Structural and Functional Assessment of Gray Matter and White Matter Microstructure in Late Middle-Age: New Insights on Connectivity, Memory Networks, and the Effects of Psychological Stress on the Aging Brain.

By

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CHAPTER 1
Introduction

Introduction

Aging involves substantial changes to the brain, some of which are expected, while other processes may reflect pathological change. To a large extent, knowledge on brain changes over the lifespan come from post mortem studies, but developments in brain imaging have facilitated new insight into the temporal dynamics of brain aging, as well as the factors that affect brain structure and function. This thesis comprises observational studies that document structural and functional changes in aging, as well as providing new information on the impact of psychological stress, a potentially negative modifier of brain aging.

Gray matter, comprising neuronal cell bodies shows age related atrophy and may underlie some age-related changes in cognitive function, however several studies suggest that the age-related structural deterioration of white matter (comprising myelin and axons) may play an even greater role in age-related cognitive decline due to disruption of neural networks. The first study in this thesis examined whether white matter loss precedes, and may even predict subsequent gray matter loss. Examining participants in late-middle age who received brain imaging over two time points separated by four years, this study showed substantial white matter atrophy in frontal brain regions over a four year period, a process that was largely independent of gray matter change.

Given the changes in white matter observed in healthy aging, the next study examined the extent to which white matter changes may impact function. Participants in late-middle age underwent both diffusion tensor imaging to assess white matter microstructure, as well as functional MRI to examine memory network activation, and

functional connectivity. This study provided novel insight into the role of white matter connectivity in functional connectivity, demonstrating that age-related variation in episodic memory recognition performance is related to the microstructure of the fornix, a crucial connection between the medial temporal lobe and both prefrontal and subcortical regions involved in memory. Interestingly, the magnitude of dynamic connectivity between recognition-elicited regions covaried across subjects with performance and fornix microstructure.

Several factors have been identified as impacting memory performance over the lifespan, both in the context of healthy aging, and pathological conditions such as Alzheimer's disease. The hippocampus in particular is a structure in the medial temporal lobe that is vulnerable to disease, and is known to be negatively impacted by chronic psychological stress, which triggers low-grade systemic inflammation. In this final study, the impact of a potentially modifiable risk factor for pathological aging—psychological stress—was examined to determine the effects on brain structure, specifically, the impact on hippocampus. In addition to examining main effects of stress on the hippocampus, this study examined whether psychological stress-induced hippocampal neurodegeneration may be mediated by pro-inflammatory processes. Psychological stress is an important modulator of human immune function, including processes related to inflammation. In this final study, I present evidence that psychological stress over the past five years is associated with elevated levels of soluble interleukin-6 receptor and smaller anterior hippocampal volume. In addition, high cumulative psychological stress

correlates with poor hippocampal-dependent behavior, specifically delayed verbal memory.

In summary, this body of work sheds light on temporal dynamics of brain aging, as well as demonstrating that a potentially modifiable factor may negatively impact brain regions vulnerable to age-related diseases such as Alzheimer's. Taken together these novel results contribute to a more precise understanding of the vulnerability of frontal and medial temporal lobes in aging midlife adults. These findings have strong implications for further characterizing possible diverging aging-trajectories and risk for dementia, and may provide support for identifying a therapeutic treatment window for age-related neurodegenerative diseases.

Specific Aim 1. Determine whether white matter integrity predicts subsequent gray and white matter volume loss during middle age

Although it is well established that white matter integrity, as indexed by fractional anisotropy (FA), decreases with age, the relationship between these microstructural changes and volume loss remains to be fully elucidated. Previous research suggests that microstructural FA changes may precede and predict white matter atrophy. The hypothesis of this aim is that baseline FA from pre-selected white matter tracts would predict volume loss in related white matter regions and related gray matter structures, including frontal, parietal, basal temporal, and parahippocampal regions. To test this, cognitively healthy middle age adults will undergo baseline diffusion tensor imaging (DTI) and longitudinal T1-weighted magnetic resonance imaging. Tensor Based Morphometry methods will be used to evaluate volume change over time. FA values will

be extracted from regions of interest that include the cingulum subjacent to the posterior cingulate, cingulum adjacent to the hippocampus, entorhinal white matter, and the genu and splenium of the corpus callosum. Baseline FA will be the predictor variable, while gray and white matter atrophy rates as indexed by Tensor Based Morphometry will be the dependent variables.

Specific Aim 2. Determine whether age-related changes in white matter integrity mediate task-related functional brain connectivity during episodic memory retrieval

Episodic memory, which is defined as the conscious recollection of events, shows substantial decline as a function of aging, and functional neuroimaging studies have demonstrated that differences in recruitment of the prefrontal cortex and medial temporal lobe are involved. However, knowledge about the relationship between white matter pathway integrity and task-related functional connectivity, defined as the correlation between regions recruited in an fMRI task, as mediators of age-related differences in performance is limited. The hypothesis of this specific aim is that higher integrity of white matter connections between frontal cortex and subcortical structures as well as connections between frontal and temporo-parietal cortex would be associated with stronger task-related functional connectivity and better episodic memory performance. To test this, a task-related functional connectivity analysis using as seeds the regions recruited during successful retrieval during an episodic memory task will be conducted. White matter integrity will be indexed as fractional anisotropy and will be

selected from specific tracts connecting regions implicated in episodic retrieval processing.

Specific Aim 3. Determine the effect psychological stress on hippocampal volume and peripheral inflammation.

Repeated exposure to psychological stress alters the homeostatic environment of the brain, contributing to susceptibility to immune-related diseases and elevating levels of chronic low-grade inflammation. Increased concentrations of pro-inflammatory cytokines such as interleukin-6 (IL-6) have been reported to negatively affect hippocampal structure and cognitive function. However, the relationships between psychological stress, peripheral inflammation, and the hippocampus are unknown. The hypothesis of this specific aim is that higher psychological stress is associated with increased levels of IL-6, smaller hippocampal volume, and poor hippocampal-dependent behavior. To test this, hippocampal volumes will be manually traced on structural T1 MRI images and IL-6 will be assayed from serum. Psychological stress will be measured from self-report scales that have domains such as early life stress, cumulative stress, etc. The Word List Immediate and Delayed recall will be used for the hippocampal-dependent behavior. Interaction analyses will be conducted to assess whether individuals experiencing high psychological stress will present with elevated levels of IL-6 and smaller hippocampal volumes. To investigate whether IL-6 mediates the relationship between psychological stress and hippocampal volume, a mediation analysis will be performed.

CHAPTER 2

Midlife measurements of white matter microstructure predict subsequent regional white matter atrophy in healthy adults

Midlife measurements of white matter microstructure predict subsequent regional white matter atrophy in healthy adults

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Abstract

Objectives: While age-related brain changes are becoming better understood, midlife patterns of change are still in need of characterization, and longitudinal studies are lacking. The aim of this study was to determine if baseline fractional anisotropy (FA), obtained from diffusion tensor imaging (DTI) predicts volume change over a four-year interval. **Experimental design:** Forty-four cognitively healthy middle-age adults underwent baseline DTI and longitudinal T1-weighted magnetic resonance imaging. Tensor Based Morphometry methods were used to evaluate volume change over time. FA values were extracted from regions of interest that included the cingulum, entorhinal white matter, and the genu and splenium of the corpus callosum. Baseline FA was used as a predictor variable, while gray and white matter atrophy rates as indexed by Tensor Based Morphometry were the dependent variables. **Principal observations:** Over a four-year period, participants showed significant contraction of white matter, especially in frontal, temporal, and cerebellar regions ($p<0.05$, corrected for multiple comparisons). Baseline FA in entorhinal white matter, genu, and splenium, was associated with longitudinal rates of atrophy in regions that included the superior longitudinal fasciculus, anterior corona radiata, temporal stem, and white matter of the inferior temporal gyrus ($p<0.001$, uncorrected for multiple comparisons). **Conclusions:** Brain change with aging is characterized by extensive shrinkage of white matter. Baseline white matter microstructure as indexed by DTI was associated with some of the observed regional volume loss. The findings suggest that both white matter volume loss and

microstructural alterations should be considered more prominently in models of aging and neurodegenerative diseases.

Introduction

Normal aging is accompanied by a progressive cognitive decline and neural degeneration; however, the mechanisms for these changes are not fully understood. Histological studies have established that there is a decrease in the number and dendritic extent of cortical neurons (Coleman and Flood, 1987) and shrinkage of neurons (Terry et al., 1987), with consequent cerebral atrophy. While gray matter loss is evident in aging, several studies suggest that the age-related structural deterioration of white matter (Tang et al., 1997) is central in the brain aging process (O'Sullivan et al., 2001; Pfefferbaum et al., 2005) and may be involved in disruption of neural networks underlying normal cognitive function (Grady, 2008; Greenwood, 2007). Moreover, human white matter development is thought to be heterochronic and regionally heterogeneous; specifically, axons from the prefrontal and other association areas continue to myelinate temporally longer than, for example, sensory or motor areas (Bartzokis et al., 2004; Benes, 2004). Fundamental questions still remain, however, about the temporal relationship between white and gray matter changes in normal aging. Recently, it has been proposed that white matter alterations may precede gray matter changes (Bartzokis, 2004; Bartzokis et al., 2004).

Longitudinal *in vivo* brain imaging of white and gray matter may help define the temporal relationship of brain tissue change. Diffusion tensor imaging (DTI) in particular has allowed investigators to examine white matter in a way that was previously not possible (Basser, 1995; Basser and Pierpaoli, 1996b). Derived from DTI, fractional anisotropy (FA) (Giorgio et al., 2010) is a quantitative index of the directionality of water

diffusion reflecting the integrity of the brain tissue (Basser and Pierpaoli, 1996a). Alterations in the microstructure environment, such as demyelination of axons and loss of axonal structure, reduce directional water diffusion and thus reduce FA (Englund, 1998). Several DTI studies of healthy aging have shown widespread age-related reductions in FA and elevations in diffusivity in white matter (Ardekani et al., 2007; Benedetti et al., 2006; Charlton et al., 2008; Grieve et al., 2007); however, across studies there is regional variability, suggesting that the effect of aging on white matter is still in need of clarification. Decreases in FA have been consistently reported in large cerebral white matter regions such as the centrum semiovale, corona radiata, frontal and parietal pericallosal areas, and periventricular regions, whereas less consistent findings have been detected in the splenium of the corpus callosum, parietal white matter, and limbs of the internal capsule (Hugenschmidt et al., 2008; Madden et al., 2004; Pfefferbaum et al., 2005; Salat et al., 2005a).

Although it is well established that white matter integrity as indexed by FA, decreases with age, the relationship between these microstructural changes and volume loss remains to be fully elucidated. In a review article evaluating voxel-based morphometry (VBM) and DTI studies of prefrontal white matter (Salat et al., 2005b), a positive correlation between FA and volume was observed only in participants over age 40. Whereas another study (Fjell et al., 2008) found moderately correlated regional white matter volume and FA in a similar cohort, the Salat et al. result is consistent with previous research (Bartzokis et al., 2001; Courchesne et al., 2000; Raz et al., 2004), showing that anterior white matter myelination peaks during middle age and

subsequently declines. The authors, Salat et al., suggest that FA may be a microstructural marker of volumetric measures and thus, reduced FA may reflect decreased white matter volume. In contrast, a previous study (Benedetti et al., 2006) used whole-brain histograms and found no correlation between mean diffusivity and volume, as measured through magnetization transfer MRI. However, this inconclusive result may be due to averaging whole-brain FA and volume, a method that may overlook regional variability. Using DTI and VBM, Hugenschmidt and colleagues (Hugenschmidt et al., 2008) showed that regions exhibiting decreased FA in middle age were the same areas that exhibit white matter volume loss in older age, suggesting that microstructural FA changes may precede and predict white matter atrophy, although proving temporal ordering is difficult.

Testing the extent to which microstructural alterations precede volume loss requires a within subject longitudinal approach and was a primary focus of this study. Using imaging data acquired in a sample of healthy middle-aged adults, the aim of this study was to understand if microstructural alterations, as indexed by FA, were related to gray and white matter volume change over time. Baseline white matter health was assessed in regions of interest (ROIs) within multiple white matter tracts where age-related declines in tissue integrity have been found previously. The selected white matter regions included the cingulum adjacent to hippocampus, entorhinal white matter, and the cingulum subjacent to the posterior cingulate, all association fibers observed to be susceptible to age-related deterioration in comparison to projection fibers (Stadlbauer et al., 2008). The ROIs also included the genu and splenium of the corpus

callosum, both of which have been reported to have age-related FA decreases (Bhagat and Beaulieu, 2004; Head et al., 2004; Ota et al., 2006; Pfefferbaum et al., 2005; Pfefferbaum et al., 2000; Sullivan et al., 2006). FA was chosen based on its reliable relationship with age related white matter alterations (Pfefferbaum et al., 2000; Salat et al., 2005a; Westlye et al., 2010). The relationship between FA and white matter alteration has been observed to be stronger in adults over age 40 (Salat et al., 2005a), similar in age range to the present sample. Thus, we expected that the FA signal from the pre-selected white matter tracts would predict volume loss in related white matter regions and gray matter structures, including frontal, parietal, basal temporal, and parahippocampal regions.

Methods

Participants

Forty-four cognitively healthy participants underwent two magnetic resonance imaging (MRI) sessions (baseline and follow-up) as part of previous functional MRI studies of memory and aging. All participants were from the Wisconsin Registry for Alzheimer's Prevention (WRAP) (Sager et al., 2005), which is a registry of healthy middle-aged adults who have at least one parent with late onset Alzheimer's disease (AD) or no parental family history of AD. The sample included participants with parental family history and genetic risk for AD, specifically, positive Apolipoprotein E ε4 (APOE4) status. All participants underwent a baseline MRI and a follow-up MRI approximately 4 years later. In addition to MRI, participants received a neuropsychological assessment. Demographics and cognitive performance scores are shown in Table 1.

Inclusion criteria for all subjects consisted of the following: normal cognitive function determined by neuropsychological evaluation, no current diagnosis of major psychiatric disease or other major medical conditions (e.g., diabetes, myocardial infarction, or recent history of cancer), no history of head trauma, and no contraindications for a MRI scan. Study procedures were approved by the University of Wisconsin Health Sciences Institutional Review Board and were in accordance with U.S. federal regulations. All participants provided written informed consent.

MRI acquisition

Participants were imaged on a General Electric 3.0 Tesla SIGNA (Waukesha, WI) MRI system with a quadrature birdcage head coil at baseline and after four years. At baseline, cardiac-gated diffusion-weighted echo planar magnetic resonance images were acquired using twelve optimum non-collinear encoding directions (obtained by minimum energy numerical optimization) with a diffusion weighting of 1114 s/mm2 and a non-DWT2-weighted reference image. The effective TR was 10–13 heartbeats (~10–15 s) dependent upon the subject's heart rate. Other imaging parameters were TE=78.2 ms, 3 averages (NEX: magnitude averaging), and an image acquisition matrix of 120×120 over a field of view of 240×240mm2. Three averages were acquired and the cerebrum was covered using 39 contiguous 3-mm thick axial slices. The acquired voxel size of 2 x 2 x 3 mm was interpolated to 0.9375 mm isotropic dimensions (256 x256 in plane image matrix). The total acquisition time was between 6.5 and 8 min dependent upon the heart rate. High order shimming was performed prior to the DTI acquisition to optimize the homogeneity of the magnetic field across the brain and to minimize EPI distortions.

3D T1-weighted volumes were obtained at baseline and follow-up using an inversion recovery prepared fast gradient echo pulse sequence. The whole brain was imaged in the axial plane with the following parameters: TI = 600 ms; TR = 9 ms; TE = 1.8 ms; NEX = 1; flip angle = 20°; acquisition matrix = 256 × 192 × 124, interpolated to 256 × 256 × 124; FOV = 240 mm; slice thickness = 1.2 mm (124 slices), receiver bandwidth = ± 16 kHz; acquisition time ~ 7.5 min.

Diffusion Tensor Imaging (DTI) and Tract-Based Spatial Statistics (TBSS) preprocessing

Diffusion-weighted DICOM images acquired at baseline were converted into NIFTI format using AFNI (http://afni.nimh.nih.gov/). FA maps were generated via the FMRIB Software Library (FSL) (http://www.fmrib.ox.ac.uk/fsl/fdt/index.html (Behrens et al., 2003) using the following procedures: (1) image distortions in the DTI data caused by eddy currents were corrected; (2) estimation of diffusion tensors was achieved using DTIFIT; (3) three-dimensional maps of FA images were computed from the tensors from step (2). The FA maps were then aligned using registration methods based on the Tract-Based Spatial Statistics (TBSS: http://www.fmrib.ox.ac.uk/fsl/tbss/index.html) processing scheme. TBSS methods were employed because the method is known to provide accurate registration of FA maps, the method allowed us to confidently position ROIs for extraction of FA values, and this method of registration reduces the inclusion of CSF voxels in the final extracted FA estimates. TBSS performs alignment of all FA data by projecting the original FA maps onto a mean FA skeleton. The main steps of the procedure we employed were as follows: a) FA images were eroded slightly and the end slices were zeroed to remove outliers from the diffusion tensor fitting; b) A non linear registration was estimated to align the FA images to a 1x1x1mm standard space. The target image was affine transformed to Montreal Neurological Institute (MNI) space and each subject's FA image had its nonlinear transform to the target and an affine transform to MNI space applied, resulting in a transformation of the original FA image into MNI space. c) The mean of all FA images was created and the image was

skeletonized. d) The mean FA skeleton was then thresholded to produce a binary skeleton mask that defined the set of voxels used in all subsequent processing. e) A "distance map" was then created from the skeleton mask. This was used in the projection of the subjects' FA maps onto the skeleton. f) All of the subjects' aligned FA data were projected onto the mean FA skeleton using warping methods that are based on free-form deformations and B-Splines (Rueckert et al., 1999). The process is achieved by filling the skeleton with FA values from the nearest relevant tract center. This was performed for each skeleton voxel, by searching perpendicular to the local skeleton structure for the maximum value in the subject's FA image. g) After projection onto the mean FA skeleton, the skeletonized data in standard space were used for the ROI analyses.

Regions of Interest

Each ROI was drawn on a common space skeleton mask in FSLview (http://www.fmrib.ox.ac.uk/fsl/fslview/index.html) and was then applied to the normalized individual maps. ROIs drawn on the template were individually checked to ensure correct placement on the single-subject normalized FA maps. Individual FA values for each ROI were extracted by acquiring the mean value across the tract labels of interest. ROIs were drawn bilaterally and included the cingulum subjacent to the posterior cingulate, cingulum adjacent to hippocampus, entorhinal white matter, and the genu and splenium of the corpus callosum (Figure 1). Corticospinal tract was included

as a control ROI based on literature suggesting these tracts, along with primary sensory and motor cortices are preserved during aging relative to association cortices.

Tensor-Based Morphometry

In order to produce estimates of volume change from baseline to follow-up, we employed Tensor-Based Morphometry (TBM) methods implemented in the SPM5 software package (http://www.fil.ion.ucl.ac.uk/spm/software/spm5/). First, bias correction with eight iterations, a FWHM of Gaussian smoothing set at a 60mm cutoff, and a medium level of regularization was applied to both the baseline and follow-up scans to correct for intensity non-uniformity. TBM procedures followed those described by Kipps and colleagues (Kipps et al., 2005). Briefly, a high-dimensional deformation field was used to warp the corrected late image to match the baseline scan within subject (Ashburner and Friston, 2000). The amount of volume change was indexed by the determinant of the gradient of deformation at a single-voxel level (Jacobian determinants). The Jacobian image represented a measure of the brain specific volume change between the first and the second scan. The maps were converted to annual rate of change maps using the formula: Annual Rate = ((Jacobian determinant)^(1/Interscan duration)-1), where “Interscan duration” was the number of years between baseline and follow-up scans. In order to warp the final TBM maps to template space, normalization parameters were estimated by matching the brain-volume images from the baseline scan with the MNI brain-volume template, these were then applied to the Jacobian

image (Ashburner and Friston, 1999). Finally, the normalized TBM maps were smoothed using an 8 mm isotropic Gaussian kernel.

Statistical analyses

In order to test the extent to which participants showed tissue change over a four year period, tissue maps were thresholded to either 1) values above zero to reflect tissue atrophy, or 2) values below zero to reflect contraction. One-way t-tests were used to determine significant regions of change. In order to test the extent to which FA obtained at baseline predicted volume change, individual FA estimates from each ROI were entered into a multiple regression analysis, where the independent predictor variable was baseline FA, and the dependent variable (volume change) was the participant's TBM map thresholded above zero (reflecting tissue atrophy). For all models, the covariates were baseline age and gender. Results were considered significant at $p < 0.001$ (uncorrected).

Results

Participants with parental family history of AD or positive APOE4 status did not differ with respect to demographic characteristics or neuropsychological test performance compared to those with no AD risk factors.

There was a significant change in volume over four years. As detailed in Table 2 and shown in Figure 2, significant volume contraction was observed predominantly in frontal, temporal, and cerebellar regions. These results survived family wise error correction (FWE) $p < 0.05$. In contrast, a voxel-wise analysis revealed no areas of significant expansion ($p < 0.001$, uncorrected).

With regard to FA, there was no significant effect of AD risk factors. The mean FA values extracted from bilateral ROIs were as follows: .70 (± .06) from the corticospinal tract, 0.71 (± .03) from the genu, 0.54 (± .06) from the entorhinal white matter, 0.53 (± .03) from the cingulum subjacent to the posterior cingulate bundle, 0.51 (± .04) from the cingulum adjacent to hippocampus, and 0.87 (± 0.03) from the splenium. No significant differences were found between the left and right hemisphere per each of the investigated ROIs and thus left and right were averaged for the remaining analyses.

As detailed in Table 3, baseline FA in entorhinal white matter, and genu and splenium of the corpus callosum predicted atrophy ($p < 0.001$, uncorrected) as indexed by the TBM maps (Figure 3). With the exception of the right cerebellar hemisphere volume loss predicted by splenium FA, the majority of tissue contraction was primarily in white matter regions, specifically within the superior longitudinal fasciculus, anterior

corona radiata, and the temporal stem, all regions predicted by baseline entorhinal FA. In addition, atrophy in the white matter of the inferior temporal gyrus was predicted by baseline genu FA. Baseline FA in the corticospinal tract, cingulum adjacent to hippocampus, and cingulum subjacent to the posterior cingulate did not predict any regions of volume loss.

Discussion

The results of this study indicate that over a four year period, middle-aged adults show significant shrinkage of white matter. Further, midlife measures of FA—a putative marker of white matter integrity—predict longitudinal rates of white matter atrophy. While T1-weighted imaging in this study and other studies appears to be sensitive to gross volume loss, techniques such as DTI are sensitive to microstructural alterations, and the results suggest that they may in fact be predictive of subsequent volume loss.

One of the largest areas of atrophy was observed in the white matter of the inferior temporal gyrus, as predicted by genu FA. Additionally, entorhinal FA was associated with atrophy in temporal stem. This is in accord with prior findings, with both volume (Raz et al., 2004) and anisotropy (Head et al., 2004; Salat et al., 2005a) in the temporal lobes showing moderate decline with age. This decline is second to the frontal cortices and is followed by smaller decreases in the parietal and occipital lobes, suggesting an anterior to posterior gradient. A volumetric cross-sectional study in men showed a quadratic relationship between age and white matter volume in the temporal lobes, with white matter volume increasing to the age of 47 years and declining subsequently (Bartzokis et al., 2001). The mean age of the current cohort was 56 years at baseline, suggesting that the majority of the cohort had crossed over the peak of myelination in this brain region, and was on a downward trajectory over the subsequent four years. The non-linear nature of white matter development over the life-span contrasts with the linear decline of gray matter volume throughout most of adulthood, and this is important to take into consideration in studies of midlife white matter change.

Another significant region of atrophy, predicted by splenium FA, was located in the right cerebellar hemisphere, which is consistent with several findings of age-related decreases in total cerebellar volume, cerebellar white matter, and other cerebellar structures (Jernigan et al., 2001; Liu et al., 2003; Luft et al., 1999; Raz et al., 2001; Sullivan et al., 2000; Walhovd et al., 2005). Moreover, longitudinally, the cerebellum shows pronounced longitudinal shrinkage with advancing age (Raz et al., 2010; Raz et al., 2005), possibly beginning to decline during the fifth decade of life, reflecting an exponential fit (Luft, 1999).

We observed a region of volume loss in the superior longitudinal fasciculus (SLF), predicted by baseline entorhinal FA. This tract is a heavily myelinated white matter bundle that connects the anterior and posterior regions of the cerebrum, sending projections to the temporal lobes (Wakana et al., 2004). Findings of longitudinal changes in the SLF converge with a longitudinal DTI study showing reduced FA in the SLF in the healthy elderly subjects (Teipel et al.), as well as in older individuals with mild cognitive impairment (Cho et al., 2008). Age-related decreases in FA of the SLF has been shown to be associated with poorer performance in a number of cognitive tasks involved in set-shifting (Perry et al., 2009), episodic memory (Lockhart et al.), executive function (Sasson et al.), and word finding (Stamatakis et al.). In addition, late-life depressed individuals exhibited greater white matter hyperintensity burden in this region (Sheline et al., 2008). Although atrophy reflected by the TBM maps was not correlated with neuropsychological performance (likely due to limited variability in this cognitively healthy sample), this volume loss may predict cognitive changes as the cohort ages,

and will be tested at future follow-ups. In addition to the SLF, entorhinal FA was associated with atrophy in anterior corona radiata. These results replicate findings in a multimodal imaging study in younger individuals where significant quadratic relationships between white matter volume and age were observed in the superior corona radiata bilaterally and in the left SLF (Giorgio et al., 2010). Overall, baseline entorhinal FA was associated with the majority of regions of longitudinal atrophy observed in this study. While specific pathways in humans have not been well-characterized, work in non-human primates suggest that the entorhinal region is widely connected with association cortices (Insausti et al., 1987), linking the hippocampus to the association areas of the frontal, parietal, temporal, and occipital lobes (Van Hoesen and Pandya, 1975).

Interestingly, baseline corticospinal FA, the control ROI, did not predict any areas of atrophy. The results are in accord with observations that primary motor and sensory cortices are relatively spared during aging. A recent study (Jang, 2011) has shown corticospinal tract FA decreases with age, whereby participants who are 50 and older show lower FA compared to participants in the third decade of life. The results of our study suggest that changes in microstructural parameters of the corticospinal tract may occur in the absence of volume loss and may not predict downstream volume change, at least during a 4-year interval in middle-aged years. Moreover, baseline FA within the cingulum adjacent to hippocampus and cingulum subjacent to the posterior cingulate did not predict any volume loss. These negative findings may suggest that the microstructural integrity of the cingulum does not decline as rapidly during middle age.

The largest areas of atrophy were primarily in frontal and temporal white matter, findings which complement a cross-sectional study from Hugenschmidt, et al. 2008, where FA had significant relationships with several areas of white matter volume loss, including temporal and parietal regions of the corona radiata, the length of the corpus callosum, and centrum semiovale (Hugenschmidt et al., 2008). In addition, our results are consistent with the convergence of research showing cerebral white matter to have an anterior-posterior gradient of decline (Buckner, 2004; Head et al., 2004; Raz, 2000). This decline in prefrontal white matter follows an inverted-U trajectory, with a linear increase in young adulthood, a plateau in middle age and significant contraction starting in the fifth decade of life (Bartzokis et al., 2001; Courchesne et al., 2000; Raz et al., 2004), the average age of our participants. This rate of decline increases with age (Raz et al., 2005), which is in line with other age-related acceleration in other indices of white matter integrity, such as MRI relaxation times (Bartzokis, 2004; Bartzokis et al., 2003) and ratio of small to large myelinated axons (Tang et al., 1997). These studies suggest that certain brain regions that are late to mature and which contain a high ratio of thinly myelinated fibers (e.g. prefrontal cortex) may be more susceptible to age-related atrophy.

Several of the white matter changes found in aging are likely to affect measurements of water diffusion anisotropy. Histopathological studies have shown that aging is associated with white matter deterioration that include myelin pallor (Kemper, 1994), loss of myelinated fibers (Marner et al., 2003; Meier-Ruge et al., 1992; Pakkenberg and Gundersen, 1997), and in non-human primates, malformation of myelin

sheaths (Peters and Sethares, 2002). Further, non-human primate studies also show that age is associated with decreases in synapses, dendritic spines, and myelin sheath degradation in the upper layers of neocortex (Peters, 2002a; Peters, 2002b). These histological studies reveal localized splitting of myelin lamellae causing spherical cytoplasmic cavities or 'balloons' within the myelin sheath, and continued myelin production constructing double myelin sheaths, where fluid may build up between layers. Furthermore, as suggested by Bartzokis et al (Bartzokis, 2004) this later myelination is more vulnerable, and age-related declines in membrane cholesterol—a hydrophobic molecule—make myelin more water permissive. All of these changes are candidates for influencing measurements of diffusion anisotropy, in addition to being candidates for predicting later volume loss. Additional histopathological studies will be needed to determine how closely microstructural changes link to overt volume loss; however, only brain imaging studies—while limited in their ability to directly measure pathology—are currently the sole approach to mapping out *in vivo* changes longitudinally.

Our findings must be interpreted in light of several limitations. Since our sample incorporates individuals with varying risk for Alzheimer's disease, we cannot rule out the possibility that our findings are Alzheimer's risk specific. However, participants carrying risk factors for Alzheimer's did not differ with regards to demographics, neuropsychological scores, or baseline FA measures. While our results showing white matter contraction over fours years survived correction for multiple comparisons, our models showing the predictive value of FA are reported at an uncorrected threshold and

thus, we can not rule out the possibility of Type 1 error. Despite this, the exploratory analyses converge on white matter volume loss and thus are less likely to be due to chance. We should also note that while we used DTI measures as the predictor variables in our study design, we can not definitively conclude that microstructural alterations precede volume loss. The temporal ordering of microstructural and volumetric changes over the lifespan is still in need of further characterization. Finally, although other DTI indices, such as axial or radial diffusion, do inform about axonal morphology and myelin characteristics, respectively, we decided to only use FA due to its consistency in the normal aging literature and reflection of several factors, such as changes in axon density, myelination, axonal membrane integrity, fiber orientation, and other alterations.

To our knowledge, this is the first study demonstrating that white matter alterations collected at baseline are associated with future longitudinal white matter volume loss in cognitively normal adults. Following these individuals as they enter the "golden years" will help in further fleshing out the time course of structural brain changes and also determine whether any of the individual variability in atrophy in middle age is due to preclinical diagnosis of age-related neurodegenerative disease. Although longitudinal studies on age-related neurodegenerative diseases including AD are still needed to evaluate patterns of degeneration in pathological processes, this study suggests that DTI may be useful for characterizing the distribution and time course of alterations that occur in the brain with normative aging, improving models of disease

progression, and will likely be important for early diagnosis and for monitoring the efficacy of treatments.

Acknowledgements

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Table Legends

Table 1. Demographic features and cognitive performance.

All neuropsychological scores reported above are raw scores. AD: Alzheimer's Disease; APOE4: Apolipoprotein E ε4; BNT: Boston Naming Test (Kaplan et al., 2001); BVMT-R: Brief Visuospatial Memory Test-Revised (Benedict, 1997); Digit Span (from WAIS-III) (Wechsler, 1987); MMSE: Mini Mental State Examination (Folstein et al., 1975); RAVLT: Rey Auditory Verbal Learning Test (Rey, 1964); TMTA/B: Trail Making Test A and B (Reitan RM, 1993); WRAT-III: Wide Range Achievement Test-III reading subtest (Jastak, 1993).

Table 2. Regions of tissue contraction over four years ($p < 0.05$, FWE corrected).

L = Left, R = Right, WM = white matter. Cluster size is expressed in number of voxels.

Table 3. Regions of tissue contraction ($p < 0.001$, uncorrected) predicted by baseline FA.

L = Left, R = Right, WM = white matter. Cluster size is expressed in number of voxels.

Table 1. Demographic features and cognitive performance.

Total N	44
Female: N (%)	27 (61%)
Parental family history AD: N (%)	25 (56%)
APOE4 positive: N (%)	20 (45%)
Baseline Age: years, SD (range)	56.3 ± 6.9 (42-75)
Baseline Education: years, SD (range)	15.9 ± 2.4 (12-20)
Time from baseline scan to follow-up: years, SD (range)	3.48 ± 0.88 (2.17-4.92)
MMSE: Mean, SD	29.6 ± .66
WRAT-III reading: Mean, SD	52.2 ± 3.5
BVMT-R total: Mean, SD	24.9 ± 7.1
BVMT-R delayed recall: Mean, SD	9.7 ± 2.0
RAVLT delayed recall: Mean, SD	10.7 ± 2.9
Digit Span: Mean, SD	18.0 ± 3.4
TMT A: Mean, SD	28.4 ± 8.5
TMT B: Mean, SD	58.7 ± 20.9
BNT: Mean, SD	56.0 ± 7.8

All neuropsychological scores reported above are raw scores. AD: Alzheimer's disease; APOE4: Apolipoprotein E, ε4; BNT: Boston Naming Test (Kaplan, et al. 2001); BVMT-R: Brief Visuospatial Memory Test-Revised (Benedict 1997); Digit Span (from WAIS-III) (Wechsler 1987); MMSE: Mini Mental State Examination (Folstein, et al. 1975); RAVLT: Rey Auditory Verbal Learning Test (Rey 1964); TMTA/B: Trail Making Test A and B (Reitan RM 1993); WRAT-III: Wide Range Achievement Test-III reading subtest (Jastak 1993).

Table 2. Regions of tissue contraction over four years (p <0.05, FWE corrected).

Location	Cluster size	MNI coordinates of peak voxel			Peak-level T-statistic
		x	y	z	
R Medial Orbital Gyrus	12841	16	32	-28	11.10
R Cingulate Gyrus	1263	6	-22	48	9.22
R Superior Frontal Gyrus WM	498	8	-4	66	8.56
L Posterior Orbital Gyrus WM	502	-32	38	-20	8.37
L Posterior Corona Radiata	492	-18	-48	30	8.20
R Cerebellar Hemisphere	318	20	-26	-22	8.06
L Superior Frontal Gyrus WM	346	-10	4	62	7.84
CSF space ventral to L Inferior Temporal Gyrus	299	-50	-30	-32	7.81
R Cuneus	99	4	-78	40	7.11
R Precentral Gyrus	69	6	-30	72	7.01
CSF space ventral to R Fusiform Gyrus	35	18	4	-46	6.77
L Fusiform Gyrus WM	55	-32	-42	-16	6.77
L Lyngual Gyrus WM	179	-16	-68	-8	6.58
L Cerebellar Hemisphere	94	-26	-72	-26	6.45
R Precentral Gyrus WM	32	14	-18	64	6.38
R Middle Temporal Gyrus	67	62	-48	-4	6.19
R Fusiform Gyrus	21	60	-60	-20	6.17

R Supramarginal Gyrus	76	62	-40	38	6.16
CSF space anterior to L Cerebellar Hemisphere	58	-20	-30	-46	6.16
R Middle Frontal Gyrus	71	46	38	24	6.07
R Middle Frontal Gyrus	34	28	62	20	5.93

L = Left, R = Right, WM= white matter. Cluster size is expressed in number of voxels.

Table 3. Regions of tissue contraction ($p < 0.001$, uncorrected) predicted by baseline FA.

Baseline ROI where FA was extracted	Cluster size	Location	MNI coordinates of peak voxel			Peak-level T-statistic	r^2
			x	y	z		
Entorhinal	36	R Anterior Corona Radiata	28	18	12	4.0	0.25
Entorhinal	22	R Temporal Stem	32	4	-14	3.9	0.21
Entorhinal	27	L Superior Longitudinal Fasciculus	-30	-46	30	3.66	0.21
Genu	91	R Inferior Temporal Gyrus WM	36	4	-28	4.59	0.33
Splenium	42	R Cerebellar Hemisphere	16	-72	-36	4.6	0.25

L = Left, R = Right, WM= white matter. Cluster size is expressed in number of voxels.

Figure Legends

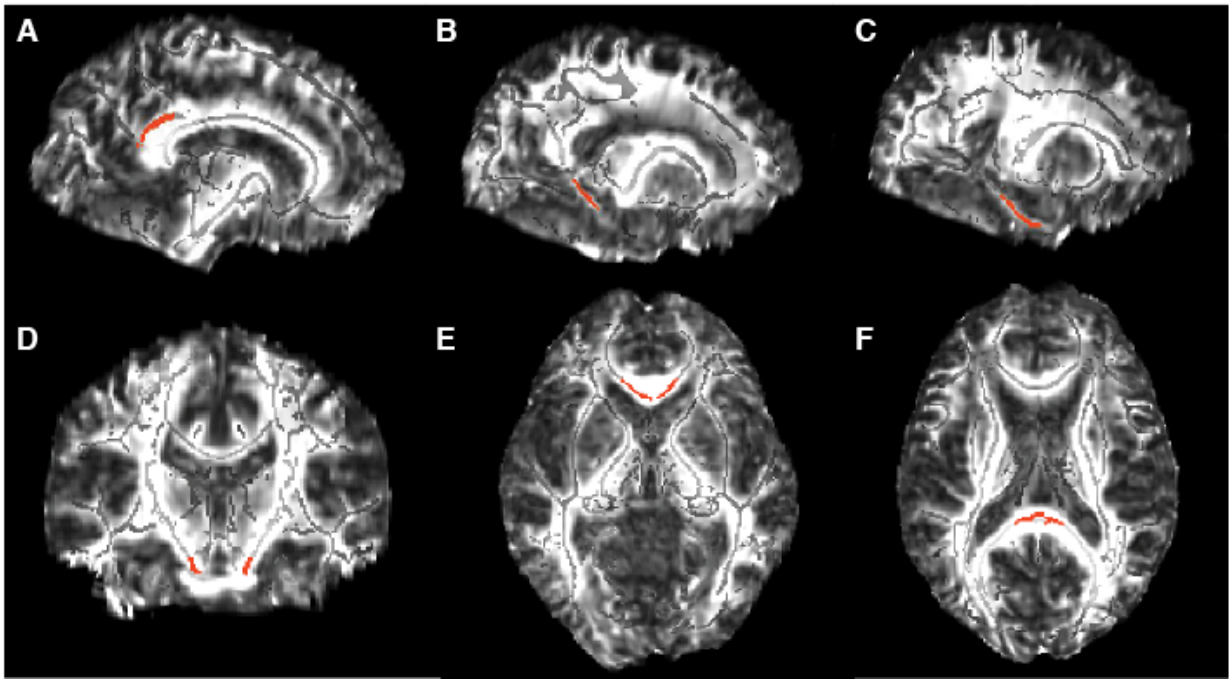

Figure 1. White matter ROIs (shown in red) overlayed on the FA template image (skeletonized FA underlayed in light gray). The bilateral ROIs included: A. cingulum bundle subjacent to posterior cingulate (119 voxels, MNI coordinates ±11 -45 28), B. cingulum adjacent to hippocampus (60 voxels, MNI coordinates ± 20 -42 -2), C. entorhinal white matter (96 voxels, MNI coordinates ±24 -26 -19), D. corticospinal tract (31 voxels, MNI coordinates ±10 -20 -24), E. splenium (49 voxels, MNI coordinates ±1 -35 14) and F. genu (48 voxels, MNI coordinates ±4 23 -1) of the corpus callosum. A-C are shown in sagittal view, D is shown in coronal view, and E-F are shown in an axial view.

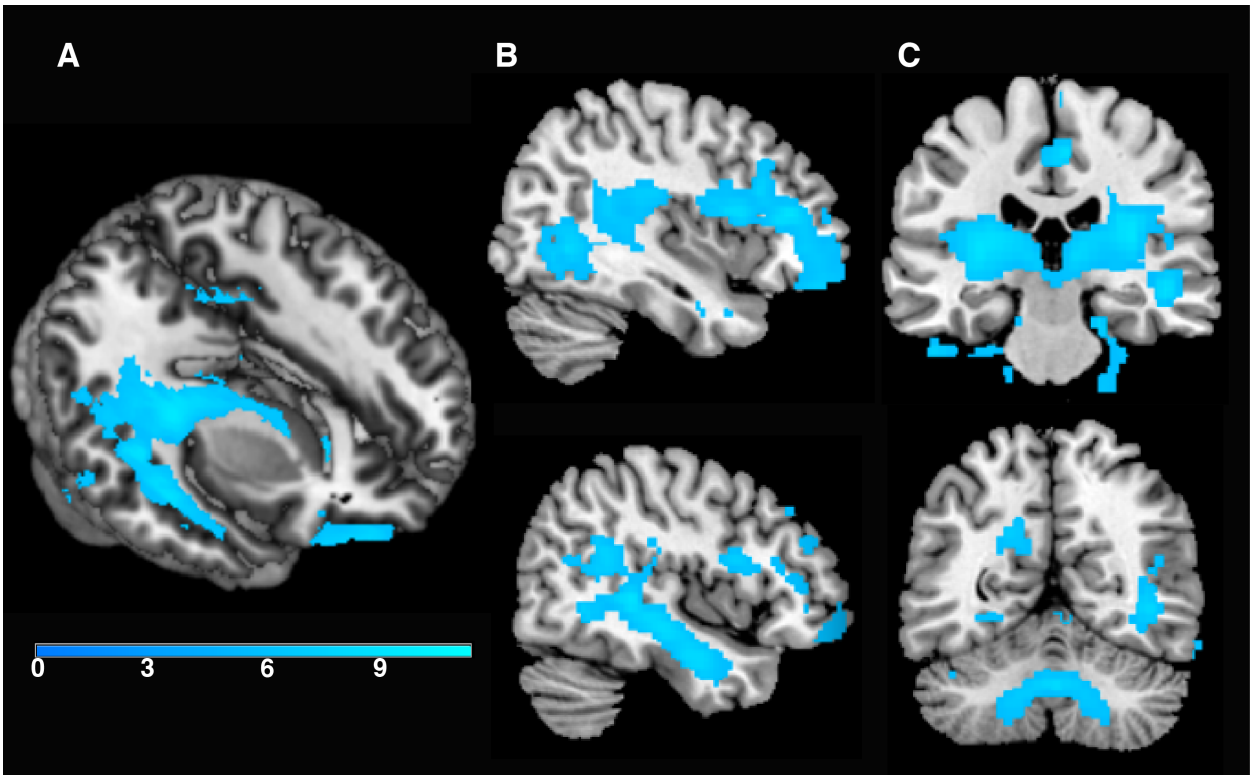

Figure 2. Regions of tissue contraction over four years ($p < 0.05$, FWE corrected). As shown in the 3D render (A) and sagittal cross-section (B) there was significant contraction in temporal stem white matter over four years. Additionally, as shown in the coronal sections (C) there was significant contraction in large portions of bilateral sub-cortical white matter and the cerebellum. The color bar represents T values.

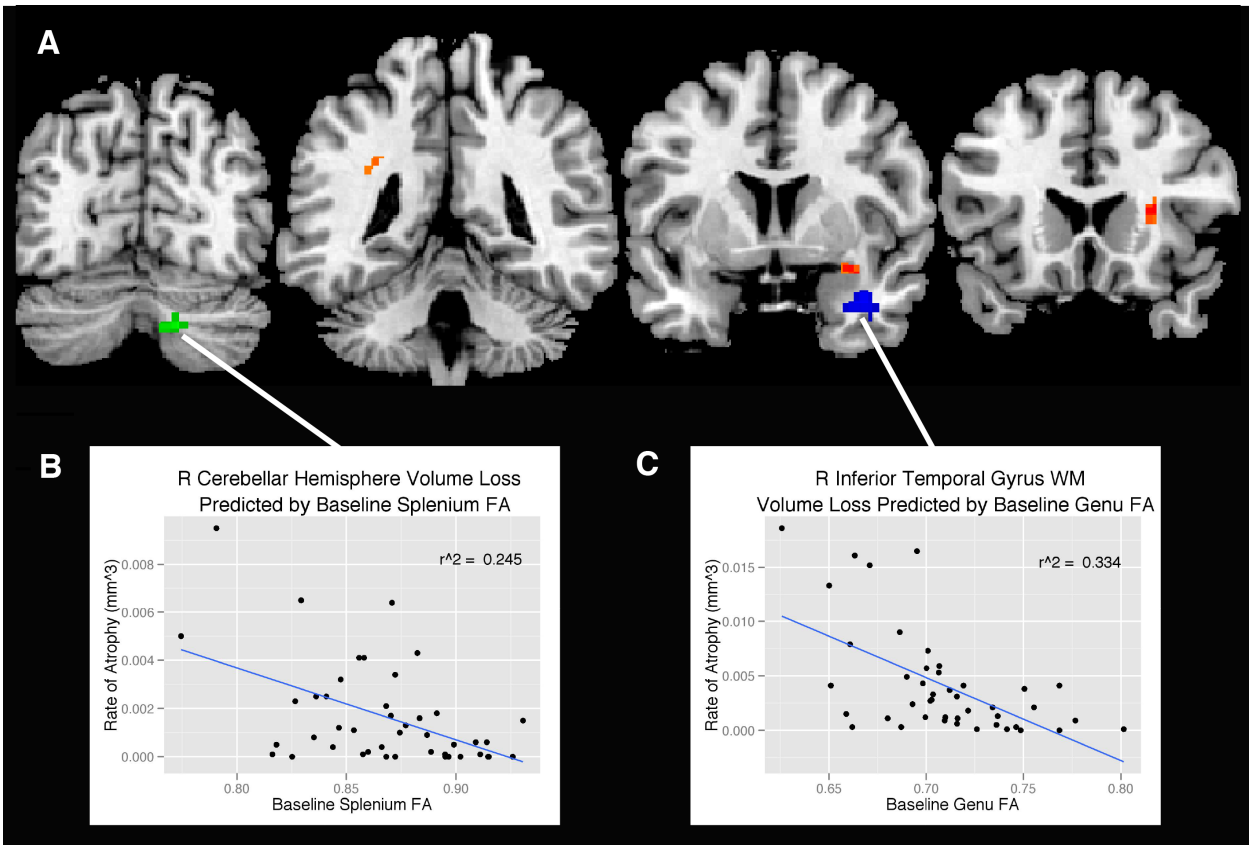

Figure 3. Regions (A) where baseline FA from the splenium (green), entorhinal white matter (orange), and genu (blue) predict volume loss from baseline to follow up ($p < 0.001$, uncorrected). The statistical map is overlaid on coronal sections of the "CH2" template available in MRIcron (Rorden, 2007). The correlation between (B) baseline splenium FA and cerebellar hemisphere volume loss was $r^2 = 0.25$, $p < 0.001$ and (C) baseline genu FA and inferior temporal gyrus WM volume loss was $r^2 = 0.33$, $p < 0.001$. Data points represent individual participants.

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CHAPTER 3

Fornix microstructure and memory performance is associated with altered neural connectivity during episodic recognition

Fornix microstructure and memory performance is associated with altered neural connectivity during episodic recognition

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ABSTRACT

Objective: The purpose of this study was to assess whether age-related differences in white matter microstructure are associated with altered task-related connectivity during episodic recognition. **Method:** Using functional magnetic resonance imaging and diffusion tensor imaging from 282 cognitively healthy middle-to-late aged adults enrolled in the Wisconsin Registry for Alzheimer's Prevention, we investigated whether fractional anisotropy (FA) within white matter regions known to decline with age was associated with task-related connectivity within the recognition network. **Results:** There was a positive relationship between fornix FA and memory performance, both of which negatively correlated with age. Psychophysiological interaction analyses revealed that higher fornix FA was associated with increased task-related connectivity amongst the hippocampus, caudate, precuneus, middle occipital gyrus, and middle frontal gyrus. In addition, better task performance was associated with increased task-related connectivity between the posterior cingulate gyrus, middle frontal gyrus, cuneus, and hippocampus. **Conclusions:** The findings indicate that age has a negative effect on white matter microstructure, which in turn has a negative impact on memory performance. However, fornix microstructure did not significantly mediate the effect of age on performance. Interestingly, dynamic functional connectivity was associated with better memory performance. The results of the psychophysiological interaction analysis further revealed that alterations in fornix microstructure explain–at least in part– connectivity among cortical regions in the recognition memory network. Our results may

further elucidate the relationship between structural connectivity, neural function, and cognition.

Keywords: Aging; Task-related functional connectivity; fMRI; gPPI; Fractional anisotropy; Episodic recognition

INTRODUCTION

Advancing age is associated with decline in multiple cognitive domains, including episodic memory (Tulving, 1984), particularly the encoding of recent events (Grady, McIntosh, & Craik, 2003; Morcom, Good, Frackowiak, & Rugg, 2003). Recognition memory, the ability to recognize previously encountered events, objects, or people, has also been shown to display an age-related decline (Duverne, Motamedinia, & Rugg, 2009; Grady et al., 2005). Recent studies have identified a network of cortical regions – each interconnected with the medial temporal lobe – that are consistently engaged during successful episodic retrieval (Rugg & Vilberg, 2013). Older age has been associated with alterations to this network, including greater frontal recruitment in conjunction with decreased occipital and parietal activation during episodic memory performance, (Anderson et al., 2000; Cabeza, Anderson, Houle, Mangels, & Nyberg, 2000; Cabeza, 2004; Davis, Dennis, Daselaar, Fleck, & Cabeza, 2008). Other studies have found age-related decreases in prefrontal cortex activity, resulting in a more bilateral prefrontal activation pattern in older adults (Anderson et al., 2000; Cabeza et al., 1997, 2000; Schacter, Savage, Alpert, Rauch, & Albert, 1996; Schiavetto, Köhler, Grady, Winocur, & Moscovitch, 2002). Likewise, functional connectivity—the temporal correlation of neural activity between spatially remote regions—also shows age-related changes during episodic memory, including decreased functional coupling between the hippocampus and the retrosplenial and parietotemporal cortices (Daselaar, Fleck, Dobbins, Madden, & Cabeza, 2006).

A postulated mechanism underlying age-associated changes in episodic memory is structural alterations in cerebral white matter, with subsequent changes in functional brain activity, particularly coordinated activity among distal regions. Older age has long been associated with structural deterioration of white matter (Tang et al., 1997), a process which in turn is likely associated with disruption of neural networks underlying normal cognitive function, including episodic memory function (Grady, 2008; Greenwood, 2007; O'Sullivan et al., 2001; Pfefferbaum, Adalsteinsson, & Sullivan, 2005). Age-related reductions in white matter fractional anisotropy (FA) have been found to correlate with memory decline (Charlton, Schiavone, Barrick, Morris, & Markus, 2010; Gunning-Dixon & Raz, 2000; O'Sullivan et al., 2001; Persson et al., 2006) and recent diffusion MRI tractography studies (Lockhart et al., 2012; Metzler-Baddeley, Jones, Belaroussi, Aggleton, & O'Sullivan, 2011) have found age-related decline in recall to be associated with microstructural degradation in several white matter tracts including the fornix, which connects the hippocampal formation to the prefrontal cortex (Poletti and Creswell, 1977), the uncinate fasciculus, connecting the anterior temporal lobe with the orbital and medial prefrontal cortex (Crosby, 1962; Schmahmann & Pandya, 2007), and the inferior longitudinal fasciculus, a major fiber bundle linking the anterior temporal lobe to the occipital lobe (Catani, Howard, Pajevic, & Jones, 2002; Schmahmann & Pandya, 2007). Changes to myelin and axonal fibers in aging may cause less stable flow of electrical currents in dendrites and axons, resulting in disruptions in conduction of action potentials and degradation of coordinated activity across the brain.

The importance of white matter microstructure for cognitive function is rooted in the spatial distribution of cognitive processes in the brain, involving a complex interplay between multiple areas (Gläscher et al., 2010). Thus, declines in white matter microstructure are associated with disrupted network functional connectivity between brain regions interconnected with compromised structural fibers (Antonenko et al., 2012, Davis et al., 2012, Steffener et al., 2012). In contrast, greater integrity of white matter fibers is associated with stronger functional connectivity (Davis et al., 2012). However, no studies have yet investigated whether the relationship between white matter microstructure, behavior, and task-related functional connectivity explains episodic recognition performance and whether these relationships are modulated by a common influence of age.

In this study, we examine whether intact tract connectivity between cortical regions within the recognition network would be associated with better memory performance and greater task related functional connectivity among cortical regions in the episodic recognition network. Combining task-related connectivity analyses with measures of white matter microstructure may provide greater insight into the mechanisms underlying age-related differences in memory function.

METHODS

Participants

Two hundred and ninety two healthy, right-handed adults aged 44 - 75 years (mean ± SD = 61.15 ± 6.71 years) participated in one MRI session (fMRI and DTI) as part of brain imaging studies on memory and aging. All participants were from the Wisconsin Registry for Alzheimer's Prevention (WRAP) (Sager, Hermann, & La Rue, 2005), which is a registry of healthy middle-aged adults who either have no parental family history of Alzheimer's disease (AD) or at least one parent with late onset AD. Positive parental family history of AD classification was defined as having one or both parents with AD as determined by a validated interview (Kawas, Segal, Stewart, Corrada, & Thal, 1994) or autopsy-confirmed or probable AD as outlined by research criteria (McKhann et al., 1984, 2011), and reviewed by a multidisciplinary diagnostic consensus panel. Absence of family history of AD required that the participant's father survive to at least age 70 years and the mother to age 75 years without diagnosis of dementia or cognitive deterioration. Family history was classified as a binary variable. *APOE ε4* genetic testing was performed by the Wisconsin Alzheimer's Disease Research Center. Participants were categorized using a binary variable as an *APOE ε4* carrier or non-carrier.

Inclusion criteria for all subjects consisted of the following: normal cognitive function determined by neuropsychological evaluation, no current diagnosis of major psychiatric disease or other major medical conditions (e.g., diabetes, myocardial infarction, or recent history of cancer), no history of head trauma, and no

contraindications for a MRI scan. Three participants were removed from the analysis based on Mini-Mental State Exam (MMSE) below 28 and seven participants were removed due to abnormal findings on radiological read of the MRI. A final sample size of two hundred and eighty two participants (94 males and 188 females) was included in the analyses. Study procedures were approved by the University of Wisconsin Health Sciences Institutional Review Board and were in accordance with U.S. federal regulations. All participants provided written informed consent.

In addition to MRI, participants received a neuropsychological assessment (Table 1). The MMSE (Folstein, Folstein, & McHugh, 1975) was used as a global measure of cognition. Episodic memory was assessed with the Rey Auditory Verbal Learning Test (RAVLT) (Schmidt, 1996) and Brief Visuospatial Memory Test (BVMT) (Benedict, 1997). Verbal generation and fluency were measured with the Wide Range Achievement Test-III reading subtest (WRAT-III) (Jastak & Wilkinson, 1984) and the Boston Naming Test (BNT) (Kaplan, Goodglass, & Weintraub, 1983). Different aspects of executive functions included: response speed and focused attention, measured with the Digits Symbol Substitution test from the Wechsler Adult Intelligence Scale – third Edition (WAIS-III) (Baddeley, 1996), attention switching, examined with the Trail Making Test A and Trail Making Test B, which requires alternation between letters and digits (TMT-A, TMT-B) (Reitan & Wolfson, 1993), and suppression of response-incongruent information, measured with the Stroop test (Trenerry, Crosson, Deboe, & Leber, 1989).

[INSERT TABLE 1 HERE]

MRI Acquisition

Participants were imaged on a General Electric 3.0 Tesla Discovery MR750 (Waukesha, WI) MRI system with an 8-channel head coil. During the fMRI task, for each participant and for each run, 167 whole-brain volumes were acquired to measure the T2* -weighted blood oxygenation level dependent (BOLD) effect with the following parameters: gradient-recall echo-planar imaging (EPI), TR = 2000 ms, TE = 30 ms, flip angle = 90°, 64 x 64 matrix, FOV = 240 mm, thirty 4 mm thick sagittal slices with a 1 mm gap acquired within each TR, resulting in voxel dimensions of $3.75 \times 3.75 \times 5$ mm^3. Three additional volumes were acquired at the beginning of the first run to allow for steady-state magnetization (discarded from analysis). Head movement during scanning was minimized with padding. Parallel imaging was employed using an array spatial sensitivity encoding technique (ASSET).

DTI was acquired using a diffusion-weighted, spin-echo, single-shot, echo planar imaging (EPI) pulse sequence in 40 encoding directions at $b = 1300$ s/ mm^2, with eight non-diffusion weighted ($b = 0$) reference images. The cerebrum was covered using contiguous 2.5 mm thick axial slices, FOV = 240 mm, TR = 8000 ms, TE = 67.8 ms, 96 x 96 mm matrix, resulting in 2.5 mm isotropic voxels. High order shimming was performed prior to the DTI acquisition to optimize the homogeneity of the magnetic field across the brain and to minimize EPI distortions.

Behavioral Task

A schematic diagram of the fMRI task is provided in Figure 1. This paradigm and a variant has been previously published by our group in adults with memory decline (Nicholas et al., 2014; Xu et al., 2009). Thirty minutes prior to fMRI, participants completed one run (48 trials) of an implicit encoding task where images of neutral faces were visually presented for 3000 ms with a 500 ms interstimulus interval. Using a labeled keyboard, participants rated each image on a Likert scale of 1 (“not at all”) to 4 (“very much”) based on the following encoding contexts: age, energy, distinctiveness, attractiveness, and likeability. The order of the encoding contexts and the presentation of the 48 faces were randomized for each participant. During fMRI, using an event-related design, participants completed two runs (96 trials, 11 minutes and 8 seconds) of a recognition task where images from the encoding phase, randomly mixed with novel images, were each presented for 2200 ms. Images presented in one run were not presented in the other run. Participants discriminated via button press between images previously viewed during the encoding phase or novel images resulting in their respective selection being highlighted on-screen. Images presented in one run were not presented in the other run. The average stimulus onset asynchrony was 6.8 s (range 4 – 11 s) with most stimulus onset asynchrony being 5 s, 6 s or 7 s. The order of the runs was counterbalanced across participants.

Reaction time for correctly identified images previously viewed and novel images was calculated. Sensitivity was defined as the statistic d’, which is a measure of the distance between the signal and the signal plus noise. d’ is interpreted as a measure of discrimination sensitivity and was calculated according to signal detection theory

(Harvey, 1992): $d' = Z_{\text{Hit Rate}} - Z_{\text{False Alarm Rate}}$, where the raw hit rate and raw false alarm rate are Z-score transformed from probabilities. Participants who performed at a hit rate < 50% were excluded. Moreover, trials that received either no response or multiple responses were not included in any of the analyses.

[INSERT FIGURE 1 HERE]

fMRI Data Preprocessing and Analysis

fMRI echo-planar imaging data were slice-time corrected using Analysis of Functional NeuroImages (AFNI). The first three fMRI volumes acquired while the participant viewed the task instructions were discarded from the analysis. Motion correction to the first functional scan was performed using a six-parameter rigid-body transformation using Statistical Parametric Mapping (SPM8) (http://www.fil.ion.ucl.ac.uk/spm/software/spm8). For each individual, the mean of the functional images was spatially normalized to the Montreal Neurological Institute (MNI) template by applying a 12–parameter affine transformation followed by nonlinear warping (Ashburner & Friston, 1999). The computed transformation parameters were applied to all of the functional images, interpolated to a final voxel size of 3 x 3 x 3 mm^3. Images were subsequently spatially smoothed with an 8 mm Gaussian kernel.

A mixed–effects, event-related statistical analysis was performed in a two-level procedure. At the first-level, a separate general linear model was specified for each participant. BOLD responses time-locked to the onset of previously viewed images,

novel images, and error trials were modeled separately, by convolving the onset vectors with a synthetic hemodynamic response function as implemented by SPM8. Trials were modeled for two TRs duration or 4000 ms duration. At the model estimation, the data were high-pass filtered (128 s cut-off) to remove low-frequency drifts and serial correlations were accounted for by an autoregressive model of the first order. Other regressors entered into the first-level models included six parameters that represented the motion-related variance in the data (3 for rigid-body translation and 3 for rotation). Contrast images comparing activity associated with identifying previously viewed images to activity associated with identifying novel images were created. The individual contrast images were then entered into a second-level random-effects analysis, using a one-sample t test, to assess the group effect. The resulting summary statistical map was thresholded at $p < 0.05$ (topological FDR-corrected for multiple comparisons across the whole brain, voxel extent = 20) (Figure 2) and was used as an inclusive mask for the functional connectivity analysis.

[INSERT FIGURE 2 HERE]

Task-related functional connectivity analysis

We conducted generalized form of context-dependent psychophysiological interaction (gPPI) analyses with the Generalized PPI toolbox (McLaren, Ries, Xu, & Johnson, 2012) (http://www.nitrc.org/projects/gppi/), which was implemented in MatLab R2011b and utilizing functions in SPM8. Six regions of interest (ROIs) were defined as seed regions. We selected ROIs based on brain regions known to be part of the

recognition network (Rugg & Vilberg, 2013) and showing group peak activation during recognition (previous viewed images > novel images). Seed ROIs were created as 6 mm spheres centered on peak activations in the left precuneus (MNI coordinates: -8 -62 28), left posterior cingulate (MNI coordinates: -8 -54 32), bilateral hippocampus (MNI coordinates: 34, -34, 10; -30 -18 -22), right cuneus (MNI coordinates: 12 -90 22), left angular gyrus (MNI coordinates: -40 -74 42), and left middle frontal gyrus (MNI coordinates: -44 32 4).

The gPPI model involved general linear modeling of the seed timecourse (physiological regressors), the task conditions (psychological regressors), and an interaction term for each task condition x the seed timecourse. Although errors were modeled at the first level univariate analysis, due to the low percentage of errors (accuracy on the task was 89.4%) they were not included in the gPPI model. For each subject, the physiological activity was computed as the deconvolved mean time series of all voxels within each ROI. The psychological regressors were created by separately convolving 2 task vectors: (1) correct previously viewed images and (2) a regressor for correct novel images, with the canonical hemodynamic response function. The interaction terms (PPIs) were then computed by multiplying the time series from the psychological regressors with the physiological activity. A whole-brain analysis (single-subject level) was performed using the general linear model in SPM8 with 5 regressors modeling the BOLD signal per run: 2 PPI regressors, 2 psychological/task regressors and the mean time course in the seed region. Additional nuisance covariates included

the same motion and session parameters that were also entered into the mean signal analyses, and the data were again subjected to a high-pass filter with a cutoff of 128 s.

For each of the six seed regions and for each subject, a PPI contrast was created and identified recognition-related changes in connectivity. Subsequently, these first-level PPI contrast images were entered into second-level analyses.

DTI Data Preprocessing and Analysis

The processing stream is reported in Adluru et al., 2014 and is briefly repeated here. A robust processing pipeline was employed, based on methods in Zhang, Avants, et al., 2007. First, head motion and image distortions (stretches and shears) due to eddy currents were corrected with affine transformation in the FSL (FMRIB Software) package (http://www.fmrib.ox.ac.uk/fsl/). Geometric distortion from the inhomogeneous magnetic field applied was corrected with the $b = 0$ field map and PRELUDE (phase region expanding labeler for unwrapping discrete estimates) and FUGUE (FMRIB's utility for geometrically unwarping EPIs) from FSL. Brain tissue was extracted using FSL's BET (Brain Extraction Tool). Tensor fitting was performed using a nonlinear least squares method in Camino (http://cmic.cs.ucl.ac.uk/camino/).

Individual maps were registered to a population specific template constructed using Diffusion Tensor Imaging Toolkit (DTI-TK), http://www.nitric.org/projects/dtitk/, which is an optimized DTI spatial normalization and atlas construction tool (Wang et al., 2011; Zhang, Yushkevich, Alexander, & Gee, 2006; Zhang, Yushkevich, Rueckert, & Gee, 2007). A subset of 80 diffusion tensor maps was used to create a common space

template. All diffusion tensor maps were normalized to the template with a rigid, affine, and diffeomorphic alignments and interpolated to 2 x 2 x 2 mm^3 voxels. With DTI-TK, FA maps were calculated in the normalized space and were visually inspected to rule out inclusion of maps with missing data in regions of interest or other artifacts. White matter alignment was performed using a diffeomorphic (topology preserving) registration method (Zhang, Avants, et al., 2007) that incrementally estimates the displacement field using a tensor-based registration formulation (Zhang et al., 2006).

The Johns Hopkins International Consortium for Brain Mapping (ICBM) FA template was warped to the study's template space using Advanced Normalization Tools (ANTS). Using ANTS, the Johns Hopkins ROIs (Wakana, Jiang, Nagae-Poetscher, van Zijl, & Mori, 2004) were individually warped to the study's template space. Seven bilateral white matter ROIs were chosen for analysis: the genu and splenium of the corpus callosum, the fornix (body and column), the cingulum bundles adjacent to the cingulate cortex (cingulum-CC, left and right averaged), the cingulum bundle projections to the hippocampus (cingulum-HC, left and right averaged), the superior longitudinal fasciculi (SLF, left and right averaged), and the uncinate fasciculi (left and right averaged). The FA map for each subject in normalized template space was masked by the chosen ROIs. The resulting FA ROIs were thresholded at 0.2 to reduce inclusion of gray matter voxels in the white matter masks. The thresholded FA ROIs were binarized and used as ROI masks to isolate the white matter ROIs of each subject's standard space FA maps. Finally, the average values of FA within each subject's ROI-masked maps were calculated.

Statistical Analyses

Statistical analyses were performed using R version 3.1.3 statistical package (http://www.r-project.org/). Age correlations with reaction time, recognition performance, and white matter measures were assessed. In addition, regression analyses were done to investigate relationships between recognition performance and white matter measures. Analyses included sex and education as covariates.

One-sample t-tests at the group level identified voxels where connectivity with a given seed was higher during correct responses to previously viewed images versus novel images. In addition, using FA and d', regression analyses were performed to investigate the influence of white matter microstructure and behavior on task-related functional connectivity within the recognition network. Sex and education were covaried. Statistical images were thresholded at $p < 0.001$ uncorrected, and were masked inclusively by the group-level univariate recognition contrast. In order to exclude small clusters and increase the anatomical plausibility of the results, a cluster size threshold of 25 contiguous voxels was used and analyses were restricted to gray matter using a binary mask.

RESULTS

Behavioral results

Demographics and neuropsychological test performance are in Table 1. The participants' performance, as measured by d', ranged from 0.79 to 4.07 (mean ± standard deviation [SD] = 2.76 ± 0.68) and was negatively correlated with age (t_{281} = -5.65, r^2 = 0.12, $p < 0.05$). Hit rates (z-transformed) ranged from 0.13 to 2.03 (mean ± SD = 1.49 ± 0.4) and false alarm rates (z-transformed) ranged from -2.03 to 0.37 (mean ± SD = -1.27 ± 0.48). On average, participants responded significantly more quickly when responding to a previously viewed face (mean ± SD = 1145.71 ms ± 284.32) compared to a novel face (mean ± SD = 1313.11 ms ± 308.63) (paired t test, t_{281} = -16.31, $p < 0.05$). In addition, participants who responded faster had significantly better performance (t_{281} = -8.55, r^2 = 0.22, $p < 0.05$), and higher age was correlated with slower reaction time (t_{281} = 5.76, r^2 = 0.11, $p < 0.05$).

Correlations between age, performance, and white matter measures

Task behavior (d', hit rates, and false alarm rates) by decade of age is presented in Table 2. Table 3 lists white matter ROI mean FA values and their zero order correlations (r) with age and performance. There were negative correlations between age and FA in several ROIs: the cingulum subjacent to the posterior cingulate (t_{281} = -2.93, r = -0.172, p = 0.003), fornix (t_{281} = -5.41, r = -0.307, $p < 0.001$), genu (t_{281} = -6.99, r = -0.385, $p < 0.01$), splenium (t_{281} = -1.99, r = -0.118, p = 0.046), and SLF (t_{281} = -2.64, r = -0.155, p = 0.008). In addition, fornix FA was positively correlated with performance

(t_{281} = 2.15, r = 0.127, p = 0.03). Given that there were significant effects of age on fornix FA and performance, we assessed whether the relationship between fornix FA and performance remained significant after controlling for age. When we control age on the relationship between fornix FA and performance, we find the following partial correlation r = -0.09, p = 0.099. In addition, we conducted a mediation analysis to assess whether fornix FA mediated the relationship between age and d' using bootstrap procedures. Unstandardized indirect effects were computed for each of the 10,000 bootstrapped samples, and the 95% confidence interval was computed by determining the indirect effects at the 2.5th and 97.5th percentiles. The bootstrapped unstandardized indirect effect was -0.00133, and the confidence interval ranged from -0.00689 to 0.004. Thus, fornix FA did not significantly mediate the effect of age on recognition performance.

[INSERT TABLE 2 HERE]

gPPI Results

Using each of the a-priori ROIs as seed regions, we then performed gPPI analyses. Since the fornix was the only white matter ROI to significantly correlate with performance, we chose to investigate whether task-related function connectivity during recognition was influenced by fornix FA, together with d'.

The imaging regression analysis revealed that fornix FA was positively correlated with dynamic connectivity of the right hippocampus seed and the right caudate during

the recognition task (difference between viewing responses to previously viewed images versus novel images) (peak: 8 16 6, t_{281} = 3.94, k = 41); of the left precuneus seed and the left precuneus (peak: -14 -52 42, t_{281} = 4.37, k = 80), bilateral middle occipital gyrus (left peak: -22 -76 4, t_{281} = 4.02, k = 25; right peak: 22 -72 2, t_{281} = 3.98, k = 80), and the left middle frontal gyrus (peak: -40 30 28, t_{281} = 3.87, k = 32) (Table 4, Figure 3). Data are not shown for one outlier with a parameter estimate greater than 3 standard deviations from the mean in the left middle frontal gyrus seed PPI connectivity difference with the left precuneus seed (Figure 3A).

d' was positively correlated with dynamic connectivity of the left posterior cingulate seed and left middle frontal gyrus during recognition memory (peak: -30 20 54, t_{281} = 3.52, k = 44); and of the right cuneus seed with the left hippocampus (peak: -24 -36 -6; t_{281} = 3.86, k = 62) (Table 4, Figure 4). Data are not shown for one outlier with a parameter estimate greater than 3 standard deviations from the mean in the left middle frontal gyrus PPI connectivity difference with the left posterior cingulate seed (Figure 4B).

One-sample t-tests at the group level ($p < 0.001$, uncorrected; k>25 voxels) revealed no other region in which there was dynamic connectivity with each of the six seed regions during the recognition task.

[INSERT TABLE 3 HERE]

[INSERT FIGURE 3 HERE]

[INSERT FIGURE 4 HERE]

DISCUSSION

Age-related variation in episodic recognition performance in middle-to-late aged individuals was found to correlate specifically with the microstructure of the fornix, a crucial connection between the medial temporal lobe and both prefrontal and subcortical regions involved in memory. In addition, the magnitude of dynamic connectivity between recognition-elicited regions covaried across subjects with performance and fornix microstructure. Consistent with previous work in our group (Adluru et al., 2014), there were aging effects on fractional anisotropy of the fornix, genu and splenium of the corpus callosum, the cingulum subjacent to the posterior cingulate, and the SLF. Our results complement previous literature pointing to the relationship between higher connectivity in the context of successful episodic memory retrieval (King, de Chastelaine, Elward, Wang, & Rugg, 2015; Schedlbauer, Copara, Watrous, & Ekstrom, 2014; Staresina, Cooper, & Henson, 2013; Watrous, Tandon, Conner, Pieters, & Ekstrom, 2013); however, to the authors' knowledge, this is the first study to determine that recognition-related connectivity is influenced by white matter microstructure.

Mean fornix fractional anisotropy values positively correlated with recognition performance. This finding is consistent with the view that the fornix, together with the hippocampus, is critically and specifically involved in specific facets of episodic memory (John P. Aggleton & Brown, 2006; Lockhart et al., 2012; Metzler-Baddeley et al., 2011; Rudebeck et al., 2009). Aggleton & Brown (2006) propose a model that places emphasis on hippocampal interactions with the mammillary bodies and thalamus, also via the fornix, which provides an indirect, alternative route to influence the prefrontal

cortex. Across studies of young and older adults, higher fornix FA was found to be associated with better working memory (Zahr, Rohlfing, Pfefferbaum, & Sullivan, 2009), episodic memory (Rudebeck et al., 2009), and with both verbal and visual recall tasks (Rudebeck et al., 2009; Zahr et al., 2009). Moreover, recent diffusion MRI tractography studies (Lockhart et al., 2012; Metzler-Baddeley et al., 2011) have found age-related decline in recall to be associated with microstructural degradation in the fornix.

We observed that dynamic connectivity between posterior regions (posterior cingulate cortex and cuneus) and frontal (middle frontal gyrus) and the MTL (hippocampus) during the recognition task was associated with better memory performance. This finding supports a recent study (King et al., 2015) that found across three experiments that the magnitude of recollection-related increases in connectivity predicted recollection accuracy. Task-related functional connectivity studies have shown that during episodic encoding higher performing older adults have greater functional connectivity between hippocampal activity and activity in the dorsolateral prefrontal and parietal cortex (Grady et al., 2003). Daselaar and colleagues (Daselaar et al., 2006) found that age-related decline in recollection-elicited activity in the hippocampus was associated with decreased functional coupling between the hippocampus and the retrosplenial and parietotemporal cortices. Moreover, greater dynamic connectivity with the middle frontal gyrus supports compensation models that posit older adults may be exhibiting greater top-down modulation from the frontal cortex in order to account for task demands and diminishing neural function (Cabeza & Dennis, 2012). Our present

findings suggest that recognition-related modulation of connectivity can also serve as a predictor of individual differences in recognition accuracy.

Although prior studies have reported relationships between fornix fractional anisotropy and low-frequency fluctuations in the BOLD signal during sustained periods of task engagement or rest (Kehoe et al., 2015; Salami, Pudas, & Nyberg, 2014), we present novel findings indicating that inter-individual variation in fornix microstructure is related to dynamic connectivity between regions within the recognition network. We observed that more preserved fornix white matter microstructure predicted dynamic connectivity between hippocampus and the caudate during a recognition task. The fornix is a major efferent and afferent white matter conduit from the hippocampi with precommissural projections to subcortical structures such as the striatum, which consists of the caudate and putamen. Although most studies examining the role of the striatum have focused on its involvement in motor response learning in anticipation of action-outcome contingencies (Faure, Haberland, Condé, & El Massioui, 2005; Williams & Eskandar, 2006; Yin, Ostlund, Knowlton, & Balleine, 2005), it has been argued that the striatum functions are not exclusively motoric, and that they are important for episodic memory behavior. Specifically, it has been suggested that the dorsal striatum supports processes in which certain stimuli need to be filtered out, whereas others need to be actively maintained in working memory (McNab & Klingberg, 2008). Interestingly, a neuroimaging study found that successful memory was associated with greater activity in both the hippocampus and the striatum during encoding and that this activity correlated for items that were later remembered, but not for items that were forgotten

(Sadeh, Shohamy, Levy, Reggev, & Maril, 2011). Moreover, episodic memory impairments are found in patients with focal lesions to the striatum, mainly the caudate (Vakil, Blachstein, & Soroker, 2004).

In addition, we observed that higher fornix fractional anisotropy predicted dynamic connectivity of the precuneus with the bilateral middle occipital gyrus and left middle frontal gyrus during the recognition task. Although the fornix does not directly project to the precuneus, Zhuang et al., 2012 found that lower FA values within the fornix coupled with altered white matter microstructure in other brain regions critical to memory including the precuneus, predicted conversion to amnestic mild cognitive impairment. Additionally, the fornix carries direct projections to the medial prefrontal cortex (Poletti & Creswell, 1977); although we should caution that our results cannot inform the directionality of dynamic connectivity between the precuneus and the middle frontal gyrus in our study. Altered connectivity as predicted by fornix FA in our study may also reflect common underlying changes due to age, given that during normal aging, the earliest morphological changes occur within medial temporal lobe gray matter, specifically the hippocampus and entorhinal structures with secondary effect to the efferent outflow tract through the fornix and mammillary bodies (J P Aggleton & Brown, 1999; Braak & Braak, 1995; Cassel, Duconseille, Jeltsch, & Will, 1997). Needless to say, the underlying age-related processes affect an interconnected network of regions rather than single regions.

This functional connectivity study and others shed light on how networks interact to subserve numerous cognitive processes. Anatomical and resting-state connectivity

research suggest that brain regions are not part of single networks but are part of multiple networks that have preferred connections or 'coordinated states' (Hellyer et al., 2014; McLaren, Sperling, & Atri, 2014). Importantly, the brain can rapidly switch between these coordinated states to perform different cognitive functions. The transition from one state to another enables the brain to integrate information from a multitude of brain regions. FA is sensitive to multiple microstructural properties of white matter, including axon density, myelination, and possibly diameter (Beaulieu, 2002; Takahashi et al., 2002). Dynamic connectivity-FA correlations might be caused by variation in axon density or diameter. Electromyography (EMG) amplitude and area both increase with fiber density in physiological studies of peripheral nerves; likewise, EMG amplitude and area increase with fiber density and axon diameter in computer simulations (Finsterer & Fuglsang-Frederiksen, 2000; Nandedkar & Stalberg, 1983). It is possible that increased myelination or axon diameter across individuals led to more-synchronized axonal conduction velocities, affecting the degree of interregional modulation observed.

These findings join a body of literature suggesting that microstructural white matter differences influence both cognitive performance and functional connectivity in aging. Microstructural white matter alterations have been associated with slower processing speed, declines in executive function, and lower memory performance (Antonenko, Meinzer, Lindenberg, Witte, & Flöel, 2012; Davis, Kragel, Madden, & Cabeza, 2012; Steffener, Habeck, & Stern, 2012). With regard to functional connectivity, older adults with higher corpus callosum fractional anisotropy show stronger functional connectivity during lateralized word matching, a task requiring cross-hemispheric

communication (Davis et al., 2012). Life experiences, such as bilingualism, have been shown to be associated with higher white matter microstructure as well as with more distributed patterns of functional connectivity in older adults (Luk, Bialystok, Craik, & Grady, 2011). Moreover, compared to monolinguals, the bilingual older adults show greater cognitive control and display increased functional connectivity in a frontal posterior network. While age itself has a robust effect on white matter microstructure, white matter development over the lifespan is influenced by several factors, both environmental and genetic.

There are a few limitations to the current study. Although the sample was enriched for Alzheimer's disease risk factors, we did not find an effect of parental family history or *APOE ε4* genotype on demographic characteristics, behavior, or any of the imaging measures. Given that the participants in the study were screened for memory impairment, and the close association between our findings and other findings in the field, it is more likely that the results represent age-related changes rather than pathological changes. The participants in this study are followed longitudinally, eventually permitting the separation of participants experiencing pathological aging from the group. In our study the strength of the relationship between white matter measures with age and performance is smaller than what has been reported in the literature. However, our results are consistent with other studies reporting links between fornix white matter, age, and memory performance (Lockhart et al., 2012; Metzler-Baddeley et al., 2011) and are reported from a much larger sample size, which may provide a more precise estimate. We should also note that in addition to age, functional connectivity,

and white matter microstructure, other factors not measured in the current study may influence face recognition performance, including the perceived age of presented faces in relation to the participants' age (Lamont, Stewart-Williams, & Podd, 2005). Moreover, given that age-related gray matter atrophy has also been associated with memory performance (Du et al., 2003; Head, Kennedy, Rodrigue, & Raz, 2009; Persson et al., 2006; Rajah, Languay, & Grady, 2011; Raz et al., 2003; Rodrigue & Raz, 2004; Yonelinas et al., 2007), future work will aim at investigating the contribution of gray matter decline to changes in functional connectivity of memory networks. Lastly, although the gPPI results are uncorrected, which could result in Type I error, these findings are still informative and useful for the aging literature, especially in light of the dearth of studies with large sample sizes investigating dynamic connectivity and their relationship to structural connectivity and behavior.

In conclusion, the findings suggest that variation in white matter microstructure is associated with memory performance, and functional connectivity among cortical regions in the recognition memory network. While age had a significant impact on microstructure across several white matter regions, the differences in fornix microstructure had the greatest impact on both functional connectivity and memory performance. The results provide further evidence that age-related cognitive differences can be better understood by incorporating measures reflecting white matter tract connectivity.

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Table 1. Demographic features and neuropsychological measures

Total N	282
Female: N (%)	188 (66%)
Parental family history AD: N (%)	199 (70%)
APOE ε4 positive: N (%)	104 (36%)
Age: years ± SD (range)	61.1 ± 6.71 (44-75)
Education: years ± SD (range)	16.3 ± 2.3 (12-20)
MMSE: Mean ± SD	28.2 ± 1.7
Episodic Memory	
RAVLT Total: Mean ± SD	51.3 ± 8.5
RAVLT Short Delay: Mean ± SD	10.7 ± 2.7
RAVLT Recognition: Mean ± SD	10.6 ± 2.9
RAVLT Delayed Recall: Mean ± SD	13.8 ± 1.5
BVMT-R Total: Mean ± SD	25.1 ± 5.6
BVMT-R Delayed Recall: Mean ± SD	9.8 ± 1.9
BVMT-R Recognition: Mean ± SD	5.7 ± 0.5
Language	
WRAT-III Reading: Mean ± SD	51.9 ± 4.0
BNT Total: Mean ± SD	57.8 ± 2.4
Executive Function	
WAIS-III Digit Symbol: Mean ± SD	57.61 ± 10.2
Trail Making Test A: Mean ± SD	25.2 ± 7.8
Trail Making Test B: Mean ± SD	59.9 ± 21.3
Stroop Color-Word, Words Named: Mean ± SD	114.9 ± 56.6

Neuropsychological scores reported above are raw mean scores and standard deviations (SD). AD = Alzheimer's disease; *APOE ε4* = Apolipoprotein E, ε4; BNT = Boston Naming Test (Kaplan et al., 1983); BVMT-R = Brief Visuospatial Memory Test-

Revised (Benedict, 1997); Digit Span (from WAIS-III) (Baddeley, 1996); MMSE = Mini Mental State Examination (Folstein et al., 1975); RAVLT = Rey Auditory Verbal Learning Test (Schmidt, 1996); TMTA/B = Trail Making Test A and B (Reitan & Wolfson, 1993); WRAT-III = Wide Range Achievement Test-III reading subtest (Jastak & Wilkinson, 1984).

Table 2. Mean task performance by age group

Age (years)	Hit Rate (z score)	False Alarm Rate (z score)	d’
40-49 (N=10)	1.65	-1.61	3.26
50-59 (N=115)	1.58	-1.33	2.92
60-69 (N=129)	1.46	-1.23	2.67
70-79 (N=28)	1.31	-1.06	2.37

Table 3. Zero order correlations between FA and age or performance (d')

Region of Interest	Correlation with FA	
	Age	Performance (d')
Cingulum subjacent to posterior cingulate FA	**-0.172****	0.048
Parahippocampal cingulum FA	-0.105	0.076
Fornix FA	**-0.307*****	**0.127***
Genu FA	**-0.385*****	0.102
Splenium FA	**-0.118***	0.059
SLF FA	**-0.155****	-0.021
Uncinate FA	-0.081	0.024

FA = fractional anisotropy; SLF = superior longitudinal fasciculus.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Table 4. Regions of dynamic connectivity ($p < 0.001$, uncorrected, k>25)

Seed Region	Location	Cluster size	MNI coordinates of peak voxel			Peak-level T-statistic
			x	y	z	
Fornix FA						
R Hippocampus	R Caudate	41	8	16	6	3.94
L Precuneus	L Precuneus	80	-14	-52	42	4.37
L Precuneus	L Middle Occipital Gyrus	25	-22	-76	4	4.02
L Precuneus	R Middle Occipital Gyrus	80	22	-72	2	3.98
L Precuneus	L Middle Frontal Gyrus	32	-40	30	28	3.87
d'						
L Posterior Cingulate	L Middle Frontal Gyrus	44	-30	20	54	3.52
R Cuneus	L Hippocampus	62	-24	-36	-6	3.86

L = Left, R = Right. Cluster size is expressed in number of voxels.

Figure Titles

Figure 1. Task schematic

Figure 2. Main effect of recognition (previously viewed images > novel images)

Figure 3. Regions of dynamic functional connectivity during the recognition task related to fornix fractional anisotropy

Figure 4. Regions of dynamic functional connectivity during the recognition task related to task performance

Figure Legends

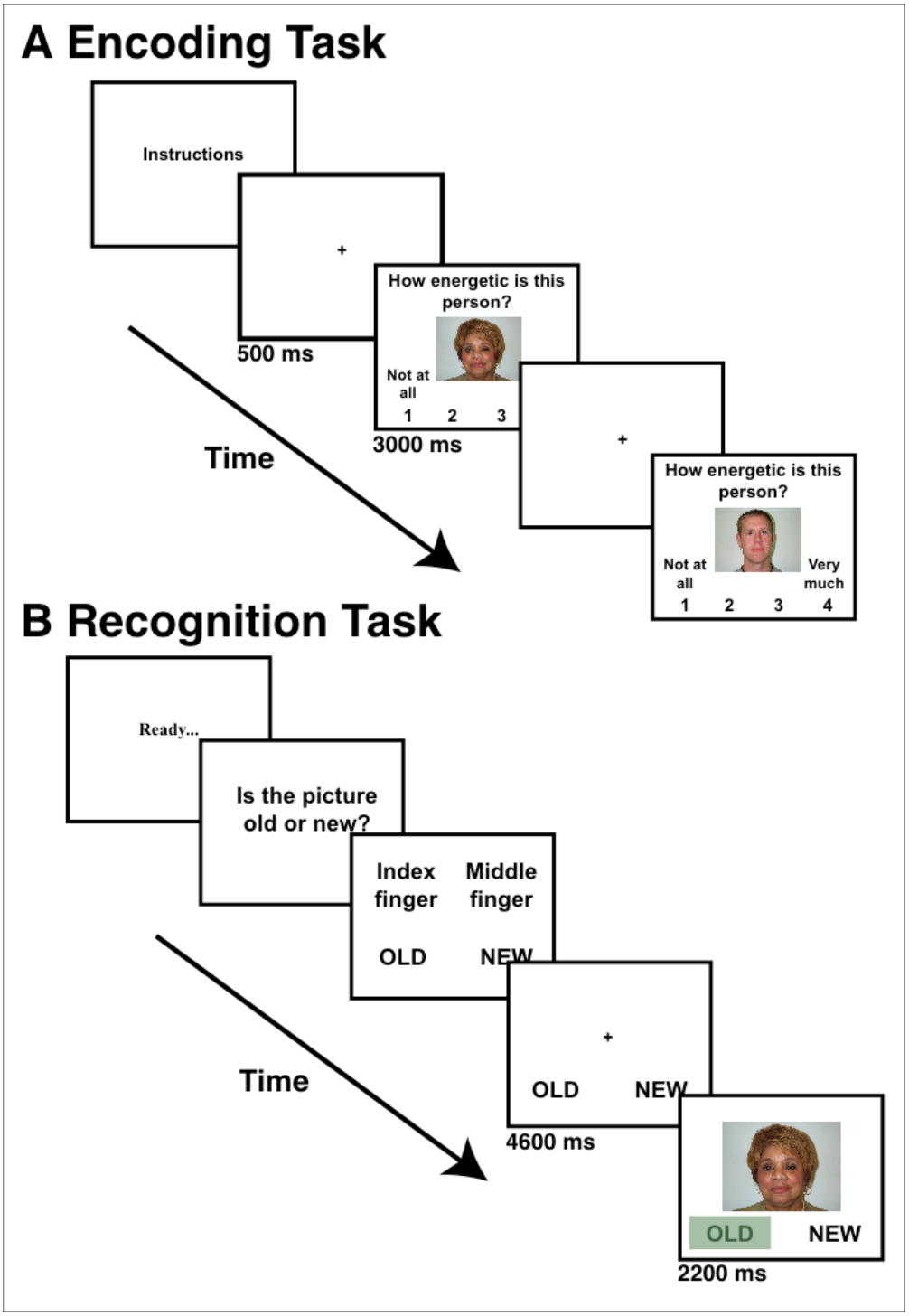

Figure 1. Outside the scanner, participants completed an implicit encoding task **(A)** where neutral faces were presented. Images were rated on a Likert scale of 1-4 based on the following characteristics: age, energy, distinctiveness, attractiveness, and likeability. During scanning, participants completed the recognition task **(B)** that consisted of discriminating via button press between images from the encoding task and randomly mixed novel images.

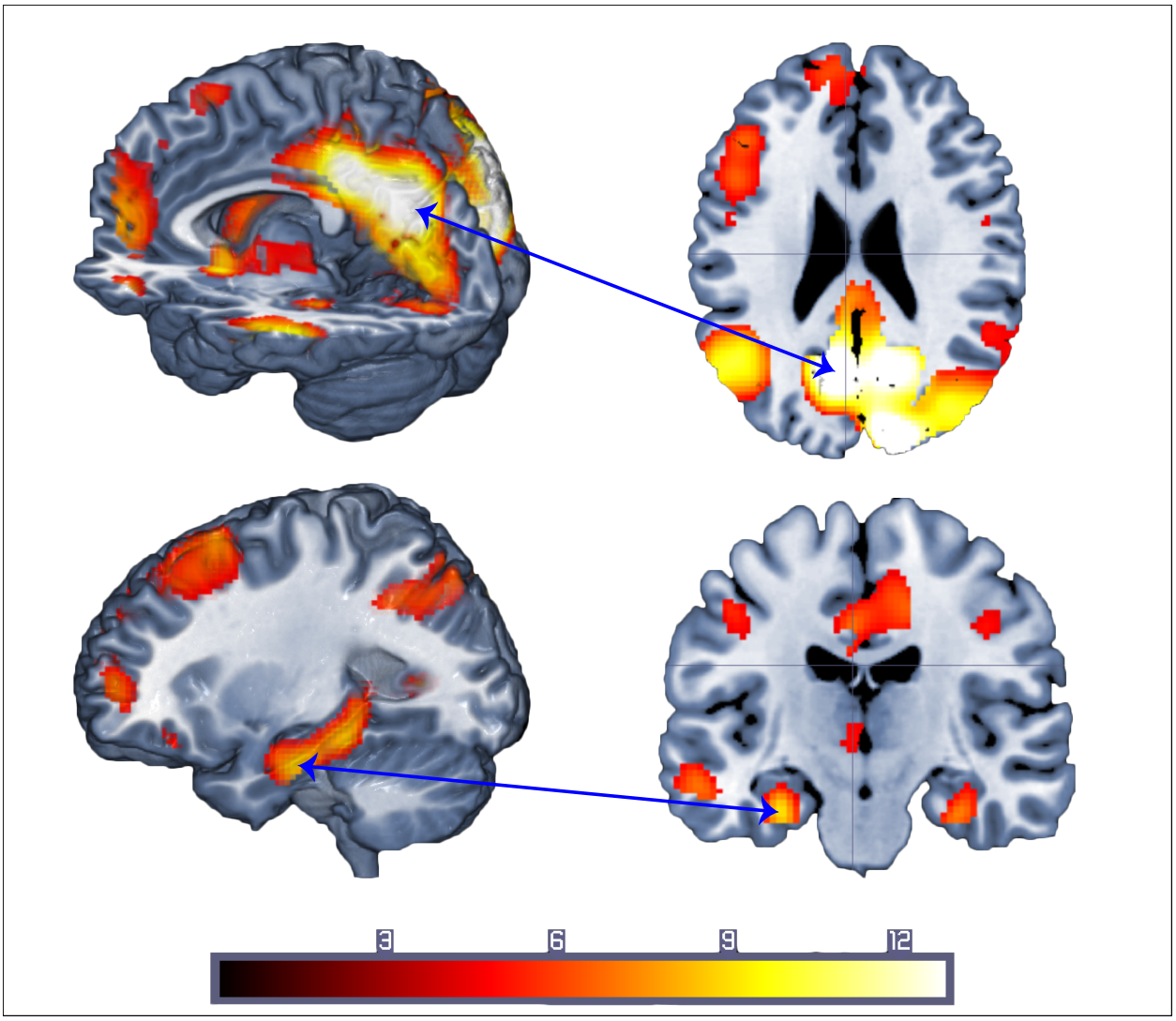

Figure 2. Regions recruited during recognition (previously viewed images > novel images). Areas included bilateral posterior cingulate, precuneus, cuneus, angular gyrus, middle frontal gyrus, and bilateral hippocampus. $p < 0.05$, corrected for multiple comparisons. The statistical map is overlaid on a structural template. The color bar represents t values, ranging from 0 to 13.

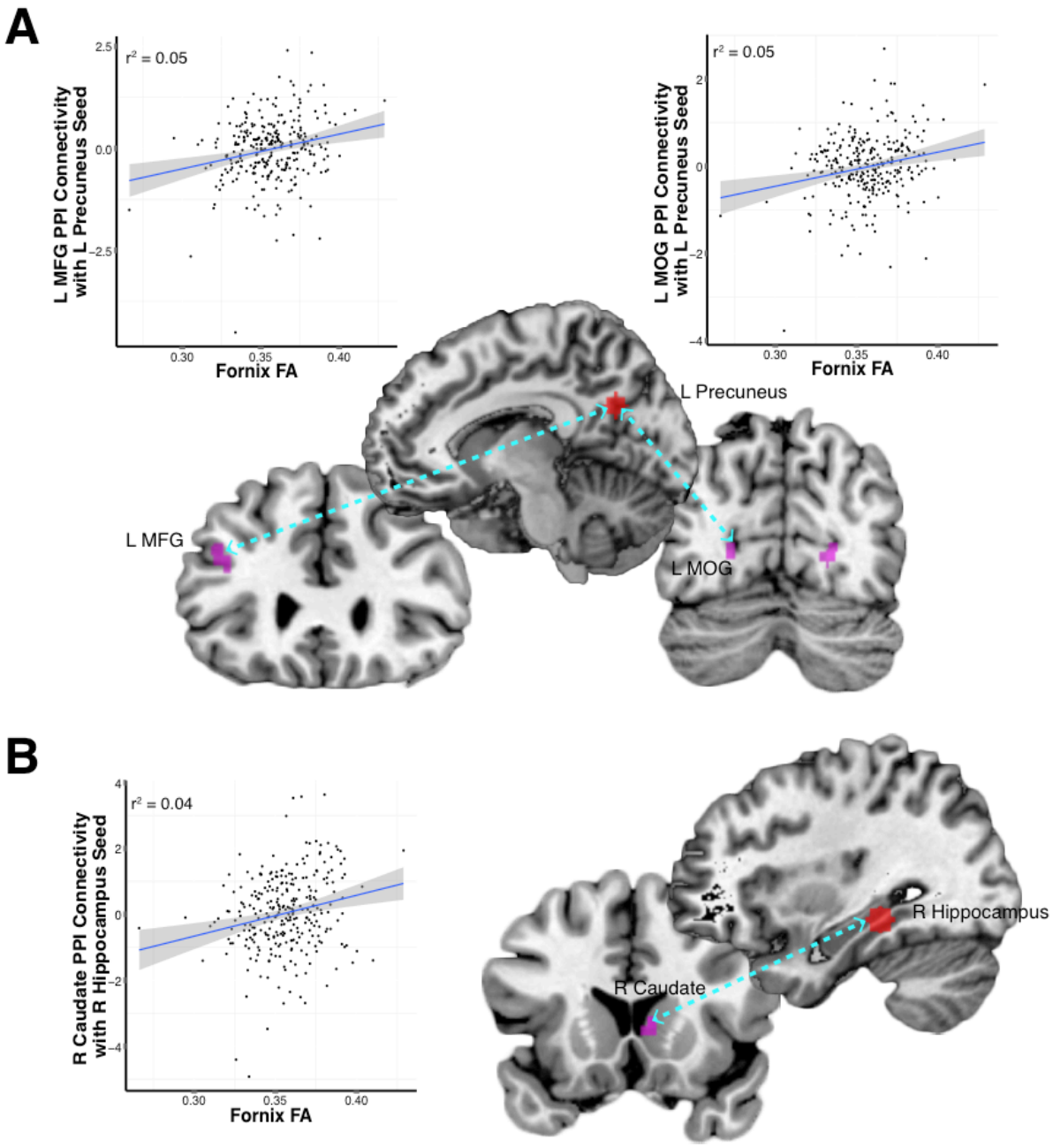

Figure 3. Regions of increased dynamic connectivity during the recognition task associated with greater fornix FA ($p < 0.001$, uncorrected; k>25). Linear regressions of parameter estimates of cluster (in magenta) gPPI connectivity with seed regions (in red) and fornix FA are plotted ($p < 0.05$). The statistical map is overlaid on a structural template. FA = fractional anisotropy; L = left; R = right; MFG = middle frontal gyrus; MOG = middle occipital gyrus; gPPI = generalized psychophysiological interaction.

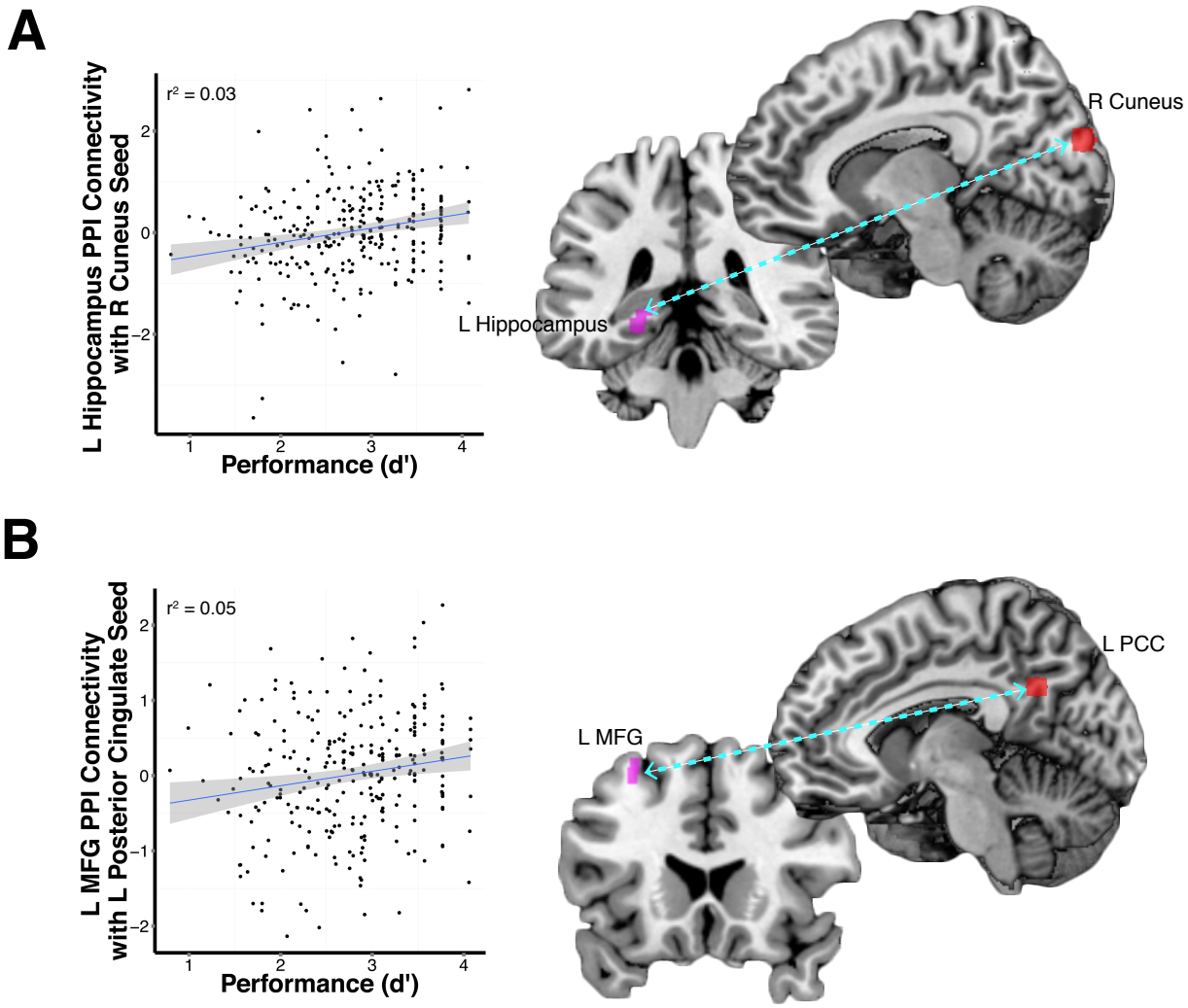

Figure 4. Regions of increased dynamic connectivity during the recognition task associated with greater performance (d') ($p < 0.001$, uncorrected; k>25). Linear regressions of parameter estimates of cluster (in magenta) gPPI connectivity with seed regions (in red) and performance (d') are plotted ($p < 0.05$). The statistical map is overlaid on a structural template. L = left; R = right; MFG = middle frontal gyrus; PCC = posterior cingulate cortex; gPPI = generalized psychophysiological interaction.

CHAPTER 4

Psychological stress is associated with elevated serum soluble interleukin-6 receptor levels & smaller anterior hippocampi

Psychological Stress is Associated with Elevated Serum Soluble Interleukin-6 Receptor Levels & Smaller Anterior Hippocampi

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ABSTRACT

Objective: Repeated exposure to psychological stress alters the homeostatic environment of the brain, contributing to susceptibility to immune-related diseases and elevating levels of chronic low-grade inflammation. Increased concentrations of pro-inflammatory cytokines such as interleukin-6 (IL-6) have been reported to negatively affect hippocampal structure and cognitive function. However, the relationships between psychological stress, peripheral inflammation, and the hippocampus are unknown. **Method:** In this study, we investigate the impact of interactions between psychological stress and peripheral IL-6 and its soluble receptor (sIL-6R) on hippocampal volume and hippocampal-dependent behavior. **Results:** Individuals who experienced greater stress in the past 5 years had higher serum sIL-6R levels and smaller bilateral hippocampal head volume. Delayed memory recall did not have a relationship with sIL-6R levels but was associated with bilateral hippocampal head volume and cumulative stress. The relationship between cumulative stress and delayed memory recall remained significant after controlling for hippocampal head volume. **Conclusions:** Psychological stress has a negative effect on peripheral inflammation and hippocampal volume. The mediation analysis reveals that psychological stress has a strong negative effect on memory behavior independent of hippocampal volume. There were no relationships with IL6. Given that pro-inflammatory IL-6 signaling is mediated by the sIL-6R, these results suggest that psychological stress is an important modulator of human immune function, when dysregulated can impact hippocampal structure.

Keywords: soluble IL-6 receptor, serum IL-6, psychological stress, hippocampal head

INTRODUCTION

Psychological stress is a strong risk factor in the etiology of numerous mental health complications (McEwen, 2012), including mood disorders and cognitive impairments. Recent work reveals that chronic stress triggers immune dysfunction that is causally implicated in mental health disturbances (Glaser & Kiecolt-Glaser, 2005; Reader et al., 2015; Wohleb, McKim, Sheridan, & Godbout, 2015). Individuals exposed to chronic stress show persistent cognitive and emotional dysregulation that contribute to deterioration of overall mental health and quality of life (Baum, Cohen, & Hall, 1993; McEwen, 2013). For instance studies with caregivers (Caswell et al., 2003; Mackenzie, Smith, Hasher, Leach, & Behl, 2007) and college-age students (Keinan, Friedland, Kahneman, & Roth, 1999) demonstrate that chronic stress is associated strongly with cognitive impairments and accelerated cognitive decline (Vitaliano, Murphy, Young, Echeverria, & Borson, 2011). Additional studies also report associations between stress-induced neuroinflammatory activation and psychological disturbances. For instance, elevated pro-inflammatory cytokines (Janelidze, Mattei, Westrin, Träskman-Bendz, & Brundin, 2011), increased microglia activation (Schnieder et al., 2014), and increased brain macrophages (Torres-Platas, Cruceanu, Chen, Turecki, & Mechawar, 2014) were all detected within specific brain regions of depressed suicide victims. Thus, stress-induced neuropsychiatric disturbances may involve impaired neuroplasticity caused by microglia activation, monocyte recruitment, and enhanced neuroinflammatory signaling.

Animal studies of chronic stress reveal that sympathetic activation promotes the production of proinflammatory monocytes (Heidt et al., 2014; Powell et al., 2013; Wohleb et al., 2011) that traffic to the brain (Wohleb et al., 2011) and promote the development of prolonged and recurring anxiety (McKim et al., 2015; Wohleb et al., 2014; Wohleb, Powell, Godbout, & Sheridan, 2013). In addition, chronic stressors activate microglia within specific stress-responsive brain regions (Tynan et al., 2010; Wohleb et al., 2011) that are spatially coupled to neurovascular facilitation of monocyte recruitment (Sawicki et al., 2015). These experimental findings reveal a profound, bidirectional interplay between stress circuitry and neuroimmune signaling that controls behavioral responses to chronic stress.

Peripheral proinflammatory cytokines, such as interleukin 6 (IL-6), are likely mediators of many of these effects, penetrating the blood-brain barrier directly through active transport mechanisms (Banks & Kastin, 1991) or indirectly through activation of the afferent vagus nerve (Maier, Watkins, & Maier, 1998; Tracey, 2002) to stimulate the production of central proinflammatory cytokines, including IL-6 in discrete brain regions (Maier et al., 1998). Recent research suggests that elevated levels of IL-6 are associated with decreased cognitive performance (A L Marsland et al., 2006) and neurodegeneration (Anna L. Marsland, Gianaros, Abramowitch, Manuck, & Hariri, 2008); however, the impact of psychological stress on these relationships in humans has yet to be investigated.

IL-6 is produced in the brain by activated glia, in response to peripheral immune challenge, and is also expressed in neuronal populations (Frank, Barrientos, Watkins, &

Maier, 2010; Ghorbel et al., 2003; Jankord et al., 2010; Vallières & Rivest, 1997). IL-6 signals through a trans-membrane receptor (IL-6Rα) and the ubiquitous tranducer molecule glycoprotein 130 (gp130). The IL-6Rα is also present as a soluble protein that acts agnostically and confers IL-6 responsivity to cells that do not express gp130 (Schöbitz et al., 1995). The shedding of soluble IL-6R (sIL-6R) cleavage from infiltrating immune cells allows IL-6 signaling to occur in cells that do not normally express the IL-6Rα (Michael Maes, Anderson, Kubera, & Berk, 2014). This process known as IL-6 trans-signaling mediates the pro-inflammatory effects of IL-6. This may be an especially important mechanism in the CNS, where sIL-6R has been detected.

There is compelling evidence that psychological stress can induce profound increases in circulating IL-6. This has been demonstrated in laboratory animals after open field exposure (LeMay, Vander, & Kluger, 1990), foot shock, restraint, and exposure to a conditioned aversive stimulus (Zhou, Kusnecov, Shurin, DePaoli, & Rabin, 1993). Similarly, studies in humans have shown that a variety of laboratory stress paradigms have the potential to induce increases in circulating IL-6, as summarized in a set of meta-analyses by Steptoe et al. (Steptoe, Hamer, & Chida, 2007). For instance, a study found that a chronic stressor may provoke long-term changes in the circulating levels of IL-6, increasing mortality risk (Kiecolt-Glaser et al., 2003). Likewise, emerging evidence suggests that indicators of well-being–such as positive affect, purpose in life, and positive social relations—are associated with lower levels peripheral inflammation (Brouwers et al., 2013; Deverts et al., 2010; E M Friedman et al., 2005; Elliot M Friedman, Hayney, Love, Singer, & Ryff, 2007; Elliot M.

Friedman & Ryff, 2012; Prather, Marsland, Muldoon, & Manuck, 2007; Steptoe, O'Donnell, Badrick, Kumari, & Marmot, 2008; von Kanel et al., 2012) and less inflammatory reactivity during acute stress tasks (Aschbacher et al., 2012; Steptoe, Wardle, & Marmot, 2005).

Studies indicate that peripheral inflammatory mediators, such as IL-6, modulate central inflammatory processes that affect cognitive function (Vitkovic et al., 2000). Notably, receptors for IL-6 are concentrated in the hippocampus and prefrontal cortex (Van Wagoner & Benveniste, 1999; Vitkovic et al., 2000). In addition, studies have shown that higher IL-6 levels are associated with smaller hippocampal and prefrontal gray matter volume and with lower executive function and memory performance in otherwise healthy, middle-aged community dwelling adults (Marsland et al., 2006; Marsland et al., 2008; Sudheimer D. et al., 2014).

In this study, we investigated whether chronic psychological stress would be associated with elevated levels of IL-6 and its soluble receptor sIL-6R, and altered hippocampal volume and hippocampal-dependent behavior. Anatomically, the hippocampal head, body, and tail are connected to separate regions of the entorhinal cortex, which conveys processed information from the association cortices to the hippocampus. Given that lower hippocampal head volume has been found to associate with poor verbal memory performance (Hackert et al., 2002), which has been shown to correlate with elevated levels of IL-6 (Marsland et al., 2006), we hypothesized that psychological stress would negatively impact IL-6 levels and the anterior hippocampus structure.

METHODS

Participants

The Survey of Midlife Development in the United States (MIDUS) began in 1995 with a national sample of Americans (N = 7,108) aged 25-74 (32). The majority (59.71%) was recruited through random digit dialing (RDD). The remaining respondents included siblings of the RDD sample and a large sample of twins (N = 1,914). Data collection focused on sociodemographic and psychosocial assessments obtained through phone interviews and self-administered questionnaires. In 2004, these survey assessments were repeated (MIDUS II). The retention rate from MIDUS I to MIDUS II was 75% (adjusted for mortality).

MRI data were collected on a subset of MIDUS II participants who underwent physiological and functional magnetic resonance imaging (fMRI). As part of these procedures, 74 participants (49 female) aged 37—76 (mean (SD) = 56.44 (10.86) years) underwent structural magnetic resonance imaging and agreed to participate in our experiment. Participants were admitted to the University of Wisconsin-Clinical and Translational Research Core (UW-CTRC).

Ethical approval for telephone and mail surveys was obtained from the Social and Behavioral Science Institutional Review Board at the University of Wisconsin – Madison. All participants gave verbal consent, which included assurance of voluntary participation and confidentiality of data. The ethics committee approved the waiver of written consent. Such passive consent is customary for survey research by telephone and mail questionnaire. Ethical approval for the follow-up psychophysiological session

was obtained from the Health Sciences Institutional Review Board at the University of Wisconsin – Madison and all participants provided written consent.

Data and documentation for MIDUS I and II, including all MIDUS projects, are publically available at the Inter-university Consortium for Political and Social Research (ICPSR; www.icpsr.umich.edu/icpsrweb/landing.jsp).

Psychosocial Stress Domains

Our first, and primary, independent variable was a single summary index measure of overall stress. The remaining eleven, secondary, independent variables were each of the component psychosocial stress domains that, together, comprised our summary index measure of overall stress. These eleven component psychosocial stress domains included (1) psychological work stress, (2) physical work stress, (3) work-family spillover stress, (4) perceived inequality, (5) relationship stress, (6) neighborhood stress, (7) discrimination, (8) current financial stress, (9) past year problems in the immediate family, (10) stressful life experiences and (11) early life stress. Drs. David Williams and Natalie Slopen from the Harvard School of Public Health compiled and scored each domain (as described below).

Psychological Work Stress

The first component psychosocial stress domain was psychological work stress. This component domain was comprised of questions from five composite scales, with each composite scale created based on formerly published scientific studies that

examined the issue of psychological work stress (Bosma et al., 1997; Karasek, R., & Theorell, 1992; R. Karasek, Baker, Marxer, Ahlbom, & Theorell, 1981; Robert a Karasek, 1979; Schwartz, Pieper, & Karasek, 1988). These composite scales included skill discretion, decision authority, demands scale, coworker non-support and supervisor non-support. The skill discretion composite scale had three questions that included "How often do you learn new things at work?", "How often does your work demand a high level of skill or expertise?" and "How often does your job provide you with a variety of things that interest you?". The decision authority composite scale had six questions that included "On your job, how often do you have to initiate things – such as coming up with your own ideas, or figuring out on your own what needs to be done?", "How often do you have a choice in deciding how you do your tasks at work?", "How often do you have a choice in deciding what tasks you do at work?", "How often do you have a say in decisions about your work?", "How often do you have a say in planning your work environment – that is, how your workplace is arranged or how things are organized?" and "How often do you control the amount of time you spend on a task?". The demands composite scale had five questions that included "How often do you have to work very intensively – that is, you are very busy trying to get things done?", "How often do different people or groups at work demand things from you that you think are hard to combine?", "How often do you have too many demands made on you?", "How often do you have enough time to get everything done?" and "How often do you have a lot of interruptions?". The co-worker non-support composite scale had two questions that included "How often do you get help and support from your co-workers?" and "How

often are your co-workers willing to listen to your work-related problems?". Lastly, the supervisor non-support composite scale had three questions that included "How often do you get the information you need from your supervisor or superiors?", "How often do you get help and support from your immediate supervisor?" and "How often is your immediate supervisor willing to listen to your work-related problems?". The response options for each of these questions, within these five psychological work stress composite scales, were "All of the time", "Most of the time", "Sometimes", "Rarely" and "Never". A point value between 1-5 was assigned to each question response with 1 point for "All of the time" to 5 points for "Never". The questions from within the first three composite scales (skill discretion, decision authority and demands scale, with the exception of the demands scale question "How often do you have enough time to get everything done?"), were reverse coded so that a higher score reflected a higher stress standing on that question. If a question from within one of the composite scales was missing a score, the mean value, obtained from the completed questions within that designated scale, was imputed for that question. Composite scale scores were computed for individuals who had valid, non-imputed, responses to at least half of the questions within that particular composite scale. Composite scale scores were not computed for individuals who had valid, non-imputed, responses to fewer than half of the questions within that particular composite scale and those individuals composite scale scores were set to missing. Each of the five composite scale scores was calculated by summing together the question scores from within each of the scales. These composite scale scores were then standardized using a z-score to have a mean

of zero and a standard deviation of one. Cronbach's alpha values, for each of the five composite scales, were calculated and ranged from 0.61 to 0.89 (skill discretion MIDUS II resurvey = 0.70, Milwaukee = 0.76; decision authority MIDUS II resurvey = 0.87, Milwaukee = 0.89; demands scale MIDUS II resurvey = 0.73, Milwaukee = 0.61; co-worker non-support MIDUS II resurvey = 0.67, Milwaukee = 0.68; supervisor non-support MIDUS II resurvey = 0.87, Milwaukee = 0.87). A single score for psychological work stress was then computed by taking the sum of the five standardized composite scale scores to create one numeric value per participant.

Physical Work Stress

The second component psychosocial stress domain was physical work stress. This component domain was comprised of questions from two composite scales: occupational physical strain and risk of injury on the job. The occupational physical strain composite scale had nine questions that included "The following items ask about the types of physical activities that you engage in while at your job. Please indicate how often, during your work-shift, you do each of the following. If you are not currently working, but were employed over the past 10 years, please tell us about your most recent job: (1) How often does your job require a lot of physical effort? (2) How often does your job require you to lift loads weighing 50 pounds or greater? (3) How often does your job require you to lift loads weighing less than 50 pounds, but greater than 10 pounds? (4) How often does your job require you to lift loads weighing up to 10 pounds? (5) How often does your job require you to crouch, stoop or kneel? (6) How often does

your job require you to stand for long periods of time? (7) How often does your job require you to use stairs or inclines? (8) How often does your job require you to walk? and (9) How often does your job require you to reach?". The risk of injury on the job composite scale had one question that asked "To what extent, over the past ten years, have you been exposed to the risk of accidents or injuries on your job?". The response options for each of the questions on the occupational physical strain composite scale were "All of the time", "Most of the time", "Some of the time", "Little of the time" and "Never". The response options for the question on the risk of injury on the job composite scale were "A lot", "Some", "A little" and "Not at all". A point value between 1-5 was assigned to each question response, for the questions within the occupational physical strain composite scale, with 1 point for "All of the time" to 5 points for "Never". A point value between 1-4 was assigned to the question from the risk of injury on the job composite scale with 1 point for "A lot" to 4 points for "Not at all". All of the questions, from both composite scales, were reverse coded so that a higher score reflected a higher stress standing on that question. The occupational physical strain composite scale score was computed by calculating the mean of the(Greenhaus & Beutell, 1985; Gutek, Searle, & Klepa, 1991; Sieber, 1974; Wayne, Musisca, & Fleeson, 2004) valid, non-imputed, question scores, from within the scale, then multiplying by 9 (the total number of questions within the scale) to account for any missing question values. Both composite scale scores were then standardized using a z-score to have a mean of zero and a standard deviation of one. A Cronbach's alpha value, for the occupational physical strain composite scale, was calculated with a score of 0.92 for the MIDUS II

resurvey participants and 0.94 for the Milwaukee supplemental sample participants. A single score for physical work stress was then computed by taking the sum of the two standardized composite scale scores to create one numeric value per participant.

Work-Family Spillover Stress

The third component psychosocial stress domain was work-family spillover stress. This component domain was comprised of items from two composite scales, with both composite scales created based on the existing work-family scientific literature and previously created theoretical frameworks and scales (Greenhaus & Beutell, 1985; J G Grzywacz & Marks, 2000; Joseph G Grzywacz & Marks, 2000; Joseph G. Grzywacz, 2000; Gutek et al., 1991; Kopelman, Greenhaus, Connolly, Greenhaus, & Kopelman, 1983; Sieber, 1974; Wayne et al., 2004). These composite scales included negative work to-family spillover and negative family-to-work spillover. The negative work-to-family spillover composite scale had four items that included “Your job reduces the effort you can give to activities at home.”, “Stress at work makes you irritable at home.”, “Your job makes you feel too tired to do the things that need attention at home.” and “Job worries or problems distract you when you are at home.”. The negative family-to-work spillover composite scale had four items that included “Responsibilities at home reduce the effort you can devote to your job.”, “Personal or family worries and problems distract you when you are at work.”, “Activities and chores at home prevent you from getting the amount of sleep you need to do your job well.” and “Stress at home makes you irritable at work.”. The response options for each of these items, within both

composite scales, were “All of the time”, “Most of the time”, “Sometimes”, “Rarely” and “Never”. A point value between 1-5 was assigned to each item response with 1 point for “All of the time” to 5 points for “Never”. All of the items were then reverse coded so that a higher score reflected a higher stress standing on that item If an item within one of the composite scales was missing a score, the mean value, obtained from the completed items within that designated scale, was imputed for that item. Composite scale scores were then computed for individuals who had valid, non-imputed, responses to at least half of the items within that particular composite scale. Composite scale scores were not calculated for individuals who had valid, non-imputed, responses to fewer than half of the items within that particular composite scale and those individuals composite scale scores were set to missing. Both of the composite scale scores were then computed by summing together the item scores from within each of the scales. These composite scale scores were then standardized using a z-score to have a mean of zero and a standard deviation of one. Cronbach’s alpha values, for both of the composite scales, were calculated and ranged from 0.80 to 0.82 (negative work-to-family spillover MIDUS II resurvey = 0.82, Milwaukee = 0.82; negative family-to-work spillover MIDUS II resurvey = 0.80, Milwaukee = 0.80). A single score for work-family spillover stress was then computed by taking the sum of the two standardized composite scale scores to create one numeric value per participant.

Perceived Inequality

The fourth component psychosocial stress domain was perceived inequality. This component domain was comprised of items from three composite scales, with each composite scale created to assess the extent to which individuals have awareness of unequal distribution of life resources (C. D. Ryff, Magee, Kling, & Wing, 1999). These composite scales included perceived inequality in the family, perceived inequality in the home and perceived inequality in the work place. The perceived inequality in the family composite scale had six items that included “I feel good about the opportunities I have been able to provide for my children.”, “It seems to me that family life with my children has been more negative than most people’s.”, “Problems with my children have caused me shame and embarrassment at times.”, “As a family, we have not had the resources to do many fun things together with the children.”, “I believe that I have been able to do as much for my children as most other people.” and “I feel a lot of pride about what I have been able to do for my children.”. The perceived inequality in the home composite scale also had six items that included “I live in as nice a home as most people.”, “I’m proud of my home.”, “Most people live in a better neighborhood than I do.”, “I don’t like to invite people to my home because I do not live in a very nice place.”, “I feel very good about my home and neighborhood.” and “It feels hopeless to try to improve my home and neighborhood situation.”. Lastly, the perceived inequality in the work place composite scale also had six items that included “I feel cheated about the chances I have had to work at good jobs.”, “When I think about the work I do on my job, I feel a good deal of pride.”, “I feel that others respect the work I do on my job.”, “Most people have more rewarding jobs than I do.”, “When it comes to my work life, I’ve had

opportunities that are as good as most people's." and "It makes me feel discouraged that other people have much better jobs than I do.". The response options for each of the items, within the perceived inequality in the family composite scale, were "Not at all true", "A little true", "Moderately true" and "Extremely true". The response options for each of the items, within the two remaining composite scales, perceived inequality in the home and perceived inequality in the work place, were "A lot", "Some", "A little" and "Not at all". A point value between 1-4 was assigned to each item response with 1 point for "Not at all true", for the perceived inequality in the family composite scale, or "A lot", for the remaining two composite scales, to 4 points for "Extremely true", for the perceived inequality in the family composite scale, or "Not at all", for the remaining two composite scales. Where needed, some of the items were reverse coded so that a higher score reflected a higher stress standing on that item. If an item within one of the composite scales was missing a score, the mean value, obtained from the completed items within that designated scale, was imputed for that item. Composite scale scores were then computed for individuals who had a valid, non-imputed, response to at least one of the items within that particular composite scale. Composite scale scores were not calculated for individuals who had a valid, non-imputed, response to zero items within that particular composite scale and those individuals composite scale scores were set to missing. Each of the three composite scale scores was then computed by calculating the mean score of the items within each scale. These composite scale scores were then standardized using a z-score to have a mean of zero and a standard deviation of one. Cronbach's alpha values, for each of the three composite scales, were calculated and

ranged from 0.56 to 0.78 (perceived inequality in the family MIDUS II resurvey = 0.71, Milwaukee = 0.56; perceived inequality in the home MIDUS II resurvey = 0.78, Milwaukee = 0.65; perceived inequality in the work place MIDUS II resurvey = 0.75, Milwaukee = 0.64). A single score for perceived inequality was then computed by taking the sum of the three standardized composite scale scores to create one numeric value per participant.

Relationship Stress

The fifth component psychosocial stress domain was relationship stress. This component domain was comprised of questions from four composite scales, with each composite scale created based on formerly published scientific studies and scales addressing the issue of social support and relationship strain (Booth, Johnson, & Edwards, 1983; Schuster, Kessler, & Aseltine, 1990; Turner, Frankel, & Levin, 1983; Walen & Lachman, 2000). These composite scales included family strain, friend strain, marital risk and spouse/partner strain. The family strain composite scale had four questions that included “Not including your spouse or partner, how often do members of your family make too many demands on you?”, “How often do they criticize you?”, “How often do they let you down when you are counting on them?” and “How often do they get on your nerves?”. The friend strain composite scale had the same four questions that included “How often do your friends make too many demands on you?”, “How often do they criticize you?”, “How often do they let you down when you are counting on them?” and “How often do they get on your nerves?”. The marital risk composite scale had five

questions that included “During the past year, how often have you thought your relationship might be in trouble?”, “Realistically what do you think the chances are that you and your partner will eventually separate?” and “How much do you and your spouse or partner disagree on the following issues: (1) Money matters, such as how much to spend, save or invest? (2) Household tasks, such as what needs doing and who does it? (3) Leisure time activities, such as what to do and with whom?”. The spouse/partner strain composite scale had six questions that included “How often does your spouse or partner make too many demands on you?”, “How often does he or she argue with you?”, “How often does he or she make you feel tense”, “How often does he or she criticize you?”, “How often does he or she let you down when you are counting on him or her?” and “How often does he or she get on your nerves?”. The response options for each of the questions, within the family strain, friend strain and spouse/partner strain composite scales, were “Often”, “Sometimes”, “Rarely” and “Never”. The response options for the first question in the marital risk composite scale were “Never”, “Once”, “A few times”, “Most of the time” and “All of the time”. The response options for the second question in the marital risk composite scale were “Very Likely”, “Somewhat likely”, “Not very likely” and “Not likely at all”. The response options for the remaining three questions in the marital risk composite scale were “A lot”, “Some”, “A little” and “Not at all. A point value between 1-4 or 1-5, depending on the number of response options for the question being asked, was assigned to each question response with 1 point for the first listed response option and 4 or 5 points, respectively, for the final listed response option. All of the questions in the family strain, friend strain and spouse/partner strain

composite scales and questions two through five on the marital risk composite scale were reverse coded so that a higher score reflected a higher stress standing on that question. Composite scale scores were computed for individuals who had a valid response to at least one of the questions within that particular composite scale. Composite scale scores were not computed for individuals who had a valid response to zero questions within that particular composite scale and those individuals composite scale scores were set to missing. Each of the four composite scale scores was then computed by calculating the mean score of the questions within each scale. These composite scale scores were then standardized using a z-score to have a mean of zero and a standard deviation of one. Cronbach's alpha values, for each of the four composite scales, were calculated and ranged from 0.64 to 0.87 (family strain MIDUS II resurvey = 0.79, Milwaukee = 0.80; friend strain MIDUS II resurvey = 0.79, Milwaukee = 0.80; marital risk MIDUS II resurvey = 0.77, Milwaukee = 0.64; spouse/partner strain MIDUS II resurvey = 0.87, Milwaukee = 0.83). A single score for relationship stress was then computed by taking the sum of the four standardized composite scale scores to create one numeric value per participant.

Neighborhood Stress

The sixth component psychosocial stress domain was neighborhood stress. This component domain was comprised of items from one composite scale, which was obtained from an article on social well-being written by Dr. Corey Keyes in the journal of Social Psychology Quarterly (Keyes, 1998). This composite scale was titled personal

beliefs on neighborhood and 47 incorporated the use of four items that included “Please indicate how much each of the following statements describes your situation: (1) “I feel safe being out alone in my neighborhood during the daytime.” (2) “I feel safe being out alone in my neighborhood at night.” (3) “I could call on a neighbor for help if I needed it.” and (4) “People in my neighborhood trust each other.” The response options for each of these four items were “A lot”, “Some”, “A little” and “Not at all”. A point value between 1-4 assigned to each item response with 1 point for “A lot” to 4 points for “Not at all”. The composite scale score was computed for individuals who had a valid response to at least one of the items within that composite scale. Composite scale scores were not calculated for individuals who had valid responses to no items within that composite scale and those individuals composite scale scores were set to missing. The personal beliefs on neighborhood composite scale score was then computed by calculating the mean score of the four items within the scale. This composite scale score was then standardized using a z-score to have a mean of zero and a standard deviation of one. A Cronbach’s alpha value for the personal beliefs on neighborhood composite scale was calculated with a score of 0.64 for the MIDUS II resurvey participants and 0.59 for the Milwaukee supplemental sample participants. Given that there was only one composite scale score for neighborhood stress, that score from the standardized composite scale was also the single score for neighborhood stress that was assigned to each participant.

Discrimination

The seventh component psychosocial stress domain was discrimination. This component domain was comprised of items from three composite scales, with each composite scale developed by Dr. David Williams and colleagues for use in a study examining racial discrimination in Detroit based on results from previously published qualitative studies assessing discrimination (Collins & Essed, 1992; Feagin, 1991; Williams, Yu, Jackson, & Anderson, 1997). These composite scales included lifetime discrimination, daily discrimination and chronic job discrimination. The lifetime discrimination composite scale had eleven questions that included "How many times in your life have you been discriminated against in each of the following ways because of such things as your race, ethnicity, gender, age, religion, physical appearance, sexual orientation or other characteristics: (1) You were discouraged by a teacher or advisor from seeking higher education? (2) You were denied a scholarship? (3) You were not hired for a job? (4) You were not given a promotion? (5) You were fired? (6) You were prevented from renting or buying a home in the neighborhood you wanted? (7) You were prevented from remaining in a neighborhood because neighbors made life so uncomfortable? (8) You were hassled by the police? (9) You were denied a bank loan? (10) You were denied or provided inferior medical care? (11) You were denied or provided inferior service by a plumber, car mechanic, or other service provided?". The daily discrimination composite scale had nine items that included "You are treated with less courtesy than other people.", "You are treated with less respect than other people.", "You receive poorer service than other people at restaurants or stores.", "People act as if they think you are not smart.", "People act as if they are afraid of you.", "People act as

if they think you are dishonest.", "People act as if they think you are not as good as they are.", "You are called names or insulted." and "You are threatened or harassed.". The chronic job discrimination composite scale had six questions that included "How often do you think you are unfairly given the jobs that no one else wanted to do?", "How often are you watched more closely than other workers?", "How often does your supervisor or boss use ethnic, racial or sexual slurs or jokes?", "How often do your co-workers use ethnic, racial or sexual slurs or jokes?", "How often do you feel that you are ignored or not taken seriously by your boss?" and "How often has a co-worker with less experience and qualifications than you gotten promoted before you?". The response options for each of the questions in the lifetime discrimination composite scale were a count, indicating the number of times the event occurred. The response options for each of the items in the daily discrimination composite scale were "Often", "Sometimes" "Rarely" and "Never". The response options for each of the questions in the chronic job discrimination composite scale were "Once a week or more", "A few times a month", "A few times a year", "Less than once a year" and "Never". For the lifetime discrimination composite scale, a raw count was reported for each of the questions in the scale. For the remaining two composite scales, daily discrimination and chronic job discrimination, a point value between 1-4 or 1-5, depending on the number of response options for the item/question being asked, was assigned to each item response with 1 point for the first listed response option and 4 or 5 points, respectively, for the final listed response option. All of the items in the daily discrimination and chronic job discrimination composite scales were reverse coded so that a higher score reflected a higher stress

standing on that item. If an item was missing a score, in either the daily discrimination or chronic job discrimination composite scales, the mean value, obtained from the completed items within that designated scale, was imputed for that item. Composite scale scores were computed for individuals who had a valid, non-imputed, response to at least one item, for the lifetime discrimination composite scale; five items, for the daily discrimination composite scale; or three items, for the chronic job discrimination composite scale. Composite scale scores were not computed for individuals who had valid, non-imputed, responses to fewer than the required number of responses for that particular composite scale and those individuals composite scale scores were set to missing. Each of the three composite scale scores was calculated by summing together the item scores from within each of the scales. These composite scale scores were then standardized using a z-score to have a mean of zero and a standard deviation of one. Cronbach's alpha values, for the daily discrimination and chronic job discrimination composite scales, were calculated and ranged from 0.76 to 0.92 (daily discrimination MIDUS II resurvey = 0.92, Milwaukee = 0.88; chronic job discrimination MIDUS II resurvey = 0.76, Milwaukee = 0.83). A single score for discrimination was then computed by taking the sum of the three standardized composite scale scores to create one numeric value per participant.

Current Financial Stress

The eighth component psychosocial stress domain was current financial stress. This component domain was comprised of questions from one composite scale. This

composite scale was titled financial stress and incorporated two questions that included “In general, would you say you (and your family living with you) have more money than you need, just enough money for your needs, or not enough money to meet your needs?” and “How difficult is it for you (and your family) to pay your monthly bills?”. The response options for the first question were “More money than you need”, “Just enough money for your needs” and “Not enough money to meet your needs”. The response options for the second question were “Very difficult”, “Somewhat difficult”, “Not very difficult” and “Not at all difficult”. A point value between 1-3 or 1-4, depending on the number of response options to the question being asked, was assigned to each question response with 1 point for the first listed response option and 3 or 4 points, respectively, for the final listed response option The second question was reverse coded so that a higher score reflected a higher stress standing on that question. The composite scale score was computed for individuals who had a valid response to at least one of the two questions within the scale. The composite scale score was not computed for individuals who did not have a valid response to at least one of the two questions within the scale and those individuals composite scale score was set to missing. The financial stress composite scale score was calculated by summing together the two question scores from within the scale. This composite scale score was then standardized using a z-score to have a mean of zero and a standard deviation of one. A Cronbach’s alpha value for the financial stress composite scale was calculated with a score of 0.79 for the MIDUS II resurvey participants and 0.66 for the Milwaukee supplemental sample participants. Given that there was only one composite scale score

for current financial stress, that score from the standardized composite scale was also the single score for current financial stress that was assigned to each participant.

Past Year Problems in the Immediate Family

The ninth component psychosocial stress domain was past year problems in the immediate family. This component domain was comprised of questions from three composite scales, with each composite scale created based on formerly published scientific studies and scales examining stress as it relates to the experience of major life events, traumatic events and overall chronic stress (Henderson, 1981; Holmes & Rahe, 1967; Sarason, Johnson, & Siegel, 1978; Turner, Wheaton, & Lloyd, 1995; Wheaton, 1994). These composite scales included past year problems for spouse/partner, past year problems for parents and past year problems for children. The past year problems for spouse/partner composite scale had ten questions that included “Please indicate whether the following problems have happened to your spouse/partner in the past 12 months: (1) Chronic disease or disability? (2) Frequent minor illness? (3) Emotional problems (such as sadness, anxiety)? (4) Alcohol or substance problems? (5) Financial problems, such as low income or heavy debts? (6) Problems at school or at work (such as failing grades, poor job performance)? (7) Difficulty finding or keeping a job? (8) Marital or partner relationship problems? (9) Legal problems (such as involved in lawsuits, police charges, traffic violations)? and/or (10) Difficulty getting along with people?”. The past year problems for parent’s composite scale asked the same ten questions, but instead of asking about the spouse/partner, asked about the parents. The

past year problems for children composite scale, again, asked the same ten questions, but instead of asking about the spouse/partner, asked about the children. The response options for each of these questions, within the three past year problems in the immediate family composite scales, were “no” and “yes”. A point value between 0-1 was assigned to each question response with 0 points for “no” to 1 point for “yes”. Composite scale scores were computed for individuals who had a valid response to at least one of the questions within that particular composite scale. Composite scale scores were not computed for individuals who had no valid responses to the questions within that particular composite scale and those individuals composite scale scores were set to missing. Each of the three composite scale scores was calculated by summing together the question scores from within each of the scales. These composite scale scores were then standardized using a z-score to have a mean of zero and a standard deviation of one. A single score for past year problems in the immediate family was then computed by taking the sum of the three standardized composite scale scores to create one numeric value per participant.

Stressful Life Experiences

The tenth component psychosocial stress domain was stressful life experiences. This component domain was comprised of questions from two composite scales, with each composite scale created based on formerly published scientific studies and scales examining stress as it relates to the experience of major life events, traumatic events and overall chronic stress (Henderson, 1981; Holmes & Rahe, 1967; Sarason et al.,

1978; Turner et al., 1995; Wheaton, 1994). These composite scales included past events within five years and lifetime stress events (6+ years ago or no age identified). The past events within five years composite scale had twenty questions. These questions asked about events that occurred within the last five years and included "Flunked out of school?", "Fired from a job?", "Did not have a job for a long time when you wanted to be working?", "A parent died?", "Parents divorced?", "Spouse/partner engaged in (marital) infidelity?", "Significant difficulty with in-laws?", "Brother or sister died?", "Child died?", "Child experienced life threatening accident or injury?", "Lost your home to a fire, flood, natural disaster, etc.?", "Physically assaulted or attacked?", "Sexually assaulted (ex. forced sexual intercourse or other unwanted sexual contact)?", "Serious legal difficulties/prison?", "Detention in jail or comparable institution?", "Declared bankruptcy?", "Suffered a financial or property loss unrelated to work?", "Went on welfare?", "Entered the armed forces?" and "Experienced combat?". The lifetime stress events composite scale asked the same twenty questions, along with three additional questions that included "Homeless in the past 15 years?", "No telephone in home or apartment in the past 15 years?" and "Marriage ended in divorce or widowhood?", but asked about events that occurred more than 6 years ago or had no identifiable age. The response options for each of these questions, within both of the stressful life experiences composite scales, were "no" and "yes". A point value between 0-1 was assigned to each question response with 0 points for "no" to 1 point for "yes". Composite scale scores were computed for individuals who had a valid response to at least one of the questions within that particular composite scale. Composite scale

scores were not calculated for individuals who had no valid responses to the questions within that particular composite scale and those individuals composite scale scores were set to missing. Both of the composite scale scores were calculated by summing together the question scores from within each of the scales. These composite scale scores were then standardized using a z-score to have a mean of zero and a standard deviation of one. A single score for stressful life experiences was then computed by taking the sum of the two standardized composite scale scores to create one numeric value per participant.

Early Life Stress

The eleventh component psychosocial stress domain was early life stress. This component domain was comprised of questions from three composite scales, with each composite scale created based on formerly published scientific studies and scales examining stress as it relates to the experience of major life events, traumatic events and overall chronic stress (Henderson, 1981; Holmes & Rahe, 1967; Sarason et al., 1978; Turner et al., 1995; Wheaton, 1994). These composite scales included count of child-adolescent specific stress experiences, relationship with parents and verbal or physical assault by parents (conflict tactics inventory) (Straus, 1979). The count of child-adolescent specific stress experiences composite scale had nine questions that included “The following questions are about experiences that you may have had as a child or teenager. Did you have any of the following experiences as a child or teenager: (1) Repeated a year of school? (2) Sent away from home because you did something

wrong? (3) Father or mother did not have a job when they wanted to be working? (4) One or both of your parents drank so often that it caused problems? (5) One or both of your parents used drugs so often that it regularly caused problems? (6) Dropped out of school? (7) Expelled or suspended from school? (8) Ever received welfare or ADC for a period of 6 months or more during childhood or adolescence?" and (9) "Moved to a totally new neighborhood or town 2 or more times during childhood?". The relationship with parent's composite scale had two questions that included "How would you rate your relationship with your mother during the years you were growing up?" and "How would you rate your relationship with your father during the years you were growing up?". The verbal or physical assault by parents composite scale had six questions that included "During your childhood, how often did your mother, or the woman you raised you, (1) insulted you or swore at you, sulked or refused to talk to you, stomped out of the room, did or said something to spite you, threatened to hit you or smashed or kicked something in anger? (2) pushed, grabbed or shoved you, slapped you, threw something at you? (3) kicked, bit, or hit you with a fist, hit or tried to hit you with something, beat you up, choked you, burned or scalded you?" and "During your childhood, how often did your father, or the man who raised you, (4) insulted you or swore at you, sulked or refused to talk to you, stomped out of the room, did or said something to spite you, threatened to hit you or smashed or kicked something in anger? (5) pushed, grabbed or shoved you, slapped you, threw something at you? (6) kicked, bit, or hit you with a fist, hit or tried to hit you with something, beat you up, choked you, burned or scalded you?". The response options for each of the questions within the count of child-adolescent

specific stress experiences composite scale were “no” and “yes”. The response options for each of the questions within the relationship with parent’s composite scale were “Excellent”, “Very good”, “Good”, “Fair” and “Poor”. The response options for each of the questions within the verbal or physical assault by parents composite scale were “Never”, “Rarely”, “Sometimes” and “Often”. A point value between 0-1 was assigned to each question response within the count of child-adolescent-specific stress experiences with 0 points for “No” to 1 point for “Yes”. A point value between 1-5 was assigned to each question response within the relationship with parent’s composite scale with 1 point for “Excellent” to 5 points for “Poor”. A point value between 1-4 was assigned to each question response within the verbal or physical assault by parent’s composite scale with 1 point for “Never” to 4 points for “Often”. Composite scale scores were computed for individuals who had a valid response to at least one of the questions within that particular composite scale. Composite scale scores were not computed for individuals who had no valid responses to the questions within that particular composite scale and those individuals composite scale scores were set to missing. Each of the three composite scale scores was calculated by summing together the question scores from within each of the scales. These composite scale scores were then standardized using a z-score to have a mean of zero and a standard deviation of one. Cronbach’s alpha values, for the relationship with parents and verbal or physical assault by parent’s composite scales, were calculated and ranged from 0.24 to 0.84 (relationship with parents MIDUS II resurvey = 0.56, Milwaukee = 0.24; verbal or physical assault by parents MIDUS II resurvey = 0.82, Milwaukee = 0.84). A single score for early life stress

was then computed by taking the sum of the three standardized composite scale scores to create one numeric value per participant. Missing Data Imputation For each of the composite scales, within our eleven component psychosocial stress domains, there were a number of individuals without an overall composite scale score due to missing data (range: 5.35%, missing the relationship with parents composite scale score, from the early life stress psychosocial stress domain, to 35.46%, missing the count of child-adolescent specific stress experiences composite scale score, also from the early life stress psychosocial stress domain, percentages based on the overall MIDUS II sample (n=5,555; MIDUS II resurvey n = 4,963, Milwaukee n = 592)). Therefore, imputation measures for the items missing values were created using IVEware software, to avoid having to exclude participants due to their missing data. This imputation method allowed for the participant's composite scale scores to be calculated, followed by the calculation of the component psychosocial stress domain scores and, ultimately, the summary index measure of overall stress value.

Summary Index Measure of Overall Stress

Following the item imputation, calculating the composite scale scores, standardizing the composite scale scores using a z-score and then computing the overall eleven component psychosocial stress domain values, each of the eleven component psychosocial stress domain values were then standardized using a z-score to have a mean of zero and a standard deviation of one. A single score for the summary index measure of overall stress was then computed by taking the sum of the eleven

standardized component psychosocial stress domains to create one numeric value per participant. This summary index measure of overall stress score was then, also, standardized using a z-score to have a mean of zero and a standard deviation of one.

Magnetic Resonance Image Acquisition and Preprocessing

Participants were imaged on a General Electric SIGNA 3.0 Tesla (Waukesha, WI) MRI system with an quadrature head coil. High resolution T1-weighted inverse recovery fast gradient echo MRI was acquired in 124 contiguous 1.2 – mm axial slices with the following parameters: TE = 1.8 ms, TR = 8.9 ms, flip angle = 30°, FOV = 240 mm, 256 x 256 data acquisition matrix, resulting in voxel dimensions of .9375 x .9375 x 1.2 mm. Head movement during scanning was minimized with padding.

Raw T1-weighted images were reconstructed using in-house software. Skull stripping was performed with AFNI's 3dSkullStrip (Cox, 1996), and inhomogeneities were smoothed with a multispectral segmentation/bias correction algorithm FAST (Zhang, Brady, & Smith, 2001). Images underwent skull and vessel correction, contrast-adjustment, and reorientation to the pathological plane in accordance with previously published work (Nacewicz et al., 2006).

Hippocampal Volume of Interest

Volume of interest (VOI) drawing of the hippocampus was done using in-house software (Spamalize; available at http://brainimaging.waisman.wisc.edu/~oaks/spam/spam_frames.htm) that allows for

simultaneous visualization in the three cardinal planes. Hand tracing of the whole hippocampal volume, as well as the three hippocampal segments (heady, body, and tail), were completed by LCP, after extensive training in hippocampal neuroanatomy. VOI definitions were completed according to protocols informed by the Duvernoy (1995) and Mai, Paxinos, & Voss (2008) neuroanatomical atlases. The hippocampus was defined as including the cornu Ammon, gyrus dentatus, subiculum and presubiculum, alveus, and fimbria, but excluding the fornix. Tracing began in the sagittal plane where the gray matter of the entire hippocampus was easily identified running inferiorly to the temporal horn of the lateral ventricle, and otherwise surrounded by white matter. Axial and coronal views were used to refine the initial VOI, though final corrections were done in the coronal plane.

Starting in the sagittal view, the anterior HH was differentiated from the amygdala by the white matter of the alveus. The superior border was defined by the temporal horn of the lateral ventricle (THLV) and white matter superior to temporal cortex provided an inferior border. The posterior border was defined by the trigone of the lateral ventricle and white matter immediately inferior to the ventricle. This initial VOI was refined on an individual pixel-by-pixel basis in all three views, with the coronal view providing the most informative comparison with the atlases.

In the coronal plane, the anterior HH was drawn either inferior to or adjacent to the anterior portion of the THLV, which also provided the lateral border. The medial border was identified by the shape of the white matter of the parahippocampal gyrus, forming a “V” inferior to the hippocampus. The uncus was included in the hippocampal

VOI when the uncul sulcus began to extend medially into the ambient cistern. The superior border of the HH was differentiated from the amygdala by following, medially, the arch of lateral ventricle. The superior border was also informed by either following (and including) the white matter of the alveus or using an approximated horizontal line starting at the superior edge of the THLV extending medially through to the ambient cistern. The inferior border was identified by white matter differentiating hippocampus from the parahippocampal gyrus.

When identifying the most posterior aspect of the HC (the HT) the crus of the fornix, fasciolar gyrus, Andreas Retzius gyrus, fasciola cinerea, and gyrus fasciolaris were included. These structures were differentiated from the pulvinar complex of the thalamus by the difference in gray scale, as the thalamus is a paler shade of gray, interlaced by many fibers.

Hippocampal Sub-region VOI Segmentation

The hippocampus was segmented into the HH, HB, and HT. The protocol for the delineation of HH/HB and HB/HT borders was informed by relevant literature (Malykhin et al., 2007; Pruessner et al., 2000) and neuroanatomical atlases (Duvernoy, 1995; Mai et al., 2008). The HH is the anterior-most segment of the hippocampus followed posteriorly by the HB and HT, respectively (Figs. 1 and 2). Our HH/HB and HB/HT borders are based on numerous landmarks in the coronal plane identified primarily anterior-posterior, but also reconfirmed through posterior-anterior, navigation; the sagittal plane was used as a reference during border identification. We have established

a set of aggregate criteria (Table 2) based on coronal plane landmarks to identify the HH/HB border and a single criterion to identify the HB/HT border.

Head/Body Border Identification

In accordance with previously outlined criteria for whole hippocampus VOI segmentation, the most anterior aspect of the HH was defined as the coronal slice where the THLV appeared and/or the alveus was present. Navigating posteriorly from the most anterior HH slice, we defined the head/body border based on five aggregate criteria (Table 2) due to individual differences in MRI that made it difficult to differentiate some neuroanatomical landmarks across all brains. Based on two primary criteria, the most anterior slice of the HB was defined as the coronal slice in which the uncus was no longer present and the ventricle was clear of tissue. The uncus forms a medial hump in the HH, but separates from the HC as one nears the HB. Uncal sulcus or other tissue can be visualized in the ambient cistern, located inferior and medial to the HC, or the THLV, which is located superior and lateral to the HC. Until navigating posteriorly to a slice in which the “ventricle” (ambient cistern and THLV) is clear of tissue, the hippocampal sub-region was defined as the HH. It is important to note that the uncus takes many forms when separating from the HC. It may appear as a clearly separate ball of tissue or a peninsula-like extension, or arm, of the superomedial HC. Identification of the uncus is facilitated by following its posterior progression beginning in the anterior slices of the HH. The shape of the HC is also valuable to identify HH and HB slices. The HH is generally wide and shaped like a kidney bean, indented slightly on

its superior border due to the medial separation of the uncal sulcus. The superomedial border could also be described as stair-shaped in contrast with a smooth, straight, and angled superomedial border in the HB. The HB is located more laterally within the medial temporal lobe and generally possesses a circular shape. The change in shape is, in part, to the thinning of the inferomedial grey matter, which can be visualized and used as a neuroanatomical reference. Finally, the sagittal slice can be referenced to verify correct identification of the HH/HB border based on whether the border slice of interest falls within the large, bulbous HH, or narrowed, long HB. Strict attention was given to the superior HB border in order to avoid inclusion of the tail of the caudate, lateral geniculate and stria terminalis.

Body/Tail Border Identification

Based on the protocol used in Malykhin et al. (2007), we used a single criterion to identify the HB/HT border in the coronal plane. Navigating posteriorly from the HB, we defined the most anterior HT slice as that in which the tracer could visualize the crus of the fonix in full profile (Fig. 3). The fornix emerges superior to the HT. In the coronal plane, the crus of the fornix (full profile) is formed by a merging of fornix tissues superiolateral and inferiolateral to the pulvinar complex of the thalamus. Due to anatomical differences and differences in MRI quality, we considered the anterior border of the HT as the most anterior slice in which at least 75% of the crus of the fornix is visualized. The HT is bordered laterally by the lateral ventricle, medially by either CSF or white matter, inferiorly by white matter, and superiorly by either lateral ventricle or white

matter. Care was taken to avoid including the pulvinar complex of the thalamus and tail of caudate on the superior border. White matter of the fornix was generally excluded, whereas the Fasciolar gyrus and Andreas Retzius gyrus were included (Amaral & Lavenex, 2007). The posterior slice of the HT was identified as the most posterior coronal slice in which grey could be visualized inferiomedial to the lateral ventricle (Pruessner et al., 2000).

VOI Reliability Procedure

LCP met reliability standards before beginning manual segmentation of hippocampal VOIs for this study. With a reliability sample of 9 VOIs, the intra-class correlation between the tracer's VOIs and a previously trained expert rater's was $r = 0.78$, $p < 0.05$, overlap >90%. The intersection over the union was >80%. For more detailed information on these reliability methods please reference previous lab publications (Dalton et al., 2005; Nacewicz et al., 2006).

Measurement of inter-rater and intra-rater reliability for HC segment segmentation was performed using reliability samples of 6 brains (12 hippocampi, 24 border identifications) and 10 brains (20 hippocampi, 40 border identifications), respectively. Reliability calculations were based on the number of identical borders chosen for the hippocampal HH/HB and HB/HT transitions along the longitudinal axis of the hippocampus in the coronal plane. Inter-rater reliability was 92%; intra-rater reliability was 88%. All hippocampal volumes were corrected for whole cerebrum volume.

Interleukin-6 and Soluble Interleukin-6 Receptor

Serum IL-6 levels were measured by high-sensitivity enzyme-linked immunosorbent assay (ELISA) (Quantikine, R&D Systems, Minneapolis, MN), with a lower sensitivity of detection at 0.16 pg/mL. All values were quantified in duplicate; any value over 10 pg/mL was re-run with sera diluted to fall on the standard curve. The laboratory intra-assay coefficient of variance (CV) was 4.1% and the inter-assay CV was 12.9% (generated by inclusion of a low and high IL-6 serum pool in each assay). Sandwich ELISA kits were also employed to quantify sIL-6r levels (Quantikine, R&D Systems). Sera was diluted 1:100 so values would fall on the standard reference curve from 7 to 2000 pg/mL. Thus, the effective assay range for sIL-6r was 0.7-200 ng/mL. The intra-assay and inter-assay CVs were 2.0% and 6.9%, respectively.

Cognitive Behavior

Cognition was assessed using the Brief Test of Adult Cognition by Telephone (BTACT), which includes six accuracy measures of key domains of cognitive aging. These are immediate and delayed 15-word-list free recall measures of episodic verbal memory. An episodic memory summary measure was created based on exploratory and confirmatory factor analysis of BTACT item scores. The episodic memory measure is comprised of scores of immediate and delayed word recall. Each summary score was computed as the mean of standardized-z-scores of component items, which was then also standardized to mean zero and standard deviation (SD) of one.

Statistical Analyses

Statistical analyses were performed using R version 3.1.3 statistical package (http://www.r-project.org/). Two-way interaction between psychosocial stress measures and IL-6 and sIL-6R, both as continuous variables, on hippocampal volumes was assessed. Since the psychosocial stress measures are not necessarily a linear scaled variable, two-way interaction analysis with psychosocial stress measures as a categorical variable was also performed. Regression analyses were done to investigate relationships between verbal memory performance and hippocampal volumes.

Mediation analyses were conducted to investigate whether IL-6 or sIL-6 levels mediated the relationship between psychosocial stress and hippocampal volumes or hippocampal-dependent behavior. Mediation analyses were conducted using a product-of-coefficient regression approach with nonparametric bootstrapping (5000 iterations) to attain 95% confidence intervals (CIs) for mediation effects (Preacher & Hayes, 2008). Unstandardized indirect effects were computed for each of the 10,000 bootstrap procedures, and the 95% confidence interval was computed by determining the indirect effects at the 2.5^{th} and 97.5^{th} percentiles.

Lastly, all analyses included sex and gender as covariates. IL-6 analyses also controlled for BMI.

RESULTS

Descriptive statistics on all variables are provided in Table 1. Missing data were limited: 1 individual had missing BMI data and 6 individuals had missing behavioral data. The degrees of freedom vary slightly among analyses, reflective of the missing data.

Age positively correlated with bilateral hippocampal head volume and negatively correlated with bilateral hippocampal body volume. Specifically, increasing age was associated with larger left (t_{71}= 3.12, r^2 = 0.15, p = 0.004) and right (t_{71}= 2.04, r^2 = 0.11, p = 0.02) hippocampal head volume, and smaller left (t_{71}= -2.61, r^2 = 0.09, p = 0.03) and right (t_{71}= -2.49, r^2 = 0.09, p = 0.03) hippocampal body volume. There were no significant main effects of either IL-6 or sIL-6R on age, hippocampal volumes, and psychosocial stress measures.

Interactions between psychosocial stress, hippocampal volumes, and inflammation

Interaction analyses revealed significant interactions between stress events from the past 5 years and sIL-6R on the left ($F(5, 68) = 4.30$, $p = 0.002$) and right ($F(5, 68) = 2.79$, $p = 0.02$) hippocampal head. Specifically, individuals with higher stress events in the past 5 years and higher levels of sIL-6R had lower bilateral hippocampal head volumes. Since stress events from the past 5 years isn't a linearly scaled measure, interaction analyses were conducted transforming this stress variable into an ordinal measure where individuals were grouped by low stress (mean – 1SD, N = 41), medium stress (within 1SD of the mean, N = 21), and high stress (mean + 1SD, N = 12). The

interaction remains significant for the left ($F(7, 66) = 3.0$, $p = 0.009$) and right hippocampal head ($F(7, 66) = 2.44$, $p = 0.03$) (Figures 1 & 2). There were no significant interactions between IL-6 and psychosocial stress on hippocampal volumes.

Behavioral results

One behavioral outlier was removed from the analysis. As hypothesized, episodic behavior correlated with hippocampal head volume. The factor score positively correlated with left hippocampal head volume ($t_{63}= 2.00$, $r^2 = 0.19$, $p = 0.004$). When individually looking at the tests that comprise the episodic factor score, this correlation was most likely driven by the number of total unique items recalled from the Word List Delayed, which positively correlated with bilateral hippocampal head volume (left: $t_{63}= 2.61$, $r^2 = 0.22$, $p = 0.001$; right: $t_{63}= 2.37$, $r^2 = 0.16$, $p = 0.009$) (Figures 3 & 4). In addition, stress from 6+ years was inversely correlated with both the episodic factor ($t_{63}= -2.86$, $r^2 = 0.38$, $p < 0.05$) and word list delayed total unique items ($t_{63}= -3.60$, $r^2 = 0.40$, $p < 0.05$) (Figure 5). There were no significant relationships between behavior and IL-6, sIL-6R, or stress events from the past 5 years.

Given that there were significant relationships of behavior with stress from the past 6+ years and bilateral hippocampal head volume, we assessed whether hippocampal head volume mediated the relationship between stress from the past 6+ years and word list delayed total unique items. The bootstrapped unstandardized mediation effect was (left head: 0.02, right head: -0.01) and the confidence interval ranged from (left head: -0.19 to 0.23; right head: -0.17 to 0.14). Thus, hippocampal head

volume did not significantly mediate the effect of cumulative stress on verbal memory. However, the relationship between cumulative stress and verbal memory remained significant after controlling for left (indirect effect = -1.22 ; CI: -2.00 to -0.44) and right (indirect effect = -1.21 ; CI: -2.00 to -0.42) hippocampal head volume

DISCUSSION

In this study, we investigated the relationships between psychosocial stress, inflammation, and hippocampal volumes. The main findings are individuals who experienced high stress in the past 5 years have elevated levels of sIL-6R and smaller bilateral anterior hippocampi. In addition, poor episodic memory behavior was associated with smaller bilateral anterior hippocampi and high cumulative stress. The relationship between high cumulative stress and poor episodic memory behavior remains after controlling for hippocampal head volume.

Interestingly, there were only relationships with sIL-6R and not IL-6. The membrane-bound IL-6 receptor is expressed predominantly by hepatocytes, neutrophils, monocytes/macrophages, and some lymphocytes (Hatziapostolou et al., 2011; Peters et al., 1996). The circulating soluble form of the IL6R (sIL6R), which can be detected in various bodily fluids and is secreted by monocytes, hepatocytes, and endothelial cells (Nilsson, Langley, & Fidler, 2005), is generated by two independent mechanisms, namely, limited proteolysis of the membrane-bound protein and translation from an alternatively spliced mRNA (Matthews et al., 2003). The two modes of IL-6 activation are presented as either classical IL-6 activation via membrane-bound IL6R (classical IL-6 signaling) or sIL6R-mediated cell signaling (IL-6 trans-signaling). The IL-6/sIL6R complex can activate IL-6 trans-signaling on cell types that do not express the membrane-bound IL6R; thus, sIL6R could play an important role in the systemic inflammatory response by acting as an agonist, amplifying and prolonging the actions of IL-6, and broadening the range of target cells and tissues (Rose-John, Scheller, Elson,

& Jones, 2006). It has also been argued that sIL6 is critically involved in the maintenance of a disease state, by promoting the transition from acute to chronic inflammation (Rose-John et al., 2006) and in mediating the pro-inflammatory activities of IL-6 (Scheller, Chalaris, Schmidt-Arras, & Rose-John, 2011).

There is growing evidence of a relationship between IL-6 trans-signalling via the sIL-6R and psychiatric illness, specifically stress-related disorders such as depression and anxiety. Maes et al. (1995) reported that serum sIL-6R levels were increased in both unipolar and bipolar depression with no significant differences between both groups. Serum sIL-6R concentrations are also significantly higher in patients with post-traumatic stress disorder (PTSD) with concurrent depression than in PTSD patients without depression and normal volunteers (Michael Maes et al., 1999). In different studies, Maes et al. found an inter-correlated increase in plasma IL-6 and sIL-6R levels in depressed individuals but not in controls (Maes et al., 1997; Maes et al., 1995). This association may be explained by a number of mechanisms; for example, sIL-6R and IL-6 may upregulate IL-6 synthesis (Maes et al., 1995). The lack of relationships with IL-6 and our other measures may be due to several reasons. Our participant cohort are cognitively healthy and don't present with comorbidities that can influence inflammation. In addition, the temporal dynamics of the transition from acute inflammation to chronic inflammation associated with health complications is unknown. A recent longitudinal study in humans provides the first evidence that peripheral inflammation predates the occurrence of depression. Although they did not measure sIL-6R, they found that children with higher circulating levels of IL-6 at age 9 were at a 10% greater risk of

developing MDD by age 18 than the general population or children with low levels of IL-6 (Khandaker, Pearson, Zammit, Lewis, & Jones, 2014). While these clinical data are promising, it will be important to investigate the IL-6 signaling cascade during the transition from acute to chronic inflammation.

In both classical and trans-signaling, the IL-6/IL-6R/gp130 complex activates intracellular signaling through the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway and the mitogen-activated protein kinase (MAPK) pathway. There is evidence that an imbalance away from the MAPK pathway via removal of regulation by suppressor of cytokine signaling 3 (SOCS3) towards the pro-inflammatory STAT3 signaling pathway contributes to autoimmune disease (Tanaka & Kishimoto, 2014) and therefore may also be a target for stress susceptibility. Another method through which circulating levels of IL-6 and its downstream mechanisms are altered is via the soluble form of gp130. While sIL-6R acts as an agonist, the soluble form of gp130 acts as an antagonist sequestering IL-6 and sIL-6R in blood (Garcia-Oscos et al., 2015; Wolf, Rose-John, & Garbers, 2014), thereby stopping IL-6 from activating trans-signaling but not classical signaling. Further research is needed to determine whether psychological stress alters soluble gp130, which may have potential use as an antidepressant.

We found that the interaction between peripheral sIL-6R and stress to be associated with smaller anterior hippocampi. In animal models, increased levels of IL-6 have been found in many areas of the brain as a function of psychosocial stress. Maternal deprivation leads to increased levels of pro-inflammatory cytokines, including

IL-6 in the CSF of rats (Reus et al., 2015). Increased IL-6 mRNA is found in microglia isolated directly from the brains of mice that have undergone a variant of repeated social defeat stress (Ramirez, Shea, McKim, Reader, & Sheridan, 2015). Treatment with the antidepressant imipramine blocked social avoidance behavior and reduced microglia IL-6 in animals exposed to stress or those given a systemic injection of LPS (Ramirez et al., 2015). Increased IL-6 protein was reported in the hippocampus of rats that underwent chronic unpredictable stress (Tianzhu, Shihai, & Juan, 2014) and was attenuated by chronic treatment with the antidepressant fluoxetine or treatment with an alternative medicine Cordycepin, a derivative of adenosine extracted from fungi shown to have antidepressant efficacy (Li et al., 2015). In a study examining healthy subjects, the polymorphism *rs1800795,* a genetic variant of IL-6, showed a strong main effect of genotype with the volume of the right hippocampus head. Homozygous carriers of the G-allele had significantly larger hippocampal gray matter volumes compared to heterozygous subjects (Baune et al., 2012).

As hypothesized, we found that delayed verbal memory to be associated with the anterior hippocampal volume. These results are consistent with Hackert et al. (2002) who found in a large sample selectively the anterior (head) of the hippocampus was associated with verbal memory performance. Our additional findings reveal that delayed verbal memory was also associated with cumulative stress and this relationship remained after controlling for the anterior hippocampal volume. Several studies suggest that increased neuroinflammatory signaling augments hippocampal neurobiological processes implicated in behavioral disorders involving neurogenesis (Koo & Duman,

2008) and metabolism of neurotransmitters (Miller, Maletic, & Raison, 2009; O'Connor et al., 2009). A recent study (McKim et al., 2015) found that stress from using a repeated social defeat model enhances neuroinflammation in the hippocampus, causes transient deficits in memory recall, alters neuronal cell fate, and prolongs social avoidance. Moreover, hippocampal-dependent memory deficits were caused by neuroinflammatory activation, whereas social avoidance and impaired neurogenesis were not. These findings suggest that perhaps hippocampal volume might not be sensitive enough to fully capture the effects of stress-induced neuroinflammation and that atrophy may not be occurring in this sample of cognitively healthy individuals.

In summary, recent psychosocial stress was associated with elevated levels of peripheral sIL-6R and smaller anterior hippocampi. Moreover, hippocampal-dependent behavior was related to anterior hippocampal volume and cumulative stress. Lastly, hippocampal volume did not mediate the relationship between cumulative stress and behavior. Future research with longitudinal multimodal imaging approaches will be necessary to more fully evaluate the extent to which psychosocial stress-induced inflammation impact hippocampal function and structure.

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Table 1. Demographic features

Total N	74
Female: N (%)	49 (66%)
Age (years): mean ± SD (range)	56.4 ± 10.8 (37 - 76)
BMI (kg/m2): mean ± SD (range)	28.7 ± 5.7 (19.2 – 48.4)
IL-6 (pg/ml): mean ± SD (range)	3.1 ± 3.1 (0.16 -18.4)
sIL-6R (pg/ml): mean ± SD (range)	32708.4 ± 9955.9 (17361 - 72990)
Episodic Memory	
Word List Immediate Recall: Mean ± SD	8.1 ± 11.3
Word List Delayed Recall: Mean ± SD	5.8 ± 11.7
Episodic Memory Factor: Mean ± SD	0.22 ± 1.4

BMI = body mass index; IL-6 = interleukin-6; sIL-6R = soluble interleukin-6 receptor

Word List Immediate and Delayed Recall scores reported above are raw mean scores and standard deviations (SD). Episodic Memory Factor is z-scored.

Table 2. Hippocampal head/body border identification

Criterion Summary	Criterion Satisfied?	
	Yes	**No**
A. Is there uncus? Has the uncus diminished from the superomedial aspect of the HC?	Body	Head
B. Is the ventricle clear of tissue? Are the ambient cistern and most medial part of the temporal horn of the lateral ventricle clear of tissue?	Body	Head
C. What is the shape of the HC? Is the HC wide and bean shaped, or circular and in a more lateral position? Is the superomedial border of the HC stair shaped or straight and angled?	Wide, stair shaped = Head Circular, angled = Body	
D. Has the inferomedial gray matter thinned? Has the inferiomedial grey matter thinned and receded as you navigate posteriorly toward the body?	Body	Head
E. How does the transition compare in the sagittal view? Does the most anterior body slice correspond with an obvious transition from head to body in the sagittal plane?	--	Refine border (A-D)

The segmentation protocol to differentiate the hippocampal body from hippocampal head is based on five criteria, A-E, summarized above. Criteria A and B were considered the most important, but C-E were utilized, as appropriate, depending on the visual clarity of the hippocampus and the surrounding brain structures.

Figure Legends

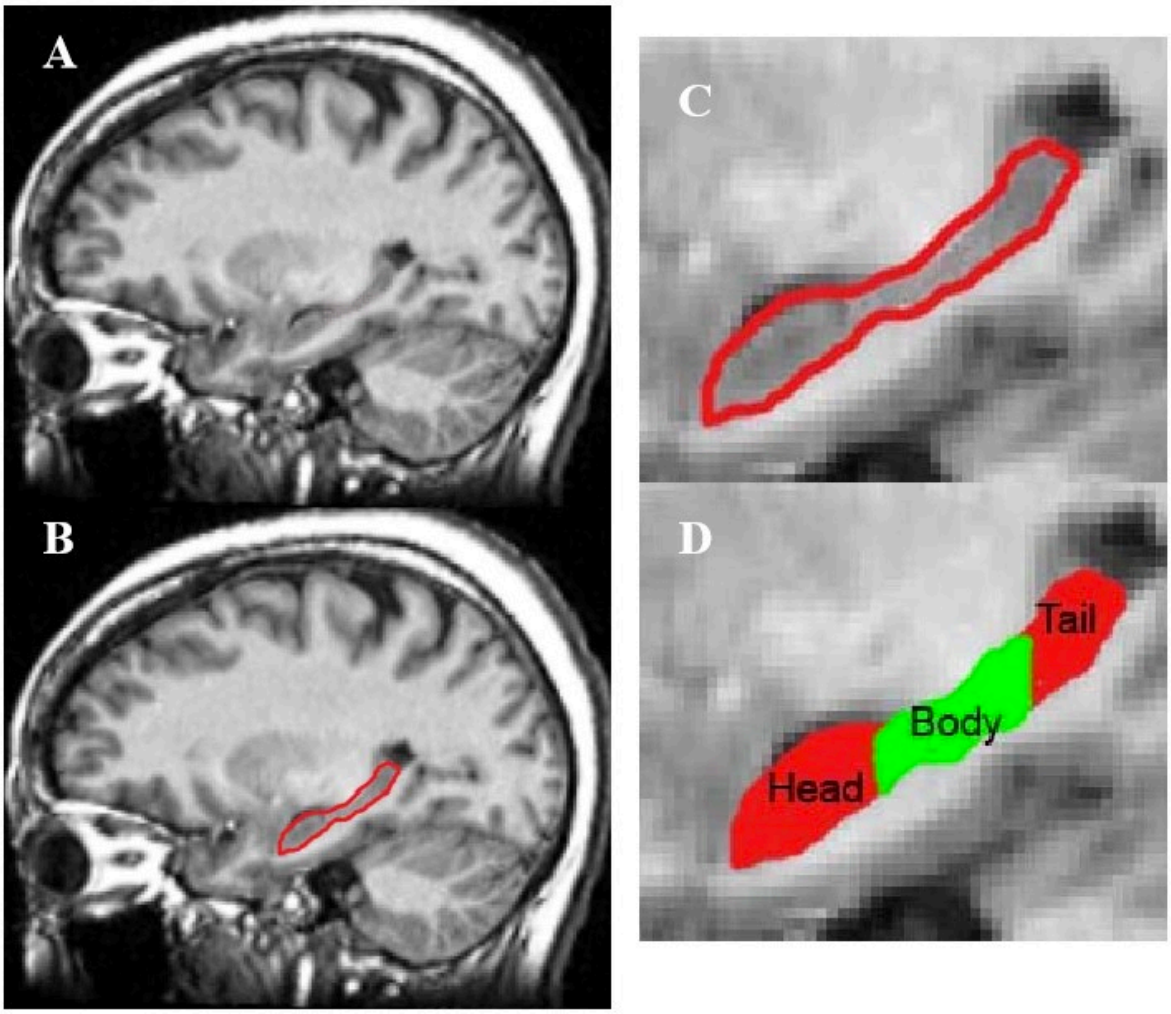

Figure 1. (A) Sagittal MRI image which displays the longitudinal axis of the hippocampus. (B) The whole hippocampus is highlighted with a red border. (C) A closer view of the hippocampus. (D) The hippocampal head, body, and tail defined by the segmentation criteria employed in this study.

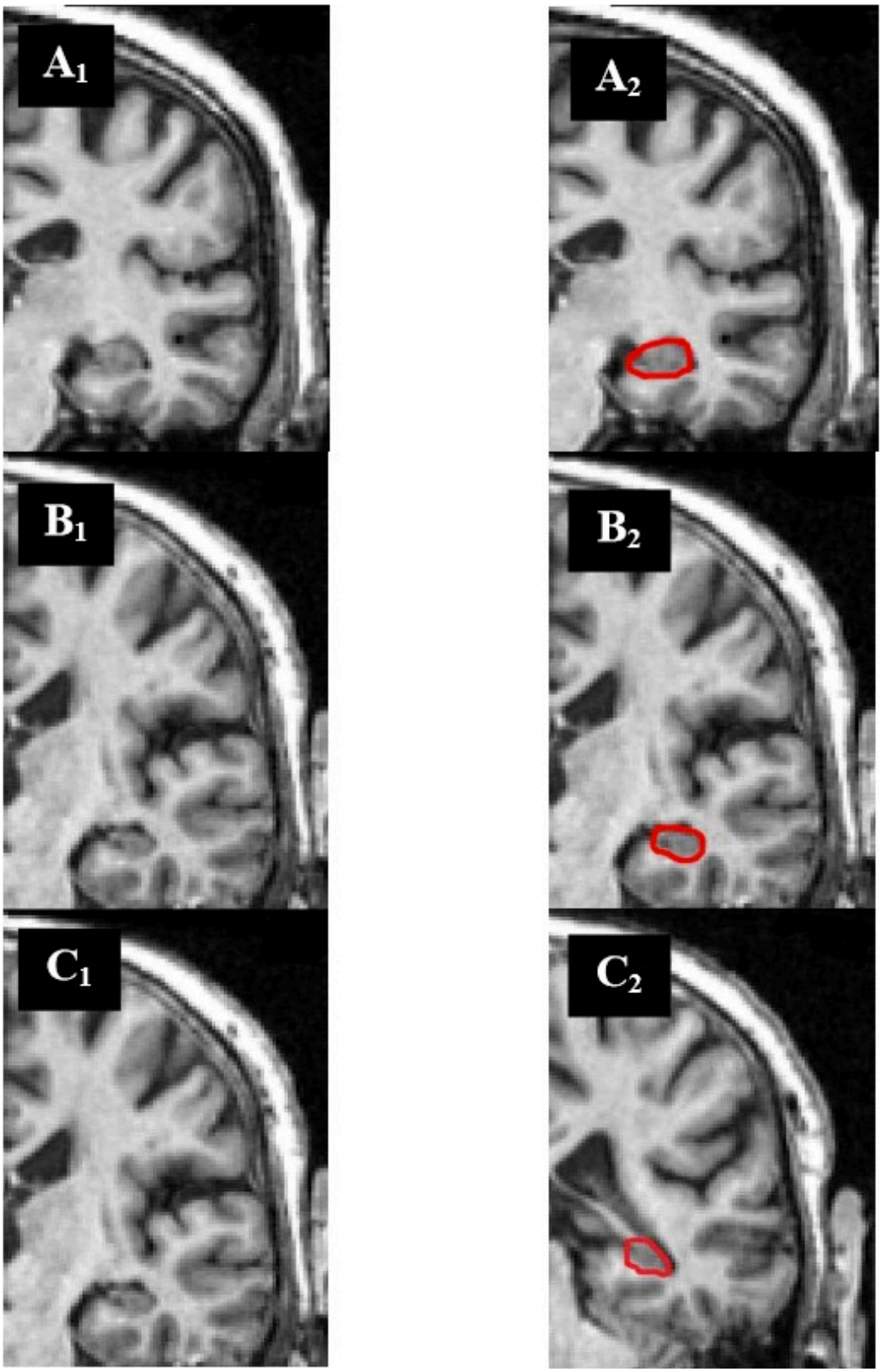

Figure 2. Coronal MRI slices. (A_1-A_2) Hippocampal head. (B_1-B_2) Hippocampal body. (C_1-C_2) Hippocampal tail.

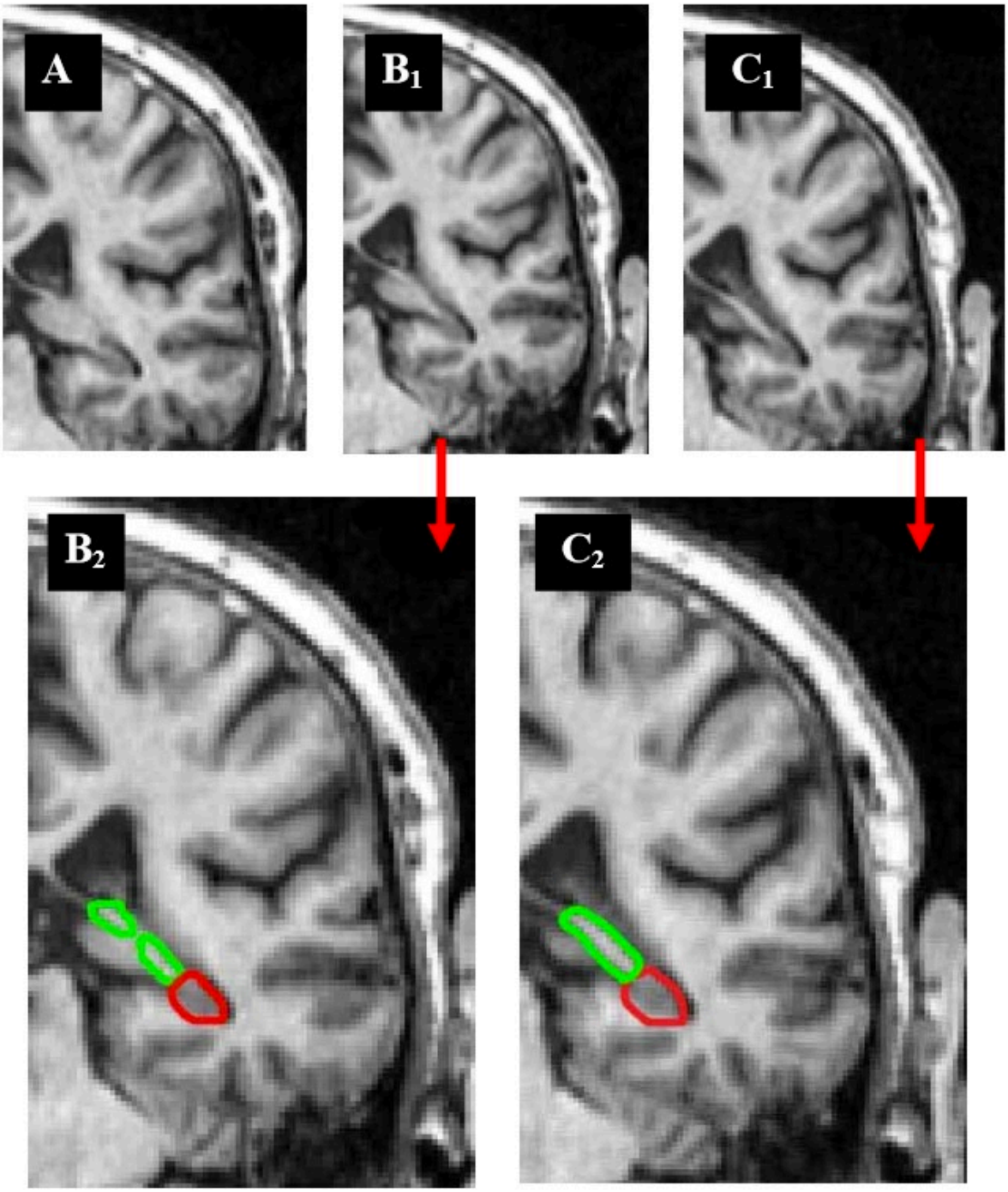

Figure 3. (A, B_1) Coronal MRI images of the hippocampal body immediately anterior to the hippocampal tail in which the crus of the fornix is not yet in full profile. (C_1) The hippocampal tail; the most anterior coronal MRI image in which the crus of the fornix appears in full profile. (B_2, C_2) The tissue from crus of the fornix (green) and the hippocampal tail (red). In identifical images C_1 and C_2, the crus of the fornix can be visualized in full profile.

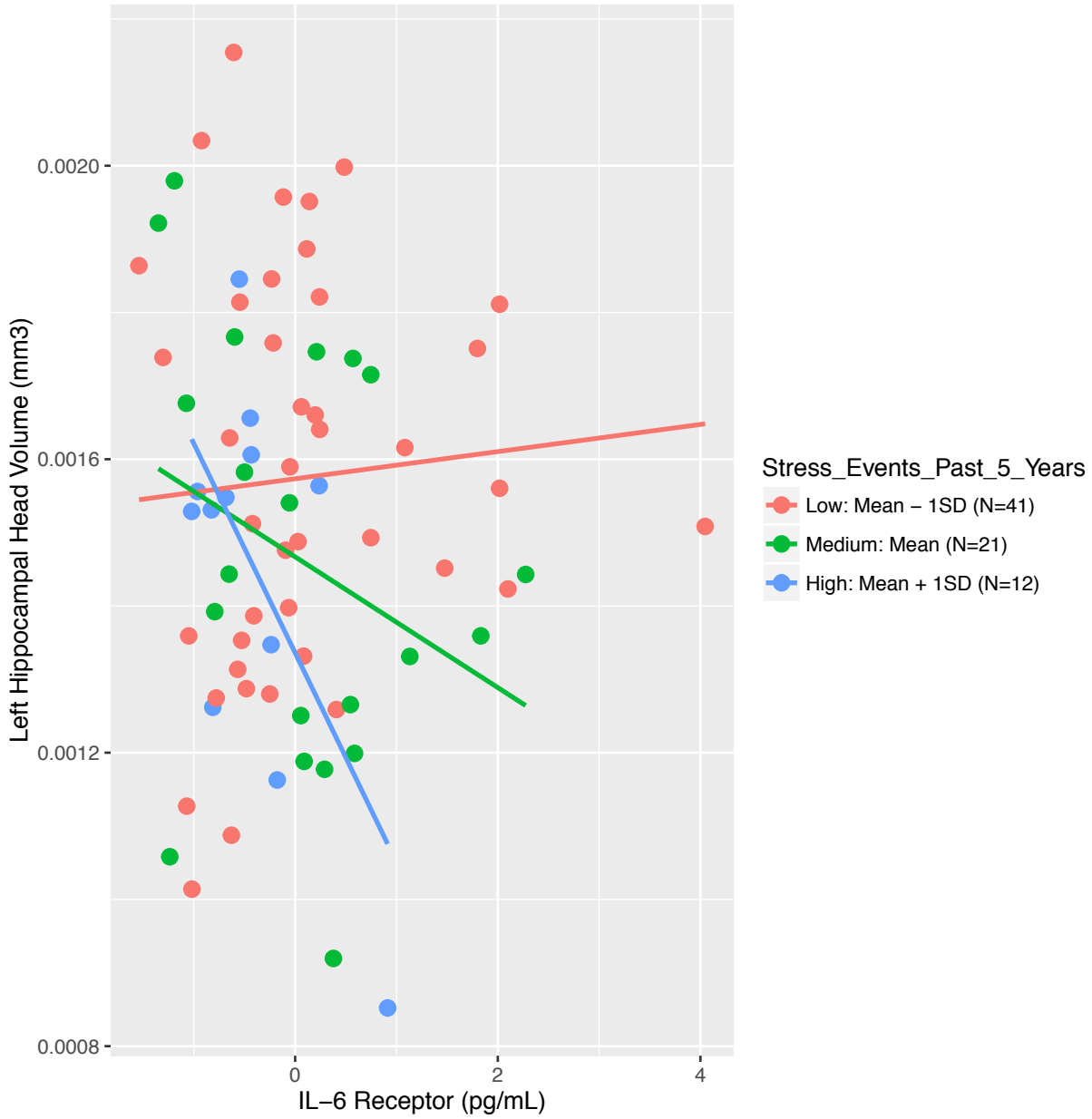

Figure 4. A significant interaction between stress events from the past 5 years and sIL-6R levels on left hippocampal head volume. Higher stress events from the past 5 years and concurrently high levels of sIL-6R are associated with smaller left hippocampal head volumes. “High” stress events was calculated as the mean + 1 standard deviation.

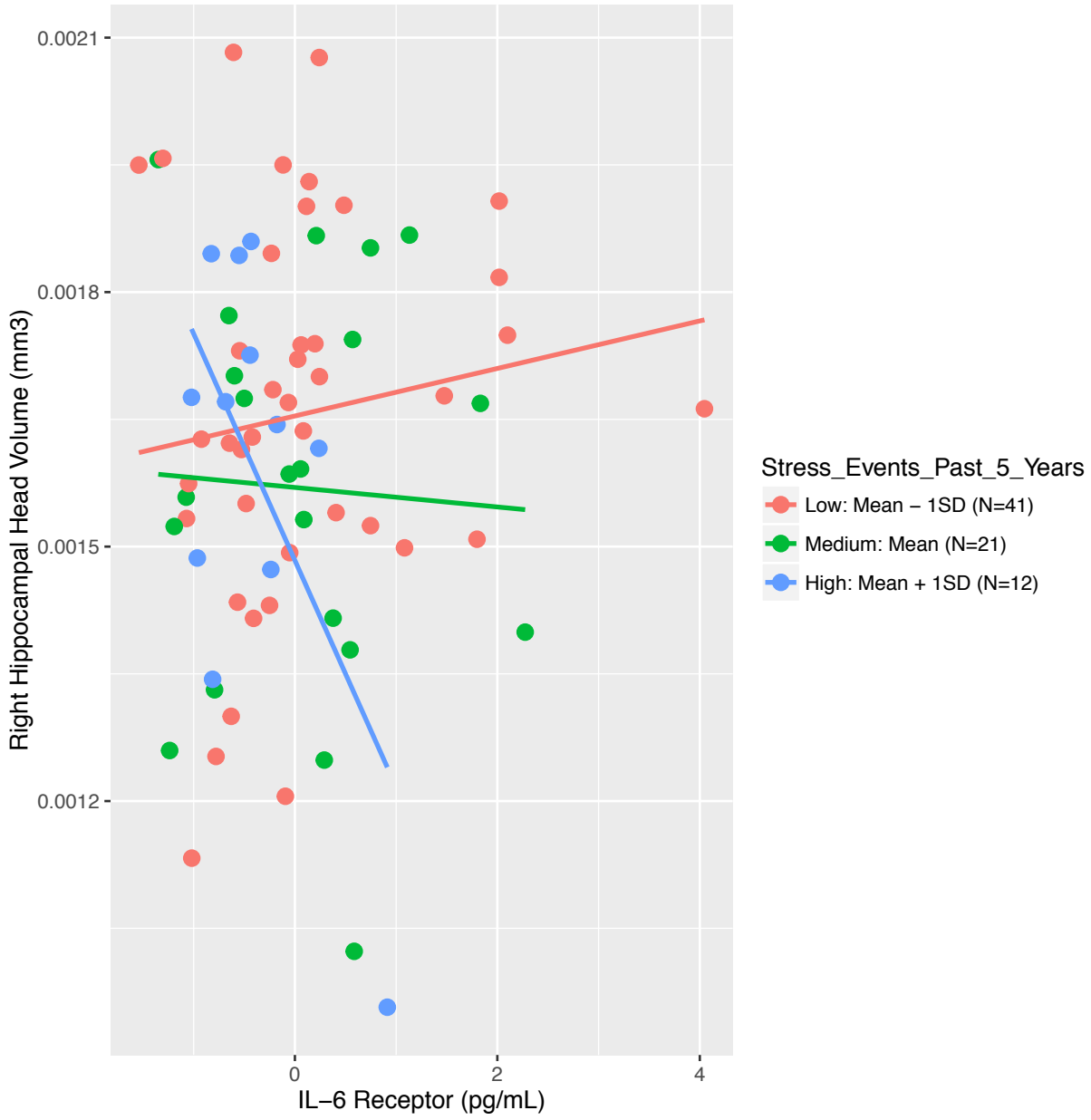

Figure 5. A significant interaction between stress events from the past 5 years and sIL-6R levels on right hippocampal head volume. Higher stress events from the past 5 years and concurrently high levels of sIL-6R are associated with smaller right hippocampal head volumes. “High” stress events was calculated as the mean + 1 standard deviation.

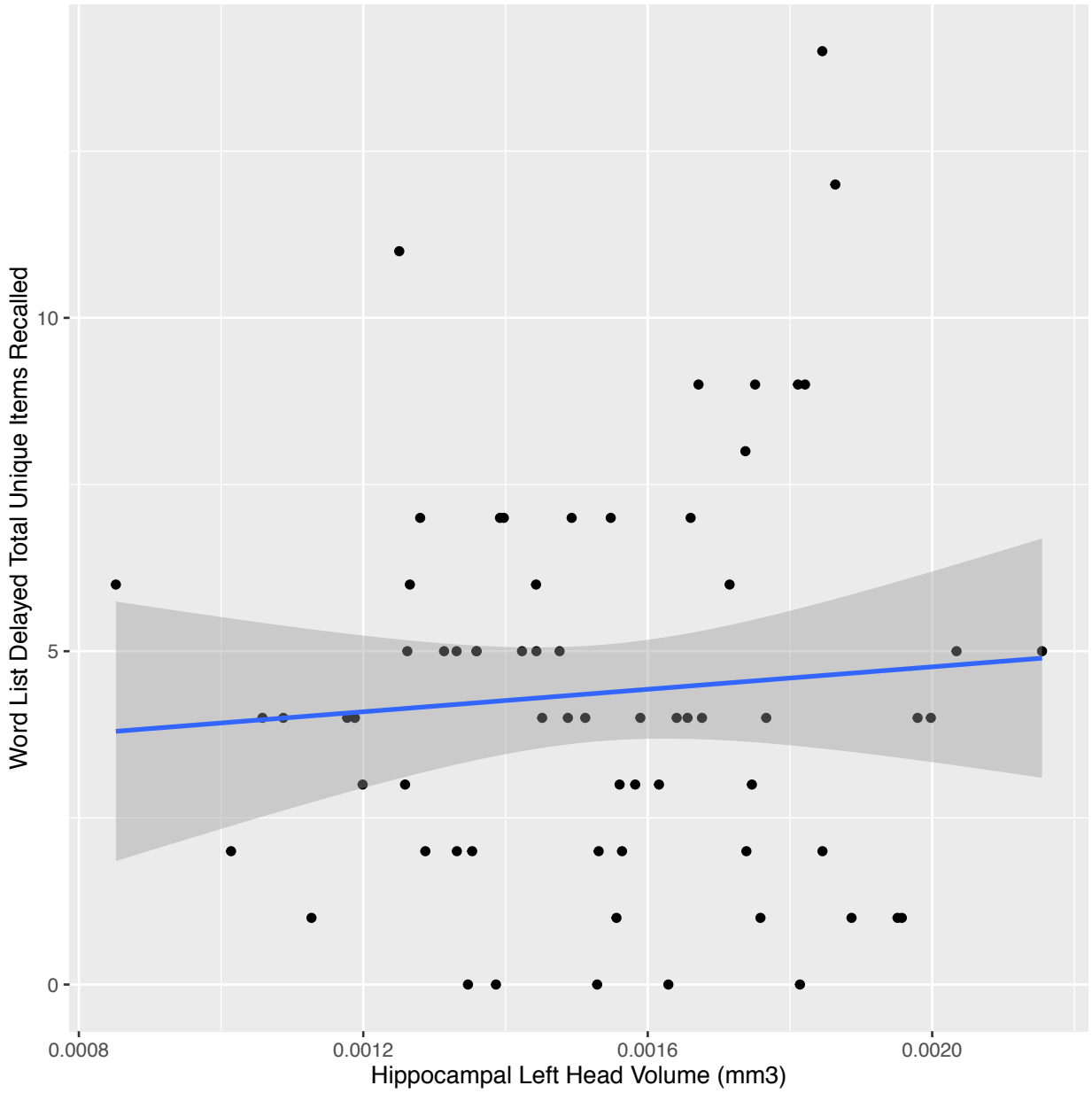

Figure 6. Left hippocampal head volume positively correlates with delayed verbal memory, specifically for the number of total unique items recalled after a delay.

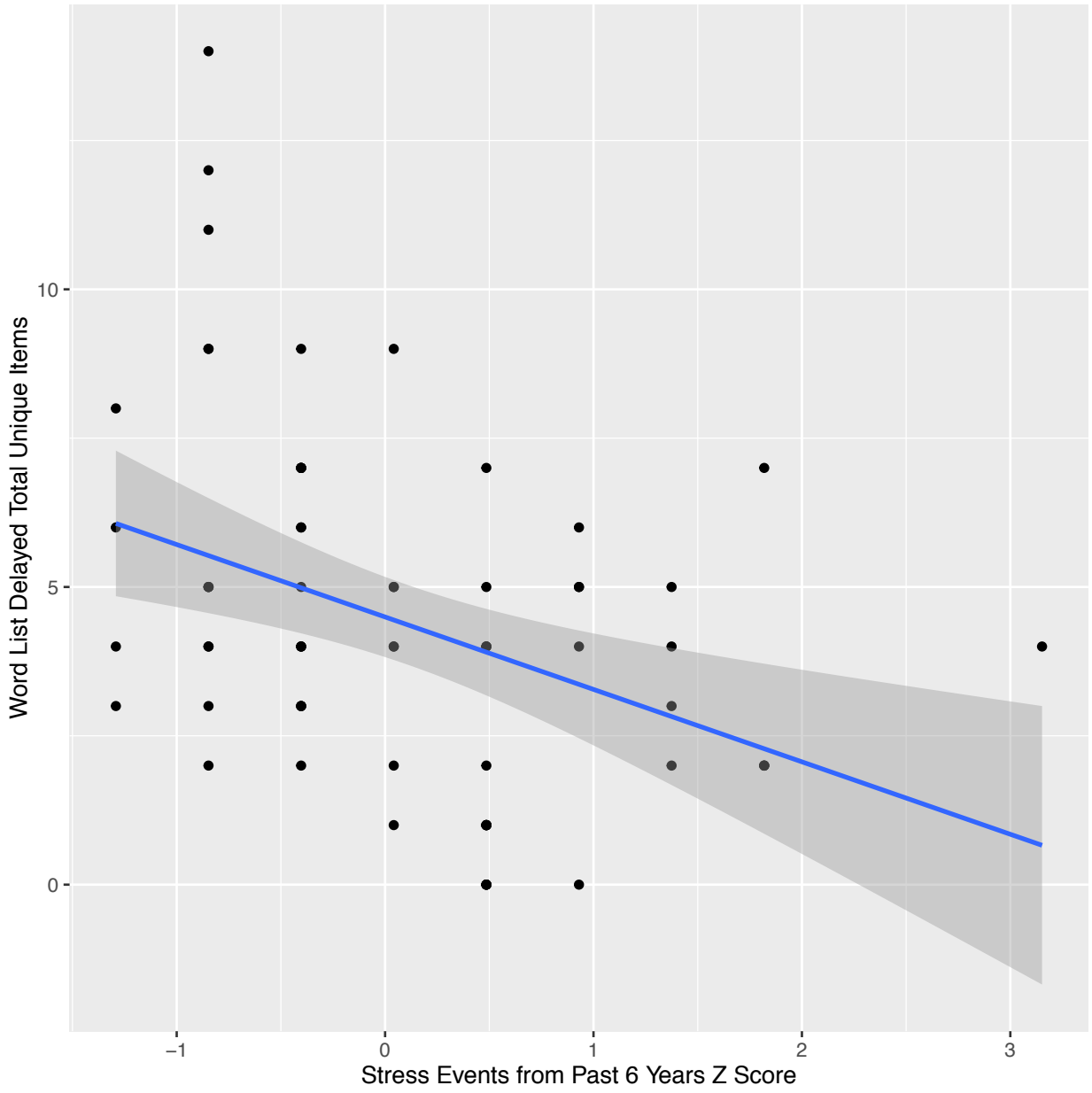

Figure 7. Cumulative stress negatively correlates with delayed verbal memory, specifically for the number of total unique items recalled after a delay.

CHAPTER 5
Conclusions & Future Directions

Conclusions

The work in this thesis provides new evidence about the temporal dynamics of brain aging, as well as demonstrating that a potentially modifiable factor may negatively impact brain regions vulnerable to age-related diseases such as Alzheimer's. Using multimodal neuroimaging methods and longitudinal analysis techniques, these findings has facilitated new insight into factors that affect brain structure and function during aging.

These findings are consistent with the convergence of research showing cerebral white matter to have an anterior-posterior gradient of decline (Buckner, 2004; Head et al., 2004; Raz, 2000). This decline in prefrontal white matter follows an inverted-U trajectory, with a linear increase in young adulthood, a plateau in middle age and significant contraction starting in the fifth decade of life (Bartzokis et al., 2001; Courchesne et al., 2000; Raz et al., 2004), the average age of our participants. This rate of decline increases with age (Raz et al., 2005), which is in line with other age-related acceleration in other indices of white matter integrity, such as MRI relaxation times (Bartzokis, 2004; Bartzokis et al., 2003) and ratio of small to large myelinated axons (Tang et al., 1997). These studies and the results from this thesis suggest that certain brain regions that are late to mature and which contain a high ratio of thinly myelinated fibers (e.g. prefrontal cortex) may be more susceptible to age-related atrophy.

The results from the functional connectivity study in this thesis and others shed light on how networks interact to subserve numerous cognitive processes. Anatomical and resting-state connectivity research suggest that brain regions are not part of single

networks but are part of multiple networks that have preferred connections or 'coordinated states' (Hellyer et al., 2014; McLaren, Sperling, & Atri, 2014). Importantly, the brain can rapidly switch between these coordinated states to perform different cognitive functions. The transition from one state to another enables the brain to integrate information from a multitude of brain regions. FA is sensitive to multiple microstructural properties of white matter, including axon density, myelination, and possibly diameter (Beaulieu, 2002; Takahashi et al., 2002). Dynamic connectivity-FA correlations might be caused by variation in axon density or diameter. Electromyography (EMG) amplitude and area both increase with fiber density in physiological studies of peripheral nerves; likewise, EMG amplitude and area increase with fiber density and axon diameter in computer simulations (Finsterer & Fuglsang-Frederiksen, 2000; Nandedkar & Stalberg, 1983). It is possible that increased myelination or axon diameter across individuals led to more-synchronized axonal conduction velocities, affecting the degree of interregional modulation observed.

Lastly, the data from the thesis reveal novel evidence for implicating a specific region of the hippocampus and soluble receptor levels for the cytokine IL-6 in individuals experiencing high psychological stress. IL-6 trans-signaling, which is conducted via sIL-6R is thought to be critically involved in the maintenance of a disease state, by promoting the transition from acute to chronic inflammation (Rose-John et al., 2006) and in mediating the pro-inflammatory activities of IL-6 (Scheller, Chalaris, Schmidt-Arras, & Rose-John, 2011). There is growing evidence of a relationship between IL-6 trans-signaling and psychiatric illness, specifically stress-related disorders such as depression

and anxiety. A recent longitudinal study in humans provides the first evidence that peripheral inflammation predates the occurrence of depression (Khandaker, Pearson, Zammit, Lewis, & Jones, 2014). Our participant cohort are cognitively healthy and don't present with comorbidities that can influence inflammation; however, it is possible that sIL-6R levels may predict subsequent peripheral inflammation that is associated with stress-related disorders.

In summary, the findings presented in this thesis fit into a larger body of work contributing to the emerging consensus for the vulnerability of frontal and medial temporal lobes in aging midlife adults.

Future Directions

The data presented in this thesis provide several avenues for continuing research. Histopathological studies have shown that aging is associated with white matter deterioration that include myelin pallor (Kemper, 1994), loss of myelinated fibers (Marner et al., 2003; Meier-Ruge et al., 1992; Pakkenberg and Gundersen, 1997), and in non-human primates, malformation of myelin sheaths (Peters and Sethares, 2002). All of these changes are candidates for influencing measurements of diffusion anisotropy, in addition to being candidates for predicting later volume loss. Additional histopathological studies will be needed to determine how closely microstructural changes link to overt volume loss, and combining structural MR imaging with postmortem data will be crucial in assessing the relationships between these molecular white matter changes and measurements of diffusion anisotropy.

Given the evidence that microstructural white matter differences influence both cognitive performance and functional connectivity in aging, it is unclear whether cognitive training interventions can improve age-related decline in white matter and decrease risk for dementia or other age-related diseases. Life experiences, such as bilingualism, have been shown to be associated with higher white matter microstructure as well as with more distributed patterns of functional connectivity in older adults (Luk, Bialystok, Craik, & Grady, 2011). An 8-week method of loci memory training increased cortical thickness (Engvig et al., 2010) and non-significantly increased FA (Engvig et al., 2012) in middle-aged and older adults and another study reported increased FA in older adults following a multidimensional program that involved working memory, episodic memory, and perceptual speed tasks. Overall the studies investigating the effect of training on the structure of the brain indicate that behavioral training can induce considerable structural plasticity in older adults; following these individuals longitudinally will further characterize who is at risk for age-related neurodegenerative diseases.

Lastly, a growing body of evidence supports immune-to-brain communication, with peripheral immune activation being associated with behavioral, affective, and cognitive disturbances. In both classical and trans-signaling, the IL-6/IL-6R/gp130 complex activates intracellular signaling through the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway and the mitogen-activated protein kinase (MAPK) pathway. There is evidence that an imbalance away from the MAPK pathway via removal of regulation by suppressor of cytokine signaling 3 (SOCS3) towards the pro-inflammatory STAT3 signaling pathway contributes to autoimmune disease (Tanaka

& Kishimoto, 2014) and therefore may also be a target for stress susceptibility. Moreover, another method through which circulating levels of IL-6 and its downstream mechanisms are altered is via the soluble form of gp130. While sIL-6R acts as an agonist, the soluble form of gp130 acts as an antagonist sequestering IL-6 and sIL-6R in blood (Garcia-Oscos et al., 2015; Wolf, Rose-John, & Garbers, 2014), thereby stopping IL-6 from activating trans-signaling but not classical signaling. Further research is needed to determine whether psychological stress alters soluble gp130, which may have potential use as an antidepressant.

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