Brain Age as a Measure of Brain Reserve in Neuropsychiatric Disorders

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Aging represents a highly heterogeneous process with highly variable clinical outcomes. Differential expression of risk and resilience factors may provide explanations for this variability. Gaining a better understanding of resilience in aging is critical as it will allow for improved individualized outcome prediction, as well as providing insight for targeted interventions that may improve the process of aging. Currently, the prevailing models of neurocognitive resilience are cognitive reserve and brain reserve. The theory of cognitive reserve suggests that those with greater cognitive reserve may better cope with loss of brain integrity through presence of more adaptable and efficient neural systems. Most studies utilize education level to assess cognitive reserve; however, many proxy measures are subjective and susceptible to hindsight bias. The concept of brain reserve overlaps with that of cognitive reserve but focuses instead on the biological characteristics that allow the brain to be resilient to the effects of aging and pathological insults. It is generally thought that with sufficient brain substrate (e.g., larger grey matter volumes, greater synaptic density, more elaborate network complexity), the brain is more capable of preserving normal functioning and maintaining homeostasis despite the presence of factors of neurodegeneration or trauma. Overall, the main goals of this dissertation are to demonstrate the impact of cognitive and brain reserve on neuropsychological outcomes and brain activation patterns (Aim 1, Chapters 2 and 3), to utilize machine learning brain age prediction as a novel proxy of brain reserve (Aim 2, Chapter 4), and to utilize brain age prediction in several

neuropsychiatric disorders to predict outcome or gain a better understanding on the disease process (Aim 3, Chapters 5, 6, 7).

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Preface

There are many people that I would like to acknowledge and thank for their support and guidance. Without them, the successful completion of this dissertation would not have been possible & my journey would not have been so enjoyable.

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1.0 General Introduction

This chapter provides a general introduction regarding brain aging, resilience, and age-related neuropsychiatric disorders. Here we will discuss the major biological processes involved in aging, the homeostatic process of maintaining normal aging over time, factors that may provide resilience in healthy aging and disease, and examples of age-related neuropsychiatric disorders.

This chapter contains excerpts from the following review papers that have been published previously:

- Mizuno, A., **Ly, M.,** Aizenstein, H.J. A Homeostatic Breakdown Model of Subjective Cognitive Decline (2018) Brain Sciences. 8(12), 228.
- **Ly, M.**, Andreescu, C. Advances and Barriers for Clinical Neuroimaging in Late-Life Mood and Anxiety Disorders (2018) Current Psychiatry Reports. 20(1): 7.

My contributions to both of these review papers were literature review, interpretation of previous work, writing the manuscript, and revising the manuscript for publication.

1.1 Aging

Due to the combination of declining fertility rates, improved survival, and increasing lifespan, the global population of older adults (60+) is rapidly increasing. It is estimated that by 2030, older adults will outnumber children aged 10 years and younger. Notably, by 2050, older adults will outnumber individuals below 24 years of age. As such, it is becoming of increasing importance to mitigate the widespread impact of age-related functional decline and disease.

1.1.1 Definitions and examples of aging

Aging can be broadly defined as a progressive, time-dependent process in which living organisms accumulate cellular and molecular damage. The cumulative burden of cellular damage to tissues and organs may lead to compromised physiological integrity, functional impairment, and increased morbidity and mortality. Common examples of physiological changes that occur with aging include decreased cardiac output, accumulation of atherosclerotic plaque, impaired pulmonic gas exchange, decreased vital capacity, decreased creatinine clearance, reduction in lean body mass, and degenerative changes in many joints (Boss 1981). Critically, increased age has been demonstrated to be the primary risk factor for a wide range of diseases and disorders, such as but not limited to cancer, type 2 diabetes mellitus, cardiovascular diseases, coronary artery disease, and neurodegenerative diseases.

1.1.2 Theories of aging

The prevailing theories of aging are generally split on the basis of the source of the accumulated cellular and molecular damage: extrinsic (stochastic) or intrinsic (developmental-genetic) (Aalami 2003 et al.). The stochastic theories of aging primarily ascribe damage to extrinsic sources, such as environmental exposure to free radicals and radiation, lifestyle factors (e.g. excessive sunbathing, cigarette smoking), and long-term accumulation of errors (e.g. faulty splicing). On the other hand, developmental-genetic theories assert that cellular and molecular damage occur as part of a "planned obsolescence"—that physiological deterioration occurs as part of an intrinsic, pre-programmed, genetic process (Finch et al. 2001). It is important to note that these theories do not necessarily represent contradictory views, as it is likely that aging encompasses aspects from both extrinsic and intrinsic sources of cellular and molecular damage.

1.1.3 The cellular and molecular hallmarks of aging

In a seminal review paper, Lopez-Otin proposed nine potential cellular and molecular hallmarks of aging that were selected with the following criteria: 1) the phenomena must be observable during the process of non-pathological aging, 2) exacerbation of the mechanism must result in an accelerated aging, and 3) reversal of the process must slow down the process of aging (Lopez-Otin 2013). The hallmarks of aging include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis (protein homeostasis), deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication (**Table 1**).

Table 1: The hallmarks of aging.

Hallmark	Putative biomarker	
Genomic Instability	Micronucleus assay	• Examples: premature aging diseases (Werner syndrome, Bloom syndrome) • Exposure to exogenous physical, chemical, biological agents, UV/IR radiation • DNA replication errors, spontaneous hydrolytic reactions, reactive oxygen species • Point mutations, translocations, chromosomal gains and losses, telomere shortening, integration of viruses or transposons
Telomere Attrition	Telomere length	• Examples: Telomerase deficiency associated with premature pulmonary fibrosis, dyskeratosis congenita, aplastic anemia • Most mammalian somatic cells lack telomerase
Epigenetic Alterations	DNA methylation clocks	• Histone modifications, DNA methylation, chromatin remodeling
Loss of Proteostasis	Clusterin	• Examples: Alzheimer's disease, Parkinson's disease, cataracts • Impaired chaperone-mediated protein folding • Decreased proteolytic quality control (autophagy-lysosomal system, ubiquitin-proteasome system)

Table 1 continued

Deregulated Nutrient Sensing	Sirtuin 1	• Converse example: dietary restriction increases lifespan • Anabolic signaling accelerates aging (GH, IGF-1, PI3K, Akt, mTOR)
Mitochondrial Dysfunction	Growth Differentiation Factor 15, Apelin	• Examples: cachexia, sarcopenia • Respiratory chain efficacy diminishes, leading to electron leakage, decreased ATP, increased ROS
Cellular Senescence	P16INK4A	• Examples: frailty, disability • Accumulation of senescent cells increases with age • Senescence may be an adaptive response to minimize proliferation of damaged tissue; however, replacement in aged systems are limited
Stem Cell Exhaustion	Circulating osteogenic progenitors	• Example: decreased hematopoiesis leads to increased anemia and myeloid malignancies • Deficient proliferation of stem cell and progenitor cells
Altered Intercellular Communication	Inflammasomes, IMM-AGE score	• Inflammation deregulates neurohormonal signaling • Immunosurveillance declines • Peri- and extracellular environment altered

1.2 Brain Aging

While age-related physiological changes may occur in all organ systems, the focus of this dissertation will be on the process of aging in the brain. The processes occurring in brain aging are consistent with the overall hallmarks of aging. Parallel cellular and molecular mechanisms include altered calcium ion signaling, decreased synaptogenesis and neurite outgrowth, excessive demyelination, inappropriate microglial activation, astrocytic hypertrophy, and decreased neural activity (Blalock 2003).

1.2.1 Structural brain aging

Aging of the brain is characterized by consistent, non-linear patterns of structural alterations that are detectable with conventional and advanced neuroimaging. In general, structural changes in the aging brain can be detected in-vivo with computed tomography (CT) and magnetic resonance imaging (MRI). However, MRI is more favored and more commonly utilized for the study of structure and function of the brain due to its low risk profile (non-ionizing, non-invasive), repeatability, high resolution, and broad range of available modalities for the study of different tissue types. Overall, structural changes that occur in the process of normal aging generally include decreased global cerebral volume with regional differences, decreased grey and white matter density, increased ventricular or cerebrospinal fluid volume, increased white matter hyperintensity burden, and decreased white matter tract integrity (Lockhart and DeCarli 2014).

Decreases in grey matter density are generally thought to represent neuronal degeneration and synaptic density reduction (Lockhart and DeCarli 2014), while decreases in white matter density may represent a change in myelinated fiber size, demyelination, expansion in perivascular

spaces (Virchow-Robin spaces), or gliosis (Meier-Ruge 1992). Alterations in grey matter and white matter density occur across the lifespan but with differing patterns. Grey matter volumes start decreasing somewhat linearly in adolescence or early adulthood and continue into late-life (Fotenos 2005). White matter volume increases until approximately age 40 and then declines with an accelerated quadratic rate (Fotenos 2005). Overall, the acceleration of whole brain volume reduction begins to be evident at approximately age 30 (Fotenos 2005).

Age-related decreases in grey matter volume have been demonstrated to affect certain regions more than others. This seeming predilection has been traditionally ascribed to regional vulnerability to age-related changes or pathologies (Fjell 2014). Alternatively, it has also been suggested that the regions developed later during development (which also developed later in the context of mammalian nervous system evolution) are more susceptible to age-related changes (Fjell 2014). In general, grey matter volumes tend to decrease most in the frontal lobe followed by the temporal lobe, while volumes in the occipital and parietal lobe are largely preserved in the absence of other neurodegenerative causes (DeCarli 2005). Furthermore, rates of atrophy in the frontal lobe demonstrate a two-phase rate of change across the lifespan, with accelerated atrophy between age 20 and 40, a period of lower atrophy from 40-60, followed by a period of accelerated atrophy after age 60 (Pfefferbaum 2013). In general, the regions that are most commonly affected by decreased grey matter volume are the caudate nucleus, cerebellum, hippocampus, prefrontal cortex, orbitofrontal cortex, inferior temporal cortex, inferior parietal cortex, and the entorhinal cortex (Raz 2005).

In comparison to the current literature on grey matter changes in aging, white matter changes are somewhat understudied in comparison. Similar to the pattern of grey matter atrophy, decreases in white matter integrity tend to follow the “last-in-first-out" hypothesis, in which

regions that myelinate later during development exhibit greater vulnerability to age-related changes or pathologies (Bennett and Madden 2014). The most prominent regions of age-related reduction of white matter integrity, as reflected by decreased fractional anisotropy (FA) and increased mean diffusivity (MD) on diffusion tensor imaging (DTI), are the genu of the corpus callosum, fornix, and the external capsule (Bennett and Madden 2014).

Reductions in white matter integrity can be explained by the presence of white matter atrophy and the formation of white matter hyperintensities (Vernooij 2008). White matter hyperintensities (WMH) are lesions that appear bright on fluid attenuated inversion recovery (FLAIR) images in deep or periventricular white matter regions. WMHs are commonly detected in asymptomatic older adults, occurring in approximately 10% - 20% of 60-year olds to approximately 90% - 100% of 90-year olds (Merino 2019). WMHs represent a manifestation of small vessel disease and result from chronic, subclinical levels of ischemia. Risk factors for WMH include diabetes, hypercholesterolemia, smoking, carotid artery disease, atrial fibrillation, and heart failure.

1.2.2 Cognitive brain aging

As part of the process of brain aging, healthy older adults demonstrate decline in some cognitive domains while maintaining normal performance in other domains. This pattern is highly consistent with the pattern of structural changes in aging. As compared with younger adults, older adults most commonly demonstrate decline in the following aspects of cognition: attention, executive functioning, non-verbal/visuospatial processing, processing speed, working memory, and episodic memory (Dumas 2015, Lockhart and DeCarli 2014). However, older adults are able to maintain their performance or even demonstrate improvement in cognitive abilities that rely on

wisdom or general knowledge (e.g. fluency, verbal ability). Cognitive ability is commonly assessed through neuropsychological testing, and a sample comprehensive neuropsychological battery can be seen in Chapter 2.

1.3 Resilience

1.3.1 Heterogeneity of brain aging

Despite the fact that the major biological processes and structural changes associated brain aging follow consistent patterns, aging represents a highly heterogeneous process with a wide range of intra-individual variability. By nature of the definition of aging, there is a large variety of potential sources of cellular and molecular damage that different individuals accumulate over the period of their lifetime. The genetic diversity of the human species also provides differential patterns of risk and resilience in different populations. In some cases, certain individuals are able to retain their cognitive ability and brain volume despite advancing into their eighth decade (Wilson 1999, DeCarli 2005b).

The wide range of heterogeneity in aging leads us to the necessary discussion of nomenclature in aging (i.e. the differentiation between healthy and normal aging). Normal brain aging is characterized by typical patterns of age-related decline in structure and function in the absence of clinically significant impairment (or neurodegeneration attributable to other causes). On the other hand, healthy brain aging represents the more uncommon pathway characterized by preserved structure and function in light of advanced age (Lockhart and DeCarli 2014). It is presumed that the individuals who experience healthy brain aging have certain characteristics that

render them resilience to the ravages of time. In the following section, we will discuss the prevailing theories of cognitive and brain reserve that contribute to resilience in the context of brain aging.

1.3.2 Reserve

Cognitive reserve represents the ability of an individual to resist functional impairment in light of brain injury (Stern 2009, Stern 2012, Stern 2018). This theory suggests that those with higher cognitive reserve may better cope with brain damage through presence of higher premorbid cognitive abilities and more efficient recruitment of compensatory processes. Traditionally, these compensatory processes have been elicited through task-based functional MRI (fMRI), as discussed below.

Cognitive reserve is commonly estimated by proxy measures, such as levels of education, occupational attainment, occupational complexity, lifetime socioeconomic status, bilingualism, or lifetime scholarly leisure activities. These proxies have been demonstrated to be associated with higher cognitive performance and/or delayed onset to Alzheimer's disease as compared with age-matched controls (Stern 1994, Scarmeas 2001, Amieva 2014, Wang 2017, Vermuri 2011, Soldan 2015).

Due to its complex nature, it is difficult to capture all aspects of cognitive reserve. It is critical to recognize that proxies of cognitive reserve only represent limited aspects of reserve itself. Most studies utilize education level and premorbid IQ to assess cognitive reserve; however, there are individuals with low education and high cognitive reserve (and vice versa). A significant limitation of many proxy measures for cognitive reserve are their subjective nature and susceptibility to hindsight bias.

Brain reserve represents the structural characteristics that allow the brain to be resilient to the effects of aging and pathological insult (Stern 2009, Stern 2012, Stern 2018). It is generally thought that with sufficient brain substrate (e.g. larger grey matter volumes, greater synaptic density, more elaborate network complexity), the brain is more able to preserve normal functioning despite the presence of factors of neurodegeneration. Theoretically, an individual with high brain reserve has more to lose before cognitive or functional deficits may become evident. Brain reserve has traditionally been estimated with different modalities of structural neuroimaging. Some common proxies for brain reserve include intracranial volume, grey matter volume, grey matter density, cortical thickness, white matter volume, and white matter integrity.

The concepts of cognitive reserve and brain reserve can sometimes be difficult to disentangle due their fundamental similarities. The most common theoretical distinctions refer to brain reserve as the "hardware," while cognitive reserve is represented as the "software." Brain reserve has clear biological substrates, while cognitive reserve refers to how the substrate may be utilized differently. However, this can be further complicated by the idea that higher structural integrity is necessary for more efficient processes.

Brain maintenance refers to the process of maintaining or improving brain reserve over time. Examples of brain maintenance include behavioral changes that improve an individual's modifiable risk factors: physical exercise, change to a healthier diet, smoking cessation, adherence to therapeutic interventions.

1.3.3 Compensatory mechanisms

Functional changes in brain aging can be thought of as compensatory mechanisms employed by the brain to maintain cognitive performance in light of age-related structural changes.

The extent and efficacy of these compensatory mechanisms can provide resilience and is represented as the concept of cognitive reserve. Age-related functional changes can be detected with a wide range of imaging modalities, such as electroencephalography (EEG), magnetoencephalography (MEG), functional MRI (fMRI), positron emission tomography (PET), or optical imaging (e.g. fNIRS).

The major themes of compensatory mechanisms in brain aging are compensatory hyperactivation, dedifferentiation, and posterior-anterior shift. Compensatory hyperactivation refers to the phenomenon where older adults demonstrate greater regional activity than younger adults in task-based fMRI studies while maintaining the same level of behavioral performance (Dumas 2015). Alternatively, dedifferentiation refers to older adults recruiting more brain regions than younger adults in task-based fMRI studies while maintaining the same level of behavioral performance (Cabeza 2002). Posterior-anterior shift refers to the tendency of older adults to increasingly activate anterior regions relative to posterior during task-based fMRI studies.

1.3.4 Homeostatic model of brain maintenance

Homeostatic regulation may serve as a potential model of brain maintenance. Homeostasis is the ability to maintain stability and equilibrium of a biological system. As a classical example, the stability of our body temperature is a consequence of homeostatic processes that coordinate the activity of muscles, blood vessels, and sweat glands. When a cold environment decreases body temperature, the hypothalamus releases a signal to the skeletal muscles, promoting shivering as a mechanism of thermogenesis and a signal to the blood vessels to increase resistance of blood flow (i.e., vasoconstriction). Both of these responses minimize heat loss, helping to maintain body temperature.

Homeostatic dysregulation in multiple systems occur in aging and may also serve as a key contributor to the biological mechanisms of aging (Li 2015). While the authors have provided evidence in the systems of lipids, immune function, oxygen transport, liver functioning, vitamin levels, and electrolyte levels, they suggest that homeostatic dysregulation is not limited to these systems and may occur in other systems in aging. Thus, compensatory hyperactivation may represent a homeostatic process that serves to maintain the stability of cognition in a changing neurobiological environment. Homeostasis in the context of cognition serves to maintain cognitive functioning, despite the presence of neurodegeneration.

However, homeostatic processes can have adverse effects. For example, extreme vasoconstriction for an extended period of time can lead to vascular cell loss. Similarly, compensatory hyperactivation may lead to glutaminergic excitotoxicity, which may lead to neuronal death (Dodd 1994) or the production of Aβ (Palop 2007). Thus, although homeostasis can slow the onset of cognitive decline, this may come at the cost of negative side effects that weaken the core cognitive infrastructure.

1.4 Neuropsychiatric Disorders

While common in older age, certain neuropsychiatric disorders, such as late-life mood and anxiety disorders and Alzheimer's disease, do not represent the normal process of aging. Those who become the oldest-old are not necessarily destined to experience these disorders. Instead, these age-related neuropsychiatric disorders may reflect different pathological branches in aging.

1.4.1 Late-life depression

Late-life depression (LLD) is a leading contributor to psychiatric and medical morbidity and mortality in older adults (Valiengo 2016). Often treatment resistant and recurrent (Lenze 2000), LLD results in highly prevalent cognitive impairment (~50%) that is persistent even after remission of depressive symptoms (Bhalla 2006, Bhalla 2009). Specifically, LLD has been associated with a two-fold increase in risk for development of multiple types of dementia, including Alzheimer's and vascular dementia (Diniz 2013, Green 2003).

Etiology of LLD is divided into sub-types based on the pattern and age of onset, namely late-onset and early-onset LLD. Late-onset refers to the first lifetime episode of depression occurring after the age of 65, while early-onset refers to the first lifetime episode of depression occurring earlier in life, with potential for longitudinally recurring episodes. The differences between late-onset and early-onset depression hold different implications with regards to etiology and pathophysiology in their respective cases (Aizenstein 2016, Taylor 2013, Riddle 2017).

More specifically, early-onset LLD has been associated with a genetic predisposition to depressive episodes, as well as potential external stressors such as adverse life events. These patients may also have a family history of lifelong depression, and also have an increased lifetime cumulative of time spent in depression compared to late-onset LLD patients, and therefore are potentially at greater risk for pathophysiologic changes such as hippocampal atrophy and decreased brain reserve.

Late-onset LLD patients on the other had may have greater vascular disease burden as explained by the vascular depression hypothesis, which proposes that changes in mood and increases in depressive symptoms may arise directly due to cerebrovascular disease leading to damage in relevant structural circuits and pathways (Aizenstein 2016, Taylor 2013). Other

pathologic processes contributing to late-onset LLD may exist as well, but generally a family history of lifelong depression is absent, although genetic predisposition to cerebrovascular disease or vascular disease in general may be present.

1.4.2 Late-life generalized anxiety disorder

Late-life generalized anxiety disorder (LLGAD) is a difficult disorder to fully characterize epidemiologically due to the diverse nature of the disorder among the wide range of experiences and presentations which older adults (especially adults in ethnic and racial minority groups) may have. However, current estimates suggest that the 1-year prevalence of LLGAD may be as high as 11.6% for all older adults in the US (Reynolds 2015, Kessler 2005). As with LLD, patients with LLGAD are at significantly greater risk for poorer quality of life including increased cognitive decline, risk for dementia, and exacerbation of other medical comorbidities. LLGAD itself is also associated with increased risk for depression, and represents a significant public health burden, all while being significantly underdiagnosed and undertreated (Zhang 2015).

Reasons for difficulties in identifying and treating LLGAD include camouflage of LLGAD symptoms as generalized physiological associations with the aging process, such as decreased sleep, difficulty focusing on tasks, and a sense of restlessness. Polypharmacy and medical comorbidities may also disguise and confound successful management of these symptoms. Other factors include the imprecise nature of patient descriptions of LLGAD symptoms, often resulting in psychological, subjective terms being used, as well as the presence of other neurocognitive disorders which share significant overlap with LLGAD in symptomology and have comorbidity rates up to 71% of all neurocognitive disorder patients (Seignourel 2008).

Current management for LLGAD includes a combination of pharmacologic and psychosocial approaches. Typical prescribed agents for LLGAD include selective serotonergic reuptake inhibitors as well as benzodiazepines, which are accompanied by serious risks for adverse events associated with a loss of motor coordination such as falls and motor vehicle accidents and are therefore recommended for short-term use only when possible. Psychosocial management of LLGAD commonly involves use of cognitive behavioral therapy, which is focused on goal-oriented, mental thought pattern changing efforts to self-correct maladaptive behaviors in the patient. This may often be accompanied with adjuvant therapies including relaxation techniques.

1.4.3 Alzheimer's disease

Alzheimer's disease (AD) is the most common form of dementia, affecting approximately 5.8 million people and is the 6th leading cause of death in the United States (Alzheimer's Association 2019). Significant public investment has been made for development of research and patient care improvements. There are currently no therapeutic interventions available to treat or delay progression of AD. Multiple promising pharmaceutical agents that have reversed the effects of AD in animal studies have failed in human clinical trials in AD patients. These trials may have been unsuccessful due to the presence of irreversible damage in later stages of AD; thus, it is possible that therapeutic intervention applied earlier in the course of AD may be more effective in disease modification and/or prevention (Sperling 2011).

Notably, the pathophysiological processes contributing to AD begin decades prior to the onset of clinical symptoms. The period of AD pathophysiological progression prior to cognitive symptom presentation is referred to as preclinical AD. Preclinical AD represents a window of opportunity for therapeutic intervention given the absence of irreversible damage as seen in the

later stages of AD. It is important to note that preclinical AD does not necessarily imply ultimate progression to an AD dementia diagnosis. However, should preclinical AD progress in severity, it is followed by early mild cognitive impairment (eMCI), late cognitive impairment (lMCI), and Alzheimer's disease (AD).

There are many reported risk factors for AD, with advanced age serving as the primary risk factor in late-onset AD. Conversely, early-onset AD is associated with genetic predispositions to AD, as represented by the mutations in amyloid precursor protein (APP), presenilin1 (PSEN1), and presenilin2 (PSEN2). Other risk factors for late-onset AD include presence of the apolipoprotein E allele, hypertension, hypercholesterolemia, physical inactivity, insulin resistance, obesity, poor sleep, traumatic brain injury, epilepsy, late-life depression, and late-life anxiety.

2.0 Demonstration of The Effect of Reserve: Late-Life Depression and Increased Risk Of Dementia

This chapter is a modified version of the following manuscript that is currently in submission:

- **Ly, M.**, Karim, H.T., Becker, J.T., Lopez, O.L., Aizenstein, H.J., Reynolds, C.F. III., Zmuda, M.D., Butters, M.A. Late-life depression and increased risk of dementia: a longitudinal cohort study.

This work was intended to support Aim 1 as a demonstration of the effect of reserve. In this study, our results suggest that the reduction of reserve in patients with late-life depression (LLD) increases their risk for developing dementia earlier than their age-matched never-depressed healthy controls. My contributions to this project were: analyses, interpretation of the results, and drafting and revising the manuscript.

2.1 Introduction

Late-life depression (LLD) is a leading contributor to psychiatric and medical morbidity and mortality in older adults (Valiengo 2016). Often treatment resistant and recurrent, LLD results in highly prevalent cognitive impairment (~50%) that is persistent even after remission of depressive symptoms (Bhalla 2006, Bhalla 2009). Specifically, LLD has been associated with a two-fold increase in risk for development of multiple types of dementia, including Alzheimer's and vascular dementia (Diniz 2013, Green 2003). However, it is not clear whether individuals with

a history of LLD experience a more rapid rate of cognitive decline in light of their increased risk of developing dementia. Clarification of the rate of cognitive decline in LLD may provide valuable clinical insight into risk stratification and possible prevention of future dementia.

The heterogeneous and multifactorial etiologies involved present a significant challenge in the process of predicting long-term neurocognitive and other outcomes in the course of LLD (Alexopoulos 2011, Morimoto 2015). Individuals with LLD present with a wide range of variability in neuropathological changes, brain structural abnormalities, and levels of cognitive functioning at baseline and following an episode of depression. It is unclear whether these abnormalities are related to the etiology of LLD or whether they represent the consequences of LLD itself. Clinical attributes, such as depression exposure (length of and number of depressive episodes), education level, and medical comorbidity, are also sources of wide variability. A potential avenue to reduce heterogeneity in LLD is through stratification of LLD into separate phenotypes.

Age of onset of the first depressive episode is highly related to depression exposure and may represent a useful phenotypic distinction. Early-onset depression (EOD) is thought to stem from genetic predisposition and adverse life events, while late-onset depression (LOD) is more associated with the accumulation of vascular burden and other pathologic aging processes in the absence of family history (Aizenstein 2016, Taylor 2013). EOD patients may experience cognitive decline due to longer time in depression or more lifetime depressive episodes, which lead to hippocampal atrophy, increased allostatic load, and decreased brain reserve. In contrast, cognitive decline in LOD patients may result directly from vascular and neurodegenerative risk factors (Aizenstein 2016, Taylor 2013). If EOD and LOD represent distinct phenotypes of LLD, it is critical to investigate whether cognitive trajectories differ between the two groups over time.

Previous investigations of the long-term cognitive trajectories in LLD have largely been cross-sectional, while longitudinal studies often did not exceed five years in duration, thereby limiting their ability to delineate longer neurocognitive trajectories. Other limitations of prior studies include small sample sizes, limited use of highly replicable neurocognitive batteries, and lack of measurement and/or statistical control of baseline cognitive functioning. In addition, many studies did not differentiate between EOD and LOD in reporting LLD subgroups. One study did stratify outcomes in EOD and LOD patients using a robust longitudinal design, but obtained baseline neuropsychological measurements while patients were depressed, which potentially confounds the interpretation of cognitive performance (Riddle 2017).

The primary purpose of this longitudinal study was to determine whether individuals with a history of LLD experience more rapid cognitive decline than those without a depression history. Participants with a history of LLD and never-depressed control (NDC) participants underwent annual neuropsychological assessments for up to ten years. Most assessments were made while LLD participants were in a state of remission. Baseline cognitive performance and rate of cognitive decline were compared between the LLD and NDC groups. We hypothesized that individuals with a history of LLD would have more cognitive impairments at baseline and exhibit a more rapid decline in multiple domains of cognitive performance compared with the NDC group. We also investigated whether dichotomization of the LLD group into EOD and LOD phenotypes revealed differing rates of cognitive decline.

2.2 Methods

2.2.1 Participants

Participants with LLD were recruited from the University of Pittsburgh Late-Life Depression Prevention and Treatment Center (N=185), while NDC (N=114) were recruited from the local Pittsburgh community. Recruitment occurred on a rolling basis which allowed for more data acquisition and longer follow-up from participants recruited in the early years of the study and higher retention rates. Inclusion criteria for LLD participants stipulated age 60 or older at baseline visit, meeting *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV) criteria for unipolar major depression, English language fluency, and visual and auditory acuity adequate to undergo neuropsychological assessment. Exclusion criteria included major unstable medical illness, diagnosis of psychiatric disorders other than unipolar major depression or anxiety disorders, neurologic disorders or injuries with direct effects on cognitive functioning, and clinical diagnosis of dementia. Control participants met the same inclusion and exclusion criteria, with the exception that they had no lifetime history of psychiatric disorder. Lifetime antidepressant exposure was reported in 93.5% (N=173) of individuals with history of LLD and 9.6% (N=11) of NDCs for indications other than depression. Over the duration of this study, approximately 70% of the LLD group and approximately 10% of the NDC group were taking antidepressant medication. LLD participants were further categorized into early-onset (EOD, N=85) and late-onset subgroups (LOD, N=100), with early-onset defined as having lifetime depressive episode at age 59 or younger and late-onset defined as first lifetime depressive episode at age 60 or older. All participants provided informed consent under a protocol approved by the University of Pittsburgh Institutional Review Board.

2.2.2 Procedures

At baseline and annual follow-up visits, participants were assessed for depressive symptoms (17-item Hamilton Depression Rating Scale, HDRS-17), medical comorbidity (Cumulative Illness Rating Scale for Geriatrics, CIRSG), and cardiovascular risk factor status (risk factors derived from Probability of Stroke Risk Profile from the Framingham Study). In order to ensure that cognitive assessments occurred during a period of reduced depressive symptoms, we aimed to test only participants whose HDRS-17 score was ≤ 10 for neuropsychological testing. Participants with HDRS-17 score > 10 were referred to treatment, with study visits postponed until symptomatic improvement had occurred (postponed by up to 3 months as needed). However, some individuals did not remit at one or more visits but were still assessed. Approximately 21% of visits [376 out of 1774 total visits across all participants] had a participant with a HDRS-17 score greater than 7 (criteria for remission). Amongst those visits, participants had on average 2.3 (SD 1.5) visits with a HDRS-17 greater than 7.

The neuropsychological battery utilized in this study has been well-validated in assessing cognitive function across multiple domains in older adults, as detailed in our prior work (Butters 2004). The raw scores of each neuropsychological test were converted to standard scores using the distribution of the comparison group. Composite scores for each cognitive domain (Attention/Processing Speed, Visuospatial Ability, Verbal Ability, Executive Functioning, and Delayed Memory) were then calculated by averaging the standard scores across tests in Table 2. Selection of cognitive domains was guided by factor analysis, conceptual groupings, and Cronbach's alphas. The Cronbach's standardized alpha values ranged from 0.55-0.75. The cognitive diagnosis for each participant was then determined through consensus conferences affiliated with the University of Pittsburgh Alzheimer's Disease Research Center.

Table 2. Neuropsychological battery

Domain	Tests	Outcome Measure
Attention/Processing Speed	Digit Symbol	Number of correct symbols in 90 seconds
	Grooved Pegboard	Time in seconds to complete for both hands
	Trail Making A	Time in seconds to completion
	Finger Tapping	Average number of taps in 10 seconds for both hands
Visuospatial Ability	Block Design	Score calculated from total number of accurate patterns and time to completion
	Clock Drawing	Number of features drawn correctly
	Modified Rey Osterreith Figure	Copy, number of features drawn correctly
	Simple Drawings	Number correct
Verbal Ability	Semantic Fluency	Number of appropriate words listed in 60 seconds
	Boston Naming	Number correct
	Spot the Word	Total number of errors
	Letter Fluency	Number of appropriate words listed in 60 seconds
Executive Functions	Trail Making B	Time in seconds to complete
	Executive Interview	Total score
	Stroop Color Word Inhibition	Number of items correct in Color-Word condition in 45 seconds
	Wisconsin Card Sorting	Percentage of errors (total, perseverative, and non-perseverative), number of categories completed
Delayed Memory	Logical Memory	Delayed recall, number of details correct
	California Verbal Learning Test	Delayed recall, number correct
	Modified Rey Osterreith Figure	Delayed recall, number of features drawn correctly

2.2.3 Statistical Analysis

Prior to any analysis, we examined data distributions to assess normality and the presence of outliers. We calculated descriptive statistics for baseline demographics and clinical measures of

the NDC and LLD groups, using t-tests to test for group differences on the continuous variables and chi-square tests for categorical variables.

We plotted cognitive domains over time for both groups to examine individual domain trajectories as well as the mean (and standard error) for each group. After reviewing the graphs, we chose to use 10 years of data in all analyses to maximize clinical relevance and to minimize bias estimates due to drop off in sample size and increased variability after 10 years. Baseline date and yearly visit dates determined the time variable for all analyses. We employed a mixed-models approach to compare domain trajectories and to test for group, time, and group by time differences. When fitting the model, we controlled for baseline domain score since the two groups differed at baseline. Baseline age, baseline medical comorbidity, education, and sex were also included in the model as covariates since these are known to affect cognitive function. Models first considered a quadratic effect to test for nonlinear trajectories. When the quadratic component was not significant, we moved to a linear model. Best fit model was determined by comparing Bayesian Information Criterion (BIC) values between models.

Attention/processing speed, verbal ability, delayed memory, and global cognitive function included both linear and quadratic time as both fixed and random effects. Executive function and visuospatial ability included only linear time as fixed and random effects. For comparing between subtypes of depression (EOD vs. LOD), only delayed memory had both linear and quadratic time as both fixed and random effects, while other domains included only linear time as fixed and random effects.

2.3 Results

The demographic and clinical characteristics of the study participants comparing LLD and NDC are displayed in table 3. The LLD group compared to NDC was older, had a greater percentage of female participants, and had greater medical comorbidity and vascular risk factors as determined by the CIRS-G and CVRF, respectively.

The demographic and clinical characteristics of the study participants comparing LOD, EOD, and NDC are displayed in table 4. The LOD group was older than both EOD and NDC; there were more women in the EOD group compared to both the LOD and NDC groups; LOD and EOD groups had greater medical comorbidity (CIRS-G) than NDC; LOD group had greater vascular risk factors than the NDC; and LOD had lower length of follow-up than NDC and EOD.

2.3.1 Comparing LLD and NDC

At baseline, the LLD group compared to the NDC performed worse in all domains except for the visuospatial domain (figure 1). The LLD declined more rapidly than the NDC only in the verbal domain, however this difference appears to be related to a lack of a practice effect among LLD compared with NDC rather than actual decline – lack of a practice effect is often due to cognitive impairment (figure 1).

2.3.2 Comparing LOD, EOD, and NDC

At baseline, the LOD group performed significantly worse than the NDC group in all domains. At baseline, all three groups differed (NDC > EOD > LOD) in the attention/processing speed and global function domains (figure 2). The LOD group declined more rapidly over time compared to the NDC group in verbal ability and delayed memory (figure 2).

Table 3. Comparison of LLD and NDC Baseline demographics/follow-up information

	NDC (N=114) Mean (std) or %(n)	LLD (n=185) Mean (std) or %(n)	LLD vs. NDC
Age	71.1 (6.5)	72.8 (6.4)	**t(297)=-2.26**
%Female	58.8 (n=67)	75.7 (n=140)	**$\chi^2(1)=9.46$**
%White	90.4 (n=103)	90.3 (n=167)	$\chi^2(1)=0.00$
Education	14.3 (2.8)	13.8 (2.6)	t(297)=1.57
Cumulative Illness Rating Scale (CIRSG)	6.8 (3.6) (n=106)	9.7 (3.6) (n=184)	**t(288)=-6.67**
Cardiovascular Risk Factor Score (CVRF)	1.7 (1.4) (n=108)	2.1 (1.2) (n=163)	**t(275)=-2.55**
Baseline Neuropsych Attention/Processing Speed**	0.02 (0.67)	-0.55 (1.1) (n=183)	**t(295)=5.70**
Verbal Ability	0.05 (0.68)	-0.18 (0.74)	**t(297)=2.71**
Delayed Memory	0.00 (0.76)	-0.28 (0.86)	**t(297)=2.87**
Visuospatial Ability	0.02 (0.74)	-0.10 (0.65)	t(297)=1.53
Executive Functions**	0.06 (0.54)	-0.27 (1.02)	**t(297)=3.15**
Global**	0.03 (0.49)	-0.27 (0.63)	**t(283)=4.61**
Age of Onset	--	56.0 (19.5) Range=10-93	--
%Single MDD episode	--	48.11 (n=89)	--
Length of follow-up*	5.7 (3.8) Range=1.0-14.8	5.1 (3.4) Range=0.9-15.8	t(297)=1.31
Pathway Status %Still Active	85.09 (n=97)	75.14 (n=139)	--
%Died	10.53 (n=12)	10.27 (n=19)	
%Terminated due to mental health	0.00 (n=0)	0.54 (n=1)	
%Moved/no transportation	0.88 (n=1)	0.54 (n=1)	
%Other Reason	0.00 (n=0)	0.54 (n=1)	
%Terminated due to physical health	0.00 (n=0)	0.54 (n=1)	
%Refused	1.75 (n=2)	8.11 (n=15)	
%Unable to contact	1.75 (n=2)	4.32 (n=8)	

*Transformation used in analyses. Means (STD) reported in original units.**Satterwaite method used due to unequal variances. Values in bold are statistically significant ($p<0.05$)

Table 4. Comparison of LOD, EOD and NDC Baseline demographics/follow-up information.

	NDC (N=114)	EOD (≤60 years) (N=85)	LOD (>60 years) (N=100)	Comparison between groups
Age	71.1 (6.5)	70.5 (5.5)	74.8 (6.5)	**F(2,296)=13.66 LOD > EOD, NDC**
%Female	58.8 (n=67)	82.4 (n=70)	70.0 (n=70)	**χ^2(2)=12.75 EOD > LOD > NDC**
%White	90.4 (n=103)	94.1 (n=80)	87.0 (n=87)	χ^2(2)=2.66
Education	14.3 (2.8)	13.9 (2.6)	13.6 (2.6)	F(2,296)=1.45
Cumulative Illness Rating Scale (CIRSG)	6.8 (3.6) (n=106)	9.7 (3.6)	9.7 (3.5) (n=99)	**F(2,287)=22.17 EOD, LOD > NDC**
Cardiovascular Risk Factor Score (CVRF)	1.7 (1.4) (n=108)	1.9 (1.2) (n=74)	2.2 (1.2) (n=89)	**F(2,268)=4.19 LOD > NDC**
Age Onset	--	38.7 (14.79) Range=10-60	70.8 (6.8) Range=61-93	--
%Single MDD Episode		12.94 (n=11)	78.00 (n=78)	**χ^2(1)=77.90**
Length of follow-up*	5.7 (3.8) Range=1.0-14.8	5.7 (3.2) Range=1.0-14.3	4.6 (3.5) Range=0.9-18	**F(2,296)=6.17 NDC, EOD > LOD**
Baseline Neuropsych Attention/Processing Speed	0.00 (0.69)	-0.35 (0.95)	-0.72 (1.1) (n=98)	**F(2,294)=17.51 NDC > EOD > LOD**
Verbal Ability	0.00 (0.70)	-0.11 (0.74)	-0.24 (0.74)	**F(2,296)=4.45 NDC > LOD**
Executive Functions	0.00 (0.58)	-0.11 (1.12)	-0.41 (0.91)	**F(2,296)=7.77 NDC > LOD**
Delayed Memory	-0.01 (0.75)	-0.13 (0.84)	-0.40 (0.86)	**F(2,296)=6.72 NDC > LOD**
Visuospatial Ability	0.00 (0.75)	0.01 (0.62)	-0.20 (0.66)	**F(2,296)=3.53 NDC > LOD**
Global Functioning	0.03 (0.49)	-0.13 (0.63)	-0.38 (0.61)	**F(2,296)=14.19 NDC > EOD > LOD**
Pathway Status %Still Active	85.09 (n=97)	78.82 (n=67)	72.00 (n=72)	--
%Died	10.53 (n=12)	7.06 (n=6)	13.00 (n=13)	
%Terminated due to mental health	0.00 (n=0)	1.18 (n=1)	0.00 (n=0)	
%Moved/no transportation	0.88 (n=1)	0.00 (n=0)	1.00 (n=1)	
%Other Reason	0.00 (n=0)	1.18 (n=1)	0.00 (n=0)	
%Terminated due to physical health	0.00 (n=0)	0.00 (n=0)	1.00 (n=1)	
%Refused	1.75 (n=2)	9.41 (n=8)	7.00 (n=7)	
%Unable to contact	1.75 (n=2)	2.35 (n=2)	6.00 (n=6)	

*Transformation used in analyses. Means (STD) reported in original units.; Values in bold are statistically significant ($p<0.05$)

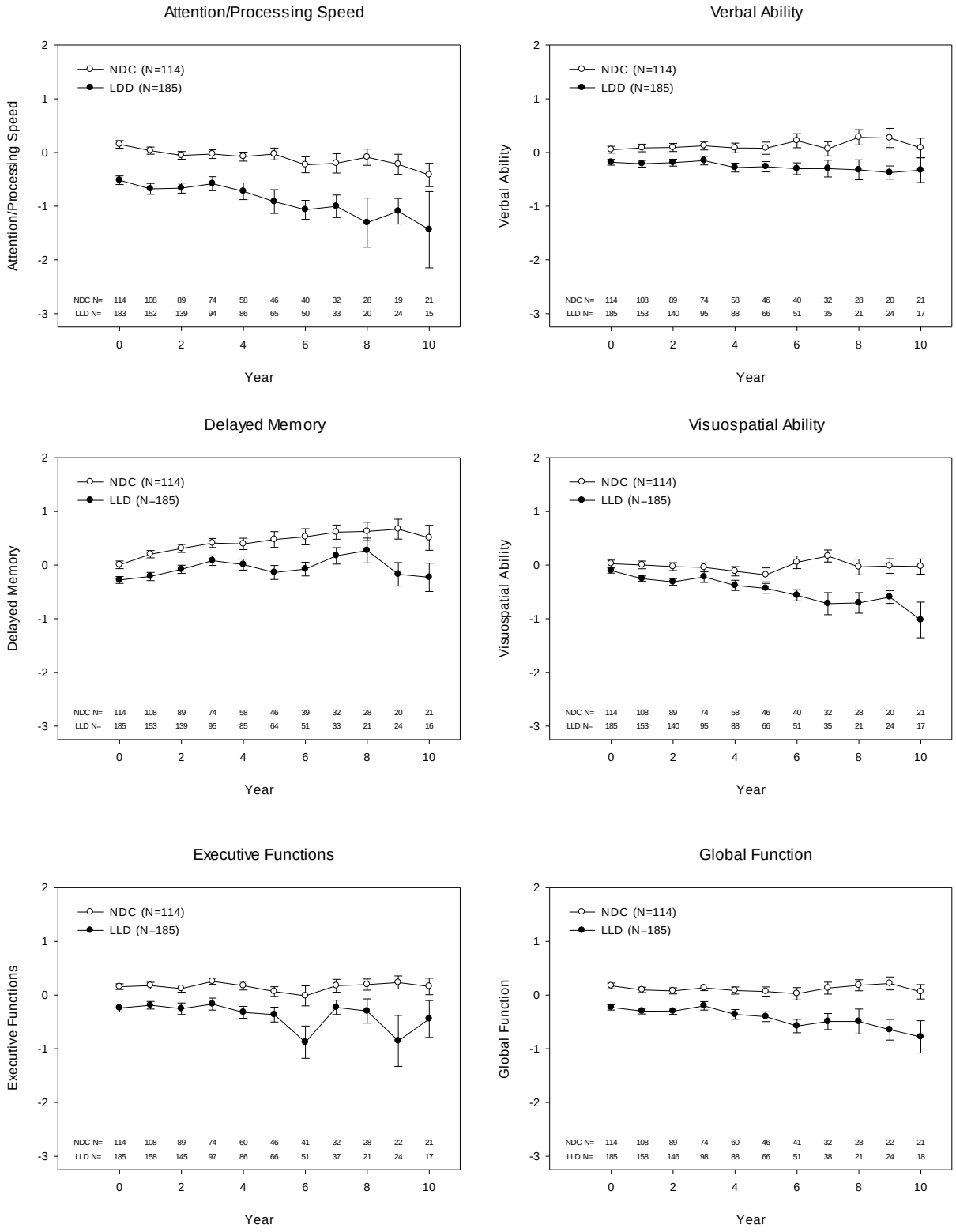

Figure 1. Graph of cognitive trajectories comparing NDC vs. LLD.

There were baseline differences between LLD and NDC in all domains except visuospatial ability. LLD group differed over time compared to the NDC in the verbal ability only – this may be due to lack of practice effect rather than cognitive decline.

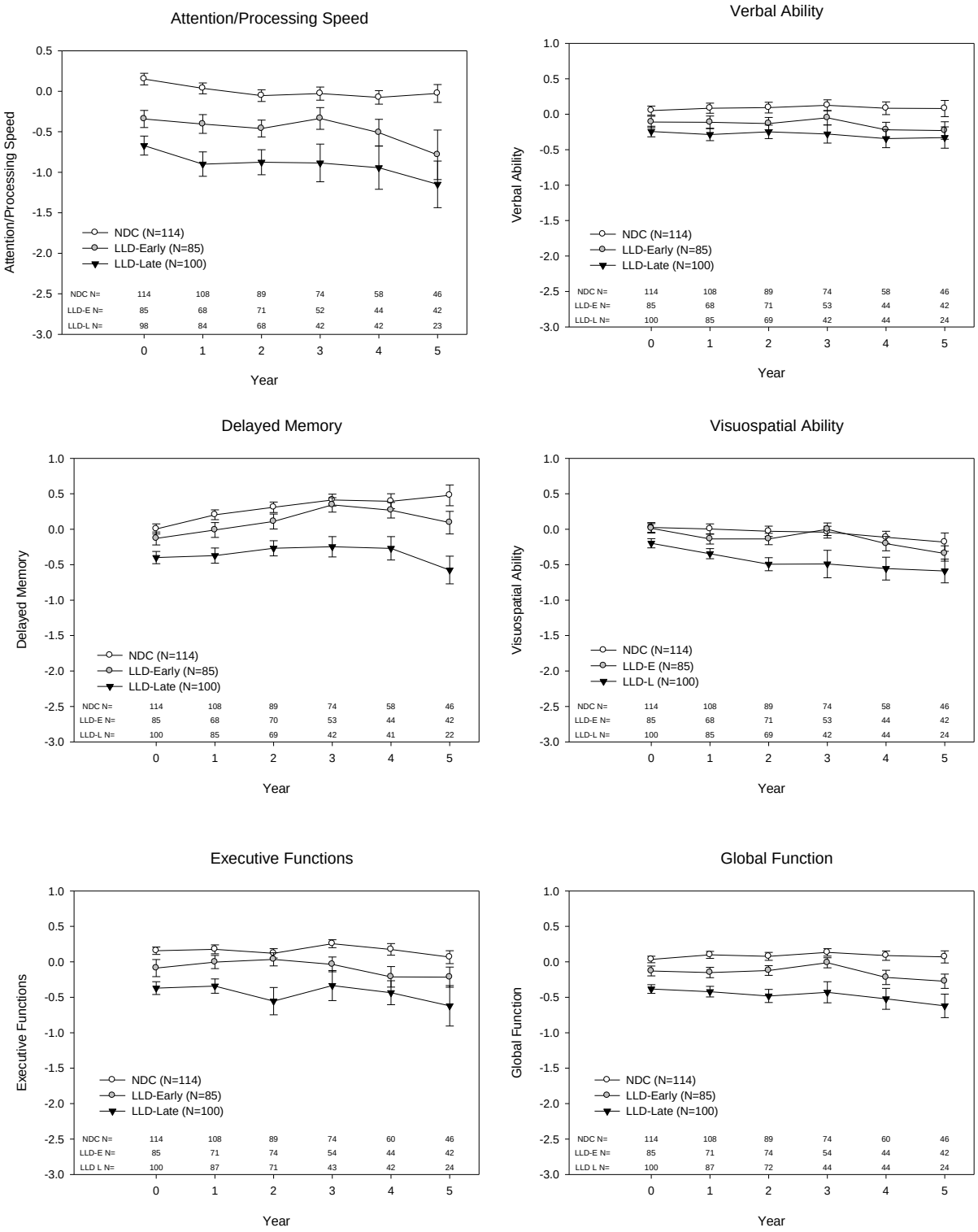

Figure 2. Graph of cognitive trajectories comparing NDC vs. LOD vs. EOD.

Graph of cognitive trajectories comparing NDC vs. LOD vs. EOD. At baseline, the LOD group performed worse than NDC in all domains while all three groups differed in the attention/processing speed and global

function domains (NDC > EOD > LOD). The LOD group declined more rapidly than both the NDC and EOD groups in the verbal ability and delayed memory domains.

2.4 Discussion

To our knowledge, this study represents one of the first longitudinal studies to utilize a broad-based, comprehensive neuropsychological battery to assess cognitive decline in remitted late-life depression. We found that at baseline, those with a history of LLD showed cognitive impairment across multiple domains and further that those with LOD showed even greater cognitive impairment compared to EOD compared to NDC. We also found that those with LOD had a more rapid rate of decline in verbal ability and delayed memory compared to NDC.

Although individuals with a history of LLD did not exhibit a steeper rate of decline compared to NDC, they did exhibit significantly greater baseline cognitive impairment. This difference could account for the increased incidence or risk of dementia and reflect mixing of EOD and LOD subtypes. The prevalent baseline impairment may reflect decreased brain and/or cognitive reserve. Brain and cognitive reserve represent protective factors, such as greater cortical thickness or high level of educational/occupational attainment, that provide resilience to age-related decline and other pathological processes (Stern 2012, Stern 2018). LLD has been associated with numerous neuropathological abnormalities, including increased inflammation and elevated glucocorticoids, which contribute to cerebrovascular injury, amyloid deposition, hippocampal atrophy, and reduced volume in the basal ganglia and prefrontal regions (Byers 2011). These pathological processes contribute to greater levels of lowered brain and cognitive reserve, thus potentially leading those with LLD to cross the threshold of clinical dementia sooner than NDCs.

Stratification of LLD into separate phenotypes based on age of onset demonstrated different patterns of cognitive impairment and decline. Individuals with EOD, while not exhibiting more rapid decline over 5-10 years, did exhibit significant impairment in the attention/processing speed and global cognitive function at baseline. Impairment in global cognitive functioning provides evidence of the neurotoxicity of depression: repeated, cumulative depression exposure can have a significant impact on brain reserve and cognitive function (Byers 2011). In contrast, individuals with LOD performed worse than NDC in all domains at baseline and experienced more rapid decline in verbal ability and delayed memory than both NDC and EOD. The progressive decline in memory performance is especially salient, as it may represent the leading clinical sign of impending dementia. Thus, our findings are consistent with the hypothesis that LOD may represent a prodromal phase of dementia (Bennett 2014). This provides support to theories that suggest LOD may be due a result of aging-related neuropathology, e.g., amyloid, gray matter atrophy, and cerebrovascular disease (Aizenstein 2016, Taylor 2013).

Our findings differ from those reported by Riddle, et al (Riddle 2017). In their study, Riddle and colleagues reported that individuals with LLD exhibited more cognitive impairment at baseline and greater subsequent decline in all cognitive domains compared with NDC, with EOD individuals experiencing greater decline than LOD and NDC groups. Of note, their participants were depressed at baseline—possibly a source of unexplained variance in subsequent measures of trajectory. Our neuropsychological battery was more broad-based and comprehensive, especially in the executive functioning and verbal domains. The Riddle et al. participants may have experienced a different cognitive trajectory than our study sample, being younger (e.g. higher brain reserve) and more educated (e.g. higher cognitive reserve) on average than our participants. We suggest that the findings in our study are complementary to those of Riddle et al., rather than

contradictory, and may capture different perspectives reflecting differences in study samples, as well as in content and timing of neurocognitive assessment.

Our study has several limitations. Although most of the neuropsychological assessments were performed while the LLD participants were in a state of remission, a small subset of participants had not achieved full remission but were still included in our analyses. Hindsight bias in self-reported age of first depressive episode may also limit the accuracy of stratification of LLD into EOD and LOD phenotypes. Incorporation of other imaging or metabolic biomarkers (e.g. white matter hyperintensities, cortisol levels) may allow for more optimal differentiation between EOD and LOD participants. Our study sample was predominantly Caucasian, and thus did not reflect the greater medical comorbidity and attendant effects on brain health and cognitive function to which African Americans are subject. Differences between groups may be due to sampling bias since the depressed participants were recruited from an academic research center, while some of the NDC were recruited from the local Pittsburgh community. Consistent with prior studies, effect sizes for cognitive decline were modest. While LLD and NDC showed no differences in follow-up, the individuals with LOD (compared to EOD and NDC) had a shorter average follow-up, which may affect the results and is a limitation of our study. Further study with longer follow-up or with a lifespan approach may provide insight toward the critical stages of cognitive decline.

In conclusion, we observed that patients with a history of LLD did not experience an accelerated rate of cognitive decline over 5-10 years as compared with NDC. Instead, we observed LLD to be associated with greater baseline cognitive impairment, providing a possible explanation for the association of LLD with development of dementia. Dichotomization of LLD based on age of depression onset yielded different cognitive trajectories over time, suggesting that EOD and LOD may represent different neural substrates that increase their risk for development of

subsequent dementia. Future studies should focus on the pathophysiological changes that may lower brain reserve in individuals with EOD, when cognitive differences are small, and later-life, when they are substantial. Furthermore, elucidation of the various neurobiological mechanisms that underlie cognitive impairment in EOD (e.g., allostatic load, chronic inflammation) vs. LOD (subclinical microvascular disease, Lewy bodies, AD pathology) could inform earlier intervention to reduce risk for future dementia.

3.0 Demonstration of The Effect of Reserve: Cognitive Reserve Effect in the Extended Memory Encoding Network in Subjective Cognitive Decline

This chapter is a modified version of the following manuscript that is currently under review:

- Mizuno, A., Karim, H.T., Rangarajan, A., **Ly, M.**, Cohen, A.D., Lopresti, B.J., Mathis, C.A., Klunk, W.E., Aizenstein, H.J., Snitz, B.E. Cognitive reserve effect in the extended memory encoding network in subjective cognitive decline: a functional MRI and amyloid-PET study.

This work was intended to support Aim 1 as a demonstration of the effect of reserve. In this study, we demonstrated that level of cognitive reserve altered the patterns of functional MRI activation during a memory-encoding task in individuals with subjective cognitive decline (SCD). These results support the theory that level of cognitive reserve may be a determinant of which neural compensatory responses to pathological processes may be employed. My contributions to this study were: interpretation of the results, drafting portions of the manuscript, and revising the manuscript.

3.1 Introduction

Subjective cognitive decline (SCD) refers to self-experienced decline in cognitive capacity, despite objectively measured normal cognitive functioning. It has been suggested that SCD may represent an early transition state from normal aging to mild cognitive impairment (MCI) and eventually Alzheimer's Disease (AD) (Buckley et al., 2017; Vogel et al., 2017). SCD symptoms are associated with amyloid-β (Aβ) deposition (Amariglio et al., 2012; Perrotin et al., 2012; Snitz et al., 2015b). However, the neural characteristics of SCD and the associations with the risk of future progression are not well understood.

SCD is commonly characterized by subjective memory complaints or impairment (Jessen et al., 2014). Prior fMRI studies of SCD have largely focused on memory encoding (Erk et al., 2011; Hayes et al., 2017; Rodda et al., 2009) and have shown SCD-associated hippocampal hypoactivation (Erk et al., 2011) and dorsolateral prefrontal cortex (DLPFC) hyperactivation (Erk et al., 2011; Rodda et al., 2009). Along with positive associations between DLPFC activation and task performance (Erk et al., 2011; Rodda et al., 2009), DLPFC hyperactivation may represent a secondary resource to compensate for hippocampal hypoactivation in SCD. Neural compensation is postulated to be a process by which additional neural resources are recruited in response to some trigger (Stern, 2012). Hyperactivation in SCD may thus occur in response to early AD neuropathology (e.g., Aβ) (Garcia-Ptacek et al., 2016). However, the relationship between task-induced hyperactivation and AD neuropathology has not been directly investigated in SCD.

To explain individual differences in resilience against risks of dementia, Stern (Stern, 2009; Stern, 2012) postulated the cognitive reserve theory: individuals vary in effective maintenance of cognitive ability in the face of neural pathology. Cognitive reserve is approximated by proxy measures such as education, IQ, literacy, and occupational complexity. The role of education on

risk for disease progression in SCD has inconsistent findings in the literature. Fewer years of education in SCD was associated with risk of progressing to MCI or AD in a research clinic setting (Reisberg et al., 2010). However, in a large population-based study (van Oijen et al., 2007), higher education in SCD was associated with greater risk of progression to AD, in contrast to the protective effect of education widely observed in other studies (see Sharp and Gatz, 2011 for a review).

This study aimed to 1) characterize the neural basis of SCD symptoms using functional magnetic resonance imaging (fMRI) during a well-known memory-encoding task; 2) examine the role of Aβ using positron emission tomography (PET) to better understand brain activation in relation to emerging AD pathology; and 3) explore how education, a proxy for cognitive reserve, may moderate the relationship between brain activation and indices of AD risk (SCD symptoms and Aβ). We selected the associative memory encoding (face-name) task because this well-validated task has been extensively used to characterize (e.g., (De Vogelaere et al., 2010; Edelman et al., 2017; Sperling et al., 2009)) neural functional alterations in AD-related disease.

3.2 Methods

3.2.1 Study Design and Participants

Data for these analyses came from 66 cognitively normal older (mean age = 73.3) individuals with varying SCD symptoms. Participants came from two study samples: self-referred patient volunteers at an academic memory clinic (n=22) and community-based volunteers for a neuroimaging study (n=44). Participants from the memory clinic were approached and recruited

after they self-referred for evaluation at the University of Pittsburgh Alzheimer Disease Research Center (ADRC). The inclusion criteria for memory clinic participants were: 1) age 60 and older; 2) clinically significant subjective concern about cognitive changes; 3) normal objective cognitive function; and 4) fluent in English. Normal cognitive function was defined as no more than two scores falling one standard deviation below age-adjusted norms on a neuropsychological battery, and adjudication of normal cognitive function in a diagnostic consensus conference. Community-based volunteers were recruited through advertisements, and their inclusion criteria were: 1) age 65 and older; 2) normal objective cognitive function; and 3) fluent in English. All participants completed a multi-domain neuropsychological assessment and were reviewed by a diagnostic consensus conference that included at least two of the same investigators (authors BES and WEK) as the ADRC setting. Exclusion criteria for all participants were: 1) diagnosis of MCI or dementia; 2) history of significant neurologic or major psychiatric conditions; 3) current medical condition or medications that may affect cognitive function; 4) current clinical depression (scored above the common clinical cutoff of 15 on Geriatric Depression Scale); and 5) contraindications for MRI or PET scans. More detailed descriptions of participants' criteria were reported previously (Snitz et al., 2015a). We excluded one participant due to a poor fMRI coverage in the inferior temporal region, with the final sample of n=66. All participants provided written informed consent according to protocols approved by the University of Pittsburgh Institutional Review Board.

3.2.2 Self-Report, Neuropsychological, and Demographic Assessments

Three measures of self-reported SCD symptoms were used: the Memory Functioning Questionnaire (MFQ) (Zelinski et al., 1990); the Cognitive Failures Questionnaire (CFQ) (Broadbent et al., 1982); and the Subjective Cognitive Complaint Scale (SCCS) (Snitz et al., 2012).

Each scale was transformed into Z-scores using age-adjusted means from published studies (Gilewski et al., 1990; Knight et al., 2004; Snitz et al., 2015b) and standard deviations (SD) from current participants' responses. After inverting the MFQ (such that higher values indicate worse SCD symptoms), we computed a mean of Z-scores. We defined this composite score as "SCD symptoms" and included it in analyses as a continuous variable indicating the SCD symptom severity. This continuous measure approach was used in a previous fMRI study in SCD (Hayes et al., 2017), and we employed the same approach here to measure brain activation during memory encoding associated with the degree of SCD severity.

We employed modified Rey-Osterrieth complex figure immediate and delayed recall scores (Becker et al., 1987) as an index of objective memory performance to complement our visual memory- encoding task for the fMRI data. We transformed both scores to Z-scores using age-adjusted means from previously published studies (Wolk et al., 2009) and SD from current participants' responses. Then, we defined an objective memory score as a mean of Z-scores. Five participants were missing memory test scores due to a change in the neuropsychological test battery; these participants were excluded from the corresponding analyses but included for the main fMRI analysis.

Participants self-reported years of education. For post-hoc analysis, we created three groups: high school (≤12 years, n=24), some college (>12 and ≤16 years, n=24), and post college (>16 years, n=18). Additionally, we measured neuroticism with the NEO Five-Factor Inventory (FFI-3) (McCrae and Costa, 2007) and included it as a covariate to account for the influence of previously reported high neuroticism in SCD (Kliegel et al., 2005) (details below).

3.2.3 Face-Name Association fMRI Task

We employed the "face-name" association task, which elicits paired associative memory encoding (De Vogelaere et al., 2010; Edelman et al., 2017; Sperling et al., 2009) (Supplemental Figure S1). Participants saw a face-name pair for 5 seconds and decided whether the name "fit" the face – there is no correct answer. Participants responded with their right/left index finger if the name fit or not, respectively. Participants were given the following instructions: "Try to remember these face-name combinations; you will be quizzed after the scan."

During encoding blocks, participants were presented with eight novel face-name pairs, while recognition blocks were identical except with familiar face-name pairs (one female and one male) learned during a pre-scan session. Faces were presented for 5 seconds each and a white plus sign was presented for 1 second after each face. Blocks lasted 48 seconds each and were alternated with a 25-second fixation period. Each block repeated twice (5 minutes for 1 run). Participants repeated the task three times (i.e., 3 runs) and saw 50 face-name pairs: two familiar and 48 novel pairs.

During the post-fMRI scan test, participants saw a face with two names, one seen in the scanner and one new, and were asked to choose the name seen in the scanner. We assessed the accuracy of recognition memory as a post-scan recognition score. Two participants were missing this score, and for the participants that did not complete all sessions, we ensured that their post-scan test used only the faces they saw in the scanner.

3.2.4 PET Data Acquisition

PET data were acquired on Siemens/CTI ECAT HR+ scanner. 15mCi of high specific activity (~2.1Ci/μmol at EOS) [11C]PiB was injected intravenously over 20sec. Transmission imaging was performed using rotating $^{68}Ge/^{68}Ga$ rod sources for attenuation correction. PET emission data was reconstructed using filtered back projection with corrections for attenuation, scatter, and radionuclide decay.

3.2.5 MRI Data Acquisition

MRI data were collected using a 3T Siemens Trio TIM scanner with a 12-channel head coil. The whole brain structural sequences were collected: sagittal 3D MPRAGE, axial 2D FLAIR, axial 3D T2-weighted sequence (see methods supplement for parameters). An axial EPI BOLD (blood oxygen-level dependent) sequence during the face-name association task was collected with echo time=32ms, repetition time=2000ms, flip angle=90 , field-of-view=128x128, 2x2x4mm resolution with no gap, and GRAPPA (GeneRalized Autocalibrating Partial Parallel Acquisition) factor 2. Due to poor coverage and placement, scans covered above the cerebellum up to the motor cortex.

3.2.6 PET Data Analysis

After inspection for interframe motion, the automated image registration (AIR) algorithm (parameters optimized for PET to PET registration) was applied to the dynamic [^{11}C]PiB image. A summed PET image over the 50-70 minute post-injection interval was calculated for PET-MR

image co-registration using AIR after manual reorientation of MRI to AC-PC line (Woods et al., 1993). The resulting spatial transformation was applied to the summed PiB image and interpolated in the MR image space. Six ROIs were separately hand-drawn on a co-registered MRI (Cohen et al., 2013; Rosario et al., 2011). Regional radioactivity concentrations from ROIs were transformed into units of standardized uptake value (SUV) using the injected dose of PIB and the participant's mass. The SUV was normalized to non-specific uptake (cerebellum as reference), yielding an SUV ratio (SUVR) measure that compares favorably to fully quantitative measures of specific radiotracer retention (Lopresti et al., 2005). SUVRs were partial volume-corrected using a previously validated method (Price et al., 2005). We defined "Aβ deposition" as a global SUVR that we computed as the average of six SUVRs, resulting in a continuous variable. We also defined participants' Aβ status as a categorical variable. We classified participants as Aβ positive or negative with a sparse k-means cluster analysis method (cutoff = 1.51 global SUVR) (Cohen et al., 2013). Three participants were missing PiB-PET data, and these participants were excluded from the corresponding.

3.2.7 MRI Data Analysis

3.2.7.1 Preprocessing.

Statistical Parametric Mapping (SPM12) toolbox in MATLAB2016b (MathWorks) was used to preprocess MRI data. Structural sequences were co-registered to the MPRAGE, bias-corrected, segmented into multiple tissue classes that generated a deformation field to normalize images to MNI (Montreal Neurological Institute) space. An automatic intracranial volume mask was generated using a threshold of 0.1 on gray/white/CSF (cerebrospinal fluid) followed by image filling and closing in MATLAB and applied to the MPRAGE to remove skull.

Functional data was motion-corrected, co-registered to the skull-stripped MPRAGE, normalized (2mm isotropic resolution), and smoothed using a Gaussian kernel with FWHM of 8mm. We computed five summary measures of motion using ArtRepair toolbox (http://cibsr.stanford.edu/tools/human-brain-project/artrepair-software.html).

We computed the mean volume of the left and right hippocampus using FIRST in FSL (FMRIB Software Library) on the skull-stripped MPRAGE. FIRST uses a model-based approach to segment subcortical structures using Bayesian shape and appearance models.

3.2.7.2 Modeling task effect.

We modeled encoding and recognition tasks (convolved with the canonical hemodynamic response function; all runs input into a single model) as well as the mean of the signal and six motion parameters from the alignment (independently modeled for each session). The model included a high-pass filter (1/128Hz to account for drift, using a series of cosines) as well as an autoregressive filter to account for serial correlations due to aliased biorhythms/unmodeled activity. We computed the contrast encoding minus recognition. We then conducted a voxel-wise one-sample t-test on the parameter estimates in statistical non-parametric mapping toolbox (SnPM13). We generated a mask based on this contrast, which was used in subsequent statistical analyses to limit the number of statistical tests computed to only regions that were activated by the task.

3.2.7.3 The main effects of indices of AD risk (SCD Symptoms, Aβ deposition).

We tested voxel-wise associations between brain activation during memory encoding and both SCD symptoms and Aβ deposition separately. We used SnPM13 to conduct all voxel-wise statistical analyses. This toolbox uses a non-parametric permutation test to calculate p-values for

each voxel. We controlled the voxel-wise false discovery rate (FDR) at $\alpha<0.05$. As an additional analysis to assess the effect of task-performance, we tested the voxel-wise association between brain activation during memory encoding and post-scan recognition scores.

3.2.7.4 Moderating role of education (interaction effects).

We examined whether education moderated the relationship between brain activation during memory encoding and either SCD symptoms or Aβ deposition. We tested (voxel-wise) interactions between SCD symptoms and education (mean-centered scores) and between Aβ deposition and education (FDR at $\alpha<0.05$ for both). To test the robustness of each interaction to nuisance variables (age, objective memory scores, neuroticism, sex, post-scan recognition scores, hippocampal volume, recruitment methods, and five in-scanner motion measures), we ran regression analyses (in R, https://www.r- project.org) with extracted mean activation (encoding-recognition contrast) from significant regions with an interaction. In this analysis, participants who had missing data in any of these variables were excluded from the robustness testing.

3.2.7.5 Exploratory analyses.

By using the same extracted mean activation values, we tested the SCD symptoms by education interaction separately for participants classified as Aβ positive (n=27) and those classified as Aβ negative (n=36) to explore the effect of Aβ (and an additional 3-way interaction) in R.

To further understand the role of Aβ in SCD symptoms and memory encoding, we computed the Pearson's correlations to assess the association of Aβ deposition with SCD symptoms, objective memory, and post-scan recognition scores (and within the two recruitment method groups) in R.

Table 5. SCD Study: Demographic information

	66 total participants (female 38%)		
Demographic	mean	median	sd
Age	73.3	74	7.3
Aβ (Global PiB SUVR)	1.6	1.5	0.3
Hippocampal volume	0.5	0.5	0.3
Neuroticism	18.0	17	8.0
Education	15.7	16	3.1
SCD Symptoms	0.4	0.3	0.9
MFQ	281.6	283.5	50.5
CFQ	39.8	40	13.1
SCCS	6.0	5.5	4.7
Objective Memory	-0.3	-0.2	1.0
REY immediate	17.7	18	3.0
REY delayed	17.4	17.4	3.1
Post-Scan Recognition	70%	71%	11%

Abbreviations: Demographic variables – SUVR: standardized uptake value rate, MFQ: Memory Functioning Questionnaire, CFQ: Cognitive Failures Questionnaire, SCCS: Subjective Cognitive Complaint Scale, REY: Rey-Osterrieth complex figure test for Immediate and Delayed recall scores, Post-Scan Recognition: an accuracy of recognition memory for the post-scan task. sd: standard deviation.

3.3 Results

3.3.1 Hippocampal Activation for Memory Encoding (Task Effect)

We observed increased activation in the bilateral hippocampus in anterior (peak coordinate: right [24,-10,-12], left [-22,-10,-14]) and posterior (peak coordinate: right [30,-26,-4], left [-26,-24,-4]) during encoding compared to recognition.

3.3.2 No Main Effects of Indices of AD Risk (SCD Symptoms, Aβ deposition)

We found no significant direct associations between activation and either SCD symptoms or Aβ deposition. There was no association between activation and post-scan recognition scores.

3.3.3 Moderating Role of Education on Activation and SCD Symptoms

In participants with higher education, greater SCD symptoms were associated with greater activation; while in participants with lower education, greater SCD symptoms were associated with lower activation. The moderation effect was found in the executive control network (ECN), salience network (SN), and subcortical regions (Figure 3 left). For the ECN, we observed activations in bilateral DLPFC extending to inferior frontal gyrus (IFG), left inferior parietal lobule (IPL) extending to angular gyrus, and dorsomedial prefrontal cortex (dmPFC). For the SN, we observed three core clusters of this network [bilateral insula, dorsal anterior cingulate cortex (dACC)]. Subcortical regions included the midbrain, vermis, ventral tegmental area (VTA)/Substantia nigra (SN), caudate/pallidum, thalamus, and putamen. Education did not moderate the association between activation and Aβ deposition.

Figure 3 plots the associations between activation and SCD symptoms for each education group. The highest education group (post-college) had the highest slope, the lowest education group (high school only) had the most negative slope. This moderation effect (SCD symptoms by education) remained significant when controlling for all nuisance variables [R^2 =0.37, F(15,44)=2.21 p= 0.01]. To check the normality assumption required for the linear regression, we ran the Shapiro-Wilk test on the regression residuals, finding them to be compatible with normality (W=0.98, p=0.37).

3.3.4 Exploratory Analysis: Further Moderating Role of Aβ Deposition

We found that participants who were Aβ positive showed the same interaction [R^2 =0.43, F(3,24)=2.24 p= 0.002] with similar slopes of association between SCD symptoms and activation (Figure 2). However, participants who were Aβ negative did not show a similar interaction [R^2 =0.15, F(3,31)=0.14 p= 0.17]. The 3-way interaction with Aβ in the linear model was not statistically significant [R^2 =0.37, F(7,55)=0.06 p= 0.73].

Aβ deposition was positively associated with SCD symptoms [r(61)=0.30, p=0.02] but not objective memory [r(58)=0.05, p=0.74] (the same results per recruitment method group; Supplemental Result 1). Aβ deposition did not correlate with post-scan recognition [r(60)= -0.05, p=0.72].

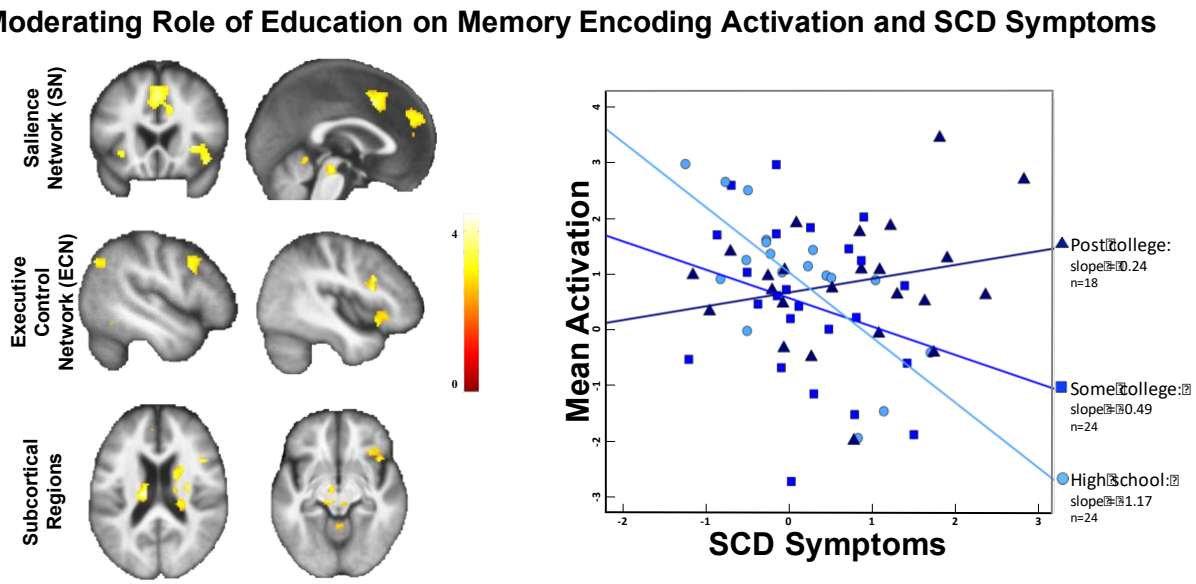

Figure 3. Moderating role of education on memory encoding activation and SCD symptoms.

Left: The significant moderation effect by education on the association between SCD symptoms and brain activation was found in the salience network, executive control network, a set of subcortical regions (threshold: p < 0.05, FDR). Right: To visualize the moderation effect by education, we plotted the results by categorizing education levels ("High School"≤12, 12<"Some College"≤16, and 16<"Post College"). The slope was positive for the highest education group, and negative for the lowest education group.

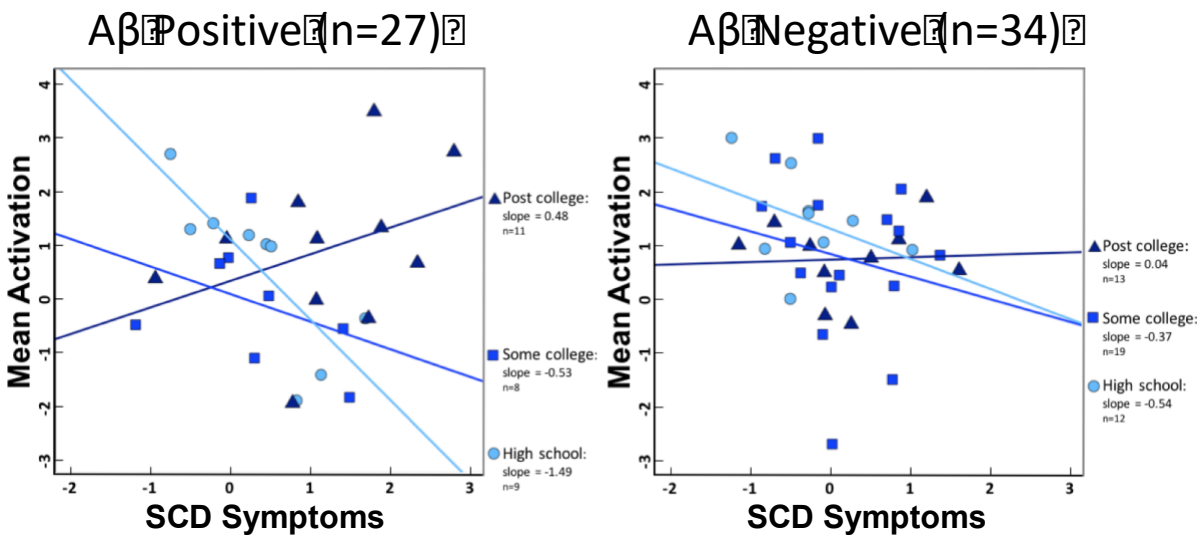

Figure 4. Moderation effect of education, separated by amyloid status.

Analyses for the moderation effect by education on the association between SCD symptoms and brain activation, separately for Aβ positive (left) and Aβ negative groups (right). The same significant interaction was found only among participants who were Aβ positive interaction [R_2 =0.43, $F(3,24)=2.24$ p= 0.002] but not in Aβ negative participants [R_2 =0.15, $F(3,31)=0.14$ p= 0.17].

3.4 Discussion

We examined brain activation during memory encoding and its association with SCD symptoms and Aβ, as well as the role of education – a proxy for cognitive reserve. In participants with higher reserve (education), greater SCD symptoms were associated with greater activation in the executive control network (ECN), salience network (SN), and subcortical regions; however, in participants with lower reserve (education), greater SCD symptoms were associated with lower activation in the same regions. This interaction was observed in participants who were Aβ positive but not Aβ negative. Aβ was not associated with activation during memory encoding. Similar to previous findings (Amariglio et al., 2012; Snitz et al., 2015b), greater Aβ deposition was associated

with greater SCD symptoms but not with objective memory. These findings suggest that individuals with higher cognitive reserve may recruit an extended neural network in the context of emerging signs of AD risk (i.e., SCD symptoms and Aβ).

3.4.1 Cognitive Reserve in Extended Neural Networks

Previous fMRI studies using this memory-encoding task have observed engagement of extended networks along with hippocampus (Edelman et al., 2017; Sperling et al., 2009). While the hippocampus plays a central role in encoding (De Vogelaere et al., 2010), we found an extended neural network that may coordinate other aspects of information processing during encoding. The ECN is involved in attention, inhibition, and shifting (Niendam et al., 2012). The SN selectively transmits subjectively relevant sensory information to higher-order cognitive regions (Menon, 2015), facilitating flexible executive functioning. Regarding subcortical regions, the thalamus may belong to an extended memory system by connecting frontal cortex and hippocampus (Jin and Maren, 2015); basal ganglia is a core structure for reward learning (Schultz et al., 2000); and both SN and VTA are involved in detection of salient signals for learning (Menon, 2015). The combinations of these regions may constitute the conflict monitoring network by integrating incoming sensory information and providing feedback control based on outcomes in order to produce successful adaptive goal-directed behaviors (2014). In SCD, the subjective experience of cognitive decline in daily cognitive activities may reflect higher cognitive load of continuously adjusting errors (i.e., conflict) between one's prediction and outcomes (Mizuno et al., 2018).

3.4.2 Moderating Role of Education and Possible Neural Compensation

We investigated the neural basis of SCD symptoms, focusing on the cognitive reserve theory to understand individual differences. Greater activation was observed among participants with greater SCD symptoms and higher education (Figure 1). This heightened neural recruitment during memory encoding may suggest a compensatory process in response to emerging pathological changes of brain (Erk et al., 2011; Rodda et al., 2009), manifested as SCD symptoms that were positively correlated with Aβ deposition in this study. It may be that lower education (lower cognitive reserve) is associated with lower capacity to mount a compensatory response.

Previous SCD studies (Erk et al., 2011; Rodda et al., 2009) described increased activation as neural "compensation" because performance was positively associated with brain activation, suggesting that greater activation facilitated memory task performance. Our study did not find an association between task performance and activation. However, due to the cross-sectional nature of these studies (including this study), it is unclear whether individuals with greater SCD symptoms would have had better performance or higher baseline activation prior to onset of SCD symptoms. Thus, we cannot distinguish whether greater activation represents neural compensation, disrupted over–activation known as dedifferentiation (Han et al., 2009) or excitotoxic neuronal damage (Palop et al., 2007). Nonetheless, our study is the first to our knowledge to report an indirect relationship between brain activation and Aβ deposition in SCD. Figure 3 displays a conceptual model of variables investigated in these analyses and theorized directions of influence.

3.4.3 Limitations

In this study, education was the sole index of cognitive reserve, which may limit the generalizability of our results. Education is commonly used as a proxy for cognitive reserve (Evans et al., 1997; Karp et al., 2004); however, other estimates of cognitive reserve (e.g., literacy (Manly et al., 2005), cognitively-engaging leisure activities (Stern, 2012)) should be investigated. Moreover, power was limited to detect a significant SCD x education x Aβ 3-way interaction. Furthermore, most participants in our 'lower' education group were high school graduates. Present results, therefore, should be confirmed with larger samples with a broader range of education levels and other measures of cognitive reserve. Finally, combining participants from two recruitment methods (memory clinic and community study settings) ignores any qualitative differences in meaning and significance of SCD symptoms between two samples (Jessen et al., 2014). Future studies can address the important factors underlying medical help-seeking behavior as an index of AD risk (Slot et al., 2018; Snitz et al., 2015a).

3.5 Conclusions

The current study investigated the neural basis of SCD symptom and Aβ deposition effects on memory encoding, observing that brain activation depended neither on SCD symptoms nor Aβ directly. Rather, level of education moderated the association between brain activation and SCD symptoms. Individuals with higher education and greater SCD symptoms displayed greater activation, whereas those with lower education and greater SCD symptoms displayed lower activation. Greater SCD symptoms may reflect a saturation of neural compensation in individuals

with greater cognitive reserve, while it may reflect diminishing neural resources in individuals with lower cognitive reserve.

4.0 Improving Brain Age Prediction Models: Incorporation of Amyloid Status in Alzheimer's Disease

This chapter is a modified version of the following manuscript that has been published:

Ly, M., Yu, G.Z., Muppidi, N.R., Karim, H.T., Mizuno, A., Klunk, W.E., Aizenstein, H.J. for the Alzheimer's Disease Neuroimaging Initiative. Improving Brain Age Prediction Models: Incorporation of Amyloid Status in Alzheimer's Disease (In press) Neurobiology of Aging.

This work was intended to support Aim 2 by creating a novel proxy measure of brain reserve. In this study, we demonstrated that amyloid status is a critical feature of brain age prediction models in the context of Alzheimer's disease. By training a brain age prediction model in amyloid negative individuals, we were able to demonstrate the greatest differences in brain age between Alzheimer's disease diagnostic groups, especially in preclinical Alzheimer's disease. My contributions to this project were: design, analyses, interpretation, and drafting and revising of the manuscript.

4.1 Introduction

Neuroimaging-based brain age prediction may serve as a promising, individualized biomarker of brain health (Cole and Franke, 2017) to understand the highly heterogeneous biological changes that occur in aging. Machine learning brain age prediction models learn the association between age and neuroimaging data in healthy individuals, where brain age is

approximately equal to chronological age (CA) in healthy individuals. Once trained, the brain age model may be used in independent samples as a marker of brain health. If the resulting brain age is lower than CA, that individual may have a "younger" brain than expected and may be more resistant to or have accumulated less pathology. Alternatively, if predicted brain age is greater than CA, that individual may have an "older" brain than expected, and may have a genetic predisposition, or have experienced a higher cumulative exposure to brain insults. These individuals may have been more impacted by pathological insults because of less effective homeostatic mechanisms compared with their age-matched peers. Brain age prediction models have demonstrated the association of increased brain age with cognitive impairment, Alzheimer's disease (AD), traumatic brain injury, Down's syndrome, HIV, and more (Beheshti et al., 2018, Cole et al., 2015, Cole et al., 2017, Gaser et al., 2013, Liem et al., 2017).

Prior brain age models have been limited because of the inclusion of amyloid-positive older participants in the training sets. It has been demonstrated that amyloid beta (Aβ) deposition, which is a hallmark of AD, may occur decades prior to the clinical onset of AD (Jack et al., 2013, Sperling et al., 2011). However, the neurotoxic effects of amyloid may be exerted on the brain for years before manifestation of overt cognitive impairment (Aizenstein et al., 2008). In this period of time before cognitive symptoms appear, Aβ(−) and Aβ(+) individuals demonstrate subtle structural or functional differences, such as gray matter (Mattsson et al., 2014) and white matter atrophy (Vipin et al., 2019), cerebral hypometabolism (Bozoki et al., 2016), disruptions in gray matter networks (Ten Kate et al., 2018), default mode network, and the central executive network (Lim et al., 2014). It is possible that brain age models not accounting for amyloid status in the training set (Aβ-insensitive models) may not detect these very subtle differences and distinguish between individuals in these stages.

Therefore, we trained a brain age model with only cognitively normal (CN), Aβ (−) individuals to potentially improve the utility of brain age as a biomarker in the context of aging and AD. We then applied multivariable regression modeling to examine for differences between AD cognitive diagnostic stages, as well as amyloid status among cognitively healthy participants. In addition, we compared our results against brain ages obtained from a well-known but amyloid-insensitive brain age model (Cole et al., 2015, Cole et al., 2017). We hypothesized that the association between brain age and CA would be moderated by the group—specifically we hypothesized that the Aβ(+) group would have a greater positive association between brain age and chronological age as compared with the Aβ(−) group indicating a more rapid aging process (cross-sectionally). We further hypothesized that more severe diagnostic groups (CN < early mild cognitive impairment [EMCI] < late mild cognitive impairment [LMCI] < AD) would have more rapid associations between brain and CA.

4.2 Methods

4.2.1 Data cohorts

This study included a total of 1256 structural magnetic resonance imaging (MRI) scans from a combination of publicly available databases. All scans were acquired using standard T1-weighted sequences.

Databases used for this study were: [*Alzheimer's Disease Neuroimaging Initiative (ADNI); Information eXtraction from Images (IXI); Open Access Series of Imaging Series: Longitudinal Neuroimaging, Clinical, and Cognitive Dataset for Normal Aging and Alzheimer's Disease*

(OASIS-3)] and data acquired at the University of Pittsburgh [*"Amyloid Pathology and Cognition in Normal Elderly" (AG025516; PI: Klunk, Aizenstein)]*.

ADNI was designed to test whether serial MRI, positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessments could be combined to measure the progression of mild cognitive impairment (MCI) and early AD (www.adni-info.org). The IXI dataset consists of nearly 600 MR images from healthy individuals across the life span and was collected as part of the project EPSRC GR/S21533/02 (https://brain-development.org/ixi-dataset). OASIS-3 is a retrospective compilation of data for over 1000 participants that were collected across several ongoing projects through the Washington University in Saint Louis Knight ADRC over the course of 30 years (https://www.oasis-brains.org).

The cohort of participants from the University of Pittsburgh were cognitively normal individuals between the ages of 60-85 recruited from the local community (previously described in Aizenstein 2008). These participants are currently followed longitudinally to assess progression in amyloid and tau deposition, alterations in structural and functional MRI, and cognitive functioning. All participants provided informed consent, with the approval of the Human Use Subcommittee of the Radioactive Drug Research Committees and the Institutional Review Board of the University of Pittsburgh.

4.2.2 Training set

The training set consisted of 757 images from healthy, Aβ(−) individuals from the ADNI (n = 92, mean age: 73.9, range: 60–85, 3T), Information eXtraction from Images (n = 264, mean age: 34.9, range: 20–49, 1.5 and 3T), and OASIS-3 (n = 401, mean age: 66.0, range: 42–85, 3T) data sets. Inclusion criteria were the age range of 20–85, normal cognitive function, and sustained

global amyloid negativity on positron-emission tomography (PET) over at least 3 years (only for participants of age 50+ years because detectable amyloid deposition is almost nonexistent in individuals without genetic mutations aged <50 years). The earliest corresponding T1 with negative amyloid status was selected for use in the training set. Participants were excluded for cognitive impairment, memory complaint, dementia, history of psychosis or neurologic disorders, and contraindications to MRI and PET imaging.

4.2.3 Test sets

The test sets (Table 6, 7) consisted of 491 3T T1 images from 6 groups: (1) CN and Aβ(−) individuals from the ADNI data set (CN-Aβ(−), n = 51); (2) CN, Aβ(−) individuals from the Pittsburgh community data set (CN-Aβ(−) PITT, n = 32); (3) ADNI CN, Aβ(+) individuals (CN-Aβ(+), n = 51); (4) ADNI EMCI individuals (n = 195); (5) ADNI LMCI (n = 88); and (6) ADNI AD individuals (AD, n = 74). The CN-Aβ(−) group was used as an independent validation set for the model. All participants were between the age of 60 and 85 years. All CN-Aβ(−) individuals sustained Aβ-PET negativity over 3 years, whereas CN-Aβ(+) individuals demonstrated Aβ-PET positivity. The CN-Aβ(−) PITT cohort was included as an additional community-based comparison against the ADNI CN cohorts. All CN cohorts were matched by age (mean age matched). EMCI, LMCI, and AD groups were all Aβ(+) and are described in ADNI protocols. No test set participants were used in training of the model.

Table 6. Diagnostic test set characteristics.

Demographic characteristics and predicted brain ages for both brain age models for cognitive stage test groups are shown. Demographic features were compared between groups with one-way analysis of variance or chi-square analysis. The EMCI group is younger than the LMCI, who are younger than the AD, who are younger than the CN groups.

	CN (N=134)	EMCI (N=195)	LMCI (N=88)	AD (N=74)	F, χ^2	p-value
Chronological Age (mean, std)	76.5 (5.0)	71.0 (6.4)	72.3 (7.1)	74.1 (6.0)	F(3,487)=21.9	<0.001*
Sex (%F)	69%	45%	52%	50%	$\chi^2(3)$=18.7	<0.001*
Education (mean, std)	15.7 (2.3)	16.2 (2.6)	16.5 (2.7)	15.1 (2.9)	F(3,487) = 5.0	<0.001*
Race (% Not White)	11.2%	5.1%	6.8%	8.1%	$\chi^2(3)$=5.1	0.17
Brain Age (Aβ(-) trained model, mean, std)	74.2 (4.3)	73.0 (5.8)	77.1 (5.0)	80.5 (4.1)	F(3,487) = 42.0	<0.001*
Brain Age (Aβ insensitive model, mean, std)	72.5 (8.9)	72.9 (9.4)	71.2 (8.7)	69.1 (8.3)	F(3,487) = 3.5	0.015*

4.2.4 Image preprocessing

Using the Statistical Parametric Mapping (SPM12) software package, structural images were segmented into tissue classes (gray, white, cerebrospinal fluid, skull, soft-tissue, and air). We used the nonlinear DARTEL (fast diffeomorphic registration) algorithm to register images to the Montreal Neurological Institute space and then generated a template per cohort, and then smoothed with a 4 mm smoothing kernel.

4.2.5 Machine learning model creation and validation

The Pattern Recognition for Neuroimaging Toolbox (Schrouff et al., 2013) was used to create the machine learning model. Whole brain, voxel-wise gray matter densities were mean-centered and then were used to compute a similarity matrix kernel—in particular we used the

simple dot product (this is an N × N matrix that estimates the distance or similarity between any 2 participants). This matrix was used in a Gaussian Processes Regression model with the similarity matrix as the independent variable and chronologic age as the dependent variable with cohort (i.e., ADNI, Information eXtraction from Images, or OASIS-3) as a covariate. Accuracy of the machine learning model was assessed by running a 10-fold cross-validation on the training set. We permuted CA (500 permutations) to assess the significance of the model prediction. Because each fold may result in slightly different models, the final overall model was an average of the 10-fold cross-validation. This average model was then tested on a separate independent test set of CN-Aβ(−), which served as a hold-out test set. We also assessed the validity of brain age prediction by comparing brain age between CN-Aβ(−), CN-Aβ(−) PITT, CN-Aβ(+), EMCI, LMCI, and AD participants. The CN-Aβ(−), CN-Aβ(−) PITT, CN-Aβ(+), EMCI, LMCI, and AD participants were not part of the training in any way.

4.2.6 Comparison against amyloid insensitive brain age model

We also computed brain age using a model that has been previously described, validated, and widely implemented in previous literature which does not account for amyloid status in its training (Cole et al., 2015, Cole et al., 2017). Code for the model was downloaded from https://github.com/james-cole/brainageR. This was used to estimate predicted brain age based on each participant's gray matter and white matter data. We used this brain age measure as an amyloid-insensitive brain age compared with our model that accounted for amyloid.

4.2.7 Statistical analysis

All statistical analyses were conducted in JMP Pro 14.1.0 (SAS Institute Inc, 2018). Multivariable regression modeling was used to determine the effects of CA, group, and CA-group interactions on brain age. This was performed for the AD cognitive diagnostic stages (CN, EMCI, LMCI, and AD), as well as CN subgroups of differing amyloid status (CN-Aβ(−), CN-Aβ(−) PITT, CN-Aβ(+)). Nonsignificant interactions were removed from the final model. Values of r, R_2, and mean absolute error (MAE) were evaluated for the goodness of fit of the model. The same analyses were performed on the results of the amyloid-insensitive model.

Difference between brain age and CA has been previously used to identify differences; however, recently it has been shown that the strength of the correlation between brain age and CA does not guarantee that the difference will be estimated accurately (Smith et al., 2019). Furthermore, the difference between brain age and CA is not orthogonal to CA—this means that factors correlated with age may be falsely associated with difference between brain and CA (Smith et al., 2019). By modeling brain age statistically with CA as a predictor, we circumvent the need to compute this brain age and CA difference. This also allows for quadratic associations with CA to be modeled (we did not do this here).

4.2.8 Cross-validated prediction of groups using logistic classifier

To help understand the predictive potential of these features, we trained a simple logistic classifier for predicting the groups (CN-β(−), CN-Aβ(+), EMCI, LMCI, and AD) using the following: CA alone; brain age alone; and CA and brain age. Because there are a high number of individuals in the EMCI group, we decided to include 30 participants from each group to help

balance our logistic classifier (as to not overfit on EMCI). We conducted following analysis 500 times: (1) choose a random set of 30 participants from each group; (2) train the logistic classifier on this training set of 150; and (3) output predictions on other individuals not in training set. We then computed the average prediction for each participant across the 500 repetitions. We then evaluated the model's performance based on area under the curve (AUC), accuracy, sensitivity, and specificity. Because the number of participants in the EMCI group is large, we needed a baseline model to help understand what performance we need to improve on. We used the ZeroR model, which predicts groups by choosing the most common category (i.e., each participant is identified as EMCI)—this is a baseline model that evaluates how good of an accuracy, sensitivity, and specificity we need to improve on chance prediction. We describe this model in Figure 5. We conducted the same predictions using the Cole model as well.

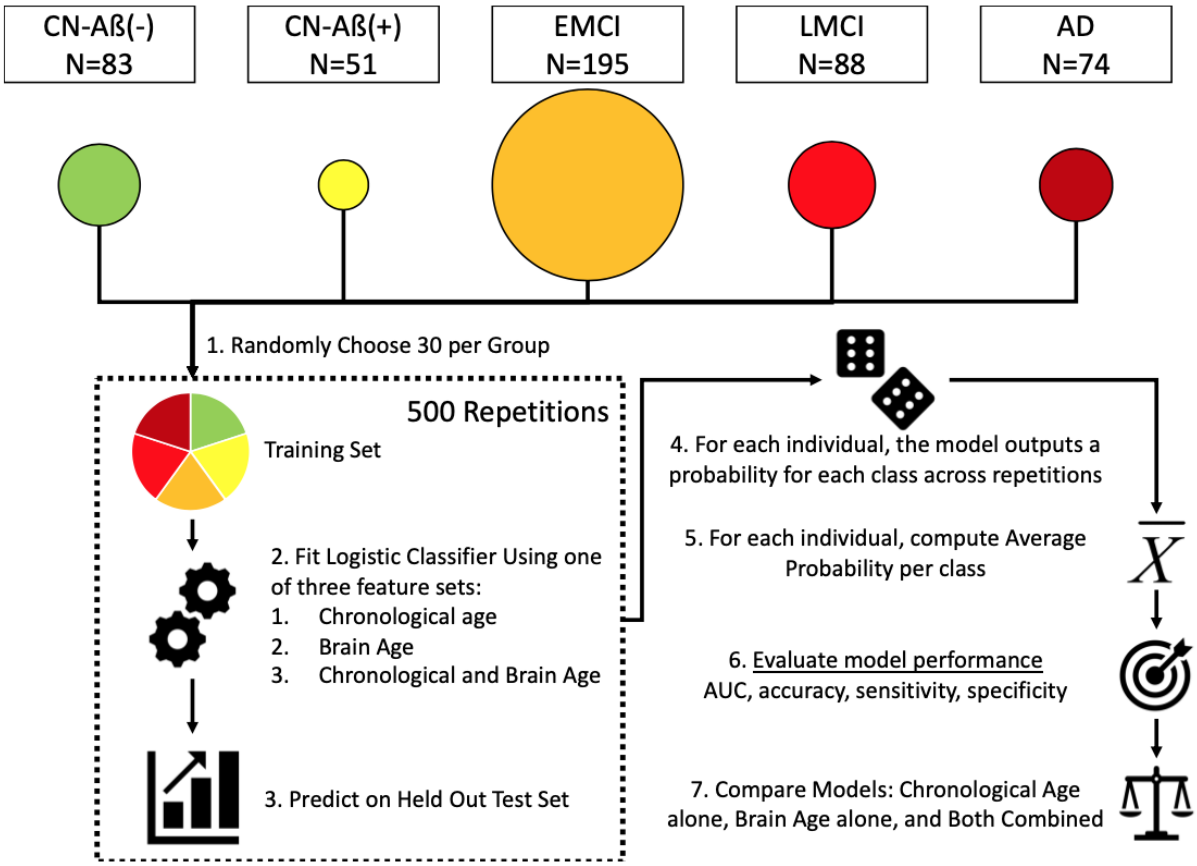

Figure 5. Cross-validated logistic classifier model.

We fit a cross-validated logistic classifier model for each of the following features: chronological age alone, brain age alone, and both features combined to evaluate their performance in predicting group. For each feature set, we conducted the following analysis 500 times: 1. choose 30 participants per group randomly (to equally represent groups); 2. Fit simple logistic classifier; 3. Predict group on held out test set; 4. Model outputs a single probability per group (class) across all repetitions (except for repetitions where participant was part of training set); 5. Calculate average probability per group (class); and 6. Evaluate model performance metrics. Once this is done for each feature set, the last step is to 7. Compare the feature sets predictive capacity (i.e., identify which features are most ideally suited for predicting groups).

4.3 Results

Cognitive groups differ by sex, CA, and education. The CN-Aβ(−), CN-Aβ(−) PITT, and CN-Aβ(+) groups do not differ by CA but do differ by education.

4.3.1 Brain age model prediction: training and independent validation sets

In our training set, our model accurately predicted brain age (r (756) = 0.94, $p < 0.002$; $R_2 = 0.88$; and MAE = 4.9 years) (Figure 6). We also show that this model does not violate any assumptions (Figure 7). In the ADNI CN-Aβ(−) independent validation set, our model also accurately predicted brain age (r (50) = 0.64, $R_2 = 0.42$, and MAE = 3.7 years). The model also accurately predicted brain age in the entire test set (r (490) = 0.60; R2 = 0.36; and MAE = 4.65 years). The voxel-wise coefficients of the model that predict CA are shown in Figure 8.

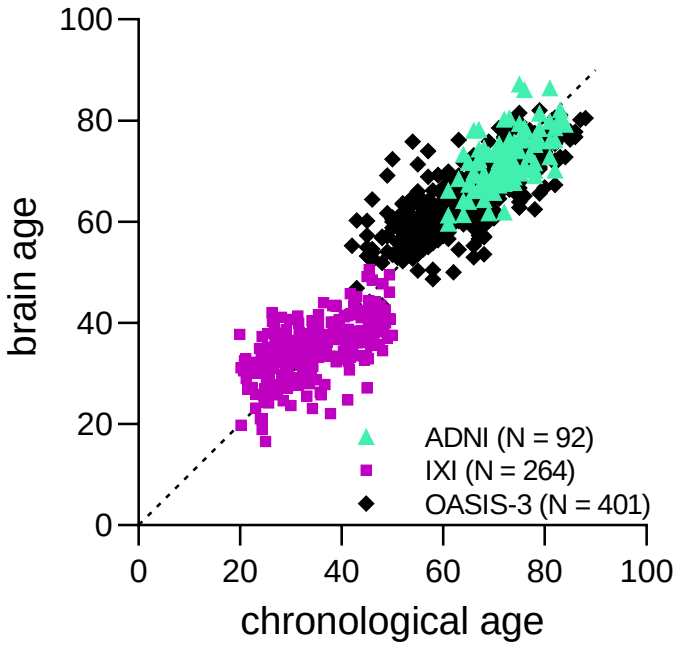

Figure 6. Cross-validated brain age predictions for the training set. Plot of 10-fold cross-validated brain age with chronological age.

Depiction of accurate brain age prediction in a training set of cognitively normal, Aβ(-) participants (n=757), with r(756) = 0.94, $R_2 = 0.88$, and mean absolute error (MAE) = 4.9 years. Dotted black line indicates identity (brain age = chronological age).

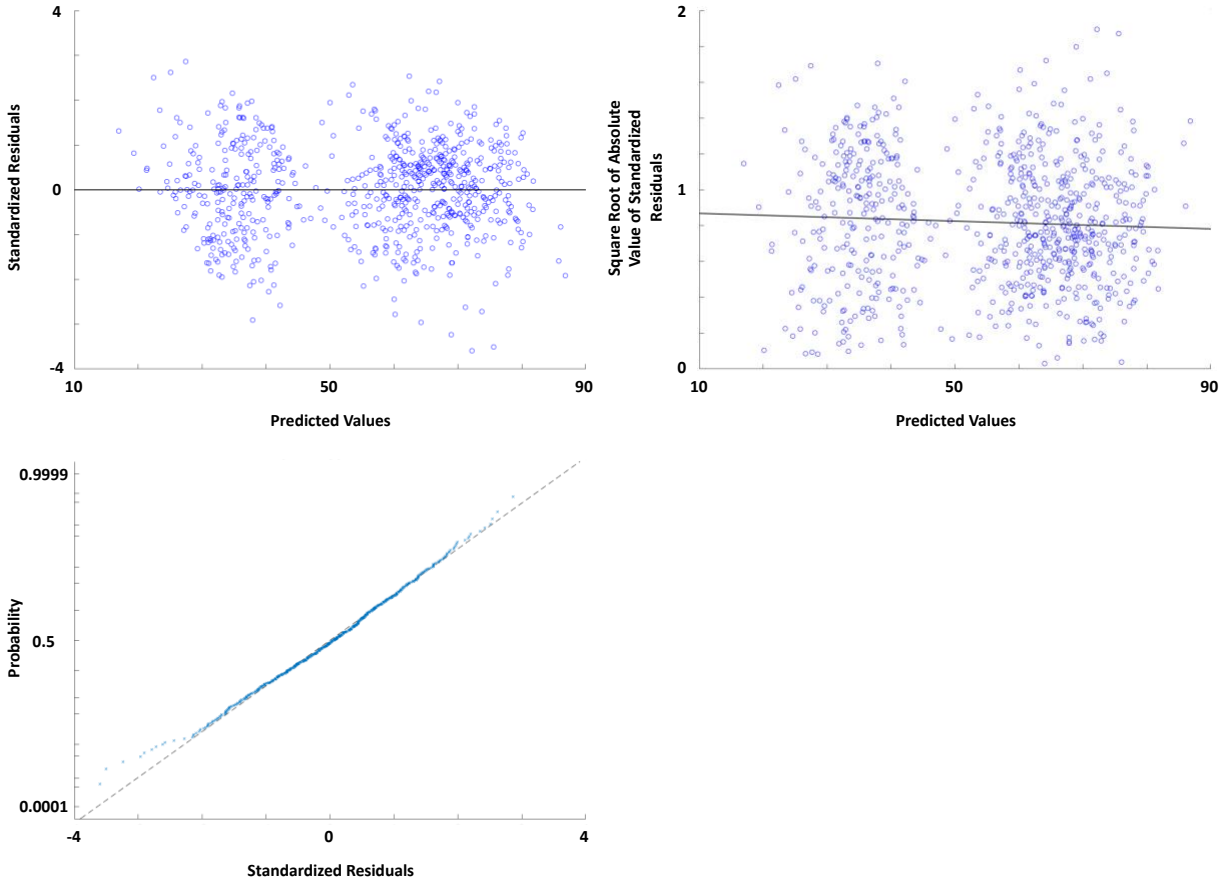

Figure 7. Diagnostic plots for association between brain age and chronological age in the training set.

(Top Left) Predicted values vs. standardized residuals indicate that our assumption for linearity is met, however there is a dip near age 50 due to the poor number of participants in that age range. (Top Right) Spread-location plot with predicted values vs. square root of the absolute value of the standardized residuals indicates homoscedasticity. (Bottom Left) Probability–probability plot (P-P plot) which indicates that our residuals are normally distributed.

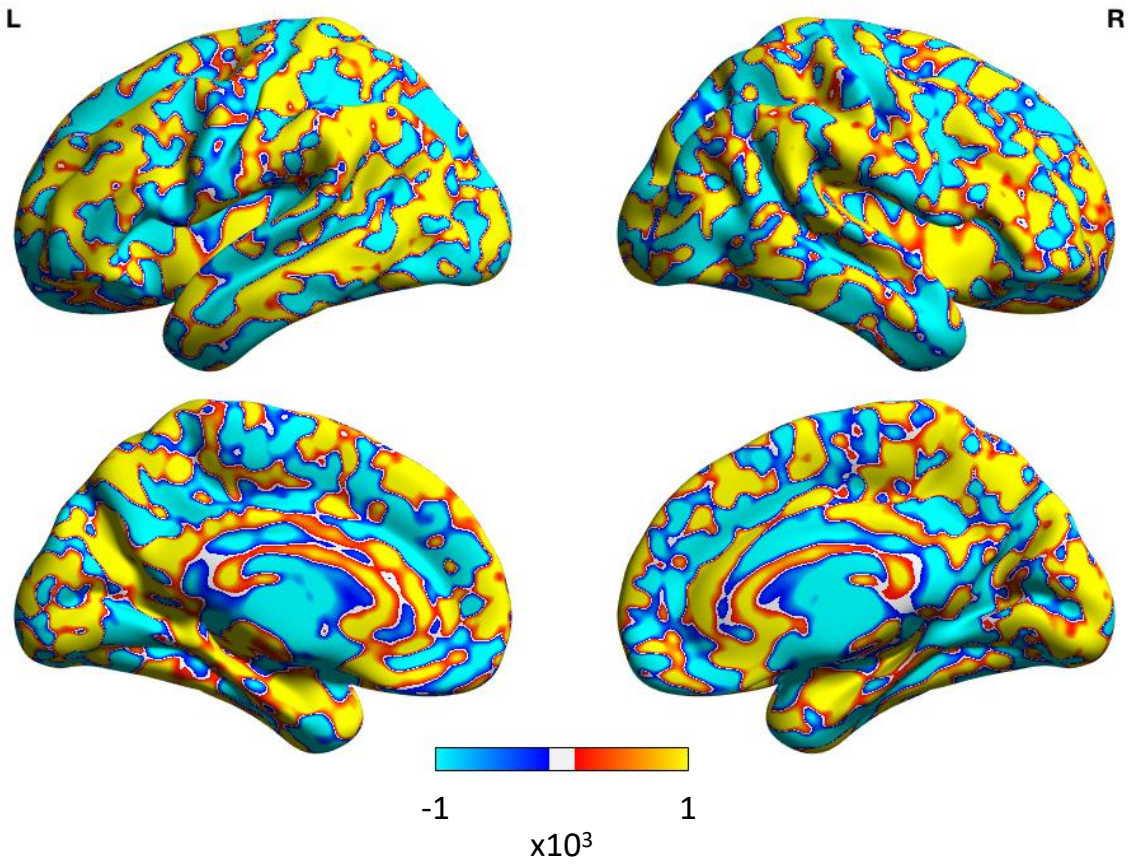

Figure 8. Cross-validated (average) coefficients for the brain age model.

Voxel-wise coefficients that predict chronological age. Positive values (red to yellow) indicate small to large positive associations with chronological age and negative values (dark blue to light blue) indicate small to large negative associations with chronological age. There is a supplementary file that contains voxel-wise coefficients that can be visualized with imaging software.

4.3.2 Multivariable linear regression model between brain age and CA with group effects

For the Aβ (−) trained model results, the diagnostic group (CN, EMCI, LMCI, and AD) was significantly associated with BA even after adjusting for race, sex, and education ($F(3, 487) = 62.3$, $p < 0.0001$, Figure 9, Table 7). Pairwise post hoc group comparisons identified significant differences between groups: (1) the CN had a lower brain age compared with EMCI, LMCI, and AD; (2) EMCI had a greater association between chronological and brain age compared with

LMCI and AD [age by group interaction]; and (3) LMCI had a lower brain age compared with AD. For the Aβ(−) trained model results, CN subgroups (CN-Aβ(−), CN-Aβ(−) PITT, CN-Aβ(+)) were significantly associated with BA even after adjusting for race, sex, and education ($F(2, 131) = 3.3$, $p = 0.04$, Figure 9, Table 7). The CN-Aβ(−) PITT had a lower brain age compared with CN-Aβ(+), but there were no differences between CN-Aβ(−) and CN-Aβ(+) (Figure 9).

For the Aβ-insensitive model results, the diagnostic group (CN, EMCI, LMCI, and AD) was also significantly associated with BA ($F(3, 487) = 6.9$, $p < 0.0001$, Figure 9). However, post hoc group comparisons showed that EMCI had greater brain age compared with CN, LMCI, and AD. Although there were significant differences between CN and EMCI, incremental differences between stages did not follow AD progression (CN to EMCI to LMCI to AD). In addition, there were no significant differences between CN subgroups ($F(2, 131) = 0.4$, $p = 0.68$).

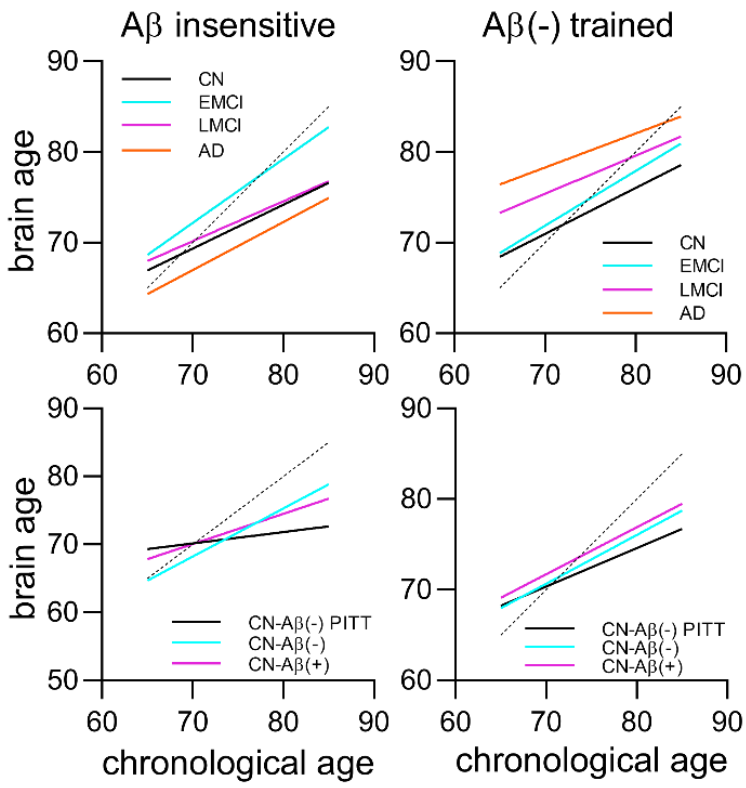

Figure 9. Multivariable linear regression for brain age models show benefits of Aβ(-) training.

Plots for the test group regression lines of brain age over chronological age for both the Aβ insensitive and Aβ(-) trained models are shown for AD diagnostic groups (top) and amyloid status in the CN group (bottom). An identity line is also provided (dotted black). The Aβ(-) trained model shows incremental differences between diagnostic groups and CN subgroups following AD progression while the Aβ insensitive model does not.

Table 7. Aβ(-) trained brain ages show significant differences between AD diagnostic groups and amyloid status in CN participants.

Results of the multivariable linear regression analysis of the Aβ(-) trained model are shown for cognitive diagnostic groups and amyloid status as a sub-analysis of the CN group. The reference test group was CN for comparison between diagnostic groups and CN-Aβ(-) PITT for CN subgroups. (CA = chronological age)

Term	Estimate	Std Error	t Ratio	p-value	Lower 95%	Upper 95%
Cognitive diagnostic groups (CN as reference)						
Intercept	35.291579	2.243782	15.73	**<.0001**	30.882867	39.70029
CA	0.5093958	0.029011	17.56	**<.0001**	0.4523937	0.5663979
EMCI	1.020452	0.467582	2.18	**0.0296**	0.1017208	1.9391832
LMCI	4.2385508	0.551762	7.68	**<.0001**	3.1544176	5.322684
AD	6.7979684	0.572472	11.87	**<.0001**	5.6731422	7.9227945
Amyloid status in CN group (CN-Aβ(-) PITT as reference)						
Intercept	34.463869	4.644971	7.42	**<.0001**	25.274351	43.653388
CA	0.5050906	0.060237	8.39	**<.0001**	0.3859185	0.6242627
CN-Aβ(-)	1.0575062	0.781668	1.35	0.1784	-0.48893	2.6039429
CN-Aβ(+)	1.9821045	0.781679	2.54	**0.0124**	0.4356468	3.5285621

4.3.3 Cross-validated simple logistic classifier

The baseline model (ZeroR), which predicts groups by choosing the most common category, had an accuracy of 42%, sensitivity of 21%, and specificity of 80%. We found that CA alone has an accuracy of 32%, sensitivity of 24%, and specificity of 82% with an AUC of 0.61. We found that brain age alone improves on this with an accuracy of 41%, sensitivity of 34%, and specificity of 84% with an AUC of 0.66. Finally, those 2 features together had an accuracy of 42%, sensitivity of 42%, and specificity of 85% with an AUC of 0.71. We have plotted receiver-operating characteristic curves in (Figure 10). Using brain age from the Cole model, we found an accuracy of 17%, sensitivity of 19%, and specificity of 80% with an AUC of 0.56. Using brain age

from the Cole model combined with CA, we found an accuracy of 32%, sensitivity of 22%, and specificity of 82% with an AUC of 0.62.

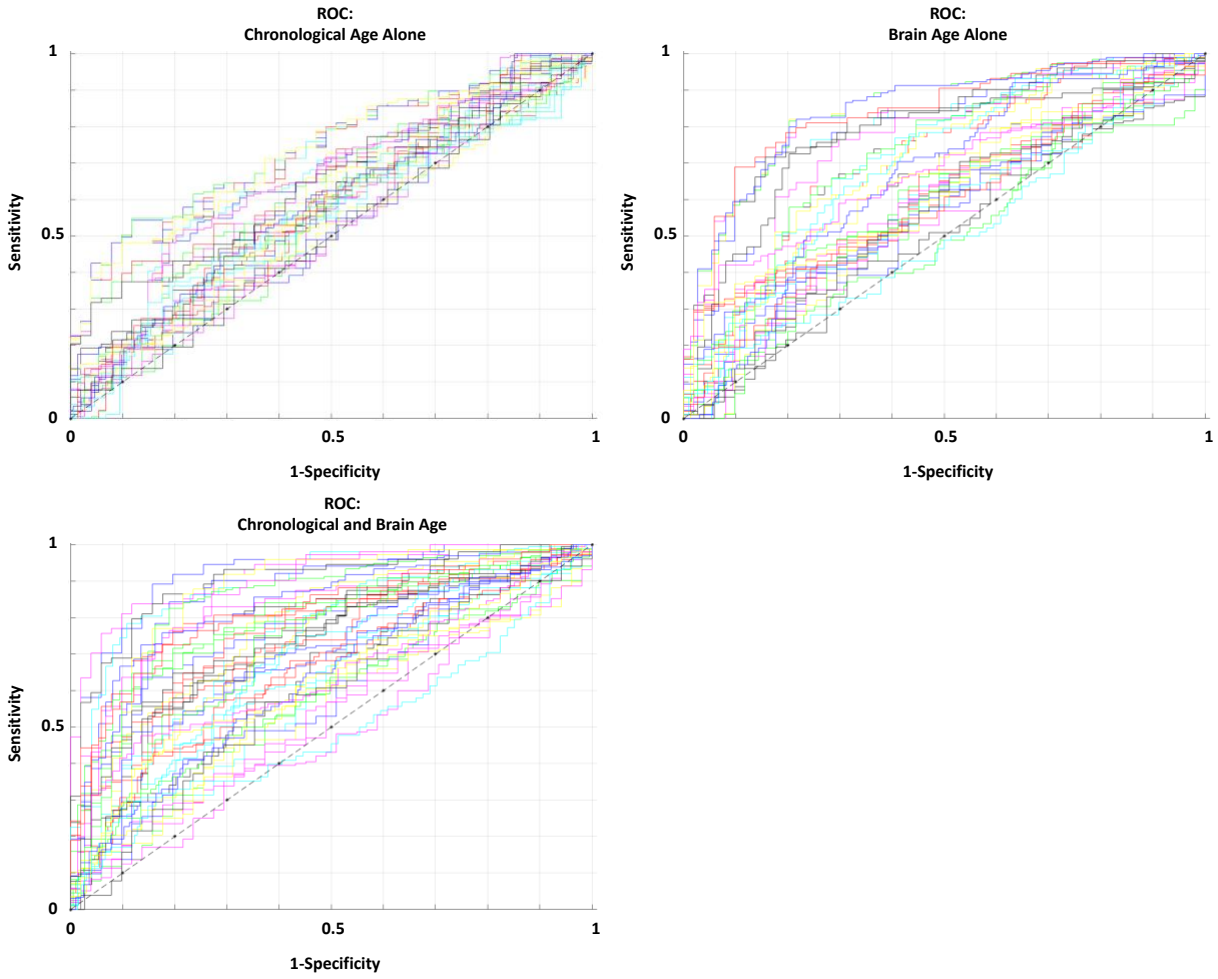

Figure 10. ROC curve.

ROC curves for the three logistic models fit: (Top Left) Chronological Age alone as a feature; (Top Right) Brain Age Alone as a feature; and (Bottom Left) Both Chronological and Brain Age as features. The dotted black line indicates uninformative classifiers. There are as many ROC curves as there are comparisons between groups (i.e., each curve represents a comparison between two groups). The total AUC is calculated by averaging across groups.

4.4 Discussion

We trained a brain age model on individuals without significant amyloid pathology to improve the utility of brain age as a potential biomarker in aging and AD. Our model predicted brain age with similar accuracy as compared with previous brain age models (Beheshti et al., 2018, Cole and Franke, 2017), both in cross-validation and in the independent test set. The resulting brain ages significantly distinguished between diagnostic stages of AD (CN, EMCI, LMCI, and AD).

Although our model distinguished between all stages of AD diagnoses, later stages showed increasing differences in BA over CA, suggesting that later AD progression results in exacerbated structural changes (as supported by Jack et al., 2013, Sperling et al., 2011).

The slope of the EMCI line was significantly higher than those of LMCI and AD, suggesting that in this stage specifically, BA reflects the greatest extent of structural change over time relative to other diagnostic stages. The other stages with similar slopes may reflect incrementally increased disease burden or diminished reserve rather than "accelerated aging," although a longitudinal study would be needed for further interpretation.

For the CN groups, our model was able to significantly distinguish between the CN-Aβ(−) PITT and CN-Aβ(+) groups, but not the CN-Aβ(−) and CN-Aβ(+) groups. This may be attributed to the Pittsburgh cohort being recruited from community-dwelling older adults for a normal aging study, whereas the ADNI cohort has been recruited from Alzheimer's Disease Research Centers Alzheimer A, which may include individuals with subjective cognitive decline or other factors not accounted for in exclusion criteria, which may warrant additional study.

To our knowledge, our model demonstrates the greatest incremental differences in BA over CA between diagnostic stages of AD disease progression. In addition, it is the first to consider amyloid status in defining the BA prediction model. Notably, a prevailing amyloid-insensitive brain age model was not able to correctly order the diagnostic stage test groups or distinguish between any CN subgroups. Although comparisons between additional amyloid-insensitive models are warranted, these preliminary results show strong potential for the consideration of amyloid status in training of brain age models.

In addition, our results demonstrate that there is potential clinical utility of machine-learning brain age models in the monitoring of AD. When considering that MRI is relatively inexpensive and noninvasive relative to PET and is commonly obtained in cases of subjective cognitive concern without objective memory impairment, more developed models may offer benefits in tracking disease progression and informing decision making regarding PET imaging. Our two-feature (CA and brain age) simple logistic classifier was capable of predicting groups, indicating their capacity as predictive features. We noted that brain age improved classification of groups with CA.

One limitation of this study is the diminished correlation coefficient in the independent validation set ($r = 0.64$, compared with $r = 0.94$ in the training set). The diminished value may be explained by the lower number of participants in the test set (51 vs. 757 in the training set) and the restricted age range of the test sample (60–85 years compared with 20–85 years in the training sample), which reduced the total variability that can be explained by the model (Bland and Altman, 2011). We also did not evaluate longitudinal changes in cognitive function or amyloid positivity—future longitudinal studies are needed to evaluate these associations.

Regardless, the MAE of our independent validation set is comparable to previously published brain age models (Cole and Franke, 2017), which is a better indicator of model accuracy. Furthermore, a prior study demonstrated a similar diminished correlation coefficient and preserved MAE (Beheshti et al., 2018) with a similar age range. Another limitation of this study is the sparsity of participants between the ages of 45 and 55 years as compared with other age groups. This is expected, as amyloid-PET is not often acquired in those younger than 60 years.

4.5 Conclusions

Our Aβ(−) trained model performed superior to a contemporary Aβ-insensitive model in both fitting BA for CA and distinguishing between groups of different stages of AD progression. Overall, incorporation of amyloid status in brain age prediction models may improve model performance and the utility of brain age as a biomarker of aging and AD.

5.0 Application of Brain Age: Accelerated Brain Aging in Chronic Low Back Pain

This chapter is a modified version of the following manuscript that is currently in submission:

Yu, G.Z.*, **Ly, M.***, Karim, H.T., Muppidi, N., Aizenstein, H.J., Ibinson, J.W. Accelerated brain aging in chronic low back pain. *co-first authors.

This work was intended to support Aim 3 by applying the brain age prediction model in cohorts with age-related neuropsychiatric disorders. In this study, we demonstrated that with increasing participant age, greater differences in brain age were found between individuals with chronic low back pain (CLBP) and healthy participants. These results suggest that CLBP may be associated with a form of accelerated brain structural aging. My contributions to this study were: design, analyses, interpretation, drafting and revising the manuscript.

5.1 Introduction

Low back pain (LBP) is the leading cause of disability worldwide. Most adults are likely to suffer from LBP during some point in their lives, and the number of years lost to disability from this condition has increased by 54% since 1990 (Hartvigsen et al., 2018). LBP is highly prevalent and challenging to manage clinically. In most cases, the specific source of pain cannot be identified, resulting in classification as non-specific LBP (Buchbinder et al., 2018). In addition, LBP is often accompanied by and exacerbates medical comorbidities, requiring additional care for

poorer treatment response (Foster et al., 2018). Lastly, LBP is highly persistent, with approximately two-thirds of patients still reporting pain after twelve months (Meucci et al., 2015).

There is a mounting body of literature suggesting that chronic LBP (CLBP) may have detrimental effects on brain structure. These alterations in brain structure may result in symptoms that extend beyond nociception, leading to impairment in attention, mental flexibility, language skills, and emotional decision making (Buckalew et al., 2010; Ivo et al., 2013; Malfliet et al., 2017; Wand et al., 2011).

Specifically, CLBP has been associated with changes in gray matter density in multiple regions, namely the prefrontal cortex, thalamus, brainstem, corpus callosum, and total gray matter volume, although the direction of these changes has been conflicting in various studies (Apkarian et al., 2004; Buckalew et al., 2010; Ivo et al., 2013; Kregel et al., 2015; Schmidt-Wilcke et al., 2006). Past studies have shown that these differences in gray matter are normalized following treatment (Seminowicz et al., 2013; Seminowicz et al., 2011).

We have previously developed and validated a machine-learning based approach to analyze global gray matter density to generate a predicted brain age (BA). BA indicates the relative structural discrepancy between the subject compared to age-matched healthy peers and has recently been shown to have significant promise as a surrogate measure of brain health and structural integrity (Ly et al., 2019). This measure may be more sensitive to small changes in anatomy and may help in the monitoring and treatment of patients.

Previously, application of brain age prediction to general chronic pain has shown significant differences in predicted age discrepancies between chronic pain patients and healthy participants (Cruz-Almeida et al., 2019). However, whether these differences hold true specifically in CLBP patients is yet unexplored. Therefore, in this study we applied our brain age prediction

model to a cohort of CLBP patients without depression. Since it has been suggested that chronic pain may result in “accelerated aging” of the brain, we hypothesized that CLBP patients would present with higher BA for their actual chronologic age than healthy controls. In addition, we investigated the association between BA and factors of CLBP duration and pain severity at the time of imaging for CLBP group.

5.2 Methods

5.2.1 Study Design and Participants.

This study included data from 63 participants, with 31 having CLBP and 32 healthy controls (HCs), from the Pain and Interoception Imaging Network (https://www.painrepository.org/repositories/). Participants with CLBP were included if they (state inclusion and exclusion criteria). (symptoms, other comorbidities, medications, substance abuse, volunteer consenting information).

5.2.2 Data Collected.

CLBP duration was self-assessed in years. The visual analog pain scale was used to assess pain on the day of the MRI scan. Depressive symptoms were self-scored using the Beck Depression Inventory (Beck et al., 1996).

5.2.3 MRI Data Collection.

All scanning was conducted at the Northwestern University Feinberg School of Medicine by the Apkarian lab on a 3T Siemens Trio TIM research-dedicated scanner (Munich, Germany) with an 8-channel head coil. An axial whole brain high-resolution (1mm3 isotropic) T1-weighted sequence (magnetization prepared rapid gradient echo, MPRAGE) was collected (TR = 2300 ms, TE = 3.43 ms, TI = 900 ms, FA = 9°) with a field of view 256 × 256 with 160 slices.

5.2.4 MR processing.

All processing was conducted in SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/). Structural MRI scans skull signal had been manually removed for de-identification. After bias correction, we conducted segmentation into three tissues: gray matter, white matter, and cerebrospinal fluid. We then used the nonlinear DARTEL (fast diffeomorphic registration) algorithm to register images to the Montreal Neurological Institute (MNI) space then generated a template for this cohort, and then smoothed with a 4 mm smoothing kernel (Ashburner, 2007). This process generates a gray matter density map – a factor associated with both gray matter volume and cortical thickness.

5.2.5 Brain Age Model and Estimation.

We have previously developed a BA estimation algorithm that estimated chronological age from gray matter density maps (Ly et al., 2019). Additional details regarding the model and databases used in the model training set may be found in the supplement. Brain age for each

participant in the CLBP and HC groups was calculated using our algorithm and mean-centered for each group.

5.2.6 Statistical Analysis.

All statistical analyses were conducted in JMP Pro 14.1.0 (SAS Institute Inc., 2018). Outliers were identified as values outside 1.5 interquartile ranges for age or having an absolute studentized residual of predicted brain age greater than 3.5 years. As a result, three participants in the CLBP group and two in the healthy controls (HC) group were not included for statistical modeling. To test for the effect of CLBP on the association between chronological age and predicted brain age, a multivariable linear regression was used. We also tested if sex moderated this association, as sex has been a significant distinguishing factor in the etiology, prevalence, and risk of disability from CLBP (DePalma et al., 2012; Dixon and Gatchel, 1999; Munce and Stewart, 2007). Additionally, due to possible interactions of sub-clinical depressive symptoms with CLBP, we tested if pain duration in years, current pain (VAS), or depressive symptoms (as characterized by the Beck Depression Inventory) moderated the association between chronological age and predicted brain age specifically within the CLBP group.

5.3 Results

Compared to the healthy control group, the CLBP group was not significantly different in participant age or sex, but had significantly greater current pain, pain duration, and depressive symptoms (Table 8).

Overall, the multivariable linear model predicted BA well (r(62) = 0.57, R2 = 0.32, RMSE = 3.46). Group moderated the association between chronological age and BA (corrected model – $F(5,62) = 6.93$, $p < 0.001$, Table 9). There was a significant interaction effect between CLBP status and chronological age on predicted brain age ($p = 0.031$, Table 9, Figure 11). Sex was not associated with BA and did not moderate the association between chronological and brain age.

Within the CLBP group, none of these factors were directly associated with BA: sex, current pain, pain duration, and depressive symptoms (Table 10).

Table 8. Demographic information and differences between experimental groups are shown.

CLBP – Chronic low back pain; HC – healthy controls; VAS – Visual analog scale for pain; BDI – Beck Depression Inventory.

	CLBP ($n = 31$)	HC ($n = 32$)	t or χ^2 (df)	p-value
Chronological Age (years)	50.7 (6.5)	50.8 (7.1)	$t(62)=0.04$	0.967
Sex (% Female)	45.2	43.8	$\chi^2(1)=0.01$	0.910
Current Pain (VAS)	6.7 (1.8)	0 (0)	$t(62)=21.3$	<0.001*
Pain Duration (years)	16.3 (11.7)	0 (0)	$t(62)=7.9$	<0.001*
BDI	5.5 (5.2)	1.6 (2.7)	$t(62)=3.8$	<0.001*

Table 9. Statistical results for multivariable linear regression model testing the effect of group on the association between chronological age and predicted brain age.

The dependent variable was brain age and the healthy control group was used as reference.

	ß with 95% CI			t-statistic	p-value
	ß	Lower Bound	Upper Bound		
Intercept	48.393	47.493	49.292	107.72	<0.001*
Chronological Age	0.283	0.152	0.414	4.32	<0.001*
Sex [F]	-0.68	-1.559	0.199	-1.55	0.127
CLBP	0.789	-0.085	1.662	1.81	0.076
CLBP * Chronological Age	0.145	0.014	0.276	2.22	0.031*

Table 10. Statistical results for multivariable linear regression model testing the association between the difference between brain and chronological ages and factors of sex, current pain, pain duration, and depressive symptoms for the CLBP group.

	ß 95% CI			*t*-statistic	*p*-value
	ß	Lower Bound	Upper Bound		
Intercept	0.537	-7.260	8.336	0.14	0.888
Sex [F]	-0.300	-2.384	1.783	-0.30	0.769
Current Pain (VAS)	-0.036	-1.334	1.262	-0.06	0.954
Pain Duration (years)	-0.064	-0.265	0.137	-0.65	0.520
BDI	-0.063	-0.526	0.401	-0.28	0.782

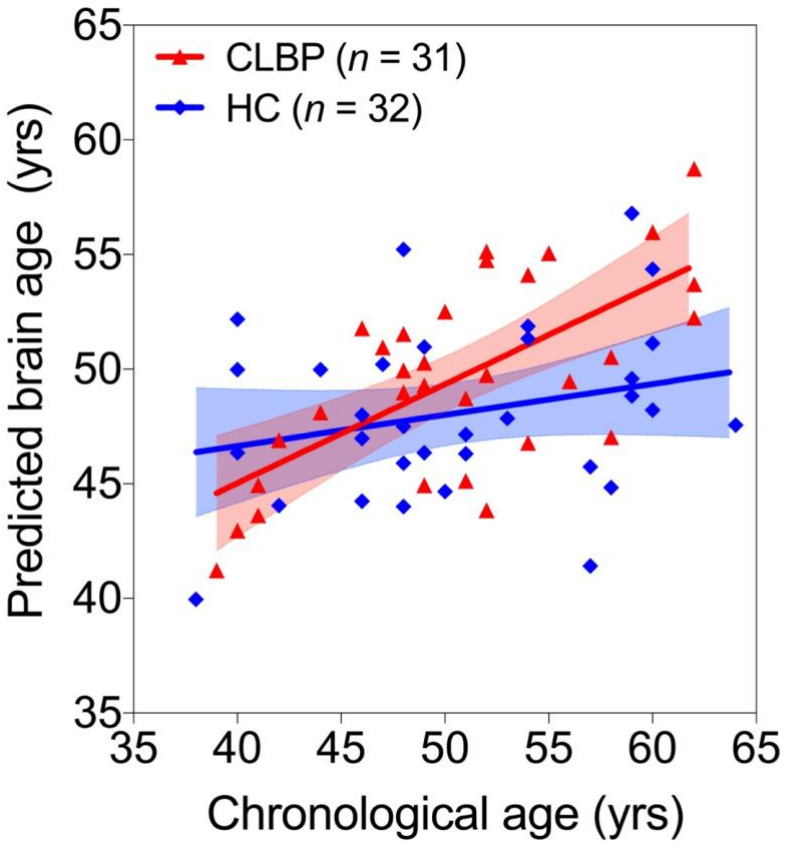

Figure 11. Association between chronological age and predicted brain age in healthy controls (HC, blue) and those with chronic lower back pain (CLBP, red).

Trendlines and 95% confidence intervals (shaded areas) are shown.

5.4 Discussion

In this study we sought to apply a machine learning-based brain age prediction model to a CLBP cohort without depression with age and sex matched healthy controls. Given the various documented deleterious effects of CLBP on brain structure, we hypothesized that the CLBP group would have older predicted BA for a given chronological age compared to the HC group. Our results supported this hypothesis, as CLBP participants showed an additional 0.145 years in predicted brain age per chronological year of life compared to their healthy counterparts.

Although the precise source of this difference is not known, the greater slope of the CLBP brain age to chronological age trendline against the HC line (and therefore a greater discrepancy between the two at greater ages) supports previous models regarding CLBP as a form of accelerated aging. Many of the changes in brain structure seen in CLBP, such as decreased gray matter density in the prefrontal cortex, thalamus, and brainstem, are also seen in the natural aging process (Apkarian et al., 2004; Ivo et al., 2013; Kregel et al., 2015). In addition, the upward translational shift in the CLBP relationship between brain age and chronological age likely indicates the greater disease burden similar to that previously seen in our application of the model to cognitive stages in Alzheimer's disease (Ly et al., 2019).

In addition, due to the greater slope of the CLBP trendline, the discrepancy in brain age between the two groups would theoretically be larger at greater chronological ages, although this was not followed longitudinally in this study. This possibly suggests that older adults with CLBP are at risk for the greatest brain morphometric changes given their longitudinal pain burden. As emphasized by previous studies, there are numerous significant differences in brain structure and function in older adults with CLBP compared to their healthy counterparts (Buckalew et al., 2010). Especially relevant to the present study is that changes in brain structure associated with late-onset

depression were seen even in non-depressed CLBP participants, suggesting that an absence of depression does not preclude the structural changes and increased vulnerability to psychiatric comorbidities. Previous literature has also shown that degenerative brain changes in older CLBP patients are distinct from younger CLBP patients, and that older adults are unable to effectively respond to pain due to age-related changes in areas of central pain regulation (Apkarian et al.; J. Karp et al.).

We found that sex, depressive symptoms, duration of pain, and current pain were not significantly associated with brain age, suggesting an alternative driving factor not encompassed by these variables. One aspect to consider is that total duration of pain and current pain level may be imperfect quantifiers of a patient's trajectory with CLBP. Not only is it possible for pain intensity to change over time, the duration in which a patient experiences higher levels of pain may also be significant factor for the activation and possible enhanced response of various pain-related brain regions (Flor et al.; Wand et al., 2011). Previous literature has also suggested that both normal and pathologic structural brain changes themselves may contribute toward a patient's experience of CLBP due to impairment of descending inhibition, implicating a bi-directional relationship between structural brain changes and chronic pain (J. Karp et al.). All these factors suggest that the trajectory of CLBP and its relationship to brain changes are more complex than may be characterized by measurements of only duration and pain at one instance (Flor et al.).

Additionally, while BDI scores were taken as a measure of depressive symptoms in participants, most scores for CLBP participants were below the clinical threshold for major depressive disorder (BDI = 16). Although there is an extensive relationship between CLBP and depression, with overlap in their underlying neurobiology and impact on brain structure, our results suggest that there are also significant effects of CLBP on brain structure in the absence of Major

Depressive Disorder (Gerhart et al.; Hung et al.; J. F. Karp et al.). However, two participants excluded as outliers had BDI scores of 17 and 19, with brain ages 15 and 21 years older than their chronological ages, respectively. A general positive trend between BDI scores in the major depressive disorder levels and greater brain aging in CLBP patients is suggested by the few participants which meet the clinical BDI threshold in the present study; however, further investigation of CLBP patients with major depressive disorder would be needed to draw a more definitive conclusion regarding brain age.

A recent study of chronic pain and the discrepancy between predicted brain age and actual age has shown significant differences between chronic pain patients and healthy participants (Cruz-Almeida et al., 2019). While our results are overall corroboratory, there are several key distinctions to note, aside from this study's specific focus on CLBP. Our present study features a much younger patient cohort, with mean ages of 50 years, versus the previous study's mean age of 70 years. In addition, we investigated a larger HC group (n = 32) versus the previous study's sample size of 14 individuals without chronic pain. However, many of the additional parameters investigated by the previous study, including therapeutic interventions for pain, psychological function, and somatosensory function, were not available in the present participant cohort, and therefore may further modulate the relationship between predicted brain age and chronological age in this study.

The main limitations of this study are the limited sample sizes for CLBP and HC participants, as well as its cross-sectional nature. Conclusions regarding trends in brain age would be strengthened by a longitudinal analysis with multiple instances of participant imaging to construct trajectories with the development and treatment of CLBP. Additional measures of both pain intensity and duration at multiple time points would also allow for more sophisticated

measures of a cumulative pain burden. A limitation of the brain age model used is its holistic mode of analysis of overall gray matter density. In addition, the training set of the brain age model was not specifically screened for CLBP status in its participants. Therefore, the predicted brain age is not generated against a healthy control population, but rather a general, mixed population. A potential direction of future investigation may be to delineate the contributions of specific brain regions to accelerated aging.

In this study we have demonstrated that brain age prediction using a machine-learning based model shows accelerated brain aging in CLBP patients. This measure may serve as a future clinical tool in tracking and possibly predicting a patient's trajectory in brain changes with CLBP as well as an indicator for treatment response.

6.0 Application of Brain Age: Increased Brain Age in Non-Remitters Compared To Remitters Following Open-Label Treatment Of Late-Life Depression

This chapter is a modified version of the following manuscript that is currently in submission:

Yu, G.Z.*, Karim, H.T.*, **Ly, M.,** Andreescu, C., Karp, J.F., Butters, M.A., Reynolds, C.F. III., Aizenstein, H.J. Increased brain age in non-remitters compared to remitters following open-label treatment of late-life depression. *co-first authors.

This work was intended to support Aim 3 by applying the brain age prediction model in cohorts with age-related neuropsychiatric disorders. In this study, we investigated pre- to post-treatment changes in brain age in a cohort of participants with late-life depression (LLD) who received 12 weeks of open-label venlafaxine. We demonstrated that non-remitters demonstrated a significant increase in brain age over the intervention period while remitters showed no significant change in brain age over the same period. My contributions to this study were: design, analyses, interpretation, drafting and revising the manuscript.

6.1 Introduction

Late life depression is a leading source of disability in older adults, occurring in up to 38% of the population [1]. In addition to being highly prevalent, the clinical trajectory of LLD is often complicated by increased time to treatment response and higher rates of treatment resistance [2,

3]. This results in worsened patient outcomes and increased risk for suicide, dementia, exacerbations of medical comorbidities, and overall mortality [4, 5]. The biological mechanisms linking LLD with these health consequences have previously been attributed to chronic stress and inflammatory hypotheses in which high systemic levels of glucocorticoids and pro-inflammatory cytokines mediate neurodegenerative changes in brain structure [6-8].

In support of these hypotheses, many cross-sectional studies have established relationships between changes in brain structure with LLD and its treatment response. Compared to healthy controls, LLD individuals have lower grey matter volumes in regions contributing to cognitive performance [9-11]. Furthermore, severity of grey matter volume differences in LLD patients have been associated with LLD symptomatic severity, decreased cognitive function, and decreased likelihood of LLD remission after treatment [12, 13]. However, these previous investigations have mostly been cross-sectional, using brain structure as a predictor rather than a longitudinal measure of disease progression or improvement. Few studies have examined structural brain changes over the course of treatment. ECT studies have reported only scarce evidence for the reverse of this disease-structure relationship, with increases in grey matter volume reported after electroconvulsive therapy [14]. However, no volumetric differences were reported between remitters and non-remitters following antidepressant monotherapy [14-16].

Given that the pathophysiology of LLD as explained by the chronic stress and inflammatory hypotheses has been likened to accelerated aging, and that aging-associated brain genetic profiles have been found to contribute to LLD vulnerability, we sought to investigate the relationship between longitudinal structural brain changes and treatment response in LLD using our previously developed brain age prediction model [17-19].

Our brain age prediction model contextualizes whole brain structural information of a test cohort against structural information from a large healthy participant cohort spanning a wide range of ages (20 to 85) to generate a machine learning-based prediction of the test participant's chronological age. In this way, discrepancies between actual chronological age and predicted brain age in test groups may indicate pathological disruption or acceleration of the aging process [20]. Past studies have also shown that other neuropsychiatric diseases, including traumatic brain injury, schizophrenia, and epilepsy, have been associated with increases in brain age [21-23]. In our previous work, we have demonstrated the capacity of our grey matter density-based brain age model in distinguishing between the various cognitive stages of Alzheimer's disease given the pivotal connection between structural changes and disease progression [24].

Given the potential for brain age prediction to provide insight into the nature of structural changes in LLD, we aimed to investigate whether changes in brain age differed between remitters and non-remitters to treatment in LLD patients. We investigated pre- to post-treatment changes in brain age in a cohort of LLD participants (N = 46) who received open-label treatment with venlafaxine. Change in brain age pre- and post-treatment was compared between remitters and non-remitters. To our knowledge, this is one of the first studies applying the brain age metric to LLD following treatment and also one of the first studies to utilize longitudinal changes in brain age in a clinical context. We hypothesized that non-remitters may show an increase in brain age (pre- to post-treatment) greater than remitters. We further hypothesized that the extent of symptomatic persistence in non-remitters would correlate with greater increases in brain age.

6.2 Methods

6.2.1 Participants and Study Design

As part of a treatment study of LLD (NCT00892047 and NCT01124188), we collected neuroimaging data, which has also been described elsewhere [25, 26]. Data was collected from January 2012 until June 2016. Participants were included if they were 55 years and older, met Diagnostic and Statistical Manual of Mental Disorders IV criteria of major depression, and had a Montgomery-Asberg Depression Rating Scale (MADRS) of at least 15 at baseline. Participants were excluded if they had a history of mania or psychosis, alcohol or substance abuse within last 3 months, or dementia or neurodegenerative disease as well as conditions that affect mood or the brain (e.g., stroke, vasculitis, unstable hypertension etc.). All participants gave written informed consent. The study was approved by the University of Pittsburgh Institutional Review Board.

Participants underwent an open-label phase of treatment with venlafaxine XR – a serotonin-norepinephrine reuptake inhibitor. During this phase, we collected neuroimaging data at five time points: pre-treatment, after a placebo lead-in, after a single dose of venlafaxine (37.5mg), a week after starting venlafaxine, and post-treatment (~12 weeks). Structural MRI scans were only collected at pre- and post-treatment therefore we will only discuss processing of this data.

During the first 6 weeks, participants returned for weekly/biweekly visits and dosage as increased up to 150mg/day per severity and tolerability. Participants who were still symptomatic (i.e., MADRS > 10) by week 6, the dose was increased per protocol to 300mg/day. Remission was defined as MADRS<10 for at least two visits during course of treatment. Participants were treated for 12-14 weeks but protocol guidelines allowed for a longer trial (up to 24 weeks) to clarify remission status.

We recruited a total of 63 participants into our study: 1 did not meet criteria for major depression, 2 had metallic implants, and 1 did not fit in the scanner. A total of 59 participants were treated with open-label venlafaxine XR: 2 discontinued communication and 4 discontinued treatment due to side effects (n=2), worsening of symptoms (n=1), or other medical conditions not related to treatment (n=1). To be included in our analysis, a pre- and post-treatment MRI scan was need, however some participants were excluded due to: claustrophobia in MR scanner after one scan (n=1), discomfort in MR scanner after one scan (n=1), continued treatment but refused follow-up imaging after second scan (n=1) or fourth scan (n=1). This resulted in a sum total of 49 participants who completed treatment and MR scanning and who were included in our analysis.

We also collected following data: demographic (age, sex, education, race), recurrent/single episode depression, and cumulative illness rating scale for geriatrics (CIRSG).

6.2.2 MRI Data Acquisition

Scanning was conducted at the University of Pittsburgh Medical Center on a 3T Siemens Trio TIM scanner (Munich, Germany). Structural MRI scans were collected at pre-treatment and post-treatment visits, while functional imaging scans (not described here) were collected at each visit. We collected an axial, whole brain 3D magnetization prepared rapid gradient echo (MPRAGE) was collected with repetition time (TR)=2300ms, echo time (TE)=3.43ms, flip angle (FA)=9 degrees, inversion time (TI)=900ms, field of view (FOV)=256x224, 176 slices, 1mm isotropic resolution and with GeneRalized Autocalibrating Partial Parallel Acquisition (GRAPPA) factor=2. An axial, whole brain 2D fluid attenuated inversion recovery (FLAIR) was collected

with TR=9160ms, TE=90ms, FA=150 degrees, TI=2500ms, FOV=256x212, 48 slices, and 1x1x3 mm resolution.

6.2.3 Structural Processing

Processing was conducted using statistical parametric mapping (SPM12 https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) in MatLab (2016b, The MathWorks, Natick). Interpolation was done with 4th degree B-spline interpolation and normalized mutual information similarity metric was used for coregistration between different image types. The FLAIR was coregistered to the MPRAGE, and both were then input into a multispectral segmentation which bias corrects the images and segments them into gray matter, white matter, cerebrospinal fluid, skull, soft-tissue, and air [27]. Due to high white matter hyperintensity burden the number of Gaussians used to identify white matter was set to two to improve identification of gray and white matter [28]. The gray and white matter maps are input into a process to generate a study specific template for estimation of gray matter density.

We used DARTEL (Diffeomorphic Anatomical Registration using Exponentiated Lie Algebra) to generate study specific gray and white matter templates – we conducted a specialized pipeline for longitudinal data analysis [29, 30]. DARTEL uses an iterative process of averages templates and coregistration to improve normalization to a standard anatomical space within a study. We leveraged longitudinal data by first generating a gray and white matter template for each participant using the pre- and post-treatment MRI. Those templates are then used to generate study-specific templates of the gray and white matter. We can then multiply the Jacobian of the transformations to this study-specific template space to generate a gray matter density image [29].

The gray matter density images were smoothed using a Gaussian kernel of full-width at half-maximum of 6mm.

6.2.4 Brain Age Estimation

We have previously developed a brain age estimation algorithm for late-life populations [24]. Briefly this used the Pattern Recognition for Neuroimaging Toolbox (PRoNTo) to predict chronological age using gray matter density and machine learning [31]. Whole brain, voxel-wise grey matter densities were mean-centered and used to calculate a similarity matrix kernel (dot product), which was input into a Gaussian Processes Regression model with the similarity matrix as the independent variable and chronologic age as the dependent variable. The training set (n=757 individuals) and inclusion criteria have been described previously [24]. Our current dataset was not a part of the training of this model. We then used this previously validated model to estimate brain age per time point in the 49 participants who were included in our analysis.

6.2.5 Statistical Analysis

All statistical analyses were performed in JMP Pro 14.1.0 (SAS Institute Inc., 2019). Three outliers were identified and removed on the basis of having change in brain age or MADRS score greater than 1.5 interquartile range above the third quartile (for a final cohort size of N = 46). Paired *t*-tests were used to compare pre- and post-treatment brain ages for the entire cohort followed by remitters and non-remitters separately. Multivariable regression modeling was used to determine the effect of remission status on change in brain age while adjusting for chronological age, sex, race, education, disease burden (CIRSG), and pre-treatment depression severity

(MADRS). This was also performed for all LLD non-remitters to determine the effect of change in brain age on change in depression severity (MADRS). As an additional exploratory analysis, baseline brain age was regressed against baseline depression severity (MADRS), age, and their interaction effect.

6.3 Results

Table 11 shows demographic and clinical information for both remitters and non-remitters. Non-remitters had lower pre- and post-treatment brain age as compared to remitters ($t(44)=2.44$, $p=0.019$ and $t(44)=2.19$, $p=0.034$ respectively). There were no differences in chronological age, sex, race, education, depression type (recurrent/single episode), cumulative illness burden (CIRSG), or pre-treatment depression severity (MADRS). As expected, remitters have a lower post-treatment MADRS.

We found no change between pre- and post-treatment in brain age when we looked at the entire sample ($t(45)=1.6$, $p=0.123$, mean difference 0.09 and standard error of difference 0.06). However, we found that while remitters show no change in brain age pre- to post-treatment ($t(23)=-0.5$, $p=0.602$, mean difference -0.04 and standard error of difference 0.08), non-remitters showed an increase in brain age from pre- to post-treatment ($t(21)=2.9$, $p<0.01$, mean difference 0.23 and standard error of difference 0.08) (see figure 12). A change in 0.23 years corresponds to approximately 11.96 weeks, the approximate duration between pre- and post-treatment MRI scans.

We found that the change in brain age was associated with remission status and chronological age, but not sex, race, education, cumulative illness burden (CIRSG), and pre-

treatment depression severity (MADRS) ($F(7, 37)=2.45$, $p=0.036$, $r_2=0.32$) (see table 12). Individuals with the lowest improvement in depression severity following treatment had the largest increases in brain age (see figure 13).

In addition, there was a strong association between change in brain age and change in depression severity (MADRS) for non-remitters ($F(6, 14)=2.51$, $p=0.073$, $r_2=0.52$) (see table 13). Non-remitted individuals with less improvement in depression severity following treatment had larger increases in brain age.

There was also a strong moderating effect of baseline depression severity on the relationship between age and baseline brain age for all participants ($F(3,42)=3.20$, $p=0.033$). The interaction effect between baseline depression severity and age was trending on significance as shown in table 14.

Table 11. Demographic and clinical differences between remitters and non-remitters.

	remitters (N = 24)	**non-remitters (N = 22)**	***t* or χ^2**	***p*-value**
age (years)	66.5 ± 7.0	64.5 ± 6.6	$t(44) = 1.04$	0.306
sex (% F)	70.8	54.5	$\chi^2(1) = 1.31$	0.252
race (% non-White)	16.7	18.2	$\chi^2(1) = 0.018$	0.892
education (years)	14.5 ± 2.6	15.6 ± 2.6	$t(44) = 1.37$	0.177
depression type (% recurrent)	58.3	59.1	$\chi^2(1) = 0.014$	0.907
CIRSG	9.4 ± 3.9	8.4 ± 4.8	$t(43) = 0.798$	0.429
pre-treatment MADRS	23.4 ± 7.9	26.9 ± 5.2	$t(44) = 1.73$	0.091
post-treatment MADRS	4.8 ± 4.4	19.3 ± 6.2	$t(44) = 9.19$	**<0.0001**
pre-treatment brain age (years)	77.9 ± 2.7	75.8 ± 3.3	$t(44) = 2.44$	**0.019**
post-treatment brain age (years)	77.9 ± 2.7	76.0 ± 3.2	$t(44) = 2.19$	**0.034**

Table 12. Association between change in brain age and remission status adjusting for pre-treatment chronological age, sex, race, education, cumulative illness burden.

Term	Estimate	Std Error	Lower 95%	Upper 95%	*t*-ratio	*p*-value
intercept	0.26	0.40	-0.54	1.07	0.66	0.5109
age	-0.02	0.01	-0.04	0.00	-2.50	**0.0169**
sex	0.05	0.07	-0.08	0.18	0.74	0.4654
race	0.05	0.08	-0.12	0.21	0.59	0.5616
education	-0.02	0.02	-0.06	0.03	-0.71	0.4801
CIRSG	0.01	0.01	-0.02	0.04	0.80	0.4282
pre-treatment MADRS	-0.11	0.07	-0.24	0.03	-1.62	0.1142
Remission Status	0.18	0.06	0.05	0.30	2.82	**0.0076**

Table 13.Association between change in depression severity (MADRS) for non-remitters and change in brain age adjusting for pre-treatment chronological age, sex, race, education, and cumulative illness burden (CIRSG).

Term	Estimate	Std Error	Lower 95%	Upper 95%	*t*-ratio	*p*-value
intercept	-1.20	0.93	-3.19	0.79	-1.30	0.2151
age	-0.04	0.02	-0.08	0.01	-1.60	0.1324
sex	0.49	0.27	-0.08	1.06	1.83	0.0889
race	0.46	0.34	-0.28	1.19	1.34	0.2027
education	0.04	0.05	-0.07	0.15	0.75	0.4651
CIRSG	-0.01	0.03	-0.08	0.05	-0.51	0.6165
Δ brain age	-1.14	0.35	-1.88	-0.39	-3.27	**0.0056**

Table 14. Association between baseline brain age and participant chronological age, baseline depression severity (pre-MADRS), and their interaction effect.

Term	Estimate	Std Error	Lower 95%	Upper 95%	*t*-ratio	*p*-value
intercept	0.04	0.45	-0.86	0.96	0.11	0.9110
age	0.09	0.07	-0.05	0.23	1.30	0.2009
pre-MADRS	-0.60	0.45	-1.51	0.32	-1.32	0.1938
age*pre-MADRS	0.11	0.06	-0.01	0.24	1.82	**0.0765**

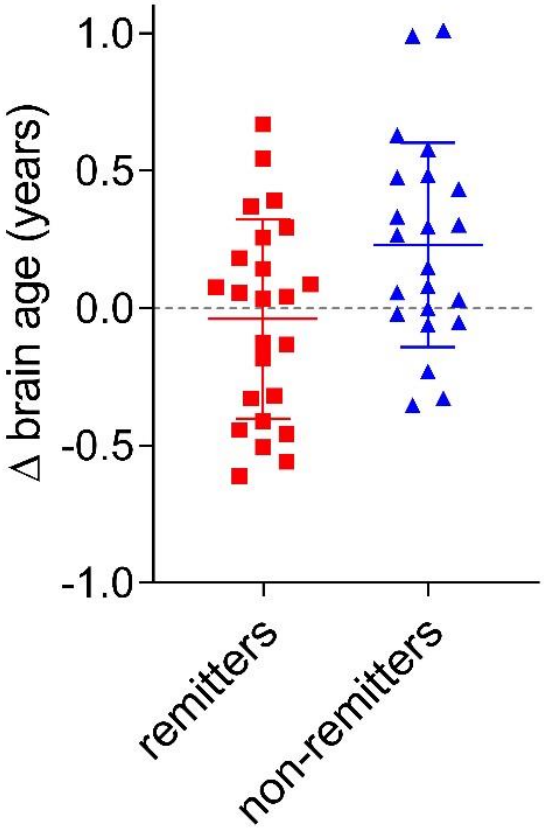

Figure 12. Change in brain age in remitters (red, square) and non-remitters (blue, triangle). Median and standard errors plotted as well as each individual participant's change.

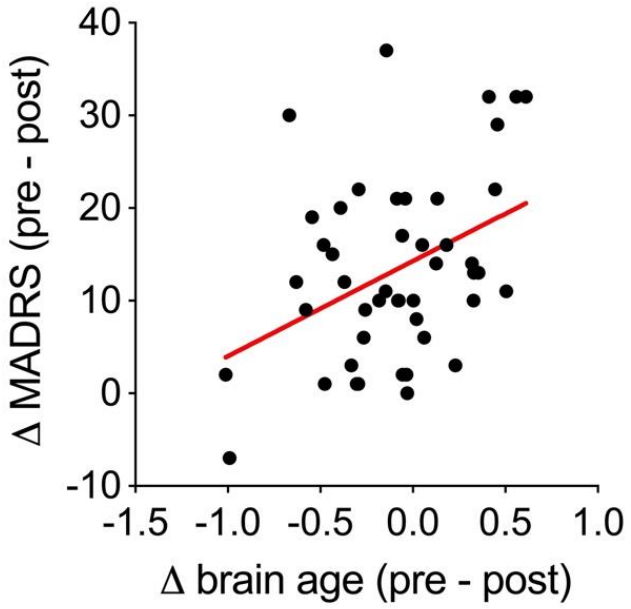

Figure 13. Association between change in depression severity (pre- minus post-treatment MADRS) and change in brain age (post- minus pre-treatment).

Note that positive values in Δ MADRS indicate improvement of depression severity while negative values in Δ brain age indicate increased brain age from pre- to post-treatment. All participants (including remitters are shown).

6.4 Discussion

To our knowledge, this is the first study applying brain age prediction to LLD. In this study, brain age prediction was applied pre- and post- venlafaxine treatment in LLD patients. Our results showed that non-remitters demonstrated a significant increase in brain age over the intervention period while remitters showed no significant change in brain age over the same period. We found that change in brain age was significantly associated with remission status even after adjusting for

demographic and clinical features. For non-remitters, less improvement in depression severity (MADRS) was associated with larger increases in brain age.

As previous literature has established a connection between regional grey matter atrophy and LLD, our results suggest that there may be subacute processes affecting brain structure on the same timescale as remission over the 12-week period of intervention [32-38]. The mean difference in brain age for non-remitters was approximately 0.23 years, or ~12 weeks older (matching the intervention period), while there was not a statistically significant change in remitters.

One manner of interpretation is that LLD patients experience brain aging at the same rate as chronological aging, which in perspective with our previous experience with our brain age model, is a pathologically accelerated rate compared to the healthy population. The remitted group, in comparison, would have brains that have not appeared to have aged, as the intervention period would be too short to detect significant changes associated with normal aging.

An alternative interpretation is that the non-remitted group experienced a rate of brain aging closer to normal, while the remitted group actually experienced a pause in brain aging (due to their brain ages having no significant change over the 12-week intervention period). This may be representative of a recovery respite from gray matter atrophy associated with LLD pathology (and in this case, normal aging as well), and therefore appear relatively younger as a result of remission. Current study design and data analysis does not allow us to distinguish between these interpretations.

Both interpretations are suggestive of different processes in the context of LLD. Degenerative structural changes have been well documented and even associated with disease severity, with many past studies demonstrating lower gray matter volumes in LLD individuals compared to healthy controls, and even LLD non-remitters against remitters [32-38].

However, a 4-year study using serial MR imaging showed that the longitudinal increases in grey and white matter lesion sizes of LLD individuals did not differ significantly from the trajectory seen in normal aging, albeit being larger in size at baseline [39]. Whether the trajectory observed belonged to remitted or non-remitted individuals is unclear, however, as the authors note that remission status and symptomatic improvement were not followed in a controlled trial environment.

Another longitudinal study which evaluated hippocampal volume at multiple time points after electroconvulsive therapy showed a transient increase in volume one week after treatment which normalized to baseline levels at 6 months post-treatment with no coevolution against symptomatic improvement [40]. It is also possible that our remitted group results are following a similar trend in which increases in gray matter volume are translated into apparently arrested brain aging which may not persist over a longer time course, for which a longer observation interval would be required to confirm. Potential mechanisms for these structural changes include increased neurogenesis in the dentate gyrus, increased neurotrophic factors such as brain-derived neurotrophic factor: BDNF), and reduction of stress/allostatic burden which in turn reduces HPA-axis-mediated suppression of neurogenesis.

While the association between treatment response and change in brain age seen in the linear modeling is expected given the increase in brain age exclusive to the non-remitted group, there was still a statistically significant improvement in disease severity (MADRS) for non-remitters ($t(21) = -5.51$, mean difference = -7.55, standard error of the mean difference = 1.37, $p < 0.0001$). Not only did individual non-remitters differ in the lessening of their disease severity, but there was a strong association between the extent of this change and the change in their individual brain age, suggesting that brain age may also serve as a quantitative measure of improvement. An alternative

interpretation is that the extent of remaining LLD pathology for those patients could be explained by the increase in brain age experienced by each individual.

In all, these results suggest there is significant potential in the utility of brain age prediction model when applied to LLD, and that structural brain changes seen in LLD may indeed be contextualized by the aging process. Brain age prediction is in some ways a simplification or aggregate measure of overall morphometric brain changes and loses the regional specificity of many previous studies. However, its quantitative nature provides a longitudinal metric that may help consolidate morphometric brain changes that are sensitive to even a 12-week period. Our results also offer additional supporting evidence for potentially significant morphometric changes accompanying symptomatic improvement in LLD, although a longer follow-up period would help confirm that these changes are not transient.

6.4.1 Limitations

There are several limitations and areas for future improvement. In our study, the remitters and non-remitters were not matched for pre-treatment brain age. As mentioned earlier, interpretation of our results, while showing significant relative differences between remitters and non-remitters, lacks an absolute comparison which longitudinal brain age measurements of a healthy cohort would provide. Our brain age model was trained on cross-sectional measurements of a healthy population and shares this limitation.

Generalizability of our results and utilization of brain age differences for prediction of disease remission are also limited by the small sample size of this treatment trial. Our study is an open-label trial and it is unclear whether these changes would be present in a randomized placebo-controlled study. It is unclear whether this generalizes to mid-life samples or is characteristic of

late-life depression. Potential future improvements may also include intervention with other antidepressant interventions and an extended period of evaluation or follow-up imaging. Other factors potentially contributing to changes in disease severity and brain age not considered in this study may include neurocognitive testing and functional MR imaging, which have previously been investigated elsewhere in the current cohort [3, 25, 26, 41].

6.4.2 Conclusions

This study demonstrated the potential utility of brain age prediction in resolving the differences in structural trajectories between remitted and non-remitted LLD individuals and shows a strong relationship between the extent of persisting symptomology and brain age changes in non-remitted individuals. This marker could help us understand the potential long-term consequences of extended periods of depression. Future studies should investigate whether brain age predicts incident depression; whether depression history (i.e., number of episodes and extent of symptoms) predicts greater brain age; and whether these changes indicate long-term changes (i.e., brain age continues to increase in non-remitters and is slowed to the rate of remitters in never-depressed individuals).

7.0 Application of Brain Age: Brain Aging Associated With Greater Worry And Rumination In Late-Life

This chapter is a modified version of the following manuscript that is currently in submission:

Karim, H.T., Ly, M., Yu, G., Khan, F., Krafty, R., Tudorascu, D.L., Aizenstein, H.J., Gross, J., Andreescu, C. Brain aging associated with greater worry and rumination in late life.

This work was intended to support Aim 3 by applying the brain age prediction model in cohorts with age-related neuropsychiatric disorders. In this study, we demonstrated that worry and rumination may drive accelerated aging in late-life generalized anxiety (LLGAD). My contributions to this study were: analyses, interpretation, and drafting and revising the manuscript.

7.1 Introduction

"Age is an issue of mind over matter. If you don't mind, it doesn't matter." Mark Twain

In the last decade, several studies have reported an independent effect of anxiety on aging. Clinically, anxiety and its disorders have been described as risk factors for multiple age-related medical conditions [1-4]. More specifically, pathologic worry in particular is associated with the development of coronary heart disease [3] and a higher burden of anxiety symptoms was associated prospectively with increased risk for incident stroke, independent of other risk factors (including

depression) [4]. In the Nurses' Health Study, a 4-year longitudinal study of community-dwelling older women (N=16,351), higher midlife anxiety was related to worse later-life overall cognition and verbal memory[5]. Chronic anxiety has been also associated with a higher beta amyloid burden[6], as well as with a moderating effect of the impact of beta amyloid on cognitive decline [7,8]. In a 2-year observational study, older adults with mildly elevated worry symptoms performed worse on measures of visual learning and memory than older adults with no/minimal worry symptoms[9]. Multiple animal studies reported impaired neurogenesis in anxiety [10], and several human studies described brain structural changes associated with anxiety in midlife (e.g. reduced hippocampal volumes and reduced gray matter density in the amygdala and hippocampus[11]). Our previous reports in a geriatric anxiety sample describe structural grey matter changes such as thinning of the orbital frontal cortex and rostral anterior cingulate cortex in late-life Generalized Anxiety Disorder (GAD)[12] and a potential effect of cerebrovascular burden in impairing emotion regulation in late-life GAD [13].

All these studies indicate that anxiety and/or worry contribute to accelerated aging. The putative mechanisms enlist molecular aging markers [e.g. shorten telomere [14]] and increased stress-response [chronic inflammatory stress, increased HPA activity and excessive autonomic responses [15-17]]. However, research in this area is still in early stages and the pathway linking anxiety or worry with brain aging remains unclear.

Most of the studies available regarding the potential effect of late-life anxiety in accelerated aging use heterogenous and often non-specific measures for anxiety. Anxiety and its disorders encompass multiple clinical constructs such as worry, rumination, somatization [ref] and it is highly comorbid with both depression and neuroticism [refs]. It is thus more difficult to detangle the specific effect of various phenotypes on accelerated aging [18]. Additionally, the highly

heterogenous changes that occur in aging make it difficult to interpret various correlation studies that point toward an association between anxiety and aging.

Brain age prediction is a machine learning method that estimates chronological age from brain scans. Thus, brain age may indicate a potential discrepancy between biological and chronological age, suggesting that pathological neuroprogression (combination of neurodegeneration, neurotoxicity and lowered neuroplasticity) is associated with accelerated aging [15]. These models have been used recently to demonstrate the associated of increased brain age with cognitive impairment, Alzheimer's disease, schizophrenia or traumatic brain injury (refs). In a previous report [19], our group has described a machine learning method for estimating brain age from neuroimaging scans while accounting for amyloid status. Our brain age prediction model contextualizes whole brain structural information of a test cohort against structural information from a large healthy participant cohort spanning a wide range of ages (20-85) to generate a machine learning-based prediction of the test participant's chronological age. In this way, discrepancies between actual chronological age and predicted brain age in test groups may indicate pathological disruption or acceleration of the aging process. We reported that our model was able to delineate significant differences in the brain age relative to chronological age between cognitively normal individuals with and without amyloid beta deposition in the brain [19].

In the current study, we aimed to test if any of the multiple anxiety phenotypes (worry, global anxiety, rumination) as well as their more frequent comorbidities (depression severity, neuroticism) are predictive of brain aging. Given the hypothesis regarding the role of increased stress response we also included the Perceived Stress Questionnaire[20] in the model. Also, as our previous reports regarding emotion regulation deficits in late-life anxiety [13,21,22], we also included

in the current model the Emotion Regulation Questionnaire (ERQ), a self-report measure of two emotion regulation strategies (cognitive reappraisal and expressive suppression) [23].

7.2 Methods

7.2.1 Participants and Study Design

We recruited participants (n=78) who were 50 years and older and were recruited in along the spectrum of worry, such that worry was normally distributed. We recruited individuals with and without anxiety (generalized anxiety disorder, panic disorder, social phobia, etc.) and/or mood disorders (e.g., major depressive disorder, persistent depressive disorder, or unspecified depressive disorder). Participants were excluded if they were: diagnosed with autism spectrum disorders, intellectual development disorder, or any form of psychosis or bipolar disorder. Other exclusion criteria were: a diagnosis of major neurocognitive disorder (e.g., dementia), a 3MS (modified mini-mental) score < 84, a diagnosis of personality disorder, have suicide risk, use of antidepressants within the last five to fourteen days (participants were allowed to washout), history of drug/alcohol abuse within last six months, use of high doses of benzodiazepines (greater than equivalent to 2mg of lorazepam), uncorrected vision problems to would preclude neuropsychiatric testing, below 6th grade level of reading, clinical diagnosis of cerebrovascular accident or Multiple Sclerosis or vasculitis or significant head trauma, ferromagnetic objects in body, claustrophobia, or too large to fit in MR scanner.

When appropriate, participants underwent an adequate washout on antidepressants determined by the primary psychiatrist on the study (CA). For fluoxetine, the washout interval was

6 weeks. Participants who are prescribed low dose psychotropics for pain, sleep disturbances, and/or medical conditions were allowed to continue them in most circumstances. The following common antidepressants were allowed at particular doses due to medical reasons: amitriptyline (50mg/day), doxepin (50mg/day), trazodone (100mg/day), and imipramine (50mg/day). Participants were recruited from the Pittsburgh area via Pitt+Me (website resource from the university), in-person recommendations, flyers around the city, and radio/television announcements. This study was approved by the University of Pittsburgh Institutional Review Board. All participants gave written informed consent prior to participating in the study.

7.2.2 Assessments

Along with demographic information (age, sex, race, and education), we assessed the following: worry (PSWQ, Penn State Worry Questionnaire), overall anxiety (HARS, Hamilton Anxiety Rating Scale), depression (MADRS, Montgomery-Asberg Depression Rating Scale), rumination subscale (RSQ, Response Style Questionnaire), neuroticism subscale (FFI, Five-Factor Inventory), perceived stress (PSS, Cohen's Perceived Stress Scale), and the habitual use of cognitive reappraisal and suppression subscale (ERQ, Emotion Regulation Questionnaire). We also collected data on illness severity (CIRS-G, cumulative illness rating scale for geriatrics).

7.2.3 MRI Data Acquisition

MRI scans were obtained at the MR Research Center of the University of Pittsburgh using a 3T Siemens MAGNETOM Prisma scanner and a 32-channel head coil. A sagittal, whole-brain T1-weighted magnetization prepared rapid gradient echo (MPRAGE) was collected with repetition

time (TR)=2400ms, echo time (TE)=2.22ms, flip angle (FA)=8deg, field of view (FOV)=320x300 with 208 slices, 0.8mm^3 isotropic resolution, 0.4mm slice gap, and GeneRalized Autocalibrating Partial Parallel Acquisition (GRAPPA) with acceleration factor of 2 (total time 6.63min). A sagittal, whole-brain T2-weighted Sampling Perfection with Application optimized Contrasts using different flip angle Evolution (SPACE) was also collected with TR=3200ms, TE=563ms, FA=120deg, FOV=320x300 with 208 slices, 0.8mm^3 isotropic resolution, no slice gap, and GRAPPA with acceleration factor of 2 (total time 5.95min). An axial, whole-brain T2-weighted Fluid Attenuated Inversion Recovery (FLAIR) was also collected with TR=10,000ms, TE=91ms, FA=135deg, FOV=320x320 with 104 slices, 0.8mm x 0.8mm x 1.6mm resolution, no slice gap, and GRAPPA with acceleration factor of 2 (total time 5.95min).

7.2.4 Structural Processing

Processing was conducted in statistical parametric mapping toolbox (SPM12)[24] in MatLab 2018b (MathWorks, Natick, MA). All interpolation was done with a 4th degree B-spline and the similarity metric used for coregistration between different image types was normalized mutual information. The T2-SPACE and FLAIR were first independently coregistered to the MPRAGE. All three were input into a multispectral segmentation which bias corrects each image and segments them into gray matter, white matter, cerebrospinal fluid, skull, soft-tissue, and air[25]. Due to high white matter hyperintensity, we adjusted the number of Gaussians used to identify white matter to two to improve identification of gray and white matter[26]. The gray and white matter maps are inputs into a process to generate a study-specific template to estimate gray matter density.

We used DARTEL (Diffeomorphic Anatomical Registration using Exponentiated Lie Algebra) to generate study-specific templates[27]. DARTEL uses an iterative process of averages

across participants and iterative coregistration to improve normalization to a standard anatomical space. Once a study-specific template is generated (an iterative average across participants), each image is normalized and then transformed into a gray matter density image by multiplying the Jacobian of the transformations[27]. The gray matter density images were smoothed using a Gaussian kernel of full-width at half-maximum of 6mm. These gray matter density images are input into the brain age estimation model.

7.2.5 Brain Age Estimation

We have previously validated a brain age estimation algorithm that predicts chronological age with gray matter density maps[19] using the Pattern Recognition for Neuroimaging Toolbox (PRoNTo)[28]. Whole brain, voxel-wise gray matter densities were mean-centered and used to calculate a similarity matrix kernel (dot product) that was input into a Gaussian processes regression to predict chronological age. The training set, which includes 757 adult MRI's of individuals without any psychiatric or neurological disorder as well as Alzheimer's pathology as measured by positron emission tomography, has been previously described[19]. The current study's participants were not part of the training set. Using this pre-trained model, we can estimate the brain age of each participant in the current study.

7.2.6 Statistical Analysis

We conducted a linear regression analysis in SPSS 26 (IBM, Armonk, NY). We used brain age as the outcome and the following as independent predictors: chronological age, sex, education (years), worry (PSWQ), anxiety (HARS), depression severity (MADRS), rumination (RSQ),

neuroticism (FFI-Neuroticism), reappraisal (ERQ, reappraisal subscale), suppression (ERQ, suppression subscale), and stress (PSS). The models conducted all had variance inflation factor (VIF) below 5, showed normally distributed standardized residuals (based on a histogram and QQ-plot), and did not violate the assumption of homoscedasticity.

A total of 69 participants (88.5%) had all data available, however there were missing values for: HARS (2 lost questionnaires), MADRS (3 not collected, 2 lost questionnaire), RSQ (1 participant error, 1 not collected), FFI (2 refused, 4 participant error), ERQ (1 refused, 3 participant error), and PSS (1 refused, 3 participant error). We conducted multiple imputations analysis[29,30] (500 imputations) to impute missing values using the Markov Chain Monte Carlo method[31] and fully conditional specification with linear regression since our values were missing at random and showed no structure in the way the data were missing.

Every variable used in the regression as well as the outcome (brain age) was used in the model, as this has been shown to improve the imputation and is not 'self-fulfilling prophecy,' but rather "replays the strength of associations between predictors and outcomes present in the complete cases, to enable valid analyses[32]." All variables were constrained to their appropriate values (e.g., HARS ranges from 0 to 56 thus values may not be imputed outside this range). We report both the imputed pooled results as well as the estimates from the original model with missing data (n=68).

Each variable was inspected for outliers and the following variables had some outliers: HARS (n=1), MADRS (n=4), RSQ (n=1), brain age (n=2), and reappraisal ERQ subscale (n=1). We conducted the regression with those participants removed (not shown) and found that the estimates did not differ from when they were included in the model.

7.3 Results

We report the characteristics of the sample in table 1. Of note worry is normally distributed around a mean worry severity of 47.6. Racial demographics match that of the surrounding Pittsburgh area.

We found that brain age was significantly associated with several factors that explained 72% of the variance in brain age [$F(11,57)=13.3$, $p<0.001$, $r_2=0.72$]. We found the following: (1) for every one chronological year participant's brain age went up by approximately 0.57 years (~6.8 months); (2) women were younger by ~3.4 years compared to men; and (3) for every one point greater on the RSQ, brain age was greater by 0.14 years (~1.7 months) (see table 2 and figure 1).

However, after imputing values that were missing for 9 participants (see table 1), we reconducted our regression and found the following (pooled results): (1) for every one chronological year participant's brain age went up by approximately 0.53 years (~6.4 months); (2) women were younger by ~4.1 years compared to men; (3) *for every one point greater on the PSWQ, brain age was greater by 0.11 years (~1.3 months); (4) for every one point greater on the RSQ, brain age was greater by 0.11 years (~1.3 months);* and (5) for every one point greater on the ERQ suppression scale, brain was lower by 0.17 years (~2.0 months). The imputed models explained 68 to 72% (range) of the variance in brain age across imputations (variance is not a pooled metric; thus, we report the range). We show associations between these factors in figure 1 using non-imputed data.

Table 15. Characteristics of the LLGAD sample

-Means and standard deviations are reported unless otherwise noted
-Means for both the original data and imputed values (see number of missing data) are reported

Variable Name	Total Sample (n=78) Mean	Std.	Number Missing	Imputed Mean (pooled)
Age, years	61.2	8.5	0	N/A
Sex, number female	53 (68%)		0	N/A
Race, W/B/HPI/MR	63 (81%); 13 (17%); 1 (1%); 1 (1%)		0	N/A
Education, years	15.6	2.6	0	N/A
Cumulative Illness (CIRSG)	3.0	2.3	1	N/A
Worry (PSWQ)	47.6	14.7	0	N/A
Anxiety (HARS)	8.5	6.9	2	8.5
Depression (MADRS)	8.2	8.1	5	8.6
Rumination (RSQ)	37.7	12.6	2	37.7
Neuroticism (FFI Subscale)	19.5	10.7	6	19.9
Reappraisal (ERQ Subscale)	29.4	7.8	4	29.4
Suppression (ERQ Subscale)	13.8	5.4	4	13.8
Stress (PSS)	15.5	8.6	4	15.6
Brain Age, years	63.6	6.1	0	N/A

Table 16. Regression model explaining variance in brain age using imputed data.

-B indicate unstandardized coefficients. We also report 95% confidence intervals and indicate significant associations in bold.

Variable	B	Std. Error	Lower B 95% CI	Upper B 95% CI	t-statistic	p-value
Constant	30.51	5.31	20.11	40.91	5.8	0.000
Age	**0.53**	**0.06**	**0.42**	**0.64**	**9.4**	**0.000**
Sex (Male Reference)	**-4.14**	**1.00**	**-6.10**	**-2.17**	**-4.1**	**0.000**
Education	-0.13	0.18	-0.48	0.23	-0.7	0.485
Worry (PSWQ)	**0.11**	**0.05**	**0.01**	**0.21**	**2.1**	**0.035**
Anxiety (HARS)	-0.17	0.14	-0.44	0.10	-1.2	0.217
Depression (MADRS)	0.05	0.10	-0.15	0.25	0.5	0.613
Rumination (RSQ)	**0.11**	**0.06**	**0.00**	**0.23**	**1.9**	**0.055**
Neuroticism (FFI-N)	-0.06	0.08	-0.22	0.10	-0.8	0.451
Reappraisal (ERQ)	0.06	0.06	-0.06	0.18	1.0	0.329
Suppression (ERQ)	**-0.17**	**0.09**	**-0.34**	**0.01**	**-1.9**	**0.060**
Stress (PSS)	-0.08	0.09	-0.25	0.09	-1.0	0.333

7.4 Discussion

Our results indicate that worry and rumination drive the accelerated aging effect of anxiety in late-life. Surprisingly, there was no effect of perceived stress and the propensity to use suppression rather than reappraisal seems to have a protective effect on brain aging.

Although sharing common phenomenological features (difficult to control repetitive thinking), worry and rumination have been usually described as two distinct symptoms, one (worry) usually associated with generalized anxiety and the other one (rumination) usually associated with depression[33]. Classically, rumination theories consider rumination is triggered by sad mood and it maintains depressive symptoms by promoting negative cognitive biases [34]. Similarly, classic worry theoretical models such as Borkovec's cognitive avoidance model, posit that worry serves a cognitive avoidance strategy that inhibits the emotional processing of highly

anxiogenic material [35]. However, newer theories propose a transdiagnostic approach that 1) includes both worry and rumination under the umbrella of negative repetitive thoughts (NRT) and 2) describe the detrimental effect of NRTs throughout multiple categorical diagnoses including major depression, GAD, Social Phobia, Bipolar disorder, Obsessive compulsive Disorder, Eating disorders and PTSD[36,37]. Several authors have proposed NRT as the core of anxiety-depression comorbidity[38,39], while others emphasized the association of NRT with worse psychological, physical and cognitive health in older adults [40].

Recently, NRTs have been "imported" in the aging and dementia field. Thus, in 2015, Marchant & Howard have advanced a model of Cognitive Debt that would involve certain symptoms/disorders actively depleting cognitive reserve and increase vulnerability to AD [41]. Thus, there is building evidence that depression, anxiety, sleep disorders, neuroticism and PSTD increase risk for AD and the authors suggest that RNT are the process common to these factors which may drive the acquisition of Cognitive Debt through diverting cognitive and emotional resources to distressing thought processes[41]. The neurobiological signature of Cognitive Debt and AD might rely on the relationship between hippocampus, PFC and the amygdala, and the HPA stress response [41].

Our results, that single out both worry and rumination as predictive of accelerated aging, would fit well in the overall model of NRTs as contributing to increased Cognitive Debt. These results also emphasize the need for preventative interventions targeting NRTs in older adults (e.g. mindful meditation, cognitive behavioral therapy or positive reappraisal therapy – a newer attempt to incorporate mindful meditation into cognitive therapy [42]).

Regarding the protective role of expressive suppression, a response-focused form of emotion regulation that seeks to prevent the outward expression of an already-generated emotion[43],

several studies have indicated the positive association between expressive suppression and volumes of the anterior insula, dorsomedial PFC and dorsal ACC [44-47]. Although there is data linking expressive suppression to anxious and depressive symptoms [23] as well as memory impairment[48], we may cautiously interpret these results through the use-dependent brain plasticity theory [44,49] that posits a 'use it or lose it' approach. Thus, chronic preferential use of expressive suppression may maintain a higher volume in prefrontal brain regions counterbalancing thus the thinning effect of aging. An additional explanation involves the age group used in the current study – emotion regulation strategies effective in younger adults may become less effective with age[50] and although older adults report using cognitive reappraisal more than younger adults, it is possible that older adults may rely less on a resource-demanding strategy such as reappraisal and use simpler techniques such as distraction or suppression [51].

Our study has several limitations: we do not have longitudinal data to follow-up on the effect of the predictive factors described above, we do not have any other biological markers of aging to corroborate the current results (e.g. inflammatory cytokines, cortisol levels, cerebral beta-amyloid burden). Most participants had mild if any depressive symptoms, thus we cannot make inferences about the effect of clinical depression on accelerated aging.

In conclusion, we present novel data suggesting a deleterious effect on aging of both worry and rumination in older adults as well as a potential protective effect of using expressive suppression. There results also emphasize the role of preventative interventions in reducing accelerated aging by targeting modifiable factors such as worry and rumination in late-life.

8.0 Summary and Discussion

The work of this dissertation mainly involves exploring the role of brain reserve and cognitive reserve in neuropsychiatric pathologies and then utilizing brain age prediction as a means of quantifying and exploring relationships between brain reserve and pathologic progression or severity. Although currently we have a variety of neuropsychiatric batteries and clinical biomarkers for various diseases, there is still significant variation between individuals which cannot be explained for by existing biomarkers alone. This was demonstrated in the work involving LLD such that LLD patients and healthy controls did not have significant differences in the rate of their cognitive decline, but LLD patients had decreased baseline levels of cognitive function which are thought to represent the diminished brain or cognitive reserve associated with disease burden. This was also demonstrated in functional imaging of participants with subjective cognitive decline, where higher educated individuals (representing individuals with increased cognitive reserve) had significantly different patterns of neural activation when faced with a memory encoding task compared to less educated individuals, suggesting that cognitive reserve is a significant factor in differentiating compensatory mechanisms of neural activation in light of age-related cognitive decline. Both of these studies demonstrate different aspects of significance for cognitive reserve.

In light of these conclusions and the overarching potential for cognitive and brain reserve to contribute toward clinical understanding of neuropsychiatric disease progression, we developed a novel model of brain age prediction through machine learning-based training on an amyloid negative healthy population. Not only did this novel model result in significantly improved

performance in delineating between different cognitive stages of AD progression compared to previously published models, it also provided an opportunity for more sophisticated analysis of pathologic contexts relevant to brain reserve.

As such, we applied our brain age prediction model to settings of LLGAD, CLBP, and depression. In these works, we demonstrated that there is significant capacity for brain age prediction to quantify changes in brain reserve associated with these pathologies and various pathologic features. In LLGAD, we found that increased brain age (representing diminished brain reserve) was associated with increased worry and rumination patient characteristics, while suppression, a defense mechanism of LLGAD, was associated with mitigation of the effects of increased brain age. In CLBP, we found that LBP patients had significantly increased trends of brain age versus chronological age compared against healthy participants, representative of accelerated brain aging that was independent from commonly utilized traits of CLBP such as pain duration and pain severity. Given the importance and implications of brain structural reserve in cognitive function and mood, brain age prediction offers a potential biomarker to track CLBP progression and increased risk for psychiatric comorbidities common to chronic pain such as depression. In depression we found that longitudinal measurement of brain reserve showed significant differences between disease remitters and non-remitters in a direct link between structural brain changes and successful reduction of symptomatic disease severity. As a predictive measure, brain age holds significant potential as a distinct measure for evaluation of antidepressant therapy efficacy as well as disease progression given its successful longitudinal application.

In all, brain age prediction remains a promising concept for the characterization and prediction of a wide range of pathological contexts. Brain age prediction also offers a holistic impression of the entire brain and its structural changes in which pre-determined regional analysis lacks. Given the significant variation which exists between the age-related structural changes in different individuals, fitting an individual's structural imaging against a vast dataset of healthy individuals is a quantifiable measure which may identify key differences (as demonstrated) which may be missed by more localized analyses. Further refining of the model through adaptive training set selection and more extensive longitudinal data collection may not only improve its capacity for predicting disease progression and remission as well as offer additional information for clinical decision making. Of course, other future directions for brain age prediction studies include increasing sample sizes to allow for increased model complexity and power for statistical analyses of results.

Bibliography

Aalami, O. O., Fang, T. D., Song, H. M., & Nacamuli, R. P. (2003). Physiological features of aging persons. Archives of Surgery, 138(10), 1068-1076.

Aizenstein HJ, Baskys A, Boldrini M, et al. Vascular depression consensus report - a critical update. BMC Med 2016; 14(1): 161.

Aldao A, Nolen-Hoeksema S, Schweizer S. Emotion-regulation strategies across psychopathology: A meta-analytic review. Clin Psychol Rev. 2010;30(2):217-237.

Alexopoulos GS, Morimoto SS. The inflammation hypothesis in geriatric depression. Int J Geriatr Psychiatry 2011; 26(11): 1109-18.

Amariglio, R.E., Becker, J.A., Carmasin, J., Wadsworth, L.P., Lorius, N., Sullivan, C., Maye, J.E., Gidicsin, C., Pepin, L.C., Sperling, R.A., Johnson, K.A., Rentz, D.M., 2012. Subjective cognitive complaints and amyloid burden in cognitively normal older individuals. Neuropsychologia 50(12), 2880-2886.

Amieva H, Mokri H, Le Goff M, Meillon C, Jacqmin-Gadda H, Foubert-Samier A, et al. Compensatory mechanisms in higher-educated subjects with Alzheimer's disease: a study of 20 years of cognitive decline. Brain. 2014;137(Pt 4):1167-75.

Andreescu C, Mennin D, Tudorascu D, et al. The many faces of anxiety-neurobiological correlates of anxiety phenotypes. Psychiatry Res. 2015;234(1):96-105.

Andreescu C, Sheu LK, Tudorascu D, et al. Emotion reactivity and regulation in late-life generalized anxiety disorder: functional connectivity at baseline and post-treatment. Am J Geriatr Psychiatry. 2015;23(2):200-214.

Andreescu C, Tudorascu D, Sheu LK, et al. Brain structural changes in late-life generalized anxiety disorder. Psychiatry Res. 2017;268:15-21.

Andreescu C, Varon D. New research on anxiety disorders in the elderly and an update on evidence-based treatments. Curr Psychiatry Rep. 2015;17(7):595.

Andreescu, C. and H. Aizenstein, MRI studies in late-life mood disorders. Curr Top Behav Neurosci, 2012. 11: p. 269-87.

Andreescu, C., et al., Gray matter changes in late life depression--a structural MRI analysis. Neuropsychopharmacology, 2008. 33(11): p. 2566-72.

Andreescu, C., et al., Resting state functional connectivity and treatment response in late-life depression. Psychiatry Res, 2013. 214(3): p. 313-21.

Apkarian, A.V., Y. Sosa, S. Sonty, R.M. Levy, R.N. Harden, T.B. Parrish, and D.R. Gitelman, Chronic back pain is associated with decreased prefrontal and thalamic gray matter density. J Neurosci, 2004. 24(46): p. 10410-5.
Asami, T., et al., Longitudinal loss of gray matter volume in patients with first-episode schizophrenia: DARTEL automated analysis and ROI validation. Neuroimage, 2012. 59(2): p. 986-996.

Ashburner, J. and K.J. Friston, Unified segmentation. Neuroimage, 2005. 26(3): p. 839-851.

Ashburner, J., A fast diffeomorphic image registration algorithm. Neuroimage, 2007. 38(1): p. 95-113.

Baldwin, R.C., et al., The prognostic significance of abnormalities seen on magnetic resonance imaging in late life depression: clinical outcome, mortality and progression to dementia at three years. International journal of geriatric psychiatry, 2000. 15(12): p. 1097-1104.

Becker, J.T., Boller, F., Saxton, J., McGonigle-Gibson, K.L., 1987. Normal rates of forgetting of verbal and non-verbal material in Alzheimer's disease. Cortex 23(1), 59-72.

Beheshti, I., Maikusa, N., Matsuda, H., 2018. The association between "Brain-Age Score" (BAS) and traditional neuropsychological screening tools in Alzheimer's disease. Brain Behav 8(8), e01020.

Bennett S, Thomas AJ. Depression and dementia: cause, consequence or coincidence? Maturitas 2014; 79(2): 184-90.

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

Bhalla RK, Butters MA, Becker JT, et al. Patterns of mild cognitive impairment after treatment of depression in the elderly. Am J Geriatr Psychiatry 2009; 17(4): 308-16.

Bhalla RK, Butters MA, Mulsant BH, et al. Persistence of neuropsychologic deficits in the remitted state of late-life depression. Am J Geriatr Psychiatry 2006; 14(5): 419-27.

Blalock, E. M., Chen, K. C., Sharrow, K., Herman, J. P., Porter, N. M., Foster, T. C., & Landfield, P. W. (2003). Gene microarrays in hippocampal aging: statistical profiling identifies novel processes correlated with cognitive impairment. Journal of Neuroscience, 23(9), 3807-3819.

Bland, J.M., Altman, D.G., 2011. Correlation in restricted ranges of data. BMJ 342, d556.

Borkovec TD. The nature, functions, and origins of worry. . In: Tallis GDF, ed. Worrying: Perspectives on theory, assessment, and treatment. Sussex, England: Wiley & Sons; 1994.

Boss, G. R., & Seegmiller, J. E. (1981). Age-related physiological changes and their clinical significance. Western Journal of Medicine, 135(6), 434.
Bouckaert, F., et al., Grey matter volume increase following electroconvulsive therapy in patients with late life depression: a longitudinal MRI study. Journal of psychiatry & neuroscience: JPN, 2016. 41(2): p. 105.

Bouckaert, F., et al., Relationship between hippocampal volume, serum BDNF, and depression severity following electroconvulsive therapy in late-life depression. Neuropsychopharmacology, 2016. 41(11): p. 2741.

Broadbent, D.E., Cooper, P.F., FitzGerald, P., Parkes, K.R., 1982. The Cognitive Failures Questionnaire (CFQ) and its correlates. Br J Clin Psychol 21 (Pt 1), 1-16.

Buchbinder, R., M. van Tulder, B. Öberg, L.M. Costa, A. Woolf, et al., Low back pain: a call for action. The Lancet, 2018. 391(10137): p. 2384-2388.

Buckalew, N., M.W. Haut, H. Aizenstein, L. Morrow, S. Perera, H. Kuwabara, and D.K.J.P.M. Weiner, Differences in brain structure and function in older adults with self-reported disabling and nondisabling chronic low back pain. 2010. 11(8): p. 1183-1197.

Buckalew, N., M.W. Haut, L. Morrow, and D. Weiner, Chronic pain is associated with brain volume loss in older adults: preliminary evidence. Pain Med, 2008. 9(2): p. 240-8.

Buckley, R.F., Hanseeuw, B., Schultz, A.P., Vannini, P., Aghjayan, S.L., Properzi, M.J., Jackson, J.D., Mormino, E.C., Rentz, D.M., Sperling, R.A., Johnson, K.A., Amariglio, R.E., 2017. Region-Specific Association of Subjective Cognitive Decline With Tauopathy Independent of Global beta-Amyloid Burden. JAMA Neurol 74(12), 1455-1463.

Butters MA, Whyte EM, Nebes RD, et al. The nature and determinants of neuropsychological functioning in late-life depression. Arch Gen Psychiatry 2004; 61(6): 587-95.

Butters, M.A., et al., Pathways linking late-life depression to persistent cognitive impairment and dementia. Dialogues in clinical neuroscience, 2008. 10(3): p. 345.

Byers AL, Yaffe K. Depression and risk of developing dementia. Nat Rev Neurol 2011; 7(6): 323-31.

Cabeza R, Anderson ND, Locantore JK, McIntosh AR. Aging gracefully: compensatory brain activity in high-performing older adults. NeuroImage. 2002;17:1394–402.

Chang, C.-C., et al., Reduction of dorsolateral prefrontal cortex gray matter in late-life depression. Psychiatry Research: Neuroimaging, 2011. 193(1): p. 1-6.

Chen, P.S., et al., White matter and subcortical gray matter lesion volume changes and late-life depression outcome: a 4-year magnetic resonance imaging study. International psychogeriatrics, 2006. 18(3): p. 445-456.

Classen J, Liepert J, Wise SP, Hallett M, Cohen LG. Rapid plasticity of human cortical movement representation induced by practice. J Neurophysiol. 1998;79(2):1117-1123.
Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. Journal of health and social behavior. 1983;24(4):385-396.

Cohen, A.D., Mowrey, W., Weissfeld, L.A., Aizenstein, H.J., McDade, E., Mountz, J.M., Nebes, R.D., Saxton, J.A., Snitz, B., Dekosky, S., Williamson, J., Lopez, O.L., Price, J.C., Mathis, C.A., Klunk, W.E., 2013. Classification of amyloid-positivity in controls: comparison of visual read and quantitative approaches. Neuroimage 71, 207-215.

Cohen, A.D., Weissfeld, L.A., Mathis, C.A., Klunk, W.E., De-Kosky, S.T., 2009. Amyloid imaging in mild cognitive impairment subtypes. Ann Neurol 65(5), 557-568.
Woods, R.P., Mazziotta, J.C., Cherry, S.R., 1993. MRI-PET registration with automated algorithm. J Comput Assist Tomogr 17(4), 536-546.

Cohen, B.E., D. Edmondson, and I.M. Kronish, State of the art review: depression, stress, anxiety, and cardiovascular disease. American journal of hypertension, 2015. 28(11): p. 1295-1302.

Cole, J.H., Annus, T., Wilson, L.R., Remtulla, R., Hong, Y.T., Fryer, T.D., Acosta-Cabronero, J., Cardenas-Blanco, A., Smith, R., Menon, D.K., Zaman, S.H., Nestor, P.J., Holland, A.J., 2017. Brain-predicted age in Down syndrome is associated with beta amyloid deposition and cognitive decline. Neurobiol Aging 56, 41-49.

Cole, J.H., et al., Prediction of brain age suggests accelerated atrophy after traumatic brain injury. Annals of neurology, 2015. 77(4): p. 571-581.

Cole, J.H., Franke, K., 2017. Predicting Age Using Neuroimaging: Innovative Brain Ageing Biomarkers. Trends Neurosci 40(12), 681-690.

Cole, J.H., Leech, R., Sharp, D.J., Alzheimer's Disease Neuroimaging, I., 2015. Prediction of brain age suggests accelerated atrophy after traumatic brain injury. Ann Neurol 77(4), 571-581.

Cutuli D. Cognitive reappraisal and expressive suppression strategies role in the emotion regulation: an overview on their modulatory effects and neural correlates. Frontiers in systems neuroscience. 2014;8:175.

De Vogelaere, F., Santens, P., Achten, E., Boon, P., Vingerhoets, G., 2010. Hippocampal activation during face-name associative memory encoding: blocked versus permuted design. Neuroradiology 52(1), 25-36.

DeCarli, C., Massaro, J., Harvey, D., Hald, J., Tullberg, M., Au, R., ... & Wolf, P. A. (2005). Measures of brain morphology and infarction in the framingham heart study: establishing what is normal. Neurobiology of aging, 26(4), 491-510.
DeCarli, C., Massaro, J., Harvey, D., Hald, J., Tullberg, M., Au, R., & Wolf, P. A. (2005b). Measures of brain morphology and infarction in the framingham heart study: establishing what is normal. Neurobiology of Aging, 26(4), 491–510.

DePalma, M. J., Ketchum, J. M., & Saullo, T. R. (2012). Multivariable analyses of the relationships between age, gender, and body mass index and the source of chronic low back pain. Pain Medicine, 13(4), 498-506.

Diniz BS, Butters MA, Albert SM, Dew MA, Reynolds CF, 3rd. Late-life depression and risk of vascular dementia and Alzheimer's disease: systematic review and meta-analysis of community-based cohort studies. Br J Psychiatry 2013; 202(5): 329-35.

Diniz, B.S., et al., Plasma biosignature and brain pathology related to persistent cognitive impairment in late-life depression. Molecular psychiatry, 2015. 20(5): p. 594.

Dixon, A. N., & Gatchel, R. J. (1999). Gender and parental status as predictors of chronic low back pain disability: a prospective study. Journal of Occupational Rehabilitation, 9(3), 195-200.

Dodd, P.R.; Scott, H.L.; Westphalen, R.I. Excitotoxic mechanisms in the pathogenesis of dementia. Neurochem. Int. 1994, 25, 203–219.

Donovan NJ, Locascio JJ, Marshall GA, et al. Longitudinal Association of Amyloid Beta and Anxious-Depressive Symptoms in Cognitively Normal Older Adults. Am J Psychiatry. 2018:appiajp201717040442.

Droppa, K., et al., Association between change in brain gray matter volume, cognition, and depression severity: Pre- and post- antidepressant pharmacotherapy for late-life depression. J Psychiatr Res, 2017. 95: p. 129-134.

Dumas, J. A. (2015). What is normal cognitive aging? Evidence from task-based functional neuroimaging. Current behavioral neuroscience reports, 2(4), 256-261.

Edelman, K., Tudorascu, D., Agudelo, C., Snitz, B., Karim, H., Cohen, A., Mathis, C., Price, J., Weissfeld, L., Klunk, W., Aizenstein, H., 2017. Amyloid-Beta Deposition is Associated with Increased Medial Temporal Lobe Activation during Memory Encoding in the Cognitively Normal Elderly. Am J Geriatr Psychiatry 25(5), 551-560.

Ehring T, & Watkins, E.R. Repetitive negative thinking as a transdiagnostic process. International Journal of Cognitive Therapy. 2008;1(3):192-205.

Erk, S., Spottke, A., Meisen, A., Wagner, M., Walter, H., Jessen, F., 2011. Evidence of neuronal compensation during episodic memory in subjective memory impairment. Arch Gen Psychiatry 68(8), 845-852.

Evans, D.A., Hebert, L.E., Beckett, L.A., Scherr, P.A., Albert, M.S., Chown, M.J., Pilgrim, D.M., Taylor, J.O., 1997. Education and other measures of socioeconomic status and risk of incident Alzheimer disease in a defined population of older persons. Arch Neurol 54(11), 1399-1405.

Finch, C. E., & Ruvkun, G. (2001). The genetics of aging. Annual review of genomics and human genetics, 2(1), 435-462.

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

Flor, H., C. Braun, T. Elbert, and N.J.N.l. Birbaumer, Extensive reorganization of primary somatosensory cortex in chronic back pain patients. 1997. 224(1): p. 5-8.

Flor, H., C. Denke, M. Schaefer, and S.J.T.L. Grüsser, Effect of sensory discrimination training on cortical reorganisation and phantom limb pain. 2001. 357(9270): p. 1763-1764.

Foster, N.E., J.R. Anema, D. Cherkin, R. Chou, S.P. Cohen, et al., Prevention and treatment of low back pain: evidence, challenges, and promising directions. The Lancet, 2018. 391(10137): p. 2368-2383.

Fotenos, A. F., Snyder, A. Z., Girton, L. E., Morris, J. C., & Buckner, R. L. (2005). Normative estimates of cross-sectional and longitudinal brain volume decline in aging and AD. Neurology, 64(6), 1032-1039.

Franklin, T.B., B.J. Saab, and I.M. Mansuy, Neural mechanisms of stress resilience and vulnerability. Neuron, 2012. 75(5): p. 747-761.

Frodl, T. and V. O'Keane, How does the brain deal with cumulative stress? A review with focus on developmental stress, HPA axis function and hippocampal structure in humans. Neurobiology of disease, 2013. 52: p. 24-37.

Garcia-Ptacek, S., Eriksdotter, M., Jelic, V., Porta-Etessam, J., Kareholt, I., Manzano Palomo, S., 2016. Subjective cognitive impairment: Towards early identification of Alzheimer disease. Neurologia 31(8), 562-571.

Gaser, C., Franke, K., Kloppel, S., Koutsouleris, N., Sauer, H., Alzheimer's Disease Neuroimaging, I., 2013. BrainAGE in Mild Cognitive Impaired Patients: Predicting the Conversion to Alzheimer's Disease. PLoS One 8(6), e67346.

Gerhart, J.I., J.W. Burns, S. Bruehl, D.A. Smith, K.M. Post, et al., Variability in negative emotions among individuals with chronic low back pain: relationships with pain and function. Pain, 2018. 159(2): p. 342-350.

Gilewski, M.J., Zelinski, E.M., Schaie, K.W., 1990. The Memory Functioning Questionnaire for assessment of memory complaints in adulthood and old age. Psychol Aging 5(4), 482-490.

Giuliani NR, Drabant EM, Bhatnagar R, Gross JJ. Emotion regulation and brain plasticity: expressive suppression use predicts anterior insula volume. Neuroimage. 2011;58(1):10-15.

Giuliani NR, Drabant EM, Gross JJ. Anterior cingulate cortex volume and emotion regulation: is bigger better? Biological psychology. 2011;86(3):379-382.

Green RC, Cupples LA, Kurz A, et al. Depression as a risk factor for Alzheimer disease: the MIRAGE Study. Arch Neurol 2003; 60(5): 753-9.

Gross JJ, John OP. Individual differences in two emotion regulation processes: implications for affect, relationships, and well-being. J Pers Soc Psychol. 2003;85(2):348-362.

Gross JJ. Antecedent- and response-focused emotion regulation: divergent consequences for experience, expression, and physiology. J Pers Soc Psychol. 1998;74(1):224-237.

Gunning, F.M., et al., Anterior cingulate cortical volumes and treatment remission of geriatric depression. International Journal of Geriatric Psychiatry: A journal of the psychiatry of late life and allied sciences, 2009. 24(8): p. 829-836.

Gustavson DE, du Pont A, Whisman MA, Miyake A. Evidence for Transdiagnostic Repetitive Negative Thinking and Its Association with Rumination, Worry, and Depression and Anxiety Symptoms: A Commonality Analysis. Collabra Psychol. 2018;4(1).

Han, S.D., Bangen, K.J., Bondi, M.W., 2009. Functional magnetic resonance imaging of compensatory neural recruitment in aging and risk for Alzheimer's disease: review and recommendations. Dement Geriatr Cogn Disord 27(1), 1-10.

Hanley AW, Garland EL. Dispositional Mindfulness Co-varies with Self-Reported Positive Reappraisal. Pers Individ Dif. 2014;66:146-152.

Hartvigsen, J., M.J. Hancock, A. Kongsted, Q. Louw, M.L. Ferreira, et al., What low back pain is and why we need to pay attention. The Lancet, 2018. 391(10137): p. 2356-2367.

Hayes JP, Morey RA, Petty CM, et al. Staying cool when things get hot: emotion regulation modulates neural mechanisms of memory encoding. Frontiers in human neuroscience. 2010;4:230.

Hayes, J.M., Tang, L., Viviano, R.P., van Rooden, S., Ofen, N., Damoiseaux, J.S., 2017. Subjective memory complaints are associated with brain activation supporting successful memory encoding. Neurobiol Aging 60, 71-80.

Hermann A, Bieber A, Keck T, Vaitl D, Stark R. Brain structural basis of cognitive reappraisal and expressive suppression. Soc Cogn Affect Neurosci. 2014;9(9):1435-1442.

Hsieh, M.H., et al., Hippocampal volume and antidepressant response in geriatric depression. International journal of geriatric psychiatry, 2002. 17(6): p. 519-525.
Hung, C.I., C.Y. Liu, and T.S. Fu, Depression: An important factor associated with disability among patients with chronic low back pain. Int J Psychiatry Med, 2015. 49(3): p. 187-98.

Ivo, R., A. Nicklas, J. Dargel, R. Sobottke, K.S. Delank, P. Eysel, and B. Weber, Brain structural and psychometric alterations in chronic low back pain. Eur Spine J, 2013. 22(9): p. 1958-64.

Jack, C.R., Jr., Knopman, D.S., Jagust, W.J., Petersen, R.C., Weiner, M.W., Aisen, P.S., Shaw, L.M., Vemuri, P., Wiste, H.J., Weigand, S.D., Lesnick, T.G., Pankratz, V.S., Donohue, M.C., Trojanowski, J.Q., 2013. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. Lancet Neurol 12(2), 207-216.

Janssen, J., et al., Cerebral volume measurements and subcortical white matter lesions and short-term treatment response in late life depression. International Journal of Geriatric Psychiatry: A journal of the psychiatry of late life and allied sciences, 2007. 22(5): p. 468-474.

Jessen, F., Amariglio, R.E., van Boxtel, M., Breteler, M., Ceccaldi, M., Chetelat, G., Dubois, B., Dufouil, C., Ellis, K.A., van der Flier, W.M., Glodzik, L., van Harten, A.C., de Leon, M.J., McHugh, P., Mielke, M.M., Molinuevo, J.L., Mosconi, L., Osorio, R.S., Perrotin, A., Petersen,

Jin, J., Maren, S., 2015. Prefrontal-Hippocampal Interactions in Memory and Emotion. Front Syst Neurosci 9, 170.

Joshi, S.H., et al., Structural plasticity of the hippocampus and amygdala induced by electroconvulsive therapy in major depression. Biological psychiatry, 2016. 79(4): p. 282-292.

Just N, Alloy LB. The response styles theory of depression: tests and an extension of the theory. J Abnorm Psychol. 1997;106(2):221-229.

Karim H, Tudorascu DL, Aizenstein H, Walker S, Good R, Andreescu C. Emotion Reactivity and Cerebrovascular Burden in Late-Life GAD: A Neuroimaging Study. Am J Geriatr Psychiatry. 2016;24(11):1040-1050.

Karim HT, Andreescu C, MacCloud RL, et al. The effects of white matter disease on the accuracy of automated segmentation. Psychiatry Research: Neuroimaging. 2016;253:7-14.

Karim HT, Tudorascu DL, Butters MA, Walker S, Aizenstein HJ, Andreescu C. In the grip of worry: cerebral blood flow changes during worry induction and reappraisal in late-life generalized anxiety disorder. Translational psychiatry. 2017;7(8):e1204.

Karim, H.T., et al., Acute trajectories of neural activation predict remission to pharmacotherapy in late-life depression. Neuroimage Clin, 2018. 19: p. 831-839.

Karim, H.T., et al., Intrinsic functional connectivity in late-life depression: trajectories over the course of pharmacotherapy in remitters and non-remitters. Mol Psychiatry, 2017. 22(3): p. 450-457.
Karim, H.T., et al., The effects of white matter disease on the accuracy of automated segmentation. Psychiatry Research: Neuroimaging, 2016. 253: p. 7-14.

Karp, A., Kareholt, I., Qiu, C., Bellander, T., Winblad, B., Fratiglioni, L., 2004. Relation of education and occupation-based socioeconomic status to incident Alzheimer's disease. Am J Epidemiol 159(2), 175-183.

Karp, J.F., B.L. Rollman, C.F. Reynolds III, J.Q. Morse, F. Lotrich, et al., Addressing both depression and pain in late life: the methodology of the ADAPT study. 2012. 13(3): p. 405-418.

Kessler R, Berglund P, Demler O, et al. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the national comorbidity survey replication. Arch Gen Psychiatry 2005;62(6):593–602.

Khalaf, A., et al., Altered Functional Magnetic Resonance Imaging Markers of Affective Processing During Treatment of Late-Life Depression. Am J Geriatr Psychiatry, 2016. 24(10): p. 791-801.

Kliegel, M., Zimprich, D., Eschen, A., 2005. What do subjective cognitive complaints in persons with aging-associated cognitive decline reflect? Int Psychogeriatr 17(3), 499-512.

Knight, R.G., McMahon, J., Green, T.J., Skeaff, C.M., 2004. Some Normative and Psychometric Data for the Geriatric Depression Scale and the Cognitive Failures Questionnaire from a Sample of Healthy Older Persons. New Zealand Journal of Psychology 33(3).

Koen, J. D., & Rugg, M. D. (2019). Neural dedifferentiation in the aging brain. Trends in cognitive sciences.

Kregel, J., M. Meeus, A. Malfliet, M. Dolphens, L. Danneels, J. Nijs, and B. Cagnie, Structural and functional brain abnormalities in chronic low back pain: A systematic review. Semin Arthritis Rheum, 2015. 45(2): p. 229-37.

Kumar, A., et al., Atrophy and high intensity lesions: complementary neurobiological mechanisms in late-life major depression. Neuropsychopharmacology, 2000. 22(3): p. 264-274.

Lambiase MJ, Kubzansky LD, Thurston RC. Prospective study of anxiety and incident stroke. Stroke. 2014;45(2):438-443.

Lenze EJ, Mulsant BH, Shear MK, Schulberg HC, Dew MA, Begley AE, et al. Comorbid anxiety disorders in depressed elderly patients. Am J Psychiatry. 2000;157(5):722–8.

Lenze, E.J., et al., Incomplete response in late-life depression: getting to remission. Dialogues in clinical neuroscience, 2008. 10(4): p. 419.

Li, Q.; Wang, S.; Milot, E.; Bergeron, P.; Ferrucci, L.; Fried, L.P.; Cohen, A.A. Homeostatic dysregulation proceeds in parallel in multiple physiological systems. Aging Cell 2015, 14, 1103–1112

Liem, F., Varoquaux, G., Kynast, J., Beyer, F., Kharabian Masouleh, S., Huntenburg, J.M., Lampe, L., Rahim, M., Abraham, A., Craddock, R.C., Riedel-Heller, S., Luck, T., Loeffler, M., Schroeter, M.L., Witte, A.V., Villringer, A., Margulies, D.S., 2017. Predicting brain-age from multimodal imaging data captures cognitive impairment. Neuroimage 148, 179-188.

Livingstone KM, Isaacowitz DM. The roles of age and attention in general emotion regulation, reappraisal, and expressive suppression. Psychol Aging. 2018;33(3):373-383.

Lockhart, S. N., & DeCarli, C. (2014). Structural imaging measures of brain aging. Neuropsychology review, 24(3), 271-289.

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., & Kroemer, G. (2013). The hallmarks of aging. Cell, 153(6), 1194-1217.

Lopresti, B.J., Klunk, W.E., Mathis, C.A., Hoge, J.A., Ziolko, S.K., Lu, X., Meltzer, C.C., Schimmel, K., Tsopelas, N.D., DeKosky, S.T., Price, J.C., 2005. Simplified quantification of Pittsburgh Compound B amyloid imaging PET studies: a comparative analysis. J Nucl Med 46(12), 1959-1972.

Ly M, Andreescu C. Advances and Barriers for Clinical Neuroimaging in Late-Life Mood and Anxiety Disorders. Curr Psychiatry Rep 2018; 20(1): 7.

Ly M, Yu GZ, Karim HT, et al. Improving brain age prediction models: incorporation of amyloid status in Alzheimer's disease. Neurobiol Aging. 2019.

MacKay DJ, Mac Kay DJ. Information theory, inference and learning algorithms. Cambridge university press; 2003.

Mackin, R.S., et al., Patterns of reduced cortical thickness in late-life depression and relationship to psychotherapeutic response. The American Journal of Geriatric Psychiatry, 2013. 21(8): p. 794-802.

Manly, J.J., Schupf, N., Tang, M.X., Stern, Y., 2005. Cognitive decline and literacy among ethnically diverse elders. J Geriatr Psychiatry Neurol 18(4), 213-217.

Marano, C.M., et al., Structural imaging in late-life depression: association with mood and cognitive responses to antidepressant treatment. The American Journal of Geriatric Psychiatry, 2015. 23(1): p. 4-12.

Marchant NL, Howard RJ. Cognitive debt and Alzheimer's disease. J Alzheimers Dis. 2015;44(3):755-770.

McCrae, R.R., Costa, J., Paul T, 2007. Brief versions of the NEO-PI-3. Journal of individual differences 28(3), 116-128.

McEvoy PM, Watson H, Watkins ER, Nathan P. The relationship between worry, rumination, and comorbidity: evidence for repetitive negative thinking as a transdiagnostic construct. J Affect Disord. 2013;151(1):313-320.
McKinney, B.C. and E. Sibille, The age-by-disease interaction hypothesis of late-life depression. The American Journal of Geriatric Psychiatry, 2013. 21(5): p. 418-432.

Meier-Ruge WI, Ulrich J, Brühlmann M, Meier E. Age-related white matter atrophy in the human brain. Annals of the New York Academy of Sciences. 1992 Dec;673(1):260-9.

Menon, V., 2015. Salience network, In: Arthur W. Toga, editor. Brain Mapping: An Encyclopedic Reference. Academic Press: Elsevier, pp. 597-611.

Merino, J. G. (2019, March). White Matter Hyperintensities on Magnetic Resonance Imaging: What Is a Clinician to Do?. In Mayo Clinic Proceedings (Vol. 94, No. 3, pp. 380-382). Elsevier.

Meucci, R.D., A.G. Fassa, and N.M. Faria, Prevalence of chronic low back pain: systematic review. Rev Saude Publica, 2015. 49.

Mizuno, A., Ly, M., Aizenstein, H.J., 2018. A Homeostatic Model of Subjective Cognitive Decline. Brain Sci 8(12).

Moons KG, Donders RA, Stijnen T, Harrell FE, Jr. Using the outcome for imputation of missing predictor values was preferred. J Clin Epidemiol. 2006;59(10):1092-1101.

Morimoto SS, Kanellopoulos D, Manning KJ, Alexopoulos GS. Diagnosis and treatment of depression and cognitive impairment in late life. Ann N Y Acad Sci 2015; 1345: 36-46.

Munce, S. E., & Stewart, D. E. (2007). Gender differences in depression and chronic pain conditions in a national epidemiologic survey. Psychosomatics, 48(5), 394-399.

Newgard CD, Haukoos JS. Advanced statistics: missing data in clinical research--part 2: multiple imputation. Acad Emerg Med. 2007;14(7):669-678.

Niendam, T.A., Laird, A.R., Ray, K.L., Dean, Y.M., Glahn, D.C., Carter, C.S., 2012. Meta-analytic evidence for a superordinate cognitive control network subserving diverse executive functions. Cogn Affect Behav Neurosci 12(2), 241-268.

Nolen-Hoeksema S. The role of rumination in depressive disorders and mixed anxiety/depressive symptoms. J Abnorm Psychol. 2000;109(3):504-511.

O'Donovan A, Hughes BM, Slavich GM, et al. Clinical anxiety, cortisol and interleukin-6: evidence for specificity in emotion-biology relationships. Brain, behavior, and immunity. 2010;24(7):1074-1077.

O'Donovan A, Slavich GM, Epel ES, Neylan TC. Exaggerated neurobiological sensitivity to threat as a mechanism linking anxiety with increased risk for diseases of aging. Neurosci Biobehav Rev. 2013;37(1):96-108.
Okereke OI, Grodstein F. Phobic anxiety and cognitive performance over 4 years among community-dwelling older women in the Nurses' Health Study. Am J Geriatr Psychiatry. 2013;21(11):1125-1134.

Okereke OI, Prescott J, Wong JY, Han J, Rexrode KM, De Vivo I. High phobic anxiety is related to lower leukocyte telomere length in women. PLoS One. 2012;7(7):e40516.

Palop, J.J., Chin, J., Roberson, E.D., Wang, J., Thwin, M.T., Bien-Ly, N., Yoo, J., Ho, K.O., Yu, G.Q., Kreitzer, A., Finkbeiner, S., Noebels, J.L., Mucke, L., 2007. Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. Neuron 55(5), 697-711.

Pardoe, H.R., et al., Structural brain changes in medically refractory focal epilepsy resemble premature brain aging. Epilepsy research, 2017. 133: p. 28-32.

Penny WD, Friston KJ, Ashburner JT, Kiebel SJ, Nichols TE. Statistical parametric mapping: the analysis of functional brain images. Elsevier; 2011.

Perna G, Iannone G, Alciati A, Caldirola D. Are Anxiety Disorders Associated with Accelerated Aging? A Focus on Neuroprogression. Neural Plast. 2016;2016:8457612.

Perrotin, A., Mormino, E.C., Madison, C.M., Hayenga, A.O., Jagust, W.J., 2012. Subjective cognition and amyloid deposition imaging: a Pittsburgh Compound B positron emission tomography study in normal elderly individuals. Arch Neurol 69(2), 223-229.

Pfefferbaum, A., Rohlfing, T., Rosenbloom, M. J., Chu, W., Colrain, I. M., & Sullivan, E. V. (2013). Variation in longitudinal trajectories of regional brain volumes of healthy men and women (ages 10 to 85 years) measured with atlas-based parcellation of MRI. Neuroimage, 65, 176-193.

Pietrzak RH, Lim YY, Neumeister A, et al. Amyloid-beta, anxiety, and cognitive decline in preclinical Alzheimer disease: a multicenter, prospective cohort study. JAMA psychiatry. 2015;72(3):284-291.

Pietrzak RH, Maruff P, Woodward M, et al. Mild worry symptoms predict decline in learning and memory in healthy older adults: a 2-year prospective cohort study. Am J Geriatr Psychiatry. 2012;20(3):266-275.

Pietrzak RH, Scott JC, Neumeister A, et al. Anxiety symptoms, cerebral amyloid burden and memory decline in healthy older adults without dementia: 3-year prospective cohort study. Br J Psychiatry. 2014;204:400-401.

Price, J.C., Klunk, W.E., Lopresti, B.J., Lu, X., Hoge, J.A., Ziolko, S.K., Holt, D.P., Meltzer, C.C., DeKosky, S.T., Mathis, C.A., 2005. Kinetic modeling of amyloid binding in humans using PET imaging and Pittsburgh Compound-B. J Cereb Blood Flow Metab 25(11), 1528-1547.

R.C., Rabin, L.A., Rami, L., Reisberg, B., Rentz, D.M., Sachdev, P.S., de la Sayette, V., Saykin, A.J., Scheltens, P., Shulman, M.B., Slavin, M.J., Sperling, R.A., Stewart, R., Uspenskaya, O., Vellas, B., Visser, P.J., Wagner, M., Subjective Cognitive Decline Initiative Working, G., 2014. A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. Alzheimers Dement 10(6), 844-852.

Reisberg, B., Shulman, M.B., Torossian, C., Leng, L., Zhu, W., 2010. Outcome over seven years of healthy adults with and without subjective cognitive impairment. Alzheimers Dement 6(1), 11-24.

Revest JM, Dupret D, Koehl M, et al. Adult hippocampal neurogenesis is involved in anxiety-related behaviors. Mol Psychiatry. 2009;14(10):959-967.

Reynolds K, Pietrzak RH, El-Gabalawy R, et al. Prevalence of psychiatric disorders in US older adults: findings from a nationally representative survey. World Psychiatr 2015;14:74–81.

Ribeiz, S.R., et al., Structural brain changes as biomarkers and outcome predictors in patients with late-life depression: a cross-sectional and prospective study. PloS one, 2013. 8(11): p. e80049.

Riddle M, Potter GG, McQuoid DR, Steffens DC, Beyer JL, Taylor WD. Longitudinal Cognitive Outcomes of Clinical Phenotypes of Late-Life Depression. Am J Geriatr Psychiatry 2017; 25(10): 1123-34.

Rodda, J.E., Dannhauser, T.M., Cutinha, D.J., Shergill, S.S., Walker, Z., 2009. Subjective cognitive impairment: increased prefrontal cortex activation compared to controls during an encoding task. Int J Geriatr Psychiatry 24(8), 865-874.

Rosario, B.L., Weissfeld, L.A., Laymon, C.M., Mathis, C.A., Klunk, W.E., Berginc, M.D., James, J.A., Hoge, J.A., Price, J.C., 2011. Inter-rater reliability of manual and automated region-of-interest delineation for PiB PET. Neuroimage 55(3), 933-941.

Sacuiu, S., et al., Chronic depressive symptomatology in mild cognitive impairment is associated with frontal atrophy rate which hastens conversion to Alzheimer dementia. The American Journal of Geriatric Psychiatry, 2016. 24(2): p. 126-135.

Scarmeas N, Levy G, Tang MX, Manly J, Stern Y. Influence of leisure activity on the incidence of Alzheimer's disease. Neurology. 2001;57(12):2236-42.

Schafer JL. Multiple imputation: a primer. Stat Methods Med Res. 1999;8(1):3-15.

Schmidt-Wilcke, T., E. Leinisch, S. Ganssbauer, B. Draganski, U. Bogdahn, J. Altmeppen, and A. May, Affective components and intensity of pain correlate with structural differences in gray matter in chronic back pain patients. Pain, 2006. 125(1-2): p. 89-97.

Schnack, H.G., et al., Accelerated brain aging in schizophrenia: a longitudinal pattern recognition study. American Journal of Psychiatry, 2016. 173(6): p. 607-616.

Schrouff, J., Rosa, M.J., Rondina, J.M., Marquand, A.F., Chu, C., Ashburner, J., Phillips, C., Richiardi, J., Mourao-Miranda, J., 2013. PRoNTo: pattern recognition for neuroimaging toolbox. Neuroinformatics 11(3), 319-337.

Schultz, W., Tremblay, L., Hollerman, J.R., 2000. Reward processing in primate orbitofrontal cortex and basal ganglia. Cereb Cortex 10(3), 272-284.

Segerstrom SC, Roach AR, Evans DR, Schipper LJ, Darville AK. The structure and health correlates of trait repetitive thought in older adults. Psychol Aging. 2010;25(3):505-515.
Seignourel P, Kunik M, Snow L, et al. Anxiety in dementia: a critical review. Clin Psychol Rev 2008;28(7):1071–82.

Sharp, E.S., Gatz, M., 2011. Relationship between education and dementia: an updated systematic review. Alzheimer Dis Assoc Disord 25(4), 289-304.

Simpson, S.W., et al., Regional cerebral volume measurements in late-life depression: relationship to clinical correlates, neuropsychological impairment and response to treatment. International journal of geriatric psychiatry, 2001. 16(5): p. 469-476.
Sivertsen, H., et al., Depression and quality of life in older persons: a review. Dementia and Geriatric Cognitive Disorders, 2015. 40(5-6): p. 311-339.

Slot, R.E.R., Verfaillie, S.C.J., Overbeek, J.M., Timmers, T., Wesselman, L.M.P., Teunissen, C.E., Dols, A., Bouwman, F.H., Prins, N.D., Barkhof, F., Lammertsma, A.A., Van Berckel, B.N.M., Scheltens, P., Sikkes, S.A.M., Van der Flier, W.M., 2018. Subjective Cognitive Impairment Cohort (SCIENCe): study design and first results. Alzheimers Res Ther 10(1), 76.

Snitz, B.E., Lopez, O.L., McDade, E., Becker, J.T., Cohen, A.D., Price, J.C., Mathis, C.A., Klunk, W.E., 2015a. Amyloid-beta Imaging in Older Adults Presenting to a Memory Clinic with Subjective Cognitive Decline: A Pilot Study. J Alzheimers Dis 48 Suppl 1, S151-159.

Snitz, B.E., Weissfeld, L.A., Cohen, A.D., Lopez, O.L., Nebes, R.D., Aizenstein, H.J., McDade, E., Price, J.C., Mathis, C.A., Klunk, W.E., 2015b. Subjective Cognitive Complaints, Personality and Brain Amyloid-beta in Cognitively Normal Older Adults. Am J Geriatr Psychiatry 23(9), 985-993.

Snitz, B.E., Yu, L., Crane, P.K., Chang, C.C., Hughes, T.F., Ganguli, M., 2012. Subjective cognitive complaints of older adults at the population level: an item response theory analysis. Alzheimer Dis Assoc Disord 26(4), 344-351.

Sperling, R.A., Aisen, P.S., Beckett, L.A., Bennett, D.A., Craft, S., Fagan, A.M., Iwatsubo, T., Jack, C.R., Jr., Kaye, J., Montine, T.J., Park, D.C., Reiman, E.M., Rowe, C.C., Siemers, E., Stern, Y., Yaffe, K., Carrillo, M.C., Thies, B., Morrison-Bogorad, M., Wagster, M.V., Phelps, C.H., 2011. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 7(3), 280-292.

Sperling, R.A., Laviolette, P.S., O'Keefe, K., O'Brien, J., Rentz, D.M., Pihlajamaki, M., Marshall, G., Hyman, B.T., Selkoe, D.J., Hedden, T., Buckner, R.L., Becker, J.A., Johnson, K.A., 2009. Amyloid deposition is associated with impaired default network function in older persons

Stern Y, Arenaza-Urquijo EM, Bartres-Faz D, et al. Whitepaper: Defining and investigating cognitive reserve, brain reserve, and brain maintenance. Alzheimers Dement 2018.

Stern Y. Cognitive reserve in ageing and Alzheimer's disease. Lancet Neurol 2012; 11(11): 1006-12.

Stern, Y., 2009. Cognitive reserve. Neuropsychologia 47(10), 2015-2028.

Stern, Y., 2012. Cognitive reserve in ageing and Alzheimer's disease. The Lancet Neurology 11(11), 1006-1012.

Taylor WD, Aizenstein HJ, Alexopoulos GS. The vascular depression hypothesis: mechanisms linking vascular disease with depression. Mol Psychiatry 2013; 18(9): 963-74.

Tully PJ, Cosh SM, Baumeister H. The anxious heart in whose mind? A systematic review and meta-regression of factors associated with anxiety disorder diagnosis, treatment and morbidity risk in coronary heart disease. J Psychosom Res. 2014;77(6):439-448.

Tully PJ, Cosh SM, Baune BT. A review of the affects of worry and generalized anxiety disorder upon cardiovascular health and coronary heart disease. Psychology, health & medicine. 2013;18(6):627-644.

Ullsperger, M., Danielmeier, C., Jocham, G., 2014. Neurophysiology of performance monitoring and adaptive behavior. Physiol Rev 94(1), 35-79.

Urry HL, Gross, JJ. Emotion Regulation in Older Age. Current Dirrections inPsychological Science. 2010;19 (6): 352-357.

Valiengo Lda C, Stella F, Forlenza OV. Mood disorders in the elderly: prevalence, functional impact, and management challenges. Neuropsychiatr Dis Treat 2016; 12: 2105-14.

van Oijen, M., de Jong, F.J., Hofman, A., Koudstaal, P.J., Breteler, M.M., 2007. Subjective memory complaints, education, and risk of Alzheimer's disease. Alzheimers Dement 3(2), 92-97.

Vemuri P, Weigand SD, Przybelski SA, Knopman DS, Smith GE, Trojanowski JQ, et al. Cognitive reserve and Alzheimer's disease biomarkers are independent determinants of cognition. Brain. 2011;134(Pt 5):1479-92.

Vernooij, M. W., de Groot, M., van der Lugt, A., Ikram, M. A., Krestin, G. P., Hofman, A., ... & Breteler, M. M. (2008). White matter atrophy and lesion formation explain the loss of structural integrity of white matter in aging. Neuroimage, 43(3), 470-477.

Vogel, J.W., Varga Dolezalova, M., La Joie, R., Marks, S.M., Schwimmer, H.D., Landau, S.M., Jagust, W.J., 2017. Subjective cognitive decline and beta-amyloid burden predict cognitive change in healthy elderly. Neurology 89(19), 2002-2009.

Wand, B.M., L. Parkitny, N.E. O'Connell, H. Luomajoki, J.H. McAuley, M. Thacker, and G.L. Moseley, Cortical changes in chronic low back pain: current state of the art and implications for clinical practice. Man Ther, 2011. 16(1): p. 15-20.

Wang HX, MacDonald SW, Dekhtyar S, Fratiglioni L. Association of lifelong exposure to cognitive reserve-enhancing factors with dementia risk: A community-based cohort study. PLoS Med. 2017;14(3): e1002251.

Wilson, R. S., Beckett, L. A., Bennett, D. A., Albert, M. S., & Evans, D. A. (1999). Change in cognitive function in older persons from a community population: relation to age and Alzheimer disease. Archives of Neurology, 56(10), 1274–1279.

Zhang X, Norton J, Carriere I, Ritchie K, Chaudieu I, Ancelin ML. Generalized anxiety in community-dwelling elderly: prevalence and clinical characteristics. J Affect Disord. 2015;172:24–9.