



Degradation of Mnemonic Networks in Aging and Alzheimer's Disease

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PROJECT SUMMARY / ABSTRACT

Functional connectivity MRI (fcMRI) is a non-invasive method to assess the integrity of anatomically distributed neural networks underlying complex behaviors. In Alzheimer's disease (AD), fcMRI of the default mode network (DMN) has shown great promise as a biomarker in clinical and basic research studies, as (1) profound decreases in DMN fcMRI are seen in prodromal and clinically evident AD and (2) the DMN is among the sites of early amyloid deposition in AD. However, using fcMRI as an early AD biomarker is limited by the overlapping changes in connectivity seen in normal aging, which, in turn, limits the identification of early AD subjects to enroll in clinical trials. To address this limitation, we propose a series of studies that use fcMRI to disambiguate normal aging from early AD by focusing on the pattern of degeneration across six well-described cortical networks in two unique subject populations. The central hypothesis of these studies is that early AD and aging will show distinct patterns of network degradation, with preferential degradation of cognitive networks (especially the Default Mode and Attention Networks) in early AD as compared to aging. We test this hypothesis by comparing young and old subjects with and without evidence of AD pathology, leveraging newly available data from young subjects with dominantly inherited AD (DIAD) drawn from the Dominantly-Inherited Alzheimer's Network (DIAN). Notably, the comparison of the DIAD population and older at-risk and symptomatic patients followed in the Harvard Aging Brain Study represents a unique opportunity to disentangle age and AD pathology, as DIAD carriers have disease onset at a young age (often in the late 30s and early 40s). In addition, using PET data on tau burden in our older subjects (from F18-T807 PET, a newly-developed tau radioligand), we will explore the relative contributions of amyloid and tau pathologies to altered fcMRI. These studies will serve the dual purpose of (1) optimizing the use of fcMRI as an AD biomarker by identifying patterns of fcMRI change that distinguish aging and AD, and (2) provide novel insight into the systems-level pathophysiology that distinguishes aging and AD. Further, these studies will compare the timing and pattern of network degradation in dominantly-inherited vs. sporadic AD and provide critical context for the interpretation of fcMRI data currently being gathered in (at least) three major AD prevention trials in older individuals at-risk for sporadic AD and dominantly-inherited AD.

SPECIFIC AIMS AND HYPOTHESES

Alzheimer's disease (AD) affects more than one of every ten individuals over the age of 65, and is projected to affect over 12 million Americans by the year 2050. Unfortunately, recent clinical trials for disease-modifying therapies in mild-to-moderate AD dementia have yielded disappointing results. These results underscore the need for earlier identification of incipient AD and intervention prior to widespread neuronal and synaptic loss, a move that will require greater reliance on biomarkers (especially non-invasive imaging biomarkers) to identify individuals in the early stages of AD.

Functional connectivity MRI (fcMRI) is a non-invasive imaging technique used to assess the integrity of large-scale, anatomically distributed neural networks thought to underlie complex behaviors. In addition to its uses in systems neuroscience, fcMRI of the default mode network (DMN) is being developed as an AD biomarker, and fcMRI data are now being collected in major AD prevention trials in both sporadic, late-onset at-risk for AD (LOAD) and dominantly-inherited AD (DIAD). However, while DMN connectivity is profoundly degraded in AD, it is also degraded to a lesser extent with normal aging, clouding interpretation in clinical trial settings and limiting the identification of individuals in the earliest stages of AD.

We propose a series of studies that use fcMRI to disambiguate normal aging from early AD by focusing on the pattern of degeneration across six well-described cortical networks in two unique subject populations. **The central hypothesis of these studies is that early AD and aging will show distinct multi-network patterns of degradation, with preferential degradation of cognitive networks (especially the DMN and attention networks) in early AD as compared to normal aging.** We test this hypothesis by comparing young and old subjects with and without evidence of early AD from the Harvard Aging Brain Study (HABS), leveraging newly available data from young subjects with DIAD mutations from the Dominantly Inherited Alzheimer's Network study (DIAN). Notably, the DIAN population presents a unique opportunity to disentangle age and AD pathology, as mutation carriers become symptomatic at a relatively young age (often by the early 40s). Lastly, utilizing PET data on tau and amyloid burden in our older subjects, we will examine the contributions of these pathologies to disrupted network connectivity.

These studies will (1) help to optimize fcMRI as an AD biomarker by identifying patterns of fcMRI change that distinguish aging and AD, (2) provide insight into the systems-level pathophysiology underlying aging and AD, and (3) provide context for the interpretation of fcMRI data collected in ongoing clinical trials.

Aim 1: Defining patterns of age-related changes fcMRI in the absence of AD markers. Using a set of network templates derived out-of-sample, we will extract connectivity values from a predetermined set of six cortical networks (Fig. 2) and compare large groups of normal young and cognitively normal elderly subjects. To better isolate the effects of aging from early AD, we will only include elderly subjects with normal hippocampal volume, normal temporoparietal FDG PET and low amyloid burden in this comparison.

Hypothesis 1: We expect that cognitive networks (DMN and attention networks) will not show preferential degradation with age in the absence of imaging biomarker evidence of preclinical AD.

Aim 2: Identifying patterns of fcMRI network degradation indicative of AD. We will identify fcMRI alterations indicative of AD through (1) comparison of asymptomatic and symptomatic DIAN mutation carriers, and (2) comparison of cognitively normal elderly (CNE) subjects with no imaging evidence of AD pathology (CNE AD-) to similar CNE subjects with imaging evidence of preclinical AD (CNE AD+) and to participants with Mild Cognitive Impairment (MCI). Additionally, we will correlate individual network connectivity with a subset of neuropsychological measures to better understand the neural correlates of cognitive change. Lastly, in the large subset of CNE subjects with tau PET imaging from HABS, we will also compare the relative contributions of tau and amyloid burden to multi-network fcMRI measures.

Hypothesis 2: Young DIAN participants and elderly with imaging evidence of AD pathology (both CNE and MCI) will show preferential degradation of key cognitive networks (DMN and attention networks).

Aim 3: Validating cross-sectional patterns of fcMRI change in longitudinal data. Focusing on early symptomatic DIAN mutation carriers, CNE AD+, and MCI subjects (groups in whom fcMRI decreases are likely to be most rapid), we will assess whether longitudinal fcMRI decreases conform to the specificity pattern identified in Aims 1 and 2. In addition to validating Aims 1 and 2, this aim will assess the utility of fcMRI as a longitudinal biomarker and secondary outcome measure.

Hypothesis 3: We predict that DIAN mutation carriers will show progressive, preferential decreases in cognitive network connectivity (esp. the DMN and attention networks). In MCI and CNE AD+, we predict a similar pattern of progressive and preferential decreases in the DMN and attention networks on a background of more modest age-related changes in sensory and motor networks.

Background and Significance:

The morbidity and public health burden of AD are truly staggering in scope, underscoring the urgent need for clinical and late-stage translational research aimed at slowing AD progression. Recent negative clinical trials in AD strongly suggest that intervention prior to irreversible neuronal and synaptic loss is likely critical to success in altering the AD disease course¹⁻³. Early intervention, especially in the preclinical stages of AD, is highly dependent on imaging biomarkers to identify and enroll those at risk into clinical trials. Resting-state functional connectivity MRI (fcMRI) is a non-invasive method used to assess the large-scale synaptic integrity of anatomically distributed neural networks that underlie complex behaviors. Notably, fcMRI does not involve radiation, is much less expensive than PET imaging, can be performed during MRI “safety scans” required in many clinical trials, does not require performance of a task (making it usable across a wider range of impairment than task fMRI), and is repeatable multiple times over the course of a clinical trial.

In AD, fcMRI of the default mode network (DMN) has shown promise as a biomarker in clinical and basic research studies, as (1) profound decreases in DMN fcMRI are seen in prodromal and clinically evident AD⁴⁻⁶ and (2) the DMN is among the sites of early amyloid deposition in AD^{7,8}. These features of fcMRI have led to its inclusion in several ongoing clinical trials in sporadic and autosomal dominant AD, including the Dominantly Inherited Alzheimer’s Network (DIAN) treatment unit, Alzheimer’s Prevention Initiative (API), and the Anti-Amyloid Treatment in Asymptomatic AD Trial (A4; led by the applicant’s mentor, Dr. Sperling). However, using fcMRI as an AD biomarker is limited by the overlapping changes in connectivity seen in normal aging and AD^{9,10}, which, in turn, limit the identification of early AD subjects to enroll in clinical trials. Additionally, the majority of studies of functional connectivity in AD focus on one or two networks, rather than examining the integrity of cortical networks more broadly and addressing whether particular multi-network patterns of degradation occur early in AD. Further, the present literature is extremely limited on the similarities and differences of functional connectivity alterations between dominantly inherited AD (DIAD) and sporadic, late-onset AD (LOAD).

To address these limitations, we outline studies comparing newly available fcMRI data from a large cohort of young mutation carriers from DIAN to normal young individuals, cognitively normal elderly individuals with no imaging evidence of AD pathology, and elderly subjects with imaging evidence of early AD pathology (stratified by hippocampal volume, FDG and PiB amyloid PET). In making these comparisons, we examine three cognitive networks: the heavily studied DMN^{4,5,11}, the ventral attention network (VAN)¹², and the dorsal attention network (DAN)¹²⁻¹⁴. In addition, we examine connectivity in three non-cognitive networks: the Motor^{13,15-17}, Extrastriate Visual^{18,19}, and Primary Visual^{18,20-22} networks. The inclusion of relatively young DIAN subjects with dementia will allow us (1) to better separate the effects of elderly age and AD than is possible in LOAD, and (2) to more confidently model changes across the Alzheimer’s disease spectrum, as the high penetrance of DIAD mutations means that there is little ambiguity as to whether mutation carriers (even those at the very early stages of the disease) will progress to AD dementia.

The major underlying hypothesis of these studies is that the pattern of networks involved and the magnitude of fcMRI change will differ between normal aging and AD, suggesting that the profound memory deficits seen with AD are mechanistically distinct from those seen with so-called “age-related cognitive decline”. While our preliminary data supports this differentiation, both the support and refutation of this underlying hypothesis provide scientifically meaningful information regarding whether aging and AD can be considered separable^{10,23,24}, especially given the association of DMN integrity to associative memory^{5,25,26}.

More broadly, the systems level approach we take in these studies allows us to explore the provocative hypothesis by Seeley, Greicius, Rabinovici, Miller and colleagues that neurodegenerative diseases are differentiable based on the networks they degrade^{30 27-29} and that symptomatic differences between neurodegenerative dementias reflect the observed pattern of network degeneration^{27,28}. From a clinical and translational perspective, the proposed studies will inform the interpretation of fcMRI data already being collected in clinical trials in LOAD and DIAD. Indeed, we explicitly explore the possibility that a better understanding of which networks change with age as opposed to AD will allow for the development of multi-network composite measures that better account for the confounding effects of age²⁴ while retaining sensitivity to AD-related changes (see Fig. 4 for a composite of this type). From a more fundamental perspective, the inclusion of a wider array of cortical networks will allow a better pathophysiologic understanding of which networks are most susceptible to early disruption in AD. In turn, this may hint at the

anatomic specificity that underlies the AD disease process, opening up new avenues of research and furthering the systems level understanding of mnemonic dysfunction in AD.

Innovation: Multiple aspects of the proposed research are highly innovative:

- The inclusion of rare cross-sectional and longitudinal data from a large group of families harboring dominantly inherited Alzheimer disease (DIAD) mutations. Relatively young subjects with DIAD mutations offer a rare opportunity to study AD in a non-elderly population. With the appropriate comparisons, this will allow for the applicant to better separate the effects of age and AD on fcMRI networks than has been possible in prior studies.

- As a counterpoint to the DIAD population, the applicant will leverage data from a large sample of cognitively normal elderly and impaired subjects who are followed in the mentors' research studies. These exceptionally well-characterized subjects undergo longitudinal neuropsychological testing and imaging measures (including resting state and task fMRI, structural MRI, PiB and FDG PET). This characterization will allow for careful stratification of subjects based on preclinical biomarkers of AD pathology (especially hippocampal volume, FDG hypometabolism and amyloid burden). This finer separation will help better distinguish age-related changes from early AD-related changes in fcMRI²⁴, as shown in preliminary data (Early Sporadic AD, Fig 3).

- A large subset of these elderly normal and impaired subjects will be imaged with a newly developed PET ligand (F18-T807), a putative tau imaging agent^{30,31}. The applicant's co-mentor, Dr. Johnson, has played a central role in the development and on-going validation of this ligand. Early results from T807 PET have been highly encouraging, and the ability to incorporate tau information with existing amyloid data will allow the applicant to examine the relative contributions of tau and amyloid burden to the degradation of fcMRI networks and to a core set of neuropsychological measures.

- The study design will allow for novel comparisons of network change between autosomal dominant and sporadic AD. These comparisons are quite relevant to the interpretation of forthcoming data from AD prevention trials and to a systems-level understanding of AD pathophysiology.

- Template-based rotation (TBR), which builds on the strengths of independent components analysis (ICA) and seed-correlation analysis (SCA), is a novel fcMRI analytic technique that was developed in the mentor's group with the applicant's close involvement³². TBR is optimized for use in clinical trial settings, and compares favorably with SCA and ICA in terms of reliability and effect size. TBR uses out-of-sample network descriptions that can be carried across clinical trial populations to facilitate uniform multi-network comparisons and eliminates the problem of merged or missing network components common in group ICA. Though ICA and SCA will also be used in parallel to ensure results are not methodology dependent, it is hoped that these studies will demonstrate TBR's potential to improve the use of fcMRI as an AD biomarker.

Approach:

Overall Approach: The proposed studies are organized into three parts: **(Aim 1)** Determine the pattern of network degeneration seen with aging by comparing cognitively normal young subjects to cognitively normal older individuals without imaging biomarker evidence of preclinical AD (CNE AD- subjects). For stratification, we will take an approach similar to of Jack, Knopman and colleagues^{33,34}. **(Aim 2)** Determine the pattern of network degeneration seen in AD by (A) comparing asymptomatic mutation carriers (CDR of 0)

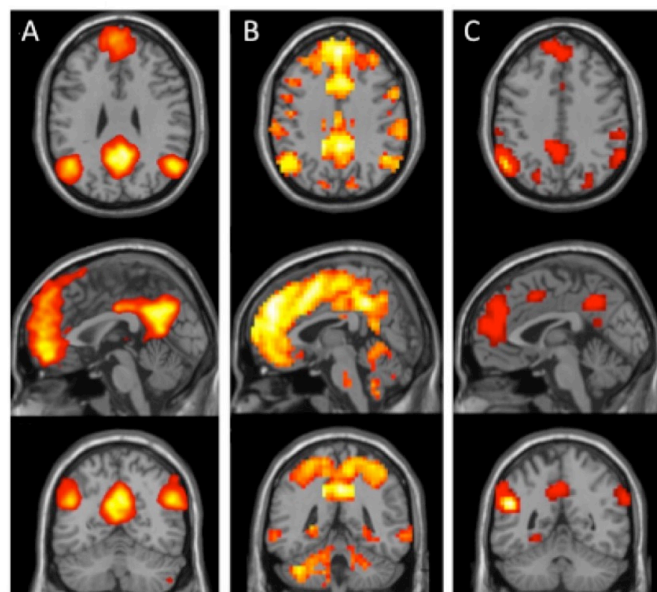


Figure 1: Age-related changes in fcMRI are present in multiple networks, whereas DIAD mutation carriers may show preferential decreases in DMN and DAN. (A) Representative depiction of the DMN: Independent component analysis of fcMRI data from 31 young subjects yielded a network component representative of the DMN, including the posterior cingulate/precuneus, medial prefrontal cortex, and regions within the lateral parietal lobes. (map threshold $p < 0.0001$; Chhatwal and Sperling, JAD, 2012) **(B) Effects of Age:** Voxel-wise, network-agnostic correlation analysis in 164 normal young subjects compared to 164 normal older subjects (young different than old contrast, $p < .001$) **(C) Effects of DIAD:** Similar to analysis to (B) but comparing 83 carriers of dominantly-inherited AD mutations (ranging from clinically normal to moderate dementia) to 37 non-demented, non-mutation carriers from the same families (non-mutation different than mutation carrier contrast, $p < .005$. Chhatwal, Schultz, Sperling, in preparation)

to early (CDR 0.5) and late (CDR $\geq$ 1) symptomatic DIAN mutation carriers; (B) comparing CNE AD- subjects to subjects with imaging biomarker evidence of early AD pathology (CNE AD+) and subjects with Mild Cognitive Impairment (MCIAD); and (C) in the large subset of CNE and MCI participants with available tau imaging, we will assess the relative contributions of amyloid and tau burden to degradation of fcMRI networks. (**Aim 3**) Validate the cross-sectional pattern of fcMRI change seen in Aims 1 and 2 using longitudinal data from DIAD and early AD subjects. In addition to determining the trajectory of fcMRI change with increasing age in these groups, the relationship of a limited subset of neuropsychological measures (Mini-Mental Status Exam – MMSE, Logical Memory Score, delayed recall performance in the Selective Reminding Task - SRT, and Clinical Dementia Rating Scale Sum of Boxes- CDR SOB) to changing fcMRI will also be assessed.

Subject populations: (approximate sample sizes are given as data collection is on-going in all groups):

- Normal Young (**NY**) subjects are drawn from the Harvard Aging Brain Study (HABS; P01AG036694; Sperling PI). We have collected data from 87 NY subjects, with an average age of 26.

The following subject groups are drawn from HABS and from an ongoing study of MCI in the mentor's research group (R01 AG027435; Sperling/Johnson Co-PI; R01 AG046396; Johnson PI). In addition to the R01 support listed above, we are very hopeful that the P01 grant for HABS will be renewed as well (priority score of 15, top 2 percentile on first submission). In these studies, functional and structural MRI, PiB amyloid PET, and FDG-PET imaging are obtained within the first six months of enrollment and then again at Year 4. Tau PET is collected at Year 4. A detailed neuropsychological battery is administered annually, and subjects in both studies will be followed longitudinally for a minimum of five years. All CNE subjects have a global Clinical Dementia Rating (CDR) of 0.

- Cognitively normal elderly subjects with no imaging biomarker evidence of AD pathology (**CNE AD-**) have baseline data demonstrating low amyloid burden, and hippocampal volume and temporoparietal FDG that are unlikely to be within an AD distribution. Cross-sectional data from 160 CNE AD- subjects with a mean age of 72 collected thus far. 80 subjects have completed at least one longitudinal follow-up visit, with the remainder expected by Year 2 of the funding period.
- Cognitively normal elderly subjects with imaging biomarker evidence of AD pathology (preclinical AD stage 2³³⁻³⁵; **CNE AD+**) are derived from the same study, but fall below the cutoff for hippocampal volume or FDG metabolism, and above the cutoff value for amyloid burden. 35 CNE AD+ subjects have completed their baseline assessments (mean age 76) with an additional 5-10 new subjects expected. Longitudinal assessment of these individuals should be complete by funding year 3.
- Impaired elderly subjects have a global CDR of 0.5 and are above cutoff for amyloid burden and below cutoff for hippocampal volume or temporoparietal FDG metabolism (high likelihood MCI due to AD by NIA-AA clinical research criteria³⁶; **MCIAD**). 60 total MCI subjects have completed baseline assessment with an additional 15-20 subjects expected by funding year 2. Longitudinal data from these subjects will be available starting in funding year 1, and complete by year 4.

Subjects from families harboring pathogenic mutations leading to AD are drawn from the Dominantly Inherited Alzheimer's Network (DIAN; U01AG032438; Morris PI; Sperling MGH/BWH site PI), a consortium of 13 institutions in the US, UK, Germany, and Australia. Participants are grouped based on CDR rating:

- Asymptomatic DIAD Mutation carriers – CDR 0, abbreviated **M+ CDR0**
- Early Symptomatic DIAD Mutation Carriers – CDR 0.5, abbreviated **M+ CDR0.5**
- Symptomatic DIAD Mutation Carriers – CDR greater than or equal to 1, abbreviated **M+ CDR1+**

Approximately 345 DIAN subjects have completed their baseline visit and >100 subjects have completed at least one longitudinal follow-up MRI visit. Baseline visit demographics are: M+ CDR0: n= 116, mean age = 35yo; M+CDR0.5: n=49, mean age= 43yo; M+CDR1+: n=31, mean age=48yo.

Methodological Summary:

In both HABS and DIAN, participants undergo eyes open, resting state fMRI with head motion restrained by foam-padded clamps using a 12-channel phased-array head coil on a 3T Siemens Trio TIM or Verio scanner. HABS subjects undergo 2 resting state fcMRI scans (each approximately 6 minutes in length) as part of 2 separate scanning sessions during the first six months of enrollment, and again at Year 4. DIAN participants undergo one 5-minute resting-state session at each MRI visit. Acquisition parameters are similar across the two studies and have previously been described in detail^{6,37}.

We plan to use Template Based Rotation (TBR)³² as the primary analytic technique for fcMRI data. TBR is an optimization of independent components analysis^{21,22,38} (ICA) that uses out-of-sample, *a priori* network descriptions (termed templates), and has several features that are beneficial for use in clinical trial settings. As compared to conventional group ICA, the use of predefined network descriptions in TBR allows for more uniform comparisons of connectivity across groups collected in different trials and clinical populations. Unlike group ICA, TBR network descriptions (e.g., Figure 2) are determined *a priori* and are not impacted by unequal sample sizes across clinical groups.

Additionally, these templates can be used on incomplete data sets without having to

re-derive network components when new subjects are added (in distinction to conventional group ICA). Based on our recent studies, TBR appears more sensitive to young/old group differences and shows greater within subject, across session reliability than seed based correlation analysis (SCA), and shows slightly better reliability and effect size as compared to dual-regression ICA³². In a recent publication, we quantitatively compared ICA, SCA, and TBR methods in the assessment of fcMRI changes in young vs. normal older adults³². In addition to TBR, all data will be analyzed in parallel using ICA and SCA techniques^{13,37,40,41} to ensure that the results are not methodology dependent.

Preliminary Data: Examining the pattern of fcMRI in a network-agnostic, voxel-wise fashion, we first explored whether the pattern of connectivity change was grossly different in normal aging as compared to AD (Figure 1). These preliminary analyses demonstrate a more widespread pattern of fcMRI change in aging as compared to DIAD; in examining the spatial patterns, this voxel-wise analysis suggests that the DMN, DAN, and VAN may show preferentially decreased connectivity in young DIAD carriers relative to their non-mutation carrying family members. A follow-up analysis in the DIAN sample also revealed that the DMN and DAN showed earlier change in the DIAD disease course than other networks (Chhatwal et al., AAIC 2014).

These observations offer qualitative support to the hypothesis that the cognitive networks may be preferentially degraded early in the DIAD disease course, and that perhaps the pattern of changing fcMRI in aging is less focused on cognitive networks than in DIAD. Leveraging the ability of the TBR to use a uniform set of network descriptions across multiple sample sets, we next quantitatively explored the patterns of network change in early symptomatic (CDR 0.5) and later symptomatic (CDR $\geq$ 1) DIAD, as well as in normal aging vs. preclinical AD. Notably, these preliminary analyses directly address the feasibility of using TBR in the studies proposed in Aims 1 and 2.

Network Degradation in Aging: To isolate changes in connectivity with aging, we performed preliminary analyses comparing cognitively normal elderly with no imaging biomarker evidence of preclinical AD (CNE AD-) to normal young subjects. We observed fairly uniform age-related changes across the three cognitive networks (DMN, DAN, VAN, all $p < 0.001$; Fig. 3), with slightly greater degradation in the two visual networks (both $p < 0.0001$). No change in Motor network connectivity was observed ($p = 0.44$). ANOVA and pairwise post-hoc tests demonstrated that the DMN ($p < 0.005$), DAN ($p < 0.005$), and VAN ($p < 0.001$) showed significantly less degradation with age than the Primary Visual network (Fig. 3). We also observed that cognitive networks as a group showed less degradation compared to the visual networks ($p < 0.005$; Fig. 4).

These preliminary analyses suggest that cognitive networks are not preferentially degraded with aging in the absence of imaging biomarker evidence of AD pathology.

Network Degradation in DIAD: Using identical network descriptions to the aging comparison, we next performed preliminary analyses on connectivity changes in asymptomatic mutation carriers (M+ CDR0) as compared to symptomatic mutation carriers at a global CDR of 0.5 (M+ CDR 0.5), and CDR 1 or greater (M+ CDR1+). Controlling for age, ANOVA across M+ CDR0, M+ CDR 0.5, and M+ CDR1+ showed significant

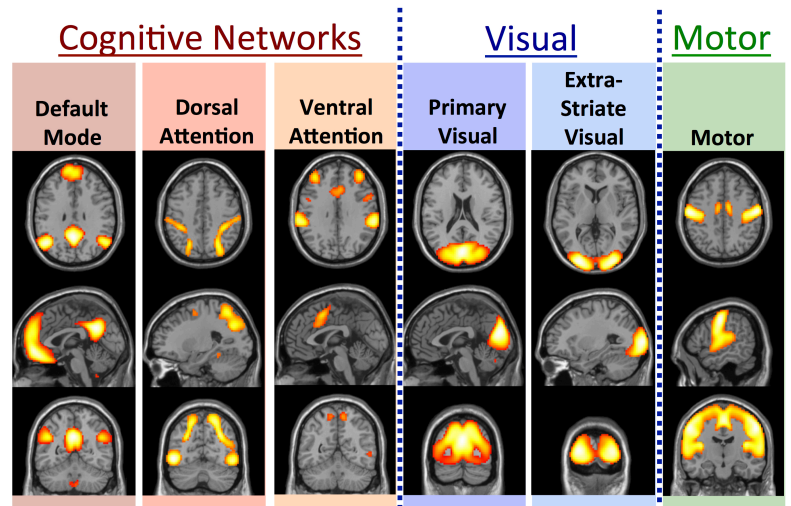


Figure 2: Six cortical intrinsic connectivity networks were chosen for analysis. These networks fall into three broad classes: Three cognitive networks (DMN, DAN, VAN) thought to be involved in directed attention, memory and other aspects of cognition; two networks centered on primary and higher order visual cortices (Primary Visual and Extrastriate Visual); and a motor network including the pre-central gyrus and supplementary motor regions.

differences in the three cognitive networks (DMN, DAN, and VAN, all $p < 0.0001$), trend-level changes in the Primary Visual ($p = 0.052$) and Motor networks ($p = 0.122$), and no significant differences in the Extrastriate Visual network ($p = 0.76$). A repeated measures ANOVA demonstrated that certain networks were preferentially degraded compared to others (Network * CDR Interaction $F_{(10,545)} = 3.461$, $p < 0.001$). Post-hoc pairwise comparisons showed that DMN and DAN connectivity decreased with increasing CDR to a

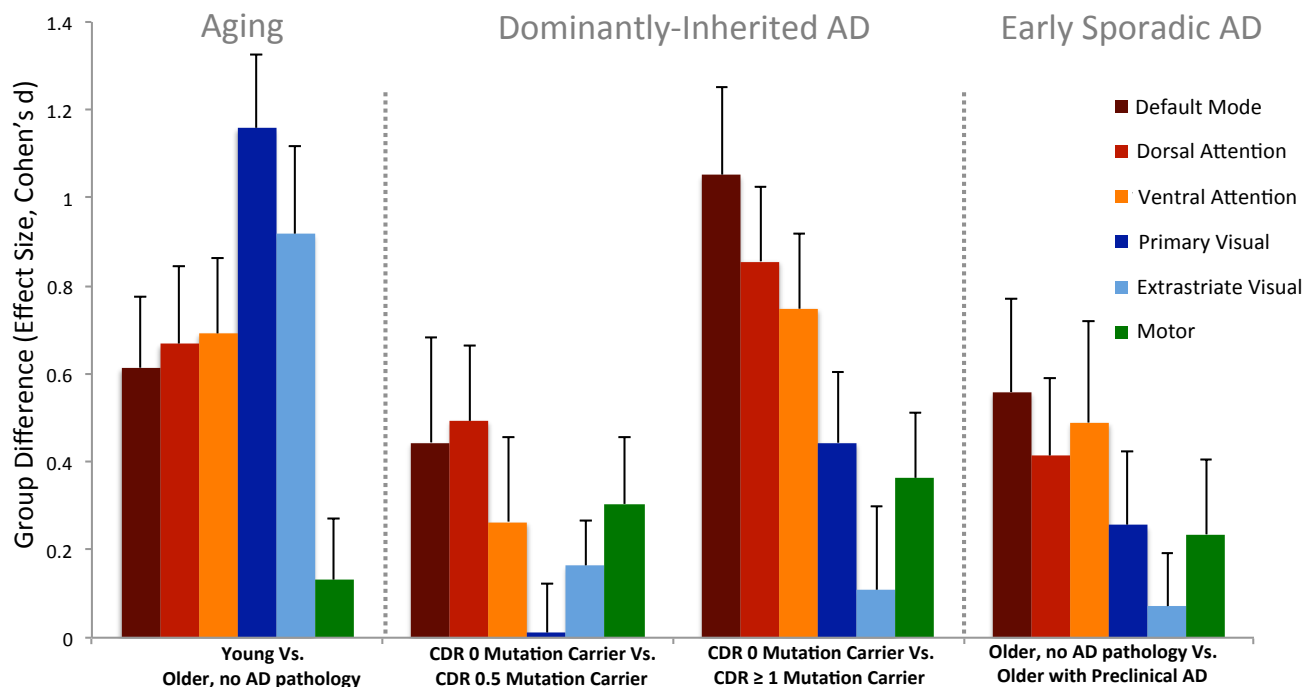


Figure 3: Patterns of Network Change in Normal Aging, Early Sporadic AD, and Dominantly-Inherited AD. Using the same set of network descriptions across all groups, we compared: (1) **Aging**: 87 normal young (mean age 26y) to 59 normal elderly (mean age 72y) subjects with no evidence of AD pathology (CNE AD-; low amyloid burden, normal range hippocampal volume and temporoparietal FDG); (2) **Dominantly-Inherited AD**: Asymptomatic DIAD mutation carriers (n=66) to early symptomatic mutation carriers (left, CDR 0.5, n=29) and to later symptomatic mutation carriers (right, CDR ≥ 1, n=17); (3) **Early Sporadic AD**: Cognitively normal elderly with no evidence of AD pathology (CNE AD-, n=59) to cognitively normal individuals with imaging biomarker evidence of preclinical AD (CNE AD+, n=30). Corrected for fMRI QA measures (movement and # of unusable volumes). Corrected for participant age in all comparisons except aging comparison. **Importantly, as age and disease progression in DIAD mutation carriers are partially co-linear in DIAD mutation carriers, age correction diminishes the apparent effect sizes for dominantly-inherited AD comparisons**, though the across network pattern is not affected. Error bars are +/- 1 SD for each effect size measurement. (Chhatwal, Schultz, Sperling, et al., in preparation)

significantly greater extent than connectivity in the Primary Visual (DMN: $p < 0.005$; DAN: $p = 0.005$), Extrastriate Visual (DMN: $p < 0.001$; DAN: $p = 0.01$) and Motor networks (DMN: $p = 0.001$; DAN: $p < 0.05$; Fig. 3). The VAN showed trend-level greater degradation with increasing CDR as compared to the Extrastriate Visual ($p = 0.0823$) and Motor networks ($p = 0.0756$). As a group, the three cognitive networks (DMN, DAN, VAN) showed significantly greater degradation with increasing CDR as compared to non-cognitive networks ($p < 0.001$; Fig. 4).

Taken together, these preliminary results suggest that the DIAD disease process leads to preferential degradation of cognitive networks (DMN, DAN, and VAN) with advancing CDR. This pattern is observable early in the disease course, and still discernible in later stages of the disease.

Network Degradation in Preclinical and early AD: We compared cognitively normal elderly with (CNE AD+) and without (CNE AD-) imaging biomarker evidence of AD pathology. After controlling for age, participants with signs of preclinical AD showed significantly decreased DMN ($p = 0.012$) and VAN ($p = 0.028$) connectivity, and trend-level decreases in DAN connectivity ($p = 0.062$). No significant differences in Primary Visual, Extrastriate Visual, or Motor network connectivity were observed between CNE AD- and AD+ subjects ($p > 0.2$). Grouping networks into cognitive (DMN, VAN, DAN), visual (Primary and Extrastriate Visual), and motor (Motor Network), repeated measures ANOVA demonstrated a significant network type by group interaction ($F_{(2,525)} = 3.115$, $p < 0.05$). Post-hoc pairwise analysis demonstrated that cognitive networks showed greater degradation than the visual networks ($F_{(1,438)} = 5.331$, $p = 0.021$). We are currently performing parallel analyses in MCI due to AD.

The pattern of degradation in preclinical AD is similar to that seen in DIAD, with cognitive networks (esp. the DMN and VAN) showing preferential degradation.

Multi-Network Composite Measures: We are in the initial stages of using the observed patterns of connectivity change to develop multi-network composite measures that improve the utility of fMRI biomarkers. The AD composite shown in Figure 4 combines connectivity values from the six networks analyzed above to yield a positively scaled connectivity measure that is highly sensitive to the AD pattern of degradation while minimizing sensitivity to age-related changes. This composite was derived from the patterns seen in DIAD and aging, and shows greater change in the DIAD groups than any single network measure or the general grouping of cognitive and non-cognitive networks (Fig. 4). This AD composite (ADC) showed little change in the aging comparison, where essentially no difference between normal young and CNE AD- subjects was observed. We then applied this composite to our preclinical AD comparison, and again observed that the ADC showed slightly greater ability to separate CNE AD- and CNE AD+ than any individual network measure or the cognitive/non-cognitive groupings (Fig. 4). Though a great deal of additional work is needed to determine the most suitable composite measure(s), these preliminary results support the idea that an **understanding of age- and AD-related patterns of changing connectivity can improve the use of fMRI as an AD biomarker.**

Neuropsychological Measures, fMRI in DIAD: We are in the initial stages of examining the relationship between connectivity and neuropsychological measures (NP) in DIAD, and our group has submitted paper examining the relationship between NP and functional connectivity in cognitively normal elderly using the TBR technique. In DIAD, preliminary analyses of mutation carriers who are at or younger than their the expected age of symptom onset⁴², fMRI from the DMN, VAN, and DAN all show significant correlations with measures of semantic fluency and MMSE. In this same subset of mutation carriers, performance on Logical Memory delayed recall significantly correlated with DMN and DAN connectivity, but not with connectivity in other networks (DMN: $r = 0.27$, $p = 0.016$; DAN $r = 0.32$, $p = 0.003$).

Tau-PET, Connectivity, and NP: As part of the development of T807 PET as a putative tau imaging agent in the co-mentor's (Dr. Johnson) research group, data from approximately 250 subjects have been collected at our site (Fig. 5). Going forward, the mentor and co-mentor's groups will acquire T807 PET data in a large subset of the CNE and MCI groups described above. We recently presented an abstract examining the relationship between T807 binding and performance in the delayed recall portion of the Selective Reminding Task (drSRT; Johnson...Chhatwal et al. AAIC 2014). Briefly, these results demonstrated that longitudinal decline in the drSRT was associated with greater entorhinal and inferior temporal T807 binding. As part of Aim 2 of this proposal, we will use amyloid and tau PET measures (and their interaction) to predict functional connectivity in the same six networks discussed above. In addition, we will use single network connectivity values along with PiB and

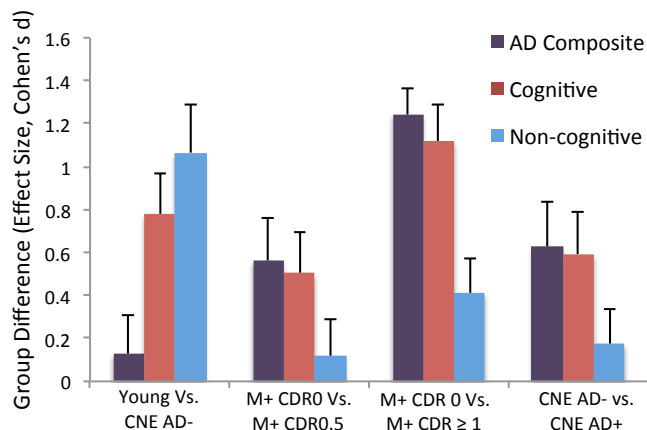


Figure 4: Multi-network Composite better differentiates aging and early AD. A multi network composite was derived based on the pattern of network change seen in DIAD and aging comparisons. This AD composite was insensitive to age-related change (Young vs. CNE AD-), but retained or exceeded the group differences based on cognitive or non-cognitive composites (shown here) or single network measures in genetic and preclinical AD comparisons (Fig. 3).

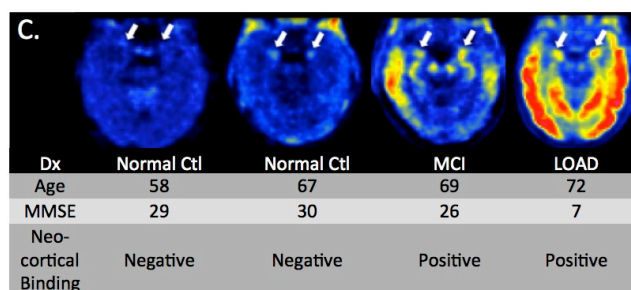
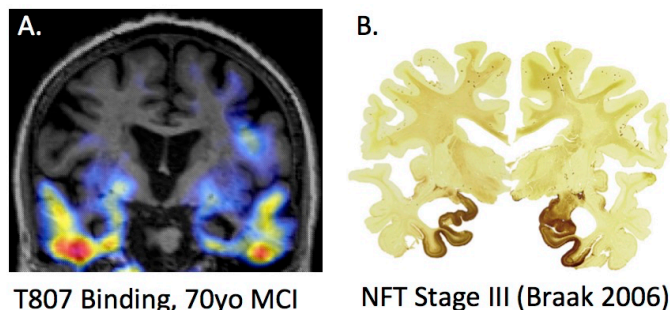


Figure 5: Preliminary categorization of neurofibrillary tangle (Tau) burden using T807 PET : (A) Coronal F-18 T807 PET image from a 70 year MCI subject. (B) For qualitative comparison only, a representation of Braak Stage III pathology using tau (AT8) immunostaining is shown (from Braak et al., 2006). (C) Transaxial F-18 T807 PET images across a spectrum of impairment, age, and neurofibrillary tangle burden. (Johnson, et al., in preparation)

T807 binding and their interaction as predictors of NP (specifically, MMSE, drSRT, Logical Memory, CDR-SOB).

Impact of microvascular disease on connectivity: A great deal of evidence supports the hypothesis that microvascular disease (MVD) has a profound impact on cognition. Using a well-validated, automated measure of white matter disease burden (WMD), we performed a series of preliminary analyses in CNE examining the interaction of WMD with changes in connectivity the six cortical networks tested above. Using the same models as above (including age as a covariate), the addition of WMD did not show a significant effect on connectivity in individual networks (DMN, VAN, Primary Vis, Extrastriate Vis all $p > 0.4$; Motor $p = 0.22$) though a statistical trend toward decreased connectivity with increasing WMD was observed in the DAN ($p = 0.078$). No significant network or network type by WMD interactions were present using the HABS CNE dataset (both $p > 0.25$), indicating that WMD does not lead to preferential patterns of network change in our preliminary dataset. These findings may be due to the circumscribed range of WMD in our present sample. Through collaboration with Dr. Lewis Lipsitz (an advisor on this K23 project), we will soon have access to a connectivity dataset in which there is a much wider range of WMD burden. We will examine the interactions of connectivity and vascular disease as our data set expands and we gain access to these new data sets.

Statistical Methods:

Overall Approach: Connectivity for each network in each subject will be derived using TBR³². Confirmatory analyses will be performed using connectivity values from ICA or SCA methods. Independent samples t-tests and ANOVA will be used to compare single or multi-network connectivity across groups (e.g., CNE AD- vs. CNE AD+) and to derive effect sizes for each comparison. No comparisons will be made across clinical cohorts (e.g., direct comparisons between the DIAN and HABS sample) to avoid confounds of slightly different acquisition parameters across cohorts, variability in testing, etc. To statistically examine the differential pattern of network degradation we will use repeated measures ANOVA with network (e.g., DMN) or Network Type (e.g., Cognitive or Visual or Motor) as a repeated measure and include a Network or Network Type by Group interaction term in the model. To examine significant interactions, pairwise comparisons of each network or network type pair will be performed to determine the direction and significance of each comparison. Similar analyses will be performed on longitudinal data using mixed effects models.

Selection of Covariates: Measures of subject movement and the number of unusable fMRI volumes will be included to control for quality of fcMRI measurement. For the preliminary analyses shown, clinical group and these quality measures were included as covariates. Additionally, age was included as a covariate in all comparisons except the young / CNE AD- comparison, where the aim was to explicitly assess age-related change. Several other potentially relevant covariates - APOE $\epsilon 4$ status, sex, family membership and mutation type (in DIAN subjects), and white matter hyper-intensity burden - did not significantly affect model fit in the preliminary analyses shown here, but we will re-examine these covariates as more data is acquired.

Amyloid and tau as predictors of network connectivity: Focusing on the subset of CNE and MCI subjects on whom we have T807 tau and PiB amyloid PET, we will examine the contributions of amyloid (PiB FLR⁴³) and tau (inferior temporal + entorhinal T807 binding) to fcMRI metrics in each network and to a subset of neuropsychological measures (see below).

Connectivity and Neuropsychological Measures: As part of SA2, we will assess the relationship of a subset of neuropsychological measures (performance on MMSE, drSRT, Logical Memory, and CDR-SOB) to connectivity in the same set of cognitive, visual, and motor networks. In addition to the covariates listed above, we will test measures of cognitive reserve as relevant covariates.

Cross-Sectional Power: Power calculations for the cross-sectional comparisons of DMN, VAN, and DAN fcMRI for three key comparisons are shown below (assuming $\alpha=0.05$, $\beta=0.80$; uncorrected effect size).

Network	NY vs. CNE AD-		CNE AD- vs. CNE AD+		DIAN Comparison (ANOVA)		
	Effect Size (d)	N	Effect Size (d)	N	# of groups	Effect Size (f^2)	N
DMN	0.61	43	0.56	51	3	0.28	35
DAN	0.67	36	0.41	94	3	0.19	51
VAN	0.69	34	0.49	66	3	0.14	69

As these sample sizes fall within the expected sample sizes for the DIAN and HABS cohort, this analysis suggests that comparisons shown above will be well powered to detect group differences when using DMN, DAN, and VAN whole-network metrics for Aims 1 and 2. We are presently in the process of analyzing data from MCIAD subjects, and estimate that we will need 34 MCIAD subjects to detect an effect size of 0.7

(assuming $\alpha=0.05$, $\beta=0.80$). Approximately 30 MCIAD subjects have been collected thus far, and we expect to collect an additional 15-20 subjects in this category by Year 2 of the funding period.

Longitudinal power: Longitudinal analyses will focus on three clinical groups that we expect to show the greatest yearly rate of network degradation: M+ CDR0.5 from DIAN, and CNE AD+ and MCIAD from HABS. Of these three groups, we have the best estimate of year-to-year change in the DIAN subjects based on preliminary data. Over a three-year period, we estimate reductions of 28.1%, 25.2%, and 13.1%, in the DMN, DAN, and VAN, respectively. Using an r of 0.4 between first and last measures, an average of 2.5 time points per subject, $\alpha=0.05$, $\beta=0.80$, and the SDs calculated from preliminary data, this indicates that we will need 13 (DMN), 16 (DAN), and 36 (VAN) subjects per group to detect change over three years. Similar analyses suggest that we will have a sufficient number of CNE AD+ and MCIAD subjects (18/20/38 and 14/20/30 for the DMN/DAN/VAN in CNE AD+ and MCIAD, respectively) by the end of funding year 3.

Constraining Multiple Comparisons: To minimize multiple comparisons, we will avoid using voxel-wise comparisons to determine connectivity, and instead reserve whole brain analyses for qualitative/illustrative purposes (e.g., Fig. 1). The six fcMRI networks we use in our TBR analysis were chosen *a priori* from previous studies using samples that do not overlap with DIAN or HABS^{12,13,18,32,41}. Additionally, our group comparisons are pre-determined as described above. Depending on the independence of the measures in question, alpha values for exploratory analyses beyond these pre-specified networks and group comparisons will be adjusted using either a family-wise error rate or bonferroni correction.

Potential Pitfalls and Alternative Approaches:

Longitudinal Analysis of fcMRI Data: We acknowledge that longitudinal analyses of fcMRI data have been difficult to perform, largely due to the poor reliability of fcMRI measures across scanning sessions. We have recently shown that TBR analysis improves short-term fcMRI reliability in CNE subjects³², and our group is actively engaged in improving cross-session normalization to facilitate longitudinal analysis. Especially in DIAD comparisons, we are fortunate to have a fairly large sample size and frequent fcMRI imaging from subjects we expect to show the most rapid fcMRI change.

fcMRI as a biomarker: Though the preliminary data support the feasibility of using fcMRI in the present studies, fcMRI is a relatively new biomarker and systems neuroscience research tool. It is possible that analytic difficulties and relatively low inter-session measurement reliability may limit the fcMRI technique's ultimate utility in clinical trials settings. Accordingly, the mentorship and advisory team and training plan will help the applicant obtain broad training in the use of imaging biomarker measures in neurodegenerative disease. Aside from MRI-based measures, the applicant will work closely with Dr. Johnson (co-mentor) to train in the development of PET based measures (esp. T807/tau PET) as AD biomarkers. This diversified training in image biomarker development mitigates the risk involved in focusing on fcMRI for these studies.

Lack of similarity between DIAD and LOAD: While we hypothesize that LOAD and DIAD will show similarities in their patterns of decreasing fcMRI (consistent with our preliminary analyses and recent analyses of CSF biomarkers in DIAD⁴²), it is possible that the patterns of changing fcMRI in DIAD are too distinct from LOAD to generate a clear picture of aging vs. AD-specific changes in fcMRI. While this would make the interpretation of the proposed comparisons in Aims 1 and 2 less clear, such a result would itself be an important finding, as it suggests that the disease processes underlying DIAD and LOAD may be more distinct than previously thought. On a somewhat related note, though acquisition parameters are similar across the HABS and DIAN, the possibility of subtle but systematic differences between cohorts is present. Though this will remain a concern, keeping group comparisons within the same cohort and expressing the results as an effect size lessens the impact of across cohort differences in measurement (Fig. 3 and 4), and the preliminary data argue against the presence of major differences in fcMRI measurements across cohorts.

Lack of a discernible difference between AD and aging: Though the central hypothesis of this proposal rests on the existence of differential AD- and age-related effects on connectivity (supported by our preliminary studies), it remains possible that fcMRI measures (whether regional, whole-network, or inter-network) will be insufficiently sensitive to distinguish these two overlapping phenomena. Though we hypothesize that this will not be the case, both the support and refutation of this hypothesis will have important implications for the use of fcMRI in clinical trial settings and perhaps on our basic understanding of the AD disease process. On a related note, we recognize that the use of age as a dichotomous variable is not optimal for describing fcMRI related changes across the lifespan. Through collaboration and local data acquisition, we are working to acquire resting state data in middle-aged individuals such that we can use age as a continuous variable.

Concluding Remarks and Future Directions:

The proposed studies involve several key innovations, most notably (1) the inclusion of young subjects with dementia (DIAD) to help disentangle the usually overlapping processes of aging and AD; (2) the use of a large, well characterized group of young and older normal subjects (HABS), a population in which we can effectively separate elderly subjects with and without signs of early AD pathology based on biomarker data; (3) the use of a novel analytic approach (TBR) that was developed to be particularly useful in clinical trial settings; (4) the inclusion of T807 PET based measures. The proposed studies, coupled with a supportive training environment and an excellent team of mentors, will allow the applicant to develop the skills to lead an independent research program focused on using neuroimaging to further our pathophysiologic understanding of AD and providing tools for AD clinical trials and drug discovery.

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