UNIVERSITY OF CALIFORNIA,
IRVINE

Investigation of the human neural correlates of memory for sequences of events and their changes in typical aging

DISSERTATION

Submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSPHY

in Biological Sciences

by

Veronique Kristin Boucquey

Dissertation Committee:
Professor Craig E.L. Stark, Chair
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2016

DEDICATION

To my husband, family, and friends

For your encouragement and inspiration

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ACKNOWLEDGEMENTS

First, I would like to thank my advisor, Dr. Craig Stark, for being an incredible teacher. You inspire curiosity in science and excitement for learning. You have challenged me to think critically and imaginatively with your patient guidance. I am exceptionally proud to say I received my PhD training under your mentorship.

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Education

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Bava, S., **Boucquey, V**., Goldenberg, D., Thayer, R.E., Ward, M., Jacobus, J., & Tapert, S.F. (2010). Sex Differences in Adolescent White Matter Architecture. *Brain Research 1375,* 41-48.

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August 2015 "Why should we study memory for sequences of events?" Elevator Pitch given at the UCI Neurobiology and Behavior 50th Anniversary Retreat.

January 2015 "Memory for sequences of events shows bilateral hippocampal and medial prefrontal activity in humans." Talk given at *Neuroblitz* at UC Irvine, Dept. of Neurobiology and Behavior.

May 2014 "A functional signal for memory of sequences in the human hippocampus." Talk given at the *Center for Neurobiology and Behavior Spring Meeting* at UC Irvine.

April 2014 "Activity in the medial temporal lobe decreases with age during performance of a learning and memory task." Talk given at the *Associated Graduate Student Symposium* at UC Irvine.

February 2014 "Age-related Decreases in Mnemonic Activity in the Medial Temporal Lobe." Talk given at *Neuroblitz* at UC Irvine, Dept. of Neurobiology and Behavior.

March 2013 "Age-related Changes in Mnemonic Activity in the Medial Temporal Lobe." Talk given at *Neuroblitz* at UC Irvine, Dept. of Neurobiology and Behavior.

May 2012 "Can Zero Sometimes Be Zero? Effects of Age on Baselines in FMRI." Talk given at *Neuroblitz* at UC Irvine, Dept. of Neurobiology and Behavior.

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Fall 2013 – Summer 2015

Graduate Student Representative

UC Irvine, Dept. of Neurobiology and Behavior

Elected to position. Brought student concerns and interests to faculty and members of the administration's attention, created and maintained daily calendar of events, served on NB&B 50th Anniversary Retreat committee, co-organized a 2015 Graduate Student Retreat focused on career development

Fall 2012 – Present

Volunteer Outreach Docent

UC Irvine, Center for the Neurobiology of Learning and Memory

Lead elementary, middle and high school student tours of the Center for the Neurobiology of Learning and Memory and teach hands-on neuroscience activities such as comparative neuroanatomy

Spring 2012 – Summer 2015

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UC Irvine, Center for the Neurobiology of Learning and Memory

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Laboratory Leader & Instructor Trainer, Neurobiology Laboratory Course
UC Irvine, Dept. of Neurobiology and Behavior
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Teaching Assistant, 'From DNA To Organisms'
UC Irvine, School of Biological Sciences
Prepared and led an independent discussion section

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Graduate Student Instructor, Neurobiology Laboratory Course
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Curriculum Developer, Neurobiology Laboratory Course
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Co-developed new experimental designs and wrote lab chapters involving electrophysiology; currently implemented in all Neurobiology Laboratory sections

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ABSTRACT OF THE DISSERTATION

Investigation of the human neural correlates of memory for sequences of events and their changes in typical aging

By

Veronique Kristin Boucquey

Doctor of Philosophy in Biological Sciences

University of California, Irvine, 2016

Professor Craig E.L. Stark, Chair

Memory for sequences of events, an ability present in humans, nonhuman primates, and rodents, is a critical component of episodic memory and is known to decline in typical aging (Allen and Fortin, 2013; Allen et al., 2015; Fabiani and Friedman, 1997; Pinto-Hamuy and Linck, 1965; Roberts et al., 2014; Tolentino et al., 2012; Tulving, 1984). The use of a cross-species task allows for complementary approaches in the rat and human in order to provide a greater understanding of the neural mechanisms underlying sequence memory ability. Using a non-spatial sequence memory paradigm in combination with blood-oxygen-level-dependent (BOLD) functional magnetic resonance imaging (fMRI), we present evidence that memory for sequences of events activates the hippocampus and medial prefrontal cortex in humans, paralleling recording studies and temporary inactivations in rats performing the cross-species task. In addition, these areas show functional connectivity over the course of the task to a greater degree than other regions, supporting the hypothesis that sequence memory in the human is subserved by the hippocampus and medial prefrontal cortex and their functional interactions. After demonstrating homology for the neural substrates of sequence memory in the rat and human, we sought to investigate the behavioral and neural changes associated with typical aging.

We found that typically aging older adults showed behavioral impairments on the sequence memory task. Equating performance between young and older adults, using BOLD fMRI we found evidence for similar neural substrates, but decreased activity in older adults. In addition, we found that functional connectivity between hippocampus and medial prefrontal cortex decreased with age, as did connectivity between other regions that in young adults showed high connectivity. Future studies using variants of the cross-species task in both rats and humans, combined with additional imaging modalities, will further elucidate the underlying neurobiological changes in typical aging.

INTRODUCTION

Typical aging is associated with declines in declarative memory function, including episodic memory (Craik and Simon, 1980). This dissertation focuses on memory for sequences of events, a critical component of episodic memory, that has also shown to decline with age (Tulving, 1984; Roberts et al., 2014; Allen et al., 2015). Using a cross-species paradigm, previous research has shown memory for sequences of events relies on the hippocampus and prefrontal cortex in rats (Allen et al., 2016; Quirk et al., 2013). The current research aimed to identify the neural correlates of sequence memory in the human using this cross-species paradigm, expecting to observe homology between the rat and human. We then sought to determine the changes to the neural substrates supporting sequence memory in typical aging. Identifying the neural changes that occur with typical aging are also of vital importance if we hope to not only mitigate memory declines in our growing older population, but also to differentiate healthy aging from dementia with the goals of earlier diagnosis and development of effective treatments.

Aim 1: Determine the neural correlates of sequence memory in young adults

Episodic memory is known to decline with typical aging and memory for sequences of events is a key feature of episodic memory (Craik and Simon, 1980; Tulving, 1984). However, episodic memory is a complex cognitive process that is difficult to study empirically. A critical aspect of episodic memory is the ability to remember the order of a sequence of events. Such sequence memory is known to rely upon both the hippocampus and the prefrontal cortex (Davachi and DuBrow, 2015; Devito and Eichenbaum, 2011; Fortin et al., 2002; Jenkins and Ranganath, 2010; Milner et al., 1985). However, there remains much to be learned about the

neurobiological mechanisms within each region, the specific role of each region, and how these regions interact to produce memory for sequences of events. A cross-species approach can provide a powerful means to address these outstanding questions. Therefore, this aim seeks to identify the neural correlates of sequence memory in humans by employing a non-spatial, non-verbal sequence memory task during BOLD fMRI scanning. The utilization of this particular task has the advantage of a cross-species complementary approach, as reversible lesion work and physiological recordings have been done in rats in the hippocampus and medial prefrontal cortex (Allen et al., 2016; Quirk et al., 2013). Using BOLD activity levels and functional connectivity (correlations in activity levels between regions over time), we found homologous underlying neural substrates supporting performance on the sequence memory task between the rat and human.

Aim 2: Investigate age-related changes to the neural substrates underlying sequence memory performance

After investigating the neural correlates of sequence memory performance in young human adults, this aim seeks to identify the functional changes that occur with age. Memory for sequences of events has shown declines in typical aging (Allen et al., 2015; Roberts et al., 2014; Tolentino et al., 2012). Therefore, we sought to understand the changes that occur to the neural substrates supporting sequence memory performance. Using BOLD fMRI, we found that highly similar neural substrates were used to support sequence memory performance in young and older adults, however older adults showed reduced activity levels in many regions. Functional connectivity results demonstrated that the hippocampus and mPFC showed reduced interaction in aged adults, as did other regions that in young adults that showed high connectivity. The

cross-species nature of the sequence memory task, combined with other imaging modalities, will allow future studies to provide a deeper understanding of the underlying neurobiological changes occurring with age.

1 Background and Significance

1.1 Discussion of long-term declarative memory and its neural correlates

The attempt to differentiate between forms of memory and their corresponding biological substrates began over a century ago. William James hypothesized in his 1890 work "Principles of Psychology" that there are habits (non-declarative memory), primary memory (short-term memory) and memory proper (long-term declarative – conscious fact or event – memory), while based on experimentation in the early 1900's Ivan Pavlov argued that all learning is stimulus-response (James, 1890; Pavlov, 1927). In the early 1800's Gall postulated that distinct brain regions had unique functions, while Flourens proposed there was no localization of brain function (Gall, 1835; Tizard, 1959). The seminal work by Scoville and Milner in 1957 settled some controversy by demonstrating that patients with medial temporal lobe (MTL) lesions had intact short-term and habit memory, but were unable to form new declarative (fact or event) memories. This demonstrated that there are indeed different memory systems that can be to some degree localized within the brain. Of course there remain debates today regarding different forms of memory and their neural substrates (e.g., are recollection and familiarity both supported by the hippocampus or is recollection supported by the hippocampus and familiarity supported by the cortex? See Ranganath, 2010; Wixted and Squire, 2010 for discussion). For the purposes of this dissertation, I will focus on the forms of long-term memory that are thought to employ hippocampal and/or the surrounding medial temporal cortical as well as prefrontal cortical processing, specifically declarative memory. On a cognitive level, declarative memory can been divided into semantic memory (memory for facts) and episodic memory (memory for the 'what',

'when', and 'where' of an event). Both types of memory are thought to be supported by the medial temporal lobes and prefrontal cortices.

The MTL (Figure 1.1) in humans, non-human primates, and rodents consists of the hippocampus, which includes CA1, CA3, dentate gyrus (DG), and subiculum (SUB), and the surrounding cortices, which include the entorhinal cortex (EC), perirhinal cortex (PRC), and parahippocampal cortex (PHC or postrhinal cortex in rats; Clark and Squire, 2013). Briefly, information from unimodal and polymodal association areas enters the PRC (primarily 'visual' or 'object' information because the main inputs are from area TE) or PHC (primarily 'spatial' or 'context' information because the main inputs come from area V4; Aggleton and Brown, 1999; Diana et al., 2007; Lavenex and Amaral, 2000). Information flows between these two areas as well as into the entorhinal cortex, where it maintains to some extent its division of information in medial EC (from PHC) and lateral (from PRC) EC (Suzuki and Amaral, 2004). From the EC, one main pathway through the hippocampus, the "trisynaptic loop" (Andersen et al., 1971) consists of

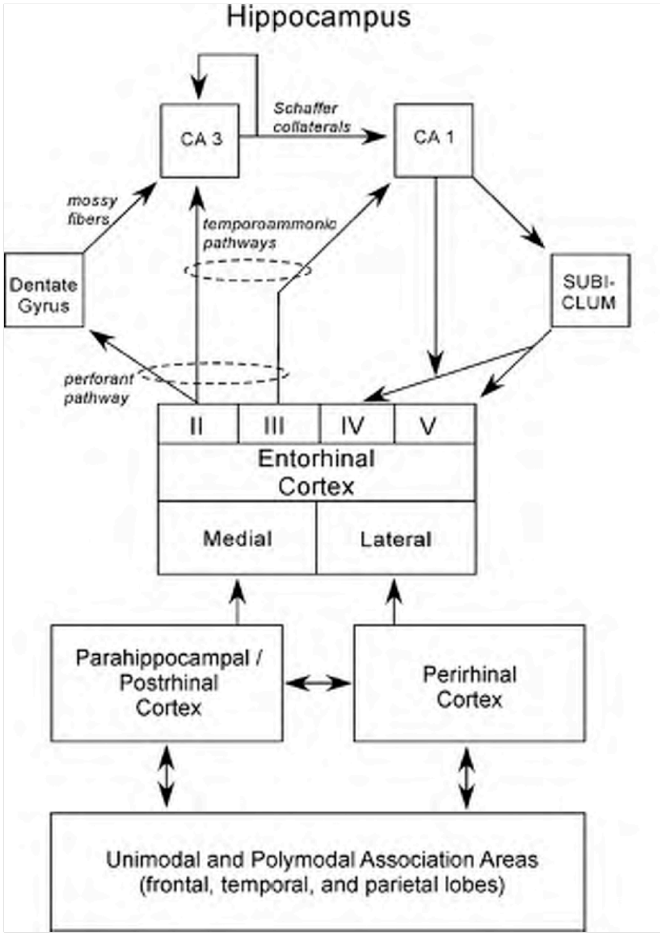


Figure 1.1. Schematic of the medial temporal lobe. Figure from Clark & Squire, 2010.

axons from EC layer II synapsing onto granule cells in the dentate gyrus (this bundle of EC axons is labeled the perforant path and also includes the EC layer II axons that synapse onto CA3 and EC layer III axons that synapse onto CA1). DG axons (mossy fibers) extend and synapse onto neurons of CA3, named "detonator synapses," because of the ability of these strong synapses to enforce a pattern of activity in CA3 (McNaughton and Morris, 1987). CA3 recurrent collaterals synapse onto neurons of CA3, while CA3 Schaffer collaterals synapse onto neurons of CA1. Axons of CA1 then synapse onto neurons in the subiculum, which then synapse onto layer IV and V of the entorhinal cortex. This is merely a sketch of what is currently known of the anatomy, as there is more complexity than 1.1 shows (see Van Strien et al., 2009 for detail).

Based on this known anatomy, computational models demonstrated how the hippocampus could be important to the formation of new episodic memories by demonstrating how the hippocampal circuit could rapidly bind arbitrary information (a feature necessary to store a memory of a single event; McClelland et al., 1995; Treves and Rolls, 1994). Specifically, a computation known as pattern separation, or the ability to disambiguate similar information into non-overlapping information, could be performed by the DG; it has 4-5 times as many neurons as there are axonal projections from the EC (Amaral et al., 1990; Boss et al., 1985); the DG has sparse coding properties (very few granule cells are active at the same time; Chawla et al., 2005, and DG cells have been shown to change their firing pattern in response to small changes in input (Leutgeb et al., 2007; Neuneubel and Knierim, 2014). These properties would lend themselves to the ability to rapidly disambiguate similar events that would be essential for establishing episodic memories, as the daily events in our lives have much overlapping information. These models also show how CA3 pyramidal cells could function as an auto-

associative network whereby an entire episode could be recalled when only a fragment of the episode is presented (pattern completion).

Researchers also developed tests of declarative memory in rodents and non-human primates in order to study the structure-function relationship (Gaffan, 1974; Mishkin, 1978). Lesions of the MTL in monkeys demonstrated the MTL in necessary to perform well on a single-trial-learning recognition memory test – the ingenious "delayed nonmatching to sample" test which requires memory for a single learning event, much like episodic memories are formed in a single trial (Mishkin, 1982). Another form of declarative memory test, the novel object recognition (NOR) test, was used to demonstrate that rodents need an MTL to form new declarative memories (see Clark and Squire, 2010). This test relies on the innate preference for novelty, and like the DNMS task, requires learning in one trial. In humans, blood-oxygen-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) has demonstrated that the MTL shows activation in declarative memory tasks, both at encoding and at retrieval, and that activity at encoding predicts subsequent memory (Brewer et al., 1998; Stark and Okado, 2003; Wagner et al., 1998). Taken together, the converging methods and model systems have demonstrated the importance of the MTL in declarative memory.

While not appreciated for as many years as the MTL, the prefrontal cortex has also been demonstrated to be important to declarative memory function (Badre and Wagner, 2007; Blumenfeld and Ranganath, 2007; Bunge et al., 2004; Cabeza and St Jacques, 2007; Milner et al., 1985). The human prefrontal cortex (Figure 1.2) can be very roughly divided into medial PFC (mPFC: Broca area (BA) 24/25/32/10; BA area 10 is sub-classified as anterior PFC in the diagram below), dorsolateral PFC (dlPFC: BA 46/9), and ventrolateral PFC (vlPFC: BA 44, 45, 47,12), although even these rough classifications are not completely consistent throughout the

literature. In addition, there are many more subdivisions hypothesized in regards to structure-function relationships, and these divisions are being researched and debated (Badre and Wagner, 2007; Blumenfeld and Ranganath, 2007; Fuster, 2008; Miller and Cohen, 2001; Passingham and Wise, 2012; Rugg and Vilberg, 2013; Simons and Spiers, 2003). Most regions of the PFC are connected reciprocally with most other regions of the PFC, allowing information to be easily transferred between the regions of the PFC. The dlPFC and vlPFC are particularly well-connected (Miller and Cohen, 2001), which is not surprising given their hypothesized roles in cognitive control of memory (see discussion below of the suspected roles of each of these areas in memory encoding and retrieval). As for connections between the PFC and MTL, there are several direct and indirect connections. Using the monkey as a model system, studies have

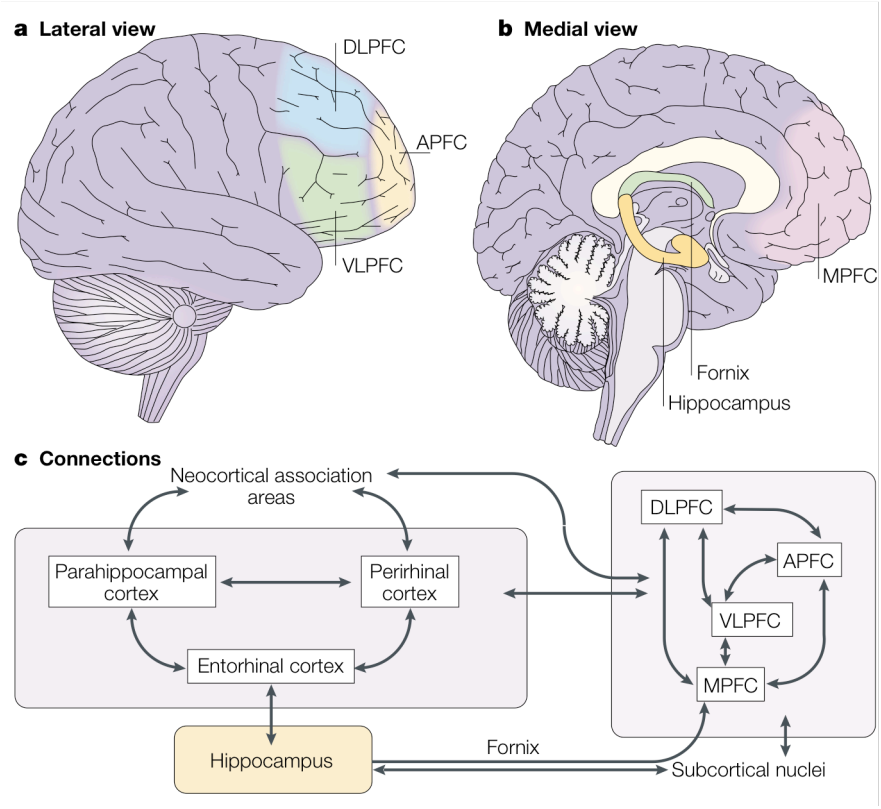


Figure 1.2. Schematic of the medial temporal lobes, prefrontal cortices, and connections between them. Figure from Simons & Spiers 2003.

revealed that there are reciprocal connections between the mPFC and parahippocampal gyrus through the uncinate fasiculus, a large white matter tract (Mark et al., 1995; Schmahmann and Pandya, 2009). The PFC and MTL are also reciprocally connected through the anterior temporal stem and anterior corpus collosum (Tomita et al., 1999). In addition, the fornix connects the ventral mPFC with the hippocampal complex reciprocally (Mark et al., 1995; Schmahmann and Pandya, 2009). The dlPFC and ventral mPFC cortices have reciprocal connections with the entorhinal and perirhinal cortices (Rempel-Clower and Barbas, 2000). The dlPFC has reciprocal connections with the hippocampus via the fronto-occipital fasiculus (Goldman-Rakic et al., 1984). There are also reciprocal connections from the hippocampus and EC to mPFC via the superior cingulum (Goldman-Rakic et al., 1984; Schmahmann and Pandya, 2009). Finally, there are also indirect connections between the MTL and PFC via the medial dorsal thalamus (Miller and Cohen, 2001).

On a cognitive level, the prefrontal cortex is thought to be important for memory encoding and retrieval because of its role in cognitive control processes (for review see Blumenfeld and Ranganath, 2007; Badre and Wagner, 2007). Cognitive control processes such as selection of information to be encoded and organization of said information is key to establishing a memory. In addition, in order to retrieve specific memories, a goal-directed search for relevant information and the monitoring of said information is necessary, which are also thought to be cognitive control processes supported by the PFC (Blumenfeld and Ranganath, 2007; Cabeza and Nyberg, 2003; Simons and Spiers, 2003).

Based on lesion work, functional MRI, and computational models, there are some gross structure-function mappings that can be postulated (for review see Simons and Spiers, 2003; Blumenfeld and Ranganath, 2007). First, in humans the vlPFC is hypothesized to support

selection processes. Using functional BOLD imaging, studies of interference resolution, response inhibition and semantic classification, which are all part of *selecting* goal-relevant information by either attending to relevant information or inhibiting goal-irrelevant information, show vlPFC activation (Aron et al., 2004; Davachi et al., 2001; Jonides and Nee, 2006). Patients with dlPFC damage do not cluster words that are similar semantically during either encoding or recall, likely reflecting a deficit in *organization*. However, if given more specific instruction regarding organization (such as telling them to cluster words by category) they can perform closer to the level of healthy adults (Gershberg and Shimamura, 1995). In addition, computational models support the hypothesis that dlPFC works to organize information (Botvinick, 2008) and work in the rhesus monkey has shown the dlPFC to be important in goal-directed behavior such as organizing information relevant to performance (see Fuster, 2008). The medial PFC is also thought to be important to memory, as BOLD fMRI activation has been shown in recollection (Rugg and Vilberg, 2013) the anterior cingulate (ACC, part of the mPFC) has been proposed to act as a "conflict detector," working to disambiguate similar memories (Botvinick et al., 2001), and areas of the ventromedial PFC are thought to be involved linking stimuli within an event (Ezzyat and Davachi, 2011) or linking items-in-context (Ritchey et al., 2015).

The hippocampus shows remarkable homology between rats and humans (Insausti, 1993; Manns and Eichenbaum, 2006). While there are greater differences between humans, non-human primates, and rodents in the composition of the PFC versus the evolutionarily older hippocampus, there can still be meaningful comparisons made. Although vastly expanded in humans, the prefrontal cortex demonstrates homology to the prefrontal cortex in rats. Specifically, the agranular medial prefrontal cortex in rats shows homology to Brodmann areas 25, 32, and 24 in the human (Passingham and Wise, 2012). Lesions of the fornix (a main

connection from hippocampus to mPFC) in rats (Olton et al., 1982), monkeys (Gaffan, 1994), and humans (Aggleton and Brown, 1999) all demonstrated deficits in episodic and episodic-like memory function. These results show that while some functions performed by the mPFC in rodents may have expanded to other regions in humans and non-human primates (e.g. dlPFC, vlPFC), some likely have remained in the mPFC. Therefore, meaningful conclusions about long-term declarative memory and its neural substrates can be made from work with these model organisms in both the MTL and PFC.

1.1.1 A subtype of declarative memory: a discussion of sequence memory and its neural correlates

Memory for events in our lives and their temporal order is a critical component of episodic memory, a subtype of declarative memory (Tulving, 1984). Memory for the sequence of events combines the what and where with the *when* of our lives. This allows us to not only recall a sequence of events from the past, but to accurately predict future events.

Many of the same brain areas that have been implicated in declarative memory are also implicated in memory for sequences of events. For example, rodent studies have shown that the hippocampus and prefrontal cortex are necessary for sequence memory (Agster et al., 2002; Devito and Eichenbaum, 2011; Fortin et al., 2002; Hannesson et al., 2004; Kesner and Holbrook, 1987; Kesner et al., 2002). Nonhuman primate studies have shown hippocampus and prefrontal cortex contribute to sequence memory (Charles et al., 2004; Naya and Suzuki, 2011; Petrides, 1995; Pinto-Hamuy and Linck, 1965). Humans with lesions to either hippocampus or prefrontal cortex have impairments in sequence memory (Mayes et al., 2001; Milner et al., 1985; Shimamura et al., 1990), and research using fMRI in healthy young adults has shown hippocampal and prefrontal activation during both encoding and retrieval of temporal

information (Cabeza et al., 1997; Chen et al., 2015; Davachi and DuBrow, 2015; Ekstrom et al., 2011; Hayes et al., 2004; Jenkins and Ranganath, 2010; Kumaran and Maguire, 2006; Ranganath and Hsieh, 2016).

In the hippocampus, events could become bound into associative memories through the recurrent collaterals of CA3 – the intrinsic activity of CA3 neurons could become associated with CA3 cells that fire in response to input from entorhinal cortex holding information about the current stimulus, therefore an indirect association could be made between the stimuli in a sequence. After several trials, exposure to that stimulus could then drive activation of the next stimulus in the sequence, a process of pattern completion in CA3 (see Davachi and Dubrow, 2015 Ranganath and Hsieh, 2016 for reviews and other proposed models). In CA1, a comparison could be made between the predicted stimulus via CA3, and the current stimulus, via the entorhinal cortex (Allen et al., 2016; Colgin et al., 2009; Lisman and Grace, 2005). Notably, studies in both the rodent and the human have provided evidence for these hypotheses. In the human, Shapiro et al. (2012) found that after several presentations, the pattern of activity in CA3 to item N became increasingly similar to the pre-learned pattern of activity to item N+1. In the rodent, a study conducting electrophysiological recordings from CA1 found that on correct trials of recency discrimination, there was greater pattern dissimilarity between items with greater lags, suggesting a slowly changing representation of temporal context (Manns et al., 2007). Allen et al. (2016) found that in the CA1 of rats, coding for sequence was present at the level of single units, ensembles, and local field potential. In this non-spatial sequence task, the authors showed that the number of cells responding to the temporal context (in this case temporal context refers to items presented “in sequence” versus “out of sequence”), as well as the modulation of the magnitude of slow gamma in CA1, was related to sequence memory performance. Therefore, it

appears the hippocampus holds information about elapsed time as a form of temporal context as well as ordinal coding as a form of temporal context (Manns et al., 2007, Allen et al., 2016).

The role of the prefrontal cortex is thought to include goal-directed retrieval search, maintenance of previous stimuli, and response selection based on the retrieved information. In support of this, Allen et al. (2011) found that the mPFC of rats displayed item-specific activity between item presentations in a sequence, ordinal coding of items, and response selection. In the human, there has been evidence the prefrontal cortex contributes to sequence memory performance as well. Humans with either hippocampal or prefrontal cortex lesions have been shown to have impairments in memory for sequences (Mayes et al., 2001; Milner et al., 1985; Shimamura et al., 1990), and fMRI research has shown hippocampal and prefrontal activation during performance of temporal memory tasks (Cabeza et al., 1997; Chen et al., 2015; Davachi and DuBrow, 2015; Ekstrom et al., 2011; Hayes et al., 2004; Jenkins and Ranganath, 2010; Kumaran and Maguire, 2006; Ranganath and Hsieh, 2016). However, there has been a general lack of consensus for localization of function within the human prefrontal cortex in support of sequence memory, which may be due to difference in task design, type of information to be processed, analysis methods, encoding instructions, among others. Therefore, there remains much to be learned about the role of each region and how the regions interact to produce memory for sequences of events. Employing a cross-species sequence memory paradigm can help to bridge the human and rodent literature in regards to neural substrates supporting sequence memory and their respective functions. The current dissertation research utilizes this task design to study the neural substrates of sequence memory in humans.

1.2 Long-term declarative memory abilities decline with typical aging

On a cognitive level, the ability to form new long-term declarative memories is known to decline in typical aging. Source memory (identifying not only the stimulus, but the source of the stimulus as well; (Craik et al., 1990; Glisky et al., 2001; Henkel et al., 1998), episodic memory (the 'what', 'when' and 'where' of an event; Craik and Simon, 1980; Mayeux et al., 2001), spatial memory (Head and Isom, 2010; Uttl and Graf, 1993), and temporal context memory (Allen et al., 2015; Roberts et al., 2014; Tolentino et al., 2012) have all been shown to decline with normal aging in humans, using cross-sectional and longitudinal study designs. Studies of item recognition memory have shown mixed results; mostly showing intact recognition memory (Bartlett et al., 1989; Naveh-Benjamin et al., 2009; Pierce et al., 2005; Prull et al., 2006), but occasionally have shown some declines with age (Craik and McDowd, 1987; Naveh-Benjamin et al., 2009). This lack of decline with age has likely been observed because in item recognition memory tasks the participant is shown the previously studied stimulus and asked yes/no if they have seen it, rather than more difficult forms of memory retrieval such as free recall (Craik, 1983). Of course, there are different levels of instruction to help participants, such as instructing the participant that they will be later tested on the stimuli (resulting in intentional encoding) versus simply instructing the participants to attend to the stimuli without warning of a future test (relying on incidental encoding). Indeed, the amount of explicit instruction explains some of the mixed results on whether item recognition memory is impaired in older adults (Koutstaal et al., 2003; Naveh-Benjamin et al., 2009).

In rodents and monkeys, spatial memory and episodic-like memory have been shown to decrease with normal aging across a variety of tasks, such as the Barnes maze, the Novel object recognition test, Morris water maze, delayed non-match to sample, and odor recognition tasks

(for review see Clark and Squire, 2010; Gallagher and Rapp, 1997). As described previously, all of these types of declarative memories are thought to be subserved by the MTL and PFC.

1.3 What is typical aging?

While numerous studies have shown that declarative memory abilities decline with "typical" or "healthy" aging, it is important to identify the criteria being used to identify typical versus pathological aging. Currently the field stands where clinicians can differentiate dementia (such as Mild Cognitive Impairment and probable Alzheimer's Disease) from typical aging (Kawas, 2003). Therefore, many typical aging studies ask participants if they have been diagnosed with any neurological or psychiatric disorders. Many also conduct assessments of global cognitive function such as the clinical dementia rating (CDR; Morris, 1993) or mini-mental state exam (MMSE; Folstein et al., 1975) (assessments used by clinicians to help determine dementia status), where subjects must fall into the non-demented categories to be included in the typical aging study. In addition, some studies use more detailed neuropsychological assessments that cover certain cognitive domains of interest more in-depth (examples include the Wechsler Adult Intelligence Scale (WAIS; Wechsler, 1997a) that tests processing speed, perceptual organization, verbal comprehension, and working memory, the Rey Auditory Verbal Learning Test (RAVLT; Rey, 1941) that tests long-term declarative memory, and the Wisconsin Sorting Task (Ber, 1948) that tests the executive function of set-shifting).

However, it remains a question whether there is such a thing as healthy aging, or whether what we observe is merely the early stages of some form of clinical dementia that we would all develop if our lives were long enough. Several findings indicate that this is not likely the case. Numerous studies have shown there are greater individual differences in performance on cognitive tasks in older versus younger groups in humans, non-human primates, and rodents

(Buckner, 2004; Gallagher et al., 2006; Morrison and Baxter, 2012, although see Salthouse, 2012) suggesting at least that there are likely many different trajectories in aging. Furthermore, it should be noted that rodents do not develop Alzheimer's Disease, a leading cause of dementia, but still show greater variability in performance with age and cognitive decline, indicating that these declines cannot be attributed to AD per se (Gallagher et al., 2006); although they could still be attributed to common, early precursors that only lead to AD in humans. Indeed, rather than viewing typical aging as a preclinical state, it will be important to understand what changes occurring in typical aging make the brain more vulnerable to pathology (Fjell et al., 2014).

As research continues, the construct of typical, healthy aging will continue to be refined. Longitudinal studies - such as the Nun Study (Tyas et al., 2007) and the 90+ study (Kawas et al., 2013) - will be important to validate or call into question results from cross-sectional studies. And, as more biomarkers become available (e.g., APOE genetic status – allele e4 has been shown to be associated with higher rates of dementia (Reitz et al., 2010) and PIB-PET imaging which measures the accumulation of AB plaques in the brain (a protein aggregate that characterizes AD) – see Jack et al., 2010), we can better predict which individuals will continue on the trajectory of typical aging and which will develop a form of dementia. For instance, recent studies have looked at the relationship between physical health and cognition; a meta-analysis found that high levels of physical activity significantly protected subjects from cognitive decline at follow-up (Sofi et al., 2011), while in the oldest-old physical performance measures correlated with increased odds of dementia (Bullain et al., 2013). These are cross-sectional studies and therefore it is unknown whether this represents a modifiable risk factor or rather a biomarker for future cognitive decline (Exercise intervention studies have shown mixed effects: Cancela Carral and Ayan Perez, 2007; Oken et al., 2006). These studies represent the multi-faceted issue of

defining typical aging and the progress being made to differentiate typical aging from trajectories that lead to dementia.

Currently we can differentiate dementia from typical aging, and typical aging shows commonalities in age-related decline that are worthy of study and characterization. For cross sectional studies, it is important to carefully to characterize participants, including employing neuropsychological test batteries and taking into account the aforementioned risk factors. As we continue to study healthy, typical aging, we may discover different trajectories that aging can take (some may still be healthy while others may predict dementia). Therefore, the study and characterization of typical age-related decline is an important endeavor in order to identify those variables contributing to age-related change as well as to identify those variables that underlie different trajectories in aging.

1.4 Biological changes that could underlie memory declines in typical aging

Having established that the MTL and PFC are critical to declarative memory function, and that declarative memory abilities decline with age, we can now attempt to understand what neurobiological changes in these regions are underlying the cognitive declines. Animal models of aging are key to understanding the neural changes occurring in the PFC and MTL with age; as mentioned earlier they afford techniques not available to implement in the human, such as electrophysiological recordings, they do not develop AD, and they age much more rapidly than humans. Work in rodents and non-human primates has given us much information about the biological changes with age in these regions. In addition, work in humans, mostly using neuroimaging techniques, but also postmortem and CSF studies, have shown us much about the biology of the aging human brain.

1.4.1 Anatomical and electrophysiological studies

The MTL and PFC are more vulnerable to synaptic changes with age than other areas of the brain (Morrison and Baxter, 2012). In the MTL, there is no evidence of frank cell loss with aging in the hippocampus or surrounding cortices in either rats or monkeys, even in the face of declarative memory decline (Burke and Barnes, 2006; Morrison and Baxter, 2012). However, there are synaptic changes; in the DG of older rats there are 1/3 fewer synaptic connections from EC than younger rats, representing a degradation of the perforant path (this also includes a reduction in synapses on CA3 from perforant path axons; see Wilson et al., 2006). This reduction of synapses is correlated with the degree of spatial memory ability in older rats, indicating that this reduction is likely contributing to declines in declarative memory with age. The finding that there is a degradation of the EC axons comprising the perforant path has been supported by work in humans. Yassa et al. (2010) used ultra-high resolution diffusion tensor imaging to demonstrate a decline in the perforant path integrity of humans, which correlated with a test of long-term declarative memory (delayed recall on the Rey Auditory Verbal Learning Test (RAVLT)). This decline in integrity of the major input to the hippocampus (the perforant path axons synapsing onto neurons of DG) likely contributes to the decreased ability of older adults to form new declarative memories. In particular, this likely contributes to problems with episodic or episodic-like memories that require one-trial learning, as McClelland, McNaughton, and O'Reilly (1995) demonstrated that the DG would be key to performing pattern separation, which would allow for discreet events to be disambiguated. It should be mentioned that the biological degradation observed in normal aging is in contrast to aging with Alzheimer's disease, in which frank cell loss is found in both hippocampus and entorhinal cortex (Hyman et al., 1984; Kordower et al., 2001).

Aged rats show deficits in both induction and maintenance of long-term potentiation (LTP) in DG and CA3, and decreases in the level of LTP induced in CA1 (Burke and Barnes, 2010). This reduction could underlie the observation that older rats have "remapping" effects whereby the place fields (cells showing activity correlated with an area in space; recorded with multiple single unit recording electrodes) in CA1 remap more often than in younger rats (Barnes et al., 1997). This remapping would make spatial memory tasks, such as the Morris water maze, more difficult because the original place map cannot be retrieved. Indeed, this study found deficits in Morris water maze performance in older rats (Barnes et al., 1997). This remapping effect is separate from the "rigidity" of place fields seen in CA3, whereby likely due to the decreased inputs to the DG (which results in less pattern separation in the DG which results in less pattern separated output to the CA3), *similar* locations are treated as the *same* by CA3 cells in older rats (Wilson et al., 2005). The dentate gyrus has also been shown in older rats and older rhesus monkeys to have decreased Arc expression (an immediate early gene that modulates with plasticity) (Blalock et al., 2003).

Long-term potentiation cannot be assessed under normal, non-invasive conditions in humans, but other methods have revealed disturbances in the hippocampal system in humans. A functional signal (using fMRI) showed that a decrease in signal in the DG and subiculum correlated with worse performance on a declarative memory measure (Small et al., 2002). In aged monkeys there was an observed decrease in cerebral blood volume in the DG (Small et al., 2004). Using FDG-PET, a marker for glucose metabolism, Walhovd et al. (2010) showed that the amount of metabolism in the hippocampus in humans correlated positively with performance on a declarative memory test, the RAVLT.

The prefrontal cortex also shows greater vulnerability with age than other regions of the brain. In aged rhesus monkeys, area 46 (part of the dlPFC) does not show significant neuronal loss, but does show significant decrease in volume resulting from loss of synapses (approximately 30-60% in layers 1 & 3) (Dumitriu et al., 2010; Peters et al., 2008). In addition, the particular synapses that are most affected are thin axospinous synapses. This loss of synaptic density correlates negatively with performance on the DNMS task (a test for episodic-like memory thought to employ the PFC and MTL), and in particular the loss of thin spines (thought to be the most plastic of spines) correlates negatively with performance on the DNMS task (an incredible r =.97; Peters et al., 2008). Layer 3 houses the cortico-cortical connections connecting the PFC with such areas as temporal cortex, and it is this layer (along with layer 1) that shows the most synaptic loss with age, in particular the neurons projecting from the superior temporal lobe to area 46 (Duan et al., 2003). Rodents show similar changes with age. In the mPFC, rats show layer 3 spine loss with age, and as with rhesus monkeys, this was mostly accounted for by loss of thin spines (Bloss et al., 2011). Work in human postmortem studies has shown that there is a decrease in the number and density of synapses in area 10, part of the mPFC, and synaptic gene expression has also been shown to be downregulated in human PFC (and to a lesser extent in hippocampal and entorhinal cortex) (Berchtold et al., 2013).

In terms of gross structure, Shamy et al. (2011) showed that the greater the volume of aged monkey dlPFC, the greater accuracy on the DNMS task. In humans, white matter hyperintensities (thought to arise from cerebrovascular brain injury) in the PFC have been shown to correlate with worse performance on the Wisconsin card-sorting task (a test of executive function thought to be supported by PFC), and also correlate with smaller PFC volume (Gunning-Dixon and Raz, 2003). In monkeys, fibers connecting dlPFC with other cortical areas

show alterations in myelination with age (Peters and Sethares, 2002). Functional anisotropy, a diffusion tensor imaging measure that correlates with axonal integrity, has been shown to be decreased in anterior corpus collosum tracts of the human – a main pathway between the MTL and PFC (Buckner, 2004), and frontal collosal fibers showed more age-related degradation than posterior fibers (Sullivan et al., 2006). In addition, correcting for global white matter changes, Bennett et al. (2014) showed a decrease in the integrity of the fornix, a white matter tract emerging from the hippocampus and connecting to the mPFC. The authors showed that this degradation related to a measure of pattern separation.

1.4.2 Human functional neuroimaging studies

Human functional neuroimaging has also revealed changes with age in the MTL and PFC during performance of declarative memory tasks. However, there exist conflicting results in the literature. Several studies have shown that during encoding and/or retrieval older adults show less activity in the PFC while maintaining activity levels in the MTL (Logan et al., 2002; Nyberg et al., 2003; Sperling, 2007). This has been interpreted as under-recruitment of the PFC due to older adults decrease in cognitive control abilities supported by the PFC. In support of this, Logan et al. (2002) demonstrated that with increased explicit instruction (participants were given a specific strategy to use), the same older adults that showed decreased activity in PFC now showed levels to the same degree as younger adults. However, other studies have shown in older adults an increase in activity in the PFC (or an increase in bilaterality with age), with either equivalent levels of activity in MTL or decreases (Dennis et al., 2008; Giovanello and Schacter, 2012; Gutchess et al., 2005; Morcom et al., 2003; Murty et al., 2008). This bilaterality in the PFC could be either compensatory recruitment in order to perform the task, or a result of dedifferentiation, whereby older adults lack specificity of neural activity (Grady, 2012). Indeed,

one study showed that increased bilaterality only occurred in lower-performing adults (Duverne et al., 2009) while another showed the increase only in higher-performing adults (Cabeza et al., 2002). In terms of activation levels, generally decreases have been interpreted as degradation, and increases in activity as compensatory, dedifferentiation, or degradation. However, correlations with behavior have been used to differentiate the possibilities (see Grady, 2012; Maillet & Rajah, 2013). If over-activation in the older group correlates with better performance, compensation is usually ascribed, while if over-activation correlates with poorer performance, degradation or less efficient us of neural resources is ascribed. If the area of activation in older adults is not present in the young adults and does not correlate with behavior, dedifferentiation may be ascribed.

Regardless of the ascribed explanation for the activation (whether compensatory or a result of degradation or dedifferentiation), the general finding of increased prefrontal bilaterality in older adults was termed HAROLD (hemispheric asymmetry reduction in older adults) to describe a trend in the neuroimaging aging literature across several cognitive domains, including episodic memory (Cabeza, 2002). Another general finding was termed PASA (posterior-anterior shift in aging), describing reduced occipital activity concurrent with increases in frontal activity, argued by the authors to be compensatory (Davis et al., 2008). A third descriptive model is termed CRUNCH (compensation-related utilization of neural circuits hypothesis), developed in an attempt to reconcile both the decreases and increases in activity seen with age (Reuter-Lorenz and Cappell, 2008; Figure 1.3). This model predicts that older adults will have increases in activity compared to younger adults during tasks with low amount of cognitive load, but with higher cognitive load, the older adults cannot increase their activity to the level of young adults.

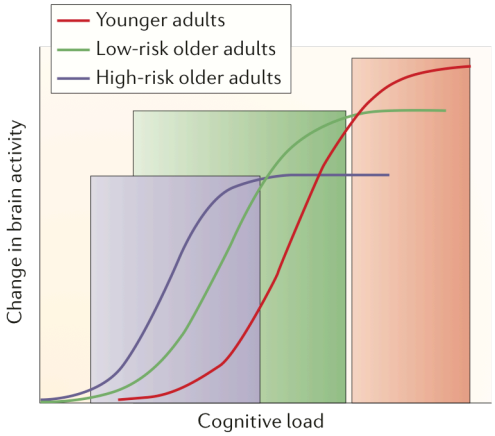


Figure 1. 3. The "CRUNCH" model. Figure from Grady, 2012.

While these models describe some of the findings in the literature, they lack a neurobiological mechanism. In addition, not all studies follow these observed patterns. Therefore, what are explanations for the differing results? First, it has been demonstrated there is greater variability in older adults' performance, and therefore likely underlying neural activity (Buckner, 2004; Gallagher et al., 2006; Morrison and Baxter, 2012; although see Salthouse, 2012). There is also the potential that different strategies are being used between groups, such that older (or some of the older) are solving the task differently from the younger participants, and therefore employ different neural resources (Hedden and Gabrieli, 2004). Task design that shows differential behavioral outcomes when participants are engaging in different strategies can ameliorate this issue. Another important aspect is the choice of contrast in BOLD fMRI. There is no inherent baseline in BOLD fMRI, therefore the signal depends on a contrast of task conditions. If the task of interest is compared to a task that may also tap into the same cognitive domain supported by the same region of interest, this could result in spurious results. For example, a memory-encoding task contrasted with a "resting" baseline in the hippocampus could show very different activation than a memory-encoding task contrasted with a non-mnemonic perceptual task (Stark and Squire, 2001). To compound this, older adults could differentially change their responses to rest, encoding, and the non-mnemonic perceptual task, which would result in different changes with age observed depending on which contrast was used. There is also evidence that the type of fMRI paradigm is important; Dennis et al. (2007)

demonstrated that in the same participants the use of event-related versus block design affected the results, wherein the event-related design older adults showed a decrease in activity in the MTL, but not in the block design. This could relate to a different strategy use, or to an inherent issue with the methodology of BOLD fMRI (see section 1.5 below for discussion). In addition, differences in task difficulty could lead to different neural outcomes due to other cognitive processes (e.g. executive control) being engaged between the groups. There is also the issue of changes to the vasculature with age, which can impact the neurovascular coupling upon which the BOLD fMRI signal relies (D'Esposito et al., 2003). See the next section for discussion of how we can attempt to mitigate these effects. In sum, there is much research that must still be done in order to understand the neural changes with age that underlie memory declines with typical aging.

1.5 Issues facing functional imaging of the aging human brain

1.5.1 General issues with BOLD fMRI

For this proposal, I will be employing functional MRI imaging that relies on blood-oxygen-level-dependent (BOLD) signal as a methodology to study the aging human brain. Therefore, the benefits and drawbacks of this methodology must be addressed. BOLD imaging is a non-invasive technique that provides an indirect measure of neural activity, and consequently is an important advancement to studying the human brain. While the hemodynamic response measured by BOLD is clearly correlated with neural activity (Logothetis, 2003) it must be acknowledged that the exact physiological basis of the BOLD signal is not known (Hargreaves et al., 2012). The basis of the BOLD signal is the ratio of non-paramagnetic oxygenated hemoglobin to paramagnetic deoxygenated hemoglobin; when neural activity occurs, there is an increase in blood flow to the area (CBF), which results in an increased ratio of oxygenated to

deoxygenated hemoglobin, which results in an increase in BOLD signal. However, the BOLD signal also depends on the cerebral blood volume in the area and the cerebral blood oxygen consumption (CMRO2) (Kim and Ogawa, 2012). See figure 1.4 from D'Esposito et al. (2003).

It is important to bear in mind BOLD fMRI is a contrastive measure, and therefore an increase or decrease in the BOLD signal is an increase or decrease in the relative difference between task conditions. What is more, a recent study found that while BOLD and local field

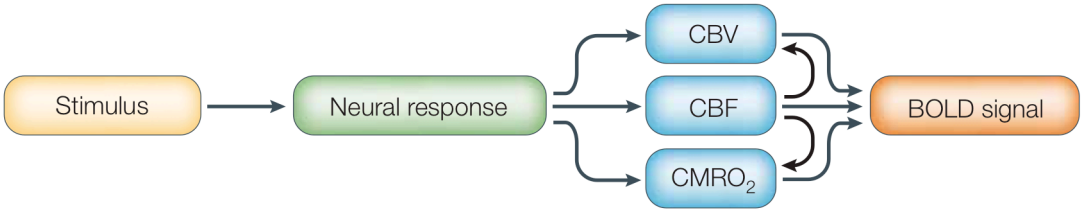


Figure 1.4. Schematic of how a stimulus evokes a neural response, eliciting a hemodynamic response, and results in the BOLD signal. Figure from D'Esposito, Deouell & Gazzaley, 2003. Modified from Buxton, 2002.

potential recordings showed very similar results for humans and monkeys completing similar tasks, the polarity was flipped for some contrasts (Hargreaves et al., 2012). Therefore, it is the change between task conditions and the change in these contrasts with age that is key, whether it be a positive or negative change. For example, reelin, a gene associated with synaptic plasticity, has been shown to be downregulated with age in the entorhinal cortex of aged rats (Stranahan et al., 2010). If local field potential (LFP) recordings were done, this decreased reelin expression would likely correlate with a decrease in LFP, but could correlate with either a decrease *or* increase in the BOLD signal (e.g. Hargreaves et al., 2012). For simplicity, I will assume a positive relationship when I predict changes between task conditions and changes with age, but a negative relationship is possible. This does not negate the findings however, as it is the change that is key, and has been shown to correlate well with LFP (Logothetis, 2003; Hargreaves et al., 2012). Therefore, regardless if the BOLD signal difference between a baseline condition and a

memory condition increases or decreases with age, it reflects a change with age for the memory vs. baseline conditions in the underlying neural signal.

1.5.2 BOLD fMRI issues specific to aging:

The relationship between neural activity and the hemodynamic response is termed neurovascular coupling. Unfortunately, this coupling is not static across brain regions and populations (D'Esposito et al., 2003). Specifically for the current dissertation, aging can impact neurovascular coupling due to changes in the vasculature with age (Ances et al., 2009; D'Esposito et al., 2003; Samanez-Larkin and D'Esposito, 2008). Increases in atherosclerosis (fatty material deposited in walls of arteries) and increases in tortuosity of vessels, which occur in aging, reduce the elasticity of the vessels, which likely results in a decreased dynamic range of the BOLD signal (D'Esposito et al., 2003). In addition, it has been demonstrated that there is reduced vascular reactivity to CO_2 in aged rats and humans. CO_2 normally increases the dilation of the arterial vessels, but studies in aged rats and humans have shown that the change from hypocapnia to hypercapnia (reduced to elevated CO_2 blood levels) resulted in a reduced range of CBF for older adults (Ito et al., 2002). Again, this will result in a decreased dynamic range of the BOLD signal. Furthermore, small lacunar infarcts (leukoariosis; termed white matter hyperintensities seen in scans of the human brain) are common in aging populations, and the amount of leukoariosis is correlated with reduced CBF as well as reduced response to hypercapnia, both of which reduce the dynamic range of BOLD (Hatazawa et al., 1997; Kuwabara et al., 1996).

Given these caveats, we can still obtain meaningful measures of brain function from BOLD fMRI. First, there are ways to obtain measures of the vascular reactivity, such as CO_2 inhalation or breathholding (Bandettini and Wong, 1997; Thomason et al., 2007). However, this

is a strenuous task that is very difficult for older populations. Kannurpatti and Biwsal (2008) have shown that measuring resting state fluctuation amplitude produces comparable results to breathholding and CO_2 inhalation, and have applied this method effectively in aging (Kannurpatti et al., 2011). We employ this method in our experiments. However, a caveat is made by Grady & Garrett (Grady and Garrett, 2014) who demonstrate that the very dynamic range which we seek to correct for could be meaningful in terms of neural activity and performance (see Grady and Garrett, 2014 for review). Therefore, we interpret results with and without RSFA correction in our experiments. Additionally, experimental design can ameliorate the issue of changes with age – contrasting task conditions within participants using percent change should be more resistant to the effects of changes to the dynamic range (because both tasks should be affected by the vascular reactivity changes with age), barring a more complex relationship between reactivity and condition. While there are changes occurring in the aging brain that can affect the BOLD signal, there are ways to design experiments and analyze the data to derive meaningful results.

2 Experiment 1: A cross-species sequence memory task reveals hippocampal and medial prefrontal cortex activity and functional connectivity in humans

2.1 Introduction

Memory for events in our lives and their temporal order is a critical component of episodic memory (Tulving, 1984). Previous research has implicated the hippocampus, surrounding medial temporal lobe cortices, and prefrontal cortex in episodic memory (Cabeza and St Jacques, 2007; Eichenbaum and Fortin, 2005; Fuster, 2008; Rugg and Vilberg, 2013; Scoville and Milner, 1957; Simons and Spiers, 2003; Squire et al., 2004). Research on learning and memory for specifically the temporal component of episodic memory has shown that these same regions are implicated. For example, rodent studies have demonstrated that the hippocampus and prefrontal cortex are necessary for sequence memory (Agster et al., 2002; Devito and Eichenbaum, 2011; Fortin et al., 2002; Hannesson et al., 2004; Kesner and Holbrook, 1987; Kesner et al., 2002) and nonhuman primate studies have shown hippocampal and prefrontal contributions to sequence memory (Charles et al., 2004; Naya and Suzuki, 2011; Petrides, 1995; Pinto-Hamuy and Linck, 1965). Humans with either hippocampal or prefrontal cortex lesions have been shown to have impairments in memory for sequences (Mayes et al., 2001; Milner et al., 1985; Shimamura et al., 1990), and fMRI research has shown hippocampal and prefrontal activation during encoding and retrieval of temporal information (Cabeza et al., 1997; Chen et al., 2015; Davachi and DuBrow, 2015; Ekstrom et al., 2011; Hayes et al., 2004; Jenkins and Ranganath, 2010; Kumaran and Maguire, 2006; Ranganath and Hsieh, 2016). Other studies in humans have shown that patterns of activity in the hippocampus hold information about sequence (Hsieh et al., 2014; Jenkins and Ranganath, 2016; Schapiro et al., 2012).

However, there remains much to be learned about the neurobiological mechanisms within each region, the specific role of each region, and how these regions interact to produce memory for sequences of events. A cross-species approach can provide a powerful means to address these outstanding questions. However, rodent and human studies have generally used different stimuli and task designs, which limits our ability to compare results across species. Some human studies have tested memory for sequences of locations in an effort to parallel studies done in rats (Fouquet et al., 2010; Hopkins et al., 2004), but these are faced with challenges of disambiguating memory for space from memory for the order of locations.

A non-spatial non-verbal cross-species task developed by Allen et al. (2014) was designed to disambiguate space from sequence as well as to establish a task that draws on similar cognitive processes in rats and humans. In this task, rats and humans are asked to learn and remember sequences of odors (rats) or fractal images (humans) using the same task design. Rats and humans perform exceptionally similarly on a range of different memory probe trials, providing evidence that the task engages the same cognitive processes between rats and humans (Allen et al., 2014). Given that parallel cognitive processes are likely occurring, we now seek to understand the neurobiology supporting these processes.

By temporarily inactivating the hippocampus and/or the prelimbic region of the medial prefrontal cortex in rats, Quirk et al., (2013) found that this task relies on both regions. Further, single-unit electrophysiological recordings in rats from the CA1 region of the hippocampus and prelimbic region of the medial prefrontal cortex show a dynamic and complementary series of changing representations that can solve the sequence task (Allen et al., 2011b, 2016). Specifically, neurons in dorsal CA1 differentially fired to odors that were in sequence versus out of sequence and ensemble activity distinguished between in and out of sequence conditions on a

trial-by-trial basis. Slow gamma power in CA1 was higher for items that were in sequence versus out of sequence. In the medial prefrontal cortex, neurons showed activity that was odor-specific between odor presentations as well as neurons that showed ordinal coding and response selection.

Our goal is to determine whether similar mechanisms exist in humans. While we cannot induce reversible lesions in the hippocampus or mPFC and the temporal resolution of BOLD fMRI preclude a number of analyses, we can determine whether the same information is present in each of these regions (as well as other areas of the brain). Specifically, we sought to determine whether we would observe differences in BOLD activity between sequences that had all items in sequence and sequences that had an item out of sequence in the hippocampus and medial prefrontal cortex. The hippocampus shows remarkable homology between rats and humans (Insausti, 1993; Manns and Eichenbaum, 2006). Although vastly expanded in humans, the prefrontal cortex demonstrates homology to the prefrontal cortex in rats. Specifically, the agranular medial prefrontal cortex in rats, the area under investigation in the cross-species task in rats, shows homology to Brodmann areas 25, 32, and 24 in the human (Passingham and Wise, 2012).

In addition to BOLD activity levels, we also predicted an interaction between mPFC and HC during sequence memory. As noted, the task appears to be solved by a dynamic interplay involving both the hippocampus and mPFC (Allen et al., 2011; Allen et al., 2016). While the time-resolution of BOLD limits it to very low frequency interactions (or "infra-slow" interactions), quasi-random patterns of bursting or coherence can be observed at these timescales (Thompson et al., 2014a, 2014b). Therefore, in humans we predicted to observe functional connectivity during task performance between mPFC and the hippocampus. Functional

connectivity was a term coined to describe temporal correlations between activity in different regions of the brain (Friston, 1994). By assessing correlations in fluctuations in activity over time between regions, it is thought to assess whether regions are interacting, although it does not provide information about direction or causation (Friston, 1994). Evidence for both activity in hippocampus and mPFC as well as functional connectivity between the regions would strongly support underlying homologous substrates for sequence memory in the rat and human, which would further our understanding of the neural substrates supporting our ability to recall the order in which events occur in our lives.

2.2 Materials and Methods

2.2.1 Participants

Exclusion criteria included MRI contraindications and/or history of neurological or psychiatric diagnoses. A total of 58 participants underwent scanning, 8 of which were excluded from analyses due to loss of behavioral data, 1 due to poor behavioral performance, and 5 due to scanner artifacts. Approximately half of these participants were scanned with whole-brain coverage and half with high-resolution coverage of only the medial temporal lobe (MTL). The remaining participants (23 whole-brain coverage: mean age = 22.04 yrs, SD = 3.52, 15 females, and 21 high-resolution of the medial temporal lobe: mean age = 20.76 yrs, SD = 2.72, 13 females) were included in the behavioral and imaging analyses. A resting state dataset that was collected in a separate set of participants was also included in the analyses: 17 participants, mean age = 27.88 yrs, SD = 5.02, 11 females. All participants gave written consented in compliance with the Institutional Review Board (IRB) at the University of California, Irvine, and all were compensated for their participation.

2.2.2 Task Description

The task developed by Allen et al., 2014 was designed to include the "what" and "when" aspects of episodic memory. Developed as a cross-species task, this task tests memory for sequences of odors in rats and fractal images in humans. Here, we modified the human variant of the task for fMRI scanning purposes. Participants were shown a sequence of six images (e.g., ABCDEF) with each image appearing only after the participant pressed and held a response button. For each image, participants indicated whether it was in or out of sequence by holding a response key for >1 second if the image was in sequence (e.g., B was presented after A) or <1 second if the image was out of sequence (e.g., C was presented after A; see Figure 2.1). To familiarize the participants with the task demands before scanning, participants completed a baseline condition of the task (e.g., presenting the actual letters ABCDEF or presenting arrows successively in a clockwise direction). In the scanner, a brief study phase was administered in which the participants were shown 6 sequences consisting of 6 images each; 4 sequences consisting of fractal images; 2 baseline sequences consisting of arrows presented successively clockwise or dots successively presented in a diagonal line from the upper left to lower right of the screen (see Figure 2.1). During scanning participants completed the test phase of the task. Participants pressed a key to initiate display of an image; if this image was in sequence they were to continue holding until the image disappeared (1s); if they image was out of sequence they were to lift their finger off the key before 1s passed (the image disappeared when the key was no longer pressed). The statement "press to begin the next sequence" was presented between each sequence. All 6 sequences were presented throughout scanning; the baseline sequences were included to provide a reminder of task demands, as no feedback was provided to participants. While in the rodent version of the sequence memory task feedback was provided on each trial, in

humans the decision to not provide feedback to participants was made to attempt to isolate the sequence memory component, rather than error or reward signals. Unlike single unit recordings in the rat where error and reward related activity can be disambiguated, the timescale of BOLD fMRI would not allow us to dissociate these signals.

2.2.3 Trial Types

There were 120 presentations of InSeq sets (sets in which all items were in the proper sequence), dispersed evenly throughout the test phase (96 fractal image sets, 24 baseline condition sets). There were 80 presentations with one image out of sequence (OutSeq). These could be either Repeat (ABCD**B**F) or Skip (AB**E**DEF) conditions (64 fractal images, 16 baseline condition images; see Figure 2.1). In the last half of trials, a different out of sequence trial type was introduced: Ordinal Transfer probe trials for the fractal image sets. These were trials in which an image from another sequence was presented in the correct position, but wrong sequence (e.g. sequence ABCDEF and UVWXYZ are known; presented is ABCD**Y**F; Y is in the correct position - 5^{th} - but wrong sequence). There were 40 Ordinal Transfer probe trials.

The different OutSeq trial types were designed to tax different cognitive processes. The Repeat type can be solved by several different strategies, including working memory ("B was already presented a few seconds ago") or item-item sequential associations ("B should not come after D") or item-in position associations ("B should not be in the 5^{th} position"). In order to correctly identify a Skip, however, participants must accurately predict the next image using either item-item or item-in-position associations, and therefore is the more sensitive measure of sequence memory. To correctly identify an Ordinal Transfer as out of sequence, participants must use sequential item-item associations, rather than item-in-position associations, or they must identify that the item does not belong to the particular set of items of that sequence.

2.2.4 Behavioral analyses

A G-test was used to compare expected and observed response frequencies. For low-frequency responses (e.g., any response indicating "out of sequence"), this test is more robust than the traditional chi-squared test (Sokal and Rohlf, 1995). A G-test was computed for both baseline and fractal images conditions. Next, to calculate overall sequence memory performance, we used the Sequence Memory Index derived in prior work (Allen et al., 2014; also see Figure 2.1). This index ranges from -1 to 1, with a 1 representing perfect performance (an individual would have RT>1s for InSeq items and <1s for OutSeq items) and 0 representing chance and is typically normally distributed. Percent correct (accuracy) was also calculated for each condition of interest (Figure 2.1).

2.2.5 BOLD fMRI acquisition

Data were collected in a 3.0T Phillips scanner with a SENSE head coil at the University of California at Irvine. A whole brain 0.75mm isotropic magnetization-prepared rapid gradient echo (MP-RAGE) structural scan was collected for each participant; repetition time (TR) / echo time (TE) = 11/4.6ms, field of view (FOV) = 240 × 231 mm, flip = 18°, 200 sagittal slices. Half of the participants underwent whole-brain functional imaging, while the other half underwent high resolution functional imaging of the medial temporal lobe. Whole-brain functional data were acquired with a T2* weighted echo planar imaging scan, at a resolution of 3 mm (isotropic), 39 axial slices, TE = 26ms, flip angle = 70°, TR = 2200ms, dynamics = 122. High-resolution functional data were acquired with a T2* weighted echo planar imaging scan, at a resolution of 1.55mm (isotropic), 39 axial slices, TE = 26ms, flip angle = 70°, TR = 2200ms, dynamics = 122. The whole-brain resting state dataset was acquired with a T2* weighted echo planar imaging scan, at a resolution of 2.5 mm (isotropic), 46 axial slices, TE = 26ms, flip angle

= 70°, TR = 2500ms, dynamics = 188. Using AFNI (Analysis of Functional NeuroImages), functional data were aligned to the participant's MP-RAGE with the script align_epi_anat.py (Saad et al., 2009). Each participant's structural scan and functional data (statistical maps) were aligned to a model template using ANTs (Advanced Normalization Tools; (Avants et al., 2008)).

2.2.6 Univariate Imaging Analyses

The inherent blur of the data were assessed using AFNI 3dFWHMx. For the whole-brain data, the inherent blur was over 4mm FWHM (mean blur across subjects = 4.5x4.5x4.1 in x,y,z planes, whole brain mask), therefore the data were not smoothed. For high-resolution acquisition of the medial temporal lobe, data were blurred to 3mm FWHM using AFNI 3dBlurToFWHM (mean blur across subjects = 2.8x3.0x3.1 in x,y,z planes). Sequences were modeled as duration-modulated blocks to account for individual timing differences using 3dDeconvolve (Ward, 2002). Sequences were modeled in a GLM as Inseq, Repeat, Skip, Ordinal Transfer, Baseline Inseq and Baseline OutSeq (repeat and skip). We performed strict motion correction with TRs with framewise displacement of >0.5, as well as 1 TR before and 2 TRs after, being removed. In addition, we regressed out the motion vectors as well as CSF and white matter (segmented using six-tissue types via ANTs' antsCorticalThickness.sh; Tustison et al., 2014) and masked images to remove extra-brain or CSF voxels. Percent signal change was computed and used in the analyses.

Univariate imaging analyses compare activity during one condition of interest to activity in another condition of interest. To test the hypothesis that sequence memory activates the hippocampus and mPFC, for both whole-brain and high resolution datasets, two-tailed, bi-directional paired t-test comparing percent signal change for the beta weights (the measure of activity derived from the GLM) for OutSeq (repeat, skip, OT combined) vs. Inseq were

completed with a voxel-wise threshold of $p<0.01$ and extent thresholds of 18 and 27 voxels in the whole-brain and high-resolution data respectively. This combined spatial threshold was determined by Monte Carlo simulation (AFNI'S 3dClustSim) to correct for multiple comparisons based on the known blur (see above). To assess the differences between OutSeq probe types, a repeated-measures one-way ANOVA was completed using the same spatial and voxel-wise thresholds. Exploratory and follow-up t-tests were all two-tailed using voxel-wise and spatial extent thresholds. Follow-up t-tests were two-tailed with a Bonferonni-corrected threshold of $p<0.05$.

2.2.7 Functional Connectivity Analyses

To assess functional connectivity during performance of the sequence memory task, we employed seed-to-seed and seed-to-brain correlation analyses as well as large-scale network analyses. The preprocessing steps included quadratic detrending, which removed up to polynomial order two. The timeseries was then normalized to have zero mean and unit variance and the motion vectors and first derivatives were regressed out of the signal. The 6 principal components computed using tCompCor (not including global signal) were also regressed out of the signal (Behzadi et al., 2007). The data were then temporally bandpass filtered (0.08 to 0.009Hz) and TRs with framewise displacement >0.5, as well as those one TR before and two TRs after were removed as recommended by Power et.al. (2012).

Seed-to-seed correlations were conducted as follows: Seed regions in the left hippocampus (HC) and medial prefrontal cortex (mPFC) were defined based on the results of the univariate contrast of InSeq vs. Outseq. A sphere within the cerebellum was used as a control region. Seed regions were warped from model space to subject space using ANTs. The mean timeseries of BOLD fMRI activity was extracted from the seed region by averaging the signal

across all voxels within the region. A Pearson's correlation coefficient was then calculated between regions and then r-to-z transformed. Seed-to-seed correlations on the GLM residual error timeseries after removing stimulus-evoked responses (aka "background connectivity"; Norman-Haignere et al., 2012) were conducted in the same way. For the seed-to-brain analyses, the same seed regions were used from the seed-to-seed analyses, but correlated to every other voxel of the brain. A t-test was then computed to determine significant correlations, using voxel-wise and spatial extent thresholds.

To conduct large-scale network analyses, seed regions were defined at 5mm spherical locations based on the graph-analyses conducted by Power et al. (2011). The graph analysis was completed by Power et al. (2011) to determine a meaningful set of functional areas across the brain, resulting in 264 regions. Power et al. (2011) refers to these regions as "nodes" therefore we will use the same term. Three additional nodes were added to the analysis: the L HC, R HC (mirror of L HC), and mPFC, each based on the center of mass of the cluster based on Inseq vs. Outseq univariate contrast. The nodes were then warped into subject space using ANTs. As was done in the seed-to-seed analyses, the preprocessed timeseries was extracted from each region and Pearson's correlation coefficient was calculated. A t-test was then computed for each z-transformed correlation. Thresholds were set as in Power et al. (2011) to assess nodes displaying the highest correlations.

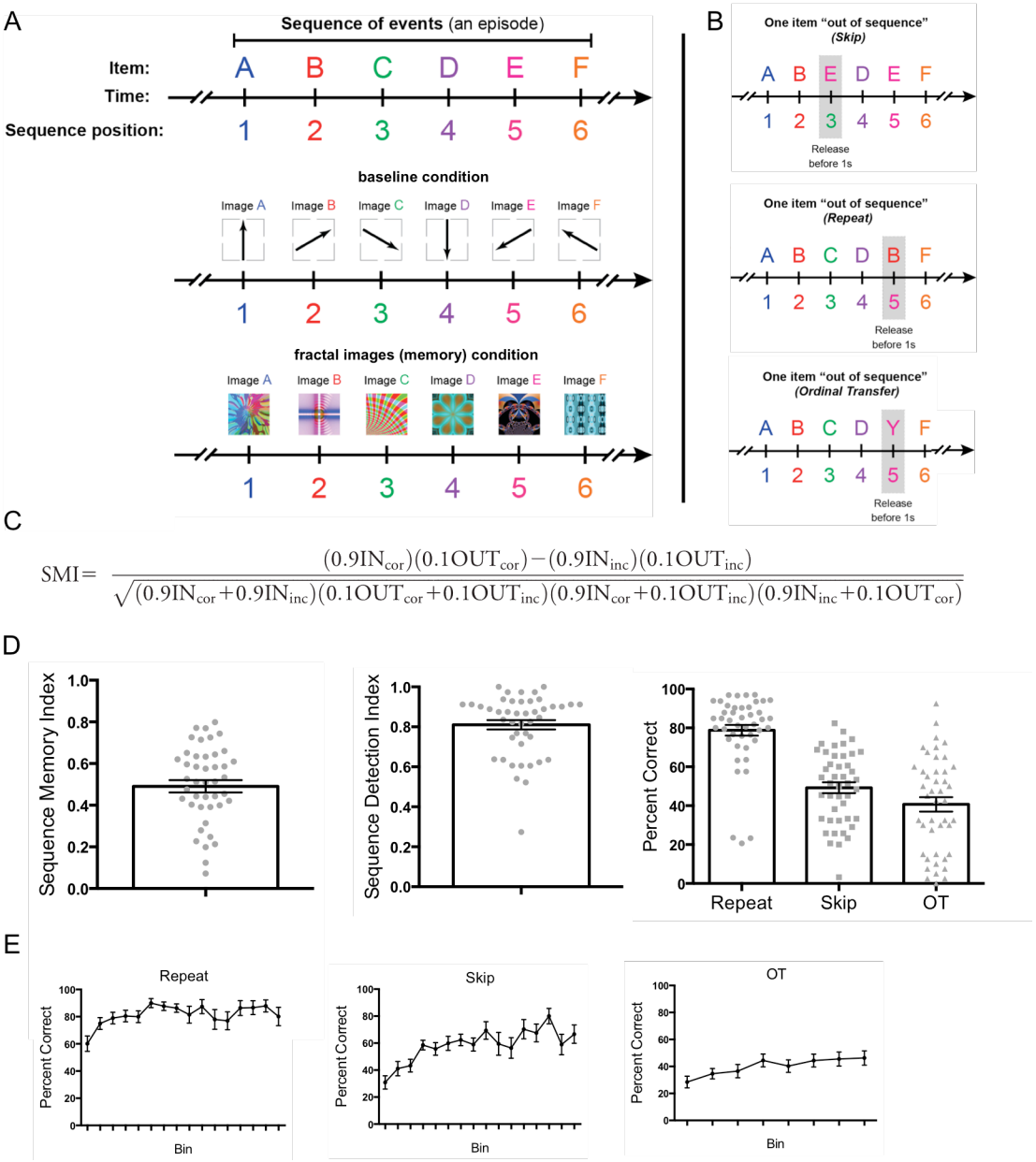


Figure 2.1. (a-b) Depiction of the sequence task modified for fMRI. (Depiction modified from Allen et al. (2014)). Participants were shown a sequence of six images (e.g., ABCDEF) and asked to indicate if the images were in or out of sequence by holding a response key for >1 second if the image was in sequence (e.g., B was presented after A) or <1 second if the image was out of sequence (e.g., C was presented after A). (a) Schematic of a baseline and fractal image condition. (b) Example of a skip, repeat, and ordinal transfer. (c) Equation used to calculate overall sequence memory performance, the Sequence Memory Index. (d) Performance on the sequence memory task replicates previous findings from Allen et al. (2014). The sequence Memory Index and Sequence Detection Index are normalized measures of sequence memory and baseline condition performance, respectively. Performance on the different memory probe types of repeat, skip, and ordinal transfer are shown, with (e) sub-graphs showing performance over the course of the task, binned every 15 sequences for a total of 16 bins. Individual subject data displayed with mean and standard error of the mean.

2.3 Results

2.3.1 Behavioral analyses

Performance on the sequence memory task replicates previous findings from Allen et al. (2014) (Figure 2.1). All participants included in the imaging analyses showed a significant G-test for the baseline condition. One participant did not show a significant G-test for the fractal image condition, but did for the sequence memory baseline condition and was therefore included in the analyses as was done previously in Allen et al. (2015).

2.3.2 Whole-brain univariate imaging analyses: Sequence memory activates the hippocampus and mPFC

To test the hypothesis that sequence memory activates the hippocampus and medial prefrontal cortex and to assess other neural correlates of sequence memory in the human, we

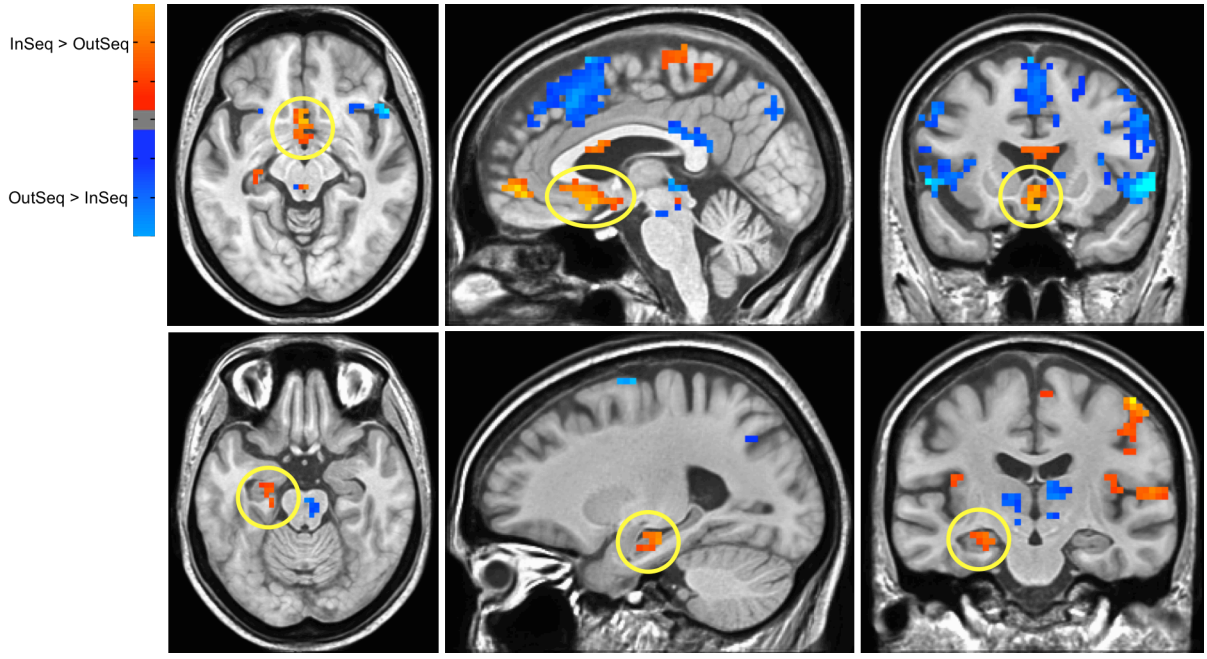


Figure 2.2. Sequence memory activates the left hippocampus and ventromedial prefrontal cortex in humans. Contrast of InSeq trials vs. all OutSeq trials (repeat, skip, ordinal transfer) (voxelwise $p<0.01$; alpha $p<0.05$).

conducted a t-test comparing InSeq vs. OutSeq trials in the whole-brain dataset. The contrast revealed clusters in the left anterior hippocampus and ventromedial PFC, with both regions showing InSeq>OutSeq (Figure 2.2). The mPFC cluster encompasses the subcallosal gyrus and anterior pericallosal sulcus, extending into an area of the anterior cingulate, corresponding to Brodmann areas 25 and 24. These regions of the mPFC have been identified as demonstrating homology to the agranular region of the medial prefrontal cortex in rats (reviewed in Passingham and Wise, 2012) that has been the focus of the electrophysiological recording and inactivation studies discussed previously (Allen et al., 2016; Quirk et al., 2013). This contrast also showed activity in an additional mPFC region including an area of the anterior cingulate (Brodmann area 32; also considered a homologous area of the mPFC; Passingham and Wise, 2012) and extending into frontal superior gyrus. Four other regions showed the InSeq>OutSeq contrast, including the right precentral gyrus extending into bilateral central sulcus, right supramarginal gyrus into lateral sulcus and transverse temporal sulcus, left lateral sulcus, and left transverse temporal sulcus. All other regions showed the reverse contrast of OutSeq>InSeq (note, interpretation of the directionality should be done with caution as there are many factors unrelated to pyramidal cell spike rate that will affect BOLD – see section 1.5.1 and Hargreaves et al., 2012). All regions are outlined in Table 2.1 (cortical structures were identified based on Freesurfer segmentation of the model template in Talairach space and the anatomical atlas as outlined in Destrieux et al. (2010); http://surfer.nmr.mgh.harvard.edu).

Table 2.1: Regions showing differential activity to InSeq vs. OutSeq

Cluster #	Peak Coordinates x	y	z	# of Voxels	Region	Direction
1	-55.5	-16.5	2.5	599	Right inferior frontal sulcus and gyrus extending into middle frontal gyrus and superior frontal sulcus, inferior precentral sulcus extending into insula	Out > In
2	-55.5	46.5	44.5	461	Right intraparietal suclus, angular gyrus, supramarginal gyrus, posterior superior temporal sulcus	Out > In
3	-40.5	25.5	59.5	404	Right precentral gyrus, central sulcus, postcentral gyrus extending medially into paracentral lobule and sulcus and into left central sulcus	In > Out
4	37.5	58.5	53.5	325	Left intraparietal suclus, angular gyrus, supramarginal gyrs, precentral sulcus	Out > In
5	1.5	-13.5	59.5	292	Bilateral superior frontal gyrus, middle anterior cingulate gyrus and sulcus	Out > In
6	-49.5	22.5	11.5	138	Right posterior segment of lateral suclus, supramarginal gyrus, subcentral gyrus, anterior transverse temporal gyrus and sulcus	In > Out
7	52.5	-7.5	35.5	115	Left inferior precentral sulcus into inferior frontal and middle frontal gyrus	Out > In
8	-1.5	76.5	41.5	81	Bilateral parieto-occipital sulcus into preucuneus	Out > In
9	49.5	-13.5	2.5	74	Left inferior frontal gyrus, circular sulcus of the insula	Out > In
10	7.5	4.5	11.5	63	Left caudate, thalamus	Out > In
11	-1.5	-13.5	-9.5	47	Bilateral subcollosal gyrus and anterior pericallosal sulcus, extending into anterior cingulate	In > Out
12	-1.5	28.5	26.5	42	Bilateral posterior-dorsal part of the cingulate gyrus into pericallosal sulcus	Out > In
13	-10.5	13.5	8.5	35	Right thalamus	Out > In
14	1.5	-46.5	-0.5	30	Bilateral anterior cingulate into superior frontal gyrus	In > Out
15	-19.5	58.5	17.5	30	Right parieto-occipital sulcus	Out > In
16	-13.5	-1.5	11.5	29	Right caudate	Out > In
17	-1.5	28.5	-0.5	28	Bilateral superior colliculli	Out > In
18	28.5	-19.5	11.5	25	Left circular sulcus of the insula	Out > In
19	19.5	19.5	-12.5	24	Left hippocampus	In > Out
20	22.5	7.5	62.5	24	Left superior frontal gyrus	Out > In
21	-1.5	19.5	-15.5	22	Bilateral pons	Out > In
22	-37.5	-43.5	26.5	22	Right middle frontal gyrus	Out > In
23	37.5	19.5	14.5	19	Left posterior segment of lateral suclus, circular sulcus of the insula	In > Out
24	61.5	16.5	11.5	18	Left transverse temporal sulcus	In > Out

In follow-up analyses, we sought to determine whether the activity observed in InSeq vs. OutSeq was related to this contrast in general or whether it was driven by differences in error rates and performance (e.g., greater errors on OutSeq trials than InSeq trials). We first re-analyzed the fMRI data excluding all error trials from the InSeq and OutSeq regressors in the GLM. The results were highly similar with the same regions exhibiting reliable InSeq vs. OutSeq activity. Three additional regions passed the threshold: left cerebellum OutSeq>InSeq, left orbital sulcus InSeq>OutSeq, and right cuneus InSeq>OutSeq, with one cluster in right insula no longer present OutSeq<InSeq. We then examined whether individual differences in performance were related to this activity. Neither the mean beta values for InSeq-OutSeq within the left anterior hippocampus cluster nor within the mPFC cluster correlated with the sequence memory index across participants (R square for L HC = 0.03; R square for mPFC = 0.12).

In an additional attempt to determine the generality of the InSeq vs. OutSeq findings, we compared activity related to this contrast during the memory trials to activity in this contrast during the baseline trials within the L HC and mPFC. In both the novel sequence memory trials and the well-known sequence baseline trials (e.g., rotating arrows), the same task must be done (determining whether the current item is either in or out of sequence). While we anticipated seeing a difference between the two, as the novel sequence memory trials are in the process of being learned, no such difference was found in directed tests (clusters defined by the univariate Memory InSeq vs. Outseq contrast; OT's were excluded from analysis because baseline trials did not include OTs) (left HC: $t_{22} = 1.60$, $p = 0.12$; mPFC $t_{22} = 1.61$, $p = 0.12$). Further, in whole-brain analyses, only the left intraparietal sulcus showed a difference in this contrast.

2.3.3 Whole brain exploratory univariate analyses

While our *a priori* hypotheses were directed at the examining in humans the strong differences observed in rodents in the in InSeq vs. OutSeq contrast, by splitting the coding of the OutSeq items by probe type (repeat, skip, or ordinal transfer), we can conduct exploratory analyses to determine whether there may be differences in the neural substrates of each. A whole-brain repeated-measures one-way ANOVA revealed three regions showing a main effect of trial type: a cluster spanning the left and right dorsal precuneus, a cluster spanning left and right ventral precuneus, and left inferior precentral sulcus (Figure 2.3). Follow-up t-tests (Bonferroni-corrected to $p<0.05$) revealed this to be a difference between ordinal transfers and both the skips and repeats (OTs > skips/repeats precuneus areas; OTs < skips/repeats in precentral sulcus) with no difference between skips and repeats in each of these three areas.

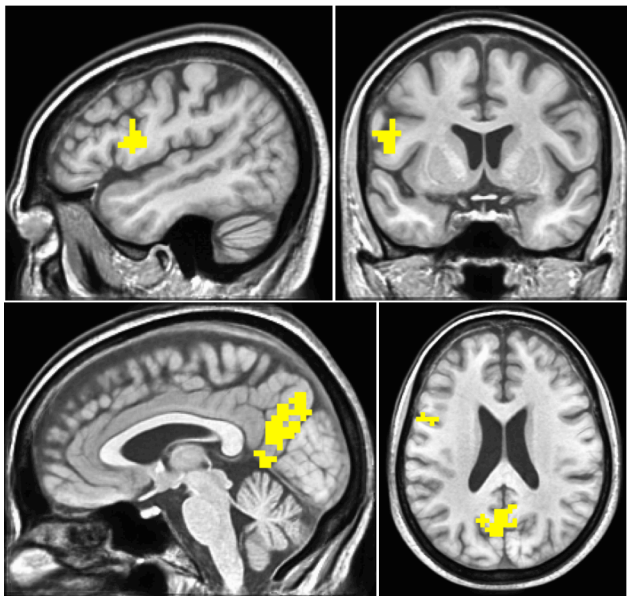


Figure 2.3. Results of one-way repeated measure ANOVA to assess regions sensitive to probe type (repeat, skip, ordinal transfer). Shown are areas demonstrating a significant F-value, including bilateral precuneus and left precentral sulcus (voxelwise $p<0.01$; alpha $p<0.05$). Subsequent t-tests Bonferroni-corrected to $p<0.05$ revealed a difference between ordinal transfers and skips/repeats (OTs > skips/repeats precuneus areas; OTs < skips/repeats in precentral sulcus), and no difference between skips and repeats in these three areas.

One potential concern is that previous research has demonstrated an "encoding/retrieval flip" in the precuneus (Huijbers et al., 2012), and OTs are only present in the second half of the dataset. To address this, a one-way repeated measures ANOVA was conducted in only the second half of the dataset for each region. Results demonstrated that the left inferior precentral sulcus and dorsal precuneus results maintained a difference between OT and both skips and repeats (Bonferroni-corrected $p<0.05$). However, it should be noted that within the ventral precuneus, only the skips remained reliably different from the OT trials when correcting for multiple comparisons. Additionally, within both precuneus regions, there was evidence of a difference in activity for the repeat trials between the first and second half of data (paired t-test $p<0.05$, uncorrected for multiple comparisons), suggesting that it is possible that the encoding/retrieval flip could be contributing to the results within the precuneus.

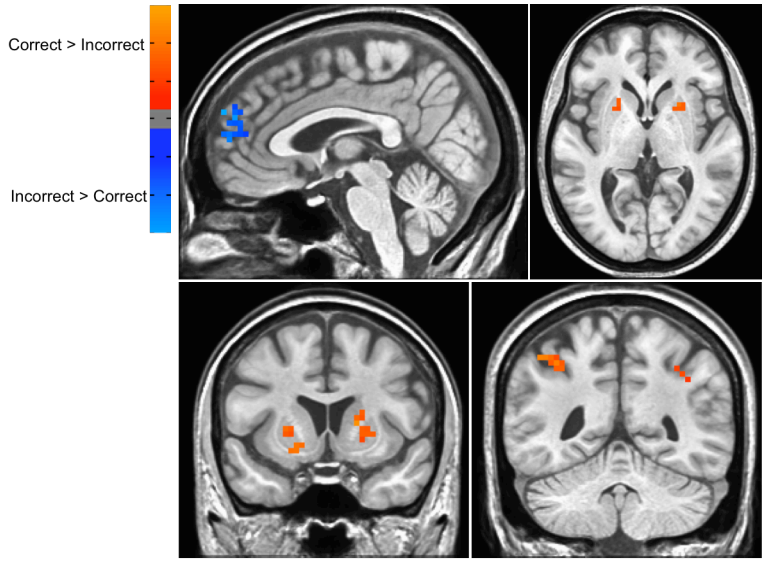


Figure 2.4. Contrast of correct Skip/OT vs. incorrect Skip/OT trials show bilateral putamen and bilateral intraparietal sulcus (Correct > Incorrect) and an anterior superior frontal gyrus region (Incorrect > Correct) (voxelwise $p<0.01$; alpha $p<0.05$).

Additionally, a repeated measures t-test was conducted to assess differences between correct and incorrect trials. Due to constraints on the number of incorrect trials, only OutSeq trials consisting of Skips and OTs could be evaluated. Bilateral putamen and bilateral intraparietal sulcus showed Correct > Incorrect, while an anterior superior frontal gyrus region showed Incorrect > Correct (Figure 2.4).

2.3.4 High resolution univariate imaging analyses: Sequence memory activates the hippocampus

An independent group of participants was scanned using high-resolution imaging of the MTL, with an orientation parallel to the long axis of the hippocampus. This can serve to replicate findings (within the limited field of view of these scans) and to better locate any activity within sub-regions of the MTL or subfields of the hippocampus. Analyses of the high-resolution data (Figure 2.5) revealed bilateral hippocampus for the contrast of InSeq vs. OutSeq, replicating the result of left hippocampus from the whole-brain analyses and extending to the right

hippocampus (which had shown just-below threshold activity in the whole-brain data). No other regions showed a difference between InSeq and OutSeq. Based upon prior work (Kirwan and Stark, 2007) and the efforts of the Hippocampal Subfields Group (Yushkevich et al., 2015), these are most likely located in the subiculum and the DG/CA3. To better determine whether the activity was located to these regions specifically, subsequent t-tests within anatomically defined hippocampal subregions (CA1, CA3/dentate gryus, and subiculum, collapsed across hemispheres) were conducted. These revealed each region showed a significant difference between InSeq and OutSeq ($p<0.01$, Bonferroni corrected). However, a significant repeated-measures one-way ANOVA revealed a difference between subregions, with post-hoc t-tests showing a difference between CA1 and the other two regions ($F = 5.168$, $p<0.05$; Bonferroni-corrected t-tests $p<0.05$: DG/CA3 > CA1 $t_{20} = 2.69$, Sub > CA1 $t_{20} = 2.66$, DG/CA3 vs. Sub n.s.

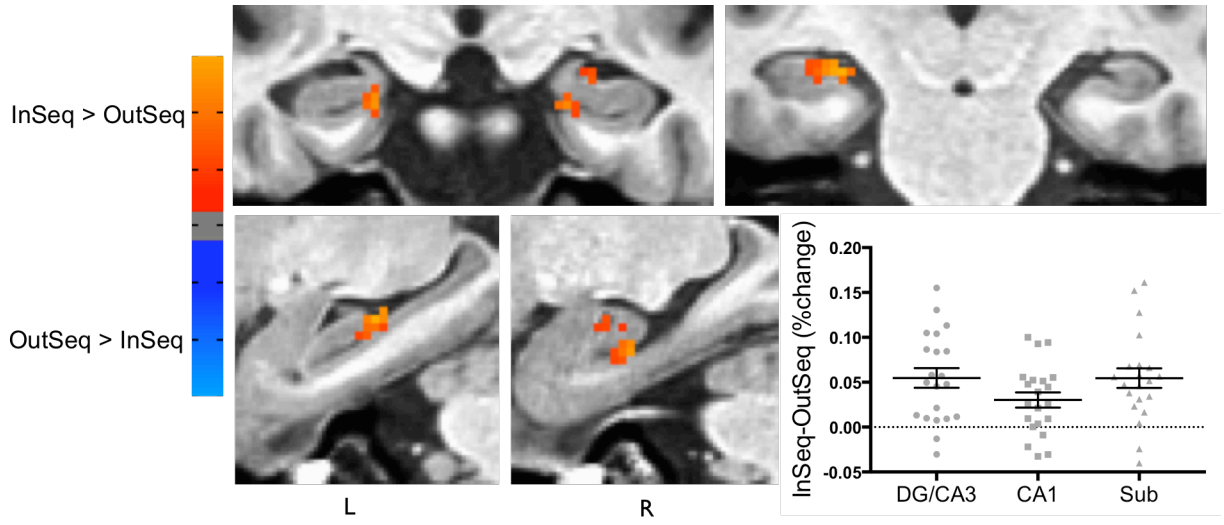


Figure 2.5. High-resolution acquisition of the medial temporal lobe reveals an InSeq vs. OutSeq contrast in bilateral hippocampus, replicating and extending results from whole-brain acquisition (voxelwise $p<0.01$; alpha $p<0.05$). Coronal and sagittal images shown. Subsequent t-tests within anatomically defined hippocampal subregions (CA1, CA3/dentate gryus, and subiculum, collapsed across hemispheres) revealed each region showed a significant difference between InSeq and OutSeq ($p<0.01$, Bonferroni corrected). Individual subject data displayed with mean and standard error of the mean

$t_{20} = 0.03$). No regions of the medial temporal lobe showed a significant F-stat in a repeated-measures one-way ANOVA conducted to assess differences between probe types (repeat, skip, OT).

2.3.5 Whole brain functional connectivity analyses: mPFC and hippocampus timeseries are correlated over the course of the sequence memory task

To test the hypothesis that there would exist an interaction between mPFC and HC during sequence memory, we conducted seed-to-seed functional connectivity analyses on both a priori seeds and on a large-scale network. Results demonstrate the left HC and mPFC show significant functional connectivity over the course of the task (t_{22}=5.318, $p<0.001$) and to a greater extent than a control seed region in the cerebellum (post-hoc $p<0.01$ Bonferroni-corrected; see Figure 2.6). Seed-to-brain functional connectivity confirms this result; the mPFC to left and right hippocampus were the highest correlations of all mPFC-to-brain correlations, and the highest correlations from left HC were right HC and mPFC (voxelwise $p<0.0001$; alpha $p<0.0001$, Figure 2.7). This high threshold was chosen to reveal the strongest correlations. Table 2.2 shows other regions significantly correlated at this threshold.

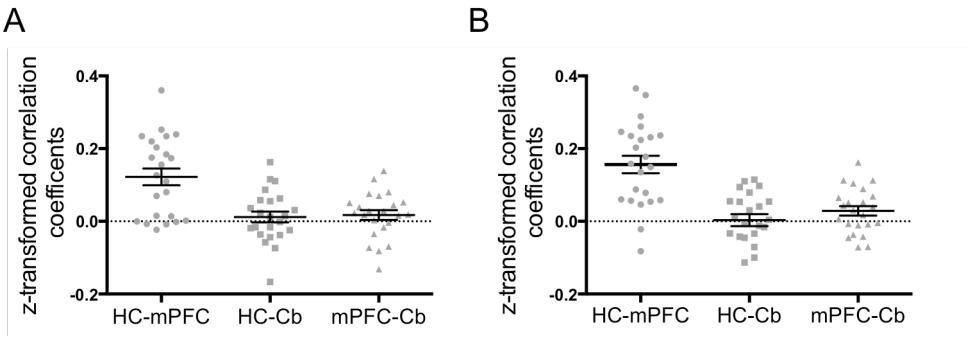


Figure 2.6. (a) Seed-to-seed functional connectivity analyses demonstrate the left HC and mPFC show significant functional connectivity over the course of the task (t_{22}=5.318, p<0.001), and to a greater extent than a seed region in the cerebellum (F=13.61, p<0.001; post-hoc t-tests showed left HC-mPFC connectivity was significantly greater than left HC-Cb and mPFC-Cb connectivity, p<0.05 Bonferroni-corrected. (b) Results maintained when analyzing the GLM residual timeseries, the remaining timeseries after removing the stimulus-evoked responses (F = 20.07, p<0.001). Individual subject data displayed with mean and standard error of the mean.

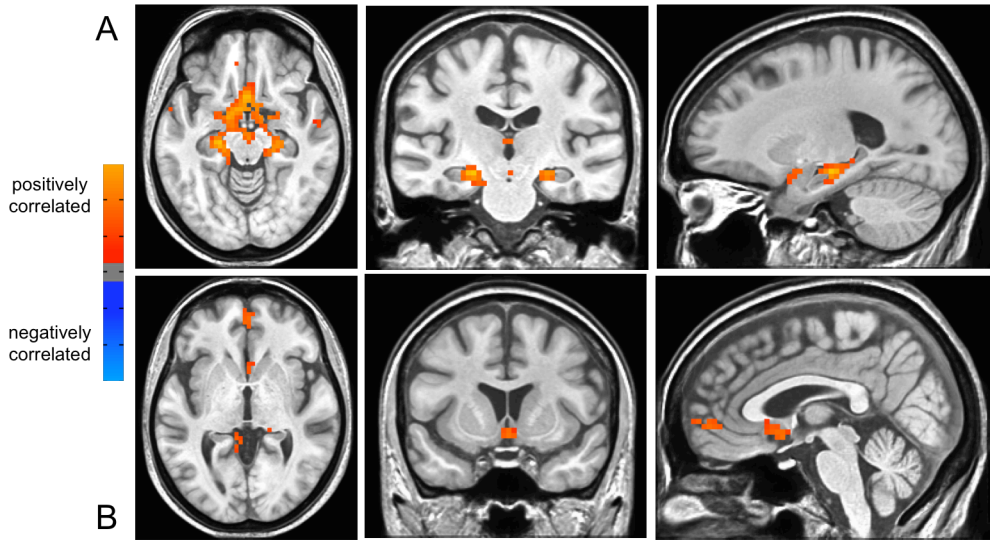


Figure 2.7. Functional connectivity analysis reveals hippocampus and mPFC are highly correlated over the course of the task. (a) Medial prefrontal time-series correlation to all voxels of the brain reveals bilateral hippocampal clusters. (b) Left hippocampal timeseries correlation to all voxels of the brain reveals medial prefrontal cluster. For (a) and (b) timeseries includes all conditions of the sequence memory task. Seed regions were defined by univariate contrast of InSeq vs. OutSeq (voxelwise p<0.0001; a<0.0001; the high threshold was chosen to reveal the strongest correlations).

Table 2.2(a): Regions showing functional connectivity with left hippocampus

Cluster #	Peak Coordinates x	y	z	# of Voxels	Region	Direction of Corr
1	22.5	16.5	-12.5	151	Seed (left hippocampus) extending into amygdala	Positive
2	-25.5	19.5	-9.5	80	Right hippocampus	Positive
3	-1.5	-7.5	-6.5	24	Bilateral subcollosal gyrus into pericallosal sulcus, anterior cingulate	Positive
4	-1.5	-49.5	-0.5	20	Bilateral ventral portion of superior frontal gyrus	Positive
5	4.5	40.5	2.5	19	Left posterior-ventral part of the cingulate gyrus, calcarine sulcus	Positive
6	-7.5	52.5	8.5	11	Right parieto-occipital sulcus	Positive
7	-22.5	-1.5	-12.5	9	Right amygdala	Positive

Table 2.2 (b): Regions showing functional connectivity with mPFC

Cluster #	Peak Coordinates x	y	z	# of Voxels	Region	Direction of Corr
1	-1.5	-13.5	-0.35	671	Seed (mPFC) extending into anterior cingulate, superior frontal gyrus	Positive
2	19.5	19.5	-9.5	347	Left and Right hippocampus connected through bilateral posterior cingulate gyrus, subparietal suclus, precuneus	Positive
3	4.5	52.5	17.5	68	Right middle frontal gyrus and sulcus	Negative
4	43.5	-25.5	35.5	30	Left middle frontal gyrus	Negative
5	49.5	67.5	20.5	22	Left posterior superior temporal sulcus	Positive
6	-49.5	-16.5	38.5	14	Right middle frontal gyrus	Negative
7	43.5	52.5	50.5	14	Right angular gyrus	Negative
8	49.5	-7.5	-15.5	12	Left anterior superior temporal sulcus into middle temporal gyrus	Positive
9	-52.5	4.5	-12.5	12	Right anterior superior temporal sulcus	Positive
10	-4.5	49.5	14.5	12	Right precuneus, Parieto-occipital sulcus	Positive
11	37.5	43.5	32.5	12	Left postcentral sulcus	Negative
12	-40.5	-4.5	53.5	10	Right middle frontal gyrus	Negative
13	-1.5	22.5	8.5	9	Bilateral thalamus	Positive

Despite these strong correlations, caution is warranted in their interpretation as there are known patterns of intrinsic connectivity in whole-brain networks (Power et al, 2011; Cole et al., 2014) and in MTL sub-networks (Lacy et al., 2012). In these intrinsic networks, there are strong patterns of connectivity regardless of task or state that are only moderately modulated by task demands. Here, we performed a large-scale network analysis augmenting the 264 nodes defined by Power et al. (2011) with the addition of HC and mPFC seeds. (The graph analysis completed by Power et al., (2011) was conducted to reveal a meaningful set of functional areas across the brain). Results showed an overall remarkable similarity between results from Power et al., 2011 and the current whole-brain sequence memory task (Figure 2.8); analysis of the high-resolution data revealed connectivity similar to previously reported in Lacy et al, 2012 and a strong correlation to the network described there - rho = 0.5044, $p<0.001$. The addition of bilateral hippocampal and mPFC nodes to the Power et al. (2011) nodes revealed the correlations between these three regions to be among the top 0.5% of all correlations. Analyses performed on the

GLM residual timeseries reveal the correlations between these regions remain in the top 3% of all correlations. Further, a generalized psychophysiological interaction (PPI) approach (McLaren et al., 2012) revealed no differences in mPFC to left HC functional connectivity between trial types (Inseq vs. Outseq t_{22}=0.78, p=0.44), and a significant correlation of mean beta weights was found between the left HC and mPFC nodes for each trial type (Inseq, OT, skip, repeat, baseline; all $p<0.05$). Therefore, the mPFC to hippocampal connectivity appeared to be present throughout all conditions of the sequence memory task. To determine if the functional connectivity between HC and mPFC was exclusive to the sequence memory task, an identical network analysis was completed in a resting state dataset in a separate group of participants. Again, and consistent with other work comparing network activity during tasks to during rest (Cole et al, 2014), results were remarkably similar to Power et al., 2011's network (Figure 2.9). Likewise, the correlations between the left and right HC and mPFC were in the top 3% of all correlations. Therefore, hippocampal–mPFC connectivity was present to a high degree during rest as well as during performance of the sequence memory task.

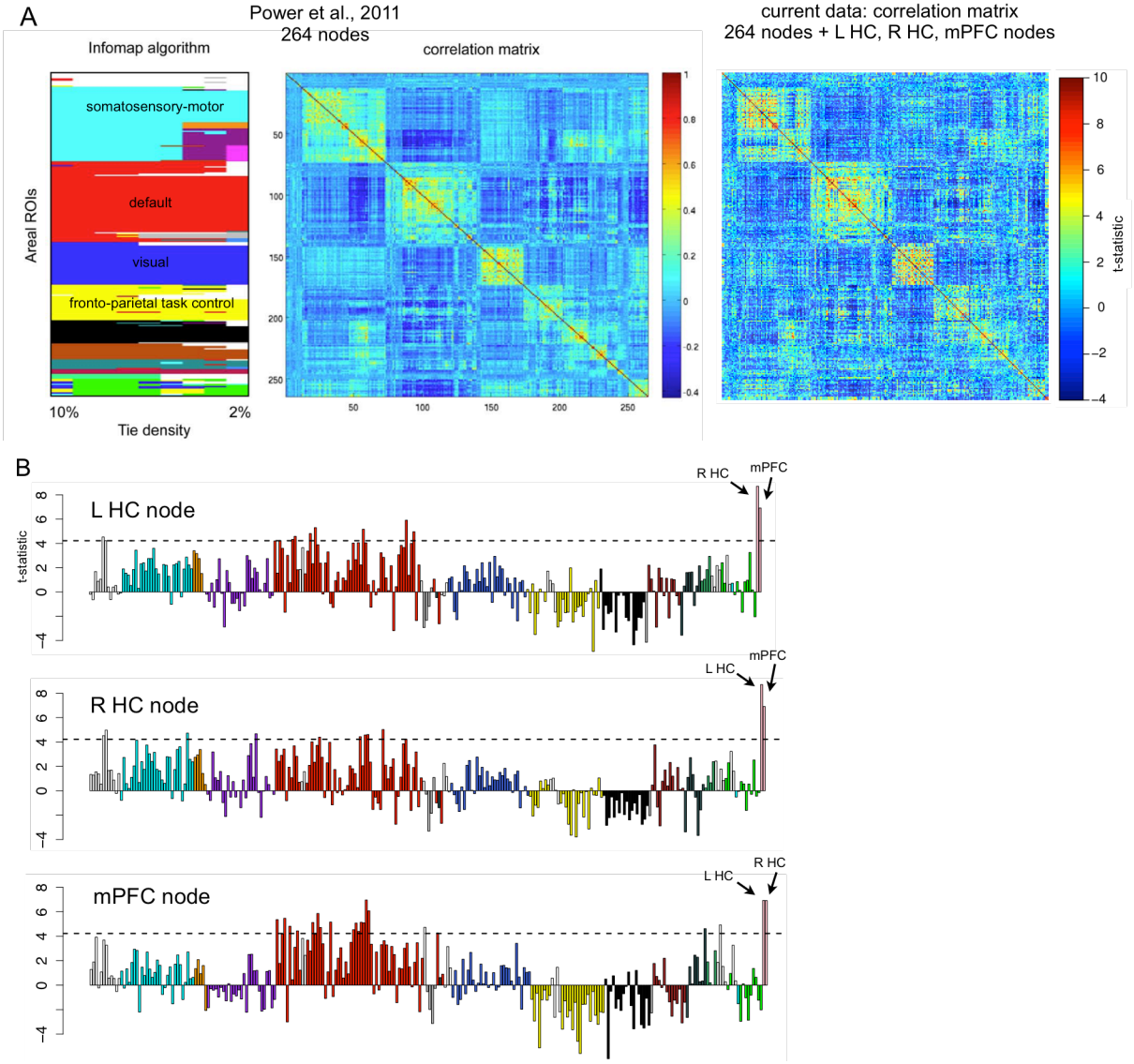


Figure 2.8. Network analyses replicate and extend network analyses from Power et al. (2011). The addition of mPFC, left HC, and right HC nodes reveals mPFC-HC connectivity beyond those of other nodes. (a) Correlation matrix from Power et al. (2011) for 264 nodes (z-transformed r's; left) and correlation matrix from current study (t-statistics; right). Thresholding at 0.5% density (top 0.05% of pairwise correlations) revealed HC-mPFC connectivity above those of other nodes to each other. Far left (from Power et al., 2011): the nodes labeled according to the functional networks to which they belong, defined by a subnetwork detection algorithm. (b) T-statistics for correlations between the left HC, right HC, and mPFC nodes to every other node. Dashed lines indicate top 4% of pairwise correlations.

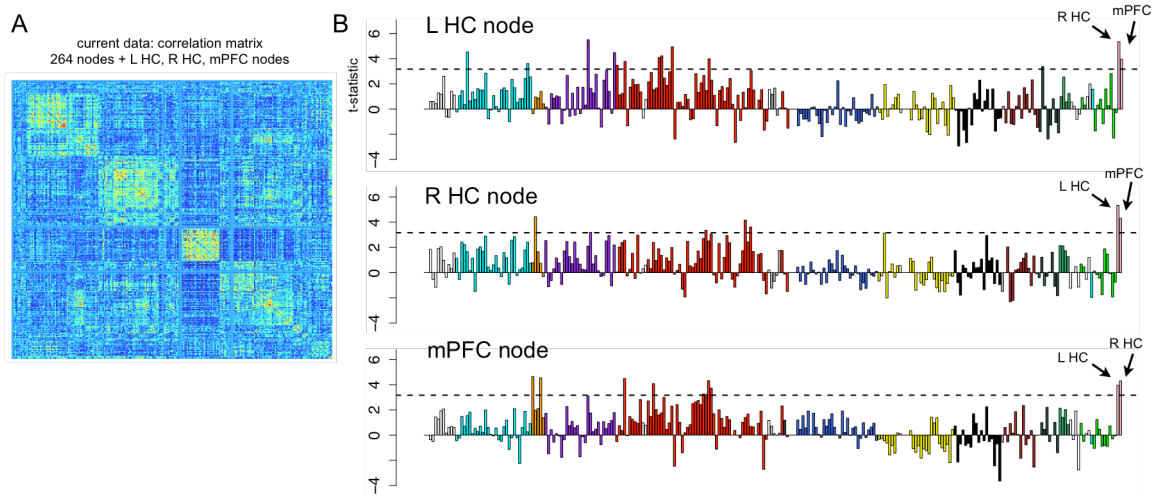


Figure 2.9. Network analysis of resting state data shows similarity to network analysis of sequence memory task data. (a) Correlation matrix of resting state data for all 267 nodes (t-statistics). (b) The t-statistic for correlations between the left HC, right HC, and mPFC nodes to every other node. Dashed lines indicate top 4% of pairwise correlations.

2.4 Discussion

The cross-species approach provides a powerful means to probe the neural substrates of sequence memory to better understand the brain areas and mechanisms supporting this memory ability. Using a cross-species sequence memory paradigm, we have shown clear homology in the neural substrates for sequence memory in the rat and human. Previous work demonstrated the hippocampus and medial prefrontal cortex support memory for sequences of events in the rat using temporary inactivations and both single unit and multi-unit electrophysiological recordings (Allen et al., 2011b, 2016; Quirk et al., 2013). Our findings show these same two areas are implicated in humans, using both univariate and functional connectivity approaches.

2.4.1 The neural substrates for memory for sequences of events are shared between the rat and human

Previous work has demonstrated that fundamental features of episodic memory are likely shared across a wide range of species including humans, non-human primates, rodents, and birds (see Allen & Fortin (2013) for review). Memory for sequences of events is a key aspect of episodic memory, encompassing the "what" and "when" features of an episodic memory. Previous research has demonstrated across rodents, non-human primates, and humans that the hippocampus and areas of the prefrontal cortex play a role in supporting memory for sequences of events (Davachi and DuBrow, 2015; Devito and Eichenbaum, 2011; Milner et al., 1985; Naya and Suzuki, 2011; Pinto-Hamuy and Linck, 1965)

However, there remains much to be learned regarding the neurobiological mechanisms supporting memory for sequences of events and the degree to which the neural substrates are shared across species. The cross-species sequence memory task developed by Allen et al. (2014) was designed to investigate the neural substrates supporting memory for sequences of events in both rats and humans. Using this non-spatial task design, the authors showed that parallel cognitive processes are likely used in both species (Allen et al., 2014). In rats, the authors demonstrate sequence memory relies on the hippocampus and medial prefrontal cortex, and electrophysiological recordings demonstrate a complementary series of changing representations that solve the task (Allen et al., 2011b, 2016; Quirk et al., 2013).

Using the cross-species task in humans, we have demonstrated homologous neural substrates supporting sequence memory. Specifically, we have shown that, in a homologous region of the medial prefrontal cortex as well as left hippocampus, activity in response to sequences of items in which all items are in sequence was greater than activity in response to

sequences of items with one item out of sequence. In a high-resolution follow-up, we replicated this result in the left hippocampus, and extended the results to the right hippocampus (which had activity at a sub-threshold level in the whole-brain data). These results parallel those from Allen et al (2016), in which they observed that slow gamma power in CA1 was higher for items that were in sequence versus out of sequence. Our finding of differential InSeq versus OutSeq activation within the hippocampus and ventromedial prefrontal cortex are also in accordance with authors suggesting that theses regions would be uniquely situated to form item-in-context representations (Ritchey et al., 2015), and work that has found the ventromedial PFC plays a role in linking stimuli within an event (Ezzyat and Davachi, 2011).

An advantage of whole-brain fMRI is the ability to simultaneously record activity throughout the brain. While we demonstrated evidence supporting our *a priori* hypothesis, that mPFC and hippocampus would show differential activity to InSeq vs. OutSeq, there were other areas showing the differential activity for InSeq vs. OutSeq as well (see Table 1.1 for all regions). Previously implicated regions in sequence memory, such as the striatum and angular gyrus (Ezzyat and Davachi, 2011; Ranganath and Hsieh, 2016; Tubridy and Davachi, 2011), show a contrast of OutSeq > Inseq. In addition, areas of the prefrontal cortex including dorsolateral, ventrolateral, and dorsal medial PFC show OutSeq > Inseq. These areas have also been previously implicated in memory for sequences, and episodic memory in general (Blumenfeld and Ranganath, 2007; Cabeza et al., 1997; Ekstrom et al., 2011; Jenkins and Ranganath, 2016; Rugg and Vilberg, 2013; Simons and Spiers, 2003).

These results highlight that while we have demonstrated homology in areas contributing to sequence memory performance between the rat and the human, there either exist other functions performed in the human PFC during sequence memory performance that rats do not

exhibit, or some of the functions performed by the mPFC in rodents have expanded to other regions of the PFC in humans. Investigating the contributing functions performed by various regions of the PFC is an important endeavor, as there exists a lack of consensus in the literature as to the localization of function within the PFC as it contributes to sequence memory performance.

Studies of sequence memory vary in the type of information to be processed (e.g., objects, words, or non-verbal stimuli), the encoding instructions (e.g., intentional or incidental), the contrast conducted (e.g., temporal vs. spatial or item vs. recency judgments), whether the sequences are repeated or single-trial, and the method of analysis (e.g., patterns of activity vs. univariate activations) which could result in different types of executive functions being taxed (or their degrees) and to differences in the sensitivity of the measures, which could contribute to the lack of a localization consensus (Cabeza et al., 1997; Ekstrom et al., 2011; Jenkins and Ranganath, 2016; Kumaran and Maguire, 2006; Tubridy and Davachi, 2011). For example, Mangels (1997) demonstrated that patients with dorsolateral frontal lobe lesions had impaired performance on a sequence memory test when using intentional encoding instructions, but were not impaired when using incidental encoding instructions, implying a deficit in strategic organization. Reviews of the literature in long-term memory also highlight the DLPFC in more organizational processing, with VLPFC engaged in goal-directed selection and maintenance of information (Blumenfeld and Ranganath, 2007; Simons and Spiers, 2003), with both processes likely playing a role in memory for sequences of events.

Future studies could systematically vary demands of the sequence memory task, such as was done by Mangels (1997), to investigate how various regions contribute to sequence memory. For example, using our sequence memory paradigm, the degree to which individuals use an

"item-item" associative memory strategy versus an "item-in-position" strategy to remember the order of events in the sequence can be manipulated by modifying the task so that InSeq items are presented as "ABCD" or "BCDA" (i.e., the sequence is circular). Likewise, we could bias the participants to adopt an "item-in-position" strategy by presenting InSeq items as "AB3D" and "12C4." Studies such as these can begin to disambiguate the roles of regions as they relate to different aspects of sequence memory performance. Cross-species work can continue to investigate the neural signals and computations performed within and between regions underlying sequence memory performance, as the task can be similarly modified for rodents. The current study provides the foundation for these future studies in the rat, as we have demonstrated homologous regions involved in the cross-species sequence memory task in the human. In addition to identifying the homologous regions we also built on those findings by using whole-brain acquisition - for instance, when analyzing regions differentially active to correct and incorrect trials of Skip/OTs, we found bilateral putamen (Correct > Incorrect). Electrophysiological recordings from the rat could identify the neurobiological mechanisms within these regions that support correct identification of out of sequence trials (and whether those signals are movement-related or learning-related). Therefore, by continuing the cross-species work using complementary approaches, we can better understand the neural substrates underlying sequence memory performance.

2.4.2 Functional connectivity: Hippocampus and medial prefrontal cortex are highly connected

Functional connectivity analyses revealed high correlations between BOLD activity in the mPFC and hippocampus over the course of the sequence memory task, using seed-to-seed, seed-to-brain, and network approaches. Using the network approach based on Power et al.

(2011), we discovered the connectivity between mPFC and bilateral hippocampus was in the top 0.5% of all connections. Even when accounting for simple stimulus or task effects (i.e., assessing "background connectivity"; Fox and Raichle, 2007; Norman-Haignere et al., 2012), the connectivity remained in the top 3% of all connections after regressing out the task-induced activation. These results are consistent with a role for hippocampal-mPFC interactions in humans that parallels the rodent (Quirk et al 2013).

To determine if the connectivity was specific to sequence memory, we conducted network analyses in a separate resting state dataset. We observed similar results, with the correlations between the left and right HC and mPFC in the top 3% of all correlations. Therefore, hippocampal-mPFC connectivity was present to a high degree during rest as well as during performance of the sequence memory task. Several explanations for the similarity in connectivity are plausible. First, rest could reflect memory-related processes, engaging the same areas as the sequence memory task, as these two regions are nodes within the default mode network (Buckner et al., 2008; Stark and Squire, 2001). Indeed, as reviewed in Lu et al. (2012) the hippocampus and mPFC show connectivity in rats during anesthetized conditions, and these regions were also correlated in the humans in resting state conditions, thought to reflect default mode processes. In addition, some authors have suggested these two regions could be areas where integration occurs between two large-scale cortical memory systems (Ritchey et al., 2015).

Second, while potentially reflecting memory-related or more general default mode processes, the connectivity could also reflect intrinsic network connectivity. For instance, it is known the mPFC and anterior hippocampus show anatomical connectivity (Barbas and Blatt, 1995; Beckmann et al., 2009; Schmahmann and Pandya, 2009; Verwer et al., 1997). Perhaps the

high degree of functional connectivity reflects intrinsic connections between these regions that are stronger than connections between other brain regions. In addition, while interpreted as reflecting default mode processes in the rat, the fact that the rats were anesthetized while showing the connectivity profile observed in Lu et al. (2012) implies the connectivity may reflect intrinsic network connections rather than default mode processes per se. Furthermore, a study using the same network analysis approach from Power et al. (2011) found that patterns of connectivity were remarkably similar across many tasks, including 'emotional,' 'language,' 'gambling,' 'relational', resting state, and others (Cole et al., 2014). In particular, there was great similarity between the resting state correlation matrix and combined task matrix (r=0.90). Therefore, it cannot be concluded that the high degree of functional connectivity between hippocampus and mPFC was exclusively driven by sequence memory task demands.

Given that the functional connectivity is similar between tasks engaging different cognitive processes, future studies that are specifically designed to test the smaller differences in functional connectivity between tasks, such as that by Norman-Haignere et al. (2012) or Geerlings et al. (2015a), can better address this question. In these task designs, separate runs of task of interest and control task allow for comparison of connectivity between tasks. In addition, continued use of complementary approaches between the rat and human will be important to identifying the role of these functional connectivity signals. For instance, dual electrophysiological recordings of the mPFC and hippocampus can be conducted in the rodent during performance of the sequence memory task as well as during rest or another task condition. The high frequency recordings will reveal what information is exchanged between regions on a moment-by-moment basis. While recording is normally done on this high frequency, millisecond by millisecond timescale, the timescale of BOLD fluctuations can also be recorded (these "infra-

slow" frequencies < 0.5 Hz are typically filtered out). These recordings will aid in elucidating what the functional connectivity signals represent in BOLD fMRI in terms of sequence memory task performance versus rest or other cognitive state. In addition, perhaps one region could be inactivated while recordings of the other region are performed, which would also help identify how the integration of signals occurs between regions.

2.5 Conclusions

The use of a cross-species task allows for complementary approaches in the rat and human in order to provide a greater understanding of the neural mechanisms underlying sequence memory ability. Using a cross-species sequence memory paradigm, we have shown remarkable homology of the neural substrates for sequence memory in the rat and human including the hippocampus and medial prefrontal cortex. Future studies using the sequence memory paradigm can seek to better understand the cognitive and neural mechanisms supporting sequence memory, as well as probe the changes within and between these homologous brain regions that may underlie declines in sequence memory ability in both healthy and pathological aging.

3 Experiment 2: Investigation of age-related changes to the neural substrates underlying sequence memory performance

3.1 Introduction

Memory for the sequence of events is a defining characteristic of episodic memory (Tulving, 1984), and is known to decline in typical aging (Allen et al., 2015; Cabeza et al., 2000; Fabiani and Friedman, 1997; Moscovitch and Winocur, 1995; Roberts et al., 2014; Tolentino et al., 2012). Here, we seek to understand the neural changes underlying these declines. Conducting aging research using fMRI has proven to be a difficult task (see section 1.4 and 1.5 for detail), because there are numerous reasons for observing differential age effects in BOLD activity and findings are often inconsistent. For instance, previous research using fMRI to explore changes in the aging brain as they relate to memory performance has revealed increases, decreases, and no changes in activity with age in the prefrontal cortex and medial temporal lobes (see Dennis and Cabeza, 2008; Grady, 2012a; Maillet and Rajah, 2013 for review and section 1.4.2). As an example, one study showed prefrontal bilateral recruitment only occurred in lower-performing adults (young showed only single hemispheric recruitment) (Duverne et al., 2009) while another showed the bilateral recruitment only occurred in higher-performing adults (Cabeza et al., 2002). Issues common to studies of young adults such as task design, task difficulty, and chosen contrast of interest can affect the results. In addition, the studies of aging are often complicated by design of between-groups comparison, impaired vs. unimpaired performance, differences in brain morphology, and changes to the vasculature with age (impacting the dynamic range of the BOLD effect), all making group comparisons difficult (Ances et al., 2009; D'Esposito et al., 2003; Samanez-Larkin and D'Esposito, 2008). Together, these factors conspire to make it difficult to adjudicate between popular models of age-related change such as compensation,

dedifferentiation, and degradation (see Grady, 2012a, Dennis and Cabeza, 2008). Descriptive models such as Hemispheric Asymmetry Reduction in Old Adults (HAROLD; Cabeza, 2002), compensation-related utilization of neural circuits hypothesis (CRUNCH; Reuter-Lorenz and Cappell, 2008), and posterior-anterior shift in aging (PASA; Davis et al., 2008) attempt to reconcile the neuroimaging aging literature, but are limited in their predictive power and scope by not being mechanistic models.

To address some of these issues presented by aging research utilizing fMRI, we use a non-spatial, non-verbal cross-species sequence memory task that allows for between-species comparisons of the neural correlates of the task, and how those neural correlates change with age. The approach will attempt to mitigate effects of the differences in performance between young and older adults, address differences in the dynamic range of the hemodynamic response with age, and leverage off of findings (and future studies) in the rodent to help provide a clearer understanding of the neural bases of age-related memory decline.

Previous research has shown that behavior declines on this task with typical aging (Allen et al 2015). We first seek to replicate this finding, then to understand what changes occur to the neural substrates supporting performance on this task. As we anticipate behavioral declines with age, we will need to address this performance confound (unimpaired vs. impaired) if we wish to determine whether aging is leading to a change in how sequence memory is accomplished, rather than simply observing neural correlates of impaired performance. Previous research has demonstrated that the behavioral declines seen in the sequence memory task are not likely due entirely to simple difficulty as conditions affecting overall difficulty could be dissociated from the age-related decline (see Allen et al., 2015 for details). Here, rather than attempt to make the task simpler for the aged group, which might alter the task demands, we will examine correct

trials only. By observing only correct responses for the entirety of the sequence (participants must correctly identify all six items within a sequence correctly as 'in' or 'out' of sequence), we can eliminate cognitive process that may be occurring more in the older group due to greater numbers of incorrect trials (i.e. error signals, behavioral response differences) and ensure we are not observing neural correlates of impaired performance.

Another consideration is what contrast of conditions is to be used. Here, we contrast sequences with all items in sequence versus sequences with one item out of sequence, which we have previously used to demonstrate homologous neural substrates shared between humans and rats (Experiment 1(Chapter 2)). Therefore, results from the current study can help inform future studies in the aging rodent using this task design. Further, this contrast is specific to evaluating the neural response to sequence memory demands, versus contrasts that may invoke different cognitive processes in each of the conditions, which could be changing differentially with age. For example, a memory-encoding task contrasted with a "resting" baseline in the hippocampus could show a different change in activation with age than a memory-encoding task contrasted with a non-mnemonic perceptual task (Stark and Squire, 2001b). In addition, resting state fluctuation amplitude correction will be applied to our dataset to attempt to account for vascular changes with age (Kannurpatti and Biswal, 2008; Kannurpatti et al., 2011). Our task design therefore will allow us to determine the similarities and differences in the neural substrates that support the task in young versus aged individuals. We can observe whether activations follow a HAROLD or PASA pattern for the older adults during sequence memory performance, and if changes are observed, we will also examine those changes in relation to behavior to help to disambiguate a compensation, dedifferentiation, or degradation hypothesis within the context of sequence memory (see section 1.4.2 for detail). In addition, relationships to behavior can

demonstrate if there exist different trajectories within our aged population, as previous research has shown greater variability with age in both rodents and humans (Buckner, 2004; Gallagher et al., 2006a; Morrison and Baxter, 2012; although see Salthouse, 2012).

We expect to observe changes in the hippocampus and mPFC with age. Previous structural research, including gross volumetric as well as at the level of synapses, in humans, nonhuman primates, and rodents, has shown the prefrontal cortex and medial temporal lobe are disproportionately impacted in typical aging (Morrison & Baxter, 2012; Burke & Barnes, 2006 Berchtold et al., 2013). Physiological studies have also shown changes – for instance, aged rats show deficits in both induction and maintenance of long-term potentiation (LTP) in the DG and CA3 of the hippocampus, and using FDG-PET, a marker for glucose metabolism, Walhovd et al. (2010) showed that the amount of metabolism in the hippocampus in humans correlated positively with a performance on a declarative memory test. Additionally, diffusion tensor imaging studies in humans have shown that connections between the PFC and MTL show greater changes with age than other connections in typical aging (Buckner, 2004, Bennett et al., 2014) (For greater detail, see section 1.4). The sequence memory task is a suitable choice for examining these age-related changes as we previously identified these regions as active during sequence memory performance (Experiment 1). Further, these regions have been shown in temporal context paradigms to be important to performance in the rodent, primate, and human (Davachi and DuBrow, 2015; Devito and Eichenbaum, 2011; Milner et al., 1985; Naya and Suzuki, 2011; Pinto-Hamuy and Linck, 1965). Here, we will examine whether typical aging leads to an alteration in activity in the hippocampus, in the mPFC, or in their interactions. For example, we may observe increased InSeq vs. OutSeq contrast in the mPFC in older individuals during correct performance, which could represent degradation or compensation; correlations

with behavior may help to disambiguate the two possibilities (see Grady, 2012; Maillet & Rajah 2013 for reviews and section 1.4.2).

We also use functional connectivity to determine changes to the interactions between brain areas in the aging brain during sequence memory performance. Previous research has shown functional connectivity changes in the aging brain during both resting state and task states. During rest, research has shown decreases within the default mode network, dorsal attention network, and fronto-parietal task control networks, while some have shown sensorimotor and subcortical networks demonstrate increases with age (Allen et al., 2011a; Grady et al., 2016; Meier et al., 2012; Wang et al., 2012). Using graph analyses, researchers have also found decreased within-network connections and increased between-network connections, demonstrating reduced network distinctiveness (Chan et al., 2014; Geerligs et al., 2015b). While researchers have found remarkable similarity in the functional connectivity profiles across a wide range of tasks and rest, they also have demonstrated that functional connectivity can vary with cognitive state (Cole et al., 2014; Geerligs et al., 2015a). Therefore, we will employ functional connectivity analyses to determine the connectivity changes with age during sequence memory performance using a seed-to-seed approach in our a priori areas of interest (hippocampus and mPFC) as well as a network-based approach.

3.2 Materials and Methods

3.2.1 Participants

Exclusion criteria included MRI contraindications and/or history of neurological or psychiatric diagnoses. A total of 50 participants were included in behavioral analyses: 25 young (age range 20-39 yrs, mean age = 27.8 yrs, SD = 5.0 yrs, 13 females), and 25 older (age range 60-84 yrs, mean age = 70.5 yrs, SD = 5.8 yrs, 15 females). A total of 50 participants underwent

scanning, 8 of which were excluded from analyses due to scanner artifacts (4 young and 4 older). The remaining participants (21 young: mean age = 27.5 yrs, SD = 5.0 yrs, 15 females; 21 older: mean age = 69.8 yrs, SD = 5.5 yrs, 13 females) were included in the imaging analyses. A resting state dataset was collected in a subset of participants (17 young: mean age = 27.9, SD = 4.9 yrs, 9 females; 16 older: mean age = 68.6 yrs, SD = 5.1 yrs, 11 females). All participants gave written consented in compliance with the Institutional Review Board (IRB) at the University of California, Irvine, and all were compensated for their participation.

In addition, to help remove concerns of dementia or other cognitive impairments, all participants must have scored 27 or higher on the Mini Mental State Exam (MMSE; Folstein et al., 1975), and scored no more than 'mild' on the Geriatric Depression Scale (older adults) or Beck Depression Inventory II (younger adults). Participants must have scored within age norms (i.e., within 1.5 standard deviations of the mean of their age group) on several tests of cognitive function, including Trail Making Test A & B – a measure of attention & task switching (Reitan and Wolfson, 1985), Wechsler Adult Intelligence Score-III (WAIS-III) digit span forward and backward – a measure of working memory, WAIS-III letter-number sequencing – a measure of working memory, the Stroop Color-Word test – a measure of selective attention (Stroop, 1935), Rey-Osterrieth Complex Figure Test – a measure of visuospatial ability and memory (Osterrieth, 1944), and the Rey Auditory Verbal Learning Test – RAVLT; a measure of immediate and long-term declarative memory (Rey, 1941). An allowance of (1) measure falling below 1.5 SD from average was accepted because it has been shown that when a battery of neuropsychological tests is completed, even in the absence of impairments, there is likelihood for a below-norm result (Russell et al., 2005). However, participants must have scored within age norms on tests of delayed memory (i.e. RAVLT delay and Rey-Osterrieth delay), as these tests are a better

predictor than other neuropsychological tests of later progression into dementia (De Santi et al., 2008). By including only those adults not impaired on these tests, we can further define typical aging and aid in identifying different trajectories in aging if we find sequence memory declines and neural changes in these older participants.

3.2.2 Task Description and Trial Types

The task developed by Allen et al., 2014 was modified for fMRI (see Chapter 2 for in-depth description). Several additional modifications were introduced for the current fMRI study. First, the OutSeq trial types were almost exclusively Repeats and Skips (with 4 trials of Ordinal Transfers to assess performance on this trial type). Participants were asked to indicate whether the item was in or out of sequence by holding a response key for >1.2 sec if the image was in sequence and <1.2 sec if the image was out of sequence. The change from 1 to 1.2 seconds was based on results by Allen et al., 2015 demonstrating older adults older participants were impaired on Repeats when a 1s threshold was used but not when a 1.2s threshold was used. This is likely due to an age-related decline in processing speed (Salthouse, 1996) rather than an impairment in working memory, because increasing the response threshold by a mere 0.2s ameliorated the impairment. Finally, participants began the task outside of the scanner (stage 1), then continued the task in the scanner (stage 2). This was done to ensure performance could reach asymptote and remain there for sufficient data collection while at the same time limiting the amount of time older participants had to remain in the scanner.

3.2.3 Behavioral analyses

As was done in Experiment 1, a G-test was used to compare expected and observed response frequencies for both baseline and fractal image conditions. The Sequence Memory

Index and Sequence Detection Index, for fractal and baseline conditions respectively, were also computed (Allen et al 2014). Percent correct (accuracy) was calculated for each condition of interest (Figure 3.1).

3.2.4 BOLD fMRI acquisition

Data were collected in a 3.0T Phillips scanner with a SENSE head coil at the University of California at Irvine. A whole brain 0.75mm isotropic magnetization-prepared rapid gradient echo (MP-RAGE) structural scan was collected for each participant; repetition time (TR) / echo time (TE) = 11/4.6ms, field of view (FOV) = 240 × 231 mm, flip = 18°, 200 sagittal slices. Whole-brain functional data were acquired with a T2* weighted echo planar imaging scan, at a resolution of 2.5mm (isotropic), 46 axial slices, TE = 26ms, flip angle = 70°, TR = 2500ms, dynamics = 175. The whole-brain resting state dataset was acquired with a T2* weighted echo planar imaging scan, at a resolution of 2.5 mm (isotropic), 46 axial slices, TE = 26ms, flip angle = 70°, TR = 2500ms, dynamics = 188.

Using AFNI (Analysis of Functional NeuroImages), functional data were aligned to the participant's MP-RAGE and slice-time corrected with the script align_epi_anat.py (Saad et al., 2009). Each participant's structural scan and functional data (statistical maps) were aligned to a model template using ANTs (Advanced Normalization Tools; (Avants et al., 2008)).

3.2.5 Univariate Imaging Analyses

The data were blurred to 4mm FWHM using AFNI's 3dBlurToFWHM. Sequences were modeled as duration-modulated blocks as described in Chapter 2. Sequences were modeled in a GLM as Inseq, Repeat, Skip, Baseline Inseq and Baseline OutSeq (repeat and skip). We performed strict motion correction with TRs with framewise displacement of >0.5 mm, as well

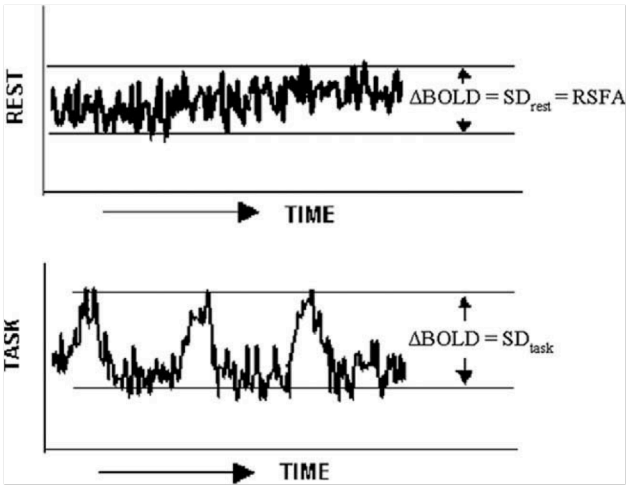
as 1 TR before and 2 TRs after, being removed (censored). In addition, we regressed out the motion vectors as well as CSF and white matter (segmented using six-tissue types via ANTs' antsCorticalThickness.sh; Tustison et al., 2014) and masked images to remove extra-brain or CSF voxels. Percent signal change was computed and used in the analyses. For analyses using correct-only sequences, all trials within the sequence must have been completed without error (e.g. a correctly identified OutSeq trial would not be included if a previous or subsequent Inseq trial was misidentified within the sequence). One subject from the Older adult group was excluded from correct-only contrasts due to too few trials (Mean number of correct Skip trials: 15 Young, 14 older; correct Repeat: 24 Young, 20 Older; correct InSeq: 48 Young, 47 Older).

We first sought to replicate our previous results by demonstrating that the hippocampus and mPFC are activate during sequence memory performance. We calculated the percent change values for InSeq vs. OutSeq within regions of the left hippocampus and mPFC in both the Young and Older datasets (regions were taken from Experiment 1 where young adults demonstrated the InSeq vs. OutSeq contrast). To further replicate, we then completed two-tailed, bi-directional paired t-test comparing beta coefficients for OutSeq (repeat and skip combined) vs. Inseq in the Young adult whole-brain dataset with correct-only sequences. We then sought to determine if older adults showed a qualitatively distinct pattern of activation during sequence memory performance by conducting the same analysis within the Older adult dataset. We then conducted a t-test including both Young and Older in the InSeq vs OutSeq contrast to observe age-invariant effects. Next, we computed a direct comparison between Young and Older adult datasets by conducting a t-test contrasting Young InSeq-OutSeq vs. Older InSeq-OutSeq for correct-only sequences to identify differences in neural substrates supporting sequence memory ability. For all contrasts, we used a voxel-wise threshold of $p<0.01$ and extent threshold of 19 voxels in the

whole-brain, which was determined by Monte Carlo simulation (AFNI'S 3dClustSim) to correct for multiple comparisons based on the blur.

3.2.6 Resting State Fluctuation Amplitude correction

The hemodynamic response that constitutes the BOLD fMRI signal can be impacted by aging due to changes in neurovascular coupling. These changes in coupling are thought to be due to changes in the vasculature, not the underlying neural activity (see Section 1.5.2). Therefore, methods to scale the BOLD response that account for these changes in vasculature could be used to correct for non-neural changes with age. We employed Resting State Fluctuation Amplitude correction, a method shown to be comparable to breathholding and CO_2 inhalation for BOLD fMRI calibration, and shown to be effective in aging (Kannurpatti and Biswal, 2008; Kannurpatti et al., 2011). RSFA is used as a hemodynamic scaling factor to calibrate both amplitude and spatial extent of the BOLD fMRI response. It works by using resting-state data to estimate the inherent dynamic range of the fMRI signal in each voxel and scaling task-derived fMRI data by this to normalize the dynamic range across participants (see Figure 3.1 from Kannurpatti et al. 2011).



BOLD signal change after scaling with RSFA;

$$B_{\text{scaled}} = \Delta\text{BOLD}_{\text{task}}/\Delta\text{BOLD}_{\text{rest}} = \text{SD}_{\text{task}}/\text{SD}_{\text{rest}}$$

Figure 3.1. Figure from Kannurpatti et al 2011. Depiction of RSFA acquisition and equation showing RSFA scaling.

For the resting state dataset, the preprocessing steps included those for functional connectivity analyses (see following section). The standard deviation was then computed for every voxel using AFNI's 3dTstat. The parameter estimates from the sequence memory GLM were then divided by the standard deviation at every voxel. Univariate analyses were completed both pre- and post-scaling.

3.2.7 Functional Connectivity Analyses

As was done in Experiment 1, to assess functional connectivity during performance of the sequence memory task, we employed seed-to-seed analyses as well as large-scale network analyses. Preprocessing steps included quadratic detrending, removing up to polynomial order two. The timeseries was then normalized to have zero mean and unit variance and the motion vectors and first derivatives were regressed out of the signal. The 6 principal components computed using tCompCor (not including global signal) were also regressed out of the signal (Behzadi et al., 2007). The data were then temporally bandpass filtered (0.08 to 0.009Hz) and TRs with framewise displacement >0.5 mm, as well as those one TR before and two TRs after were removed as recommended by Power et al. (2012).

Seed-to-seed correlations were conducted as follows: Seed regions in the left hippocampus (HC) and medial prefrontal cortex (mPFC) were defined based on the results of the Experiment 1, specifically the univariate contrast of InSeq vs. OutSeq. Seed regions were warped from model space to subject space using ANTs. The mean timeseries of BOLD fMRI activity was extracted from the seed region by averaging the signal across all voxels within the region. A Pearson's correlation coefficient was then calculated between regions and then r-to-z transformed using Fisher's technique (hyperbolic tangent) to approximate a normal distribution.

To conduct large-scale network analyses, seed regions were defined at 5 mm spherical locations based on the graph-analyses conducted by Power et al. (2011). Three additional seed regions were added to the analysis: the L HC, R HC (mirror of L HC), and mPFC, each based on the center of mass of the cluster based on InSeq vs. OutSeq univariate contrast from Experiment 1. The seed ROIs were then warped into subject space using ANTs. As was done in the seed-to-seed analyses, the preprocessed timeseries was extracted from each region and Pearson's correlation coefficient was calculated. A t-test was then computed for each z-transformed correlation within both the young and older groups. Thresholds were set as in Power et al. (2011) to assess nodes displaying the highest correlations. To assess changes from young to older in functional connectivity, a t-test was completed for each z-transformed correlation in the young vs. older groups, resulting in a difference matrix. Thresholds were set to determine the nodes displaying the greatest change between young and older. The correlation between the older and younger datasets, as well as the young dataset and Experiment 1 young dataset, was also assessed.

3.3 Results

3.3.1 Behavioral analyses

Behavioral performance on the sequence memory task was assessed in older and younger adults for both stage 1 (out of scanner) and stage 2 (in scanner) (See Figure 3.2). For stage 2, all participants showed reliable sequence performance in the form of a significant G-test for the baseline condition and fractal image condition. Therefore, all participants without substantial scanner artifacts were included in the imaging analysis.

Separate repeated-measures two-way ANOVAs were used to examine age-related effects on behavioral performance for the pre-experimentally known baseline sequence (Sequence

Detection Index) and for the newly-learned sequences (Sequence Memory Index). Sequence Detection Index scores revealed no main effects of age ($F(1,48)=0.54$, $p=0.47$) or stage ($F(1,48)=0.00001$, $p=.997$) and no interaction $F(1,48)=2.17$, $p=0.15$). Sequence Memory Index scores demonstrated a main effect of age ($F(1,48)=81.82$, $p<0.01$, Y > O) and stage ($F(1,48)=8.9$, $p<0.0001$, stage 2 > stage 1) with some evidence for an age x stage interaction ($F(1,48)=3.34$, $p=0.07$).

In a follow-up analysis, we examined the age-related effects in each stage separately. For stage 1, a repeated-measures two-way ANOVA assessing effects of age and probe type (repeat, skip) on accuracy (percent correct) revealed a main effect of age ($F(1,48)=6.83$, $p<0.05$, Y > O) and probe type ($F(1,48)= 201.5$, $p<0.0001$, Repeat > Skip) and no interaction ($F(1,48)=0.84$, $p=0.36$). For stage 2, there was a main effect of probe type ($F(1,48)=78.31$, $p<0.0001$ Repeat > Skip), no effect of age ($F(1,48)=1.58$, $p=0.22$), and no interaction ($F(1,48)=0.01$, $p=0.92$).

The behavioral results parallel those found in Allen et al., 2015 that assessed young and older performance on the sequence memory task and found decreases with age. However, we did not replicate the prior finding that older adults were exclusively impaired on skips, as evidenced by the lack of age x probe type interaction for stage 1. Our results indicate performance on both skips and repeats decrease with age, similar to Allen et al 2015 for the 1sec response window, but with increased practice the declines become less evident (as we observed a lack of a main effect of age for stage 2).

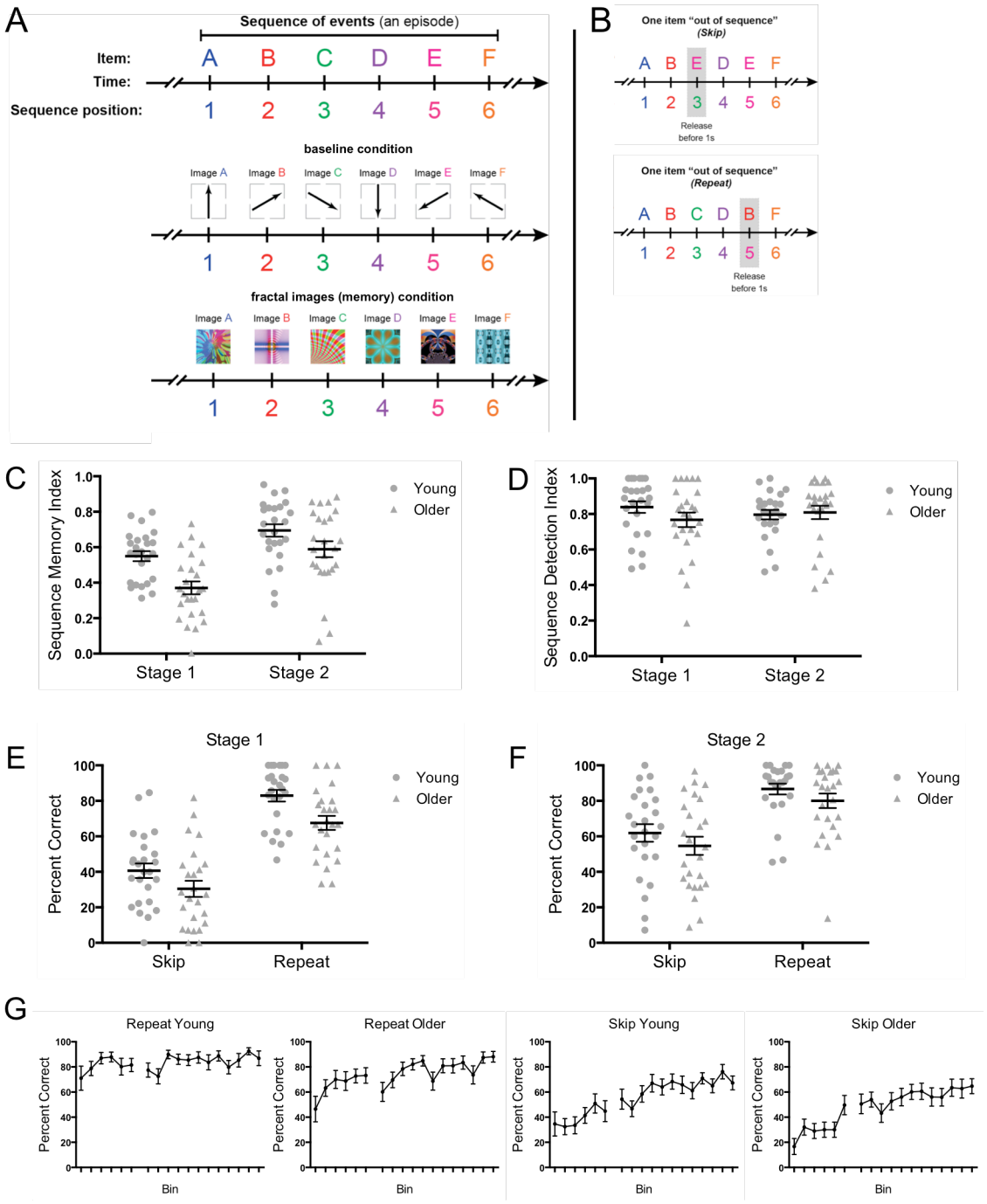


Figure 3.2. (a-b) Depiction of the sequence task modified for fMRI in aging (Depiction modified from Allen et al. (2014)). Participants were shown a sequence of six images (e.g., ABCDEF) and asked to indicate if the images are in or out of sequence by holding a response key for >1.2 second if the image is in sequence (e.g., B was presented after A) or <1.2 second if the image is out of sequence (e.g., C was presented after A). (a) Schematic of a baseline and fractal image condition. (b) Example of a skip and repeat. (c-d) The sequence Memory Index and Sequence Detection Index for young and older participants, stage 1 and stage 2. (e-f) Performance on the different memory probe types of repeat and skip for young and older participants, stage 1 and stage 2. Individual subject data displayed with mean and standard error of the mean. (g) sub-graphs showing performance over the course of the task, the break indicating the transition from stage 1 to stage 2. Detailed statistics in main text.

3.3.2 Hippocampal and mPFC univariate imaging analyses

3.3.2.1 Sequence memory activates the hippocampus and mPFC in both young and older participants, however older participants show decreased activity

In examining the fMRI data, we first sought to replicate our previous work by demonstrating that the hippocampus and mPFC are active during the sequence memory task. The left HC and two ventromedial PFC clusters from the Experiment 1 InSeq vs. OutSeq contrast were used as *a priori* regions of interest. Figure 3.3 shows the percent signal change for InSeq vs. OutSeq in young and older participants in the current study, for all trials (A), and correct-only trials (B-C). Including all trials, young adults displayed significant differences between the conditions (InSeq > OutSeq) in all of the regions, and older adults displayed a significant difference in L HC and trends in the mPFC regions (L HC young: t_{20}=3.51, p<0.01; older: t_{20}=3.29, p<0.01; mPFC cluster 1 young: t_{20}=4.35, p<0.001; older: t_{20}=1.99, p=0.06; mPFC cluster 2 young: t_{20}=2.92, p<0.01; older: t_{20}=1.86, p=0.08).

As noted in the Introduction, given the performance differences, the age-related effects observed in the overall InSeq vs. OutSeq contrast could be artefactual. For the subsequent analyses, only correct trials were included because the analysis was done to determine if neural substrates were changed from younger and older adults during sequence memory. For all three regions, both young and older adults showed a significant difference between the conditions, showing InSeq > OutSeq (L HC young: t_{20}=4.98, p<0.0001; older: t_{19}=5.0, p<0.0001; mPFC cluster 1 young: t_{20}=6.25, p<0.0001; older: t_{19}=2.37, p<0.05; mPFC cluster 2 young: t_{19}=6.17, p<0.0001; older: t_{19}=3.79, p<0.01) (One outlier that was 10 standard deviations from the mean was excluded for young mPFC cluster 2). To assess differences between the young and older groups, two-tailed t-tests were completed for each region of interest. For both mPFC regions,

there was a difference between the groups, with younger displaying a greater contrast between InSeq and OutSeq (mPFC cluster 1: t_{39}=2.69, p<0.05; mPFC cluster 2: t_{38}=4.64, p<0.0001), and the L HC displayed a trend of the same direction (t_{39}=1.69, p=0.1). For each group, we also assessed whether the InSeq vs. OutSeq percent change (first including only correct trials, then including all trials) correlated with performance as measured by the Sequence Memory Index. No regions showed a correlation in either group.

To help mitigate concerns that different dynamic ranges in the BOLD effect across groups might lead to observations of activity differences, we next examined InSeq vs. OutSeq in the RSFA corrected data. After RSFA correction, the results maintained, with both young and older showing a significant difference between the conditions (Figure 3.3 C) (L HC young: t_{17}=4.44, p<0.001; older: t_{16}=4.49, p<0.0001; mPFC cluster 1 young: t_{17}=6.18, p<0.0001; older: t_{16}=3.63, p<0.01; mPFC cluster 2 young: t_{16}=6.34, p<0.0001; older: t_{16}=3.63, p<0.01). When testing for group differences, the results using RSFA mirrored the results without RSFA except that the L HC difference became significant (mPFC cluster 1: t_{31}=3.76, p<0.001; mPFC cluster 2: t_{30}=5.35, p<0.0001; L HC: t_{30}=2.14, p<0.05).

While we could not evaluate subfields of the hippocampus (which may show differential effects of age), we divided the hippocampus into head, body, and tail to determine if there were differential changes in activity for young and older adults along the long axis of the hippocampus. Looking at correct-only trials for InSeq vs. OutSeq, we found within the head that both young and older adults showed InSeq > OutSeq (young: t_{20}=5.60, p<0.0001; older: t_{19}=2.43, p<0.05), in the body only young adults showed InSeq > OutSeq (young: t_{20}=5.07, p<0.0001; older: t_{19}=0.63, p=0.54), and in the tail neither showed a difference (young: t_{20}=1.57, p=0.13; older: t_{19}=0.39, p=0.70). In the head and body, there was a difference between older and younger

adults, with Young > Older (head: t_{39}=2.37, p<0.05; body: t_{39}=3.09, p<0.01), with no difference in the tail (t_{39}=0.34, p=0.74).

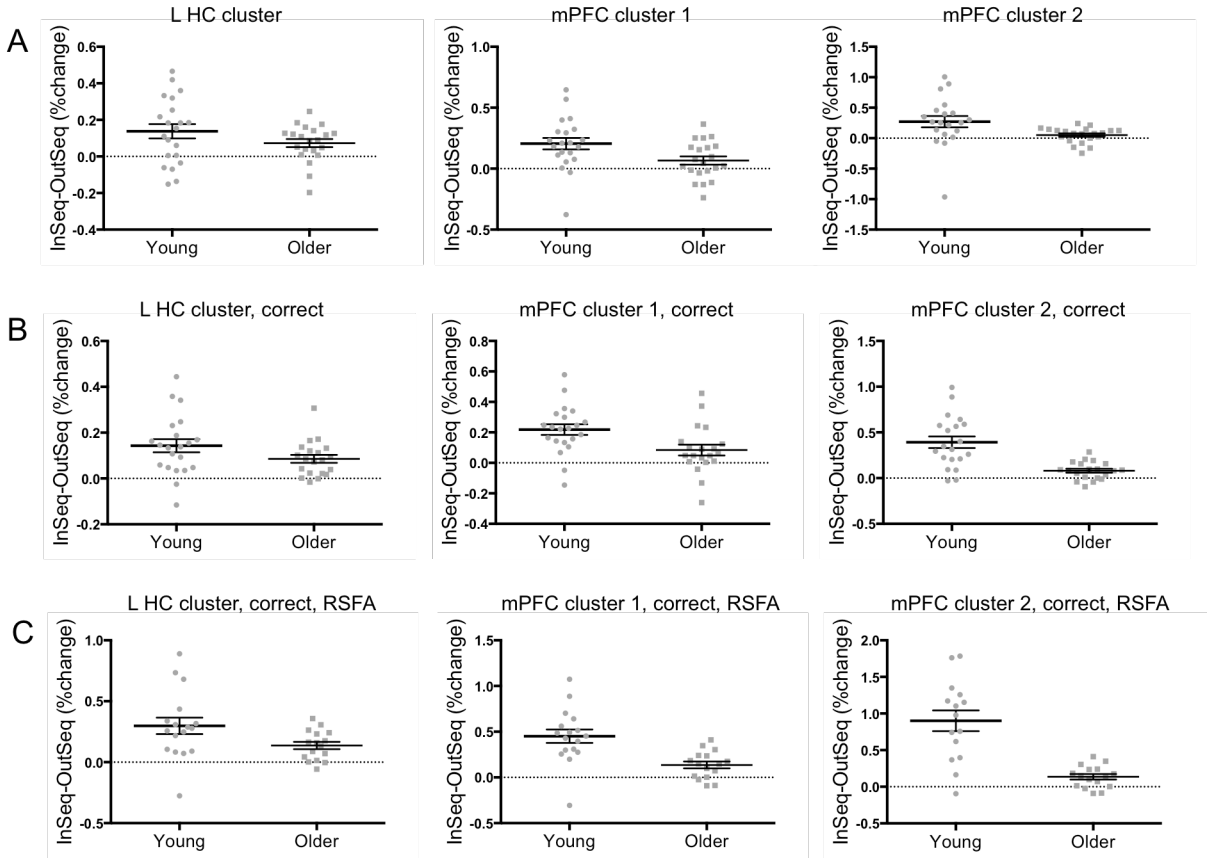


Figure 3.3. InSeq vs. OutSeq contrast for young and older participants (*a priori* regions of interest defined by Experiment 1 InSeq vs. OutSeq contrast). (a) Data plotted including all trials (b)For correct-only trials, both young and older showed a difference between InSeq and Outseq for all three regions. Young showed a greater difference between InSeq and OutSeq than Older in mPFC cluster 1 and mPFC cluster 2, with a trend in L HC. (b) After RSFA correction, all results maintained, and the difference between young and older became significant for L HC. See main text for detailed statistics.

3.3.3 Whole-brain univariate exploratory analyses

After replicating the results from Experiment 1 that showed a difference between InSeq and OutSeq in young adults in the L HC and mPFC, and demonstrating that a difference between these task conditions in the L HC and mPFC was also present in the older adults, we conducted the InSeq vs. OutSeq contrast brain-wide for both younger and older adults. This was done both

to determine whether our prior results in the young were reliable and to determine whether the age-related declines in activity would be observed only in these regions, throughout the brain, or whether there was any evidence for "compensation" in the older population. In each test, only the correct trials were used to equate performance between the groups and to examine the neural substrates supporting successful sequence memory.

3.3.3.1 Replication of brain-wide activity pattern observed in young adults; with matched performance, older adults show decreased activity but similar neural substrates

3.3.3.1.1 Young and older whole-brain contrast of InSeq vs. OutSeq

Overall, the results in young adults paralleled those seen in Experiment 1, with both left

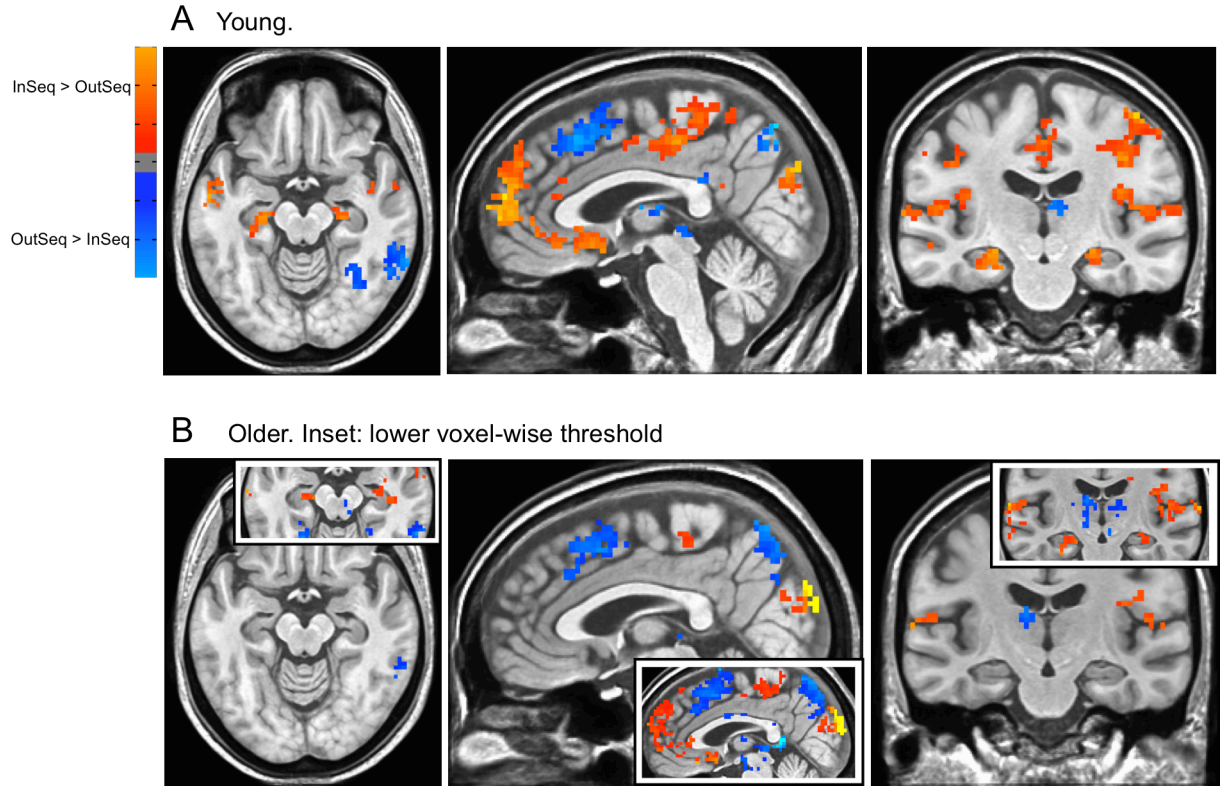


Figure 3.4. Young (a) and older (b) brain-wise t-tests for InSeq vs. OutSeq. (a) Young shows a replication of Chapter 2 results. (b) Older shows fewer significant regions, with 26 out of 29 regions overlapping with the young. Insets show similarity between young and older brain-wide maps at a lower voxel-wise threshold for older adults.

and right HC and mPFC showing InSeq > OutSeq (see Figure 3.4A) in this independent dataset. We also replicated the whole brain contrast map as outlined in Table 1 (Chapter 2), with all regions showing reliable activity again (of the three additional regions observed for correct-only in Experiment 1, right cuneus InSeq > OutSeq remained, while left cerebellum OutSeq < InSeq and left orbital sulcus OutSeq > InSeq not longer showed an effect). In addition to these regions, we also observed several additional regions: For the InSeq > OutSeq, these included anterior middle/superior frontal gyrus bilaterally; left temporal sulcus; anterior, middle, and posterior cingulate gyrus; left inferior frontal gyrus, and left central sulcus. For OutSeq > Inseq, these included left superior frontal sulcus, posterior inferior temporal sulcus, and bilateral fusiform gyrus. The additional regions may be due to the difference between the design used previously and the current sequence memory task design, as the current task design excluded ordinal transfers and allowed for a greater response window. Alternatively, the differences could be due to difference in power and thresholding effects. When including all trials (not excluding incorrect trials) we observe fewer regions, with HC and mPFC just below threshold for young adults. When we conducted the same correct-only InSeq vs. OutSeq contrast in older adults, many of the same regions showed the contrast, with 26 out of the 29 significant regions overlapping with the young (see Figure 3.4B). The three non-overlapping regions included left parieto-occipital sulcus, left lateral orbital sulcus, and left thalamus, all showing OutSeq > Inseq. Indeed, when the voxel-wise threshold is lowered, the contrast map is remarkably similar between young and older adults, suggesting the same regions are underlying sequence memory in both the older and young (see Figure 3.4B insets). When including all trials, similar to the young group, we observed several regions that were absent, and as well as several that were absent in the correct-only. However, reducing the voxel-wise threshold showed these regions were present in both

brain-wide maps. Thus, we can conclude that when looking at the young participants, the results here substantially replicate the prior work. In addition, older adults appear to use similar neural substrates, but show decreases in activity.

3.3.3.1.2 Direct comparison of young vs. older InSeq vs. OutSeq

We then completed a brain-wise t-test directly comparing young and older InSeq vs. OutSeq (correct only) to determine whether there was evidence for age-related changes in the neural bases of accurate sequence memory. We found 10 regions showing a significant difference (see Figure 3.5A for depiction of regions). All regions showed Young > Older for the contrast. From this contrast alone, however, we cannot know what changed with age. For instance, as can be seen in the hypothetical graph in Figure 3.5B, the Young > Older difference could be driven by either a greater InSeq > OutSeq difference in the young group, or by a greater OutSeq > InSeq difference in the older group.

Our results indicate that the Young > Older difference was driven primarily by a greater InSeq > OutSeq difference in the young (see Figure 3.5C). In post-hoc t-tests, all ten regions showed significant InSeq > OutSeq for young (Bonferonni-corrected $p<0.05$), while the older only showed one region with a significant difference between InSeq and OutSeq: OutSeq > Inseq (cluster located in left middle/superior frontal sulcus – cluster not present in older whole-brain map) (Bonferonni-corrected $p<0.05$). The results indicate that these regions were mainly driven by a greater InSeq > OutSeq in young, with either no difference in older or numerically OutSeq > InSeq. After RSFA correction, 6 of the 10 clusters remained and no additional clusters were revealed. Note, caution should be taken here before concluding a strong effect of RSFA correction as there is lower power here with only 17 young participants and 16 older participants.

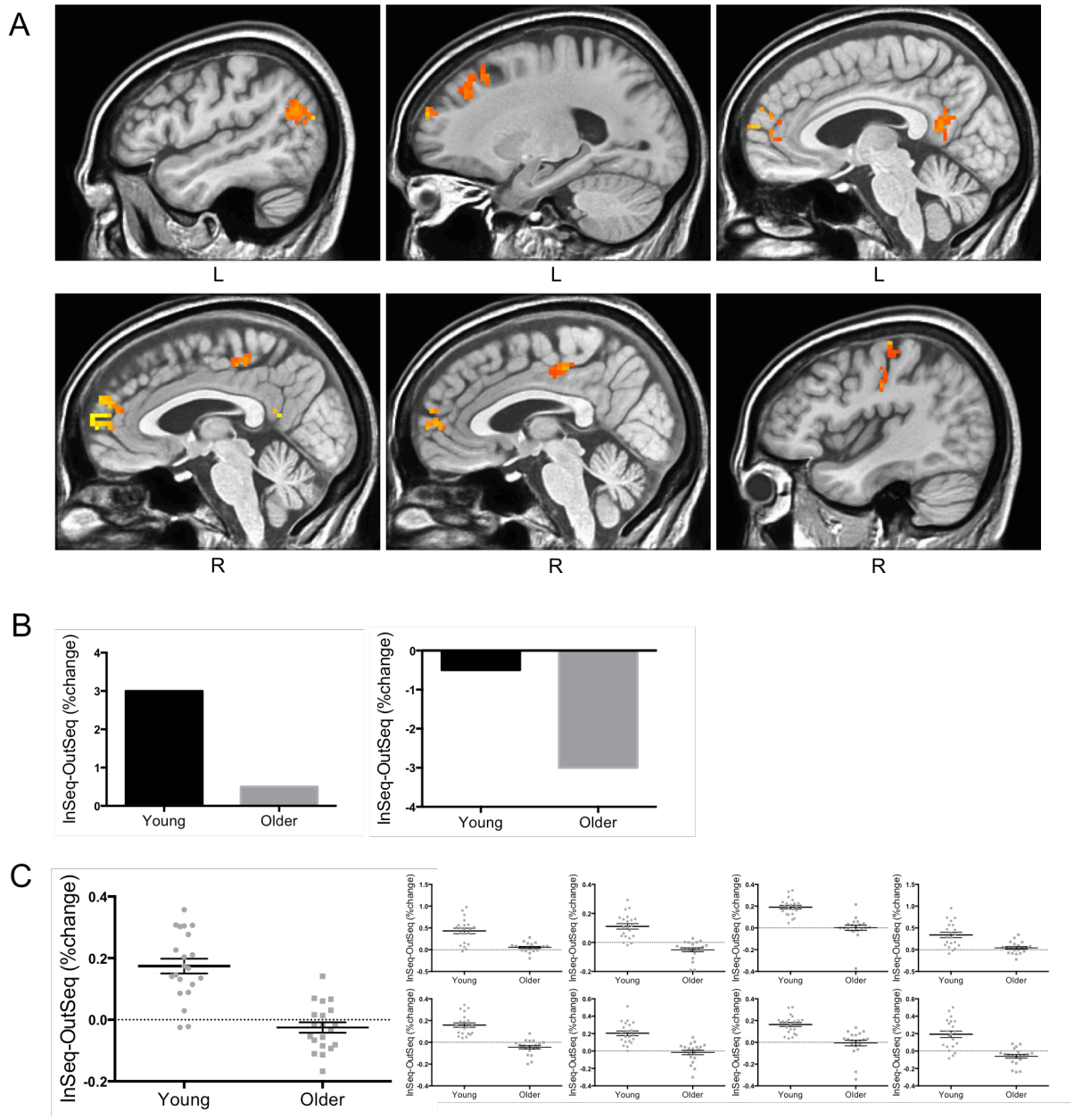


Figure 3.5. Regions demonstrating a difference between young and older for InSeq vs. OutSeq; all show Young > Older. (a) Top row shows left hemisphere, bottom row shows right hemisphere. (b) Hypothetical underlying contrasts driving the Young > Older result. (c) Actual data from the clusters demonstrating the contrast is largely driven by young InSeq > OutSeq.

3.3.3.1.3 Combined young and older InSeq vs. OutSeq contrast

We then conducted a brain-wise contrast for InSeq vs. OutSeq for young and older combined to determine age-invariant effects. We again found a strikingly similar brain-wise map to the young and older maps, with 39 significant clusters. The four clusters not present in the young included left thalamus OutSeq > InSeq (observed in the older dataset), left lateral orbital sulcus OutSeq > InSeq (observed in the older dataset), left orbital gyrus InSeq > OutSeq (not in young or older), and right middle frontal sulcus OutSeq > InSeq (not in young or older). In young, areas observed that were not in the combined map included the bilateral anterior cingulate InSeq > OutSeq, left central sulcus InSeq > OutSeq, left inferior frontal gyrus InSeq > OutSeq, and right HC InSeq > OutSeq. In older, the only region not in the combined map was the left parieto-occipital sulcus OutSeq > InSeq.

These results again indicate that the neural substrates are highly overlapping between young and older, as regions that came through as significant in the combined map that were not observed in the young (but were observed in the older) were likely sub-threshold in the young. In addition, the clusters observed only in the young (and not in the combined map) were all areas not observed in the Experiment 1 young dataset. Therefore, these areas likely represent a thresholding effect. Thus, overall, the results indicate similar neural substrates for both the young and older groups for the InSeq vs. OutSeq contrast. When we completed a brain-wise 3dRegAna in the young and older groups to identify any regions showing a correlation between SMI and InSeq vs. OutSeq activity (including incorrect trials), no regions were found to positively correlate.

3.3.4 Whole-brain functional connectivity analyses

3.3.4.1 mPFC and hippocampus timeseries are correlated over the course of the sequence memory task for both young and older adults

We first sought to replicate the connectivity results from Experiment 1, showing high connectivity between HC and mPFC during the sequence memory task in young adults. Using the L HC and mPFC as seed regions (defined by InSeq vs. OutSeq contrast in Experiment 1), we observed significant functional connectivity over the course of the task in young volunteers (t_{20}=6.16, p<0.0001). In Older adults, the regions also show significant functional connectivity over the course of the task (t_{20}=2.29, p<0.03). However, the Young adults show significantly greater connectivity than older adults (t_{40}=2.29, p<0.01) (Figure 3.6).

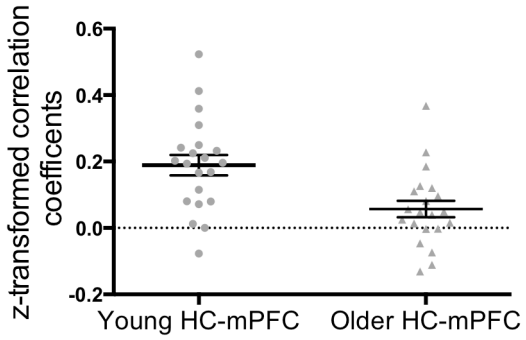


Figure 3.6. Functional connectivity between L HC and mPFC over the course of the task. Both groups show significant connectivity, with young showing greater connectivity than older.

3.3.4.2 Connectivity between mPFC and HC decreases with age, as does connectivity between other highly correlated nodes in young adults

Network analyses were completed to determine the specificity of the HC-mPFC connectivity decline with age. First, a correlation matrix was constructed for each participant using the 264 nodes from Power et al., (2011) and the additional L HC and R HC nodes. These were then z-transformed and group-level t-statistics were computed. Figure 3.7A shows the resulting correlation matrix for young adults. This matrix correlated with the Experiment 1 young adult correlation matrix (r = 0.66, p<0.0001). The older adult matrix, shown in Figure 3.7B, correlates with the young adult

matrix ($r = 0.55$, $p<0.0001$). To a first approximation at least, similar networks exit between young and older individuals, as has been shown previously (Chan et al., 2014; Geerligs et al., 2015a; Grady et al., 2016).

To assess the changes with age in network connectivity, we completed a t-test for every element of the matrix comparing young to older individuals. Figure 3.8A shows the difference matrix. The difference map is similar to the young map (correlation between the young map and the difference map: $r = 0.66$, $p<0.0001$), suggesting that the same network used by the young adults is what is changing with age. Thus, the age-related differences appear quantitative rather than qualitative.

If the age-related connectivity changes are driven to a large extent by a simple decline in observed activity and connectivity (i.e., lower signal-to-noise), we would expect there to be a correlation between the strength of the signal in the young and the amount of age-related decline. To examine this, we identified the top 4% of all matrix elements in young (following Power et al., 2011's threshold) and correlated the functional connectivity in the young with the age-related change (the difference between young and older). We observed a strong relationship here (Figure 3.8B; $r = 0.39$, $p<0.0001$). The red dots in Figure 3.8B indicate the L HC–R HC, L HC–mPFC, and R HC–mPFC correlations. As can be observed, the correlations were among those that showed the largest changes with age, but were also were among the highest correlations in the young.

Of interest, the average functional connectivity showed a slight increase with age (mean difference young-older t-statistic matrices = -0.37) (Figure 3.9). This however, could be driven by the large number of negative correlations, as we did not set negative correlations to zero (or exclude them) as some studies have done (Chan et al., 2014; Grady et al., 2016). If there exist a

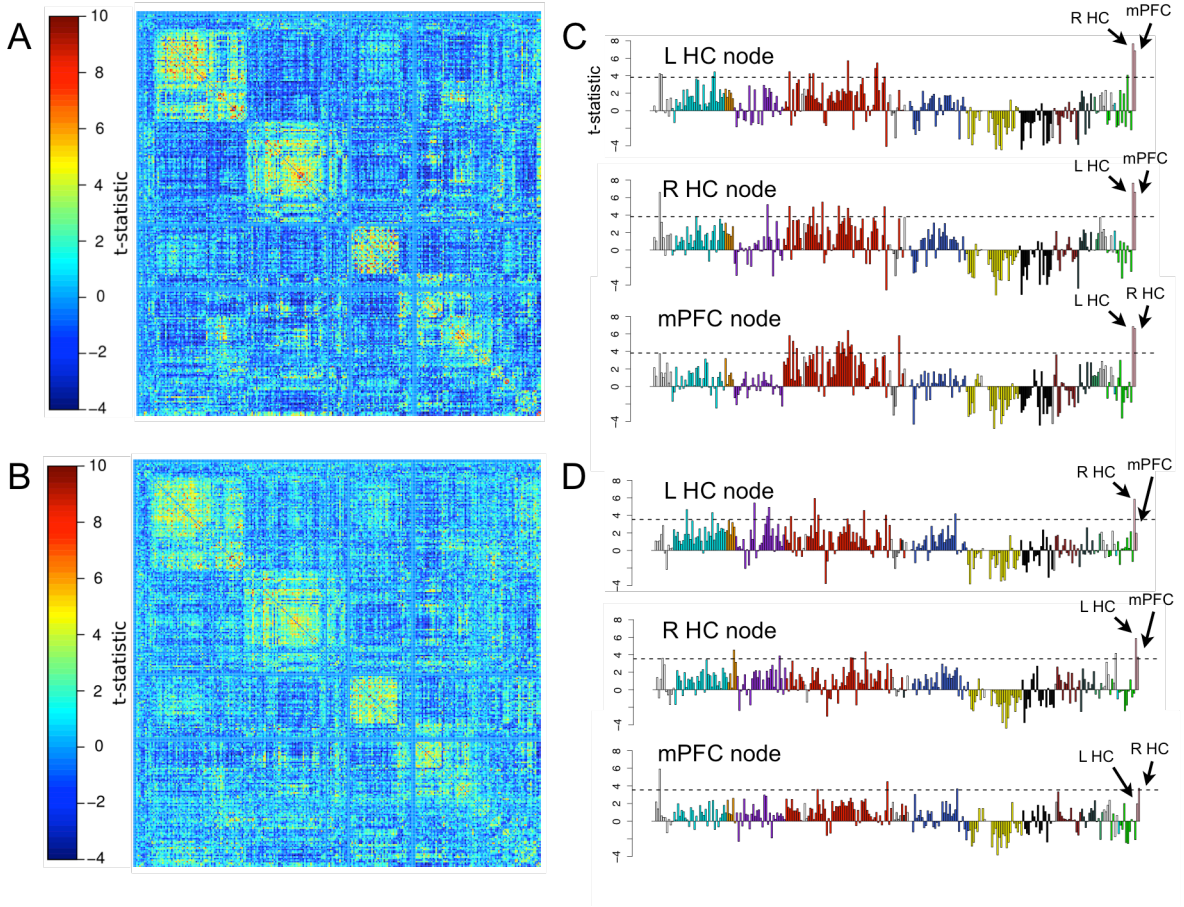


Figure 3.7. Network analyses. (a) Replication of young adult correlation matrix from Experiment 1 with current young data (t-statistics). (b) Older adults show a similar correlation matrix (correlated with the young matrix: r=0.65). (c-d) T-statistics for correlations between the left HC, right HC, and mPFC nodes to every other node for (c) young and (d) older adults. Dashed lines indicate top 4% of pairwise correlations.

greater number of negative correlations, and those are also decreased in magnitude with age, it would result in an overall increase with age (previous research has shown negative correlations decreasing in magnitude with age: Meier et al., 2012). As can be observed in Figure 3.7, many between-network connections are negative.

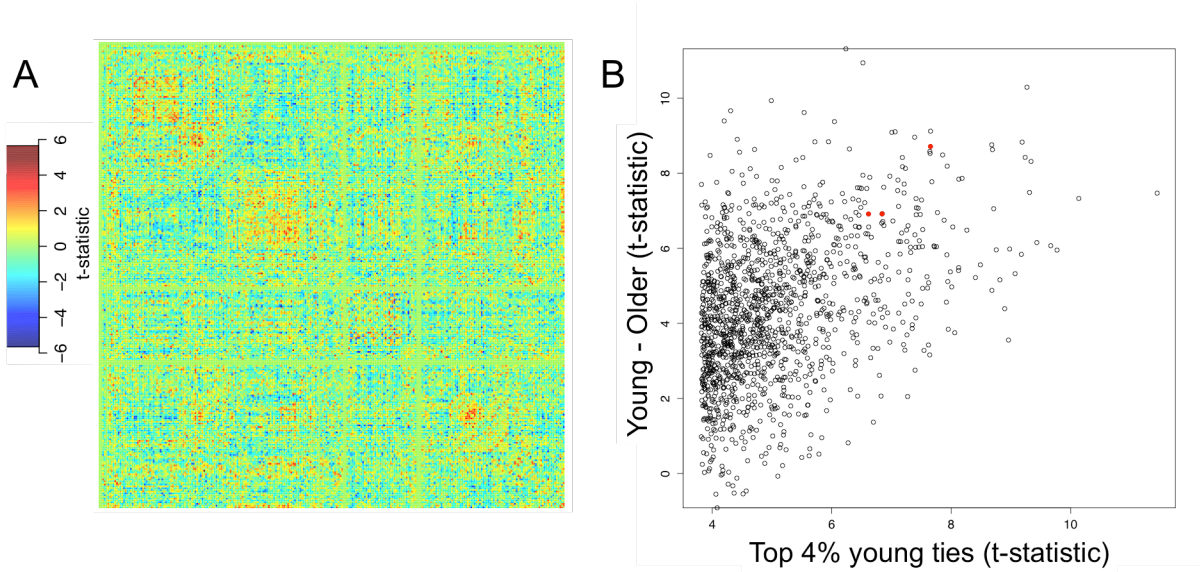


Figure 3.8. (a) Difference matrix between young and older correlation matrices (t-statistics). (b) Top 4% of pairwise correlations in the young plotted against their change with age.

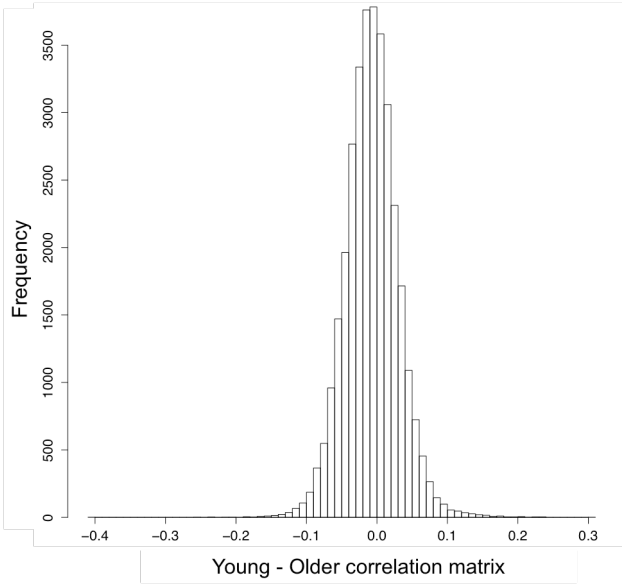


Figure 3.9. The average functional connectivity showed a slight increase with age (mean difference young-older t-statistic matrices = -0.37)

3.4 Discussion

Memory for the sequence of events is an important aspect of episodic memory. We have demonstrated using a cross-species sequence memory task that this capacity declines with age, replicating previous results (Allen et al., 2015). We then sought to understand what neural changes occurred with age during performance of the sequence memory task. Overall, we found that similar neural substrates appeared to be used in typical older individuals with decreases of activity and connectivity being widespread.

Previous studies of memory-related activity changes with typical aging have demonstrated increases, decreases, and no changes with age in the PFC and MTL. Decreases have generally been interpreted as degradation, while increases have been interpreted as compensatory activity, particularly when the behavior is matched or when greater activity correlates with better performance (although attempted compensation has been ascribed to poor performance with increased activity). These interpretations, however, are rarely tied to specific neurobiological mechanisms. Similarly, dedifferentation is invoked as a description of conditions in which older adults show less activity in the areas activated by young, but greater activity in other areas, with the further stipulation that this increase in activity does not predict improved performance (see Dennis & Cabeza, 2008; Grady, 2012, Maillet & Rajah 2013 for reviews). Indeed, if this over-activation predicts impaired performance, it may indicate degradation (Duverne et al., 2009; Yassa et al., 2011). (It should be noted that the predictions of increased activity made by Yassa et al., 2011 were motivated by specific neurobiological mechanisms that were found to have changed with age in the rodent, and they confirmed these predictions in the human, therefore providing a more specific mechanistic account of the changes in typical aging. Further, a subsequent study found that decreasing this activity in patients with amnestic mild cognitive impairment improved performance (Bakker et al., 2012).) The differing results in the literature, such as research showing that increased prefrontal activity in older adults was present only in poor performing older adults (Duverne et al., 2009; Persson et al., 2006) while other research finds increased activity only in high performing adults or increased activity predicts better performance (Cabeza et al., 2002; Rajah and McIntosh, 2008), could be due to complications such as task design, task difficulty, chosen contrast of interest, and changes to the vasculature.

To address some of the issues presented by studying aging using neuroimaging, we used a non-spatial, non-verbal, cross-species task design. This allows us to leverage complementary approaches, address performance confounds, and select a contrast specific to evaluating the neural response to sequence memory demands. Based on our results from Experiment 1 showing activation of hippocampus and mPFC during sequence memory performance and evidence of changes in typical aging preferentially targeting these regions, we hypothesized that we would observe changes in these areas with age during sequence memory performance. We sought to determine if, during a sequence memory task, we would observe evidence for compensatory activity, dedifferentiation, or degradation in the MTL and PFC, or more generally brain-wise in shifts in patterns of activation consistent with models such as HAROLD or PASA. We found that both hippocampus and mPFC were active during correct sequence memory performance in young and older adults, however, we found that activity was decreased in older adults. We also found evidence that the same neural substrates supported correct task performance in older adults brain-wise, but was decreased in older adults. Additionally, using functional connectivity analyses we found that within the highly correlated areas of the brain during sequence memory performance in the young, including hippocampus and mPFC, connectivity decreased with age. Therefore, using several analysis methods, we see a generalized pattern of decreases with age with no evidence for either compensation or dedifferentiation in older adults [1] (we have potentially observed degradation, as there were decreases in activity and connectivity with age, however this was observed during correct performance – see further discussion below). Thus, despite being a task that shows clear behavioral effects of aging and despite it relying on

[1] Although, it should be noted the older adults' decrease in *difference* between InSeq and OutSeq, such that older adults neural circuitry could be treating the two conditions more similarly, could be interpreted as a form of dedifferentiation

structures with clear, known, neurobiological effects of age, the observed data did not fit either of these explanations or fit popular descriptive models such as HAROLD or PASA.

From our results, it is clear that identifying the underlying cause of these decreases is an important future endeavor – do these decreases reflect actual degradation on a neural level, and what is the mechanism? The descriptive models have been useful in interpreting much of the neuroimaging data, however they have not accounted for the current data, as well as others in the literature (e.g. Wang et al., 2016). To aid the interpretation of the aging neuroimaging results, studies designed to investigate the neurobiological mechanisms underlying the BOLD fMRI results will be an important next step. The limitations of BOLD fMRI and PET can be addressed by using other modalities (and model species) to inform our understanding of the current results and those in the literature. Increasing our knowledge of the specificity of changes with age will aid in characterizing typical aging in order to both allow for earlier differentiation between typical and pathological aging, as well as to develop targeted interventions in the future. Below, we propose several lines of future research to address this issue.

3.4.1 Univariate Analyses: Younger and Older adults use the same neural substrates to perform the sequence memory task, however older adults show less activity

The results from the univariate activation analysis demonstrate a remarkable similarity in neural substrates supporting sequence memory performance between older and young adults. Within *a priori* regions of interest, hippocampus and mPFC, we observed a significant contrast between InSeq and OutSeq during correct performance (and during all trials) for both younger and older individuals. We found, however, that young adults activated these regions to a greater extent than older adults. To attempt to correct for the vascular changes with age that change the hemodynamic coupling ratio, and therefore decrease the dynamic range of the BOLD signal, we

employed resting state amplitude correction (RSFA; (Kannurpatti and Biswal, 2008; Kannurpatti et al., 2011); see Figure 3.3). Our results maintained, showing differences between young and older adults.

These decreases with age were not selective to the hippocampus and mPFC, however. When conducting whole-brain analyses, we found that young adults had many more regions active than older adults, with the areas active in older adults highly overlapping with the young. When the statistical threshold was lowered in the older adult analysis, they showed highly similar neural substrates to the younger adults. Therefore, we found that the older individuals were likely using the same neural substrates, but that the activity appears weaker, leading to less robust results. In addition, when we directly contrasted the young and older adults, we found several areas showed Young > Older. In order to determine the directionality of the underlying contrast, we created a scatterplot of each cluster, finding that the results were mainly driven by the young showing an InSeq > OutSeq contrast. Creating a scatterplot aids in disambiguating the meaning of the Young vs. Older contrast, as the underlying signal is always a contrast of task conditions.

Thus, we did not observe evidence of either compensatory activity or dedifferentiation in the older adult group, as our results showed an overall decrease in activity for the older compared to younger group, and there were no positive correlations between InSeq vs OutSeq activity and the Sequence Memory Index in the brain. The results of the current study support previous research demonstrating decreased activity with age in the PFC and/or HC during memory performance (Dennis et al., 2007, 2008; Grady et al., 1995; Murty et al., 2008; Rajah et al., 2010), (although some of these studies showed decreased hippocampal activity with increased prefrontal activity – Dennis et al., 2007; Murty et al., 2008), and with reports that

demonstrate that neural mechanisms remain stable with age (Wang et al., 2016). Interestingly, the current results also parallel results from a study we conducted of encoding and retrieval (recognition) of scene stimuli that show a linear decline in activity with age in the medial temporal lobe (Figure 3.10, unpublished findings). The declines were observed during both intentional and incidental encoding, with behavior equal between groups in the intentional variant.

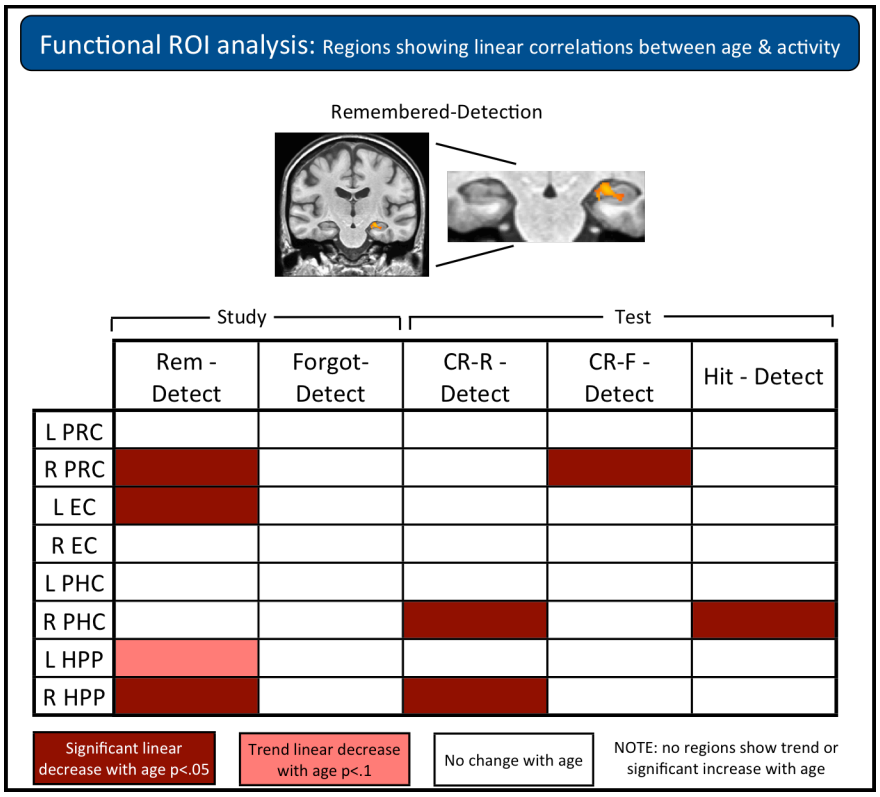


Figure 3.10. Functional regions of interest analysis reveal decreases in activity with age during a scene encoding and retrieval study with 95 adults ages 20-87 yrs. Abbreviations: PRC: perirhinal cortex. EC: entorhinal cortex. PHC: parahippocampal cortex. HPP: hippocampus. Rem = activity during encoding of scenes that will be subsequently remembered; Forgot = activity during encoding of scenes that will be subsequently forgotten; Detect = Detection-in-noise task condition (baseline), a non-mnemonic task where participants must detect differences in luminance levels; CR-R = correct rejection, later remembered; CR-F = correct rejection, later forgotten; Hit = correct recognition.

3.4.2 Potential sources of age-related decreases in activity

What could be underlying this decrease in activity with age? First, there could be residual vascular issues in our dataset. While we employed RSFA correction, there may remain a decreased BOLD dynamic signal in our aging adults (or at least a decrease in signal-to-noise). To determine if the decreases represent a vascular rather than neural change with age, a future study could employ arterial spin labeling (ASL) (Petcharunpaisan et al., 2010). ASL provides a quantitative measure of cerebral blood flow, and therefore a direct measure of blood flow could be made for each of the task conditions, along with a baseline (such as a perceptual discrimination task that is not thought to engage the same networks as a memory task (Stark and Squire, 2001). By demonstrating that the changes with age are present to a greater degree within the tasks of interest versus the baseline condition would help to rule out that the findings are purely vascular in origin. In addition, the current design contrasts the task conditions of InSeq and OutSeq, therefore we cannot determine if aging differentially affects one of the task conditions - ASL could elucidate which of the conditions show changes with age.

The decreased signal in older adults could be due to decreases in neural activity, which in turn could be driven by changes to white matter connecting the regions, gray matter changes including synaptic changes, changes to the physiological properties of the cells, and changes to the neurotransmitter and inflammatory systems, all which have been shown previously to exhibit changes with age (Bennett and Madden, 2014; Burke and Barnes, 2006; Grady, 2012b; Morrison and Baxter, 2012; Ryan and Nolan, 2016; Samson and Barnes, 2013; Yassa et al., 2010). Indeed, understanding the changes in the typically aging brain require multiple modalities. To this end, future studies can use magnetic resonance spectroscopy (MRS) imaging to examine how changes to the neurotransmitter systems relate to changes in activity during sequence memory

performance, as event-related MRS has been shown to be a viable method (Apsvalka et al., 2015). In addition, measures of white matter integrity and gray matter volume would also inform the meaning of the functional activity results, as previous research has shown correlations between these three measures (see Bennett and Madden, 2014; Maillet and Rajah, 2013; Salat, 2011). (As a preliminary analysis however, we found no correlations between white matter integrity (FA and MD) in the fornix and superior cingulum, connections between HC and mPFC, with either SMI or functional connectivity between HC and mPFC in either young or older groups).

Additionally, it is possible that the changes in activity seen in the whole brain contrast could be driven by the changes within the hippocampus and mPFC. As it has been established that these regions are critical to task performance, changes within these regions could result in changes to the entire network during task performance. And, previous research has shown that these areas are differentially impacted in typical aging with higher gray matter volume loss, synaptic changes, changes in plasticity, and degradation to white matter connections between these regions (Bennett and Stark, 2015; Burke and Barnes, 2006; Grady, 2012b; Morrison and Baxter, 2012; Samson and Barnes, 2013). Another plausible explanation is that older adults have greater variability in the cognitive strategies used to perform the task, with resulting higher neural activity variability. However, given that with lower thresholds, the same regions are shown to be active in older adults as the younger adults, this is less likely to be the case. Additionally it should be noted that confidence is not controlled for in this experimental design. Therefore, it is plausible that older adults, while performing at the level of younger adults in the correct-only contrast, may have a weaker memory which could be observed as decreases in

activity. A future variant of the sequence memory task could assess confidence level after each sequence to help address this issue, such as was done by Morcom et al. (2007).

A distinct advantage, however, of the sequence memory task is its cross-species nature. Therefore, the task can be assessed in aging rats. The ability to record directly from both single neurons and ensembles in the hippocampus and mPFC in aged rats during performance of the task can greatly inform our BOLD fMRI signal changes with age. For instance, there could exist changes to slow gamma in CA1 in aged rats (which in young rats showed higher power for InSeq vs. OutSeq) and changes to the numbers or coding properties of 'sequence cells' (as well as changes to immediate early gene expression), that may not be observed in other brain areas. Observations such as these would aid in understanding the neurobiological mechanisms underlying the BOLD activity decreases as well and increase the specificity of the observed changes with age. In addition, variants such as those including confidence judgments could also be conducted in rats, using criterion shifts such as was done by Fortin et al. (2004). Finally, studies of cognitive training or intervention could inform our results. A recent study showed that a video game intervention, as a form of environmental enrichment, improved mnemonic discrimination thought to rely on the hippocampus (Clemenson and Stark, 2015). Therefore, an intervention such as this would allow pre- and post- hippocampal imaging, demonstrating either increases or decreases in activity in the hippocampus during the sequence memory task that are concurrent with improvements in hippocampal function.

3.4.3 Functional Connectivity: Younger and older adults show similar connectivity profiles, with older presenting decreased connectivity

The functional connectivity results paralleled those of the univariate activity, showing similar but potentially degraded neural profiles with age. The connectivity results showed that

the HC-mPFC connection decreased with age, however this decrease was not selective, as the other highly connected nodes also decreased their connectivity with age. Interestingly, the overall connectivity increased with age, however this could have been driven by a higher percentage of negative correlations existing in the matrix that decreased their magnitude with age (this would be supported by Meier et al., 2012 that showed decreases in negative correlations with age). Previous research has shown that the within-network connections decrease with age (Chan et al., 2014; Geerligs et al., 2015b). Our results show a similar finding, because the highest correlations are within-network connections. The between-network connections have shown increases with age (Chan et al., 2014), however it is difficult to interpret this result with negative correlations set to zero. However, the literature suggests there is decreased distinction between brain systems (Chan et al., 2014; Geerligs et al., 2015b; Grady et al., 2016). The decreased segregation seems to be behaviorally meaningful as well, as regressing out age has shown the amount of segregation predicts episodic memory scores (Chan et al., 2014).

The question again remains what do these functional connectivity changes represent? They could represent decreased segregation between brain systems, as has been reported. However, in the current fMRI data there exists a decrease in the standard deviation (and median absolute deviation - MAD) in the gray matter in older adults compared to younger over the course of the scan (MAD gray matter: $t_{40} = 2.79$, $p < 0.01$; MAD CSF: $t_{40} = 0.094$, $p=0.93$; MAD WM: $t_{40} = 1.89$, $p = 0.07$). While a lower standard deviation would typically be thought of as less noise, importantly the overall time-course contains both variance related to the noise and variance related to the task-related neural activity. The reduced variability is only observed in the raw fMRI data and not observed when task effects are regressed out, demonstrating that aging is leading to a reduction in *signal* and therefore a reduced signal-to-noise ratio (SNR). This reduced

SNR would account for at least some of the changes observed in functional connectivity. The reduction in SNR in older adults could be due to a change in the vasculature, whereby because of the reduced dynamic range, the SNR is lowered. Therefore, if we could assess the noise inherent in older adults, and then apply these noise estimates to the younger dataset, would we observe similar decreases to the connectivity (and univariate activity)? If the young now resembled the older adults, it may indicate that the changes to the networks with age (such as reduced modularity and local efficiency; Geerligs et al., 2015a) were not neurally-driven, but rather an artifact of the vasculature. However, if there remain residual differences between the older and younger groups, these differences could indicate more specific changes with age that are observable once the reduction in SNR is corrected for (such as specific networks differentially impacted by age in functional connectivity and specific regions in univariate activity). Again, the cross-species nature of the task can help address this issue as well. Multi-site electrophysiological recording can be completing in aged rats to measure the connectivity between regions on a much shorter timescale than BOLD fMRI. However, connectivity can also be measured at lower frequencies (consistent with the sampling rate of fMRI) – termed "infra-low" interactions (Thompson et al., 2014b). Therefore, when completing the sequence memory task, the millisecond by millisecond interactions can be observed, as well as interactions at the timescale of BOLD fMRI. The changes in age to both of these measures in the rat could help inform our BOLD functional connectivity results.

3.5 Conclusion

We sought to discover changes with age in memory for sequences of events, a defining feature of episodic memory. Older adults showed declines in performance, and also decreases in neural activity and connectivity, however the neural substrates remained very similar to those of

young adults. Discovering the underlying neurobiological changes driving these results are an important future endeavor, and the cross-species nature of the task, combined with other imaging modalities, will allow for deeper understanding of the changes observed with age during sequence memory performance.

4 Conclusion

Characterizing typical aging is an important step in the endeavor to both mitigate declines in the aging population and differentiate typical from pathological aging. Previous research has shown that episodic memory declines in typical aging, and memory for sequence of events is critical aspect of episodic memory (Craik and Simon, 1980; Tulving, 1984). The research in this dissertation sought to first identify the neural correlates of sequence memory in the human and then characterize the changes with age to these neural substrates. The dissertation had two aims: (1) Determine the neural correlates of sequence memory in young adults and (2) investigate age-related changes to the neural substrates underlying sequence memory performance. Below are a review of the findings from those aims and a discussion of outstanding issues and future directions.

4.1 A cross-species sequence memory task reveals homologous neural substrates shared between the rat and human

4.1.1 Brief summary of findings

The first aim of the dissertation was to identify the neural substrates supporting memory for sequences of events, a crucial component of episodic memory. Using a cross-species non-spatial non-verbal sequence memory task combined with BOLD fMRI, we showed hippocampus and mPFC were active during sequence memory performance, showing differential activity to items presented in sequence versus out of sequence. High-resolution imaging replicated this result in the hippocampus. With functional connectivity analyses, we showed that activity in these areas was highly correlated over the course of the task. The results parallel those found in the rat, which found using temporary inactivations that hippocampus and mPFC were important

for task performance, and electrophysiological recordings from the these regions showed complementary signals that can solve the task (Allen et al., 2016; Quirk et al., 2013).

4.1.2 Outstanding issues and future directions

We demonstrated homology between the rat and human in the medial PFC and hippocampus, and therefore complementary work can continue to understand the neural mechanisms supporting memory for sequences of events. However, there remain differences particularly with regards to the prefrontal cortex between rats and humans (Passingham and Wise, 2012). Understanding the contributions of other areas of the human PFC to memory for sequences of events is an important continued endeavor, as other sequence memory paradigms, as well as the current paradigm, showed activation in prefrontal areas other than mPFC. In addition, there is little consensus as to the role of particular regions of the prefrontal cortex specifically involved in forming and retrieving temporal context memory. Reasons for these differences in activation across studies of temporal context could be due to varied task design, chosen contrast, chosen analysis method, etc., which could change the cognitive demands of the task (see section 2.4.1 for more detail). Future studies can systematically vary demands of the sequence memory task, emphasizing different cognitive strategies, which would allow for identification of how the various PFC regions contribute to sequence memory. For instance, as discussed in section 2.4.1, we could bias participants to adopt a strategy of item-in-position versus item-item associations, and the same changes could be made to the rodent version of the sequence memory task. This comparison would allow for understanding of the homology of neural signals as well as the divergence between species.

In addition, the complementary approach can also elucidate the functional connectivity findings in the human. We found that mPFC - HC functional connectivity was high during the

sequence memory task (the correlation in activity between the regions was within the top 0.5% of correlations in the entire brain), but was also was highly correlated during rest. In addition, other research (and our current research) has demonstrated that the functional connectivity across the whole brain is very similar even in different cognitive states (Cole et al., 2014). Dual electrophysiological recordings completed in the rat hippocampus and mPFC may aid in understanding the underlying neural mechanisms supporting functional connectivity, as recordings can be completed on a similar timescale to BOLD fMRI (as well as the higher frequency timescale). The relationship between signals on these timescales can help elucidate the underlying neural mechanisms of functional connectivity in general, and recordings made during performance of the sequence memory task as well as during resting and/or other cognitive states can reveal the specificity of the functional connectivity during temporal context memory.

4.2 Younger and older adults utilize highly similar neural substrates to perform a sequence memory task, however older adults show decreases in activity and functional connectivity

4.2.1 Brief summary of findings

The second aim of the dissertation was to identify the changes that occur to the neural substrates supporting sequence memory performance with age. First, we replicated results from Experiment 1, showing the hippocampus and mPFC were active during sequence memory performance in young adults. We then found that performance on the sequence memory task declined with age, supporting previous findings (Allen et al., 2015; Cabeza et al., 2000; Fabiani and Friedman, 1997; Moscovitch and Winocur, 1995; Roberts et al., 2014; Tolentino et al., 2012). We found with equated performance (to eliminate observing neural correlates of impaired performance), older adults showed very similar neural substrates to young adults, including

hippocampus and mPFC, but demonstrated overall lower activity. We also found using functional connectivity analyses that the activity in hippocampus and mPFC was highly correlated over the course of the task in both groups, but was decreased in older adults. This decrease was similarly found for other highly correlated regions in the young adults. The results indicate highly similar neural substrates support performance on the sequence memory task in young and older adults, but are potentially degraded with age.

4.2.2 Outstanding issues and future directions

We observed decreases in both activity and functional connectivity in older adults during performance of the sequence memory task. While models such as HAROLD or PASA, or explanations such as compensation or dedifferentiation, describe many neuroimaging results, the current data do not fit these models. In addition, the descriptive models do not provide an underlying neurobiological mechanism. Utilizing other imaging modalities and model species will help provide mechanistic explanations for the neuroimaging results, and aid in interpretation of the mixed results seen in the aging neuroimaging literature (such as increases, decreases, or no changes with age in activity levels - Duverne et al., 2009; Giovanello and Schacter, 2012; Gutchess et al., 2005; Liu et al., 2013; Nyberg et al., 2003; Rajah and McIntosh, 2008; Sperling, 2007).

For instance, in the current study several possible explanations for the decreases in activity and connectivity exist, including vascular confounds, synaptic and physiological changes, anatomical connectivity changes, and changes in cognitive strategy. Understanding which of these explanations accounts for the changes with age will be an important future endeavor to further characterize typical aging. Using several modalities, such as arterial spin labeling to eliminate vascular confounds, magnetic resonance spectroscopy to determine how changes to the

neurotransmitter systems affect BOLD activity changes, observing relationships with white matter tracts and gray matter volume, and performing new variants of the sequence task (such as including confidence judgments) will help to disambiguate these possibilities and provide for greater specificity as to the changes with age.

In addition, utilizing the cross-species approach will aid in interpretation of the current neuroimaging results. For example, in aged rats direct electrophysiological recording from single units and ensembles during sequence memory performance could reveal changes to oscillatory dynamics or coding properties of individual cells and these may vary between brain regions, providing increased specificity. Multi-site recording can reveal changes with age to the connectivity between regions both at higher frequencies and lower frequencies (consistent with the sampling rate of BOLD fMRI); the changes with age to these measures with age could inform the functional connectivity changes seen in the current study. In addition, variants of the task such as including confidence judgments (by using criterion shifts) can be completed in both the rat and human. The collaborative, cross-species approach will provide greater understanding and specificity of the neural mechanisms changing with typical aging.

Overall, this dissertation demonstrates the benefits of collaborative work. Experiment 1 revealed the neural substrates for sequence memory performance were shared between rats and humans. This lays the groundwork for future studies in both the rat and the human to further investigate the cognitive constructs underlying sequence memory performance and their neural substrates by using complementary techniques. In Experiment 2, we found that activity and connectivity were decreased in older adults during sequence memory performance. Again, complementary techniques will provide a greater understanding of the underlying neural mechanisms that have changed with age. For instance, electrophysiological recordings can be

completed in aged rats, and imaging modalities such as arterial spin labeling can be performed in the human. Understanding the neurobiological processes underlying memory for sequences of events and their changes with age remain an important endeavor, and the cross-species approach will allow for continued fruitful collaboration.

5 References

Aggleton, J.P., and Brown, M.W. (1999). Episodic memory, amnesia, and the hippocampal-anterior thalamic axis. Behav. Brain Sci. *22*, 425–489.

Agster, K.L., Fortin, N.J., and Eichenbaum, H. (2002). The hippocampus and disambiguation of overlapping sequences. J. Neurosci. *22*, 5760–5768.

Allen, T.A., and Fortin, N.J. (2013). The evolution of episodic memory. Proc Natl Acad Sci U A *110 Suppl 2*, 10379–10386.

Allen, E.A., Erhardt, E.B., Damaraju, E., Gruner, W., Segall, J.M., Silva, R.F., Havlicek, M., Rachakonda, S., Fries, J., Kalyanam, R., et al. (2011a). A baseline for the multivariate comparison of resting-state networks. Front. Syst. Neurosci. *5*, 2.

Allen, T.A., Jacobs, N.S., Feinberg, L.M., Bharadwaj, K.R., Wang, M.X., and Fortin, N. (2011b). Prefrontal cortex neurons code for sequences of events.

Allen, T.A., Morris, A.M., Mattfeld, A.T., Stark, C.E., and Fortin, N.J. (2014). A Sequence of events model of episodic memory shows parallels in rats and humans. Hippocampus *24*, 1178–1188.

Allen, T.A., Morris, A.M., Stark, S.M., Fortin, N.J., and Stark, C.E. (2015). Memory for sequences of events impaired in typical aging. Learn Mem *22*, 138–148.

Allen, T.A., Salz, D.M., McKenzie, S., and Fortin, N.J. (2016). Nonspatial Sequence Coding in CA1 Neurons. J. Neurosci. Off. J. Soc. Neurosci. *36*, 1547–1563.

Amaral, D.G., Ishizuka, N., and Claiborne, B. (1990). Neurons, numbers and the hippocampal network. Prog. Brain Res. *83*, 1–11.

Ances, B.M., Liang, C.L., Leontiev, O., Perthen, J.E., Fleisher, A.S., Lansing, A.E., and Buxton, R.B. (2009). Effects of aging on cerebral blood flow, oxygen metabolism, and blood oxygenation level dependent responses to visual stimulation. Hum. Brain Mapp. *30*, 1120–1132.

Andersen, P., Bliss, T.V.P., and Skrede, K.K. (1971). Lamellar organization of hippocampal excitatory pathways. Exp. Brain Res. *13*, 222–238.

Apsvalka, D., Gadie, A., Clemence, M., and Mullins, P.G. (2015). Event-related dynamics of glutamate and BOLD effects measured using functional magnetic resonance spectroscopy (fMRS) at 3T in a repetition suppression paradigm. Neuroimage *118*, 292–300.

Aron, A.R., Robbins, T.W., and Poldrack, R.A. (2004). Inhibition and the right inferior frontal cortex. Trends Cogn Sci *8*, 170–177.

Avants, B.B., Epstein, C.L., Grossman, M., and Gee, J.C. (2008). Symmetric diffeomorphic image registration with cross-correlation: evaluating automated labeling of elderly and neurodegenerative brain. Med Image Anal *12*, 26–41.

Badre, D., and Wagner, A.D. (2007). Left ventrolateral prefrontal cortex and the cognitive control of memory. Neuropsychologia *45*, 2883–2901.

Bakker, A., Krauss, G.L., Albert, M.S., Speck, C.L., Jones, L.R., Stark, C.E., Yassa, M.A., Bassett, S.S., Shelton, A.L., and Gallagher, M. (2012). Reduction of hippocampal hyperactivity improves cognition in amnestic mild cognitive impairment. Neuron *74*, 467–474.

Bandettini, P.A., and Wong, E.C. (1997). A hypercapnia-based normalization method for improved spatial localization of human brain activation with fMRI. NMR Biomed *10*, 197–203.

Barbas, H., and Blatt, G.J. (1995). Topographically specific hippocampal projections target functionally distinct prefrontal areas in the rhesus monkey. Hippocampus *5*, 511–533.

Barnes, C.A., Suster, M.S., Shen, J., and McNaughton, B.L. (1997). Multistability of cognitive maps in the hippocampus of old rats. Nature *388*, 272–275.

Bartlett, J.C., Leslie, J.E., Tubbs, A., and Fulton, A. (1989). Aging and memory for pictures of faces. Psychol Aging *4*, 276–283.

Beckmann, M., Johansen-Berg, H., and Rushworth, M.F.S. (2009). Connectivity-based parcellation of human cingulate cortex and its relation to functional specialization. J. Neurosci. Off. J. Soc. Neurosci. *29*, 1175–1190.

Behzadi, Y., Restom, K., Liau, J., and Liu, T.T. (2007). A component based noise correction method (CompCor) for BOLD and perfusion based fMRI. Neuroimage *37*, 90–101.

Bennett, I.J., and Madden, D.J. (2014). Disconnected aging: cerebral white matter integrity and age-related differences in cognition. Neuroscience *276*, 187–205.

Bennett, I.J., and Stark, C.E.L. (2015). Mnemonic Discrimination Relates to Perforant Path Integrity: An Ultra-High Resolution Diffusion Tensor Imaging Study. Neurobiol. Learn. Mem.

Bennett, I.J., Huffman, D.J., and Stark, C.E. (2014). Limbic Tract Integrity Contributes to Pattern Separation Performance Across the Lifespan. Cereb Cortex.

Ber, E.A. (1948). A simple objective technique for measuring flexibility in thinking. J Gen Psychol *39*, 15–22.

Berchtold, N.C., Coleman, P.D., Cribbs, D.H., Rogers, J., Gillen, D.L., and Cotman, C.W. (2013). Synaptic genes are extensively downregulated across multiple brain regionsin normal human aging and Alzheimer's disease. Neurobiol Aging *34*, 1653–1661.

Blalock, E.M., Chen, K.C., Sharrow, K., Herman, J.P., Porter, N.M., Foster, T.C., and Landfield, P.W. (2003). Gene microarrays in hippocampal aging: statistical profiling identifies novel processes correlated with cognitive impairment. J Neurosci *23*, 3807–3819.

Bloss, E.B., Janssen, W.G., Ohm, D.T., Yuk, F.J., Wadsworth, S., Saardi, K.M., McEwen, B.S., and Morrison, J.H. (2011). Evidence for reduced experience-dependent dendritic spine plasticity in the aging prefrontal cortex. J Neurosci *31*, 7831–7839.

Blumenfeld, R.S., and Ranganath, C. (2007). Prefrontal cortex and long-term memory encoding: an integrative review of findings from neuropsychology and neuroimaging. Neuroscientist *13*, 280–291.

Boss, B.D., Peterson, G.M., and Cowan, W.M. (1985). On the number of neurons in the dentate gyrus. Brain Res. *338*, 144–150.

Botvinick, M.M. (2008). Hierarchical models of behavior and prefrontal function. Trends Cogn Sci *12*, 201–208.

Botvinick, M.M., Braver, T.S., Barch, D.M., Carter, C.S., and Cohen, J.D. (2001). Conflict monitoring and cognitive control. Psychol. Rev. *108*, 624–652.

Brewer, J.B., Zhao, A., Desmond, J.E., Glover, G.H., and Gabrieli, J.D.E. (1998). Making memories: Brain activity that predicts how well visual experience will be remembered. Science *281*, 1185–1187.

Buckner, R.L. (2004). Memory and executive function in aging and AD: multiple factors that cause decline and reserve factors that compensate. Neuron *44*, 195–208.

Buckner, R.L., Andrews-Hanna, J.R., and Schacter, D.L. (2008). The brain's default network: anatomy, function, and relevance to disease. Ann N Acad Sci *1124*, 1–38.

Bullain, S.S., Corrada, M.M., Shah, B.A., Mozaffar, F.H., Panzenboeck, M., and Kawas, C.H. (2013). Poor physical performance and dementia in the oldest old: the 90+ study. JAMA Neurol *70*, 107–113.

Bunge, S.A., Burrows, B., and Wagner, A.D. (2004). Prefrontal and hippocampal contributions to visual associative recognition: interactions between cognitive control and episodic retrieval. Brain Cogn *56*, 141–152.

Burke, S.N., and Barnes, C.A. (2006). Neural plasticity in the ageing brain. Nat. Rev. *7*, 30–40.

Burke, S.N., and Barnes, C.A. (2010). Senescent synapses and hippocampal circuit dynamics. Trends Neurosci *33*, 153–161.

Cabeza, R. (2002). Hemispheric asymmetry reduction in older adults: the HAROLD model. Psychol. Aging *17*, 85–100.

Cabeza, R., and Nyberg, L. (2003). Functional neuroimaging of memory. Neuropsychologia *41*, 241–244.

Cabeza, R., and St Jacques, P. (2007). Functional neuroimaging of autobiographical memory. Trends Cogn Sci *11*, 219–227.

Cabeza, R., Mangels, J., Nyberg, L., Habib, R., Houle, S., McIntosh, A.R., and Tulving, E. (1997). Brain Regions Differentially Involved in Remembering What and When: a PET Study. Neuron *19*, 863–870.

Cabeza, R., Anderson, N.D., Houle, S., Mangels, J.A., and Nyberg, L. (2000). Age-related differences in neural activity during item and temporal-order memory retrieval: a positron emission tomography study. J. Cogn. Neurosci. *12*, 197–206.

Cabeza, R., Anderson, N.D., Locantore, J.K., and McIntosh, A.R. (2002). Aging gracefully: compensatory brain activity in high-performing older adults. NeuroImage *17*, 1394–1402.

Cancela Carral, J.M., and Ayan Perez, C. (2007). Effects of high-intensity combined training on women over 65. Gerontology *53*, 340–346.

Chan, M.Y., Park, D.C., Savalia, N.K., Petersen, S.E., and Wig, G.S. (2014). Decreased segregation of brain systems across the healthy adult lifespan. Proc. Natl. Acad. Sci. *111*, E4997–E5006.

Charles, D.P., Gaffan, D., and Buckley, M.J. (2004). Impaired recency judgments and intact novelty judgments after fornix transection in monkeys. J. Neurosci. Off. J. Soc. Neurosci. *24*, 2037–2044.

Chawla, M.K., Guzowski, J.F., Ramirez-Amaya, V., Lipa, P., Hoffman, K.L., Marriott, L.K., Worley, P.F., McNaughton, B.L., and Barnes, C.A. (2005). Sparse, environmentally selective expression of arc RNA in the upper blade of the rodent fascia dentata by brief spatial experience. Hippocampus *15*, 579–586.

Chen, J., Cook, P.A., and Wagner, A.D. (2015). Prediction strength modulates responses in human area CA1 to sequence violations. J. Neurophysiol. *114*, 1227–1238.

Clark, R.E., and Squire, L.R. (2010). An animal model of recognition memory and medial temporal lobe amnesia: history and current issues. Neuropsychologia *48*, 2234–2244.

Clark, R.E., and Squire, L.R. (2013). Similarity in form and function of the hippocampus in rodents, monkeys, and humans. Proc Natl Acad Sci U A *110*, 10365–10370.

Clemenson, G.D., and Stark, C.E.L. (2015). Virtual Environmental Enrichment through Video Games Improves Hippocampal-Associated Memory. J. Neurosci. *35*, 16116–16125.

Cole, M.W., Bassett, D.S., Power, J.D., Braver, T.S., and Petersen, S.E. (2014). Intrinsic and task-evoked network architectures of the human brain. Neuron *83*, 238–251.

Colgin, L.L., Denninger, T., Fyhn, M., Hafting, T., Bonnevie, T., Jensen, O., Moser, M.-B., and Moser, E.I. (2009). Frequency of gamma oscillations routes flow of information in the hippocampus. Nature *462*, 353–357.

Craik, F.I. (1983). On the transfer of information from temporary to permanent memory. IPhilosophical Trans. R. Soc. Series B302, 341–359.

Craik, F.I.M., and Simon, D. (1980). Age differences in memory: the roles of attention and depth of processing. In New Directions in Memory and Aging: Proceedings of the George A. Talland Memorial Conference, (Hillsdale, NJ: Lawrence Erlbaum Associates),.

Craik, F.L., and McDowd, J.M. (1987). Age differences in recall and recognition. J. Exp. Psychol. Learn. Mem. Cogn. *13*, 474–479.

Craik, F.I., Morris, L.W., Morris, R.G., and Loewen, E.R. (1990). Relations between source amnesia and frontal lobe functioning in older adults. Psychol Aging *5*, 148–151.

Davachi, L., and DuBrow, S. (2015). How the hippocampus preserves order: the role of prediction and context. Trends Cogn. Sci. *19*, 92–99.

Davachi, L., Maril, A., and Wagner, A.D. (2001). When keeping in mind supports later bringing to mind: neural markers of phonological rehearsal predict subsequent remembering. J Cogn Neurosci *13*, 1059–1070.

Davis, S.W., Dennis, N.A., Daselaar, S.M., Fleck, M.S., and Cabeza, R. (2008). Que PASA? The posterior-anterior shift in aging. Cereb. Cortex N. Y. N 1991 *18*, 1201–1209.

Dennis, N.A., and Cabeza, R. (2008). Neuroimaging of healthy cognitive aging. In Handbook of Aging and Cognition, (Mahwah, NJ: Erlbaum),.

Dennis, N.A., Daselaar, S., and Cabeza, R. (2007). Effects of aging on transient and sustained successful memory encoding activity. Neurobiol. Aging *28*, 1749–1758.

Dennis, N.A., Hayes, S.M., Prince, S.E., Madden, D.J., Huettel, S.A., and Cabeza, R. (2008). Effects of aging on the neural correlates of successful item and source memory encoding. J. Exp. Psychol. Mem. Cogn. *34*, 791–808.

D'Esposito, M., Deouell, L.Y., and Gazzaley, A. (2003). Alterations in the BOLD fMRI signal with ageing and disease: a challenge for neuroimaging. Nat Rev Neurosci *4*, 863–872.

DESTRIEUX, C., FISCHL, B., DALE, A., and HALGREN, E. (2010). Automatic parcellation of human cortical gyri and sulci using standard anatomical nomenclature. NeuroImage *53*, 1–15.

Devito, L.M., and Eichenbaum, H. (2011). Memory for the order of events in specific sequences: contributions of the hippocampus and medial prefrontal cortex. J Neurosci *31*, 3169–3175.

Diana, R.A., Yonelinas, A.P., and Ranganath, C. (2007). Imaging recollection and familiarity in the medial temporal lobe: A three-component model. Trends Cogn. Sci. *11*, 379–386.

Duan, H., Wearne, S.L., Rocher, A.B., Macedo, A., Morrison, J.H., and Hof, P.R. (2003). Age-related dendritic and spine changes in corticocortically projecting neurons in macaque monkeys. Cereb Cortex *13*, 950–961.

Dumitriu, D., Hao, J., Hara, Y., Kaufmann, J., Janssen, W.G., Lou, W., Rapp, P.R., and Morrison, J.H. (2010). Selective changes in thin spine density and morphology in monkey prefrontal cortex correlate with aging-related cognitive impairment. J Neurosci *30*, 7507–7515.

Duverne, S., Motamedinia, S., and Rugg, M.D. (2009). The relationship between aging, performance, and the neural correlates of successful memory encoding. Cereb. Cortex N. Y. N 1991 *19*, 733–744.

Eichenbaum, H., and Fortin, N.J. (2005). Bridging the gap between brain and behavior: cognitive and neural mechanisms of episodic memory. J Exp Anal Behav *84*, 619–629.

Ekstrom, A.D., Copara, M.S., Isham, E.A., Wang, W., and Yonelinas, A.P. (2011). Dissociable networks involved in spatial and temporal order source retrieval. NeuroImage *56*, 1803–1813.

Ezzyat, Y., and Davachi, L. (2011). What constitutes an episode in episodic memory? Psychol. Sci. *22*, 243–252.

Fabiani, M., and Friedman, D. (1997). Dissociations between memory for temporal order and recognition memory in aging. Neuropsychologia *35*, 129–141.

Fjell, A.M., McEvoy, L., Holland, D., Dale, A.M., Walhovd, K.B., and Alzheimer's Disease Neuroimaging Initiative (2014). What is normal in normal aging? Effects of aging, amyloid and Alzheimer's disease on the cerebral cortex and the hippocampus. Prog. Neurobiol. *117*, 20–40.

Folstein, M.F., Folstein, S.E., and McHugh, P.R. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J. Psychiatr. Res. *12*, 189–198.

Fortin, N.J., Agster, K.L., and Eichenbaum, H.B. (2002). Critical role of the hippocampus in memory for sequences of events. Nat Neurosci *5*, 458–462.

Fortin, N.J., Wright, S.P., and Eichenbaum, H. (2004). Recollection-like memory retrieval in rats is dependent on the hippocampus. Nature *431*, 188–191.

Fouquet, C., Tobin, C., and Rondi-Reig, L. (2010). A new approach for modeling episodic memory from rodents to humans: the temporal order memory. Behav. Brain Res. *215*, 172–179.

Fox, M.D., and Raichle, M.E. (2007). Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat. Rev. Neurosci. *8*, 700–711.

Friston, K.J. (1994). Functional and effective connectivity in neuroimaging: A synthesis. Hum. Brain Mapp. *2*, 56–78.

Fuster, J. (2008). The Prefrontal Cortex, Fourth Edition.

Gaffan, D. (1974). Recognition impaired and association intact in the memory of monkeys after transection of the fornix. J. Comp. Physiol. Psychol. *86*, 1100–1109.

Gall, F.J. (1835). On the Functions of the Brain and of Each of Its parts: With Observations on the Possibility of Determining the Instincts, Propensities, and Talents, Or the Moral and Intellectual Dispositions of Men and Animals, by the Configuration of the Brain and Head, Volume 1 (Marsh, Capen & Lyon).

Gallagher, M., and Rapp, P.R. (1997). The use of animal models to study the effects of aging on cognition. Annu. Rev. Psychol. *48*, 339–370.

Gallagher, M., Colantuoni, C., Eichenbaum, H., Haberman, R.P., Rapp, P.R., Tanila, H., and Wilson, I.A. (2006). Individual differences in neurocognitive aging of the medial temporal lobe. Age *28*, 221–233.

Geerligs, L., Rubinov, M., Cam-CAN, and Henson, R.N. (2015a). State and Trait Components of Functional Connectivity: Individual Differences Vary with Mental State. J. Neurosci. *35*, 13949–13961.

Geerligs, L., Renken, R.J., Saliasi, E., Maurits, N.M., and Lorist, M.M. (2015b). A Brain-Wide Study of Age-Related Changes in Functional Connectivity. Cereb. Cortex N. Y. N 1991 *25*, 1987–1999.

Gershberg, F.B., and Shimamura, A.P. (1995). Impaired use of organizational strategies in free recall following frontal lobe damage. Neuropsychologia *33*, 1305–1333.

Giovanello, K.S., and Schacter, D.L. (2012). Reduced specificity of hippocampal and posterior ventrolateral prefrontal acticity during relational retrieval in normal aging. J Cogn Neurosci *24*, 159–170.

Glisky, E.L., Rubin, S.R., and Davidson, P.S. (2001). Source memory in older adults: an encoding or retrieval problem? J Exp Psychol Learn Mem Cogn *27*, 1131–1146.

Goldman-Rakic, P.S., Selemon, L.D., and Schwartz, M.L. (1984). Dual pathways connecting the dorsolateral prefrontal cortex with the hippocampal formation and parahippocampal cortex in the rhesus monkey. Neuroscience *12*, 719–743.

Grady, C. (2012a). The cognitive neuroscience of ageing. Nat Rev Neurosci *13*, 491–505.

Grady, C. (2012b). The cognitive neuroscience of ageing. Nat. Rev. Neurosci. *13*, 491–505.

Grady, C.L., and Garrett, D.D. (2014). Understanding variability in the BOLD signal and why it matters for aging. Brain Imaging Behav *8*, 274–283.

Grady, C., Sarraf, S., Saverino, C., and Campbell, K. (2016). Age differences in the functional interactions among the default, frontoparietal control, and dorsal attention networks. Neurobiol. Aging *41*, 159–172.

Grady, C.L., McIntosh, A.R., Horwitz, B., Maisog, J.M., Ungerleider, L.G., Mentis, M.J., Pietrini, P., Schapiro, M.B., and Haxby, J.V. (1995). Age-related reductions in human recognition memory due to impaired encoding. Science *269*, 218–221.

Gutchess, A.H., Welsh, R.C., Hedden, T., Bangert, A., Minear, M., Liu, L.L., and Park, D.C. (2005). Aging and the neural correlates of successful picture encoding: frontal activations compensate for decreased medial-temporal activity. J. Cogn. Neurosci. *17*, 84–96.

Hannesson, D.K., Howland, J.G., and Phillips, A.G. (2004). Interaction between perirhinal and medial prefrontal cortex is required for temporal order but not recognition memory for objects in rats. J. Neurosci. *24*, 4596–4604.

Hargreaves, E.L., Mattfeld, A.T., Stark, C.E., and Suzuki, W.A. (2012). Conserved fMRI and LFP signals during new associative learning in the human and macaque monkey medial temporal lobe. Neuron *74*, 743–752.

Hatazawa, J., Shimosegawa, E., Satoh, T., Toyoshima, H., and Okudera, T. (1997). Subcortical hypoperfusion associated with asymptomatic white matter lesions on magnetic resonance imaging. Stroke *28*, 1944–1947.

Hayes, S.M., Ryan, L., Schnyer, D.M., and Nadel, L. (2004). An fMRI study of episodic memory: retrieval of object, spatial, and temporal information. Behav Neurosci *118*, 885–896.

Head, D., and Isom, M. (2010). Age effects on wayfinding and route learning skills. Behav Brain Res *209*, 49–58.

Hedden, T., and Gabrieli, J.D. (2004). Insights into the ageing mind: A view from cognitive neuroscience. Nat. Rev. Neurosci. *5*, 87–96.

Henkel, L.A., Johnson, M.K., and De Leonardis, D.M. (1998). Aging and source monitoring: cognitive processes and neuropsychological correlates. J. Exp. Psychol. *127*, 251–268.

Hopkins, R.O., Waldram, K., and Kesner, R.P. (2004). Sequences assessed by declarative and procedural tests of memory in amnesic patients with hippocampal damage. Neuropsychologia *42*, 1877–1886.

Hsieh, L.-T., Gruber, M.J., Jenkins, L.J., and Ranganath, C. (2014). Hippocampal activity patterns carry information about objects in temporal context. Neuron *81*, 1165–1178.

Huijbers, W., Vannini, P., Sperling, R., Pennartz, C., Cabeza, R., and Daselaar, S. (2012). Explaining the encoding/retrieval flip: memory-related deactivations and activations in the posteromedial cortex. Neuropsychologia *50*.

Hyman, B.T., Van Hoesen, G.W., Damasio, A.R., and Barnes, C.L. (1984). Alzheimer's disease: cell-specific pathology isolates the hippocampal formation. Science *225*, 1168–1170.

Insausti, R. (1993). Comparative anatomy of the entorhinal cortex and hippocampus in mammals. Hippocampus *3 Spec No*, 19–26.

Ito, H., Kanno, I., Ibaraki, M., and Hatazawa, J. (2002). Effect of aging on cerebral vascular response to Paco2 changes in humans as measured by positron emission tomography. J Cereb Blood Flow Metab *22*, 997–1003.

Jack, C.J., Wiste, H.J., Vemuri, P., Weigand, S.D., Senjem, M.L., Zeng, G., Bernstein, M.A., Gunter, J.L., Pankratz, V.S., Aisen, P.S., et al. (2010). Brain beta-amyloid measures and magnetic resonance imaging atrophy both predict time-to-progression from mild cognitive impairment to Alzheimer's disease. Brain *133*, 3336–3348.

James, W. (1890). The principles of psychology.

Jenkins, L.J., and Ranganath, C. (2010). Prefrontal and medial temporal lobe activity at encoding predicts temporal context memory. J Neurosci *30*, 15558–15565.

Jenkins, L.J., and Ranganath, C. (2016). Distinct neural mechanisms for remembering when an event occurred. Hippocampus *26*, 554–559.

Jonides, J., and Nee, D.E. (2006). Brain mechanisms of proactive interference in working memory. Neuroscience *139*, 181–193.

Kannurpatti, S.S., and Biswal, B.B. (2008). Detection and scaling of task-induced fMRI-BOLD response using resting state fluctuations. Neuroimage *40*, 1567–1574.

Kannurpatti, S.S., Motes, M.A., Rypma, B., and Biswal, B.B. (2011). Increasing measurement accuracy of age-related BOLD signal change: minimizing vascular contributions by resting-state-fluctuation-of-amplitude scaling. Hum Brain Mapp *32*, 1125–1140.

Kawas, C.H. (2003). Clinical practice. Early Alzheimer's disease. N Engl J Med *349*, 1056–1063.

Kawas, C.H., Greenia, D.E., Bullain, S.S., Clark, C.M., Pontecorvo, M.J., Joshi, A.D., and Corrada, M.M. (2013). Amyloid imaging and cognitive decline in nondemented oldest-old: the 90+ Study. Alzheimers Dement *9*, 199–203.

Kesner, R.P., and Holbrook, T. (1987). Dissociation of item and order spatial memory in rats following medial prefrontal cortex lesions. Neuropsychologia *25*, 653–664.

Kesner, R.P., Gilbert, P.E., and Barua, L.A. (2002). The role of the hippocampus in memory for the temporal order of a sequence of odors. Behav Neurosci *116*, 286–290.

Kim, S.G., and Ogawa, S. (2012). Biophysical and physiological origins of blood oxygenation level-dependent fMRI signals. J Cereb Blood Flow Metab *32*, 1188–1206.

Kirwan, C.B., and Stark, C.E.L. (2007). Overcoming interference: An fMRI investigation of pattern separation in the medial temporal lobe. Learn. Mem. *14*, 625–633.

Kordower, J.H., Chu, Y., Stebbins, G.T., DeKosky, S.T., Cochran, E.J., Bennett, D., and Mufson, E.J. (2001). Loss and atrophy of layer II entorhinal cortex neurons in elderly people with mild cognitive impairment. Ann. Neurol. *49*, 202–213.

Koutstaal, W., Reddy, C., Jackson, E.M., Prince, S., Cendan, D.L., and Schacter, D.L. (2003). False recognition of abstract versus common objects in older and younger adults: testing the semantic categorization account. J Exp Psychol Learn Mem Cogn *29*, 499–510.

Kumaran, D., and Maguire, E.A. (2006). An Unexpected Sequence of Events: Mismatch Detection in the Human Hippocampus. PLoS Biol. *4*.

Kuwabara, Y., Ichiya, Y., Sasaki, M., Yoshida, T., Fukumura, T., Masuda, K., Ibayashi, S., and Fujishima, M. (1996). Cerebral blood flow and vascular response to hypercapnia in hypertensive patients with leukoaraiosis. Ann Nucl Med *10*, 293–298.

Lavenex, P., and Amaral, D.G. (2000). Hippocampal-neocortical interaction: a hierarchy of associativity. Hippocampus *10*, 420–430.

Leutgeb, J.K., Leutgeb, S., Moser, M.B., and Moser, E.I. (2007). Pattern separation in the dentate gyrus and CA3 of the hippocampus. Science *315*, 961–966.

Lisman, J.E., and Grace, A.A. (2005). The hippocampal-VTA loop: controlling the entry of information into long-term memory. Neuron *46*, 703–713.

Liu, P., Hebrank, A.C., Rodrigue, K.M., Kennedy, K.M., Section, J., Park, D.C., and Lu, H. (2013). Age-related differences in memory-encoding fMRI responses after accounting for decline in vascular reactivity. NeuroImage *78*, 415–425.

Logan, J.M., Sanders, A.L., Snyder, A.Z., Morris, J.C., and Buckner, R.L. (2002). Under-recruitment and nonselective recruitment: dissociable neural mechanisms associated with aging. Neuron *33*, 827–840.

Logothetis, N.K. (2003). The underpinnings of the BOLD functional magnetic resonance imaging signal. J Neurosci *23*, 3963–3971.

Lu, H., Zou, Q., Gu, H., Raichle, M.E., Stein, E.A., and Yang, Y. (2012). Rat brains also have a default mode network. Proc. Natl. Acad. Sci. U. S. A. *109*, 3979–3984.

Maillet, D., and Rajah, M.N. (2013). Association between prefrontal activity and volume change in prefrontal and medial temporal lobes in aging and dementia: a review. Ageing Res. Rev. *12*, 479–489.

Mangels, J.A. (1997). Strategic processing and memory for temporal order in patients with frontal lobe lesions. Neuropsychology *11*, 207–221.

Manns, J.R., and Eichenbaum, H. (2006). Evolution of declarative memory. Hippocampus *16*, 795–808.

Manns, J.R., Howard, M.W., and Eichenbaum, H. (2007). Gradual changes in hippocampal activity support remembering the order of events. Neuron *56*, 530–540.

Mark, L.P., Daniels, D.L., Naidich, T.P., and Hendrix, L.E. (1995). Limbic connections. AJNR Am J Neuroradiol *16*, 1303–1306.

Mayes, A.R., Isaac, C.L., Holdstock, J.S., Hunkin, N.M., Montaldi, D., Downes, J.J., Macdonald, C., Cezayirli, E., and Roberts, J.N. (2001). Memory for single items, word pairs, and temporal order of different kinds in a patient with selective hippocampal lesions. Cogn Neuropsychol *18*, 97–123.

Mayeux, R., Small, S.A., Tang, M., Tycko, B., and Stern, Y. (2001). Memory performance in healthy elderly without Alzheimer's disease: effects of time and apolipoprotein-E. Neurobiol Aging *22*, 683–689.

McClelland, J.L., McNaughton, B.L., and O'Reilly, R.C. (1995). Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. Psychol. Rev. *102*, 419–457.

McLaren, D.G., Ries, M.L., Xu, G., and Johnson, S.C. (2012). A generalized form of context-dependent psychophysiological interactions (gPPI): a comparison to standard approaches. NeuroImage *61*, 1277–1286.

McNaughton, B.L., and Morris, R.G. (1987). Hippocampal synaptic enhancement and information storage withing a distributed memory system. Trends Neurosci. *10*, 408–415.

Meier, T.B., Desphande, A.S., Vergun, S., Nair, V.A., Song, J., Biswal, B.B., Meyerand, M.E., Birn, R.M., and Prabhakaran, V. (2012). Support vector machine classification and characterization of age-related reorganization of functional brain networks. NeuroImage *60*, 601–613.

Miller, E.K., and Cohen, J.D. (2001). An integrative theory of prefrontal cortex function. Annu Rev Neurosci *24*, 167–202.

Milner, B., Petrides, M., and Smith, M.L. (1985). Frontal lobes and the temporal organization of memory. Hum Neurobiol *4*, 137–142.

Mishkin, M. (1978). Memory in monkeys severely impaired by combined but not by separate removal of amygdala and hippocampus. Nature *273*, 297–298.

Mishkin, M. (1982). A memory system in the monkey. Philos Trans R Soc Lond B Biol Sci *298*, 83–95.

Morcom, A.M., Good, C.D., Frackowiak, R.S., and Rugg, M.D. (2003). Age effects on the neural correlates of successful memory encoding. Brain J. Neurol. *126*, 213–229.

Morcom, A.M., Li, J., and Rugg, M.D. (2007). Age effects on the neural correlates of episodic retrieval: increased cortical recruitment with matched performance. Cereb. Cortex N. Y. N 1991 *17*, 2491–2506.

Morris, J.C. (1993). The Clinical Dementia Rating (CDR): current version and scoring rules. Neurology *44*, 2412–2414.

Morrison, J.H., and Baxter, M.G. (2012). The ageing cortical synapse: hallmarks and implications for cognitive decline. Nat. Rev. Neurosci. *13*, 240–250.

Moscovitch, M., and Winocur, G. (1995). Frontal lobes, memory, and aging. Ann. N. Y. Acad. Sci. *769*, 119–150.

Murty, V.P., Sambataro, F., Das, S., Tan, H.Y., Callicott, J.H., Goldberg, T.E., Meyer-Lindenberg, A., Weinberger, D.R., and Mattay, V.S. (2008). Age-related Alterations in Simple Declarative Memory and the Effect of Negative Stimulus Valence. J. Cogn. Neurosci.

Naveh-Benjamin, M., Shing, Y.L., Kilb, A., Werkle-Bergner, M., Lindenberger, U., and Li, S.C. (2009). Adult age differences in memory for name-face associations: The effects of intentional and incidental learning. Memory *17*, 220–232.

Naya, Y., and Suzuki, W.A. (2011). Integrating what and when across the primate medial temporal lobe. Science *333*, 773–776.

Neuneubel, J.P., and Knierim, J.J. (2014). CA3 retrieves coherent representations from degraded input: direct evidence for CA3 pattern completion and dentate gyrus pattern separation. Neuron *81*, 416–427.

Norman-Haignere, S.V., McCarthy, G., Chun, M.M., and Turk-Browne, N.B. (2012). Category-selective background connectivity in ventral visual cortex. Cereb. Cortex N. Y. N 1991 *22*, 391–402.

Nyberg, L., Sandblom, J., Jones, S., Neely, A.S., Petersson, K.M., Ingvar, M., and Backman, L. (2003). Neural correlates of training-related memory improvement in adulthood and aging. Proc Natl Acad Sci U A *100*, 13728–13733.

Oken, B.S., Zajdel, D., Kishiyama, S., Flegal, K., Dehen, C., Haas, M., Kraemer, D.F., Lawrence, J., and Leyva, J. (2006). Randomized, controlled, six-month trial of yoga in healthy seniors: effects on cognition and quality of life. Altern Ther Health Med *12*, 40–47.

Olton, D.S., Walker, J.A., and Wolf, W.A. (1982). A disconnection analysis of hippocampal function. Brain Res *233*, 241–253.

Osterrieth, P.A. (1944). Le test de copie d'une figure complexe. Arch Psych *30*, 206–356.

Passingham, R.E., and Wise, S.P. (2012). The Neurobiology of the Prefrontal Cortex: Anatomy, Evolution, and the Origin of Insight (OUP Oxford).

Pavlov, I.P. (1927). Conditioned Reflexes. (London: Routledge and Kegan Paul).

Persson, J., Nyberg, L., Lind, J., Larsson, A., Nilsson, L.-G., Ingvar, M., and Buckner, R.L. (2006). Structure-function correlates of cognitive decline in aging. Cereb. Cortex N. Y. N 1991 *16*, 907–915.

Petcharunpaisan, S., Ramalho, J., and Castillo, M. (2010). Arterial spin labeling in neuroimaging. World J. Radiol. *2*, 384–398.

Peters, A., and Sethares, C. (2002). Aging and the myelinated fibers in prefrontal cortex and corpus callosum of the monkey. J Comp Neurol *442*, 277–291.

Peters, A., Sethares, C., and Luebke, J.I. (2008). Synapses are lost during aging in the primate prefrontal cortex. Neuroscience *152*, 970–981.

Petrides, M. (1995). Impairments on nonspatial self-ordered and externally ordered working memory tasks after lesions of the mid-dorsal part of the lateral frontal cortex in the monkey. J. Neurosci. Off. J. Soc. Neurosci. *15*, 359–375.

Pierce, B.H., Sullivan, A.L., Schacter, D.L., and Budson, A.E. (2005). Comparing source-based and gist-based false recognition in aging and Alzheimer's disease. Neuropsychology *19*, 411–419.

Pinto-Hamuy, T., and Linck, P. (1965). Effect of frontal lesions on performance of sequential tasks by monkeys. Exp. Neurol. *12*, 96–107.

Power, J.D., Cohen, A.L., Nelson, S.M., Wig, G.S., Barnes, K.A., Church, J.A., Vogel, A.C., Laumann, T.O., Miezin, F.M., Schlaggar, B.L., et al. (2011). Functional network organization of the human brain. Neuron *72*, 665–678.

Power, J.D., Snyder, A.Z., Schlagger, B.L., and Petersen, S.E. (2012). Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. Neuroimage *59*, 2142–2154.

Prull, M.W., Dawes, L.L., Martin, A. 3rd, Rosenberg, H.F., and Light, L.L. (2006). Recollection and familiarity in recognition memory: adult age differences and neuropsychological test correlates. Psychol Aging *21*, 107–118.

Quirk, C.R., Allen, T.A., and Fortin, N.J. (2013). Temporary inactivations of the hippocampus and prefrontal cortex impair memory for sequences of events.

Rajah, M.N., and McIntosh, A.R. (2008). Age-related differences in brain activity during verbal recency memory. Brain Res. *1199*, 111–125.

Rajah, M.N., Languay, R., and Valiquette, L. (2010). Age-related changes in prefrontal cortex activity are associated with behavioural deficits in both temporal and spatial context memory retrieval in older adults. Cortex J. Devoted Study Nerv. Syst. Behav. *46*, 535–549.

Ranganath, C. (2010). A unified framework for the functional organization of the medial temporal lobes and the phenomenology of episodic memory. Hippocampus *20*, 1263–1290.

Ranganath, C., and Hsieh, L.-T. (2016). The hippocampus: a special place for time. Ann. N. Y. Acad. Sci.

Reitz, C., Tang, M.X., Schupf, N., Manly, J.J., Mayeux, R., and Luchsinger, J.A. (2010). A summary risk score for the prediction of Alzheimer disease in elderly persons. Arch Neurol *67*, 835–841.

Rempel-Clower, N.L., and Barbas, H. (2000). The laminar pattern of connections between prefrontal and anterior temporal cortices in the Rhesus monkey is related to cortical structure and function. Cereb Cortex *10*, 851–865.

Reuter-Lorenz, P.A., and Cappell, K.A. (2008). Neurocognitive Aging and the Compensation Hypothesis. Curr. Dir. Psychol. Sci. *17*, 177–182.

Ritchey, M., Libby, L.A., and Ranganath, C. (2015). Chapter 3 - Cortico-hippocampal systems involved in memory and cognition: the PMAT framework. In Progress in Brain Research, S.O. and M. Tsanov, ed. (Elsevier), pp. 45–64.

Roberts, J.M., Ly, M., Murray, E., and Yassa, M.A. (2014). Temporal discrimination deficits as a function of lag interference in older adults. Hippocampus *24*, 1189–1196.

Rugg, M.D., and Vilberg, K.L. (2013). Brain networks underlying episodic memory retrieval. Curr Opin Neurobiol *23*, 255–260.

Ryan, S.M., and Nolan, Y.M. (2016). Neuroinflammation negatively affects adult hippocampal neurogenesis and cognition: can exercise compensate? Neurosci. Biobehav. Rev. *61*, 121–131.

Saad, Z.S., Glen, D.R., Chen, G., Beauchamp, M.S., Desai, R., and Cox, R.W. (2009). A new method for improving functional-to-structural MRI alignment using local Pearson correlation. Neuroimage *44*, 839–848.

Salat, D.H. (2011). The Declining Infrastructure of the Aging Brain. Brain Connect. *1*, 279–293.

Salthouse, T. (2012). Consequences of age-related cognitive declines. Annu Rev Psychol *63*, 201–226.

Samanez-Larkin, G.R., and D'Esposito, M. (2008). Group comparisons: imaging the aging brain. Soc Cogn Affect Neurosci *3*, 290–297.

Samson, R.D., and Barnes, C.A. (2013). Impact of aging brain circuits on cognition. Eur. J. Neurosci. *37*, 1903–1915.

Schapiro, A.C., Kustner, L.V., and Turk-Browne, N.B. (2012). Shaping of Object Representations in the Human Medial Temporal Lobe Based on Temporal Regularities. Curr. Biol. *22*, 1622–1627.

Schmahmann, J.D., and Pandya, D.N. (2009). Fiber Pathways of the brain (New York: Oxford University Press).

Scoville, W.B., and Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. J. Neurol. Neurosurg. Psychiatry *20*, 11–21.

Shamy, J.L., Habeck, C., Hof, P.R., Amaral, D.G., Fong, S.G., Buonocore, M.H., Stern, Y., Barnes, C.A., and Rapp, P.R. (2011). Volumetric correlates of spatiotemporal working and recognition memory impairment in aged rhesus monkeys. Cereb Cortex *21*, 1559–1573.

Shimamura, A.P., Janowsky, J.S., and Squire, L.R. (1990). Memory for the temporal order of events in patients with frontal lobe lesions and amnesic patients. Neuropsychologia *28*, 803–813.

Simons, J.S., and Spiers, H.J. (2003). Prefrontal and medial temporal lobe interactions in long-term memory. Nat Rev Neurosci *4*, 637–648.

Small, S.A., Tsai, W.Y., DeLaPaz, R., Mayeux, R., and Stern, Y. (2002). Imaging hippocampal function across the human life span: is memory decline normal or not? Ann. Neurol. *51*, 290–295.

Small, S.A., Chawla, M.K., Buonocore, M., Rapp, P.R., and Barnes, C.A. (2004). Imaging correlates of brain function in monkeys and rats isolates a hippocampal subregion differentially vulnerable to aging. Proc. Natl. Acad. Sci. U. S. A. *101*, 7181–7186.

Sofi, F., Valecchi, D., Bacci, D., Abbate, R., Gensini, G.F., Casini, A., and Macchi, C. (2011). Physical activity and risk of cognitive decline: a meta-analysis of prospective studies. J Intern Med *269*, 107–117.

Sokal, R.R., and Rohlf, F.J. (1995). Biometry: the priciples and practices of statistics in biological research (New York: W.H. Freemand and Company).

Sperling, R. (2007). Functional MRI studies of associative encoding in normal aging, mild cognitive impairment, and Alzheimer's disease. Ann. N. Y. Acad. Sci. *1097*, 146–155.

Squire, L.R., Stark, C.E.L., and Clark, R.E. (2004). The medial temporal lobe. Annu. Rev. Neurosci. *27*, 279–306.

Stark, C.E.L., and Okado, Y. (2003). Making memories without trying: Medial temporal lobe activity associated with incidental memory formation during recognition. J. Neurosci. *23*, 6748–6753.

Stark, C.E.L., and Squire, L.R. (2001). When zero is not zero: The problem of ambiguous baseline conditions in fMRI. Proc. Natl. Acad. Sci. *98*, 12760–12766.

Stranahan, A.M., Haberman, R.P., and Gallagher, M. (2010). Cognitive Decline Is Associated with Reduced Reelin Expression in the Entorhinal Cortex of Aged Rats. Cereb Cortex.

Stroop, J.R. (1935). Studies of interference in serial verbal reactions. J. Exp. Psychol. *18*, 643–662.

Sullivan, E.V., Adalsteinsson, E., and Pfefferbaum, A. (2006). Selective age-related degradation of anterior callosal fiber bundles quantified in vivo with fiber tracking. Cereb Cortex *16*, 1030–1039.

Suzuki, W.A., and Amaral, D.G. (2004). Functional neuroanatomy of the medial temporal lobe memory system. Cortex *40*, 220–222.

Thomason, M.E., Foland, L.C., and Glover, G.H. (2007). Calibration of BOLD fMRI using breath holding reduces group variance during a cognitive task. Hum Brain Mapp *28*, 59–68.

Thompson, G.J., Pan, W.-J., Magnuson, M.E., Jaeger, D., and Keilholz, S.D. (2014a). Quasi-periodic patterns (QPP): large-scale dynamics in resting state fMRI that correlate with local infraslow electrical activity. NeuroImage *84*, 1018–1031.

Thompson, G.J., Pan, W.-J., Billings, J.C.W., Grooms, J.K., Shakil, S., Jaeger, D., and Keilholz, S.D. (2014b). Phase-amplitude coupling and infraslow (<1 Hz) frequencies in the rat brain: relationship to resting state fMRI. Front. Integr. Neurosci. *8*, 41.

Tizard, B. (1959). Theories of brain localization from Flourens to Lashley. Med Hist.

Tolentino, J.C., Pirogovsky, E., Luu, T., Toner, C.K., and Gilbert, P.E. (2012). The effect of interference on temporal order memory for random and fixed sequences in nondemented older adults. Learn. Mem. *19*, 251–255.

Tomita, H., Ohbayashi, M., Nakahara, K., Hasegawa, I., and Miyashita, Y. (1999). Top-down signal from prefrontal cortex in executive control of memory retrieval. Nature *401*, 699–703.

Treves, A., and Rolls, E.T. (1994). Computational analysis of the role of the hippocampus in memory. Hippocampus *4*, 374–391.

Tubridy, S., and Davachi, L. (2011). Medial temporal lobe contributions to episodic sequence encoding. Cereb. Cortex N. Y. N 1991 *21*, 272–280.

Tulving, E. (1984). Precis of Elements of episodic memory. Behav. Brain Sci. *7*, 223–268.

Tustison, N.J., Cook, P.A., Klein, A., Song, G., Das, S.R., Duda, J.T., Kandel, B.M., van Strien, N., Stone, J.R., Gee, J.C., et al. (2014). Large-scale evaluation of ANTs and FreeSurfer cortical thickness measurements. Neuroimage *99*, 166–179.

Tyas, S.L., Snowdon, D.A., Desrosiers, M.F., Riley, K.P., and Markesbery, W.R. (2007). Healthy ageing in the Nun Study: definition and neuropathologic correlates. Age Ageing *36*, 650–655.

Uttl, B., and Graf, P. (1993). Episodic spatial memory in adulthood. Psychol Aging *8*, 257–273.

Van Strien, N.M., Cappaert, N.L., and Witter, M.P. (2009). The anatomy of memory: an interactive overview of the parahippocampal-hippocampal network. Nat Rev Neurosci *10*, 272–282.

Verwer, R.W., Meijer, R.J., Van Uum, H.F., and Witter, M.P. (1997). Collateral projections from the rat hippocampal formation to the lateral and medial prefrontal cortex. Hippocampus *7*, 397–402.

Wagner, A.D., Desmond, J.E., Glover, G.H., and Gabrieli, J.D. (1998). Prefrontal cortex and recognition memory: Functional-MRI evidence for context-dependent retrieval processes.

Walhovd, K.B., Fjell, A.M., Dale, A.M., McEvoy, L.K., Brewer, J., Karow, D.S., Salmon, D.P., and Fennema-Notestine, C. (2010). Multi-modal imaging predicts memory performance in normal aging and cognitive decline. Neurobiol Aging *31*, 1107–1121.

Wang, L., Su, L., Shen, H., and Hu, D. (2012). Decoding Lifespan Changes of the Human Brain Using Resting-State Functional Connectivity MRI. PLOS ONE *7*, e44530.

Wang, T.H., Johnson, J.D., de Chastelaine, M., Donley, B.E., and Rugg, M.D. (2016). The Effects of Age on the Neural Correlates of Recollection Success, Recollection-Related Cortical Reinstatement, and Post-Retrieval Monitoring. Cereb. Cortex N. Y. N 1991 *26*, 1698–1714.

Ward, D.B. (2002). Deconvolution analysis of fMRI time series data. AFNI 3dDeconvolve Documentation. Med. Coll. Wisconson.

Wilson, I.A., Ikonen, S., Gurevicius, K., McMahan, R.W., Gallagher, M., Eichenbaum, H., and Tanila, H. (2005). Place cells of aged rats in two visually identical compartments. Neurobiol. Aging *26*, 1099–1106.

Wilson, I.A., Gallagher, M., Eichenbaum, H., and Tanila, H. (2006). Neurocognitive aging: Prior memories hinder new hippocampal encoding. Trends Neurosci. *29*, 662–670.

Wixted, J.T., and Squire, L.R. (2010). The role of the human hippocampus in familiarity-based and recollection-based recognition memory. Behav Brain Res *215*, 197–208.

Yassa, M.A., Muftuler, L.T., and Stark, C.E.L. (2010). Ultrahigh-resolution microstructural diffusion tensor imaging reveals perforant path degradation in aged humans in vivo. Proc. Natl. Acad. Sci. USA *107*, 12687–12691.

Yassa, M.A., Lacy, J.W., Stark, S.M., Albert, M.S., Gallagher, M., and Stark, C.E.L. (2011). Pattern separation deficits associated with increased hippocampal CA3 and dentate gyrus activity in nondemented older adults. Hippocampus *21*, 968–979.

Yushkevich, P.A., Amaral, R.S., Augustinack, J.C., Bender, A.R., Bernstein, J.D., Boccardi, M., Bocchetta, M., Burggren, A.C., Carr, V.A., Chakravarty, M.M., et al. (2015). Quantitative comparison of 21 protocols for labeling hippocampal subfields and parahippocampal subregions in in vivo MRI: Towards a harmonized segmentation protocol. Neuroimage.