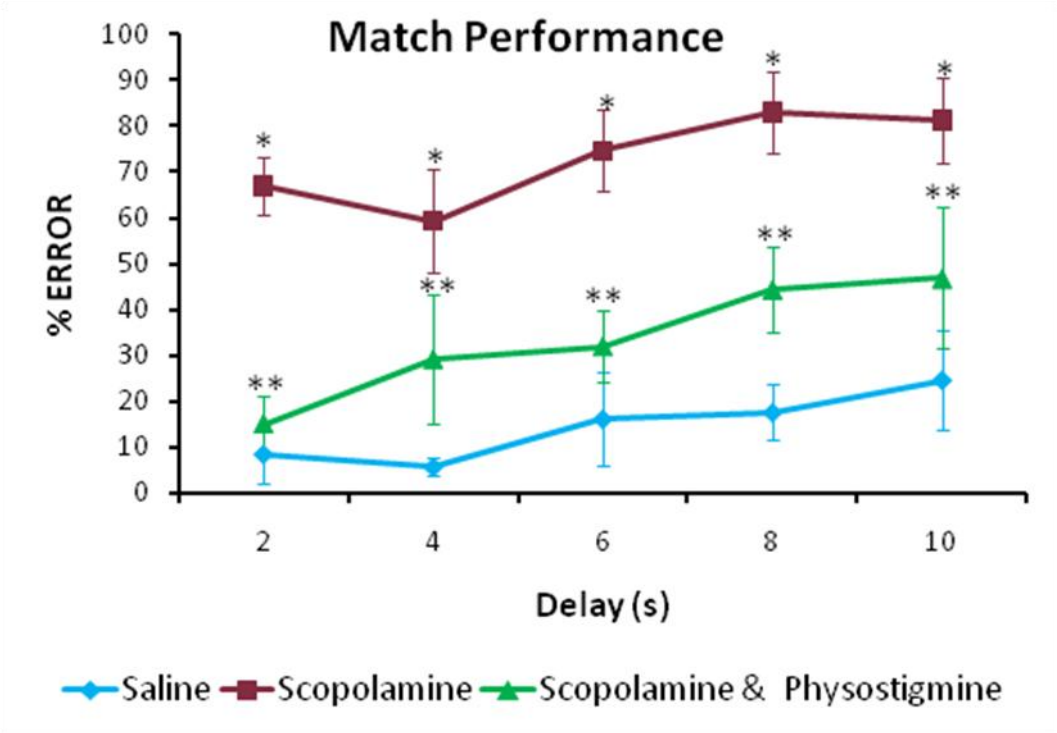

Figure 10. Performance on Match trials, animals were significantly impaired on scopolamine alone compared to performance on saline and when both scopolamine and physostigmine were administered (*$p \leq 0.05$; ≤ 0.05). Performance when both drugs were administered was also significantly different from performance on saline (**$p \leq 0.05$).

For performance on nonmatch trials there was no significant interaction, effect of treatment or delay, Figure 11. Some animals were missing latencies for response errors on nonmatch and an ANOVA was not viable. Instead we used *t*-tests, with a corrected *p*-value (0.01) for multiple comparisons to examine differences between dosages at each delay. There were no significant response latency differences for nonmatch trials, Figure 13.

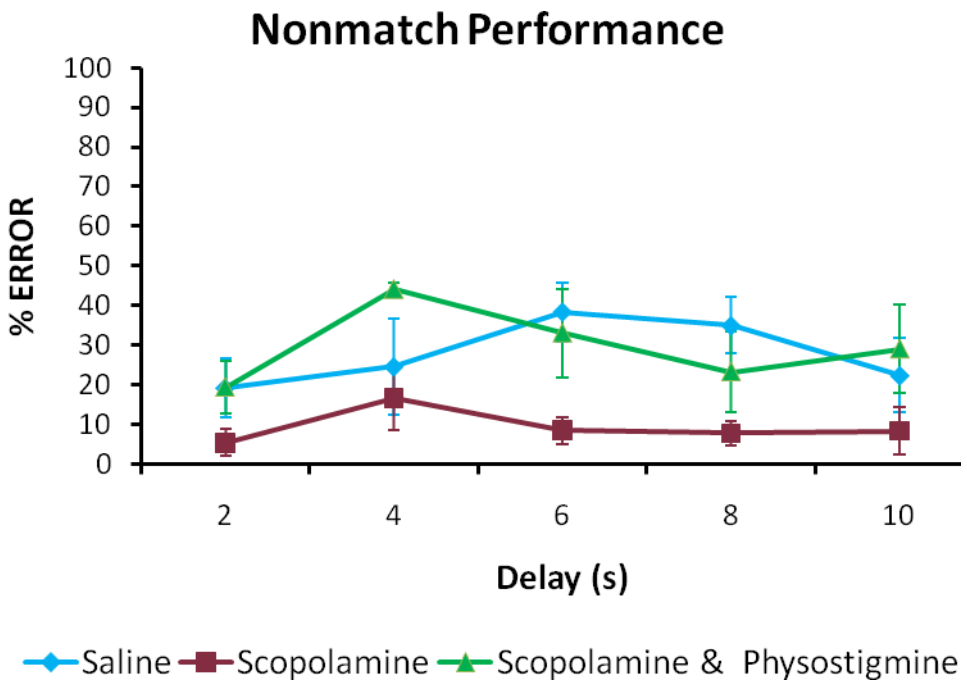

Figure 11. Performance on nonmatch trials under the influence of saline, scopolamine, or scopolamine and physostigmine, there were no significant differences.

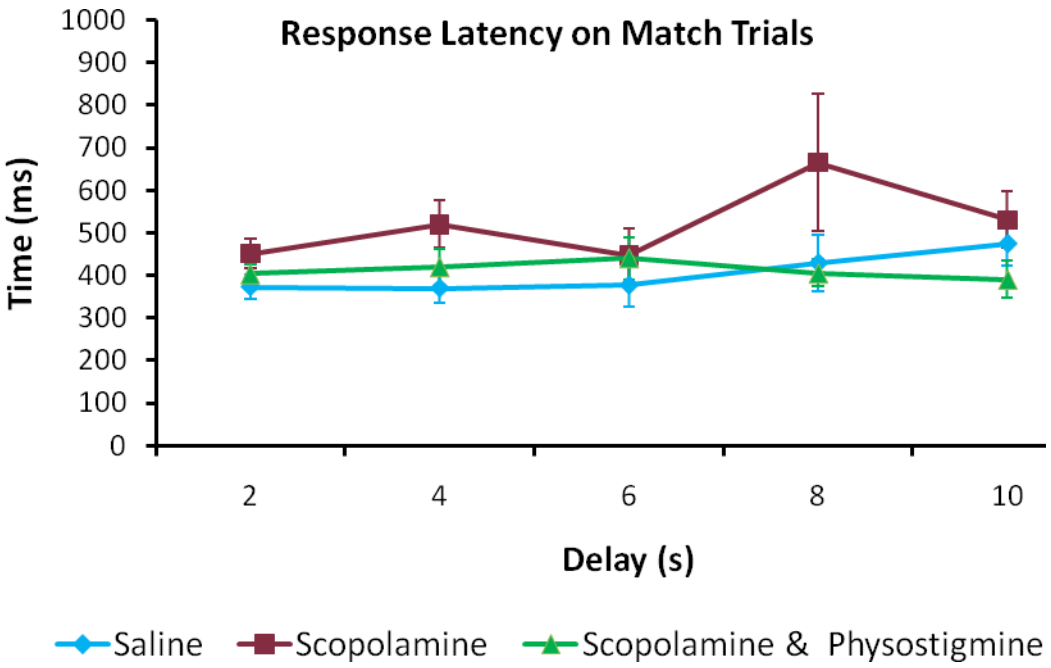

Figure 12. Response latency on match trials, under the influence of scopolamine, or scopolamine and physostigmine, there were no significant differences compared to saline.

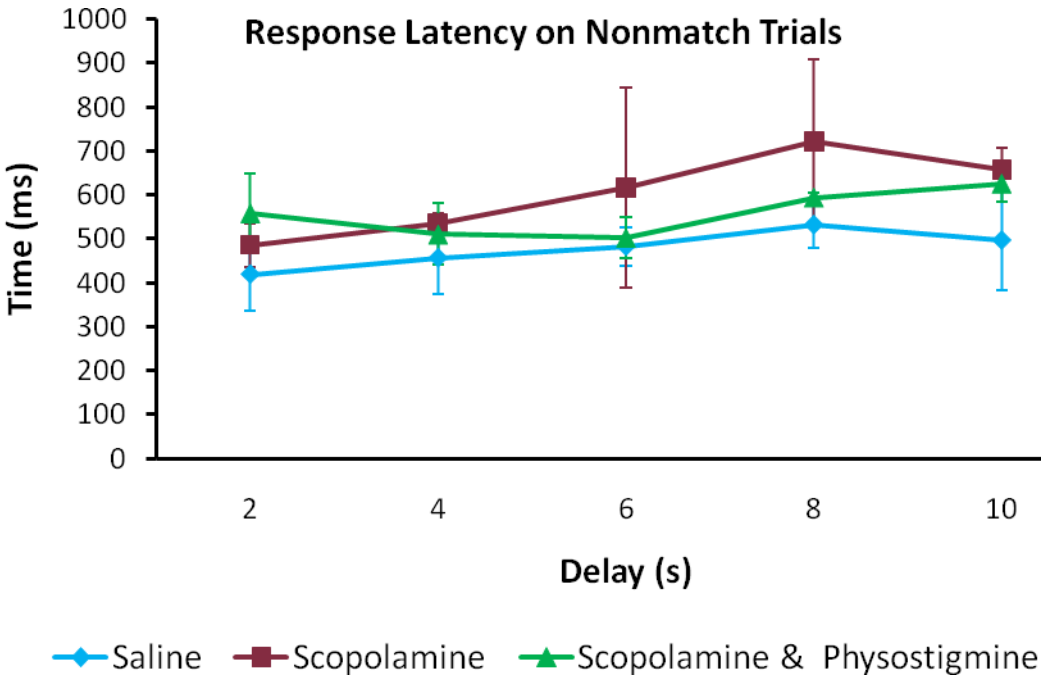

Figure 13. Response latency on nonmatch trials, under the influence of scopolamine, or scopolamine and physostigmine, there were no significant differences compared to saline.

Food Reward Control Test Outcome

During saline sessions for the food reward test monkeys reached for and obtained all 20 treats. During the scopolamine sessions the monkeys obtained on average 17 treats made obtainable throughout the food reward test. There was no significant difference between the number of rewards taken on saline versus scopolamine during the food reward test (*t*-test: $p = 0.09$).

Same Sound DMTS Control Test Outcome

Animals performed well on the same sound DMTS task during both saline and scopolamine conditions. There were no significant differences in performance between

the saline and scopolamine session for sound match trials (*t*-test: $p = 0.14$) nor on the light only trials (*t*-test: $p = 0.15$).

Low Memory DMTS Control Test Outcome

There were no significant differences on low memory demand DMTS task performance for match or nonmatch trials between saline and scopolamine sessions (*t*-test: $p = 0.11$, $p = 0.76$).

Discussion

Blocking muscarinic transmission with scopolamine impaired performance of auditory DMTS, while administration of physostigmine and scopolamine together improved performance compared to performance on scopolamine alone. Administration of physostigmine alone did not significantly change performance.

Similar results have been found in other research for this compound on short-term memory tasks (Penetar & McDonough, 1983; Rupniak, 1991). Improvement of performance has been found in visual DNMTS tasks after administration of physostigmine (Aigner & Mishkin, 1986; Ogura & Aigner, 1993). However, in some cases no improvement of performance was observed or mixed results were observed with some animals improving their performance and others not (Bartus, 1979; Rupniak et al., 1991). This could be due to task demands that differed between studies, or because acetylcholine is not the only neurotransmitter that is important for short-term visual memory.

Our animals were impaired when given scopolamine alone but their performance improved when more ACh was made available to the system. Perhaps, this higher level of ACh allowed for some compensation mechanisms within the auditory cortex or other

cortical areas. The control tasks used here demonstrated that the monkeys could hear sound and respond to matching sounds, food, as well as ignore unnecessary stimuli such as a light. This means the deficit caused by scopolamine alone cannot be solely due to problems with motivation, motor performance, or general attention. The improvement when the agonist was administered could be due to an increased attentional capacity which allowed them to better encode the stimuli, less impairment of memory areas needed for this task (ie., superior temporal gyrus) or a combination of these. In the visual domain, animals given both scopolamine and physostigmine had improved behavior for short-term memory tasks (Hironaka & Ando, 1996). The results seen here in the auditory domain appear to be analogous. Improved performance on the combination of scopolamine and physostigmine compared to performance on scopolamine alone lends support to the conclusion that acetylcholine is important for auditory memory, as it is in visual memory.

Visual short-term memory relies on several brain areas including the frontal lobe, rhinal cortex, perirhinal cortex, parietal lobe, as well as other cortical regions (Champod et al., 2007; Fritz et al., 2005; Hironaka &Ando, 1996; Malkova et al., 2001; Turchi, et al., 2005; Xu & Chun, 2007). It is possible areas such as the prefrontal cortex, which are recruited during short-term memory demands (Artchakov et al., 2007; DePisaia et al., 2007; Fuster, 2000, Miller et al., 1996) may be involved in both the auditory and visual versions of DMTS tasks. The prefrontal cortex also receives cholinergic input and blocking that input directly via infusions of scopolamine impairs visual working memory (Chudasama et al., 2004). The prefrontal cortex and its cholinergic system could be one

area where auditory and visual memory act in a parallel fashion. Thus, the cholinergic system may be used for multiple modalities when short-term memory is required.

Conservation of a neurotransmitter system for a type of particular neural processing is also seen in reward systems where dopamine (DA) is important for multiple types of reward seeking behaviors. Dopamine is an important neurotransmitter for regulating goal-directed behavior, drug addiction, and reward dependent discrimination tasks regardless of the reward type, e.g., food, liquid, or drugs (Di Chiara & Bassareo, 2007; Grace et al., 2007). Perhaps, in the same generalized way dopamine is utilized for reward; the cholinergic system can be used for short-term memory demands.

Recently, others have demonstrated a role for ACh in Pavolvian auditory conditioning, where there is an increase in ACh release in the auditory cortex of rats during learning (Butt et al., 2009). It is possible that ACh needs to be released within the auditory cortex or in other cortical regions for good auditory short-term memory performance. Our monkeys were able to respond to sound only trials and ignore light only trials correctly even when under the influence of scopolamine, so while there overall memory performance was impaired, it is not likely due to an inability to hear or process the sounds on a sensory level. Secondly, while our monkeys performed on the low memory demand control task, their performance was not as good as when given a lower dose of scopolamine in Plakke et al., 2008. Thus, it is possible scopolamine disrupts auditory cortical levels of ACh which could effect the monkey's ability to discriminate distinct sounds, which has been suggested by others (Milar & Dykstra, 1985).

The improvement seen when physostigmine was administered along with scopolamine provides more evidence that the cholingeric system supports auditory short-

term memory, in a comparable fashion as it does for visual short-term memory (Hironaka & Ando, 1996; Myers et al., 2002; Ogura & Aigner, 1993; Penetar & McDonough, 1983; Robbins et al., 1997; Taffe et al., 1999). In humans, administration of physostigmine during visual tasks led to improvements in response speed and better performance for attention than for spatial working memory (Bentley et al., 2004). Although in the current study, physostigmine alone did not improve performance; it is possible that when physostigmine was administered with the scopolamine attention was boosted. This may have allowed the animals to better encode the stimuli and perform better. Many cortical regions receive cholinergic projections and these areas could be involved in both auditory and/or visual short-term memory such as the prefrontal cortex, thalamus, rhinal cortex, and temporal cortex (Fibiger et al., 1991). Future research could focus on local blockades of ACh in areas of interest such as the prefrontal cortex. In addition, the processes involved in short-term memory from encoding stimuli to retrieval could be examined with various techniques to examine the role of ACh in auditory short-term memory and attention.

CHAPTER 4. NEURAL CORRELATES OF AREA 46 DURING AUDITORY WORKING MEMORY

The lateral prefrontal cortex (lPFC) has been associated with working and short-term memory function through a variety of techniques. Lesions studies of the lPFC, especially area 46, lead to clear impairment on visual working memory tasks (Goldman-Rakic & Rosvold, 1970). Electrophysiological studies have found that cells in lPFC are responsive to an array of visual stimulus categories; both for visual objects and visual location information across the delay time in working memory tasks (Fuster, 1973; Kojima & Goldman-Rakic, 1982; Pasupathy & Miller, 2005; Rao et al., 1997; Warden & Miller, 2007). There is also some electrophysiological evidence of auditory responsive cells within the lPFC (Bodner et al., 1996; Romanski & Goldman-Rakic, 2002). In addition, a PET study also found activation in the left ventral bank of the PS during this same DMTS task, see Figure 14 (Poremba et al., 2000). Thus, lPFC is critical for visual working memory and has auditory responsive neurons, how is this region involved in an auditory working memory task?

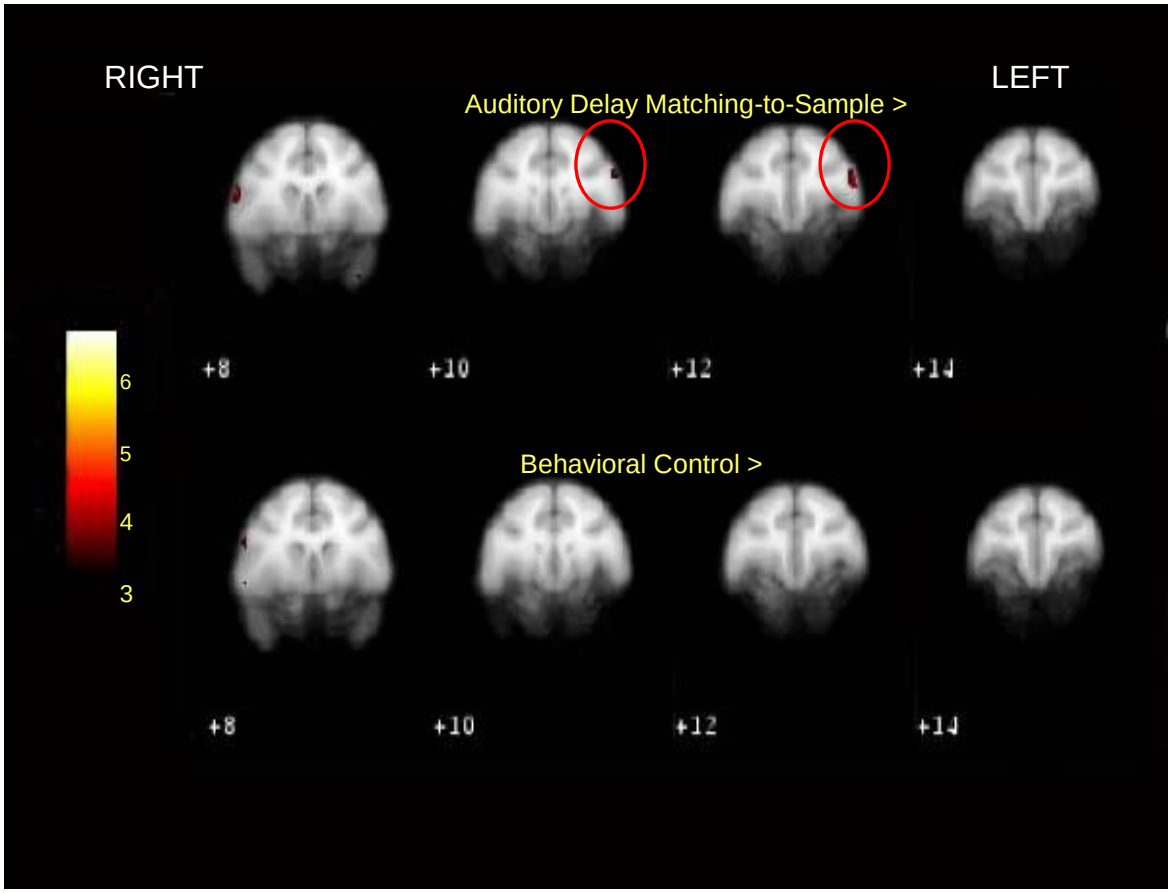

Figure 14. Adapted from Poremba et al., 2000. The figure shows images from PET scanning. The top row shows activity in PFC cortex during an auditory DMTS task above and beyond the activity found in the sound only control task. The bottom row shows activity from a behavioral response control task above and beyond activity found in the sound only control condition. The highlighted activity in the red circles on the top row shows activity related to the DMTS task demands, which is not present in the bottom panel, demonstrating that the uptake is not due to motor responses but to cognitive processes related to task demand. The image is reversed so the left hemisphere is on the right side.

The lPFC including area 46 receives projections important for conveying complex auditory sounds, from the anterolateral, medial lateral, and caudolateral parabelt areas of the superior temporal region (Romanski et al., 1999b). Passive listening activates the lPFC, and cells here respond to auditory clicks, tones, and monkey vocalizations (Newman & Lindsley, 1976; Poremba et al., 2003; Poremba et al., 2000). Spatial auditory memory cells have also been found within the lPFC, utilizing simple tones and during an oculomotor task (Kikuchi-Yorioka & Sawaguchi, 2000; Watanabe, 1992). However, there are few studies that have examined this brain area using auditory stimuli with a memory delay. Bodner et al. (1996) used auditory stimuli, but it was not with complex sounds and the auditory stimuli were paired with a visual stimulus. Thus, the animals learned to pair associated cues so that when they heard the tone, after the delay, they responded to the correct matched color, meaning the memory may have been stored visually or through a rule such as high tone means choose red (Bodner et al., 1996).

By utilizing a DMTS task with complex auditory stimuli, and recording from within area 46, we can examine how this brain region contributes to an auditory memory task. We hypothesize that auditory memory cells, (a cell which increases its firing rate over the delay period, compared to baseline firing rate) will be found in our DMTS task within this region. The lPFC is associated with more activation in tasks requiring working memory demands and lesions of the dlPFC impair visual working memory tasks (D'Esposito et al., 2000; Goldman-Rakic, 1987; Goldman & Rosvold, 1970). Consequently, for this study we hypothesize that some cells will have more activation during the memory delay. In addition, we hypothesize some cells will be responsive to a particular sound stimulus (e.g. coo vs. dog bark), and that cells will be responsive to other

events within the task, such as during the presentation of auditory cues, and when the monkey receives a reward, similar to activity found in visual working memory (Hikosaka & Watanabe, 2000; Miller, 2000).

Cell activity to sounds will be analyzed during passive listening as well. Cell activity during passive listening will be compared to activity during the DMTS task for the same sound exemplar in addition to other simple and complex sounds. This provides an opportunity to examine how a specific cell responds to a particular stimulus between different behavioral contexts. It has been demonstrated with visual stimuli that some cells within lPFC respond to the same visual cue in one behavioral context, but not in another task context (Asaad et al., 2000). In addition, cells will be examined to determine if the sounds that individual cells are responsive to are grouped by sound type, or by possible acoustic properties such as the harmonic-to-noise ratio or spectral mean.

One sound responsive region within the lPFC is located near the inferior prefrontal dimple and covers areas 45 and 12 (Romanski et al., 2005; Romanski & Averbeck, 2009; Romanski & Goldman-Rakic, 2002). Neurons in this region are particularly responsive to monkey vocalizations (Romanski & Averbeck, 2009). Finding sound responsive neurons within area 46 could provide evidence for another auditory area within lPFC and verify the functional imaging results of Poremba et al. (2003). It is hypothesized that by using multiple sound types beyond pure tones, monkey and human vocalizations more sound responsive neurons could be found.

Methods

Subjects

Two rhesus monkeys (*Macaca mulatta*), 1 female and 1 male (11 to 12 years old; 5-10 kg), were born and raised in captivity, and housed in Spence Laboratories at the University of Iowa (12-hr light/ dark cycle). Monkeys were fed standard monkey chow (Harlan Teklad Global Diet, Madison, WI, USA) with fresh fruit and vegetables. The majority of food was given after training each day. Water was provided ad libitum in the home cage with all animals given environmental enrichment. Each animal's weight was maintained above 85% of starting weight and adjusted upwards based on age. The Institutional Animal Care and Use Committee at the University of Iowa approved all procedures.

Sound Stimulus Selection

Sounds presented to monkeys included tones, music, human voices, monkey calls, other animal calls (e.g. dog bark), and manmade sounds (e.g. cars, train whistles or airplanes), totaling over 1,000 sounds. The sounds were played for both passive listening and the DMTS task. For passive listening, a subset set of sounds was played in blocks of sounds with one sound from each sound type; monkey vocalizations, human vocalization, animal, natural, synthesized, white noise, pure tones, and music represented in each block. Within each block of sounds an individual sound was repeated at least 8 times in a pseudorandom fashion. Sounds used for the DMTS task were one block of the passive listening sounds, pseudorandom selected for each day, with a new block used for each day of training. When all the blocks were used then they were repeated in a

pseudorandom fashion for training. Each sound for the DMTS task was played in both positions of the match and nonmatch trials and repeated at least 8 times in each position.

Behavioral Task- Auditory Delayed Match-to-Sample

Monkeys for this experimental chapter continued to be trained in the same go/no-go DMTS task as used in Chapters 2 and 3 of this thesis. Conditioning apparatuses were also the same as in Chapters 2 and 3 of this thesis. Training sessions were held 5 days a week and recording sessions occurred 3-5 days a week. On recording days the animal underwent 200 trials/session. For match trials the monkey was to respond by pressing the response button releasing a small chocolate candy reward. For nonmatch trials the monkey was not to respond. If the monkey pressed the button after a nonmatch trial they received a 500 ms air puff reminder not to respond (a mild punishment). The mild air puff was applied semi-randomly during training after nonmatch errors to discourage incorrect responding. If the air puff did not deter over-responding then a training delay punishment of 10-30 seconds was given after the incorrect button press.

Surgery for Headwell Implantation and Headwell Maintenance

MRI (magnetic resonance imaging) images were used to help determine stereotaxic coordinates for headwell placement, (Figure 15). The surgery was performed at the National Institutes of Mental Health by Amy Poremba and Richard Saunders. The monkey was placed under general anesthesia and surgery was conducted under aseptic conditions. All procedures were approved by the University of Iowa Committee on Animal Research guidelines and met NIH standards. The monkey was held in place with

an aluminum and plastic stereotaxic apparatus. A recording chamber and headpost were implanted and held in place with dental acrylic and small screws placed into the bone. The recording chamber was placed tangential to the cortical surface, centered over the principal sulcus. The headpost is a small stainless steel piece that allows the head to be held in a fixed position; it is attached with titanium screws and dental acrylic. After surgery the animal was given antibiotics and analgesics and received daily cleanings around the headpost and recording chamber to protect against infection, topical antibiotics were administered as needed. Before the initial recordings took place, the bone left within the recording chamber was removed to expose the dura mater. Cleaning of headwell chambers occurred at least twice a week but always occurred before and after each recording session.

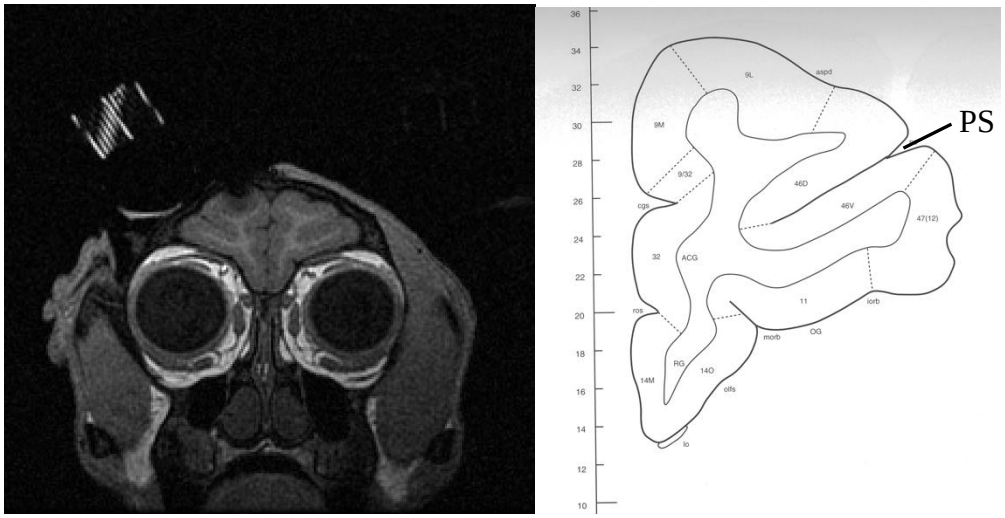

Figure 15. MRI of animal B. The recording chamber can be seen on the top left. Next to the MRI is an atlas picture at 15.93 mm from Bregma, from where the MRI slice was taken (Paxinos et al., 2000). PS = Principal sulcus; 46 = Area 46

Recording Day Protocol

On recording days animals were chaired with their head held in a fixed position, their headwell chamber was cleaned, and the electrodes were lowered. Once a cell was isolated, the monkey underwent one block of passive listening. Those specific sound stimuli from the same passive listening set were used as the sound stimuli for 200 trials of the DMTS task, followed by a block of passive listening. Upon task completion the electrodes were raised and the headwell was cleaned again.

Electrophysiological recording

Recordings were completed with sterilized, insulated tungsten microelectrodes, 1-3MΩ, (FHC Instruments, Bowdoin, ME) and stainless steel guide tubes (23 gauge) held through a grid. The electrodes were advanced and held by an electrode positioner attached to a micromanipulator (Nan Instruments, Nazareth, Israel). As the electrode was lowered and a cell was found, the individual spike wave forms were discriminated with the box sorting method and analyzed by an online-spike sorting system (Plexon Systems, Dallas, TX). Spike wave data was stored for further analysis offline. The sampling rate was 40kHz, the filter was a low cut two pole (250Hz) on the signal board of the Plexon system. Gains ranged from 12,000 to 30,000 per electrode. The software boxes select particular waveforms which can be used later for template sorting. The template sort set option allowed sorting with tolerance values and used waveforms from the collected set of waveforms and principal components were used to cut as well.

Data analysis

Behavior on the task was analyzed by examining the percent correct for both match and nonmatch trials. Response latency for each animal was computed and analyzed with a *t*-test ($p < 0.05$) to examine if the latency to respond on correct match button presses were different from incorrect nonmatch button presses (the two trial types where a response occurs). An on-line LabView program (National Instruments, software) tracked the events of the task including the stimulus presentations, the delivery of reward, and the latency of the response time. Electrode placement was estimated with the MRI, stereotaxic coordinates, recording grid and depth of the electrode. The headwell was centered over the principal sulcus to ensure the recordings were from area 46.

For neuronal data, the signal was sent from the electrode through a pre-amp and amplifier, and recorded by software (Plexon Systems, Dallas, TX). Individual waveforms were isolated by a window discriminator system in the software and recorded with storage for further offline analysis. The recorded neuronal data was further analyzed with template matching software to sort the waveforms into single-units (Offline Sorter, Plexon Systems, Dallas, TX). The spike sorting system (Plexon Systems, Dallas, TX) also tracked the timeline of events such as stimulus onset and offset, delay time and behavioral response. Trials were excluded for a number of reasons including noise, poor behavioral performance, if a cell was lost, or if there was a cue malfunction. A minimum of 10 trials were used for any trial type analysis. Single-unit mean firing rates were analyzed by trial type (correct match, correct nonmatch, incorrect match, incorrect nonmatch), with one-way ANOVAs, (Tukey post-hoc, $p < 0.05$) to examine event related activity compared to baseline (BS) (baseline = 500 ms before cue 1 onset, part of the

inter-trial-interval). Separate ANOVAs were run for sets of events compared to BS; (cue 1, cue 1 offset, cue 2, cue 2 offset, each 500 ms); (delay, divided into 9 equal 500 ms epochs) and (wait time, 500 ms and response time divided into 3 equal epochs (500 ms). After we began recording we added the wait time, where immediately after cue 2 presentations the animal had to wait for 1 s before being allowed to respond, consequently we do not have the wait time data for some cells (N = 26).

A secondary analysis was performed for cells that had a change in firing rate on correct match or incorrect nonmatch trials during the wait or response periods. Spike data for the trial types where the monkey button pressed were aligned with the button press and three epochs were compared to the original baseline: (Pre-Response 1) the first 500 ms immediately preceding the button press, (Post Response 1) during the button press and the following time up to 500 ms and (Post Response 2) the second 500 ms after button press. Histograms of the single-cell spike densities were created using 100 ms bins. Trials were averaged to generate trial type histograms (NeuroExplorer, Nex Technologies, Littleton, MA).

To examine if there was stimulus selectivity during the behavioral task, individual sounds were assessed with a one-way ANOVA for each sound, analyzed from the cue 1 position across all trials for comparison to the same sounds when played during passive listening blocks. The first cue presentation was selected for comparison to the passive listening sounds, as it was deemed the purest comparison. The second sound presentation was not selected for comparison as it could be when the animal is making the decision about whether it is a matching or nonmatching sound and the animal may start planning a choice response. Visual selectivity is also frequently accomplished by assessing cue 1

activity (Asaad et al., 2000; Asaad et al., 1998; Freedman et al., 2002; Freedman et al., 2001; Nieder et al., 2002).

Changes across the population were analyzed with individual cell spike activity sampled at 10 ms bins normalized by taking the baseline mean (500 ms epoch before cue 1 onset) and calculating the standard deviation from the baseline. Activity values for each event, were standardized [all the same events as for the single-unit data (all 500 ms epochs)] by subtracting the baseline mean, and then dividing by the standard deviation of the baseline period. The resultant standardized scores were then used for statistical analysis. Cells with correct trials (N = 215) and a sub population of cells with incorrect nonmatch trials (N = 154) were examined with three repeated measures ANOVAs in order to assess event evoked activity by trial type (Least Significant Difference (LSD) post hoc, $p < 0.05$). The means for each event were compared to baseline for these sets of events: (1) baseline, cue 1, cue 1 offset, cue 2, cue 2 offset), (2) baseline, delay 1-9, each epoch 500 ms) and (3) baseline, wait time, and the three response epochs (each 500 ms). To examine possible changes between trial types a repeated measures ANOVA was run for the delay epochs (9 epochs each 500 ms) and for the three response periods (each epoch 500 ms). Individual events (cue 1, cue2, cue2 offset, wait time), which may have contributed to encoding or possibly related to choice between match/nonmatch stimuli were examined with a repeated measures ANOVA examining trial type x interval (5, 100-ms intervals) with a between measures factor of cell. Cue 1 and cue 2 (as event) were also examined by trial type with a repeated measures ANOVA for both populations of cells. A difference score between cue 2 and cue 1 was also calculated for correct trials

a repeated measures ANOVA with factors of trial type by interval (5, 100 ms intervals) was computed.

For the sounds presented during passive listening, (a minimum of 9 sound presentations were played) the baseline was set as 500 ms before cue onset and the mean firing rate for cue presentation was assessed with a one-way ANOVA, (Tukey post-hoc, $p < 0.05$) for each sound. T-tests were used with Keppel's correction for family wise error in order to examine if the number of cells that responded to one sound type (animal vs. human) were significantly different. The 8 sound types were entered as the experimental treatment (8-1) = 7 which was multiplied by the critical value (0.05), the product was then divided by the number of planned comparisons (36) yielding a critical value of $p = .009$. During the second passive listening block the monkeys heard 12 sounds from 8 different sound types (animal vocalizations, human vocalizations, monkey vocalizations, music clips, natural sounds, pure tones, synthetic sounds and white noise). It is possible cells could fire to sounds of one sound type (e.g. to monkey vocalizations) than to another sound type. However, cells could also respond to sounds that are similar based on an acoustic property. To account for this possibility we calculated the harmonics-to-noise ratio, which can be used as an indicator of sound quality against noise, or how much acoustic energy of a signal was devoted to harmonics over time, relative to the remaining noise (i.e., representing nonharmonic, irregular or chaotic acoustic energy). The harmonics-to-noise ratio [HNR, expressed in decibels (dB)], and the degree of acoustic periodicity, were generated for each sound sample, as used in a previous study (Ng et al., 2009). The HNR algorithm determined the degree of periodicity of a sound,

x(t), based on finding a maximum autocorrelation, $\acute{r}_x(\tau_{max})$, of the signal at a time lag (T) greater than zero.

$$HNR(dB) = 10*\log_{10}[\{\acute{r}_x(\tau_{max})\}/\{1-\acute{r}_x(\tau_{max})\}]$$

Furthermore, we assessed the spectral mean of a sound. Since sound stimuli contained rapid, changing frequencies at different energy levels; frequency and intensity were taken into account for acoustic analysis. All calculations were done in MATLAB (The MathWorks, Natick, MA) programming software. First we calculated the two-dimensional Fourier transform of each sound. Sound amplitudes at each frequency were then converted to power in scales of decibels. The spectral mean of a sound stimulus was defined as the weighted mean of frequency across intensity, as follows:

$$\text{Spectral mean} = \sum(f_i \times P_i) / \sum(P_i) \quad i: \text{frequency level at 1–Hz increments}$$

Then for each cell that was responsive to sound stimuli, we compared those sound's HNR and spectral mean values, to determine if a cell was responsive to particular sound stimuli based on these acoustic properties.

Histograms of the single-unit spike densities were also created. A number of trials were averaged to generate a histogram to match a particular time event such as the onset of the stimulus, the onset of the delay period, or the onset of the behavioral response. Raster plots were also created (NeuroExplorer, Nex Technologies, Littleton, MA).

Results

DMTS Task

During recording sessions the overall average behavioral performance was 74% correct on match trials and 72% correct for nonmatch trials. Monkey A never vocalized while performing the task, and monkey B vocalized an average 10 times per session, usually at the start of the behavioral task or during the inter-trial-interval. Monkey A used the right hand to button press for greater than 99% of the time, using the left hand for 1 out every 200 button presses on average. Monkey B exclusively used the left hand to button press. Average response latency for monkey A was 171 ms for correct match trials and 237 ms for incorrect nonmatch trials, for monkey B it was 215 ms for correct match trials and 242 ms for incorrect nonmatch trials. There was a significant difference in response latency for monkey A, where he was faster to respond on correct trials vs. incorrect trials.

A total of 220 cells were recorded from (136 from Monkey A, 84 from Monkey B). A majority of the cells were responsive to at least one portion of the DMTS task (88%) and were considered task related. For the following section all percentages are out of 215 cells except where noted. Five cells were analyzed for passive listening but were not included in the behavioral DMTS memory task analysis due to poor behavioral performance.

Correct Trials

This section reports how cells responded to events within the task for performance on correct match or correct nonmatch trials, all percentages are out of 215 cells unless noted. During cue 1 presentations within correct match trials, 15% of cells had a change

in firing rate, whereas during cue 2 presentations 24% had a change in firing rate (Table 1). During cue 1 presentations within correct nonmatch trials 16% of cells had a change in firing rate, with 21% changing during cue 2 presentations (Table 1). See Figures 16, 17, and 18 for an example of cells with cue 1 and cue 2 activity. Classifications of cells within tables are not exclusive.

Table 1. Percent of cells with change in firing rate (FR) during cue events for correct match (CM) and correct nonmatch (CNM) trials (N = 215 cells).

		Cue 1	Cue 1 offset	Cue 2	Cue 2 offset
CM	↓ in FR	5	8	10	12
	↑ in FR	10	7	14	16
	Total change:	**15**	**14**	**24**	**28**
CNM	↓ in FR	8	7	9	9
	↑ in FR	8	6	12	8
	Total change:	**16**	**13**	**21**	**17**

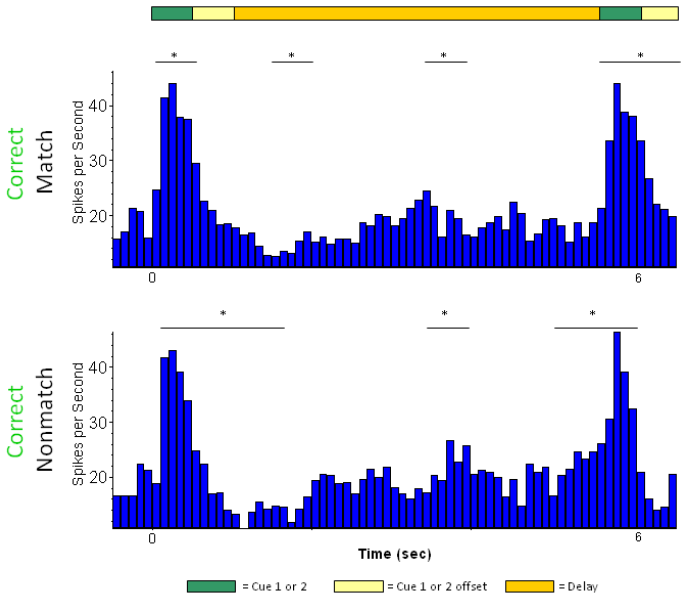

Figure 16. An example cell responsive to both cue1 and cue 2 during correct match and correct nonmatch trials. The green bars represent when the cue presentations occurred. This cell demonstrates significant delay activity as well. Bin = 100 ms * = signficant change in firing rate from baseline, is same for all figures.

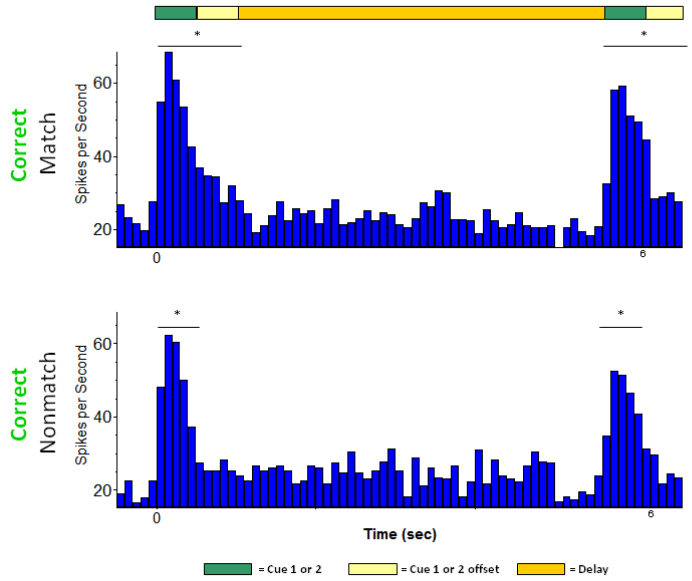

Figure 17. Another example cell responsive to both cue 1 and cue 2 during correct match and correct nonmatch trials. Bin = 100 ms.

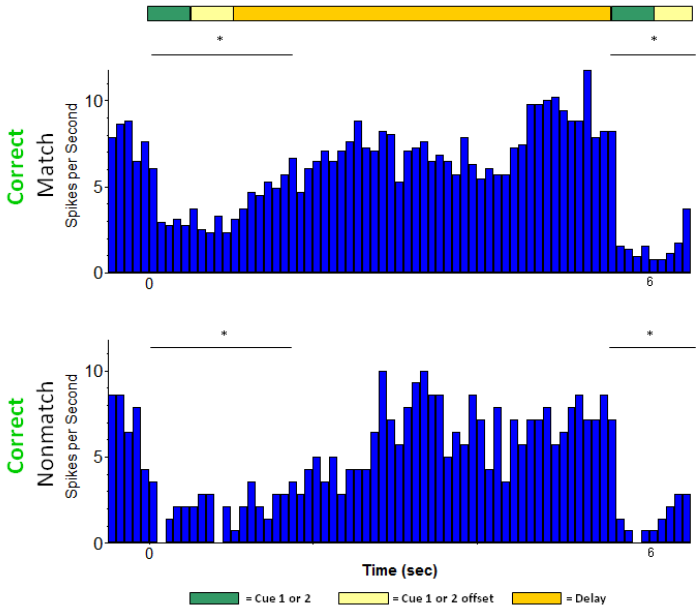

Figure 18. An example cell with a decrease in FR to both cue 1 and cue 2 during correct match and correct nonmatch trials. Bin = 100 ms.

During the delay on correct match trials 3-17% of cells had a change in firing rate for the early, middle, and late portions. During the delay on correct nonmatch trials 7-14% of cells had a change in firing rate for the early, middle and late portions (each portion three 500 ms epochs, Table 2). For example cells with early and late delay activity see Figures 19 and 20. See Figure 21 for an example cell with intermittent delay activity.

Table 2. Percent of cells with a change in firing rate (FR) during the delay for correct match (CM) and correct nonmatch (CNM) trials (N = 215 cells).

		Early	Middle	Late
CM	↓ in FR	6	8	8
	↑ in FR	7	5	8
	Total change:	**13**	**13**	**16**
CNM	↓ in FR	5	2	5
	↑ in FR	8	5	7
	Total change:	**13**	**7**	**12**

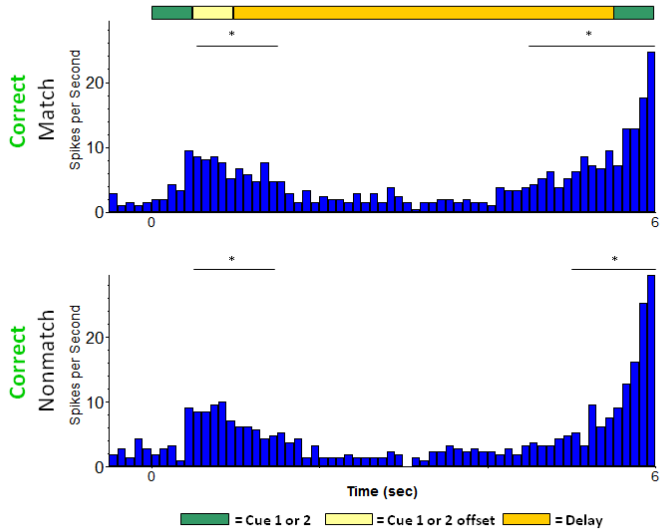

Figure 19. An example cell responsive during the early and late portions of the delay for correct match and correct nonmatch trials. Bin = 100 ms.

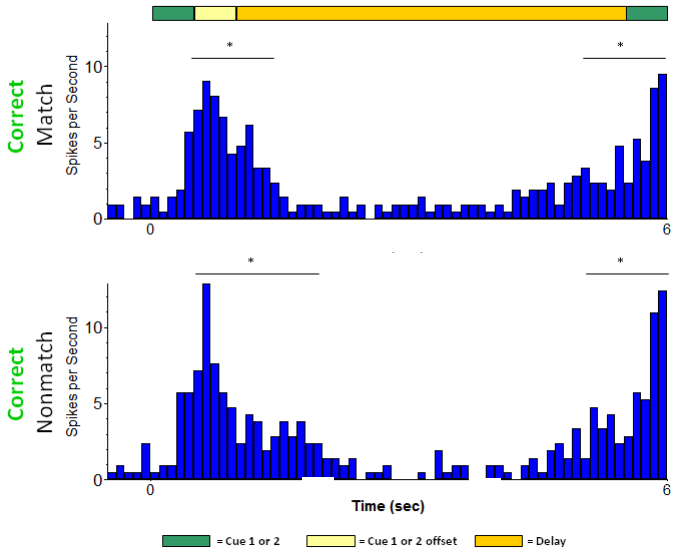

Figure 20. An example cell responsive during the early and late portions of the delay for correct match and correct nonmatch trials. Bin = 100 ms.

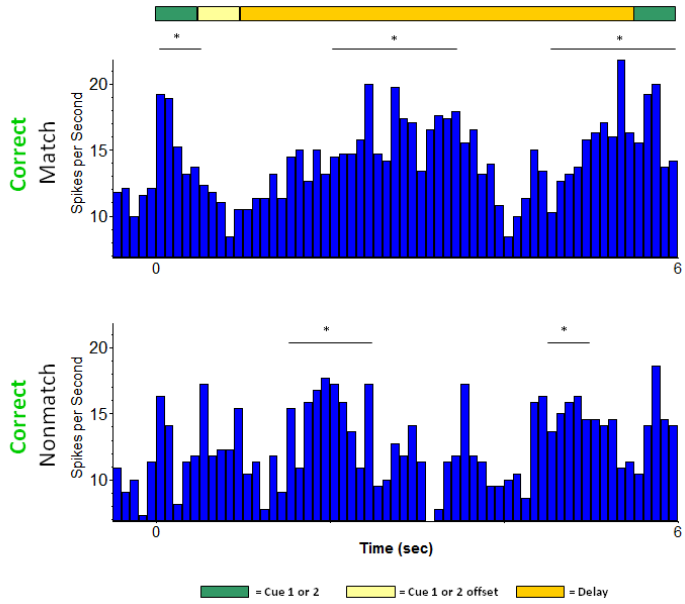

Figure 21. An example cell responsive intermittently throughout the delay for correct match and correct nonmatch trials. Bin = 100 ms.

During the wait time for correct match trials, 41% of cells had a change in firing rate, and for correct nonmatch trials only 19% of cells had a change in firing rate (out of 189 cells, Table 3). Averaged across the response periods, 37% of cells had a change in firing rate for correct match trials. Averaged across the response periods on correct nonmatch trials, 12% of cells had a change in firing rate (N = 215 cells, see Table 4). See Figures 22-24 for example cells that were responsive during the wait time and response periods.

Table 3. Percent of cells with change in firing rate (FR) during the wait time for correct match (CM) and correct nonmatch (CNM) trials (N = 189 cells).

		Wait time
CM	**↓ in FR**	19
	↑ in FR	22
	Total change:	**41**
CNM	**↓ in FR**	6
	↑ in FR	13
	Total change:	**19**

Table 4. Percent of cells with change in firing rate (FR) during the response periods (R1, R2, R3,each 500 ms) for correct match (CM) and correct nonmatch (CNM) trials (N = 215 cells).

		R1	R2	R3
CM	↓ in FR	14	20	17
	↑ in FR	27	18	15
	Total change:	**41**	**38**	**33**
CNM	↓ in FR	7	6	2
	↑ in FR	8	7	6
	Total change:	**15**	**13**	**8**

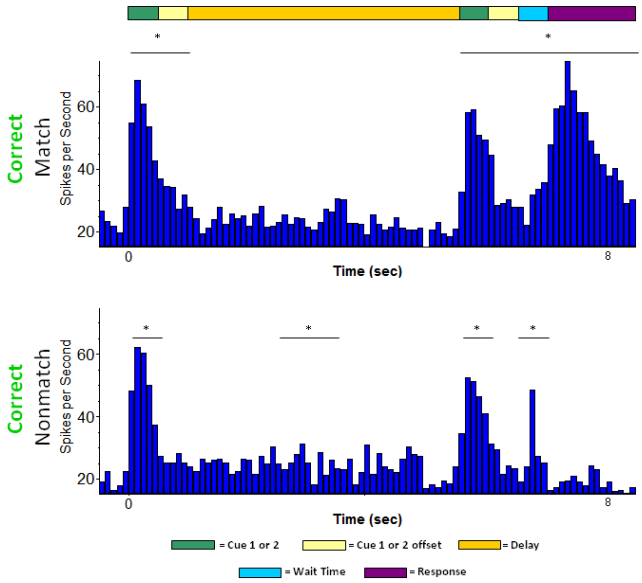

Figure 22. An example cell responsive during the wait time for correct match and correct nonmatch trials, and responsive during the response period for correct match trials. Bin = 100 ms.

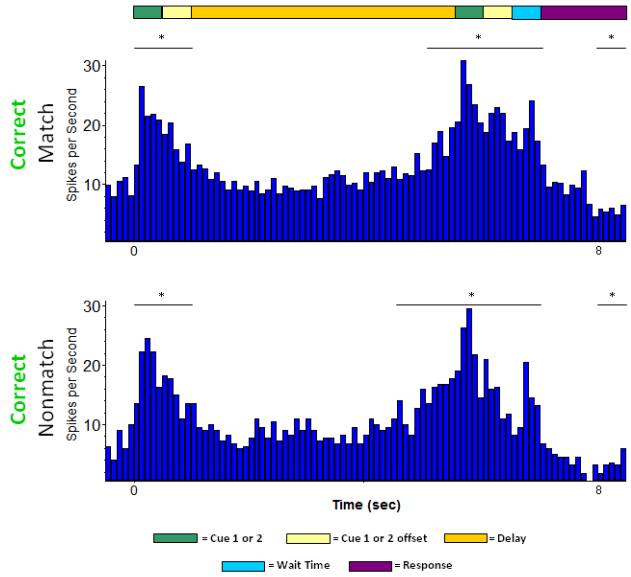

Figure 23. An example cell with increased firing rate during the wait time and decreased firing rate during response periods for correct match and correct nonmatch trials. Bin = 100 ms.

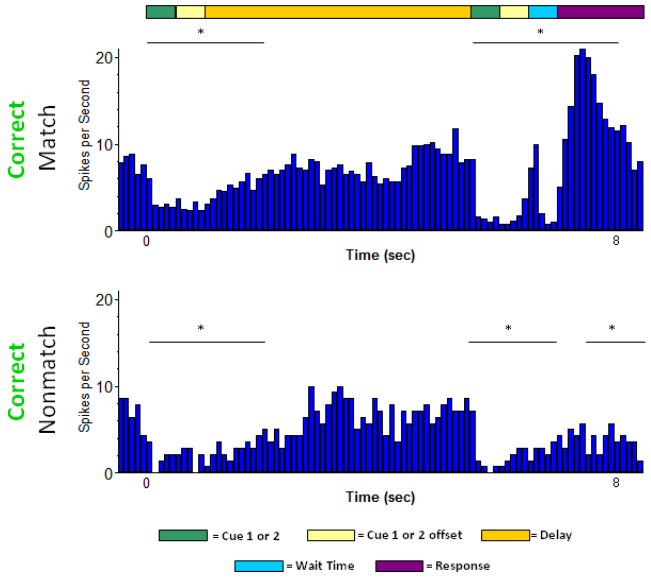

Figure 24. An example cell responsive during the wait time and response periods for correct match and correct nonmatch trials. Bin = 100 ms.

Incorrect trials

This section reports the firing rates of cells during incorrect behavioral performance which include incorrect match trials (N = 53 cells) and incorrect nonmatch trials (N = 154 cells). During cue 1 presentations on incorrect match trials, 12% of cells had a change in firing rate, and during cue 2 presentations 12% of cells had a change in firing rate (Table 5), compared to 15% during cue 1 and 24% during cue 2 for correct match trials (Table 1). During cue 1 presentations on incorrect nonmatch trials 13% of cells had a change in firing rate, and during cue 2 presentations 18% of cells had a change in firing rate (Table 5), compared to 16% during cue 1 and 21% during cue 2 for correct nonmatch trials (Table 1).

Table 5. Percent of cells with change in firing rate (FR) during cue events for incorrect match (IM); (N = 53 cells) and incorrect nonmatch (INM) trials (N = 154 cells).

		Cue 1	Cue 1 offset	Cue 2	Cue 2 offset
IM	↓ in FR	8	13	4	13
	↑ in FR	4	0	8	4
	Total change:	**12**	**13**	**12**	**17**
INM	↓ in FR	6	7	7	6
	↑ in FR	7	6	11	12
	Total change:	**13**	**13**	**18**	**19**

During the delay for incorrect match trials, 8-11% of cells had a change in firing rate for the early, middle, and late portions (Table 10), compared to 13-16% during correct match trials (Table 2). During the delay for incorrect nonmatch trials 1-8% of

cells had a change in firing rate for the early, middle, and late portions (Table 6), compared to 7-13% during correct nonmatch trials (Table 2).

Table 6. Percent of cells with change in firing rate (FR) during the delay for incorrect match (IM); (N = 53 cells) and incorrect nonmatch (INM) trials (N = 154 cells).

		Early	Middle	Late
IM	↓ in FR	9	8	6
	↑ in FR	2	2	2
	Total change:	**11**	**10**	**8**
INM	↓ in FR	4	4	4
	↑ in FR	7	4	2
	Total change:	**11**	**8**	**6**

During the wait time for incorrect match trials, 8% of cells had a change in firing rate; and for incorrect nonmatch trials, 25% of cells had a change in firing rate (Table 7) compared to 41% on correct match and 19% on correct nonmatch trials. On average across the three epochs where a response could occur on incorrect match trials, 12% of cells had a change in firing rate for the three epochs (Table 8), compared to 37% of cells on correct match trials (Table 4). On average across the three epochs where a response could occur on incorrect nonmatch trials, 18% of cells had a change in firing rate (Table 8), compared to 12% on correct nonmatch trials. For example cells see Figures 25 and 26.

Table 7. Percent of cells with change in firing rate (FR) during the wait time for incorrect match (IM); (N = 28 cells) and incorrect nonmatch (INM) trials (N = 130 cells).

		Wait time
IM	↓ in FR	4
	↑ in FR	4
	Total change:	**8**
INM	↓ in FR	8
	↑ in FR	17
	Total change:	**25**

Table 8. Percent of cells with change in firing rate (FR) during the response periods (R1, R2, R3) for incorrect match (IM); (N = 53 cells) and incorrect nonmatch (INM) trials (N = 154 cells).

		R1	R2	R3
IM	↓ in FR	11	9	9
	↑ in FR	4	2	2
	Total change:	**15**	**11**	**11**
INM	↓ in FR	8	5	8
	↑ in FR	16	10	7
	Total change:	**24**	**15**	**15**

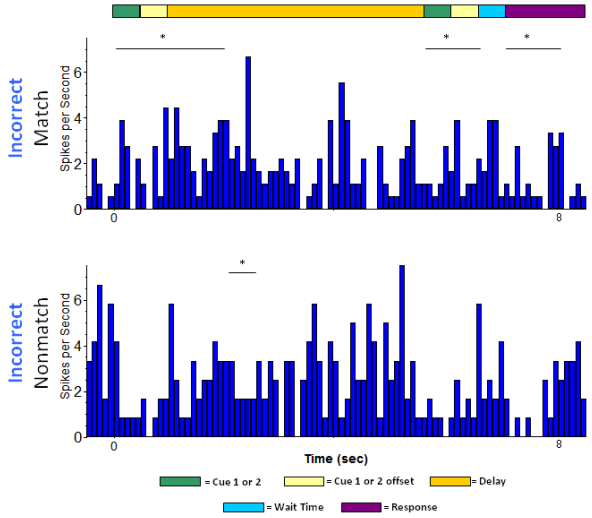

Figure 25. An example cell responsive to cue 1, cue 2, and the response period for incorrect match trials. Bin = 100 ms.

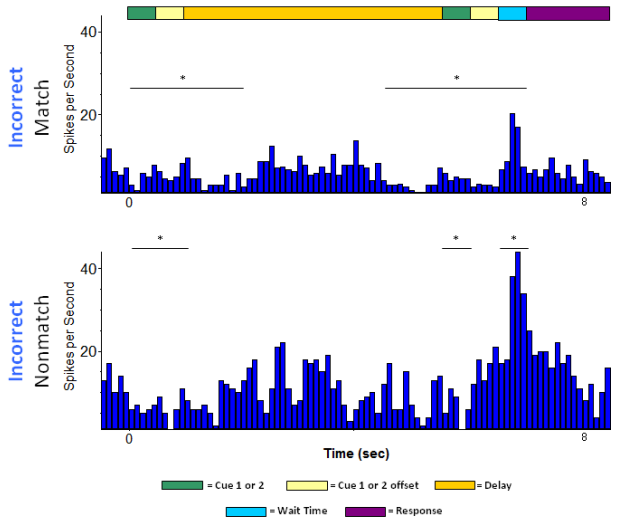

Figure 26. An example cell demonstrating decreases during cue 1 and cue 2, but increases during the wait time on incorrect match and incorrect nonmatch trials. Bin = 100 ms.

General Summary of Cell Activity

During the DMTS task, during the first cue presentation 28% of the cells had a change in firing rate, while during the second cue presentation 36% of cells had a change in firing rate, for at least one trial type (CM, CNM, IM, INM); (Table 9). In total, 64% of the cells fired to cue 1 or cue 2 across at least one of the trial types. Of the cells that fired to either cue 1 or cue 2, 21% of cells fired to both cue 1 and cue 2. For the portion of cells that responded to both cue 1 and cue 2, 15% of those cells showed an enhanced firing rate to cue 2 over cue 1 while 6% showed a suppression to cue 2 compared to cue 1. Of cells that fired to cue 1 or cue 2 for at least one trial type, 2-6% cells fired in a consistent manner to cue 1 or cue 2 across more than one trial type, such as to cue 1 and cue 2 for correct match, correct nonmatch, and incorrect nonmatch trials, (Figure 27).

Table 9. Percent of cells with change in firing rate (FR) during the cue events for at least one trial type (N = 215 cells).

	Cue 1	**Cue 1 offset**	**Cue 2**	**Cue 2 offset**
↓ in FR	13	18	16	21
↑ in FR	15	11	20	24
Total change:	**28**	**29**	**36**	**46**

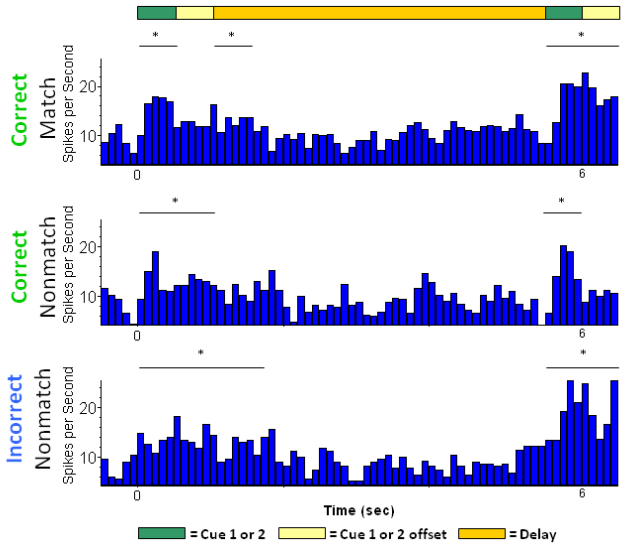

Figure 27. An example cell responsive to cue 1 and cue 2 for correct match, correct nonmatch, and incorrect nonmatch trials. Bin = 100 ms.

The delay was divided into three equal portions: early, middle, and late (each consisting of 3 epochs of 500 ms). During the delay, for at least one trial type 26% of cells had a change in firing rate for the early portion, 21% had a change in firing rate for the middle portion and 26% had a change in the late portion of the delay (Table 10). Delay activity was defined as at least one epoch having a change in FR out of three epochs. For the very first 500 ms after cue 1 offset, 20% of cells had a change in firing rate and for the very last 500 ms epoch of the delay before the onset of cue 2, 18% of the cells had a change in firing rate. There was also a smaller set of cells that fired across the delay period, with some firing in a constant manner across the delay (4%) while others were more transient across the delay period (5%).

Table 10. Percent of cells with change in firing rate (FR) during the delay for at least one trial type (N = 215 cells).

	Early	Middle	Late
↓ **in FR**	11	11	13
↑ **in FR**	15	10	13
Total change:	**26**	**21**	**26**

For at least one trial type during the wait time, 53% of the cells had a change in firing rate (N = 189 cells, Table 11). For at least one trial type averaged across the three response periods, there were 52% of cells which had a change in firing rate (Table 12). See Figures 28 and 29 for example cells.

Table 11. Percent of cells with change in firing rate (FR) during the wait time for at least one trial type (N = 189 cells).

	Wait time
↓ **in FR**	24
↑ **in FR**	29
Total change:	**53**

Table 12. Percent of cells with change in firing rate (FR) during the response periods (R1, R2, R3) for at least one trial type (N = 215 cells).

	R1	R2	R3
↓ in FR	22	27	23
↑ in FR	34	26	23
Total change:	**57**	**53**	**47**

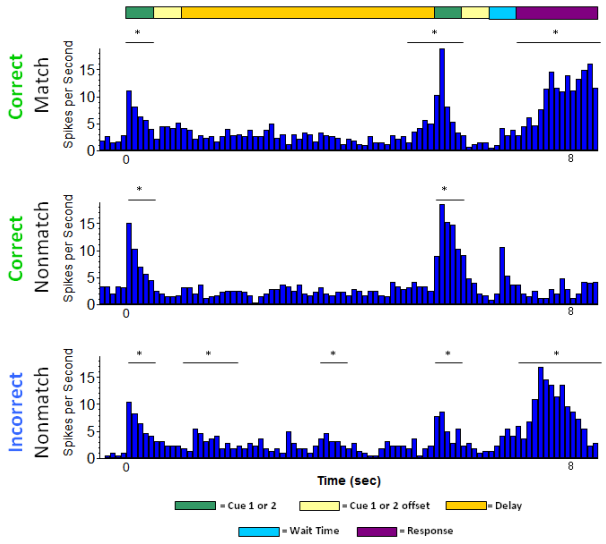

Figure 28. An example cell responsive to cue 1 and cue 2 for correct match, correct nonmatch, and incorrect nonmatch trials. This cell was also responsive during the response period for correct match and incorrect nonmatch trials. Bin = 100 ms.

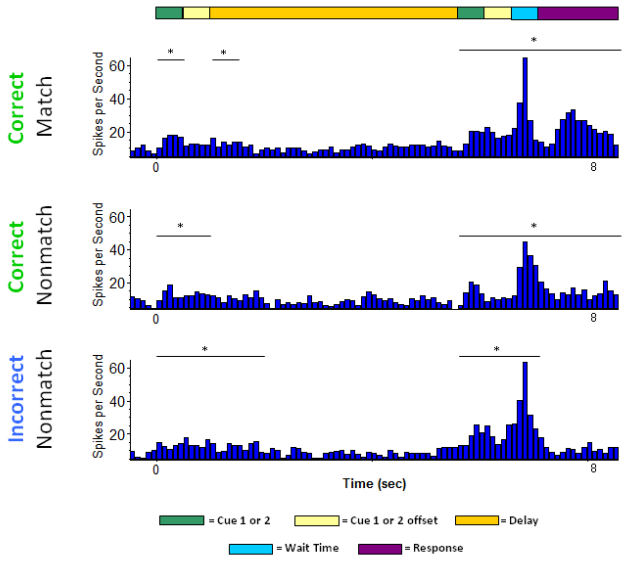

Figure 29. An example cell responsive to cue 1 and cue 2 for correct match, correct nonmatch, and incorrect nonmatch trials. This cell was also responsive during the response period for correct match and correct nonmatch trials. Bin = 100 ms.

While a large portion of cells had some event-related activity during the DMTS task, some cells also coded for more than one event throughout the task (cue 1, cue 1 offset, delay, cue 2, cue 2 offset, wait time, response periods 1-3). A majority of cells, 72% (N = 189 with all events available) coded for 2 and 9 events (Figure 30).

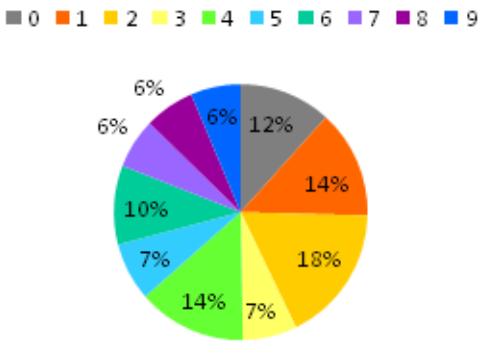

Figure 30. Percent of cells that had a change in FR for multiple events throughout the DMTS task.

Comparing Correct Match to Incorrect Nonmatch Trials

Some cells (N = 110) had a change in firing rate during the wait time or response periods for correct match or incorrect match trials. These cells were examined in a secondary analysis where spike rates were aligned with the button press. This comparison (when the behavioral response was the same for both correct match and incorrect nonmatch trials), may help explain what information the neural activity is encoding for correct versus incorrect behavior. For correct match trials, 47% of the cells had a change in firing rate directly before the button press (Pre-Response 1), 46% of cells had a change in firing rate during and right after the button press (Post Response 1) and 46% had a change in firing rate in the next 500 ms epoch (Post Response 2; Table 13). For incorrect nonmatch trials 39% of the cells had a change in firing rate directly before the button press (Pre-Response 1), 38% of cells had a change in firing rate during and right after the button press (Post Response 1) and 16% had a change in firing rate in the

next 500 ms epoch (Post Response 2; Table 13). See Figures 31-33 for examples of cells with changes in firing rate during these epochs.

Table 13. Percent of cells with change in firing rate (FR) during the pre- and post-response periods (N = 110 cells).

		Pre-Response1	Post Response 1	Post Response 2
CM	↓ in FR	22	17	26
	↑ in FR	25	29	20
	Total change:	**47**	**46**	**46**
INM	↓ in FR	14	13	6
	↑ in FR	25	25	10
	Total change:	**39**	**38**	**16**

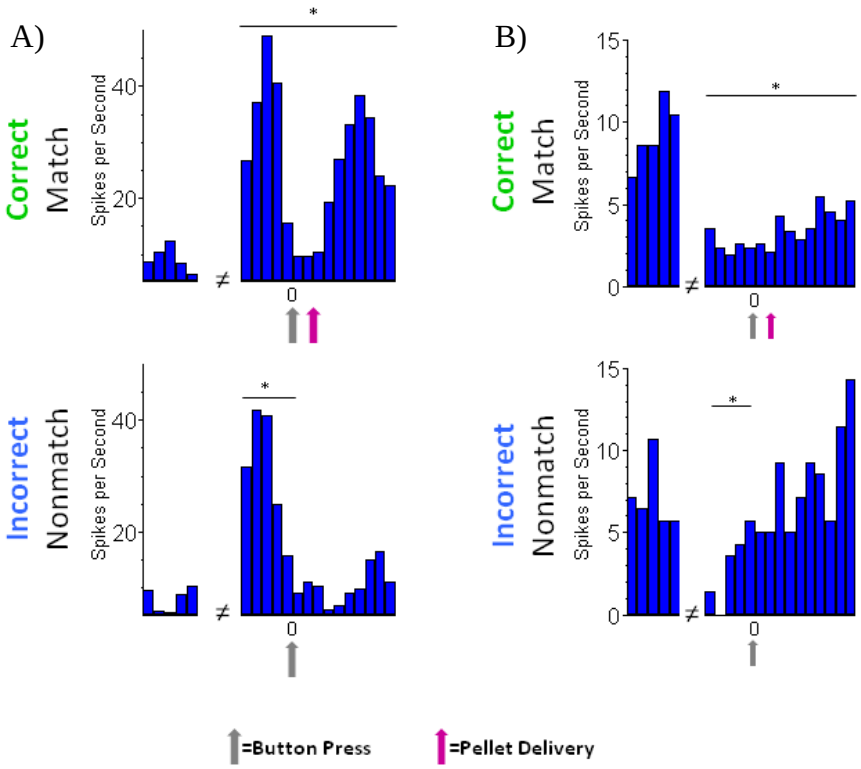

Figure 31. On the left of both panels is the baseline period which is the same for the next series of figures. Panel A) a cell with an increased firing rate directly before the button press for both correct match and incorrect nonmatch trials, and an increased firing rate after the button press only for correct match trials. Panel B) a cell with a decreased firing rate directly before the button press for both correct match and incorrect nonmatch trials and in contrast to cell A no increased firing rate after the button press on correct match trials. Bin = 100 ms.

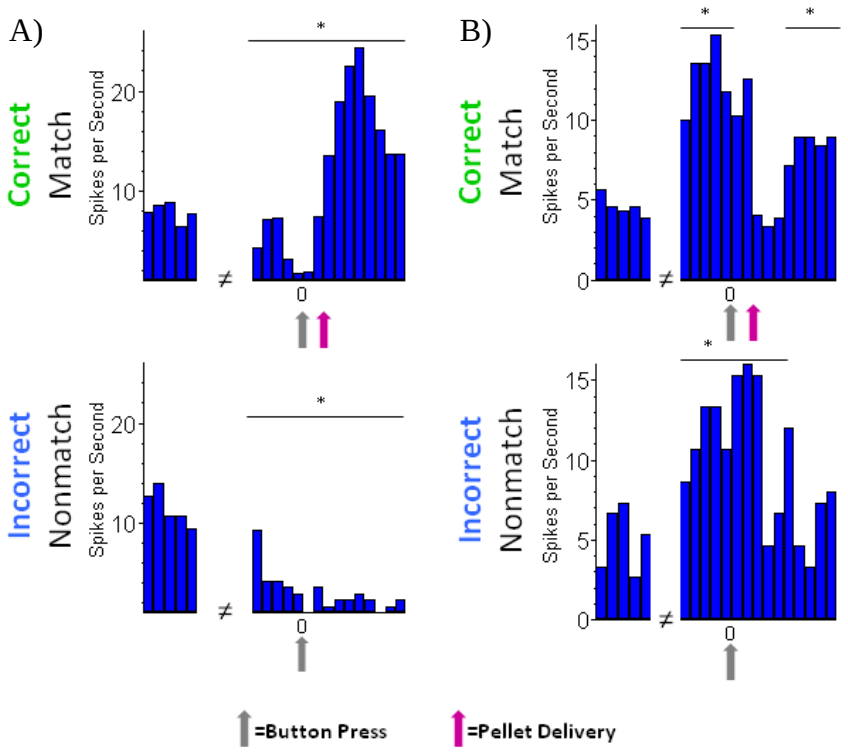

Figure 32. Two example cells. Panel A) a cell with a decreased firing rate directly before the button press for both correct match and incorrect nonmatch trials, and an increased firing rate after the button press only for correct match trials, demonstrating reward activity. Panel B) a cell with an increased firing rate directly before the button press for both correct match and incorrect nonmatch trials demonstrating response-related activity. Bin = 100 ms.

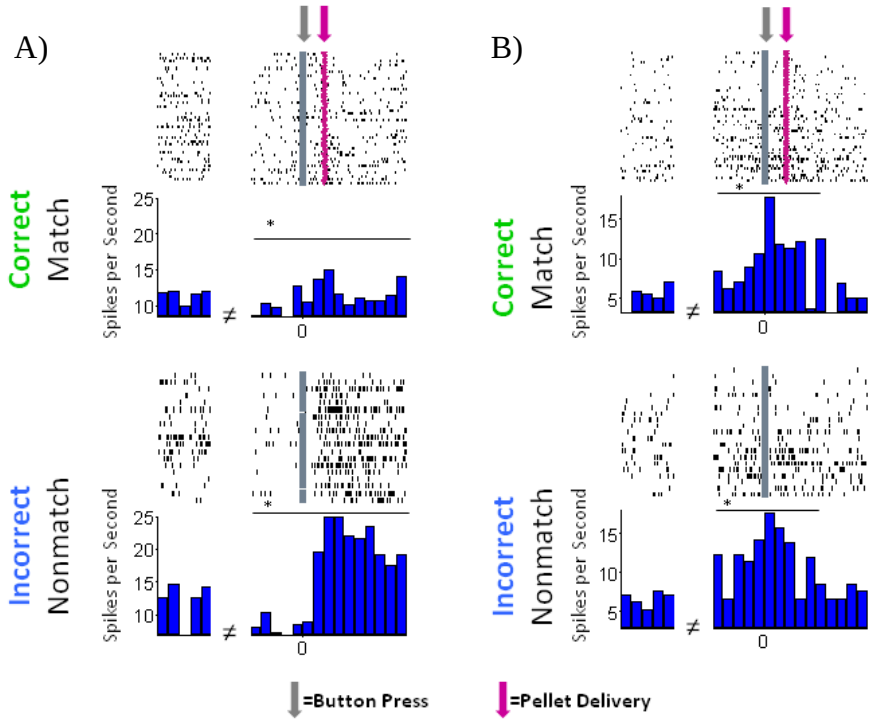

Figure 33. Raster plots with data aligned by button press. Panel A) a cell with decreased firing rates across the pre- and post response periods on correct match trials, but an increased firing rate during the post response period on incorrect nonmatch trials, perhaps signaling loss of expected reward. Panel B) a cell with increased firing rate before and during the button press for correct match and incorrect nonmatch trials, response-related activity. Bin = 100 ms.

Sound Responsiveness

Sound selectivity during the DMTS task was examined by analyzing when the sound was presented in the first cue position. Between 16-19% of cells had a change in firing rate for a particular sound stimulus by sound type indicating no preference for any one sound type (Table 14). Out of the 215 cells analyzed, 124 had a change to at least one out of the eight possible sound stimuli used in the task for that day. Of those 124

cells responsive to at least one particular sound stimulus, a majority of the cells (84%) were selective in that they fired to between 1 and 4 sounds (Figure 34). See Figures 35-37 for example cells.

Table 14. Percent of cells with a change in FR to a sound stimulus for that sound type.

	↓ in FR	↑ in FR	Total change:
Animal	4	12	**16**
Human	7	12	**19**
Monkey	5	12	**16**
Music	6	13	**19**
Natural	7	10	**18**
Pure tone	10	9	**19**
Synthetic	8	11	**19**
White noise	7	9	**16**

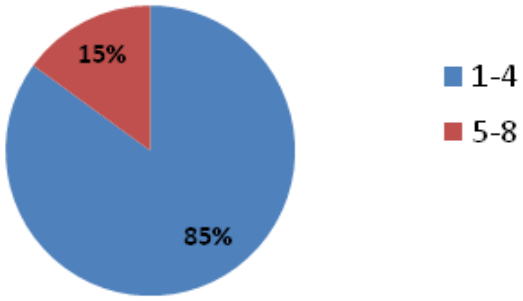

Figure 34. Percent of cells (out of 124 responsive to at least one sound stimulus) responsive to that number of sounds during first block of passive listening (across sound types).

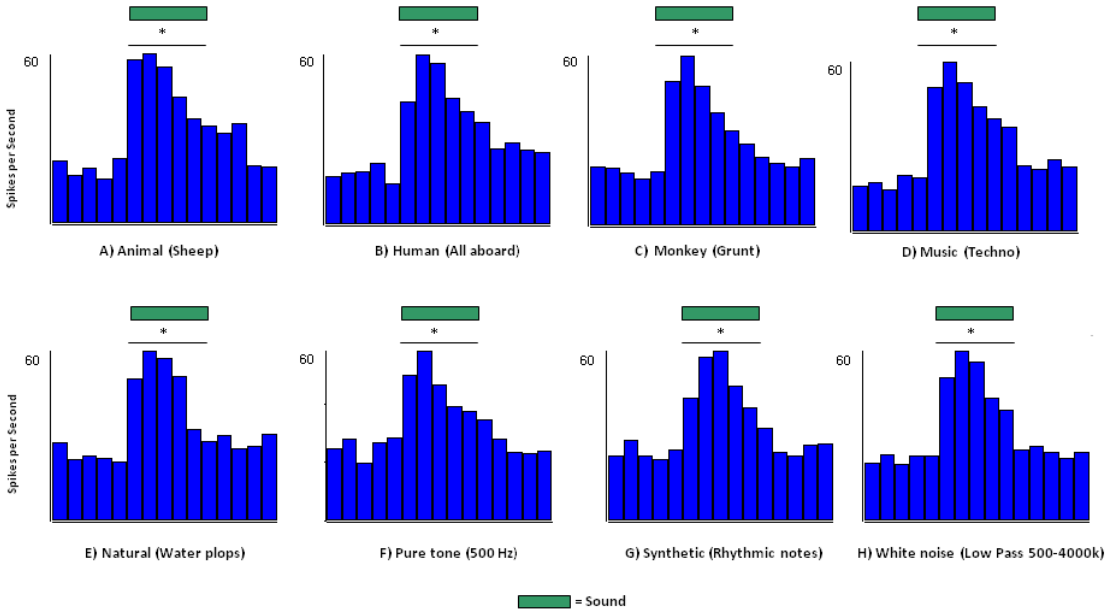

Figure 35. An example cell during DMTS showing an increased firing rate to all 8 sounds used during that recording session. Where the x-axis denotes time, green bar indicates when cue was played. Bin = 100 ms.

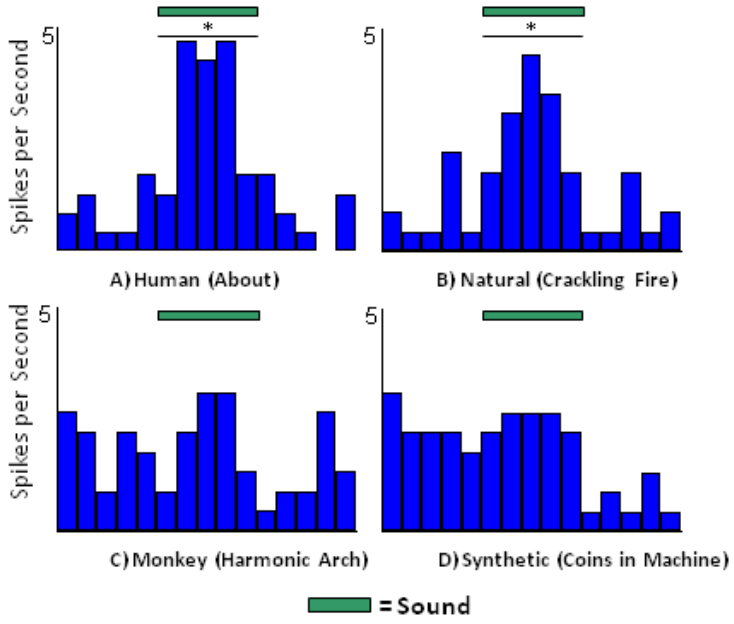

Figure 36. An example cell selective for specific sounds during DMTS. There was a significant increase in firing rate to sounds in panel A and B, but not a significant change in firing rate to sounds in panels C and D. Where the x-axis denotes time, green bar indicates when cue was played. Bin = 100 ms.

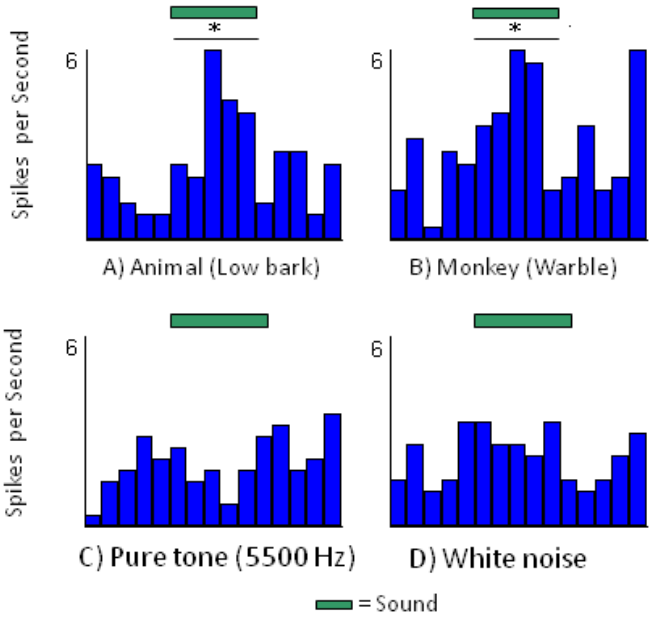

Figure 37. An example cell selective for specific sounds during DMTS. There was a significant increase in firing rate to sounds in panel A and B, but not a significant change in firing rate to sounds in panels C and D. Where the x-axis denotes time, green bar indicates when cue was played. Bin = 100 ms.

Population Results for Evoked Activity

Across the population for correct trials (N = 215) there was evoked activity above baseline. For correct match trials, all events except for the delay were significantly higher in activity compared to baseline. There was a trend for cue 2 to have significantly higher activity compared to cue 1 ($p = 0.06$). For correct nonmatch trials, cue 1, cue 1 offset, cue 2, wait time, and the last two response periods were significantly higher in activity compared to baseline. There was significantly higher activity during cue 2 compared to cue 1. There was no significant change in delay activity for correct nonmatch trials.

In order to assess evoked activity for incorrect behavior, a sub-population of cells (N =154) with incorrect nonmatch trials was examined by trial type. For the sub-population with incorrect nonmatch trials (N = 154), a similar pattern of evoked activity was found. For correct match trials within this sub-population, cue 1, cue 1 offset, cue 2, cue 2 offset, wait time and the three response periods were significantly higher in activity compared to baseline. For correct nonmatch trials cue 1, cue 1 offset, cue 2, cue 2 offset, wait time, response periods 1 and 3 were significantly higher than baseline. For incorrect nonmatch trials, cue 1 offset, cue 2, cue 2 offset, wait time, and the first two response periods were significantly higher than baseline. The delay was not significantly different for any of the trial types.

Population Results to Examine Trial Type Effects

Trial type effects for events were also examined for both the correct trials population (N = 215) and the sub-population with incorrect nonmatch trials (N = 154). For correct trials, during the three response periods there was a main effect of trial type and a significant interaction. Post-hoc tests found that for the three response periods, for each epoch (500 ms), correct match trials had significantly more activity compared to nonmatch trials. There were no delay trial type effects for either population. Within the sub-population with incorrect nonmatch trials, there was a significant main effect of trial type and post-hoc tests found that correct match trials had significantly more activity than correct nonmatch trials.

In general, activity during cue 2 was greater than during cue 1 for both correct match and nonmatch trials, where there was a significant event effect showing higher activity during cue 2 compared to cue 1. Additionally, a match ‘enhancement’ effect was

found for correct trials, demonstrated by a significant trial type effect for the difference score (between cue 2 and cue 1) showing a higher level of activity on correct match compared to correct nonmatch trials.

Population Results with Fine-grain Analyses of Events

Particular events of interest (cue 1, cue 2, cue 2 offset, wait time) were examined individually to determine trial type by time interval (5, 100 ms intervals for each event) effects for both the correct trials (N = 215) and for the sub-population with incorrect nonmatch trials (N = 154). For correct trials during cue 1 there was a significant effect of trial type, with correct match trials having more activity than correct nonmatch trials (Figure 38).

For correct trials during cue 2 there was a significant effect of trial type, interval, and a significant interaction. Post-hoc tests by interval found that there was a significant increase in activity during the 2^{nd}, 3^{rd}, and 5^{th} 100 ms intervals, for correct match trials compared to correct nonmatch trials.

For correct trials during cue 2 offset, there was a significant trial type and interval effect, with more activity occurring during correct match compared to correct nonmatch trials.

For correct trials during the wait time, there was a significant effect of trial type, interval, and a significant interaction. The post-hoc tests by interval found there was a significant increase in activity during match trials compared to nonmatch trials during the 4^{th} and 5^{th} intervals (Figure 38).

For the sub-population that included incorrect nonmatch trials during cue 1 there was a significant trial type effect, where there was a higher level of activity during correct match trials compared to both correct nonmatch and incorrect nonmatch trials.

During cue 2 there was a significant effect of trial type, interval, and an interaction. The post-hoc tests by trial type found that activity was significantly higher on correct match trials compared to both correct nonmatch and incorrect nonmatch trials (Figure 39). The post-hoc tests by interval found that during the 2^{nd}, 3^{rd}, and 5^{th} intervals correct match activity was greater than both correct nonmatch and incorrect nonmatch trials.

During cue 2 offset there was a significant trial type effect and an interaction. The main effect post hoc tests found that activity was significantly higher on correct match trials compared to correct nonmatch and incorrect nonmatch trials and that there was significantly higher level of activity on incorrect nonmatch trials compared to correct nonmatch trials (Figure 39).

During wait time there were significant trial type and interval effects as well as an interaction. The main effect post-hoc tests found a significantly higher level of activity on correct match trials compared to correct nonmatch and incorrect nonmatch trials. The activity on incorrect nonmatch trials was also significantly higher compared to correct nonmatch trials (Figure 39).

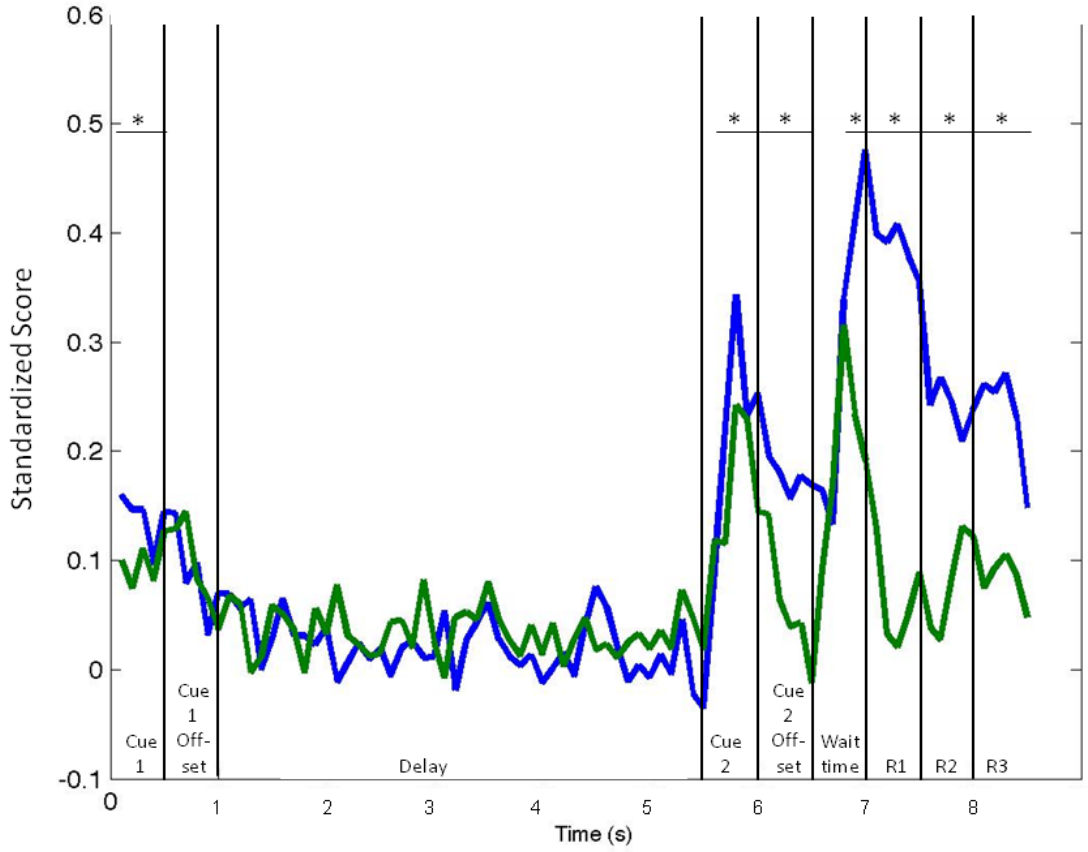

Figure 38. Standardized activity across the population of neurons for correct match trials (blue line) and correct nonmatch trials (green line). Events are listed across the bottom. * = significant trial type effect, here correct match trials had more activity across cue 1, cue 2, cue 2 offset, the last two intervals of wait time, and the three response periods. During cue 2, there is a "match enhancement" effect i.e., the absolute difference between cue 2 and cue 1 is significantly greater for correct match than correct nonmatch trials.

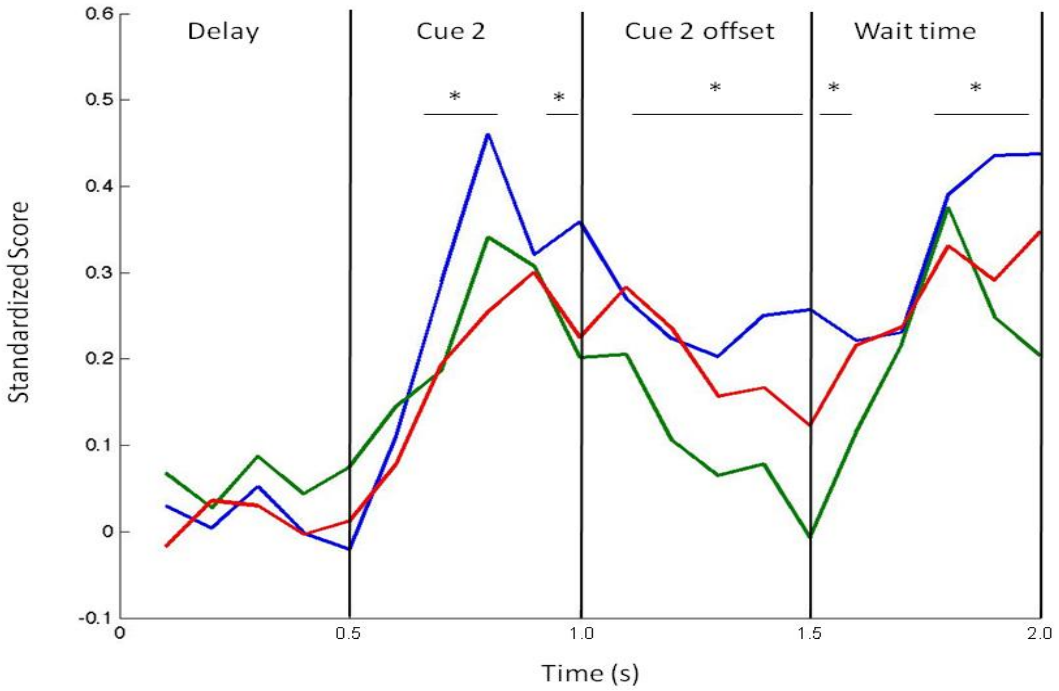

Figure 39. Standardized activity across the population of neurons for correct match trials (blue line), correct nonmatch trials (green line), and incorrect nonmatch trials (red line). Events are listed across the bottom. * = significant trial type effect. Correct match trials had significantly more activity across all three events compared to both other trial types. Incorrect nonmatch trials had significantly more activity compared to correct nonmatch trials for both cue 2 offset and wait time events.

Passive Listening

For passive listening presentations animals had one block of sounds played before the behavioral DMTS task, followed by another block (consisting of 96 sounds). For the first block of passive listening the monkeys heard 8 stimuli and there were 113 cells analyzed. Between 9 and 19% of cells responded to any one particular stimulus out of 8 possible sounds (Table 15). Of the cells that were responsive to at least one particular

sound stimulus (70 cells) 93% were fairly selective in that they were responsive to between 1 and 4 sounds (Figure 40).

Table 15. Percent of cells responsive during first passive listening block to particular sound stimulus types.

	Animal	Human	Monkey	Music	Natural	Pure	Synthetic	White
↓ **in FR**	5	4	6	11	4	8	9	3
↑ **in FR**	8	12	3	9	9	5	11	12
Total change:	**13**	**16**	**9**	**19**	**13**	**13**	**19**	**14**

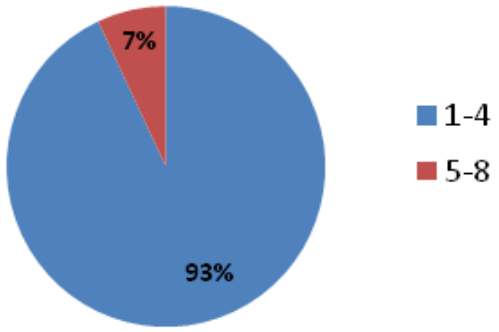

Figure 40. Percent of responsive cells firing to between 1 and 4 or 5 and 8 sound stimuli during the first passive listening block (across sound types). (N = 70)

For the second passive listening set there were a total of 96 sounds presented to the monkeys including the 8 heard during the DMTS task and 157 cells were held and analyzed. For any individual sound stimulus 4-16% of the cells had a change in firing

rate (Table 16). A majority of the cells (98%) held during these passive listening blocks were responsive to at least one particular sound stimulus. Many cells responded to a small range of possible sound stimuli; 34% responded to between 1-4 sounds and 39% of cells responded to between 5-9 sounds. A smaller number of cells (14%) fired to between 15-48 sounds (Figure 41). Some cells were responsive to particular sound stimuli (Figures 42-44). There were no patterns in firing rate by cell discerned from the acoustic properties of the harmonic-to-noise ratio or for spectral mean, meaning there were no cells that fired to a specific range for either harmonic-to-noise ratio or spectral mean. For sound type there were no significant differences except between the animal sounds and pure tones ($p = 0.004$).

Table 16. Summary of percentage of cells with change in FR during the second passive listening block (96 sounds, 157 cells). The far left label is sound type.

	Sound	↓ in FR	↑ in FR	Total change:
Animal	1 (Sheep, ba)	4	3	**7**
	2 (Kitten, mew)	4	8	**12**
	3 (Pig, squeal)	2	4	**6**
	4 (Dog, high bark)	5	4	**9**
	5 (Bird)	4	5	**9**
	6 (Dog, low bark)	5	4	**9**
	7 (Seal)	4	6	**10**
	8 (Hawk)	7	3	**10**
	9 (Cat)	8	2	**10**
	10 (Horse)	3	3	**6**
	11 (Bird tweet)	6	6	**12**
	12 (Bird song)	5	4	**9**
Human	1 (All aboard)	6	6	**12**
	2 (About)	8	2	**10**
	3 (That highest)	3	3	**6**
	4 (Whimper)	8	3	**11**
	5 (Laugh)	5	3	**8**
	6 (Oooh)	4	3	**7**
	7 (Buddy)	6	3	**9**
	8 (Go long)	6	3	**9**
	9 (Get down)	2	2	**4**
	10 (Undo)	5	7	**12**
	11(Male shouting)	6	4	**10**
	12 (Boo)	3	4	**7**

Table 16. Continued

	Sound	↓ in FR	↑ in FR	Total change:
Monkey	1 (Grunt)	5	3	**8**
	2 (Harmonic arch)	7	6	**13**
	3 (Noisy scream)	5	4	**9**
	4 (Grunt)	3	2	**5**
	5 (Harmonic arch)	4	4	**8**
	6 (Warble)	3	3	**6**
	7 (Undulating scream)	4	4	**8**
	8 (Shrill bark)	7	3	**10**
	9 (Grunt)	5	4	**9**
	10 (Coo)	3	2	**5**
	11 (Coo)	3	2	**5**
	12 (Grunt)	5	3	**8**
Music	1 (Techno)	8	3	**11**
	2 (Drums)	6	6	**12**
	3 (Plucked string)	5	4	**9**
	4 (Trumpet)	4	5	**9**
	5 (Piano notes)	4	3	**7**
	6 (Clarinet)	5	5	**10**
	7 (Piccalo)	7	4	**11**
	8 (Pan flute)	3	4	**7**
	9 (Bells)	2	1	**3**
	10 (Flute)	3	2	**5**
	11 (Recorder Whistle)	5	0	**5**
	12 (Wooden flute)	6	3	**9**

Table 16. Continued

	Sound	↓ in FR	↑ in FR	Total change:
Natural	1 (Fast water plops)	3	4	**7**
	2 (Crackling fire)	6	6	**12**
	3 (Roar of fire)	5	2	**7**
	4 (Heavy rain)	3	3	**6**
	5 (Surf of ocean)	4	6	**10**
	6 (Bubbles)	4	4	**8**
	7 (Gentle rain)	2	4	**6**
	8 (Falling rock)	1	6	**7**
	9 (Rolling wave)	4	3	**7**
	10 (Thunder)	4	5	**9**
	11 (Falling wood)	6	5	**11**
	12 (Water drops)	5	3	**8**
Pure tone	1 (500 Hz)	5	3	**9**
	2 (1500 Hz)	4	2	**6**
	3 (2500 Hz)	3	2	**5**
	4 (3500 Hz)	4	3	**7**
	5 (4500 Hz)	4	3	**7**
	6 (5500 Hz)	3	2	**5**
	7 (6500 Hz)	4	3	**7**
	8 (8500 Hz)	6	4	**10**
	9 (7500 Hz)	4	4	**8**
	10 (10500 Hz)	2	7	**9**
	11 (600 Hz)	4	3	**7**
	12 (12000 Hz)	4	3	**7**

Table 16. Continued

	Sound	↓ in FR	↑ in FR	Total change:
Synthetic	1 (Rhythmic notes)	2	3	**5**
	2 (Coins in machine)	2	4	**6**
	3 (Car accelerating)	6	4	**10**
	4 (Synthesized high note)	8	3	**11**
	5 (Object rolling)	5	2	**7**
	6 (Synthesized long note)	6	7	**13**
	7 (Machine beep)	5	5	**9**
	8 (Synthesized flute)	6	4	**10**
	9 (FM sweep)	5	4	**9**
	10 (Metallic bell)	4	4	**8**
	11 (Synthesized beep)	7	4	**11**
	12 (Synthesized low note)	3	3	**6**
White noise	1 (Low pass 500-4000k)	4	6	**10**
	2 (Low pass 500-5000k)	4	3	**7**
	3 (Low pass 500-1500k)	3	3	**5**
	4 (Low pass 900-3000k)	3	6	**9**
	5 (High pass 500)	3	8	**11**
	6 (Band stop 5000 1k)	5	4	**9**
	7 (Low pass 0-400k)	3	3	**5**
	8 (No filter)	9	7	**16**
	9 (Low pass 5,000k)	6	7	**13**
	10 (Low pass 10-400k)	5	1	**6**
	11 (Low pass 2000k)	6	4	**10**
	12 (Low pass 200-5200)	7	3	**10**

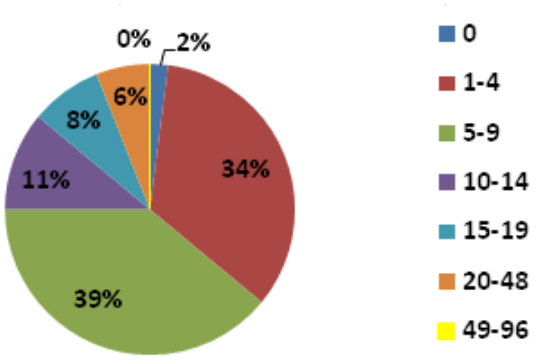

Figure 41. Percentage of cells (N = 157) that responded during the second passive listening block and the corresponding number of sounds to which they responded (across sound types).

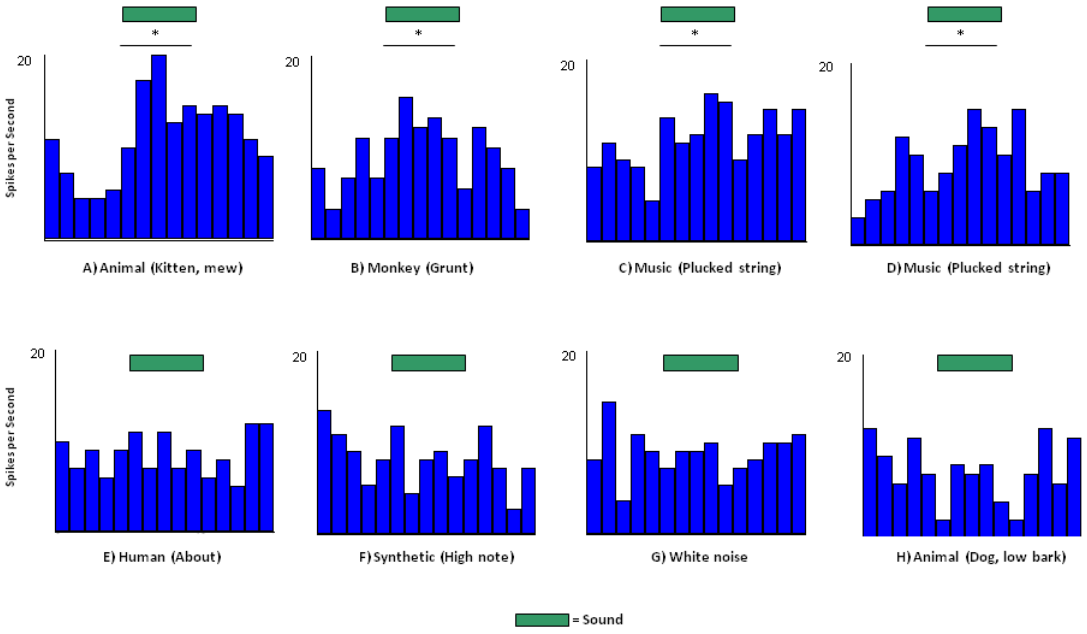

Figure 42. This example cell was selective to particular sound stimuli. There were significant increases in firing rate to sounds presented in the top panel (A-D), but no significant change to sounds in the bottom panel (E-H) during passive listening. Where the x-axis denotes time, green bar indicates when sound was played. Bin = 100 ms.

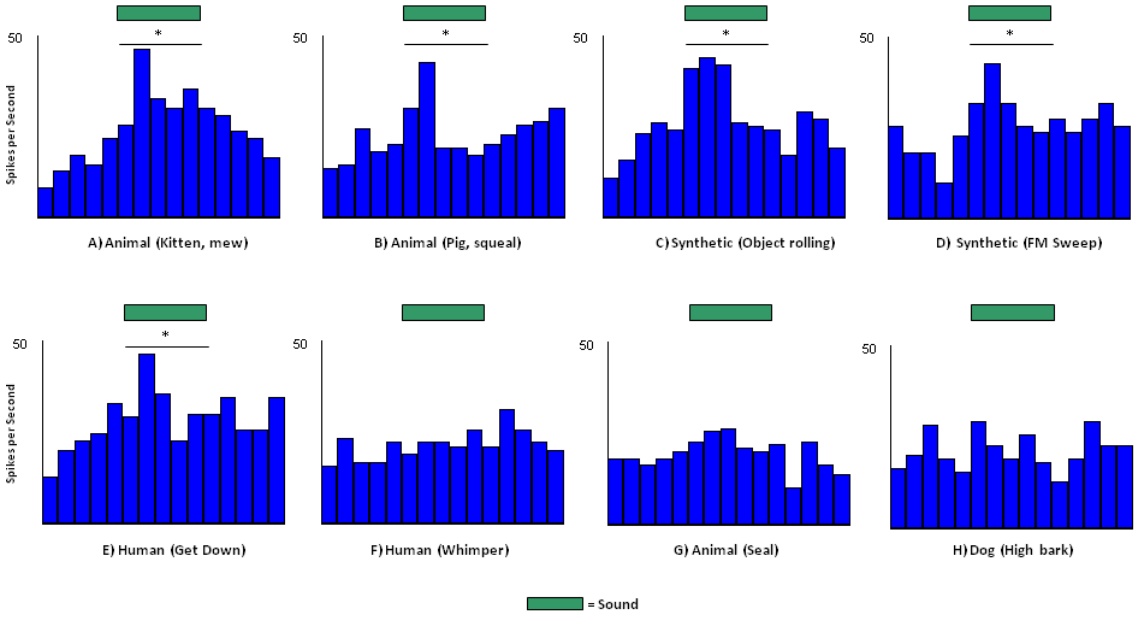

Figure 43. This example cell was selective to particular sound stimuli. There were significant increases in firing rate to sounds presented in panel (A-E), but no significant change to sounds for panel (F-H) during passive listening. Where the x-axis denotes time, green bar indicates when sound was played. Bin = 100 ms.

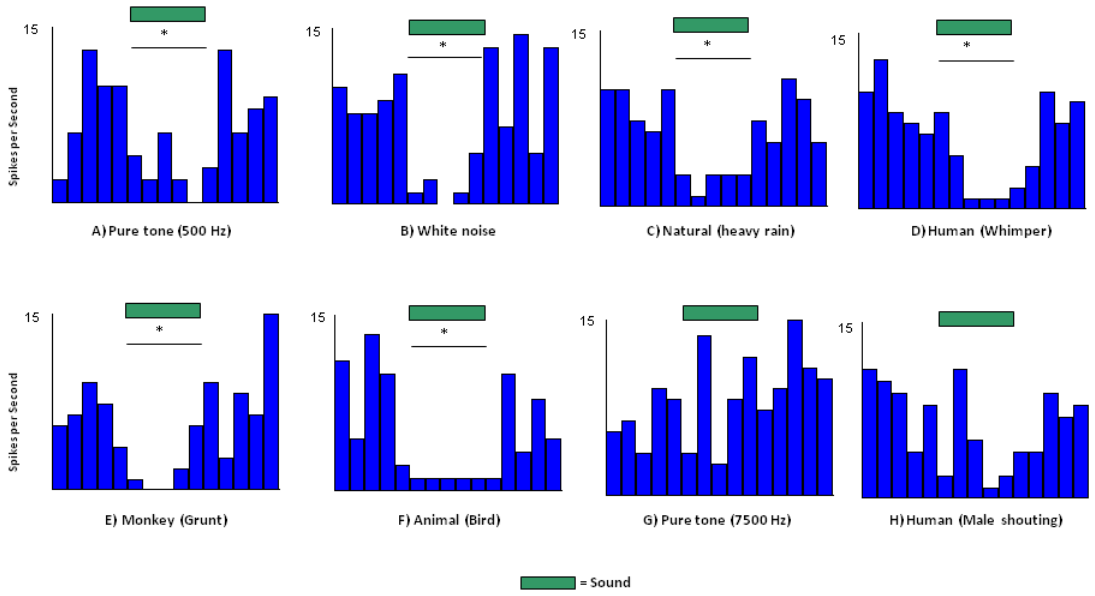

Figure 44. This example cell was selective to particular sound stimuli. There were significant decreases in firing rate to sounds presented in panel (A-F), but no significant change to sounds for panel (G-H) during passive listening. Where the x-axis denotes time, green bar indicates when sound was played. Bin = 100 ms.

Comparing Passive Listening Activity to Sound Activity during the DMTS Task

A comparison of the same cells (113 cells) that were held during the first passive listening block and during the DMTS task found that the specific sounds a cell responded to changed between the passive listening and the active memory task. Some of the cells were only responsive during passive listening (39%), while some cells were responsive to specific, but different sounds during both tasks (26%). Another set of cells fired to sounds during both tasks, but either stopped responding to some sounds and responded to

different sounds, or just started responding to different sounds during the DMTS task (see Figure 45).

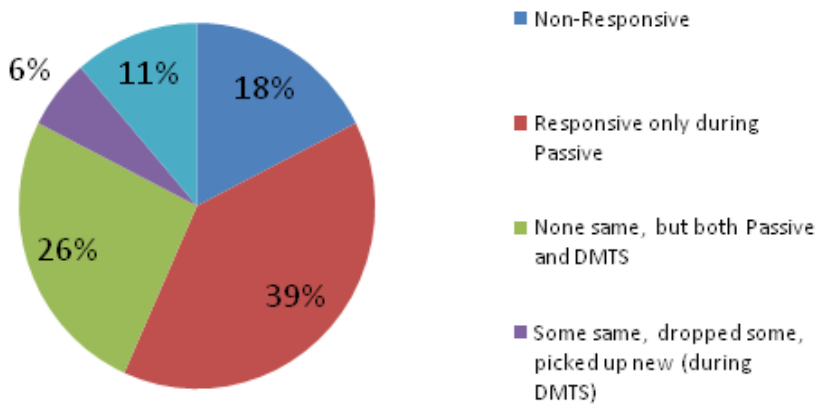

Figure 45. Percent of cells that changed which sounds they were responsive to during the first passive listening block compared to the DMTS task.

A comparison of cells held during the DMTS task and during the second passive listening (97 cells) found that in general a majority of cells that were responsive to sound stimuli during the behavioral task also were responsive to sound during the second passive listening block (67%; Figure 46). Of the cells responsive to sounds during both the DMTS task and during the second passive listening block cells changed what specific sound stimuli they fired to in several ways (66 cells). Some cells fired to the same sounds they had fired to during the DMTS task but also responded to new sound stimuli presented during passive listening (6%). However, a majority of cells did not consistently fire to that same stimulus after the task, but did fire to new sounds presented during passive listening (83%; Figure 47).

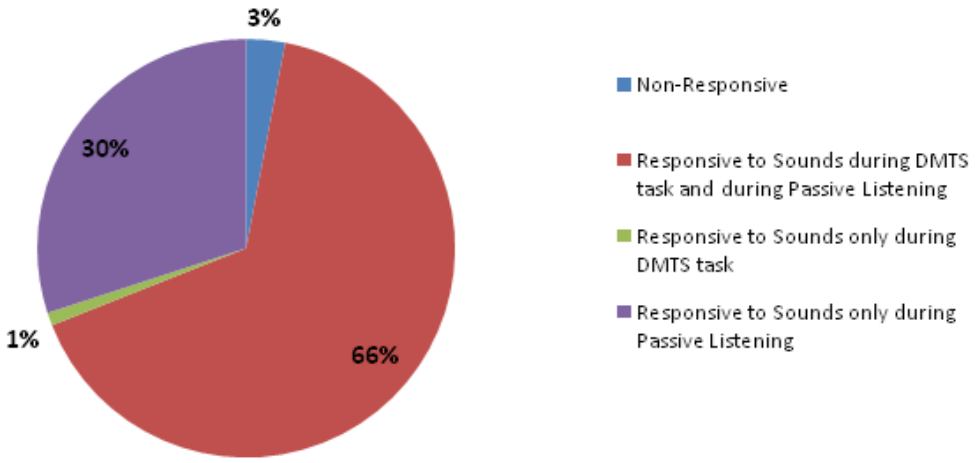

Figure 46. Percentage of cells responsive to particular sound stimuli during the DMTS task and during the second passive listening block (N = 97 cells).

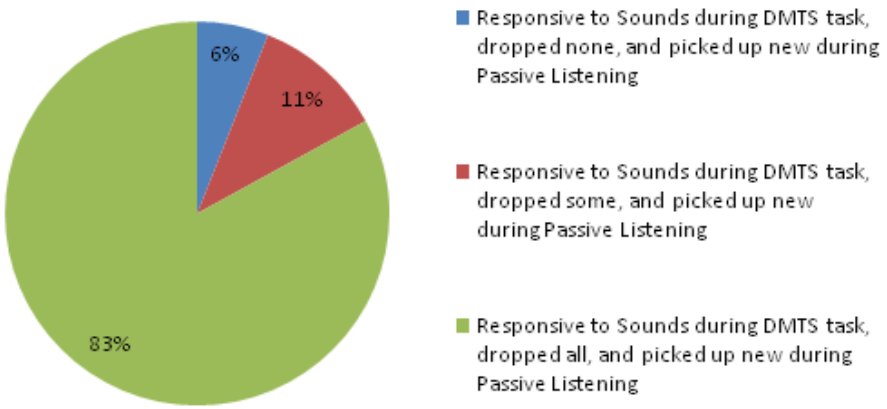

Figure 47. Percentage of cells that changed which sounds they were responsive to the during the DMTS task and during the second passive listening block (N = 66; cells responsive to at least one sound during DMTS and passive listening).

Placement

The placements of recording wells are shown in Figure 48, for closer view of recording locations see Figure 49. Recordings were mostly in area 46 (also including area 46/9). Placements were dispersed across both the dorsal and lateral banks of the principal sulcus, however, as planned, on average there were more recordings taken from the ventral bank. The recording depth ranged from .263-5.135 mm. There were 7 cells with no event activity located in the dorsal bank, and 15 with no event activity cells in the ventral bank, for a total of 55 dorsal active cells and a total of 137 ventral active cells. Both regions had neurons that were active to the various events throughout the DMTS task. For the dorsal area there was more cue 1 activity (31%) compared to ventral cells (23%) on correct trials (Table 17), the trend was similar for incorrect trials. During the delay there was more activity in the ventral bank (51%) than in the dorsal bank (40%) for correct trials. For the wait time, and response periods there was more activity in the ventral bank (44-61%) compared to the dorsal bank (42-45%) on correct trials (Table 17).

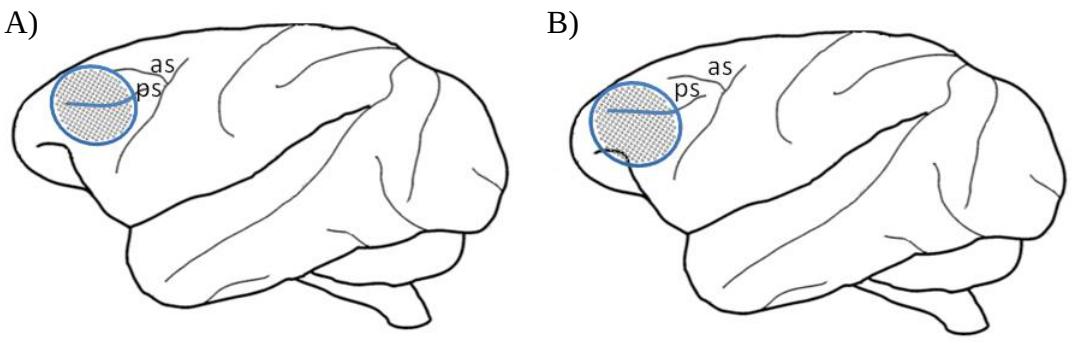

Figure 48. Left hemisphere schematics depicting headwell placement. (A) monkey A (B) monkey B. PS = Principal Sulcus; AS = Arcuate Sulcus.

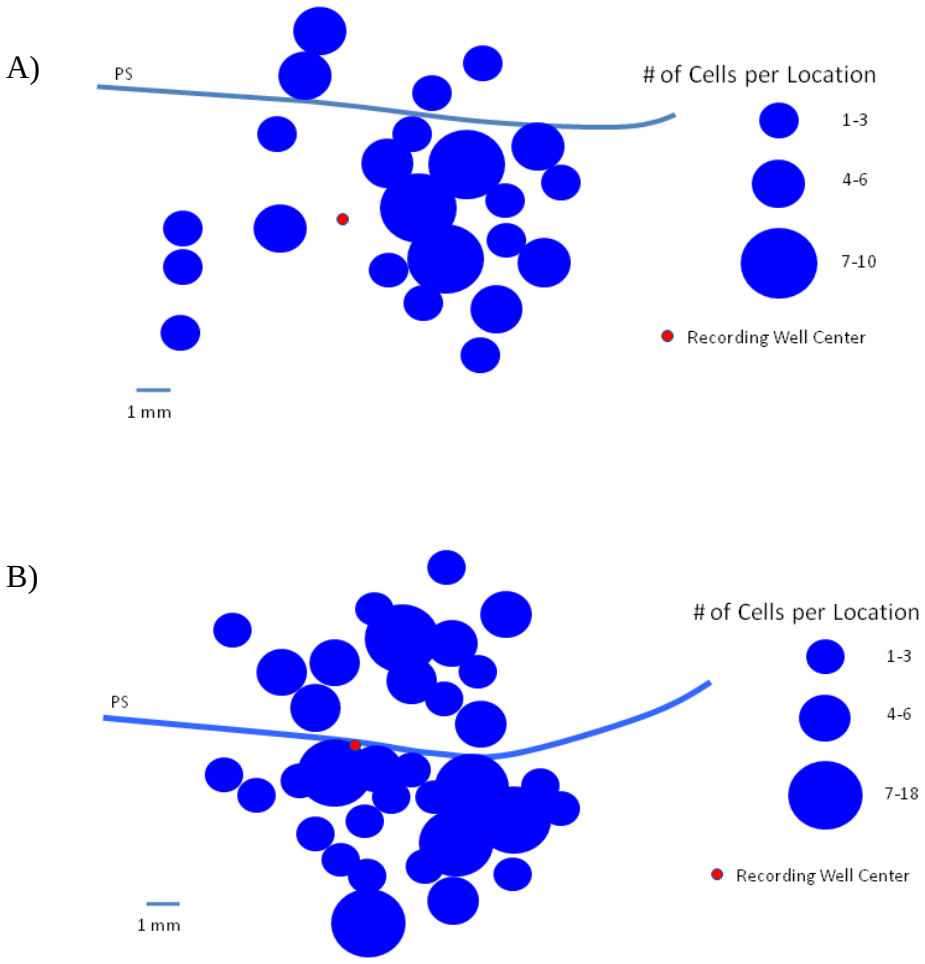

Figure 49. Recording placements where the red dot is the center of the well. Panel A) monkey A. Panel B) monkey B. Both show the range of numbers of cells from each recording location. PS = Principal Sulcus.

Table 17. Summary of percentage of cell activity by event in dorsal and ventral banks of the principal sulcus by correct and incorrect trials. (Dorsal cells = 55; ventral cells = 137).

		Cue 1	Cue 1 offset	Delay	Cue 2	Cue 2 offset	Wait time	R1	R2	R3
Correct	**Dorsal**	29	31	40	36	44	42	42	45	42
	Ventral	26	23	51	37	41	50	61	54	44
Incorrect	**Dorsal**	18	18	15	16	18	18	20	15	13
	Ventral	10	11	24	18	19	17	23	15	14

Discussion

A majority of the neurons (88%) demonstrated a significant change in firing rate to at least one event within the DMTS task and thus, are considered task related. Events that neurons responded to during the memory task include cue 1 and cue 2 presentations, the delay period, the wait time, and the response periods. Most of the neurons recorded from area 46 encoded events within the auditory working memory task, similar to its involvement during cue, delay, and response periods in visual working memory tasks (Fuster et al., 1982; Iba & Sawaguchi, 2002; Kojima & Goldman-Rakic, 1982; Miller et al., 1996; Warden & Miller 2007).

Cue activity

During the DMTS task, area 46 neurons were responsive to auditory cues. A large percentage (64%) of the neurons fired to cue 1 or cue 2 across at least one of the trial types. While a smaller percentage (28%) responded to cue 1, a larger percentage (36%) responded to cue 2 for at least one trial type. Out of the neurons responding to both cues, 23% changed the magnitude of their firing rate (a greater increase or decrease),

to the second sound presentation. This activity may represent a neural correlate corresponding to processing that goes beyond strict sound processing. Cue 2 presentations likely signal the start of the decision making process for considering if the second cue matched the first cue or not. The lPFC has been suggested to process this type of decision information during visual working memory tasks (Asaad et al., 2000; Kennerley et al., 2009; Miller et al., 1996).

In our study, across the population of neurons, activity to cue 2 was significantly greater on correct trials than to cue 1 suggesting a change in encoding upon hearing the second sound stimulus. Enhancement of activity across the population is evidence of this region weighting the second sound differently from the first. This activity could be signaling "now is the time to compare the sounds", or be related to the impending decision of whether it is a matching or nonmatching sound. The higher level of activity during correct match and nonmatch trials, suggests this region is engaged in signaling "it is time to compare the stimuli", similar to results found in visual memory studies.

In addition, neurons across the population demonstrated match enhancement as there was significantly more activity for the absolute difference between cue 2 and cue 1 on correct match trials compared to correct nonmatch trials. This suggests even though the animal was correct in both instances, that there is a difference between match and nonmatch trials regarding stimulus comparison. Similar match enhancement has been found with visual stimuli (Miller et al., 1996).

In various visual memory tasks, neurons responsive during the visual cue epoch have been found for between 17 and 73% of neurons, depending on the task and from which brain region recordings were taken (Asaad et al., 2000; Fuster, et al., 1982;

Quintana & Fuster, 1999; Rainer, et al., 1998; Rainer, et al., 1999; Warden & Miller, 2007). Our overall percentage of responsiveness to cue 1 or cue 2 is comparable, but our cue 1 activity is not as robust as what is reported in some visual working memory tasks. This is possibly due to task differences and that some of the visual memory tasks were recorded in more posterior and ventral areas such as area 45. It also could be related to the modality input differences between visual and auditory stimuli since the regions of the lPFC are more responsive to visual stimuli in general compared to auditory stimuli. For example, Romanski & Goldman-Rakic (2002) played more than 100 sound stimuli to 400 neurons in the more posterior ventral lPFC (area 45, inferior convexity) and only found 17.5% to be responsive to sound during the stimulus period. In addition, the majority of these auditory responsive neurons (81%) were localized to a 4x4 mm area within the inferior convexity, an area below the principal sulcus (Romanski & Goldman-Rakic, 2002). In a study that used tones and colors in a paired associates memory task recorded partially from area 46, it was found that only 17.2% of neurons responded to sound stimuli during the task (Fuster et al., 2000). Comparatively, the percentage of auditory responsive neurons to cues within our auditory working memory task, in a different lPFC location was larger than those recorded in previous studies. Additionally, in visual studies, it takes as long as 100 ms for cues to reach the lPFC, however we found that some neurons had onset changes within 70 ms. While this is similar to other areas of the lPFC for auditory cues (Romanski et al., 2005), it also illustrates a difference between the lPFC regions in processing visual and auditory stimuli.

Delay activity

Many neurons responded to a portion of the delay with 46% of neurons coding at least one delay epoch for at least one trial type. However, few neurons (4%) were consistently firing across the entire 5 second delay; while other neurons were responsive in an intermittent manner across the delay (5%). More neurons (20%) coded the beginning of the delay (the portion after cue 1 offset) and about the same amount (18%) coded the last 500 ms of the delay before cue 2 onset. While a considerable portion of neurons did not have large sustained changes in firing rate across the entire delay, a sizeable portion tracked the beginning and/or end of the predictable 5s delay, suggesting that as a population the neurons were tracking the delay in some fashion. Variable delay activity was also found in another working memory task, which used pure tones (Bodner et al., 1996).

In visual studies, the pattern of activity during the delay is variable depending on the task at hand, with many research groups finding delay activity similar to ours where it is intermittent or where delay activity is affected by the preceding stimulus location, stimulus characteristic, or rule of the task (Asaad et al., 1998; Funahashi et al., 1989; Wallis et al., 2001). When rewards, choice outcomes (i.e., if sample was green, choice will most likely be red), or upcoming events (another object in a series will be presented) are predictable, then there are delay neurons which show a 'climbing' pattern of delay activity where just before the next predictable event occurs, delay activity increases (Asaad et al., 2000; Iba & Sawaguchi, 2002; Miller et al., 1996; Quintana & Fuster, 1999; Rainer et al., 1999). In a similar fashion, some of our neurons demonstrated this type of activity suggesting that the role of area 46 may be the same for both auditory and visual

working memory. However, in some visual studies, more neurons with strong sustained responses across the delay have been found (Fuster et al., 1973). It is possible that we did not find a large portion of this type of delay neuron due to a difference in recording placements, modality, or task demands. It is also possible that the low percentage of neurons showing sustained activity across the delay could hinder the monkey's performance at longer delays. In a similar auditory working memory task, performance drops to near chance levels at 37.5 s (Fritz et al., 2005), which is quite different from visual memory performance at similar delay lengths. Scrutinizing the delay activity at longer delays or inserting a variable delay are manipulations that could be considered in future studies.

Wait Time Activity

The largest portion of neurons with changes in firing rate during the wait time occurred during correct match trials, where 41% of cells had a change. This event period is when the monkey is most likely making a decision about the sound and/or response choice (after the sound but before being allowed to respond) and may include initiating other brain regions to start planning for a motor response. For correct nonmatch and incorrect nonmatch trials, for which a response is made, there were respectively 19 and 25% of cells active during this time period. It is possible that the higher percentage of neurons responsive on incorrect nonmatch trials compared to the correct nonmatch is related to the eventual motor response on incorrect trials.

The time period directly after the sound presentation is considered cue 2 offset time, because it may simultaneously have activity related to the cue that was just presented, and the monkey is waiting to respond. In any case, for correct match trials,

almost a third of neurons (28%) were responsive during this time but for all other trial types only a small percentage (16-18%) of neurons was responsive. On correct match performance this activity could be associated with the prediction of a future reward; however it is difficult to differentiate between activity due to cue properties or activity in relation to anticipation/expectation. In the other three trial types, a decision to respond (or not) is also being made. If the activity were related to prediction of a future reward, then it is also related to the 'match' decision, as reward anticipation could only occur if the monkey considered it to be a matching cue. One way to examine this in the future would be to reward both correct responses or reward the correct nonmatch trials instead of correct match trials.

The event periods of cue 2 offset and wait time can also be considered 'decision making' time, as the monkey has heard the sound and now must determine if the second sound matches or not, and after the wait time press or not press the button. Across the population for neurons in area 46 there was a significant increase in activity for both events on correct match trials, a corresponding increase on incorrect nonmatch trials, but a significantly lower level of activity on correct nonmatch trials. This activity may be encoding the animals impending decision to go or not go after the wait time. The significantly higher levels of activity on correct match and incorrect nonmatch trials could correspond to the imminent button press, while the lower level of activity on correct nonmatch activity could correspond to the no button press.

Response Activity

During the three response periods of the task, after the wait time, more cells changed their firing rate on correct match performance than for correct nonmatch,

incorrect match, or incorrect nonmatch performance. For correct match trials, greater than 30% of cells responded for each period while for correct nonmatch trials it decreased from 15% in the first period to 8% in the last period. During the last period on correct match performance is when the animal would be consuming a reward, while on correct nonmatch trials there was no reward, and thus no reward-related neuronal activity.

In order to examine the activity in these time periods relating to the response, the firing rate data was aligned by button press with the time directly before and after the button press examined for correct match and incorrect nonmatch trials. In general, pre-response and first post response periods had a similar percentage of neurons change for both correct match and incorrect nonmatch trials. This pre-response activity may indicate possible initiation for sending a signal to premotor areas to prepare for a motor response, as well as reflecting the actual motor response (activity occurring in the first post response period) as it occurred on both correct match and incorrect nonmatch trials when a motor response occurred. This type of response-related activity has also been found in the lPFC during visual working memory tasks. Most often in the visual literature saccades are used for a motor response and pre-saccadic as well as post-saccadic activity has been found (Constantindis et al., 2001; Funahashi et al., 1991; Hasegawa et al., 1998). However, during the second post response period for correct match trials 51% of neurons had a change compared to 18% of neurons on incorrect nonmatch trials. Again, this period is most likely when the monkeys are consuming the food reward, and thus this could be reward-related activity on correct match trials (Figure 31 A and Figure 32 A). In addition, a few cells showed an increase in activity in this second post response period on incorrect match trials, which could be coding reward expectancy or registering the

lack of an expected reward, (Figure 33 A). Areas of the lPFC including area 46 have been found to show reward-related and reward-expectancy activity for visual working memory tasks (Hikosaka & Watanabe, 2000; Kennerley & Wallis, 2009; Leon & Shadlen, 1999; Wallis & Miller, 2003; Watanabe, 1996). This would indicate that at least some of the changes in firing rate at the end of the correct match trials seen here reflects reward-related activity, suggesting this region is encoding information in a similar way for auditory working memory task demands as it does for visual working memory.

Multi-event Coding Neurons

Many neurons (72%) responded to two or more events throughout the DMTS task. We termed these neurons multi-event coders as they were encoding for more than one part of the memory task, such as encoding cue 1, cue 2, and the response periods (Figure 24). Neurons such as these have been found in visual working memory tasks as well, (Asaad et al., 1998; Asaad et al., 2000; Freedman et al., 2002; Freedman et al., 2003; Rainer et al., 1998; Warden & Miller, 2007). This type of coding provides support for the theories that position the lPFC as a key player in working memory, whereas, regardless of modality it tracks important cues involved in the task, maintains some information during delay, and is involved in response and reward aspects of the task.

Sound Selectivity

Sounds played in the cue 1 position across all trial types were analyzed for stimulus selectivity. During the DMTS task, there did not seem to be any one particular sound type that neurons in the lPFC responded to more than others. In general, 33 to 41% of neurons responded to at least one sound type (Animal, Human, Monkey, Music, Natural, Pure tones, Synthetic or White noise, one exemplar per recording day). A

majority of those neurons (73%) were selective for one or two sounds (out of neurons responsive to at least one sound stimulus, N = 124). The other neurons (27%) were selective for between 3 and 8 sounds. This demonstrates a fairly high level of selectivity among the sounds used during the auditory working memory task. There were also a small percentage of neurons (8%) that were not selective, in that they fired to all 8 possible sounds presented on the recording day. Both types of coding seem necessary to perform this type of auditory memory task. Evidence from visual working memory suggests neurons in the lPFC are flexible and sub-populations of individual neurons represent relevant information related to a particular task including individual visual stimuli, location, and/or rules of a task (e.g., go left, go right) (Asaad et al., 1998; Asaad et al., 2000; Rainer et al., 1998; Wallis et al., 2001). Neurons responding to one or two individual sound stimuli could be encoding the particular sound stimuli in a specific way so that the matching/nonmatching comparison can be made. Another set of neurons responding to many of the cue stimuli, used for that recording day, could be encoding the general cue information. This general cue encoder could be assisting with ordering events within the task for example, 'now is cue 1'. The neural activity found here is congruent with what has been found visually.

Population and Trial Type Summary

In general across the population by trial type, neurons encoded multiple events similar to what was found with the single-unit activity. For example, there were increases in activity during cue 1 and cue 2 for the correct trial neurons (N = 215). There was also a significantly higher level of activity during cue 2 compared to cue 1 for correct trials, which suggests that during cue 2 there is an evaluation to begin determining if this

stimulus is a match or nonmatch compared to the sample. There was also a trial type effect found when a difference score between cue 2 and cue 1 was computed, indicating a possible 'match enhancement' when the second cue is presented. This 'match enhancement' could be due to the recognition of the same sound being presented. The high level of activity during cue 2 indicates that the lPFC is involved in the recognition of a same or different sound, in addition to stimulus memory and delay encoding, which supports the hypothesis of the region role as being involved in decision making.

During the event periods that occur when a comparison between the sample and cue 2 or response choice are possibly being made (cue 2, cue 2 offset, wait time), different levels of neuronal activity were observed between trial types. This indicates that as a population, neurons are encoding which response choice the monkey will make, go or no-go. During the correct match trials, there was significantly more activity during all three possible "decision" events (cue 2, cue 2 offset, wait time), compared to correct nonmatch trials. This could be evidence of a 'match' effect during correct performance. To further examine this hypothesis we examined the sub-population that had incorrect nonmatch trials where the monkeys' incorrectly respond as though they "thought" it was a match trial. In this instance during all three events, correct match activity was significantly higher than correct nonmatch or incorrect nonmatch activity. During cue 2 offset and wait time, incorrect nonmatch activity was also significantly higher than on correct nonmatch trials, indicating that the animal may be perceiving the sound stimulus as a 'match' and encoding an error. For this population of neurons the mistake does not appear to be during encoding of the sample stimulus because during cue 1 there was no difference between correct nonmatch and incorrect nonmatch activity. This suggests that

the error occurs later on, possibly during cue 2 offset or during the wait time as that is when correct nonmatch activity is less than on incorrect nonmatch trials, thus the incorrect nonmatch activity is more like the correct match activity during those two events. In general, neural activity during errors made on nonmatch trials suggested that the monkey encoded cue 2, as if it was a 'match' presentation.

On correct match trials the monkey prepares for making a motor response and could also be anticipating a reward; whereas on correct nonmatch trials, monkeys will not button press and will not receive a reward. High levels of activity across neurons on correct match and incorrect match led to button presses, whereas a lower level of activity during cue 2 offset and wait time on correct nonmatch trials led to no button press. This implies that if the overall activity of the region could be measured in real time it would predict the monkey's choice behavior. If the level of activity was high on a match trial, the monkey could be expected to button press; while if it was high on a nonmatch trial an error of button pressing could be predicted. Likewise, if the overall activity during these events was lower on a nonmatch trial, the correct no-go response would be predicted. In population analysis, neuronal activity was averaged across recording days and neurons, which indicates that the region as a whole may be encoding this decision. The higher level of activity seen during the cue 2 offset and wait time periods also supports the role of this region as being involved in decision making as well as working memory. Neurons in vlPFC were found to encode a monkey's choice decision in an auditory oddball task (Russ et al., 2008), thus the results found here are comparable. In the future using multi-unit activity or local field potential signals, which would average the activity of many

more neurons, could help determine if clusters of neurons on the same day encode a similar prediction of choice outcome.

Passive Listening

Monkeys heard 8 sound stimuli during the first passive listening block and neurons were not responsive to any one sound type over another sound type. The neurons were also fairly selective with 93% (responsive to at least one sound stimulus) and in general responded to between 1 and 4 sounds. This is comparable with sound selectivity during the DMTS task.

When a larger sound set (96 sounds) was presented to neurons in this region during the second passive listening block a pattern of selectivity similar to the DMTS task was demonstrated. The percent of neurons responsive to sounds across any one sound type (e.g.. all animal sounds) was between 7 and 9 for all sound types meaning there was no one sound type that neurons were selective for over another sound type. There were also no groupings of neurons responsive to selective stimuli based on the acoustic properties we examined including HNR or spectral mean values.

A considerable amount of neurons (34%) were selective for between 1 and 4 sounds, illustrating a high level of selectivity to specific sound exemplars. This is similar to what Romanski et al. (2005) found when playing a large set of sounds to neurons in the nearby vlPFC (within the inferior convexity) where most neurons responded to between 1 and 5 vocalizations. Even though our recording areas are more anterior, include more sound type presentations, the selectivity of the neurons still appears to be similar.

Overall 97% of neurons were responsive to at least one sound stimulus which is higher than what has been found by previous groups, however our recording area is more

anterior and dorsal than what has been previously tested (Romanski & Goldman-Rakic, 2002). Another reason we may have found more auditory responsive neurons is because we presented a greater variety of sounds besides pure tones and monkey vocalizations including music, natural sounds, synthetic, other animal and human vocalizations. Thus, this region has a portion of neurons which is very selective in that the neurons are responsive to only a few sounds (1-4) and a portion of neurons that are not selective, responding to many sounds. Both types of encoding could be important for solving working memory problems, with some neurons tracking specific stimuli and some being more broadly responsive to assist with tracking new incoming stimuli.

Comparisons Between Active and Passive Listening

The stimuli that particular neurons responded to were variable between tasks. Neurons that were held from the first passive listening block and during the DMTS task changed which sounds they responded to. Some neurons fired to different sounds during passive listening than during the DMTS task (26%). A smaller portion of neurons was responsive to some of the same sounds during both the passive listening and DMTS task but then began responding to additional different sounds during the DMTS task (17%). For neurons held from the DMTS task to the second passive listening set 66% of neurons were responsive to sound stimuli in both tasks. Of those, 83% stopped responding to the sounds they had responded to during the DMTS task but started responding to new sounds presented during passive listening. The lPFC frequently fires to a specific visual cue in one task but not the same visual cue in a different task (Miller et al., 1996). Furthermore, neurons in the lPFC have been found that encode an associative rule of a task in one condition but not in another task condition (Asaad et al., 2000; Miller et al.,

1996; White & Wise, 1999; Rainer et al., 1998). This type of neuron, which changes it selectivity (either to cues or rules) in different contexts, may be how the PFC maintains flexibility for coding information during working memory tasks (Miller et al., 1996; Fuster, 2000).

Summary of Dorsal and Ventral Placements

In general, there were neurons responsive to each event within the task in both dorsal and ventral banks of the principal sulcus. A larger percentage of neurons responsive to the delay were located within the ventral bank for both correct and incorrect trials. Both regions are associated with delay activity in a variety of tasks (Funahashi et al., 1989; Rainer et al., 1998). Furthermore, a greater percentage of neurons were responsive across the response periods within the ventral bank compared to neurons within the dorsal bank. These differences could be influenced by more recording placements in ventral tissue. Both dorsal and ventral banks have been shown to be responsive to cues, delay, reward, and planning events (Assad et al., 2000; Assad et al., 1998; Romanski & Goldman-Rakic, 2002; Wallis et al., 2001; Warden & Miller, 2007). The larger portion of neurons encoding events in the ventral region in this study corresponds with PET imaging (Poremba et al., 2000) where the greatest change in activity was found in the ventral bank.

Auditory and Visual Working Memory

The lPFC has been shown to encode various events within visual working memory tasks including, cues, delay, response, and reward events. Here, we have demonstrated that area 46 also encodes events within an auditory working memory task. Various theories describe lPFC as a ‘decision maker’ and besides tracking cues and rules

of a task, it interacts with other brain regions (IT, PPC) to perform a working memory task (Miller, 2000; Fuster, 2000). Evidently in this particular auditory working memory task, it is encoding key events such as the cues, delays, decision, and response periods of the task.

Although lPFC is utilized by visual and auditory working memory demand, the underlying circuitry sending connections to lPFC are different. The neural circuitry for visual working memory includes inputs from early visual processing regions within the occipital lobe which sends visual information through structures in the inferior temporal lobe, and parietal lobe, to the lPFC. The lPFC also sends connections to inferior temporal cortex and parietal lobe, and during working memory demand these regions have been shown to interact with lPFC (Miller et al., 1996; Fuster, 2000). The recording data in this experiment suggests lPFC is involved in auditory working memory in a similar manner, yet auditory information comes from different input regions. The early auditory processing areas from the temporal lobe also send connections through parietal cortices to lPFC, but the connections continuing through the temporal pole route through the superior temporal gyrus, instead of through the inferior temporal gyrus as for visual stimuli. One critical region for auditory working memory in monkeys is the rostral superior temporal gyrus (rSTG) (Fritz et al., 2005). In visual studies, neurons within lPFC encoded more information regarding a cue as matching the sample, while neurons in IT encoded more specifically the individual visual stimuli (Miller et al., 1996; Miller & Desimone, 1991). It is possible the rSTG performs a similar function for the auditory stimuli, representing acoustic properties (such as pitch) allowing the lPFC to encode more information about the task in general, such as timing related to cue 1 (sample) vs.

cue 2 (compare to cue 1). Recordings from areas such as rSTG and other temporal cortical regions during the task could address this question.

Our study found a lower level of delay activity for auditory working memory compared to visual working memory. In many visual tasks, there is a high level of delay activity, and although we found some; it was not as high across the entire delay. This may be one reason why the memory of monkeys is poorer for auditory tasks at slightly longer delays (~30 s) compared to no impairment at that delay length with visual stimuli (Fritz et al., 2005).

While there are some differences between auditory and visual working memory there appears to be more in common within lPFC as the region encodes cues, delay, response periods, rewards, and expresses match enhancement. The current findings support the hypothesis that lPFC is involved in working memory for at least two modalities, and that this region has more neurons which are responsive to auditory stimuli than previously demonstrated. Future studies could examine differences in delay activity between modality, as well as different delay lengths. Further work to understand auditory working memory should include recording neuronal activity in other regions that send projections to the lPFC (e.g. rSTG) and include lesions or temporary inactivation experiments to determine if lPFC is critical for performance of this task.

CHAPTER 5. GENERAL DISCUSSION

Traditionally, working memory and its neural underpinnings have been studied in the visual domain. One line of research has suggested that the cholinergic system is involved in visual working memory, and a rich and diverse amount of research has investigated the lPFC as a primary area for visual working memory. This dissertation examined auditory working memory and found similar patterns of neurophysiological activity for processing task events between visual and auditory working memory.

The first two experimental chapters demonstrated that the cholinergic system is involved in auditory recognition memory in a comparable fashion to visual recognition memory. In chapter 2, blocking ACh impaired performance on an auditory recognition/working memory task in a dose dependent manner. Analogous results have been found in multiple species, across a wide array of tasks, when using visual stimuli (Aigner & Mishkin, 1986; Aigner, Walker, & Mishkin, 1991; Elsmore et al., 1989; Flicker et al., 1990; Hironaka & Ando, 1996; Hudzik & Wenger, 1993; Myers et al., 2002; Ogura & Aigner, 1993; Penetar & McDonough, 1983; Pontecorvo, et al., 1991; Pontecorvo & Evans, 1985; Spinelli et al., 2006**;** Taffe et al., 1999). Chapter 3 investigated the specificity of the effect of blocking ACh by administering an ACh agonist (physostigmine) at the same time as an ACh antagonist (scopolamine). When both drugs were administered together performance on the DMTS task improved compared to performance on scopolamine alone. This performance reversal supports the hypothesis that the cholinergic system is complementarily engaged in auditory and visual working memory as the same type of improvement has been demonstrated in visual working memory (Hironaka & Ando, 1996). As administration of the agonist alone did

not improve performance, other neurotransmitter systems may be involved or perhaps a specific level of ACh is needed to perform working memory tasks well. Future studies should examine neural structures that are fundamental to working memory and perform direct infusions of agonists or antagonists in specific brain regions such as the lPFC to test the effects on behavioral performance. The role of ACh in working memory paradigms in both the auditory and visual domains remains to be investigated further, possibly examining different processes such as encoding versus retrieval.

Chapter 4 investigated the neural correlates of auditory working memory in area 46 and found that this region of the lPFC has neurons that are responsive to auditory working memory components similar to neural responses during visual working memory tasks. Neurons in the lPFC have been found that respond to visual cues, the delay portion of tasks, the wait time, response, and reward times. We found neurons in area 46 that had significant changes in activity to auditory cues during passive listening and the DMTS task. There were also neurons that responded to the delay, wait time, response and reward aspects of the DMTS task. All of these neuron types have been found within the visual working memory domain, lending support to the hypothesis that the lPFC, including area 46 is functionally involved in representing key pieces of information for working memory. Finding evidence of similar neurons here in the auditory domain supports the hypothesis of the lPFC as being generally engaged in working memory, regardless of modality. Lesion or temporary inactivation experiments would be necessary to verify if area 46 is essential to auditory working memory tasks.

An interesting question for the future would be to investigate how individual neurons within lPFC encode both auditory and visual stimuli in the same working

memory task. It is possible the same neuron could respond to both stimuli types within the same task. It has been suggested that for some multimodal neurons, visual stimuli enhance the responsiveness of neurons to auditory stimuli when presented together within lPFC (Sugihara et al., 2006). Another exciting comparison could be to examine the delay period for visual-visual cues compared to auditory-auditory cues (within blocks) for the same neuron. Would a neuron with a strong sustained delay response for visual cues also have the same sort of delay profile for auditory cues? This could help elucidate if a memory component between modalities differs under similar working memory demand. It has already been suggested that neurons in lPFC encode visuospatial and audiospatial memory demand in a complimentary manner (Kikuchi-Yorioka & Sawaguchi, 2000).

This study discovered a much higher level (97%) of auditory responsive neurons (responsive to at least one sound stimulus), than what has been reported previously (Azuma & Suzuki, 1984; Romanski & Goldman-Rakic, 2002). This result could be due to the greater variety of sound types presented to the monkeys that included music, natural sounds, synthetic sounds, animal and human vocalizations, which encompass many more possibilities to evoke activity compared to the traditional exemplars used, which include monkey vocalizations, white noise, and pure tones (Azuma & Suzuki, 1984; Romanski & Goldman-Rakic, 2002).

Another reason more sound-evoked activity was recorded in this study could be due to the exact recording location within lPFC. Earlier studies found fewer auditory responsive neurons in recordings taken mainly from the most posterior end of the principal sulcus and/or from below the arcuate sulcus including areas 8, 12, and 45 (Azuma & Suzuki, 1984; Romanski & Goldman-Rakic, 2002); whereas our recordings

were from more rostral locations, along the principal sulcus from the dorsal and ventral banks of area 46. Previously, in cases where many auditory responsive neurons were found, recordings were taken from a small region in area 45 near the inferior prefrontal dimple (Romanski et al., 2005; Gifford et al., 2005). Thus, the large portion of auditory responsive neurons found here is new for the general region of the lPFC, and verifies area 46 as a sound responsive region (Poremba et al., 2003).

Finally, our monkeys have been in training with some form of an auditory memory task for many years, which could influence the way neurons in the PFC perceive stimuli. All of the sounds heard during passive listening were used in the behavioral DMTS task on a different day. This could contribute to a 'familiarity' effect where neurons were responsive because at some point in the past they needed to 'encode' that sound to perform the memory task. This influence may have come from other areas involved in long term memory such as the medial temporal lobe (MTL) where 'familiarity neurons' (neurons which shift their firing rate to stimuli on long term familiarity basis) have been described (Brown & Aggleton, 2001; Yassa & Stark, 2008). This would be a wonderful hypothesis if our stimuli were visual in nature. Areas in the MTL such as the perirhinal cortex are essential for visual recognition memory (Buffalo et al., 2000), however for auditory recognition memory it has been demonstrated that when these regions are lesioned, auditory recognition memory is not affected. Instead, the superior temporal gyrus is the essential area for auditory recognition memory (Fritz et al., 2005). Perhaps, it is possible that neurons in the superior temporal gyrus encode something like 'familiarity' for auditory cues as perirhinal cortex does for visual cues (Yassa & Stark, 2008). Lastly, the neurons in PFC may not be influenced by 'familiarity'

at all and are simply responsive; an experiment using novel sounds could elucidate this further.

Visual and auditory cues appear to share the lPFC and its processing capacity for working memory in a similar fashion. It is interesting to note that while auditory and visual information are both processed within lPFC for short-term memory demand the essential brain regions for long term memory between visual and auditory domains are different. Essential cortices for visual long term recognition memory including the perirhinal, entorhinal and parahippocampal cortices (Buffalo et al., 2000; Davachi & Goldman-Rakic, 2001; Málková et al., 2001; Meunier et al., 1993; Murray & Mishkin, 1986; Suzuki et al., 1997; Suzuki et al., 1993; Yassa & Stark, 2008; Zola-Morgan et al., 1989) are not essential for auditory memory, as lesions of rhinal cortices did not impair performance on an auditory memory task (Fritz et al., 2005). Monkeys with intact cortices performed below 75% at delays up to 50s with auditory cues (Fritz et al., 2005), thus even when the rhinal cortices are available the monkeys are not utilizing them for longer auditory memory delays. This is in sharp contrast to the visual domain where monkeys can remember a visual object after one trial for 15-20 minutes (Murray & Mishkin, 1998; Zola et al., 2000). Thus, while general working memory processes for auditory and visual cues within lPFC are shared, long term memory processes must occur in different brain regions and/or operate on different time scales (auditory memory fading in seconds; visual memory lasting for minutes).

In summary, auditory working memory appears to be analogous to visual working memory in two ways: first they share a similar neural mechanism via the cholinergic system and second, the lPFC which is essential for visual working memory is involved in

auditory working memory in a congruent fashion. Neurons in lPFC encode cue, delay, response, and reward information for both auditory and visual working and recognition memory tasks. It remains to be determined if the lPFC is essential to auditory working memory as it is for visual memory. Future considerations of working memory in lPFC should examine other modalities within the region as well as single-unit contributions. Determining that a region such as area 46 performs a parallel role in working memory for two modalities greatly extends our understanding of the general role of lPFC. Despite visual and auditory information coming in through very different portals and sending connections from different sub regions (inferior temporal lobe vs. superior temporal lobe) both auditory and visual working memory demands are processed by lPFC. Future experiments should investigate other modalities and the possible role of lPFC. In addition, it will be important to investigate the multi-modal interactions between stimuli that are both auditory and visual within lPFC.

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