INVESTIGATING THE CHOLINERGIC REGULATION OF HUMAN LEARNING AND MEMORY USING FUNCTIONAL MAGNETIC RESONANCE SPECTROSCOPY

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By

Kyle F. Shattuck, B.A.

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ABSTRACT

Current theories consider the effects of acetylcholine neuromodulation in neuronal ensembles as a bidirectional network modulator: increased levels set network dynamics to process external stimuli, and decreased levels set network dynamics to process information that has been previously stored. Accordingly, invasive measurements in animal models have shown that acetylcholine drives neural activity increases during tasks of memory encoding and attention. Using noninvasive proton magnetic resonance spectroscopy ($^{1}$H-MRS) while subjects performed a configural working memory task, our first study aimed to be the first to measure increases in cholinergic neurotransmission during memory encoding in human medial temporal lobe. We found that changes in choline-containing compounds over minute-long task blocks correlated with the subjective difficulty of the task across subjects.

More recent animal work during tasks of attention have shown that increases in acetylcholine appear on much faster timescales than had previously been considered; these phasic increases occur in coordination with learned behavioral responses to sensory stimuli and last for only seconds. In addition, research employing pharmacological functional magnetic resonance imaging (fMRI) in human subjects have indicated that
acetylcholine may not only activate areas required for external focus, but also decrease ongoing neural activity in brain areas that may interfere with task performance. Converging these lines of evidence, our next experiments collected fMRI and $^1$H-MRS measurements in ventromedial prefrontal cortex while subjects performed a sustained attention task. The results show that stimulus-based phasic cholinergic activity coincides with phasic reductions in neural activity. Together, these findings support the theory that acetylcholine works in a coordinated manner on both tonic and phasic timescales to control a global brain-state switch between the processing of externally generated and internally generated information.
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# TABLE OF CONTENTS

Chapter I. Introduction .................................................................................................................. 1

A. Acetylcholine as a regulator of global brain state ................................................................. 1

B. Acetylcholine as a regulator of memory .................................................................................. 2

C. Acetylcholine as a regulator of attention ............................................................................... 4

D. Measuring acetylcholine in humans ...................................................................................... 6

E. Investigational overview ......................................................................................................... 9

Chapter II. Cholinergic regulation of memory encoding in medial temporal lobe ............... 10

A. Summary ................................................................................................................................. 10

B. Introduction ............................................................................................................................ 11

C. Experimental procedures ...................................................................................................... 14

D. Results .................................................................................................................................... 26

E. Discussion ............................................................................................................................... 31

Chapter III. Cholinergic regulation of attention in ventromedial prefrontal cortex ............ 39

A. Summary ................................................................................................................................. 39

B. Introduction ............................................................................................................................ 40

C. Experimental procedures ...................................................................................................... 44

D. Results .................................................................................................................................... 58

E. Discussion ............................................................................................................................... 67

Chapter IV. Discussion .................................................................................................................. 71

A. Summary of findings ............................................................................................................. 71

B. Interpretation of $^1$H-MRS measurements as a proxy for cholinergic neurotransmission .. 73
C. Interpretation of memory encoding results ................................................................. 74
D. Interpretation of sustained attention results ............................................................. 75
E. Integrated findings and implications for translational research ............................. 77

Bibliography .................................................................................................................. 79
LIST OF FIGURES

Figure 1. Configural working memory task (cWMT) .................................................................16
Figure 2. Example of cWMT card deck ..................................................................................19
Figure 3. Voxel position .........................................................................................................22
Figure 4. In-scanner task performance correlates with post-learning test performance ...........28
Figure 5. MRS measurements by task block .........................................................................29
Figure 6. MRS correlations with task performance ...............................................................32
Figure 7. MRS correlations with post-learning test performance ..........................................33
Figure 8. Sustained attention task: trial procedure .................................................................46
Figure 9. Regions of interest ..................................................................................................51
Figure 10. [Cho] levels across tasks .......................................................................................56
Figure 11. Tonic BOLD deactivations increase as dSAT accuracy decreases .......................61
Figure 12. Phasic BOLD decreases while phasic [Cho] increases in vmPFC .........................62

LIST OF TABLES

Table 1. Behavioral results for the sustained attention task ..................................................59
CHAPTER I
INTRODUCTION

A. Acetylcholine as a regulator of global brain state

As a neuromodulator that reaches all regions of the cortex and all major brain nuclei (Woolf 1991, Zaborszky 2002), acetylcholine has a central role in controlling brain-wide states that adapt to changes in environmental conditions (Picciotto 2012). The classic understanding of acetylcholine in the brain as an arbiter of general arousal (Phillis 1968) is still supported by observations of large diurnal fluctuations (Paolone 2012) driving accommodative behavior such as wakefulness and the enhancement of sensory perception (Jones 2005). More recent work has focused on how increases in acetylcholine play a critical role in setting neural dynamics for attention (Sarter 2006) and memory encoding (Hasselmo 2006). In addition, the notion of acetylcholine as a typical neuromodulator that works on timescales of minutes to hours regulating large neural circuits (Sarter 1997) has been augmented by studies showing that local circuitry regulates phasic cholinergic signalling on the timescale of seconds critical to attentional (Sarter 2014) and memory (Gu 2011) functions. While advances in our understanding of how acetylcholine controls network dynamics on both large and small scales have come primarily from invasive measurement techniques in animals, the studies presented in Chapters II and III aimed to translate these findings into a human model.
B. Acetylcholine as a regulator of memory

Evidence for acetylcholine’s role in human memory formation points to a bidirectional role at different stages of learning; high levels of cholinergic activity enhance memory encoding, but reduced levels are required for memory consolidation (Hasselmo 2006). The longest standing evidence has come from effects of sleep on memory, which show that the duration of slow wave sleep — during which brain acetylcholine is at its lowest daily levels — correlates with sleep-related memory improvements (McGaugh 2000). Pharmacological evidence in humans has circumscribed the consolidation benefits of sleep to acetylcholine levels rather than other processes. Subjects given post-encoding acetylcholinesterase inhibitors, which prevent the breakdown of acetylcholine and thus keep extracellular levels high, do not show sleep-related consolidation benefits compared to placebo treated controls (Gais 2004). Further, subjects who do not sleep but receive combined nicotinic and muscarinic receptor antagonists to reduce cholinergic processing, show a memory consolidation benefit compared to awake controls that receive placebo (Rasch 2006). The same subjects were impaired on encoding of new information when it was presented during periods of cholinergic antagonism, reinforcing the necessity of increased cholinergic activity for memory encoding. These results isolate cholinergic processing as a regulator of neural organization that switches between states of acquiring external information and consolidating previously acquired information. Further, they show that states of consolidation do not require sleep, per se, but are also observed during periods of quiet waking when cholinergic levels are low (Carr 2011, see also Logothetis 2012).
Physiology experiments in animals and subsequent computational modelling have provided evidence that the presence of acetylcholine in cortical and hippocampal neural circuits acts enhances the processing of afferent sensory information. For example, when acetylcholine levels are high in cortical areas, the spiking patterns of the main output cells (layer V pyramidal neurons) are increasingly driven by afferent thalamocortical inputs as opposed to cortico-cortical feedback communications (Hasselmo 2004); thalamocortical inputs represent the pathway for information coming from external stimulus representations. In addition, the presence of acetylcholine reduces fast spiking accommodation in pyramidal neurons, allowing them to maintain persistent spiking patterns for tens of minutes and possibly beyond (Heys 2012). Persistent output firing can maintain a representation of information within a neuronal circuit for extended periods, thus providing a mechanism for temporary information maintenance required in tasks such as working memory and sustained attention. Importantly, high levels of acetylcholine also act on local inhibitory interneurons in cortical circuits, causing them to selectively inhibit lateral feedback activity between pyramidal neurons (Porter 1999). Network level patterns of lateral feedback connections between cortical neurons can be conceptualized as the long-term representations of previously stored information (Hasselmo 2004). Thus, by reducing the representations of previously stored information while simultaneously enhancing sensitivity to external stimuli, the presence of acetylcholine shapes cortical networks toward behavior driven by responses to the current environment. As such, acetylcholine can act as a global state switch between the processing of external and internal mental representations, correlating with periods of memory encoding and memory consolidation.
C. Acetylcholine as a regulator of attention

Given that acetylcholine drives persistent excitatory spiking activity, it is perhaps surprising that some brain areas actually reduce overall activity in the presence of acetylcholine. For example, areas of the subgenual ventromedial prefrontal cortex (vmPFC) in macaques have higher spiking output during periods of non-REM sleep, when the cortex experiences its lowest levels of acetylcholine (Rolls 2003). Further evidence of this phenomenon has been provided by a neuroimaging meta-analysis of studies employing agonists of nicotinic acetylcholine receptors (nAChRs; Sutherland 2015), showing that this same vmPFC region, among others, deactivates across several different types of tasks during cholinergic stimulation. Interestingly, the network of areas that deactivates in response to nAChR stimulation corresponds well to brain-wide networks that deactivate during behavioral tasks which require attention to external stimuli, commonly referred to as the default mode network (DMN; Raichle 2001). From a mechanistic understanding of acetylcholine as a regulator of balance between afferent and feedback activity within neural circuits, a reduction in activity might be expected in areas with a greater weight of feedback connections compared to afferent connections — areas that would be inherently more active during processing of previously stored information; this would cause an overall dampening of activity when cortico-cortical communication is suppressed. This point of view places acetylcholine as a candidate to mediate the anti-correlations observed in functional connectivity between the DMN and areas commonly activated during externally oriented tasks, the task positive network (TPN; Fox 2005).
The behavioral importance of separation between DMN and TPN activity is exemplified by the findings that the strength of anticorrelation predicts the amount of attentional effort required to perform a task (Esterman 2014) as well as the subjective difficulty between subjects (Kelly 2008). Further, several disorders that include attentional impairments show reductions either in the strength of anticorrelations between between the DMN and the TPN or in DMN deactivation during externally oriented tasks — including schizophrenia (Garrity 2007), major depressive disorder (Sheline 2009), mild cognitive impairment (Haense 2012), Alzheimer’s disease (Celone 2006), autism (Kennedy 2006), and attention deficit/hyperactivity disorder (Liddle 2011). Interestingly, each of these disorders has also been associated with disrupted cholinergic processing and is often treated with cholinergic interventions.

Important contributions to the understanding of the cholinergic regulation of attention have come from studies measuring in vivo changes of acetylcholine in rodents during task performance. One common behavioral paradigm for attentional studies in rodents is the sustained attention task with distractors (dSAT). This task presents a sequence of infrequent trials, each of which may or may not contain a target (e.g., light flash). The animal is then prompted by a cue (e.g., an auditory tone), to indicate via lever (for rats; McGaughy 1995) or retractable nose-port (for mice; St. Peters 2011a) the presence or absence of a cue. The task difficulty can be increased with the addition of distracting stimuli, usually flickering house lights. Tonic levels of acetylcholine in prefrontal cortex of behaving rats — measured by microdialysis on the timescale of minutes —
approximately double from pre-task baseline to the first task block (Himmelheber 2000, Arnold 2002), then double again under distracting conditions (St. Peters 2011b). More recent work has used choline-sensitive microelectrodes to measure changes at subsecond temporal resolution, finding that target trials are associated with seconds-long spikes in cholinergic neurotransmission not seen in trials that do not present a target (Parikh 2007). Evidence that these phasic increases of acetylcholine are not tied to bottom-up attentional processes comes from the fact that the timing of the increases is associated with the learned timing of reward following them — the levels rise just before the anticipated reward is to appear, even if no reward is provided (Parikh 2008). The interpretation given for this target-related but reward-timed neurotransmission is that phasic acetylcholine activity switches network properties from ongoing processing toward a learned behavioral pattern associated with the target (Hasselmo 2011). A recent optogenetic study using the mouse variant of the dSAT supports this theory, finding that artificial acetylcholine release in prefrontal cortex on non-target trials increased the frequency of reported targets, and artificial acetylcholine suppression on target trials increased the frequency of reported non-targets (Gritton 2016). These results show that acetylcholine works to control circuit dynamics not only as a classic neuromodulator on tonic timescales, but also during very brief phasic periods when environmental demands elicit increased focus on external stimuli.

D. Measuring acetylcholine in humans

As microdialysis and selective amperometry are invasive methods that are not feasible for use in humans, measurements of changes in cholinergic activity seen in animals cannot
be directly replicated. Instead, the studies presented here utilize noninvasive proton magnetic resonance spectroscopy (\(^1\)H-MRS) to probe neurochemical changes associated with task demands. Conventional MRI excites, and measures energy emitted from, hydrogen protons associated with water molecules, the most abundant species of proton in the body. These protons can be selectively identified by the known frequency at which they receive and emit electromagnetic energy, a frequency directly proportional to the known strength of the MR scanner. \(^1\)H-MRS works on the same principles — identifying protons by their known frequencies — with the exception that the protons are associated with other metabolites of interest rather than water. The three proton species that are most abundant in typical \(^1\)H-MRS spectra are \(N\)-acetyl methyl groups which are represented in the molecules \(N\)-Acetylaspartate and \(N\)-acetylaspartylglutamate, methylamino groups represented in the molecules creatine and phosphocreatine, and ammonium trimethyl groups represented by the soluble choline-containing compounds: phosphocholine, glycerophosphocholine, free choline, and acetylccholine. As all of these metabolites occur far less abundantly than water, collection voxels must be large and many collections averaged in order to attain sufficient signal-to-noise ratios for accurate measurements.

\(^1\)H-MRS is most commonly used to estimate stable concentrations of metabolites; this can be useful in the clinic to characterize tumor diagnoses, or in research to describe compositional differences between groups of subjects. In addition, \(^1\)H-MRS measurements can be collected as subjects perform cognitive tasks in order to identify process-related changes in brain metabolism, a technique termed functional MRS (fMRS). At higher magnetic fields, signal to noise is sufficient to measure fluctuations in
signal from lactate (Prichard 1991, Urrila 2003), GABA (see Stagg 2014 for review), and, more recently, glutamate (Gussew 2010, Lally 2014).

In addition, Nishitani (Nishitani 2003) reported task-related changes in $^1$H-MRS signal for choline-containing compounds in medial temporal lobe (MTL) during a target detection task. These same subjects performed the task during magnetoencephalography (MEG) collection, and Nishitani found event-related synchronization in theta band activity when modeling dipoles at the location of the $^1$H-MRS voxel. Since physiological studies in animals have consistently found increases in theta band activity to be driven by acetylcholine (e.g., Vandecasteele 2014), the $^1$H-MRS signal increases were interpreted as being produced by spikes in cholinergic neurotransmission. For Nishitani’s interpretation to be reasonable, a plausible mechanism for how changes in cholinergic neurotransmission might affect the observed $^1$H-MRS signal must be established. As extracellular increases in choline and acetylcholine — released from cholinergic nerve terminals and taken back up into the cell on the order of a few seconds by a high affinity choline transporter (Yamamura 1972) — have been shown to increase by amounts in the range of 400% on both tonic and phasic timescales (Pepeu 2004, Parikh 2007), then changes in signal based on compartmentalization are possible. Compartmentalization can restrict the resonance of the chemical species contributing to the observed signal, especially through interactions with other intracellular molecules; in the case of the ammonium trimethyl groups contributing to the $^1$H-MRS signal, a dominant molecular interaction is involvement with phosphate groups of cell and vesicular membranes, to which they readily bind at near covalent strength (Yeagle 1975, Ohno 1981). Based on
Nishitani’s observations and the preceding rationale, the studies reported in Chapters II and III use $^1$H-MRS to test for task-based changes in cholinergic neurotransmission during tasks of memory encoding and sustained attention.

E. Investigational overview

The underlying hypothesis of this dissertation is that acetylcholine acts on a large scale to tune brain-wide dynamics to either external or internal information processing. As such, the presence of acetylcholine should increase neural activity in areas that process sensory stimulus information during memory encoding and attentional tasks. Additionally, it should accompany decreases in neural activity in brain areas that increase baseline activity during internal mentation. These changes are believed to occur on both tonic and phasic timescales.

The experiment reported in Chapter II employs fMRS during a configural working memory task to test the hypothesis that minute-long increases in cholinergic metabolism in MTL can be measured for the first time in humans during memory encoding. The experiments reported in Chapter III gather data using both BOLD fMRI and fMRS to test the hypothesis that cholinergic increases on both tonic and phasic timescales accompany reduced neural activity in vmPFC during a sustained attention task. Finally, Chapter IV discusses how the results of the experiments contribute to the idea that acetylcholine acts as a brain-state modulator, and explores implications of the technique for future investigations.
A. Summary

Though short-term memory has traditionally been associated with neural processing in fronto-parietal networks, recent work emphasizes the importance of medial temporal lobe structures (MTL) for working memory involving novel or configural information. Increases in MTL neuronal activity during the active maintenance period between stimulus exposure and recognition test have been shown to correlate with subsequent memory performance, indicating a role for MTL in transitioning current activity to long-term encoding. Importantly, cholinergic neurotransmission enhances both active maintenance activity in the MTL and its encoding effect. Despite the fact that task-based increases in MTL acetylcholine levels have been observed through invasive measurements in animal studies for the last two decades, changes have never been measured in humans. In this study, we use proton MR spectroscopy (\textsuperscript{1}H-MRS) to test whether cholinergic activity during active maintenance occurs locally in human MTL. \textsuperscript{1}H-MRS measurements were acquired at 3T from a 2x2x3 cm voxel in right medial temporal lobe — including portions of parahippocampal cortex, perirhinal cortex, entorhinal cortex, and hippocampus — from 36 subjects during performance of a configural visual object recognition task that can be solved only by remembering feature arrangements rather than individual features themselves. Concentration changes of
unbound choline-containing metabolites ([Cho]) were referenced to an internal standard of creatine + phosphocreatine metabolites ([Cre]) and compared between four task blocks: resting baseline, stimulus memorization, active maintenance of information, and stimulus recognition. An unannounced post-learning test of memory retention was conducted in 21 subjects. Increases from a resting baseline in the ratio of [Cho]/[Cre] were observed during 60-second blocks of active working memory maintenance across the group. Behavioral accuracy during configural working memory task performance correlated with memory retention between subjects, as measured by the post-learning test. These results show that short-timescale increases in human medial temporal lobe $^1$H-MRS [Cho]/[Cre] ratio occur during the active maintenance of configural working memory information, an activity hypothesized to drive local increases in cholinergic activity and subsequent memory formation.

B. Introduction

Working memory, classically defined as the memory system responsible for the temporary maintenance and manipulation of conscious information during a cognitive task (Baddely & Hitch 1974, Eichenbaum & Cohen 2001), has most often been attributed to neural circuits in frontoparietal regions of the brain; while, memory research on the medial temporal lobe (MTL) region has historically focused on its privileged role in the formation of declarative, long-term memories (Squire 1992, Schacter & Tulving 1994). This dichotomy occurs to such an extent that well-cited reviews of working memory may not mention the medial temporal lobe (e.g., Rottschy 2012), and reviews on functions of the medial temporal lobe may not mention working memory (e.g., Squire 2004a).
However, much research has provided evidence that particular types of information, especially novel relationships or configurations, rely on MTL processing even for short maintenance durations (see Jonides 2008 for review). A principal mechanistic proposal from this research is that cholinergic transmission tunes MTL network dynamics for active maintenance during working memory (see Hasselmo & Stern 2006 and Barry 2012 for reviews).

Direct measurements in the MTL of rats show increases of acetylcholine (ACh) during working memory tasks (Fadda 1996) that rise with task performance (Fadda 2000) as well as task difficulty (Egorov 2002), empirically independent of increases caused by motor activity (Giovannini 2001), exploration (Ihalainen 2010), or task anticipation (McIntyre 2003). Systemic administration of scopolamine, a muscarinic acetylcholine receptor antagonist, reduces spatial memory performance in rats specifically when there is a delay of more than a few seconds (Bolhuis 1988), as do selective lesions of cholinergic afferents to MTL (Johnson 2002, McGaughy 2005).

In rhesus macaques, accuracy on a delayed non-match to sample task has similarly been shown to decrease with both systemic (Penetar 1983, Aigner 1986) or local (Tang 1997) scopolamine administration to rhinal cortex, emphasizing that these working memory deficits are not driven by alterations to afferent signalling. Selective cholinergic deafferentation to rhinal cortex causes equivalent performance deficits to non-selective excitotoxic lesions under this same task paradigm (Turchi 2005).

Though invasive measurements and lesion studies are not feasible in human subjects, systemic pharmacological interventions have evidenced a similar role for MTL
cholinergic control in working memory tasks with novel or configural stimuli. Systemic scopolamine administration causes deficits in task performance (Beatty 1986, Robbins 1997, Koller 2003, Green 2005) and decreased BOLD-fMRI signal in MTL during active maintenance (Schon 2005); importantly, MTL activation during active maintenance has been correlated with subsequent performance on post-learning memory tests (Schon 2004), indicating a role for transitioning information to successful longer-term memory storage.

In order to non-invasively determine local changes in cholinergic processing in human MTL, we collected short-timescale proton magnetic resonance spectroscopy (\(^1\)H-MRS) measurements while subjects performed a configural working memory task (cWMT). Working on the same principles as common MRI techniques that identify a magnitude of signal at the resonant frequency of protons in water molecules (the most abundant chemical species in the body), \(^1\)H-MRS evaluates frequencies associated with protons from other molecules of interest. Functional magnetic resonance spectroscopy (fMRS), which collects measurements time-locked to the performance of behavioral tasks, has most commonly been used to measure metabolic changes in lactate production (e.g., Prichard 1991), GABAergic neurotransmission (see Stagg 2014 for review), and more recently, glutamatergic neurotransmission (e.g., Lally 2014). During a target detection task of emotional face stimuli, Nishitani (Nishitani 2003) showed increases in human MTL choline-containing compounds that paralleled an increase in MTL theta activity measured by magnetoencephalography. There is ample evidence that emotional stimuli, like working memory, increase levels of acetylcholine (e.g., Acquas 1996) that spur MTL
Based on Nishitani’s results for emotional stimuli, as well as previous data presented by our lab (Jones 2009), we hypothesized that we would observe an increase in MTL $^1$H-MRS signal for choline-containing compounds during active maintenance on the cWMT. Similar to rodent studies, we also expected the magnitude of increase in choline-containing compounds to correlate with task difficulty (Pepeu 2004), as determined by differences in accuracy scores between subjects.

C. Experimental procedures

Subjects

Thirty-six volunteer subjects were recruited via advertisements posted on the Georgetown University campus or using the online Georgetown Research Volunteer Program (https://georgetown.sona-systems.com) as approved by the Georgetown University Institutional Review Board. Four volunteers received course credit through the Georgetown University Psychology Department; the remaining subjects received no compensation. Data from five subjects failed to pass data quality control criteria (see MR Data Quality Control section) due to excessive movement or poor scan acquisition, resulting in data reported from 31 subjects (24 females, 7 males between 18.2 - 33.4 years; average 22.0 ± 4.0 years). All subjects self-reported as right-handed, having normal or corrected-to-normal vision with no deficiency in color perception, no history of psychiatric or neurological disorders, no use of nicotine, and no use of psychoactive medications. Subjects were screened before scanning to ensure compatibility with an MR
environment; as such, subjects were not claustrophobic, pregnant, and were able to remove all preclusive metallic or magnetic devices.

After providing informed consent according to procedures of the Georgetown University Institutional Review Board, each subject completed a standardized cWMT training module on a computer in a private office and reported feeling comfortable with task instructions. The deck of cards used during cWMT training was not one of the four decks presented during fMRS data collection (see Stimuli section below).

**Behavioral data collection**

*Configural working memory task*

Each run of the cWMT consisted of three consecutive one-minute blocks: a *memorization* block, an *active maintenance* block, and a *test* block (Fig. 1). In the scanner, the subject held a thumb-button response box in each hand — one hand designated as the *new* button and the other designated as the *old* button, the sides of which were counterbalanced across subjects. Each cWMT run was immediately preceded and followed by a one-minute *rest* block during which subjects were instructed to fixate on a centrally located fixation cross, below which appeared the word “REST”, while letting their mind wander.

During the *memorization* block, subjects were shown four serially presented cards, each card presented for four seconds, interspersed by one second of blank screen. Beneath each card, the word “OLD” appeared on the side corresponding to the subject’s response
a.  

b.  

c.  

d.  

Figure 1. Configural working memory task (cWMT)

Each subject performed the cWMT four times in the MRI scanner. Each task block lasted one minute. A rest block (a) was conducted immediately before and after each run of the three task blocks, during which subjects were instructed to keep their eyes fixed on the cross and let their minds rest. During the memorization block (b), subjects were shown four cards for four seconds each with one second between card presentations; these four cards were then repeated twice more, for twelve total presentations. Subjects pressed the button box in their hand corresponding to the “NEW” icon to indicate that it was the first time observing this card, or pressed the button in their hand corresponding to the “OLD” icon to indicate that the card had been shown before. During the remember block (c), subjects were instructed to keep their eyes fixed on the cross and keep the previously memorized cards in their mind for the subsequent test. During the test block, subjects were shown twelve different cards, four of which were the previously memorized cards. Similar to the memorize block, subjects indicated via button press if the card had been previously shown.
box for the old button, and the word “NEW” appeared on the side corresponding to the subject’s response box for the new button. The series of four cards was presented three times in identical order, for twelve total card presentations. The subject was instructed to memorize the cards and to press the new button if the card had not previously been shown, or press the old button if it had been shown; thus, the correct answers for the memorization block were always four new responses followed by eight old responses.

During the active maintenance block, subjects were shown a centrally located fixation cross, below which appeared the word “REMEMBER”, and instructed to keep their eyes on the cross while keeping in mind the four cards presented to them during the memorization block.

During the test block, subjects were shown twelve different cards with timing, appearance, and instructions comparable to the memorization block; however, eight of the cards were foils that had not been presented for memorization, and the remaining four were the cards presented during the memorization block. Presentation of the four memorized cards was pseudorandomly ordered such that one appeared within the span of each three cards presented.

Importantly, the visual and response components of the cWMT are identical between the memorization and test blocks, as well as between the remember and rest blocks; as such, comparisons between these matched blocks remove confounds for perceptual and motor representations.
In order to avoid memory interference between runs, four novel decks of cards — each with distinct colors and shapes — were presented across the four cWMT runs. The order of deck presentation was counterbalanced across subjects. Each deck of cWMT cards comprised 16 cards, including all possible combinations between four binary stimulus dimensions: shape, color, number, and orientation. For example, each card from the deck used during task training (Fig. 2) presented either one or two (number) blue or orange (color) $X$’s or $O$’s (shape) aligned vertically or horizontally (orientation). Before each run, four of the 16 cards were pseudorandomly selected for presentation during the remember block, ensuring that no pair of the selected cards matched across more than two stimulus dimensions. As such, each of the foils presented in the test block necessarily matched at least two of the remembered cards in more than one stimulus dimension, and task performance relied on the subject remembering the configural relationships between stimulus attributes. All stimulus presentation scripts were written in MATLAB (Mathworks, Natick, MA) using the Psychophysics Toolbox extensions (Brainard, 1997; Pelli, 1997; Kleiner et al, 2007). To enhance shape contrast, stimuli items were outlined in black and presented on grey cards; all screen backgrounds were black, and all text was white.

Post-learning test

Beginning 5-10 minutes after scanning, 21 of the 31 subjects performed a post-learning test to evaluate memory retention on a computer outside the scanner. Subjects were not
Five cards decks, one for task training and one for each of the four runs performed by each subject, were constructed based on the same principle. Each deck consisted of sixteen cards representing all combinations across four stimulus dimensions: number (two or three), color (e.g., orange or blue), shape (e.g., X or O), and orientation (horizontal or vertical). Each deck of cards used unique colors and shapes.
made aware that a post-learning test would take place. The instructions and screen display were identical to the test block of the cWMT task, except that each card was presented on the screen for an unlimited duration and advanced only after the subject made a new or old decision. The sixteen cards that had been presented to the subject across four cWMT memorization blocks were randomly shuffled with the sixteen unseen foils, four from each of the decks; thus, each card in the post-learning test was either one that had been memorized or one that had never been presented to the subject.

**MR data collection**

Scanning took place at the Center for Functional and Molecular Imaging at Georgetown University Medical Center in Washington, D.C., using a 3-Tesla Siemens Magnetom Tim Trio whole-body scanner (Erlangen, Germany) and a manufacturer supplied 12-channel phased array head coil. Following a high resolution structural scan (T1-weighted magnetization prepared rapid acquisition gradient echo; TR/TE = 1900/2.52ms, flip angle = 9°, 160 1mm sagittal slices, FoV = 256x160x256mm, 1mm³ resolution) used for subsequent MRS voxel positioning, each subject performed four cWMT runs while undergoing fMRS scanning (point resolved spectroscopy sequence; TR/TE= 2000/30ms, flip angle = 90°, VOI = 30x20x20mm, bandwidth = 1800Hz, water suppression bandwidth = 60Hz, acquisition duration = 284ms, timepoints = 512; 10 averages for unsuppressed collections, 30 averages for water suppressed collections). Unsuppressed spectra used for water referencing and eddy current correction were collected before cWMT run 1, after run 2, before run 3, and after run 4. Based on the methods used in Nishitani (Nishitani 2003), the rectangular MRS voxel was manually positioned along the
hippocampal fissure — guided by the high resolution structural scan — to cover a maximal amount of medial temporal lobe grey matter while avoiding the enlarged portion of the temporal horn of the lateral ventricle (Fig. 3). Thus, the voxel included portions of hippocampus as well as entorhinal, perirhinal, and parahippocampal cortices. Head movement was minimized by padding the space between the subjects’ protective headphones and the scanning coil. Linear shims of the B0 magnetic field within the voxel location were manually optimized prior to MRS scanning.

**MR data analysis**

Quantitation of metabolites contributing to the observed $^1$H-MRS spectra was estimated using LCModel software, version 6.3 (Provencher Inc., Oakville, Canada; Provencher, 1993). LCModel fits a linear combination of canonical metabolite peaks from an empirical scanner-specific basis set to estimate concentrations and uncertainties contributing to observed data with a minimum of subjective input. Unsuppressed data from the VOI — collected with identical scan parameters except without a water suppression pulse — were used to estimate eddy current effects and improve baseline fit, line shape, and zero-order phase correction within the model.

Though a full basis set of 27 metabolites was used to fit the frequency domain data (as well as common spectral contributions from four lipids and five macromolecules to produce an informed background signal), main outcome measures were estimates of two metabolite concentrations: first, ammonium trimethyl contributions dominated by a peak
Figure 3. Voxel position

The 2x3x2 cm MRS voxels was manually positioned along the right hippocampal fissure of each subject just anterior to the temporal horn enlargement of the lateral ventricle. Color coding on a normalized anatomical template shows the overlaid voxel positioning for each subject: red areas were included in at least 27 subjects, and purple areas were included in fewer than five subjects.
at 3.22 ppm calculated from the combination of fitted peaks for glycerophosphocholine and phosphocholine ([Cho]); and second, methylamino contributions dominated by a peak at 3.03 ppm (and supported by a concurrent ethyl contribution at 3.91 ppm) calculated from the combination of fitted peaks for creatine and phosphocreatine ([Cre]). For each subject, estimates of each of these metabolite concentrations, as well as [Cho]/[Cre] ratio, were calculated for each rest block and each task block across the four cWMT runs. Metabolite-to-creatine ratios have long been the standard for clinical diagnosis in MRS imaging, and have been empirically recommended as the measure of choice for accurate detection of changes in metabolite concentrations due to both the amelioration of T2* fluctuation effects as well as correlative trends in accuracy across metabolites due to model fitting (Kanowski 2004). For example, changes in local magnetic field susceptibility due to neural activity-induced increases in blood oxygenation (commonly known as the BOLD effect) may cause confounding task-related signal drift across metabolites that is regularized by utilizing metabolite ratios rather than estimates of absolute metabolite concentrations.

**MR data quality control**

*Inclusion criteria*

Each individual time domain spectrum from the acquired data was assessed for reliability based on the determined Cramér-Rao lower bounds (CRLB) of the metabolites of interest, as well as the linewidth (FWHM) and signal-to-noise ratio (SNR) of the largest representative metabolite peak, typically N-Acetylaspartate at 2.01 ppm, to twice the root
mean square of the residuals. The CRLB of the model fit represents the lowest possible standard deviation of all unbiased model parameter estimates obtained from the data and provide an evaluation for the precision of metabolite estimations (Cavassila 2001). For each CRLB, FWHM, and SNR, Chauvenet’s criterion was used on the pooled set of all collected spectra in order to determine an unbiased cut-off for inclusion in final analyses (Bol'sheev 1975); thus, the maximum CRLB for [Cho] was 17% (median = 8%), maximum CRLB for Cre was 16% (median = 8%), maximum FWHM was 0.143 ppm (median = .086 ppm), and minimum SNR was 4 (median = 9). Five subjects had at least one spectra excluded from each of their four cWMT runs and were excluded from further analysis. Among the remaining 31 subjects (682 total spectra), 21 spectra were excluded due to failed quality control measures. Subsequent statistical analyses included data only from cWMT runs with unexcluded data from all task blocks and at least one of the adjacent rest blocks.

**Determination of outcome measure**

In order to confirm that model fitting of the collected data produced correlative trends across metabolites (i.e., that higher [Cho] estimations predicted higher [Cre] estimations within the same voxel across time), the Pearson’s correlation coefficient was determined between [Cho] and [Cre] across spectra within each subject, and a z-test was performed on the Fisher transformed z-scores across subjects; the significant groupwise correlation between estimations of [Cho] and [Cre] (mean $r = 0.50$, 95% CI = [0.19 0.73], df = 30, $p = 0.0014$) determined that metabolite ratios should be used as a more sensitive outcome measure than metabolite concentrations (based on Kanowski 2004).
In order to confirm that poorer scan quality within a subject correlated with the variance of metabolite ratios between scans measured for that subject (i.e., that a subject with poorer overall scan quality showed greater changes in \([\text{Cho}] / [\text{Cre}]\) within the same voxel across time), a Pearson’s correlation coefficient was determined across subjects for the estimated average within-spectra \([\text{Cho}] / [\text{Cre}]\) variance (based on \(\text{CRLB}_{[\text{Cho}]}^2 + \text{CRLB}_{[\text{Cre}]}^2\)) and across-spectra \([\text{Cho}] / [\text{Cre}]\) variance; the significant correlation \((r = 0.46, p = 0.0085)\) determined that metabolite ratios should be z-scored within-subjects before groupwise comparisons of concentration changes across time. Thus, the main outcome of interest for task-related metabolite concentration changes was determined to be within-subject z-scored \([\text{Cho}] / [\text{Cre}]\). To characterize independent contributions of the ratio terms, within-subject z-scored \([\text{Cho}]\) and within-subject z-scored \([\text{Cre}]\) analyses were run to follow up all analyses of the main outcome measure.

**Determination of order effects**

One-way ANOVAs for each block type provided no concern for order effects of \([\text{Cho}] / [\text{Cre}]\) measurements across runs: for \(\text{rest}\) blocks \(F = 0.25, \text{df} = 5, p = 0.94\); for \(\text{memorization}\) blocks \(F = 0.23, \text{df} = 3, p = 0.87\); for \(\text{remember}\) blocks \(F = 0.71, \text{df} = 3, p = 0.55\); and for \(\text{test}\) blocks \(F = 0.59, \text{df} = 3, p = 0.63\). In addition, paired t-test between pre- and post-task \(\text{rest}\) blocks (with subject as a random effect) revealed no significant difference in resting baseline \([\text{Cho}] / [\text{Cre}]\) whether or not the measurement was taken before or after task completion \((t = 0.18, \text{df} = 24, p = 0.85)\).
D. Results

Behavioral results

Accuracy on the cWMT (d’ = 3.71 ± 1.29, acc. = 92.6 ± 11.6%) showed that the subject group was able to perform the task at ceiling level on 68 of 124 runs. Six of the 31 subjects scored perfectly across all 4 cWMT runs. Of the 3 subjects that did not score any perfect runs, 2 got only a single trial incorrect on 3 of the 4 runs; the remaining subject also scored above chance levels (d’ = 1.5 ± 0.71, acc. = 70 ± 4.8%). Reaction times for correct answers (1.70 ± .353 sec) were significantly faster than RT for incorrect answers (2.09 ± 0.658 sec) based on a paired sample t-test among subjects with incorrect responses (t = 2.9, df = 52, p = .0049). Mean subject accuracy (d’) correlated inversely with mean subject reaction time (Pearson’s r = -0.56, p = 0.0010, 95% CI = [-.76 -.26]).

Accuracy on the post-learning test (group: d’ = 0.93 ± 0.72, acc. = 78.0 ± 12.8%) showed that 20 of 21 subjects performed significantly better than chance based on binomial tests (cutoff at 20 out of 32 correct for p < .05); the remaining subject scored 13 out of 32 correct. Post-learning test reaction times for correct answers (3.30 ± 1.28 sec) were not significantly different from incorrect answers (3.33 ± 1.17 sec). There was no significant correlation between post-learning test accuracy and reaction time (r = -0.19, p = 0.42, 95% CI = [-.57 .27]).

Correlations between the cWMT and post-learning tests were significant for both subject d’ (r = .48, p = .027, 95% CI [0.063 0.76]) and subject reaction time (r = .44, p = .049, 95% CI = [.0042 .73]) based on z-tests of Fisher-transformed Pearson’s correlation
coefficients (Fig. 4), showing that subjects who performed more accurately on the in-scanner task also performed more accurately on the post-learning recognition test.

All statistical tests were two-tailed with a significance threshold of $p < .05$.

**MR data results**

The mean and standard deviation of $\text{[Cho]/[Cre]}$ values across within-subject averages was $0.31 \pm 0.036$. To identify task-related changes in metabolite measurements, modeled concentrations of each metabolite, leading to the $\text{[Cho]/[Cre]}$ ratio, were $z$-scored within each subject across all blocks that passed quality control (see *Determination of Outcome Measure* section above); subsequent analyses refer to these measurements as $z$-$\text{Cho/Cre}$.

*Increases in fMRS signal during remember blocks*

A one-way repeated measures ANOVA showed a significant effect of block type (*rest, memorization, remember, test*) on $z$-$\text{Cho/Cre}$ ($F(3, 503) = 4.46, p = .0042$). A post-hoc Tukey HSD test revealed one significant difference between block types: *remember* blocks > *rest* blocks ($p = .0025$, 95% CI = [0.11, 0.71]) (Fig. 5).

*No observed correlations between behavioral and fMRS results*

To test for correlations between cWMT behavioral measures and within-subject changes in metabolite concentrations, $z$-$\text{Cho/Cre}$ increases above resting baseline for *remember* blocks were tested for correlation with subject $d'$ scores as well as mean within-subject reaction time, with no significant correlations found for either the in-scanner task or
a.

Figure 4. In-scanner task performance correlates with post-learning test performance

After performing four runs of the in-scanner configural working memory task (cWMT), 21 subjects performed an unexpected recognition test on 32 items: the 16 memorized cards (four from each cWMT run) and 16 previously unseen foils (four from each of the card decks). Both accuracy (a) and reaction time (b) correlated between tests. d': d-prime accuracy score, RT: reaction time.
Figure 5. MRS measurements by task block

Modeled concentrations of each metabolite, as well as the [Cho]/[Cre] ratio, were z-scored within each of the 31 subjects and averaged across the group for each block type of the configural working memory task. The [Cho]/[Cre] ratio showed a significant increase while subjects performed the remember blocks compared to the rest blocks (a). The [Cho] signal showed no significant changes between blocks (b), while the [Cre] signal drove the change in ratio (c), decreasing during remember blocks compared to rest blocks. Error bars represent standard error from the mean. REST: rest blocks, MEM: memorization blocks, REM: remember blocks, TEST: test blocks.
post-learning test (in-scanner $d'$: $r = -.11, p = .57, 95\% \text{ CI} = [-.44, .26]$; in-scanner RT: $r = .11, p = .56, 95\% \text{ CI} = [-.25, .45]$; post-learning $d'$: $r = -.24, p = .30, 95\% \text{ CI} = [-.61, .22]$; post-learning RT: $r = -.27, p = .23, 95\% \text{ CI} = [-.63, .18]$).

**Exploratory MR analysis results**

In order to explore the possibility that individual metabolite concentration estimations were independently driven by task demands, similar repeated measures ANOVAs were run on within-subject z-scored [Cho] and [Cre], referred to z-Cho and z-Cre. No significant effect of block type was found for z-Cho ($F(3, 503) = 4.80, p = .69$), but a significant effect of block type for z-Cre ($F(3, 503) = 4.86, p = .0024$) was revealed by post-hoc Tukey HSD test to be driven by a significant difference for rest blocks $>$ remember blocks ($p = .0025, 95\% \text{ CI} = [0.11, 0.71]$). Pearson’s correlation tests for z-Cho and z-Cre with behavioral measures revealed a correlation between z-Cho and $d'$ scores for rest blocks ($r = .39, p = .0032, 95\% \text{ CI} = [.037, .65]$) and an inverse correlation between z-Cre and reaction time for test blocks ($r = -.41, p = .0024, 95\% \text{ CI} = [-.66, -.060]$). In order to determine which specific block-to-block changes accounted for the z-Cho correlation with accuracy, the difference between z-Cho for rest blocks and each of the other three block types was calculated and then correlated with $d'$ scores; the only significant result found was a correlation between $d'$ scores and [rest blocks $>$ remember blocks] ($r = .37, p = .041, 95\% \text{ CI} = [.018, .64]$), showing that subjects with better accuracy had lower z-Cho during active maintenance than during rest (**Fig. 6a**). A similar follow-up test to determine which block-to-block changes drove the correlation between z-Cre and reaction time also revealed only one significant result, a correlation between
reaction time and $[\text{memorization blocks} > \text{test blocks}]$ ($r = .53, p = .0024, 95\% \text{ CI} = [.21 .74]$), showing that subjects with faster reaction times had greater $z$-Cre during stimulus recognition than stimulus encoding (Fig. 6b). Similar correlation analyses between metabolite measurements and behavioral scores on the post-learning test revealed no significant correlations with $z$-Cho/Cre but did reveal correlations between $d'$ scores and test blocks for both $z$-Cho ($r = .38, p=.044, 95\% \text{ CI} = [.12 .51]$) and $z$-Cre ($r = .53, p=0.014, 95\% \text{ CI} = [.13 .78]$). Follow-up tests were performed to identify the block-to-block changes driving the significant results revealed a correlation between $d'$ scores and $z$-Cho changes for $[\text{test blocks} > \text{remember blocks}]$ ($r = .56, p=0.0086, 95\% \text{ CI} = [.17 .80]$) (Fig. 7a) as well as between $d'$ scores and $z$-Cre changes for $[\text{test blocks} > \text{memorization blocks}]$ ($r = .59, p = .0046, 95\% \text{ CI} = [.22 .82]$) (Fig 7b). These results indicate that subjects with better memory retention on the post-learning recognition test had lower $z$-Cho during active maintenance than during stimulus recognition, and higher $z$-Cre during stimulus recognition than during stimulus encoding.

**E. Discussion**

**Summary of results**

This study demonstrated that changes in short-timescale $^1$H-MRS measurements correlate with cognitive processes in human MTL during a configural working memory task; the concentration of choline-containing metabolites increased while subjects actively maintained task-relevant information that was subsequently remembered on a post-learning memory test.
Figure 6. MRS correlations with task performance

Metabolite concentrations were z-scored across all collected scans within each of the 31 subjects, then averaged for each of the four blocks of the configural working memory task (cWMT). Metabolite z-score changes between task blocks were then correlated with task behavior. **a)** Subjects with greater [Cho] during *remember* blocks compared to *rest* blocks had lower accuracy on the in-scanner task. **b)** Subjects with greater [Cre] during *test* blocks compared to *memorization* blocks had faster reaction times on the in-scanner task. **d’**: d-prime accuracy score, **z-Cho**: within-subject z-scored [Cho] concentration, **z-Cre**: within-subject z-scored [Cre] concentration.
Figure 7. MRS correlations with post-learning test performance

Differences in metabolite concentrations between blocks on the configural working memory task were correlated with performance measures on an unexpected recognition test administered 5-10 minutes after the scanning session. **a)** Subjects with greater [Cho] during *remember* blocks compared to *test* blocks had lower accuracy on the post-learning recognition test. **b)** Subjects with greater [Cre] during *test* blocks compared to *memorization* blocks had better accuracy on the post-learning recognition test. $d'$: d-prime accuracy score, **z-Cho**: within-subject z-scored [Cho] concentration, **z-Cre**: within-subject z-scored [Cre] concentration.
Relationship to studies of MTL acetylcholine in working memory

Task-related changes in $^1$H-MRS [Cho] have been hypothesized to reflect changes in cholinergic activity by Nishitani after observations of increased [Cho] paralleling increased theta-band activity in human MTL during an emotional face processing task (Nishitani 2003). However, Nishitani also observed stimulus-related increases in [Cre], leaving open the possibility that measurements could have fluctuated due to more general mechanisms such as localized magnetic field fluctuations. Results from the current study are based on increases in metabolite ratios, showing an increase specific to the [Cho] signal in comparison with [Cre] as a baseline reference. Further, Nishitani relied on raw estimations of absolute metabolite concentrations, which are susceptible to increased scan-to-scan variability in subjects with lower scan quality; this phenomenon leads to group averages weighted toward subjects with less reliable measurements. By normalizing concentrations across all scans within each subject before combining group data, the current study removed the confounding magnitude of variance associated with scan quality, conceding that conclusions can be made only for the consistency of task-related changes rather than the size of effect.

Schon, et al., investigated cholinergically driven neural activity increases in human MTL during working memory by systemically administering the muscarinic antagonist scopolamine to subjects before in-scanner task performance with BOLD-fMRI measurement (Schon 2005). The result was a decrease in local MTL activity during the active maintenance of information, activity that had previously been shown to correlate with accuracy on a post-learning recognition test (Schon 2004). Since acetylcholine in
MTL has been shown to induce persistent spiking in rats (Hasselmo 2006) and theta activity during working memory tasks in humans (Tesche 2000) — and these two processes promote memory encoding (see Hasselmo 2014 for review) — the authors propose that local cholinergic increases were driving encoding-related neural activity. However, the use of systemic drugs does not rule out distal cholinergic effects on local MTL activation. The methods of the current study were able to show task-related changes in [Cho] specifically in MTL of untreated subjects; and, though it was not determined that these changes correlated with task results, behavioral results did replicate the finding that working memory performance enhanced subsequent recall.

**Implications of exploratory results**

Subjects who showed greater [Cho] increases during active maintenance of task-relevant information (*remember* blocks) tended to have more difficulty on both in-task recognition and recognition on an unexpected post-learning test. Subjects who showed greater [Cre] increases during in-task recognition (*test* blocks) tended to have faster reaction times as well as increased recognition on the post-learning test. Estimations of [Cre] are commonly used as an internal reference in MR spectroscopy, as its main constituents (creatine and phosphocreatine) are expected to remain at constant levels due to a slow and steady conversion to creatinine (not resolvable by $^1$H-MRS), remain soluble, and not interact with other molecules via bonds strong enough to disrupt their contribution to the resonance peak. By using an internal reference and evaluating metabolite-to-[Cre] concentration ratios rather than estimations of absolute metabolite concentrations, comparisons between spectra collected at different points in time can be made more
accurate as well as robust to line broadening and reduced signal-to-noise; the improved accuracy is due to correlational changes in model fitting in the presence of collection-related changes such as magnetic field drift, inhomogeneity, and T2 fluctuations (Kanowski 2004). We showed that model-estimated [Cho] and [Cre] were correlated within-subjects — when [Cre] increased, so did [Cho] — evidencing a noise-based fluctuation in model fit that would indeed benefit from the use of an internal standard. Estimated absolute metabolite concentrations are calculated by reference to a single unsuppressed water peak collected closely in time to the water-suppressed measurements; as such, absolute estimates present greater measurement variance both within and between subjects, and provide less statistical power when testing for differences between spectra. Having determined that subjects with poorer overall scan quality also tended to have higher variance in estimated metabolite concentrations between scans, we normalized the absolute concentration estimates within each subject and compared z-scores across subjects; accordingly, our results reflect the consistency rather than the magnitude of metabolic changes measured during task performance. The interpretation of task-based changes in individual metabolite concentrations on minute-long timescales is confounded by the conflation of BOLD-induced magnetic field changes and actual metabolite concentration changes contributing to the observed signal. Since the metabolites contributing to the [Cre] estimation are known to remain relatively constant, changes in z-Cre can cautiously be understood as representing the local field fluctuations, and possibly stand in proxy for BOLD changes due to increased concentrations of oxygenated hemoglobin in nearby vasculature following neural activation.
Unlike creatine, choline-derived metabolites do bind other molecules with strengths sufficient to eliminate contributions to the \([\text{Cho}]\) resonance detected by \(^1\text{H-MRS}\). In fact, phosphatidylcholine, the choline-derived metabolite with the highest concentration in brain, does not contribute to the observed resonance due to its involvement in the non-soluble cell membrane (Miller 1996). Free choline and acetylcholine, when stored in intracellular vesicles, also interact at near-covalent strengths to the phosphate groups of cell membranes (Yeagle 1975, Ohno 1981). As extracellular MTL concentrations of choline and acetylcholine often more than double during high activity (Pepeu 2004), this release from membrane proximity can drive the observed \([\text{Cho}]\) signal change, indicating increased cholinergic neurotransmission. Changes in \(z\text{-Cho}\), therefore, are hypothesized to represent changes in a combined signal from both local magnetic field changes as well as changes in metabolite compartmentalization, which could artificially enhance correlational analyses (if both signals are changing in the same direction to task demands) or cancel each other out.

**Significance to research and disease**

Several diseases that affect cognition present abnormal cholinergic regulation in MTL – including Alzheimer’s disease (Cummings 1998), mild cognitive impairment (Haense 2012), schizophrenia (Freedman 1995), and traumatic brain injury (Arciniegas 1999) – each of which is treated with therapeutics that modulate cholinergic activity. These diseases exhibit a frustratingly heterogeneous symptomatology, and patients may take years to optimize treatment options. Measurements of functional cholinergic activity, with and without pharmacologic intervention, are currently unavailable. As more work is
done to characterize the underlying mechanisms generating task-related changes seen in the $^1$H-MRS [Cho] signal, we move closer to a replicable measurement of cholinergic function and enable insights for potential interventions at a neural systems level.

**Conclusion**

In conclusion, this study shows task-related changes in $^1$H-MRS [Cho] specific to human MTL during the active maintenance of configural working memory information. These metabolic changes are consistent with an understanding of cholinergic activity in MTL as a driver of subsequent memory encoding.
CHAPTER III

CHOLINERGIC REGULATION OF ATTENTION
IN VENTROMEDIAL PREFRONTAL CORTEX

A. Summary

Cognitive tasks that involve maintaining attention to external stimuli result in reduced activation in several brain areas, including ventromedial prefrontal cortex (vmPFC). Systemic cholinergic agonists increase this deactivation, suggesting a role for acetylcholine in task-induced deactivations. Though direct measurements in rodent studies have shown that acetylcholine increases on both tonic and phasic timescales during tasks of sustained attention, no work in humans has investigated the nature of these effects on reductions in neural activity. While subjects performed the sustained attention task with distraction (dSAT), a task that has been cross validated in both rodents and humans for addressing cholinergic effects on sustained attention, we collected BOLD fMRI and proton magnetic resonance spectroscopy ($^1$H-MRS) measurements in vmPFC. Task induced deactivations in fMRI results were observed during the task procedure on a tonic scale, as well as on a phasic scale in response to target stimuli. Results also showed that $^1$H-MRS measurements of choline-containing metabolites increased in response to target stimuli, providing evidence that cholinergic activity induces task-related deactivations in vmPFC.
B. Introduction

Brain deactivations that occur during cognitive tasks from a resting baseline have been observed since nearly the advent of neuroimaging, and have become a common focus in research under the term default mode network (DMN). Though there is no grand consensus on why particular areas of the brain would consistently reduce neural activity during the types of tasks subjects perform in research, a common hypothesis states that ongoing internal mentation during rest is dampened by switches in attention to external stimuli (Binder 2012). This view is supported by functional connectivity studies that show anti-correlated activity between brain-wide networks that consistently respond to either intrinsic or extrinsic information processing (Fox 2005). The functional importance of anticorrelations between the DMN and task positive network (TPN) is substantiated by findings that greater anticorrelation between networks predicts better attentional task performance (Kelly 2008) and that the DMN deactivations are attenuated in several diseases of cognitive impairment (Anticevic 2012).

Evidence for a mechanistic contribution to effectively switching between default and task states has been provided by a neuroimaging meta-analysis of studies employing nicotinic acetylcholine receptor (nAChR) agonists (Sutherland 2015). These data show that systemically activating nAChRs not only enhances brain activity in TPN regions but also reduces brain activity in areas of the DMN, including the ventromedial prefrontal cortex (vmPFC). As a neuromodulator that accesses nearly the entire cortex, and increases in response to externally directed attention (Picciotto 2012), it is conceivable that
acetylcholine can mediate both the activations and deactivations in brain-wide anticorrelated networks.

The regulation of attentional processes by acetylcholine has been well studied in rodents, where direct in vivo measurements are possible. One common behavioral paradigm for these studies is the sustained attention task with distractors (dSAT). This task presents a random sequence of either target (tone or light) or blank (no tone or light) trials, each followed by a brief response period. The response period commences with the appearance of two manipulanda — retractable levers for rats (McGaughy 1995) or retractable nose-ports for mice (St. Peters 2011a) — through which the animal indicates the presence or absence of a target. Correct hits and correct rejections are rewarded, while false alarms or target misses incur a delay penalty before the next trial is presented. During some blocks of trials, distracting conditions such as flickering house lights increase the level of task difficulty, marked by reduced task performance.

Tonic levels of acetylcholine in prefrontal cortex of behaving rats — measured by microdialysis on the timescale of minutes — nearly double from pre-task baseline to the first task block (Himmelheber 2000, Arnold 2002), then double again under distracting conditions (St. Peters 2011b). Phasic levels of acetylcholine — measured by selective amperometry at sub-second resolution — manifest in seconds-long cholinergic increases time-locked to the onset of correct hits (Parikh 2007). Importantly, levels of tonic cholinergic activity are not the result of summed phasic activity, and are hypothesized to originate from distinct neural processes (Sarter 2012). Tonic cholinergic activity
correlates with task difficulty and not task performance (Sarter 2006), leading to the hypothesis that tonic acetylcholine levels regulate the cognitive control of attention. On the other hand, phasic cholinergic activity correlates with item-specific responses irrespective of tonic levels; leading to the hypothesis that phasic acetylcholine is necessary for the incorporation of cues into ongoing cognitive processes (Parikh 2008).

A revised version of the dSAT has been validated for use in humans with the specific intention of translating evidence from animal-based research on the cholinergic regulation of attention (Demeter 2008). The computer-based dSAT utilizes shorter stimulus presentations (light flashes), button presses rather than levers or nose-ports, monetary rather than gustatory reward, and a flashing background computer screen rather than flashing house lights for distracting conditions. The Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia group (CNTRICS) selected the dSAT as one of only two behavioral assessments for the specific cognitive constructs that are dysfunctional in schizophrenia (Luck 2012), and recommended its use in basic science research on healthy controls. The dSAT has been adapted for use in human neuroimaging (Demeter 2011, Howe 2013), the results of which showed that increases in right middle frontal cortex increased during the more difficult task blocks that involve distraction. This finding agrees with results from rodent studies that showed tonic increases in acetylcholine during distractor blocks. Moreover, the results from Demeter, et al., (Demeter 2011) also showed that increases in activity correlated inversely with subject accuracy, indicating that an increase in attentional effort between subjects can
drive differences in neural activation. However, these studies did not report areas of
deactivation, which may also be driven by increased levels of acetylcholine.

The current study aimed to test the hypothesis that increases in cortical acetylcholine
during the dSAT drive reductions in vmPFC neural activity. Task induced deactivations
in vmPFC have been previously correlated with increased task demands (McKiernan
2003, Harrison 2011, Leech 2011), so we predicted that tonic reductions in activity would
increase for the distractor block as well as correlate inversely with subject accuracy
scores. Further, we predicted that phasic vmPFC deactivation would occur in response to
salient targets. To test these hypotheses, we collected blood-oxygen-level dependent
(BOLD) functional magnetic resonance imaging (fMRI) data while subjects performed
the dSAT task on blocks with and without distraction. Our task design allowed for block
analysis, to test for tonic changes in activity level, as well as event-related analysis, to test
for trial-locked changes in activity level.

Further, in order to test for local changes in vmPFC cholinergic metabolism during task,
we collected magnetic resonance spectroscopy (MRS) data in the same subjects
performing a similar version of the same task. While traditional functional MR-based
neuroimaging techniques such as BOLD-fMRI measure changes in blood oxygenation
and blood flow that correlate with undifferentiated neural activity, functional MRS
(fMRS) measures changes in specific brain metabolites during behavioral tasks. Only a
small subset of brain metabolites, however, provide sufficient signal-to-noise
measurements for fMRS; examples include lactate (Urrila 2003), GABA (Floyer-Lea
2006), and glutamate (Gussew 2010). In addition, Nishitani (Nishitani 2003) showed that increases in human MTL choline-containing metabolite signal ([Cho]) paralleled an increase in MTL theta activity measured by magnetoencephalography, a finding attributed to measuring increases in cholinergic neurotransmission. We employed a similar technique in vmPFC during dSAT performance, hypothesizing that increased block levels of [Cho] during distractor blocks would correlate across subjects with decreases observed from the BOLD-fMRI measurements, showing that cholinergic activity increases occurred in parallel with neural activity decreases and correlated with the subjective difficulty of the task between subjects. We also hypothesized that we would observe event-related spikes in [Cho] time-locked to target trials, indicating phasic cholinergic activity across all subjects, coinciding with event-related BOLD decreases.

C. Experimental procedures

Subjects
Thirty-one volunteer subjects (19 females, 12 males between 18.2 - 38.7 years; average 21.3 ± 4.2 years) were recruited via advertisements posted on the Georgetown University campus or using the online Georgetown Research Volunteer Program (https://georgetown.sona-systems.com) as approved by the Georgetown University Institutional Review Board. All subjects received compensation in the form of cash or course credit through the Georgetown University Psychology Department. All subjects self-reported as right-handed, having normal or corrected-to-normal vision, no history of psychiatric or neurological disorders, no use of nicotine, and no use of psychoactive medications. Subjects were screened before scanning to ensure compatibility with an MR
environment; as such, subjects were not claustrophobic, pregnant, and were able to remove all preclusive metallic or magnetic devices.

Data from one subject was excluded from all analyses due to larger than expected scanning artifacts from a dental retainer observed during scanning of Experiment 1; data for Experiment 2 was not collected for this subject. Two subjects in Experiment 1, and a different two subjects in Experiment 2, had difficulties with the button response boxes; these subjects were included in block analyses but excluded from behavioral and event-related analyses. Two other subjects failed to pass data quality control criteria for Experiment 2 (see MRS Data Quality Control section) due to excessive movement or poor scan acquisition; these subjects were excluded from analyses in Experiment 2 only.

After providing informed consent according to procedures of the Georgetown University Institutional Review Board, each subject received instructions on how to perform the behavioral task and was screened for MR scanning. Task practice was provided during collection of an anatomical scan, followed immediately by Experiment 1 (fMRI) and then Experiment 2 (MRS).

**Methods for the Sustained Attention Task**

The sustained attention task (SAT) trial procedure, performed by subjects in both Experiment 1 and Experiment 2, is depicted in Figure 8. Subjects visually monitored the center of a blank silver screen for the brief appearance of a small black box (a target). Every 6-10 seconds, subjects were prompted (low auditory tone) to indicate via button press whether a target had just appeared. Feedback (high auditory tone) was provided
Each sustained attention task (SAT) trial lasted a total of 6-10 seconds. Subjects viewed a blank screen with silver background and were instructed to monitor for a visual signal. After 4-8 seconds, 50% of trials flashed a small black box on the center of the screen for 20, 30, or 50 ms; the other 50% of trials retained a blank screen. An auditory prompt (440 Hz for 500 ms) sounded 100 ms after the onset of the possible target. Subjects had 1.4 seconds from the onset of the auditory prompt to indicate via button press if they had observed a target, at which time a feedback tone (660 Hz for 500 ms) sounded only if the response was correct. The monitoring period for the subsequent trial began immediately after the end of the feedback tone period. In task blocks with distraction (dSAT), task procedure was identical to the SAT; however, the background screen strobed between silver and black at 10 Hz. Visual signals were always presented on a silver screen.
only for correct responses. In task blocks with distraction (dSAT), the trial procedure was identical to the SAT; however, the background screen strobed between silver and black at 10 Hz. Visual targets were always presented on a silver screen.

For Experiment 1 (fMRI), there were six possible combinations for trials with a target, comprised of two different delays before target onset (4 or 6 seconds) and three different target durations (20, 30, or 50 ms). Addition of a matching set of six non-target trials that were identical but did not present a target created a full set of 12 trials that lasted 84 seconds. For each task block of Experiment 1, this full set of trial types was presented once each in randomized order. Across the experiment, four task blocks were completed, including two each for SAT and dSAT conditions. At the beginning and end of the experiment, as well as between each task block, a 30-second rest block was performed; subjects were instructed to keep their eyes on a centrally located fixation cross and let their mind relax.

Trials in Experiment 2 (1H-MRS) were similar to Experiment 1, with the addition of a third possible onset delay (8 seconds), resulting in a full set of 36 trials lasting 144 seconds. For each block of Experiment 2, the full set of trials was presented three times in randomized order, for a total block length of 432 seconds. Across the experiment, one block each was performed for SAT and then dSAT. At the beginning and end of the experiment, 3-minute rest blocks were performed with the same instructions as in Experiment 1.
Task training and practice occurred during collection of an anatomical scan at the beginning of the MR scanning session. An automated, interactive instruction procedure that described the task and introduced the visual and auditory stimuli was followed by practice on one block each of the SAT and dSAT task versions, identical to the description of task blocks described in Experiment 1. Subjects were allowed further practice if they were not yet comfortable with the task, or if the auditory volume needed to be adjusted for subject comfort.

All stimulus presentation and data collection was run on a PC running E-Prime software (Psychology Software Tools) and projected to a screen at the back of the scanner bore, which was viewed by subjects by a mirror attached to the scanning head coil. The visual target (black box) was created using ASCII code 254 (■) in 20 point Arial font. Auditory tones were presented through pneumatic headphones and adjusted in volume to the comfort level of each subject during task training. Auditory prompts (to initiate button press responses) were 440 Hz and lasted 500 ms; feedback tones (given for correct trials only) were 660 Hz and also lasted 500 ms. Button response boxes were held in separate hands and operated via thumb press. The hand that indicated a target or non-target response was counterbalanced across subjects and held constant for each subject through both Experiment 1 and Experiment 2.

In addition to compensation for participation, subjects received a bonus of 2 cents for each accuracy percentage point on the sustained attention task averaged across both experiments, for a total possible bonus of $2.00.
Behavioral responses for all trials were coded as hits, misses, correct rejections, false alarms, or omissions. Accuracy analyses for each subject on each task version (SAT and dSAT) were performed on measures of $d'$, calculated as the difference between z-transforms of the hit rate and the false alarm rate; this measure gives a sensitivity index for target detection. Since z-transforms cannot be calculated for perfect scores, hit rates and false alarm rates for 100% accurate tasks were adjusted by $1/2N$, where $N$ is the number of subjects performing the task; thereby, the maximum and minimum reported $d'$ scores for this study are $\pm 4.17$. Though reaction time data are reported, it should be noted that target trials are biased for faster reaction times than non-target trials, as the visual target appears before the prompt for a response.

**Methods for experiment 1**

*Functional MRI data collection*

Scanning took place at the Center for Functional and Molecular Imaging at Georgetown University Medical Center in Washington, D.C., using a 3-Tesla Siemens Magnetom Tim Trio whole-body scanner (Erlangen, Germany) and a manufacturer supplied 12-channel phased array head coil. Head movement was minimized by padding the space between the subjects’ protective headphones and the scanning coil. Following a high resolution structural scan (T1-weighted magnetization prepared rapid acquisition gradient echo; TR/TE = 1900/2.52 ms, flip angle = 9°, 160 1-mm sagittal slices, FoV = 256x160x256 mm, 1mm$^3$ resolution) during which subjects trained on the task procedure (see *Sustained Attention Task* section), subjects performed alternating 30-second rest blocks and 84-
second task blocks during continuous collection of BOLD weighted whole brain images (243 collections of echo-planar imaging sequence; TR/TE = 2000/30 ms, flip angle = 90°, 31 4-mm axial slices, interleaved acquisition, FoV = 192mm², 3x3x4mm resolution). Five initial functional collections were run but not collected to allow for T1 equilibration.

*Functional MRI data analysis*

*ROI selection.* In order to test for the hypothesized attentional effects of cholinergic processing on the BOLD signal in vmPFC, an a priori location of interest was selected from a reported peak coordinate of maximum nicotinic receptor induced deactivation (Talairach brain atlas space: 0, 30, -2) based on results of a meta-analysis of neuroimaging studies employing nicotinic receptor agonists (Sutherland 2015). This coordinate was transformed into the MNI space of the preprocessed images using the Yale BioImage Suite (http://bioimagesuite.yale.edu, Lacadie 2008) resulting in a center point of (0, 34, -4). A sphere with a radius of 10 mm was created around this location using the MarsBar toolbox for SPM (Brett 2002) and then masked to include only areas of grey matter tissue probability greater than 50% for the study cohort based on an average of the tissue segmented images (see *Image preprocessing* section). The result is a 2432 mm³ ROI overlapping with the posterior quarter of the larger voxel location used in Experiment 2 (Fig. 9).

*Image preprocessing.* MR images were processed and analysis models created using SPM12 software (Wellcome Department of Imaging Neuroscience, Institute of Neurology, London, UK) running on MATLAB version 9.0 (MathWorks, Natick, MA).
**Figure 9. Regions of interest**

**Blue:** Region of interest for Experiment 1, fMRI data collection. The center coordinate of this ROI is based on a peak coordinate of deactivation (Talairach brain atlas space: 0, 30, -2) reported from a meta-analysis of neuroimaging studies employing nicotinic receptor agonists (Sutherland 2015). A sphere with 10mm radius was built around this peak coordinate and then masked to include only areas of grey matter tissue probability greater than 50% for the study cohort.

**Red:** For MRS data collection, each subject had a 2 cm$^3$ square voxel of interest manually positioned to cover maximal ventromedial prefrontal cortex grey matter based on the individual anatomical scan. To show the degree of overlap across subjects, the voxel location of each was normalized to a standard brain space and summed: brighter red indicates greater voxel overlap and black indicates less voxel overlap.
T1-weighted anatomical scans were segmented into six tissue classes and normalized into MNI space using default segmentation parameters. For EPI image preprocessing, each subject’s images were slice-time corrected to the middle slice of the volume, realigned to the mean of the image series, coregistered to the native space anatomical image, and normalized into MNI space using the transformation parameters acquired from the anatomical normalization.

**Block and event-related designs.** Two first-level general linear models were constructed for each subject, one block design to test for tonic changes across task blocks, and a second event-related design to test for phasic changes between target and non-target trials. Both models divided the time series into separate sessions for evaluation, so that the first session included the time course of the two SAT task blocks and surrounding three rest blocks, and the second session included the two dSAT task blocks and surrounding three rest blocks. In order to allow for signal associated with the final SAT task block to subside, the first five scans of the third rest block were excluded from the beginning of the second session; thus, the first rest block of the dSAT session included ten scans (20 seconds). For the block design, task block onsets and durations were convolved with a canonical hemodynamic function as regressors of interest; this allowed for a contrast between estimated beta values for sustained activity during task and rest. For the event-related design, regressors of interest were entered with durations of zero for hits and correct rejections; this allowed for a contrast of interest between estimated beta values for phasic differences between these trial types. Regressors of non-interest included all incorrect trials. Both the block design and event-related design included a high pass filter
of 128 seconds for each session as well as a first order autoregressive model used during parameter estimation to account for serial correlations in the time series. For both the block and event-related designs, parameter estimates and contrasts of interest (REST > TASK for the block design, and CORRECT REJECTIONS > HITS for the event-related design) based on the mean time course of all voxels in the vmPFC ROI were calculated using the MarsBar SPM toolbox (Brett 2002).

**Methods for experiment 2**

*MR spectroscopy data collection*

A 20 mm$^3$ MRS collection voxel was positioned manually, guided by each subject’s anatomical scan, to cover a maximal amount of ventromedial prefrontal cortex grey matter, centered just anterior to the genu of the corpus callosum. A graphical representation of all voxel positions is given in **Figure 9**. Following voxel positioning, a manual shim procedure to optimize B0 field homogeneity within the voxel position was performed. All MRS scanning used a point resolved spectroscopy sequence (TR/TE=2000/30ms, flip angle = 90°, VOI = 20 mm$^3$, bandwidth = 1200Hz, water suppression bandwidth = 50Hz, acquisition duration = 853 ms, timepoints = 1024). Thus, 3-minutes rest blocks were covered by 90 spectra acquisitions, and the 7-minute and 12-second task blocks required 216 acquisitions. Unsuppressed spectra used for water referencing and eddy current correction were collected with 6 acquisitions before each rest or task block and again after the final task block.
**MR spectroscopy data analysis**

Quantitation of metabolites contributing to the observed 1-H MRS spectra was estimated using LCModel software, version 6.3 (Provencher Inc., Oakville, Canada; Provencher, 1993). LCModel fits a linear combination of canonical metabolite peaks from an empirical scanner-specific basis set to estimate concentrations and uncertainties contributing to observed data with a minimum of subjective input. Unsuppressed data from the VOI — collected with identical scan parameters except without a water suppression pulse — were used to estimate eddy current effects and improve baseline fit, line shape, and zero-order phase correction within the model. Though a full basis set of 27 metabolites was used to fit the time domain data (as well as common spectral contributions from four lipids and five macromolecules to produce an informed background signal), main outcome measures were estimates of choline-containing compounds, represented by resonance signal from ammonium trimethyl protons at a peak of 3.20 ppm ([Cho]). Changes in [Cho] concentration are used clinically as a measure of cellular density, and changes on long timescales of weeks to months can indicate rapid cellular growth, such as is seen in a metastatic tumor (Castillo 1996); however, [Cho] changes time-locked to a cognitive task have been reported as indications of cholinergic neurotransmission (Nishitani 2003). Also reported are [Cho] ratios to the estimated concentration of creatine-containing metabolites ([Cre]). Estimations of [Cre] are commonly used as an internal reference in MR spectroscopy, as its main constituents (creatine and phosphocreatine) are expected to remain at constant levels due to a slow and steady conversion to creatinine (not resolvable by $^1$H-MRS), remain soluble, and not interact with other molecules via bonds strong enough to disrupt their contribution to the
resonance peak. By using an internal reference and evaluating metabolite-to-[Cre] concentration ratios rather than estimations of absolute metabolite concentrations, comparisons between spectra collected at different points in time can be made more accurate as well as robust to line broadening and reduced signal-to-noise; the improved accuracy is due to correlational changes in model fitting in the presence of collection-related changes such as magnetic field drift, inhomogeneity, and T2 fluctuations (Kanowski 2004). As previous results (see Chapter II) identified task-related changes to both [Cho] and [Cho]/[Cre] quantifications, each are reported here.

A major hurdle for fMRS measurements is that signal-to-noise ratios in individual spectra are too low for sufficient model fitting. Without identifiable peaks in frequency-domain data, models of metabolite concentration estimates cannot accurately estimate the varying phase shift in the acquired spectrum; so, multiple spectra are typically collected in a block, averaged, and then analyzed as a single spectrum. This technique is prohibitive for event-related analyses, since effects of interest are spread out in time, and summing the spectra without phase correction results in line broadening. To overcome the problem of independent phase shifts, we averaged blocks of 5 adjacent spectra across all time points in a sliding window fashion. Estimates of [Cho] concentration from the sliding window time course is depicted in Figure 10a. Though the variance of metabolite estimations for these scans is high, they do provide sufficient signal-to-noise for an accurate localization of the evolving phase shift in the voxel location across time. Thus, for the main dependent variable of tonic [Cho] across full task blocks, we averaged the phase corrected spectra from all sliding window averages created during the block. This way,
MRS data collections in vmPFC were collected every 2 seconds across rest blocks (3 minutes each at the beginning and end of the scanning period) and task blocks (7 minutes and 12 seconds). As individual scans do not have sufficient signal-to-noise for appropriate model fitting, sliding window blocks of 5 scans (10 seconds) were averaged and estimates of choline-containing metabolites were estimated across the time series. These time series were scaled by z-score within each subject for presentation. (a) Group average of z-scored 10-second sliding window [Cho] across the group with time labeled by task on the x-axis. Estimated phase corrections from the sliding window analysis were used to coherently average all scans within each task block for each subject, resulting in a single block metabolite concentration estimate of choline-containing metabolite, tonic [Cho]. (b) Average tonic [Cho] across all subjects, with error bars representing standard error from the mean. Units on the y-axis are mM, but should taken as accurate relative concentrations rather than absolute concentrations. No significant differences were found between task blocks for tonic [Cho]. *SAT*: sustained attention task blocks without distraction. *dSAT*: sustained attention task with 10 Hz strobing background added for distraction.

**Figure 10. [Cho] levels across tasks**
we could ask the question of whether tonic [Cho] changed from one cognitive task block to the next. For event-related analyses, we averaged the spectra that were time-locked to hits and correct rejections (with data collection occurring 30 - 883 ms after target/non-target onset), and we overcame the phase correction hurdle by using the median phase shift of all five sliding window spectra that include each time point of interest. This way, we could ask the question of whether phasic [Cho] was different between these two types of task trials immediately following the detection of a visual target.

*MRS data quality control*

Each time domain spectrum from the averaged blocks of data was assessed for reliability based on the estimated Cramér-Rao lower bounds (CRLB) of [Cho] (described above), a creatine peak at 3.03 ppm, and a peak from N-Acetylaspartate at 2.01 ppm. The CRLB of the model fit represents the lowest possible standard deviation of all unbiased model parameter estimates obtained from the data and provide an evaluation for the precision of metabolite estimations (Cavassila 2001). Spectra quality were additionally assessed by the linewidth (FWHM) and signal-to-noise ratio (SNR) (determined as the ratio of the largest representative metabolite peak to twice the root mean square of the residuals) of the frequency domain data. For the 10-second sliding window analyses, Chauvenet’s criterion was applied to each CRLB, FWHM, and SNR on the pooled set of all collected spectra across all subjects in order to determine an unbiased cut-off for inclusion in final analyses (Bol'shev 1975); thus, 95.6% of sliding window scans passed quality control. The maximum CRLB for [Cho] was 43% (median = 11%), maximum FWHM was 0.2 ppm (median = .067 ppm), and minimum SNR was 2 (median = 4). For the higher quality
tonic and phasic spectra (with a greater number of averages), a standard maximum cutoff of CRLB <= 20% was used. In the tonic [Cho] analyses, 95% of scans passed QC with a median [Cho] CRLB of 5%, a maximum FWHM of 0.095 ppm (median = 0.057), and a minimum SNR of 3 (median = 14). In the phasic [Cho] analyses, 98% of scans passed QC with a median [Cho] CRLB of 6%, a maximum FWHM of 0.095 ppm (median = 0.064), and a minimum SNR of 4 (median = 9). Subsequent statistical analyses on tonic [Cho] included data only from subjects with no excluded data from both task blocks and at least one of the adjacent rest blocks. Subsequent phasic analyses included only scans that were not excluded for each subject.

D. Results

Experiment 1: fMRI results

Distraction impairs attention task performance

Behavioral results for dSAT performance during fMRI scanning are reported in the top two rows of Table 1. Consistent with previous reports on dSAT performance (Demeter 2008, Demeter 2012), the introduction of a distracting background strobe decreased accuracy and increased reaction times of the group: paired t-tests showed lower d’ accuracy scores ($p = 1 \times 10^{-6}$, $t = 6.1$, df = 27) and longer reaction times ($p = 2 \times 10^{-4}$, $t = 4.3$, df = 27) for dSAT compared to SAT. The omission rate was less than 1% and not significantly different between tasks ($p = .12$). Due to most subjects performing at or near ceiling on the non-distractor SAT, no correlation analyses with SAT accuracy scores are reported.
<table>
<thead>
<tr>
<th></th>
<th>*Acc. (%)</th>
<th>*d’</th>
<th>*Hit Rate (%)</th>
<th>*False Alarm Rate (%)</th>
<th>*RT Hits (ms)</th>
<th>*RT Misses (ms)</th>
<th>RT False Alarms (ms)</th>
<th>RT Correct Rejections (ms)</th>
</tr>
</thead>
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<tr>
<td>SAT (fMRI)</td>
<td>92 ± 9.1</td>
<td>3.7 ± 0.56</td>
<td>91 ± 15</td>
<td>0.89 ± 2.6</td>
<td>368 ± 171</td>
<td>365 ± 156</td>
<td>648 ± 107</td>
<td>625 ± 107</td>
</tr>
<tr>
<td>dSAT (fMRI)</td>
<td>76 ± 15</td>
<td>2.2 ± 0.97</td>
<td>68 ± 22</td>
<td>21 ± 19</td>
<td>497 ± 170</td>
<td>465 ± 150</td>
<td>633 ± 111</td>
<td>628 ± 119</td>
</tr>
<tr>
<td>SAT (MRS)</td>
<td>94 ± 5.9</td>
<td>3.6 ± 0.55</td>
<td>93 ± 10</td>
<td>0.82 ± 1.9</td>
<td>392 ± 176</td>
<td>380 ± 169</td>
<td>627 ± 103</td>
<td>612 ± 103</td>
</tr>
<tr>
<td>dSAT (MRS)</td>
<td>76 ± 14</td>
<td>1.9 ± 0.93</td>
<td>65 ± 18</td>
<td>9.6 ± 12.5</td>
<td>492 ± 168</td>
<td>467 ± 150</td>
<td>622 ± 103</td>
<td>624 ± 108</td>
</tr>
</tbody>
</table>

**Table 1.**

Behavioral results for the sustained attention task

Rows indicate whether the task was performed without distraction (**SAT**) or with distraction (**dSAT**) as well as whether the results were collected during BOLD imaging collection (**fMRI**) or proton magnetic resonance spectroscopy collection (**MRS**). Group mean and standard deviation scores are reported. **d’**: d-prime accuracy score, **RT**: reaction time, * : significant effect of distraction.
**Tonic vmPFC deactivation correlated with dSAT task difficulty**

Task-induced deactivations in the vmPFC ROI were observed for attentional task performance compared to a resting baseline ([Fig. 11a](#)). A 2-way ANOVA to compare the main effects of attention (task or rest) and block (SAT or dSAT) on beta values from the block design, controlling for subject as a random effect, revealed a main effect of attention ($p = .037$, $F(1,87) = 4.5$). Post hoc paired $t$-tests showed a marginal deactivation from rest during SAT ($p = .063$, $t = 1.6$, df = 29, 95% CI = [-0.038 0.30]) and deactivation from rest during dSAT ($p = .036$, $t = 1.9$, df = 29, 95% CI = [0.21 0.72]).

These results indicate that BOLD activity was significantly reduced during attentional performance. The ANOVA resulted in no main effect of block ($p = .16$, $F(1,87) = 2.0$), nor an interaction between attention and block ($p = .27$, $F(1,87) = 1.3$), indicating that there was not significantly more deactivation from rest during performance of the dSAT than during the SAT. During the dSAT block, behavioral scores were lower for subjects with greater deactivation in the vmPFC ROI ([Fig 11b](#)), indicated by a Pearson correlation followed by Fisher transformation ($p = .012$, $r = -.47$, 95% CI = [-0.72 -0.11]). This shows that subjects having a more difficult time on the task had greater tonic task-induced deactivation.

**Phasic vmPFC deactivation on target trials**

Short timescale, target-induced deactivations in the vmPFC ROI were observed for correct trials with a target compared to correct trials without a target ([Fig. 12a](#)). A 2-way ANOVA comparing the main effects of target (hits or correct rejections) and block (SAT or dSAT) on beta values from the event-related design, controlling for subject as a
Figure 11. Tonic BOLD deactivations increase as dSAT accuracy decreases

a: Group averaged percent mean BOLD signal (detrended and low pass filtered) within the vmPFC ROI across 5 rest blocks (shaded areas) and four task blocks (white areas). The first two task blocks were performed without distraction (SAT), and the second two task blocks were performed with distraction (dSAT). Neural activation in vmPFC was driven higher by rest blocks than by task blocks across the group.

b: Subjective task difficulty increased task deactivation from rest between subjects. During dSAT blocks, d-prime accuracy scores (d') inversely correlated with increased task induced deactivations in the vmPFC ROI (y-axis: parameter contrast estimates for dSAT > REST).
Figure 12. Phasic BOLD decreases while phasic [Cho] increases in vmPFC

a: In Experiment 1, short-timescale decreases in BOLD signal were observed in response to trials in which subjects detected a signal compared to when no signal was presented. Units are estimated parameter contrasts between [CORRECT REJECTIONS > HITS].

b: In Experiment 2, short-timescale increases in [Cho] were observed in response to trials in which a signal was detected compared to when no signal was presented. Units are on the scale of mM for the contrast [HITS > CORRECT REJECTIONS].
random effect, revealed a main effect of target ($p = .033$, $F(1,81) = 4.7$). Post hoc paired $t$-tests showed greater deactivation for target events in both SAT and dSAT task blocks (SAT $p = .0039$, $t = 3.15$, df = 27; dSAT $p = .027$, $t = 2.3$, df = 27), indicating that the phasic decreases for events with a visual target deactivated to a greater extent than for non-target events. The ANOVA found no main effects of block ($p = .58$, $F(1,81) = 0.31$), nor was there an interaction between target and block ($p = .23$, $F(1,81) = 1.45$), indicating that phasic deactivations were not significantly different between SAT and dSAT.

No correlation was found between phasic deactivation to target events and $d'$ scores ($p = .31$, $r = -.20$, 95% CI = [-0.54 0.19]).

**Experiment 2: MRS results**

*Behavioral scores correlated between experiments*

Accuracy and reaction times for Experiment 2 are shown in rows three and four of Table 1.

Accuracy was better across experiments for SAT blocks compared to dSAT blocks. A 2-way ANOVA testing the main effect of block (SAT or dSAT) and experiment (Experiment 1 or Experiment 2) on $d'$, with subject entered as a random effect, showed a main effect of block ($p = 2 \times 10^{-17}$, $F(1,77) = 120$). Follow-up paired $t$-tests showed that average $d'$ scores were higher for SAT blocks than dSAT blocks for both Experiment 1 ($p = 1 \times 10^{-6}$, $t = 6.1$, df = 27) and Experiment 2 ($p = 1 \times 10^{-10}$, $t = 8.2$, df = 26). The
ANOVA showed no main effect for experiment, nor an interaction between block x experiment, indicating that the difference between SAT and dSAT scores did not change between Experiment 1 and Experiment 2.

Reaction times did not differ between experiments. A 2-way ANOVA testing the main effect of block (SAT or dSAT) and experiment (Experiment 1 or Experiment 2) on reaction time, with subject entered as a random effect, showed a main effect of block ($p = 7 \times 10^{-10}, F(1,77) = 50$), but no main effect of experiment ($p = .36, F(1,77) = .84$) nor an interaction between block and experiment ($p = 9 \times 10^{-5}, F(1,77) = .99$).

Subjects that performed more accurately on the sustained attention task in Experiment 1 also performed more accurately on Experiment 2; also, subjects that had faster reaction times on Experiment 1 had faster reaction times on Experiment 2. Correlation analyses for $d'$ scores between experiments showed significant between-subject consistency for both SAT ($p = .023, r = .45, 95\% \text{ CI} = [.069 0.72]$) and dSAT ($p = 7 \times 10^{-4}, r = .63, 95\% \text{ CI} = [.31 0.82]$). Correlation analyses for reaction times between experiments also showed significant between-subject consistency for both SAT ($p = 2 \times 10^{-11}, r = .93, 95\% \text{ CI} = [.84 0.97]$) and dSAT ($p = 5 \times 10^{-8}, r = .85, 95\% \text{ CI} = [.69 0.93]$) as well.

*No task-based changes in tonic [Cho] were observed*

Tonic levels of [Cho], based on the single averaged spectrum for each task block, did not change between rest, SAT, or dSAT (Fig. 10b). A 1-way ANOVA testing for the effect of block (rest, SAT, or dSAT) on estimated tonic [Cho] concentrations, with subject
entered as a random effect, showed no significant difference between means ($p = .33$, $F(2,76) = 1.1$). In order to test for consistent within-subject task-based changes in tonic [Cho], the SAT and dSAT tonic [Cho] measurements were scaled within-subject as percent change from rest and submitted to a paired $t$-test ($p = .49$, $t = 0.69$, df = 25); this result indicates that there was no consistent change in the group average from resting baseline between SAT and dSAT blocks. Similar negative results were obtained from an analysis of tonic [Cho]/[Cre] (1-way ANOVA $p = .16$, $F(2,76) = 1.9$; paired $t$-test $p = .72$, $t = 0.37$, df = 25).

Tonic [Cho] measurements were not found to correlate with task accuracy. No significant correlation was found between tonic [Cho] and $d'$ scores within the dSAT block ($p = 0.65$, $r = .092$, 95% CI = [-.30 .46]); neither was a correlation found between tonic [Cho] scaled as percent change from resting baseline and $d'$ ($p = .68$, $r = .08$, 95% CI = [-.30 .44]). Results were similarly negative for [Cho]/[Cre] ($p = .36$, $r = .18$, 95% CI = [-.21 .53]).

To test the hypothesis that tonic changes in [Cho] correlate with tonic task induced deactivations between subjects, correlations were calculated between tonic [Cho] measurements from Experiment 2 and task deactivation beta values from Experiment 1 for each the SAT and dSAT task blocks. These tests showed no significant results (SAT: $p = .31$, $r = .22$, 95% CI = [-.21 .58]; dSAT: $p = .64$, $r = -.10$, 95% CI = [-0.48 0.31]). Results were similarly negative for [Cho]/[Cre] (SAT: $p = .75$, $r = .07$, 95% CI = [-.33 .44]; dSAT: $p = .51$, $r = -.13$, 95% CI = [-0.48 0.26]).
**Phasic vmPFC [Cho] increased on target trials**

Phasic increases in [Cho] on target trials, based on each subject’s single averaged spectrum for all measurements taken at trial onset, were greater than similar averages coinciding with non-target trials (Fig. 12b). A 2-way ANOVA testing the effect of target (hits or correct rejections) and block (SAT or dSAT) on [Cho] levels revealed a main effect of target ($p = .039, F(1,73) = 4.4$). Follow-up paired $t$-tests showed an increase in target-locked phasic [Cho] for the SAT ($p = .028, t = 2.3, df = 25$), a trend for dSAT ($p = .12, t = 1.6, df = 25$), and no significant difference between SAT and dSAT ($p = .70, t = .39, df = 24$). The interaction term between target and block of the ANOVA was not significant ($p = .80, F(1,73) = 0.07$), indicating that phasic responses to target trials were not significantly greater than non-target trials between SAT and dSAT. A similar 2-way ANOVA for [Cho]/[Cre] did not return a significant effect of target ($p = .14, F(1,73) = 2.2$).

A correlation between phasic [Cho] magnitude and $d'$ accuracy scores on the dSAT was not observed ($p = .50, r = -.14, 95\% \text{ CI} = [-.51, .27]$). A correlation test for [Cho]/[Cre] during dSAT and $d'$ accuracy scores was similarly negative ($p = .62, r = -.10, 95\% \text{ CI} = [-.48, .30]$).
E. Discussion

Summary of results

This study demonstrates that short-timescale neural deactivations occur in vmPFC in response to target stimuli during sustained attention; in addition, similarly-timed increases in $^1$H-MRS [Cho] signal provide support for the theory that cholinergic regulation mediates phasic task induced deactivations. Results of this study also reinforce previous findings that the magnitude of vmPFC deactivation correlates with measures of attentional effort; in this case, reduced task accuracy on the distractor task.

Consistency of the behavioral task

Behavioral results from the sustained attention task were consistent with previous studies in both rodents and humans, showing reduced accuracy and increased reaction times in the presence of visual distraction. Though the task format was altered between experiments to accommodate data collection of different scanning measures, the longer task duration and longer average trial length in Experiment 2 did not result in differences between accuracy or reaction time. Task similarity was further validated by a strongly significant correlation between experiments for both subject accuracy and reaction time.

Tonic vmPFC deactivations

We did observe the expected tonic deactivation from rest while subjects performed SAT blocks, in addition to finding that subjects who scored lower on the dSAT — interpreted as requiring more attentional effort to perform the task — tended to show larger deactivations. However, we did not find a significant increase in average deactivation
across the group with the introduction of distraction: dSAT blocks did not deactivate more from rest than SAT blocks for the group. This may have to do with the reduced power in our task design, which did not compare the blocks directly; rather, each task was analyzed as a separate session and compared to rest. Alternating the task blocks during acquisition would provide a more powerful statistical analysis to compare differences between deactivations. The reasoning for not alternating task blocks in the task design stems from the unknown time course of acetylcholine increases in humans during the dSAT. Rats performing the dSAT show increases in acetylcholine when they are put into the operant chamber, further increases during SAT performance, further increases again during dSAT performance, and then maintain high levels of cortical acetylcholine for minutes after task completion (Himmelheber 2000). As the time course of acetylcholine release in humans is not known, our $^1$H-MRS dSAT task was designed to be able to replicate the results from rodent studies, and the fMRI dSAT format followed suit in order to provide the opportunity to draw conclusions between the two task variations without the confound of possible tonic acetylcholine carryover between blocks.

However, our tonic MRS results showed no significant changes between task blocks, nor did they show an expected correlation with task performance. Though we account for frequency drift in our voxel by summing phase-corrected spectra, we assume that our effects of interest are greater than any field susceptibility changes due to changes in oxygenation of hemoglobin concentrations from nearby blood vessels. Whereas the BOLD effect in fMRI scans includes signal from high water concentration within vasculature, MRS metabolites of interest are almost entirely confined to brain
parenchyma and nearby intracellular space. Still, findings at higher magnetic fields have shown BOLD effects on MRS metabolites (Zhu 2001), indicating that local field fluctuations due to vascular susceptibility changes are sufficient to effect measurement. Since we expected to observe increases in [Cho] during periods of lower deoxyhemoglobin levels (as indicated by the reduction in BOLD fMRI signal), effects of interest would have to outweigh BOLD-induced changes in signal to provide the anticipated results. One way to overcome BOLD contamination with effects of interest would be to use metabolite ratios, as is often recommended (Kanowski 2004). However, this assumes that signal from the metabolite used as an internal reference (usually signal from creatine) is not independently affected by task conditions, an assumption we have empirically observed to not always hold (see Chapter II). Alternatively, future studies may consider employing alternating water suppressed/unsuppressed scan sequences (Apšvalka 2015) in order to measure ongoing BOLD effects on the water signal and use them as covariates in analyses. Though this method would halve the number of available data points, it may prove to greatly benefit the power of the technique.

Phasic BOLD and MRS changes for target trials

Event-related reductions in BOLD signal were found time locked to correct target events compared to correct non-target events; in addition, short timescale increases in [Cho] were found for the same contrast. This supports our hypothesis that cholinergic activity mediates the reduction of neural activity in vmPFC even on a phasic timescale. Since our phasic MRS analysis compares multiple within-subject trials that occur within the first two seconds upon target onset — unlike the delayed hemodynamic response to target
onsets by the BOLD signal — they are not confounded by BOLD effects on metabolite measurements as the tonic measurements may be. Amperometric microelectrode recordings in rats performing the dSAT have shown seconds-long increases in acetylcholine in response to successful target trials (Parikh 2007), the timing of which is task-dependent and occurs time-locked to the initiation of a rewarded behavioral response. In our case, the rewarded response (a button press) was controlled by a 100ms delay between target onset and auditory prompt such that our collection window (30 - 883 ms following target onset) coincided with the effect of interest. The role of brief cholinergic increases in response to salient cues is theorized to be a switch in mental state away from monitoring and toward a stimulus initiated response (Sarter 2016). Our hypothesis is that this switch in mental state requires not just the initiation of activity in neural areas required for the behavioral response, but also the reduction of activity in neural areas that are not required. Previous work using the dSAT showed brief target-related BOLD increases in right middle frontal cortex (Howe 2013), and the current study provides evidence that target-related BOLD decreases in one part of an anticorrelated brain network, the vmPFC, may be driven by cholinergic activity as well.
A. Summary of findings

The driving hypothesis underlying this dissertation is that acetylcholine acts on a large scale to tune brain-wide dynamics to either external or internal information processing. As such, it should increase neural activity in areas that process sensory stimulus information during memory encoding and attentional tasks. Additionally, it should mediate decreases in neural activity across brain areas that primarily function during internal mentation. These changes are believed to occur on both tonic and phasic timescales. In order to test these hypotheses in humans for the first time, $^1$H-MRS was used to measure changes in observed concentrations of choline-containing metabolites, standing as a proxy for cholinergic neurotransmission.

The experiment presented in Chapter II tested the hypothesis that increases in cholinergic activation occur in human MTL during encoding of external information. A configural working memory task was constructed to engage MTL activity that has previously been shown to induce long term memory encoding, and a post-learning recognition test affirmed memory retention. $^1$H-MRS measurements showed that concentrations of choline-containing metabolites increased during the active maintenance of task information that was subsequently remembered.
Exploratory analyses interested in determining whether task-related correlations occurred independently for individual metabolite concentrations estimations found that subjects with greater task difficulty showed higher observed concentrations of choline-containing metabolites than subjects with less task difficulty. This result supports the hypothesis that the magnitude of cholinergic neurotransmission during memory encoding is mediated by task difficulty. An important coincidental finding was that concentrations of creatine-containing metabolites, which are often used as a purportedly stable internal standard during $^1$H-MRS analysis, correlated independently with task demands. Though speculation about the nature of this finding was discussed at the end of the chapter, the implication for future fMRS research is that different information may be available to us through individual metabolites and metabolite ratios.

The experiments presented in Chapter III tested the hypothesis that increases in cholinergic activation mediate task-related deactivation in neural activity on both tonic and phasic timescales. These experiments collected BOLD fMRI and then $^1$H-MRS measurements in vmPFC while participants performed a validated human subject version of a sustained attention task that has been used extensively to study cholinergic processing in animals. BOLD fMRI results showed both tonic deactivations during task performance, as well as phasic deactivations in response to target stimuli. The magnitude of tonic BOLD deactivations correlated with subjective task difficulty as determined by behavioral accuracy scores. These results corroborate previous neuroimaging results that task induced deactivations in vmPFC scale with task difficulty, and they add the finding that task induced deactivations occur in response to stimuli on an event-related time
scale. $^1$H-MRS results showed phasic increases in levels of observed choline-containing metabolites in response to target stimuli, providing evidence that the observed phasic BOLD decreases may be the results of cholinergic control.

B. Interpretation of $^1$H-MRS measurements as a proxy for cholinergic neurotransmission

In both Chapters II and III, significant task-based changes in concentrations of choline-containing metabolites were observed, each in the direction hypothesized to coincide with extracellular concentrations of acetylcholine. When using a relatively young technique, it certainly is encouraging to find some consistent validation. However, no tonic changes in choline signal were observed during the sustained attention task presented in Chapter III. On the one hand, it is possible that these changes indicate fluctuations in cholinergic activity that occur on timescales shorter than the 432-second task blocks; thus, the variance in measurements between task blocks would not be driven by task-based processes. This would be great news for clinical measurements and research studies interested in static metabolite levels, which typically collect many averages over blocks longer than those used in this study and work under the assumption that the type of video, audio, or other stimulation their subject uses to bide time does not significantly affect measurements. As discussed in Chapter III, it is also possible that BOLD effects on the metabolite signals are significant enough to preclude the use of long-duration blocks when collecting fMRS data, similar to standard fMRI data. Future research may solve the question of how relevant BOLD fluctuations are for MRS data by employing scan
sequences that collect alternating water suppressed/unsuppressed collections (Apšvalka 2015), which would allow for tracking of both signal changes over time.

The incidental observation of task-related changes in $^1$H-MRS signal for creatine-containing metabolites in the experiment presented in Chapter II lends caution to future analyses. Observed creatine-containing concentrations during the task block significantly decreased from a resting baseline, contributing to increases in the [Cho]/[Cre]. Observed creatine-containing concentrations during the testing blocks correlated both with subject reaction time as well as with subsequent accuracy on the post-learning recognition test. After recognizing that changes in the observed signal for creatine-containing metabolites significantly correlated with multiple task-based measures, the use of estimated creatine concentrations as an internal standard was not used as a sole measure for the subsequent $^1$H-MRS experiment presented in Chapter III. Determining whether task-based creatine signal changes are based on BOLD-related effects, as yet unknown resonance contributions, or a combination of factors will be informative to future fMRS research as well as possibly opening new lines of investigation.

C. Interpretation of memory encoding results

Behavioral results from the experiment presented in Chapter II validated the assumption that subject performance on the configural working memory task was, in fact, related to longer-term memory encoding; subjects that performed more accurately on the working memory task also performed more accurately on the unexpected post-learning recognition test outside of the MR scanner. Prior studies identified the MTL BOLD-fMRI response
during active maintenance as a predictor of subsequent memory (Schon 2004), and also that pharmacologically inhibiting cholinergic activity reduced this response (Schon 2005); this implicates cholinergic activity in MTL as a driver of memory encoding related activity. Additionally, MTL BOLD activity during active maintenance has been shown to depend on working memory load, theoretically also driven by graded increases in cholinergic activity (Schon 2016). This theory is corroborated by findings from the experiment presented in Chapter II, in which MTL cholinergic activity during active maintenance of task information increased more for participants with greater subjective task difficulty, another measure of relative working memory load. The increases in acetylcholine during active maintenance are hypothesized to induce persistent spiking for memory maintenance, and to filter out background processing of task-irrelevant information (Hasselmo 2006). Though the average increase in cholinergic activity for the active maintenance block was not observed to increase significantly across the group, it is possible that the subjects performing at ceiling did not require a significant cholinergic circuit enhancement to complete the task. As such, future research should consider varying levels of task difficulty in order to identify group-averaged changes beyond those observed between subjects.

D. Interpretation of sustained attention results

Although the fMRI experiment reported in Chapter III did find that tonic neural activity in vmPFC deactivated more for subjects with greater subjective difficulty on the task, no conclusion can be made about the role of acetylcholine in driving these changes since tonic levels of observed choline-containing metabolites were not found to increase. On
the other hand, event-related neural deactivations for target trials observed with fMRI did have a corroborating increase in target-related cholinergic activity. The region of interest selected for the BOLD analysis was based on an area expected to reduce activity given nicotinic stimulation (Sutherland 2015). The selected region falls into a hub of the DMN, which is expected to reduce activity during externally oriented task performance. The fMRI results indicate that this deactivation occurs at multiple timescales, including event-related deactivations in response to salient sensory stimuli. The phasic $^1$H-MRS results show that deactivations may occur not just for nicotinic pharmacological agonism, but also under the influence of endogenous acetylcholine increases as well. Though the results do not show a causative relationship, they do inform future modeling of network dynamics. The fact that both neural activity changes and cholinergic activity changes occurred for target trials when compared to non-target trials — these trials did not differ in reward or in auditory stimuli — indicates a privileged role for the target in neural processing (Hasselmo 2011).

A recent study in rodents has indicated that not all targets respond to acetylcholine similarly. Rather, target trials following either non-target trials or misses (incongruent targets) elicit a phasic acetylcholine increase, and target trials that follow other target trials (congruent targets) do not (Howe 2013). As the present study was designed and data collection commenced before results based on congruency effects were published, it did not ensure a sufficient number of congruent target trials for statistical power to interrogate these effects. Certainly, future investigations in human subjects should aim to corroborate this finding.
E. Integrated findings and implications for translational research

The combined results of the experiments presented above support a functional role for acetylcholine as a brain network modulator that increases information processing of external stimuli and diminishes processing for previously stored information. They have provided evidence that acetylcholine increases are found for both memory encoding as well as attentional performance, and on both tonic and phasic timescales. Adding to previous research, these results contribute to the theory that acetylcholine may play a role in regulating brain-wide state switches represented by global anticorrelated networks involved in cognitive task performance (Sutherland 2015). Importantly, the use of $^1$H-MRS to identify cholinergic changes in humans opens the door for use in studying disorders and treatments associated with cholinergic functioning. As our understanding of acetylcholine evolves from thinking of it as a general arousal modulator to a dynamic regulator of cognitive tasks, we can identify new treatments that aim to restore cholinergic control to the individual. Recent pharmaceutical advances, especially positive allosteric modulators targeting receptor subtypes that have long eluded drug specification, aim to more precisely personalize and adjust cholinergic activity related to cognition (Hurst 2005, Shirey 2008, Shirey 2009, Taly 2009, Apostol 2012). These advances have generated calls from basic science researchers to establish more targeted treatment strategies (Deutsch 2010, Hasselmo 2011, Sarter 2015, Yegla 2015). In order to evaluate new treatments, we must first delineate typical cholinergic dynamics in humans, and from them establish protocols for evaluating interventions. The studies presented
above are an early step towards replicable measurements of neurodynamic cholinergic function in humans.


80


Sutherland, Matthew T., Kimberly L. Ray, Michael C. Riedel, Julio A. Yanes, Elliot A. Stein, and Angela R. Laird. 2015. “Neurobiological Impact of Nicotinic Acetylcholine Receptor


