Published in final edited form as:

Hippocampus. 2013 December ; 23(12): 1179–1188. doi:10.1002/hipo.22156.

Hippocampal Network Connections Account for Differences in Memory Performance in the Middle-Aged Rhesus Monkey

Bang-Bon Koo¹, Adrian L. Oblak^{1,2}, Yansong Zhao³, Chad W. Farris¹, Bethany Bowley¹, Douglas L. Rosene¹, and Ronald J. Killiany^{1,*}

¹Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, Massachusetts

²Department of Pathology & Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN

³Philips healthcare, Cleveland, Ohio

Abstract

Recent neurophysiological and functional neuroimaging studies suggest that the memory decline found with normal aging is not solely due to regional disruptions in the hippocampus, but also is brought about by alterations in the functional coupling between the hippocampus and long-distance neocortical regions. However, the anatomical basis for this functional “dyscoupling” has not been fully revealed. In this study, we applied a multimodal magnetic resonance imaging technique to noninvasively examine the large-scale anatomical and functional hippocampal network of a group of middle aged rhesus monkeys. Using diffusion spectrum imaging, we have found that monkeys with lower memory performance had weaker structural white matter connections between the hippocampus and neocortical association areas. Resting state functional imaging revealed somewhat of an opposite result. Monkeys with low memory performance displayed elevated coupling strengths in the network between the hippocampus and the neocortical areas. Taken together with recent findings, this contradictory pattern may be the result of either underlying physiological burden or abnormal neuronal decoupling due to the structural alterations, which induce a neuronal compensation mechanism for the structural loss or interference on task related neuronal activation, respectively.

Keywords

chemical LTP; TEA; glycine; spine remodeling; chemical LTD

INTRODUCTION

The rhesus monkey has been an important animal model for the study of the neurobiological basis of memory function since they can be used to obtain both micro- and macroscopic

biological observations without confounding factors inherent to disease (Roth et al., 2004). High resolution magnetic resonance imaging (MRI) has been used to show that memory loss in the rhesus monkey may strongly relate to metabolic disruptions in the hippocampus (Small et al., 2004), which is consistent with what has also been reported in humans (Small, 2003). These structural alterations in the rhesus monkey were dominantly found at the subneuronal level rather than reflecting significant neuronal loss (West, 1993; Keuker et al., 2003), and opened a window to investigating a variety of possible memory related neurobiological mechanisms that are linked to each other. However, the hippocampus is a complex structure made up of distinct subregions with extensive connections to a broad range of cortical and subcortical sites (Rosene and Van Hoesen, 1977; Insausti, et al., 1987; Condé et al., 1995; Friedman et al., 2002). This suggests that the hippocampus should be considered to be a node in a memory network rather than an isolated structure. By looking at the broader network of interconnected brain regions that contribute to mnemonic function, we may be able to understand more fully how alterations in a node of this network such as the hippocampus can have a widespread impact.

Emerging evidence for the complementary roles between the hippocampus and neocortex on memory functioning has been reported in the recent studies on both humans and rodents (Wang and Morris, 2010; Poch and Campo, 2012). An important but not fully addressed issue in these studies is the neuroanatomical basis for the functional interactions between the hippocampus and neocortical regions. While the rhesus monkey may be an optimal target to scrutinize the issue, such large-scale hippocampal–neocortical connections have not been fully confirmed in the rhesus monkey.

The central aim of this report was, therefore, to investigate the role of hippocampal–neocortical network to memory functioning in the rhesus monkey. We used the spatial condition of the delayed recognition span (DRST-spatial) test as a marker of the memory performance on monkeys. DRST-spatial test was chosen among other cognitive measures because of its sensitivity to hippocampal damage (Beason-Held et al., 1999) and age-related declines of memory in rhesus monkeys (Moss et al., 1997). Also, the DRST-spatial test used with monkeys has possible advantages to provide useful insights into the process of normal cognitive aging in humans, since it has been used to examine memory dysfunction in normal aging with healthy aged humans (Inouye et al., 1993) and participants in the early stages of Alzheimer's disease (Moss et al., 1986).

Here, investigation of the relationship between hippocampal–neocortical network and DRST-spatial test performance on the rhesus monkey was pursued by assessing the hippocampal network alterations based on the novel *in vivo* neuroimaging framework, which include both diffusion spectrum imaging (DSI, Wedeen et al., 2008) and resting-state functional MRI (rs-fMRI, Scholvinck et al., 2010).

DSI provides structural information about the intravoxel axonal arrangement, making it possible to investigate brain anatomical circuitry in a large-scale (Wedeen et al., 2008). In this study, DSI was used for imaging white matter (WM) structural connections to examine whether a disruption of structural connections between the hippocampus and the neocortical structures are related to memory performance in the same monkeys.

The blood oxygen level dependent (BOLD) signals from the rs-fMRI correlates to actual neuronal activity in gamma frequency range (Scholvinck et al., 2010), and its temporal correlation between different brain regions provide a nice estimation of distinct functional networks in both human and monkeys (Mantini et al., 2011). From the current *in vivo* imaging techniques, it is now clear that the brain spends a considerable energy during its resting state. While its metabolic energy consumption is remarkably higher than the task reflexive functional status of the brain, spontaneous activity during the resting-state has become a key index to understand the underlying mechanism of brain (Raichle, 2010). Together with DSI, rs-fMRI was applied in the imaging framework to determine the independent or synergic effects of structural and functional network properties centered on the hippocampus in the age-related memory decline that tends to start in middle age.

MATERIALS AND METHODS

Subjects

Multimodal imaging was conducted on 10 physically intact healthy, female, middle aged rhesus monkeys (age: 17–20). Each of these monkeys was a part of a larger ongoing study of aging and cognition taking place in our lab. Each had known birth dates and complete health records. As a part of our studies of aging and cognition, each of the monkeys was administered the DRST-spatial task to assess their visual short-term memory performances. During the MRI scanning session the monkeys were anesthetized with an initial dose of ketamine and then given propofol IV using a syringe pump in order to maintain anesthesia throughout the session. In the scanner, the monkeys were put in the stereotactic MRI-compatible head holder specially designed to fit within the 8-channel phase array head coil and held the head in a fixed position prevented any inadvertent movement from respiration. Respiration and oxygenation levels were monitored throughout the imaging session. Body temperature was maintained through a combination of a circulating warm water pad and a blanket.

Cognitive Testing: DRST-Spatial Test

A board which has 18 small wells, arranged in a 3×6 matrix was used for DRST-spatial test. All of the stimuli were identical brown disks. For each trial, a disk was placed over one of the 18 wells, which was baited with a reward. The screen was then raised and the animal was allowed to displace the disk and obtain the reward. The screen was lowered and a second identical disk was placed on the board and was baited, while the first disk was replaced over the original but now unbaited well. If a correct response was made, the screen was lowered and a third disk was added to the matrix and baited. Every successive correct response was followed by the addition of a new disk until the animal made an error. Ten such trails were presented each day for a total of 10 days. The experimental endpoint was the recognition span, or the mean number of stimuli the monkey was able to correctly identify before committing an error.

MRI Acquisition

All MRI scans were performed on an Achieva 3T whole body MRI scanner (Philips Healthcare, Best, The Netherlands). We used the eight-channel synergy head coil that is

compatible with the use of sensitivity encoding (SENSE) parallel imaging, allowing for shortening acquisition time and alleviating distortions in echo planar images (EPI). RF transmission was through the quadrature body coil.

T1 structural imaging—High resolution T1-weighted images were acquired using a T1-weighted 3D-turbo field echo (TFE) sequence that was fully optimized to provide high signal to noise ratio (SNR) and high gray-WM contrast at high resolution with following parameters: TR/TE 7ms/3ms, flip angle 8°, NEX 6, intershot delay 2,800 ms, TFE factor 200, voxel size: 0.6/0.6/0.6 mm, sagittal plane acquisition.

Diffusion spectrum imaging—DSI scan was collected in the sagittal plane. DSI parameters were as follows: voxel size of 2 mm iso-cubic, FOV = 160 mm, TR/TE 3590/50, number of directions 128, multiple high *b*-values, half Q-space acquisition, NSA = 1, flip angle = 90°, sense factor of 2 in the AP direction, with a total scan time of 35 min.

Resting state functional imaging—rs-fMRI was collected from T2*-weighted echo planar (EPI) sequence (2 mm slice thickness and slice gap = 0.4 mm, repetition time (TR) = 2.0 s, echo time (TE) = 30 ms, flip angle = 80°, slice thickness = 2 mm, 75 slices, FOV = 240 mm, matrix = 240 × 240) and 155 volumes acquired per subject.

Defining Anatomical Structures

Defining anatomical structures in the cortex was the first step to reconstruct hippocampal–neocortical connections on both structural and functional level. To provide a unified analysis framework on both structural and functional level connections, we applied the same anatomical definitions to both DSI and rs-fMRI analysis. Here, the average brain atlas of the rhesus monkey (Frey et al., 2011) with Paxinos anatomical definitions (Fig. 1, colors in MNI_paxinos) was used for defining anatomical regions. The average brain template was fully optimized to exclude ROIs that were too small to be applied to the rs-fMRI and DSI (~2 mm cubic voxel) scans. Ninety-two region of interests (ROI, Table 1) were included in the final atlas. Noisy values in the anatomical definitions were also extracted (Fig. 1A). The template also had tissue probability information based on Wisconsin rhesus tissue probability map (McLaren et al., 2009).

There were two different nonlinear coregistration steps to apply the ROIs from the average brain atlas to each individual monkey scans. First, the high resolution T1-weighted images from each monkeys were coregistered to the MNI Paxinos template based on nonlinear transformation algorithm (Greve and Fischl et al., 2009). Then, inverse of the coregistration parameters were calculated and applied to the template ROIs for corresponding ROI delineations in each monkey's T1-weighted image (Fig. 1B). An average tissue probability map was also transformed into each monkey's T1-weighted image based on the same parameters and used as tissue prior information for segmenting each tissue class (Zhang et al., 2001). In the second step, ROIs in each monkeys T1-weighted images were transformed to their DSI and rs-fMRI based on the nonlinear transformation algorithm. DSI and rs-MRI scans were separately transformed to each subject's T1-weighted scans, and then, ROIs in the T1-weighted scans were inversely transformed to the DSI and rs-MRI images (Fig. 1C).

Structural Connectivity Reconstruction from DSI

Structural connectivity was defined based on the existence and amount of WM tracts between hippocampus and rest of the ROIs. Here, motion and eddy current distortions on DSI were initially corrected using the FMRIB's software library (Jenkinson et al., 2012). After the preprocessing, DSI studio (<http://www.dsi-studio.labsolver.org>) software package was used for carrying out the WM tracts.

To reconstruct WM tracts from DSI, a hanning filter and three-dimensional Fourier transformation were applied to the DSI dataset to estimate WM orientation distribution functions (ODF) at each voxel (Fig. 2A) was used. WM tracts were then reconstructed from streamline fiber tracking based on the ODF (Wedeen et al., 2008; Yeh et al., 2010) with the following parameters: 0.5 mm step size, 0.6 in turning angle threshold, random seeding with primary direction tracking initiation and 150 mm in maximum length restrictions. Both anterograde and retrograde hippocampal connections were reconstructed based on the previously defined ROIs. For the anterograde hippocampal connection estimation, the hippocampus was selected as the seed regions for fiber-tracking and 10,000 iterative fiber tracking were performed (Fig. 2B). Other ROIs, which were not used for seeding ROIs were defined as target ROIs. After the fiber-tracking, reconstructed fibers, which failed to connect between source and target ROIs were excluded from the ROI-to-ROI connectivity reconstructions. The number of fibers existing between the source and target ROIs were counted for each of the targets. For the retrograde hippocampal connection estimation, all other nonhippocampal ROIs were iteratively applied as a seeding region for fiber tracking under the same tracking scheme used in anterograde tracking. WM tracts connecting to the hippocampus were then counted for each of seeding ROIs. Here, the resulting proportion of fibers from both anterograde and retrograde fiber-tracking (from 20,000 tracking in total) were used for defining local connection strength between the hippocampus and each of nonhippocampal ROIs (local hippocampal connection strength: LCS).

Functional Connectivity Reconstruction from rs-fMRI

Functional connectivity was defined as the temporal correlations between the previously defined ROIs. Using FMRIB's software library package (Jenkinson et al., 2012, FSL), rs-fMRI data was preprocessed to correct followings: differential slice timing (*slicetimer* in FSL), motion correction (*MCFLIRT* in FSL), and spatial smoothing. After that, resting functional connectivity between hippocampus and each nonhippocampal ROIs were extracted using REST software library package (Song et al., 2011): (1) Low-frequency fluctuations (0.009–0.08 Hz) were extracted from band-pass filter to remove high frequency noise, (2) Average time course of the low-frequency fluctuations was extracted from each ROIs (Fig. 2C), (3) WM and cerebrospinal fluid as well as global fluctuations were detrended to avoid any nonneuronal fluctuations in the data (Fox et al., 2009), (4) Pearson correlation coefficients were computed between the time course of a given pair of regions, and used to assess functional connectivity (Biswal et al., 1995). All statistics were computed on Fisher Z-transformed Pearson correlation values. Similar to the structural connectivity analysis described in previous section, local functional connection strength between the hippocampus and each ROIs (**f-LCS**) was defined by Fisher Z-transformed Pearson correlation values between hippocampus and neocortical ROIs.

Statistical Analysis

All analysis calculated Pearson's partial correlation coefficients between DRST-spatial and hippocampal network, separately for structural and functional connectivity. To explore cognitive memory significance of the local structural hippocampal connections, LCS on each of hippocampal–neocortical ROIs was separately correlated with DRST-spatial test score while controlling for age. For functional network analysis, separate partial correlation analyses were done under replacing LCS to f-LCS. All results were regarded significant at $p < 0.05$, corrected for multiple comparisons. Here, permutation testing (Anderson and Robinson, 2001) using 10,000 permutations was used for multiple comparison correction on each correlation analysis.

RESULTS

DRST-Spatial Test Performance

Performance on the DRST-spatial test is shown in Figure 3A. The mean score was 2.06 (standard deviation: 0.14). There was no significant relationship between age and performance on the DRST-spatial test in this group of monkeys. Three among 10 rhesus monkeys had scores 1 standard deviation over the mean DRST-spatial score while 2 monkeys had scores 1 standard deviation below the mean score. The pattern of variation in the monkeys in this report is consistent to that seen in previous reports from our group (Herndon et al., 1997). The DRST-spatial score of five out of the ten monkeys reported on here were above the previously reported minimum DRST-spatial score of young monkeys (Herndon et al., 1997) and can be regarded as weakened but not impaired status.

Hippocampal Structural Connectivity

The rhesus monkeys showed widespread structural connection patterns between the hippocampus and neocortical regions. Structural connectivity reconstructions consistently confirmed hippocampal to medial temporal, hippocampal to posterior cingulate, and hippocampal to parietal connections. However, the spatial distribution of LCS was different across the monkeys. Figure 3B shows an example on such intersubject variation of LCS. As shown in Figure 3B, a monkey with highest DRST-spatial test score had dominant connection patterns in inferior temporal, superior parietal ROIs, and minor connections in prefrontal ROIs. When compared to the pattern, LCS patterns on the other monkey with lowest DRST-spatial test score showed differences. Lowest DRST score monkey showed dominant inferior temporal connections rather than medial temporal, and also, it had wide spread frontal connection patterns, which were not confirmed in highest DRST score monkey.

As we described in the method section, intersubject variances on LCS were statistically as compared to DRST-spatial test score and the results are shown in Figure 3C. Partial correlation analyses revealed that the monkeys with lower DRST-spatial performance have significantly weaker connections (lower LCS) in various ROIs as compared to others (Fig. 4, and Table 1 for regional details). Both ipsi- and contra-lateral connections in the left hippocampus showed more significant results as compare to the right hippocampus connections, but most of the significant results were centered in the inferior temporal and the

occipital connections in both left and right hippocampal network. Highest significance was confirmed in the right hippocampus to parietal area cingulate connection (PECg, $P < 0.001$, corrected), while the left hippocampus connection showed less significant pattern under the corrected $P < 0.05$ level. There were also prefrontal connections significantly correlated with DRST-spatial score. The left hippocampus to the frontal connections in lower memory performance monkeys had weaker hippocampal connections in the medial frontal, the orbitofrontal and in area 46v. The right hippocampus showed significant alterations only in the orbito-prefrontal connections.

Hippocampal Functional Connectivity

Similar to what we found from the structural network analysis, hippocampal connections to the medial prefrontal, inferior temporal, and parietal cortices were also partly confirmed from the rs-fMRI data. Resting functional hippocampal network also showed variable patterns in f-LCS across the monkeys. Figure 5A shows functional hippocampal network reconstructions on same monkeys shown in Figure 3B. Similar to the structural network, topological patterns of the overall hippocampal connections were different between the monkeys with highest and lowest DRST-spatial test score. However, unlike structural network properties shown in the previous section (Fig. 3B), monkey with lowest DRST-spatial test score showed higher f-LCS with more spatially divergent patterns than the monkey with highest DRST-spatial test score.

Partial correlation analyses between functional connectivity and DRST-spatial scores showed overall elevated f-LCS in the monkeys with lower memory performances, compared to the monkeys with better memory performances (Fig. 5B). Statistically, significant correlations were only confirmed in positive f-LCS and most elevated f-LCS did not show bivariate alterations in their structural connections (i.e., LCS). For example, significant elevations in the functional connections were found between the left hippocampus and superior temporal sulcus area two-gyral part in monkeys with lower memory performance, although they had weaker structural connections as compared to high performance monkeys (Fig. 5C, Hip_L-ST2G_L). Bivariate patterns were found in the connections between the right hippocampus and the right parietal area PE and also in the connections between the right hippocampus to the right parietal area POa external part (Fig. 5C, Hip_R-POaE_R). Although the statistically significant bivariate patterns were not common in our results, bivariate patterns were found between the right hippocampus and its ipsilateral connections to superior part of parietal lobe though this did not reach statistical significance (see Fig. 4).

DISCUSSIONS

In this study, we investigated the relationship between hippocampal connections and memory performance in a group of healthy middle aged rhesus monkeys. Memory performance was measured by the DRST-spatial test, which has been shown to be sensitive to hippocampal damage. Hippocampal connectivity was assessed by multi-modal neuroimaging that including both DSI and rs-fMRI sequences to assess both structural and functional hippocampal connections. To our knowledge, this is the first attempt to investigate cognitive performances of rhesus monkeys in terms of both structural and

functional hippocampal–neocortical connections. The main finding of this study was that monkeys with lower memory performance had stronger resting functional hippocampal–neocortical connectivity in the face of concomitant weaker structural hippocampal connections compared to monkeys with better memory performance.

Structural Connectional Loss and Cognitive Decline

On the basis of extensive microscale observations of the rhesus monkeys, disruptions in neuronal networks induced by alterations in WM have been hypothesized as being a prominent index for understanding the cognitive decline associated with the normal aging process. Changes in WM connections may affect the topology of neuronal networks, and consequently contribute to alterations in information transfer between cortical regions, which results in cognitive decline (Kohama et al., 2012).

Our *in vivo* observations based on the DSI findings support the WM hypothesis of cognitive decline. The DSI reconstructed long-distance axonal connections that we observed in this study are consistent with those that have been reported in previous *ex vivo* axonal tracing studies on monkeys (Barbas and Blatt, 1995; Insausti and Munoz, 2001), and the statistically significant memory related variances in the LCS that we found suggest that short-term memory performance rests upon the structural integrity of these connections.

Considering the recent advances on imaging microscopic myelin structure by DSI (Takahashi et al., 2010; Wedeen et al., 2012), the weaker structural connections that we found in low memory performance monkeys may indicate a large-scale exhibition of myelin alterations resulting in a decrease of the flexibility in their hippocampal functional interactions. Similar aspect has been also confirmed in the studies on healthy cognitive aging in humans (Sullivan and Pfefferbaum 2006; Sullivan et al., 2010; Voineskos et al., 2012) and suggests that weakening or loss of structural connections may decrease the efficiency of the functional interactions, and therefore, result in lowered cognitive processes.

Enhanced Resting Functional Connectivity

Considering the observations on structural connectivity, one might expected to see lowered functional connections as well. However, the functional network analysis that we undertook showed that the underlying transitional mechanism from structural neuronal network alterations to cognition may be more complicated. The functional hippocampal–neocortical connections revealed stronger temporally correlated BOLD fluctuations in the monkeys with low memory performance and weakened structural connections.

Recent clinical findings based on rs-fMRI technique report elevated patterns in resting state functional connections in patients much like we have found in our monkeys with low memory performance. In Alzheimer's disease (AD) or subjects with strong genetic factors on AD, increased resting functional connectivity was confirmed in patients (Yao et al., 2010; Westlye et al., 2011), and aberrant hippocampal decoupling during the resting state has been consistently discussed as a possible major cause for the increased resting functional connectivity in the patients. Interestingly, a recent longitudinal fMRI study based on face-name associative memory paradigm confirmed increased baseline activation and decreased

task related activations in subjects with significant cognitive decline (O'Brien et al., 2010), which supports the abnormal functional decoupling hypothesis.

At the microscopic level, neurobiological alterations related to the memory decline in monkeys has been found mostly in the subneuronal components or physiological properties (Luebke and Barbas, 2010). Interestingly, such microlevel assessments also revealed bivariate patterns between the anatomical and physiological properties. Somewhat burdened patterns of physiological properties were shown in the monkeys with low memory performance despite the subneuronal structural deteriorations including increased number of abnormal myelin (Peters and Sethares, 2002) and decreased synapses (Peters et al., 2008). The pyramidal cells had more rapid firing rate of action potentials, and γ -aminobutyric acid (GABA)-ergic inhibitory postsynaptic currents were increased in low memory performance monkeys (Luebke et al., 2004). Such physiological burden may also lead to increases in neuronal synchronizations (Fingelkurts et al., 2004) and have an impact upon resting state functional connectivity. Considering both the functional decoupling hypothesis and recent observations of physiological burden in monkeys with low memory performance, our observation of elevated resting functional hippocampal connections in monkeys with low memory performance may indicate a disadvantageous neuronal activity when performing cognitive tasks.

Structural and Functional Connectivity

Recent *in vivo* imaging studies assessing both structural and functional networks also provide insights into our findings. As shown in the recent work of Honey et al. (2009), it is now clear that the functional network relies on the structural network. On the basis of the long range structural connections, interplay between different local neural dynamics build the final form of resting state neural activities (Cabral et al., 2011). However, robust functional connectivity can be also found in regions that have no direct structural connections. It is because of that the resting state functional connectivity indexes both polysynaptic and monosynaptic connections (Lu et al., 2011). Deterioration of axonal connection among the polysynaptic pathway may interrupt neural interplay and build unexpected increases in functional connections in terms of both strength and spatial connection patterns. Therefore, deterioration of axonal connection among the polysynaptic pathway may be an important factor for understanding the elevations in functional connections shown in the monkeys with low memory performance. Less bivariate patterns shown in our results may also indicate complicated underlying relationships between structural and functional networks.

Studies on traumatic brain injury (TBI) may provide additional insights on both structural and functional connectivity since WM connection damage is one of the most common features of TBI. In recent observation on mild TBI, patients demonstrated weaker connections within the default mode network (DMN) and hyper-connectivities between DMN and other resting networks compared to controls (Mayer et al., 2011). Moreover, such hyper-connectivities between DMN and other resting networks were related to cognitive impairment in mild TBI. However, in the chronic states of TBI, stronger connections were found only within the DMN, and such patterns also correlated with cognitive performances

(Sharp et al., 2011; Bonnelle et al., 2011). Taken together, these findings support the hypothesis of compensatory recruitment. Resting functional connectivity outside the DMN may increase in acute stages of disease to compensate for the functional loss. However, such compensatory mechanism may disappear in chronic stages due to adaptive changes occurred in the brain.

Here, what we observed from structural network analysis was reduced structural connection strength. While those patterns revealed mostly weakened rather than complete loss of structural connections, elevations in resting functional connectivity along with spatial pattern differences may also reveal compensatory mechanisms. As shown in Figure 3C, low memory performance monkeys had weaker structural connections than high performance monkeys in bilateral hippocampal connections to parietal ROIs, where the core DMN take place in. Considering the compensation hypothesis based on TBI, widespread elevations in hippocampal–neocortical functional connections, shown in Figure 5, are implicated in compensation mechanism for such structural loss in DMN.

In conclusion, we confirmed a deterioration of structural connections and elevated patterns on functional connections in the monkeys with low memory performance. Monkeys did not showed exact one-to-one correspondence between the structural and the functional hippocampal–neocortical connectional properties in terms of their memory performances. Such elevations in functional connections may be a resultant phenomenon either from underlying physiological burden or abnormal neuronal decoupling due to the structural alterations. However, at this point, it is hard to conclude whether the physiological burden or abnormal neuronal decoupling is related to a neuronal compensation mechanism for the structural loss or interference on task related neuronal activation respectively.

On the basis of the multimodal neuroimaging framework, our findings extend recent observations of local hippocampal alterations and its impact on memory function. While recent studies have reported local physiological (Luebke and Rosene, 2003) and metabolic (Small, 2003) alterations of the hippocampus itself, our results emphasize the importance of large-scale hippocampal connectional properties for understanding memory function in normal cognitive aging process and disease states (Westlye et al., 2011; Kenny et al., 2012). Further observations on neurochemical and neurophysiological properties in line with our multimodal neuroimaging framework may provide us with a better understanding of this, and including task fMRI in the imaging framework will be also important if we want to draw conclusions on the relationship between resting and task related activations.

Acknowledgments

Grant sponsor: NIH; Grant number: P01-AG000001.

REFERENCES

- Anderson MJ, Robinson J. Permutation tests for linear models. *Aust N Z J Stat.* 2001; 43:75–88.
- Barbas H, Blatt GJ. Topographically specific hippocampal projections target functionally distinct prefrontal areas in the rhesus monkey. *Hippocampus.* 1995; 5:511–533. [PubMed: 8646279]
- Beason-Held LL, Rosene DL, Killiany RJ, Moss MB. Hippocampal formation lesions produce memory impairment in the rhesus monkey. *Hippocampus.* 1999; 9:562–574. [PubMed: 10560927]

- Biswal B, Yetkin FZ, Haughton VM, Hyde JS. Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn Reson Med*. 1995; 34:537–541. [PubMed: 8524021]
- Bonnelle V, Leech R, Kinnunen KM, Ham TE, Beckmann CF, De Boissezon X, Greenwood RJ, Sharp DJ. Default mode network connectivity predicts sustained attention deficits following traumatic brain injury. *J Neurosci*. 2011; 31:13442–13451. [PubMed: 21940437]
- Cabral J, Hugues E, Sporns O, Deco G. Role of local network oscillations in resting-state functional connectivity. *NeuroImage*. 2011; 57:130–139. [PubMed: 21511044]
- Condé F, Maire-Lepoivre E, Audinat E, Crépel F. Afferent connections of the medial frontal cortex of the rat. II. Cortical and subcortical afferents. *J Comp Neurol*. 1995; 352:567–593. [PubMed: 7722001]
- Fingelkurts A, Fingelkurts A, Kivisaari R, Pekkonen E, Ilmoniemi RJ, Kähkönen S. Enhancement of GABA-related signalling is associated with increase of functional connectivity in human cortex. *Hum Brain Mapp*. 2004; 22:27–39. [PubMed: 15083524]
- Fox MD, Zhang D, Snyder AZ, Raichle ME. The global signal and observed anticorrelated resting state brain networks. *J Neurophysiol*. 2009; 101:3270–3283. [PubMed: 19339462]
- Frey S, Pandya D, Chakravarty MM, Bailey L, Petrides M, Collins DL. An MRI based average macaque monkey stereotaxic atlas and space (MNI monkey space). *NeuroImage*. 2011; 55:1435–1442. [PubMed: 21256229]
- Friedman DP, Aggleton JP, Saunders RC. Comparison of hippocampal, amygdala, and perirhinal projections to the nucleus accumbens: combined anterograde and retrograde tracing study in the Macaque brain. *J Comp Neurol*. 2002; 450:345–365. [PubMed: 12209848]
- Greve DN, Fischl B. Accurate and robust brain image alignment using boundary-based registration. *NeuroImage*. 2009; 48:63–72. [PubMed: 19573611]
- Herndon J, Moss M, Rosene DL, Killiany RJ. Patterns of cognitive decline in aged rhesus monkeys. *Behav Brain Res*. 1997; 87:25–34. [PubMed: 9331471]
- Honey C, Sporns O, Cammoun L, Gigandet X, Thiran JP, Meuli R, Hagman P. Predicting human resting-state functional connectivity from structural connectivity. *PNAS*. 2009; 106:2035–2040. [PubMed: 19188601]
- Inouye SK, Albert MS, Mohs R, Sun K, Berkman LF. Cognitive performance in a high-functioning community-dwelling elderly population. *J Gerontol*. 1993; 48:M146–M151. [PubMed: 8315227]
- Insausti R, Amaral DG, Cowan WM. The entorhinal cortex of the monkey. II. Cortical afferents. *J Comp Neurol*. 1987; 264:356–395. [PubMed: 2445796]
- Insausti R, Munoz M. Cortical projections of the non-entorhinal hippocampal formation in the cynomolgus monkey (*Macaca fascicularis*). *Eur J Neurosci*. 2001; 14:435–451. [PubMed: 11553294]
- Jenkinson M, Beckmann CF, Behrens TE, Woolrich MW, Smith SM. *Fsl. Neuroimage*. 2012; 62:782–790. [PubMed: 21979382]
- Kenny ER, Blamire AM, Firbank MJ, O'Brien JT. Functional connectivity in cortical regions in dementia with Lewy bodies and Alzheimer's disease. *Brain*. 2012; 135(Pt 2):569–581. [PubMed: 22189566]
- Keuter JI, Luiten PG, Fuchs E. Preservation of hippocampal neuron numbers in aged rhesus monkeys. *Neurobiol Aging*. 2003; 24:157–165. [PubMed: 12493561]
- Kohama SG, Rosene DL, Sherman LS. Age-related changes in human and non-human primate white matter: from myelination disturbances to cognitive decline. *Age (Dordr)*. 2012; 34:1093–1110. [PubMed: 22203458]
- Lu J, Liu H, Zhang M, Wang D, Cao Y, Ma Q, Rong D, Wang X, Buckner RL, Li K. Focal pontine lesions provide evidence that intrinsic functional connectivity reflects polysynaptic anatomical pathways. *J Neurosci*. 2011; 31:15065–15071. [PubMed: 22016540]
- Luebke J, Barbas H. Effects of normal aging on prefrontal area 46 in the rhesus monkey. *Brain Res Rev*. 2010; 62:212–232. [PubMed: 20005254]
- Luebke JI, Rosene DL. Aging alters dendritic morphology, input resistance, and inhibitory signaling in dentate granule cells of the rhesus monkey. *J Comp Neurol*. 2003; 460:573–584. [PubMed: 12717715]

- Luebke JI, Chang YM, Moore TL, Rosene DL. Normal aging results in decreased synaptic excitation and increased synaptic inhibition of layer 2/3 pyramidal cells in the monkey prefrontal cortex. *Neuroscience*. 2004; 125:277–288. [PubMed: 15051166]
- Mantini D, Gerits A, Nelissen K, Durand JB, Joly O, Simone L, Sawamura H, Wardak C, Orban GA, Buckner RL, Vanduffel W. Default mode of brain function in monkeys. *J Neurosci*. 2011; 31:12954–12962. [PubMed: 21900574]
- Mayer AR, Mannell MV, Ling J, Gasparovic C, Yeo RA. Functional connectivity in mild traumatic brain injury. *Hum Brain Mapp*. 2011; 32:1825–1835. [PubMed: 21259381]
- McLaren DG, Kosmatka KJ, Oakes TR, Kroenke CD, Kohama SG, Matochik JA, Ingram DK, Johnson SC. A population-average MRI-based atlas collection of the rhesus macaque. *Neuroimage*. 2009; 45:52–59. [PubMed: 19059346]
- Moss MB, Albert MS, Butters N, Payne M. Differential patterns of memory loss among patients with Alzheimer's disease, Huntington's disease, and alcoholic Korsakoff's syndrome. *Arch Neurol*. 1986; 43:239–246. [PubMed: 2936323]
- Moss MB, Killiany RJ, Lai ZC, Rosene DL, Herndon JG. Recognition memory span in rhesus monkeys of advanced age. *Neurobiol Aging*. 1997; 18:13–19. [PubMed: 8983028]
- O'Brien JL, O'Keefe KM, LaViolette PS, DeLuca AN, Blacker D, Dickerson BC, Sperling RA. Longitudinal fMRI in elderly reveals loss of hippocampal activation with clinical decline. *Neurology*. 2010; 74:1969–1976. [PubMed: 20463288]
- Peters A, Sethares C. Aging and the myelinated fibers in pre-frontal cortex and corpus callosum of the monkey. *J Comp Neurol*. 2002; 442:277–291. [PubMed: 11774342]
- Peters A, Sethares C, Luebke JI. Synapses are lost during aging in the primate prefrontal cortex. *Neuroscience*. 2008; 152:970–981. [PubMed: 18329176]
- Poch C, Campo P. Neocortical-hippocampal dynamics of working memory in healthy and diseased brain states based on functional connectivity. *Front Hum Neurosci*. 2012; 6:36. [PubMed: 22403534]
- Raichle ME. Two views of brain function. *Trends Cogn Sci (Regul Ed)*. 2010; 14:180–190. [PubMed: 20206576]
- Rosene DL, Van Hoesen GW. Hippocampal efferents reach widespread areas of cerebral cortex and amygdala in the rhesus monkey. *Science*. 1977; 198:315–317. [PubMed: 410102]
- Roth GS, Mattison JA, Ottinger MA, Chachich ME, Lane MA, Ingram DK. Aging in rhesus monkeys: relevance to human health interventions. *Science*. 2004; 305:1423–1426. [PubMed: 15353793]
- Sharp DJ, Beckmann CF, Greenwood R, Kinnunen KM, Bonnelle V, De Boissezon X, Powell JH, Counsell SJ, Patel MC, Leech R. Default mode network functional and structural connectivity after traumatic brain injury. *Brain*. 2011; 134:2233–2247. [PubMed: 21841202]
- Scholvinck ML, Maier A, Ye FQ, Duyn JH, Leopold DA. Neural basis of global resting-state fMRI activity. *Proc Natl Acad Sci U S A*. 2010; 107:10238–10243. [PubMed: 20439733]
- Song X-W, Dong Z-Y, Long X-Y, Li S-F, Zuo X-N, et al. REST: A toolkit for resting-state functional magnetic resonance imaging data processing. *PLoS ONE*. 2011; 6:e25031. [PubMed: 21949842]
- Small SA. Measuring correlates of brain metabolism with high-resolution MRI: A promising approach for diagnosing Alzheimer disease and mapping its course. *Alzheimer Dis Assoc Disord*. 2003; 17:154–161. [PubMed: 14512829]
- Small SA, Chawla MK, Buonocore M, Rapp PR, Barnes CA. Imaging correlates of brain function in monkeys and rats isolates a hippocampal subregion differentially vulnerable to aging. *Proc Natl Acad Sci U S A*. 2004; 101:7181–7186. [PubMed: 15118105]
- Sullivan EV, Pfefferbaum A. Diffusion tensor imaging and aging. *Neuro Sci Biobehav Rev*. 2006; 30:749–761.
- Sullivan EV, Rohlfing T, Pfefferbaum A. Quantitative fiber tracking of lateral and interhemispheric white matter systems in normal aging: Relations to timed performance. *Neurobiol Aging*. 2010; 31:464–481. [PubMed: 18495300]
- Takahashi E, Dai G, Wang R, Ohki K, Rosen GD, Galaburda AM, Grant PE, Wedeen VJ. Development of cerebral fiber pathways in cats revealed by diffusion spectrum imaging. *NeuroImage*. 2010; 49:1231–1240. [PubMed: 19747553]

- Wang S-H, Morris RGM. Hippocampal-neocortical interactions in memory formation, consolidation, and reconsolidation. *Annu Rev Psychol.* 2010; 61:49–79. C1–4. [PubMed: 19575620]
- Wedeen VJ, Wang RP, Schmahmann JD, Benner T, Tseng WYI, Dai G, Pandya DN, Hagmann P, D'Arceuil H, de Crespigny AJ. Diffusion spectrum magnetic resonance imaging (DSI) tractography of crossing fibers. *NeuroImage.* 2008; 41:1267–1277. [PubMed: 18495497]
- Wedeen VJ, Rosene DL, Wang R, Guangping D, Mortazavi F, Hagmann P, Kaas JH, Tseng WI. The geometric structure of the brain fiber pathways. *Science.* 2012; 335:1628. [PubMed: 22461612]
- West MJ. Regionally specific loss of neurons in the aging human hippocampus. *Neurobiol Aging.* 1993; 14:287–293. [PubMed: 8367010]
- Westlye ET, Lundervold A, Rootwelt H, Lundervold AJ, Westlye LT. Increased hippocampal default mode synchronization during rest in middle-aged and elderly APOE ϵ 4 carriers: Relationships with memory performance. *J Neurosci.* 2011; 31:7775–7783. [PubMed: 21613490]
- Yeh FC, Wedeen VJ, Tseng WY. Generalized q-sampling imaging. *IEEE Trans Med Imaging.* 2010; 29:1626–1635. [PubMed: 20304721]
- Voineskos AN, Rajji TK, Lobaugh NJ, Miranda D, Shenton ME, Kennedy JL, Pollock BG, Mulsant BH. Age-related decline in white matter tract integrity and cognitive performance: A DTI tractography and structural equation modeling study. *Neurobiol Aging.* 2012; 33:21–34. [PubMed: 20363050]
- Yao Z, Zhang Y, Lin L, Zhou Y, Xu C, Jiang T. Abnormal cortical networks in mild cognitive impairment and Alzheimer's disease. *PLoS Comput Biol.* 2010; 6:e1001006. [PubMed: 21124954]
- Zhang Y, Brady M, Smith S. Segmentation of brain MR images through a hidden Markov random field model and the expectation maximization algorithm. *IEEE Trans Med Imaging.* 2001; 20:45–57. [PubMed: 11293691]

FIGURE 1.

Automatic labeling of both rs-fMRI and DSI. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 2.

Structural and functional hippocampal network reconstruction. Reconstructions on one rhesus monkey are shown in this figure. Overlaid figure of local white matter orientation (colored lines) and diffusion spectrum image data is shown in panel A. Panel B shows 10,000 iterative fiber tracking from left hippocampus to its ipsi-lateral cortex. Colors other than red indicates cortical regions of interests (ROIs). Low frequency resting BOLD fluctuations in different regions are illustrated in Panel C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 3.

Short-term memory performance of monkeys and their structural connections. The spatial condition of delayed recognition span (DRST-spatial) test of monkeys are shown in Panel A. Red horizontal line indicates mean DRST-spatial score and red dotted line shows standard deviation. Monkeys with DRST-spatial over the mean are plotted in orange to red cross. Below mean DRST-spatial score are plotted in light to dark blue cross. Dotted circles indicate the monkeys used for sample figures, shown in Panel B. Panel B shows hippocampal structural connection pattern in highest and lowest DRST-spatial test score monkeys. Dominant hippocampal-neocortical connections are shown in red color while the gray and yellow color shows no or weak connections. Panel C shows the correlation analysis result on the relationship between structural connection and DRST-spatial test. Statistically significant ($P < 0.05$, corrected) memory related structural connection loss are depicted in dark blue arrow. Also, connections with high r square values are shown in light to dark blue in the cortical surface. Small figures in left and right marginal side shows contralateral connections. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 4. Regional details of the statistical analysis. Summaries on both structural (Str) and functional (Func) analysis were shown in here. Areas with blue text indicate statistically significant result from the correlation analysis in contralateral hippocampal–neocortical connections. See Table 1 for abbreviations and their full name. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 5.

Analysis on resting functional connection between hippocampus and neocortical regions. Panel A shows hippocampal functional connection pattern in highest and lowest DRST-spatial test score monkeys, same monkeys shown in Figure 3B. Dominant hippocampal–neocortical connections are shown in red color while the gray and yellow color shows no or weak connections. Panel B shows the correlations analysis on the relationship between functional connection and DRST-spatial test. Statistically significant ($P < 0.05$ corrected) memory related structural connection loss are depicted in dark red (stronger functional connection in low performance) and blue (weaker functional connection in low performance) spheres. Panel C shows graphical plot of both functional and structural connection analysis. Left y-axes and red rectangles shows functional connectivity analysis and Right y-axes and blue cross shows structural connectivity analysis. Left hippocampus to left superior temporal sulcus area 2 gyrus part is shown in the left, and right hippocampus to right parietal area POa external part (PoaE_R) is shown in the right side. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE 1

Full Name and Abbreviations of ROIs Used in This Study

ROIs		
1: area 1 of cortex (somatosensory)	30: area 30 of cortex	PPT: posterior parietal area
2/1: areas 2/1 of cortex (somatosensory)	31: area 31 of cortex	V1: visual area 1 (primary visual cortex)
2: area 2 of cortex (somatosensory)	35: area 35 of cortex	V2: visual area 2
3a: area 3a of cortex (somatosensory)	TAA: temporal area TAA	V3A: visual area 3A
3b: area 3b of cortex (somatosensory)	TEa: temporal area TEa	V3D: visual area 3, dorsal part
S2: secondary somatosensory cortex	TE: temporal area TE	V3V: visual area 3, ventral part
4(F1): area 4 of cortex (primary motor)	TE1–3: temporal area TE1–3	V4D: visual area 4, dorsal part
6: area 6	TF: temporal area TF	V4V: visual area 4, ventral part
ProM: area ProM (promotor)	TH: temporal area TH	V4T: visual area 4, transitional part
6/32: area 6/32 of cortex	TL(36): temporal area TL (area 36)	PO: parieto-occipital area
32: area 32 of cortex	TPOC: temporal parietooccipital associated area in sts, caudal part	44: area 44 of cortex
9/32: area 9/32 of cortex	ST1: superior temporal sulcus area 1	45A: area 45A of cortex
9: area 9 of cortex	ST2S: superior temporal sulcus area 2, sulcal part	45B: area 45B of cortex
9/46: area 9/46 of cortex	ST2G: superior temporal sulcus area 2, gyral part	GI: granular insular cortex
46V: area 46V of cortex	FST: fundus of superior temporal sulcus	AI: agranular insular cortex
9/46V: area 9/46 of cortex, ventral	PGa: area PG associated region of the superior temporal sulcus	DI: dysgranular insular cortex
9/46D: area 9/46 of cortex, dorsal	MST: medial superior temporal area	IPro: insular proisocortex
46D: area 46D of cortex	MT(V5): middle temporal area (visual area 5)	GP: globus pallidus
8A: area 8A of cortex	IPa: intraparietal sulcus associated area in the superior temporal sulcus	Cd: caudate nucleus
8B: area 8B of cortex	Hip: hippocampus	ProK: prokoniocortex
8AD: area 8 of cortex, anterodorsal	EC: entorhinal cortex	OPro: orbital proisocortex
8AV: area 8 of cortex, anteroventral	TPPAI and TPPro: temporopolar periallocortex and temporopolar proisocortex	OPAI: orbital periallocortex
10M: area 10 of cortex, medial part	TPO: temporal parietooccipital associated area in sts	Gu: gustatory cortex
10D: area 10 of cortex, dorsal part	PaAR: paraauditory cortex	Pul: pulvinar
11: area 11 of cortex	AK: auditory koniocortex	thalamic nucleus
47(12): area 47 (old 12) of cortex	DIP: depth intraparietal area (VIP)	Eml: external medullary lamina
13: area 13	PE: parietal area PE	AcbC: accumbens nucleus, core
14: area 14 of cortex	PECg: parietal area PE, cingulate part	APir: amygdalopiriform transition area
23: area 23 of cortex	PG: parietal area PG	Pu: putamen
24: area 24 of cortex	POaI: parietal area POa, internal part (LIPI)	VP: ventral pallidum
25: area 25 of cortex	POaE: parietal area POa, external part (LIPE)	