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Biological and Environmental Factors Impacting Risk of Cognitive Decline:Imaging ß-amyloid plaques, Tau Neurofibrillary Tangles and the 5HT1A Receptor



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# Biological and Environmental Factors Impacting Risk of Cognitive Decline: Imaging ß-amyloid plaques, Tau Neurofibrillary Tangles and the $5HT_{1A}$ Receptor

A dissertation to be submitted in partial satisfaction of the requirements for the degree

Doctor of Philosophy in Neuroscience

By

Laurel Martin-Harris

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ABSTRACT OF THE DISSERTATION

Biological and Environmental Factors Impacting Risk of Cognitive Decline:

Imaging ß-amyloid plaques, Tau Neurofibrillary Tangles and the $5HT_{1A}$ Receptor

By

Laurel Martin-Harris

Doctor of Philosophy in Neuroscience

University of California, Los Angeles, 2015

Professor Susan Y. Bookheimer, Chair

Diseases of aging, such as Alzheimer's, occur at the hands of many cumulative risk factors occurring over a lifetime. As with virtually all non-mendelian diseases, genetics and environment play essential and synergistic roles in disease development. Disentangling the relative contributions of risk factors will aide future prevention in youth to reduce an individual's risk later in life. The same risk factors may also serve as biomarkers signaling brain changes in advance of disease state. Once we have successful treatments, early identification and intervention will be the best, and perhaps only way to prevent Alzheimer's disease.

The goal of this research is to identify early cognitive and physiological changes or differences in subjects at high-risk for AD, as well as to identify pathological features that are most related to memory decline. This knowledge has strong implications for identification of both Alzheimer's disease and the optimal targets for treatment.

In chapter 1 we use the positron emission tomography (PET) ligand [$^{18}$F]FDDNP, a marker for beta-amyloid plaque and tau neurofibrillary tangle deposition, to investigate the relationship between lifestyle factors, protein deposition and severity of

memory impairment. We show that BMI and age are significant predictors of plaque and tangle deposition that correlates with memory impairment and Alzheimer's disease.

In chapter 2, we combine structural magnetic resonance imaging (MRI), cortical segmentation and the PET ligand [$^{18}$F]MPPF, which selectively binds the serotonin (5-HT) 1A receptor. We quantify hippocampal 5-$HT_{1A}$ receptor densities in older adults because the hippocampus is integral to the formation of new memories and one of the earliest brain regions to show AD-related changes. Receptor density is a proxy for neuronal loss, and can be compared to measures of hippocampal volume obtained with cortical segmentation. We find that 5-$HT_{1A}$ receptor density predicts differences in memory performance across all subjects, and is highly correlated in subjects with below-average memory performance. We also see lateralization of binding density consistent with previously suggested functional differences.

In chapter 3 we investigate the relationship between anxiety and memory loss with the $5HT_{1A}$ receptor, known to influence anxiety behavior, in regions previously shown to play a role in anxiety disorders such as the amygdala, insula and cingulate cortex. Though, surprisingly, we do not see a relationship between 5-$HT_{1A}$ receptor density and anxiety in amygdala, we do see a significant relationship between receptor density and anxiety in insula, and this relationship is also related to working memory deficits and a perceived increase in forgetfulness. Furthermore this relationship is in direct opposition in women and men. While women show a decrease in 5-$HT_{1A}$ receptor density with increased anxiety, men show the inverse. This supports previous suggestion of significant sex differences in the serotonin system, and a relationship between mood disorders such as anxiety and depression and risk of later cognitive decline.

The dissertation of Laurel Martin-Harris to be approved by

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## I. INTRODUCTION

Alzheimer's disease (AD), the most common form of dementia, is an extremely costly and devastating disease characterized by relentless deterioration in memory systems and eventually total debilitation. The population bulge of the baby boom, comprising individuals now in their 50s and 60s, will quickly cause a spike in the number of people diagnosed with AD (Doepke et al, 2007). At the same time, increasing life expectancy means a larger percentage of this same generation will live long enough to develop the disease. The Alzheimer's Association estimates the incidence rate to be 1 in every 8 people over the age of 65, with an even higher incidence for women. In the U.S, the Center for Disease Control and Prevention places the total number of Americans suffering above five million, surpassing diabetes to become the 6th leading cause of death among American adults. Because the percentage affected increases with age and the number of older adults is itself increasing, the healthcare cost of this illness is quickly becoming astronomical. In 2005 AD affected around 13% of the Medicare population, but AD related spending was responsible for 34% of Medicare spending (estimated to be $91 billion). On a personal level, the cost is equally severe. Beginning with memory loss that impairs daily function, and ultimately producing a loss of memory so complete that the individual's characteristics of self or personhood are obliterated. As the reality of this disease looms large on a personal and population level, we need a better understanding of early disease state both for early intervention, and future prevention. Ultimately any therapy will be most successful when deployed at the earliest stages, and contributing environmental factors may be some of the best targets for risk reduction while the wait for successful therapies continues.

In the early stages, mild cognitive impairment (MCI), which is typically defined as an intermediate memory deficiency between normal aging and Alzheimer's disease, may affect patients for years prior to diagnosis of AD (Morris et al 2001). MCI manifests

as a measurable impairment in cognitive function that nevertheless does not severely impair daily function (Petersen et al. 2004). It's been suggested that MCI will affect 20-30% of the population above 75. Furthermore 80% of those with amnesic MCI (MCI with memory impairment) will ultimately progress to Alzheimer's disease (Small et al. 2006). By that time the disease symptoms are severe, and neuronal loss is already extensive, making recovery more difficult no matter what future treatments become available. Consequently there is a great deal of interest in pre-clinical markers for AD. Analyses of post-mortem brain tissue (Riley et al. 2002; Morris et al. 1996), and imaging studies in at-risk subjects (Petrella et al. 2002; Smith et al. 2002), suggest that the process of brain deterioration begins many years before diagnosis. Brain imaging studies have also shown that patients exhibit cortical differences and decreases in glucose metabolism prior to diagnosis (Burggren et al. 2008; Kepe et al. 2006; Reiman et al. 2004). Identifying at-risk individuals before the onset of significant cognitive decline offers the most potential benefit for patients, who may be able to delay onset with currently available drug treatments and lifestyle changes. Maintaining as much intact brain tissue as possible ensures retention of cognitive function; likely all therapies will be dependent on very early intervention to maximize success (Bookheimer and Burggren, 2009).

In the case of neurodegenerative diseases, such as Alzheimer's, identifying early markers and risk factors is particularly challenging. These factors may be cumulative over a lifetime, and may interact with one another in unknown ways. Even the most comprehensive longitudinal studies do not follow patients for as long, or collect as many metrics as needed. There has been much recent controversy, for example, about the growing body of evidence linking traumatic brain injury (TBI) to future risk of neurodegenerative diseases. As we have come to better understand TBI, we have uncovered a number of injury related responses in the nervous system that have significant long-term impact on functioning and future risk of neurodegenerative disease

(Smith et al 2103). But precisely how severity and age of injury influence risk may be difficult to determine. What other factors may be impacting long-term risk of Alzheimer's disease? A number of lifestyle factor candidates are emerging as potential contributors. Diabetes and depression are among a few factors most consistently associated with increased AD risk (Williams et al, 2010), but evidence suggests vascular health, body mass index (BMI) and education also impact risk (Emmerzaal et al 2014). Clarifying which of these represents a true relationship that, when altered, could improve brain health and reduce risk of future disease will aide treatment and prevention (Joseph et al. 2009). Lifestyle factors may be of vital importance for ultimate management of this disease.

**Disease Hallmarks- Plaques and Tangles**

Two proteins characterize this disease neuropathologically: ß-amyloid in senile plaques and tau neurofibrillary tangles (Price et al. 1999; Braak et al. 1991), which accumulate relatively predictably in the medial temporal and frontal lobes, while accumulation in the cerebellum and motor cortex remains comparatively low. Some believe that beta-amyloid senile plaques are the primary symptom promoter in AD, and attempts have been made to develop drugs that will control formation of or clear these plaques. Evidence has been mixed, however, with reduction of plaques producing encouraging results in some cases (Leinenga & Götz, 2015) but not others (Holmes et al. 2008). Neuronal loss, which cannot be rescued, could be the reason for this discrepancy. Tau protein aggregation has also been suggested as the potential instigator of symptoms. Tau accumulates initially primarily in the hippocampal complex, while amyloid is more prominent in the frontal lobes and other areas of cortex (Frisoni et al. 2009); Precisely how these two aggregates differentially contribute to the disease remains somewhat unclear.

**Disease Hallmarks- The hippocampal complex**

Memory is typically one of the first areas of cognition to suffer with age related cognitive decline. In Alzheimer's disease memory is ultimately abolished all together. The famous patient H.M. clarified the medial temporal lobe (MTL) as the seat of declarative memory formation when he lost the ability to form new memories after a bilateral resection of the MTL to treat intractable epilepsy (Scoville et al. 1957). Today, with the advent of Magnetic Resonance Imaging (MRI) and functional MRI, the investigation has turned towards precisely which areas of the medial temporal lobe are responsible for which aspects of behavior. In the MTL, the hippocampus itself is made up of the dentate gyrus, the cornu ammonis (CA) fields 1, 2/3 and the subiculum. Its intricately coiled structure runs the length of the medial temporal lobe; the adjacent collateral sulcus, fusiform gyrus, and parahippocampal, entorhinal and perirhinal cortices together with the hippocampus make up the hippocampal complex or formation (Amaral et al. 1999). Just rostral to the head of the hippocampus sits the amygdala, and at its caudal end, the hippocampus terminates in the fornix.

While studies of patients with medial temporal lobe and hippocampal damage suggest that long-term memories are stored elsewhere, within the medial temporal lobe it is well known that different architectural components of the hippocampal complex contribute to new memory formation and retrieval uniquely (Sass et al. 1994; Rausch et al. 1993). The hippocampus itself is involved in declarative memory, which includes both semantic and episodic memory (Eichenbaum 2004). But while the hippocampus has a clear role in episodic memory (Ekstrom et al. 2007, Squire 1992), it appears that semantic memory, which refers to explicit fact separate from personal experience, requires the neighboring parahippocampal, entorhinal and perirhinal cortices (Ryan et al. 2009; Squire et al. 2004; Aggleton and Brown 1999). Recent studies using fMRI during different aspects of memory encoding and retrieval have also shown distinction between anterior and posterior activations (Zeineh et al. 2003; Fernandez et al. 1998;

Gabrieli et al. 1997). This is of particular interest because recent evidence shows subtly different patterns of memory deficit may exist in cognitively declining APOE-4 carriers versus non-carriers suggesting regional differences in disease pathology (Wolk et al 2010).

## Disease Hallmarks- Neuronal Loss

Both early and advanced AD is clearly marked by neuronal loss and atrophy in the hippocampus and entorhinal cortex, and ultimately throughout the cortex (Price et al. 2001); once significant neuronal loss has occurred recovery is made much more difficult. By the time an Alzheimer's diagnosis is made, substantial deterioration is already evident in both gray and white matter with massive structural breakdown (Bookheimer and Burggren, 2009). But it is unclear whether neuronal loss also precedes or is entirely caused by deposition of plaques and tangles.

Figure 1: Degeneration of Hippocampal Cell Types in Aging and Alzheimer's Disease (Scheibel 1979)

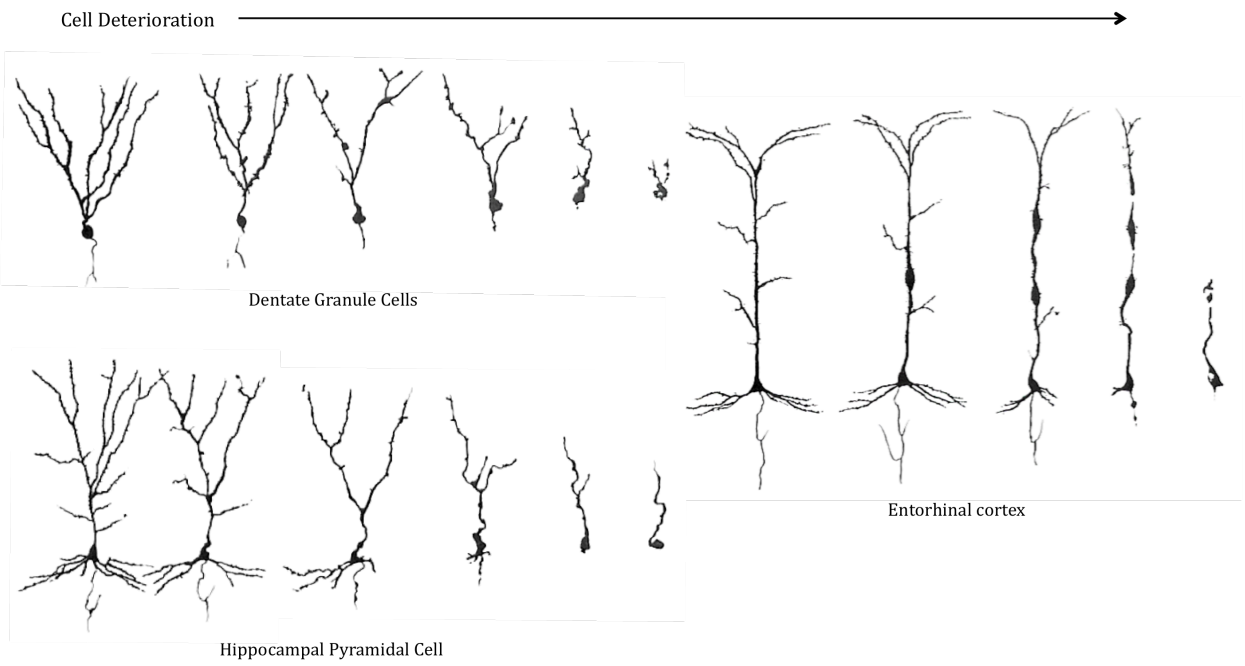
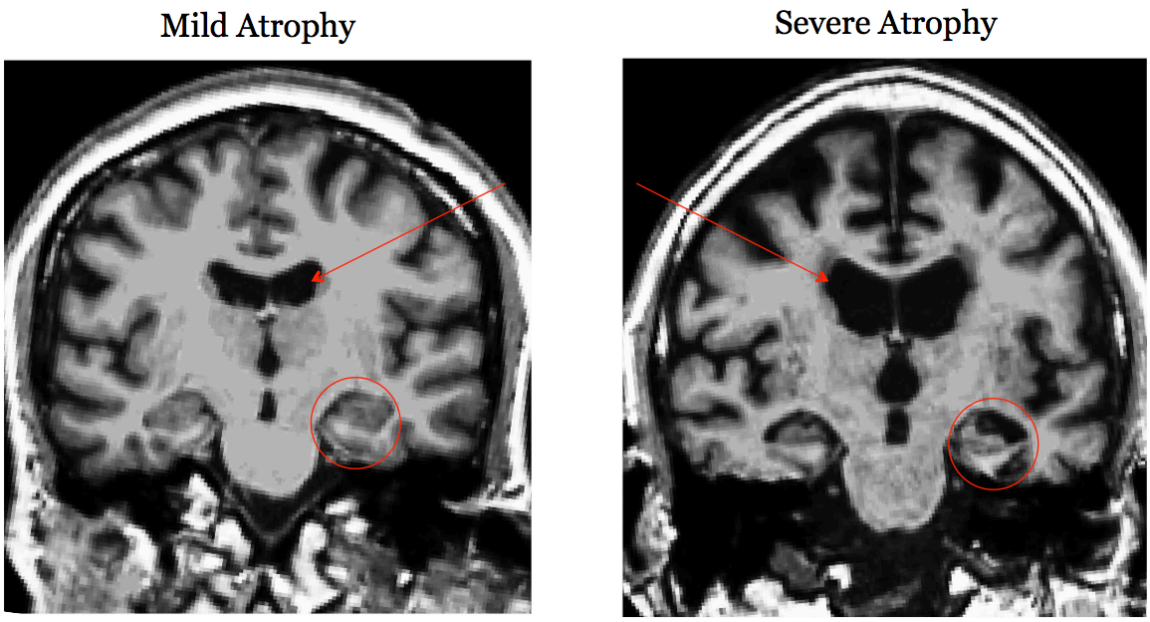


Figure 2: Overall volume loss is shown in increased size of fluid filled space; highlighted in the ventricles and hippocampus with red arrows circles respectively




## Risk Factors

Multiple potential risk factors have emerged comprising, but not limited to: pathological protein accumulation, age, genotype, family history, BMI, diabetes and cardiovascular health, diet and exercise, smoking and depression, traumatic brain injury, inflammation and overall education. Many of these factors are cumulative and interact over an individual's entire lifespan, potentially contributing to disease genesis many decades before disease onset. The relationship between variables that may act synergistically on memory, cognition and the functioning of the hippocampal complex should be carefully considered. Furthermore, lifestyle factors such as diet and exercise are known to be associated with more than one domain and, as in the case of depression and AD, new therapies that target one may also benefit the other.

## Apolipoprotein E ε4

Many studies have now shown that carriers of a lipid trafficking protein allele variant, known as apolipoprotein E ε4 (APOE-4) show specific structural and functional brain changes compared to APOE-3 carriers even in cognitively healthy individuals (Burggren et al. 2008; Ercoli et al. 2006; Reiman et al. 2004). APOE-4 has emerged as one of the most consistent risk factors associated with an increased risk of developing Alzheimer's disease (Williams et al, 2010) and therefore can be used as a factor to separate those at risk for AD from non-risk subjects. Apolipoproteins are a family of lipid trafficking proteins that regulate the metabolism and uptake of lipids throughout the body. The APOE gene is polymorphic with three variants, each of which differs by single amino acid substitutions. Roughly 55% of the population is homozygous for the APOE-3 allele while 25% carries at least one copy of the APOE-4 variant and only about 2% of the population is homozygous for the APOE-4 allele, however a single copy of the gene still confers risk (Hill et al, 2007). A number of recent studies suggest that persistent differences exist between APOE-4 carriers and non-carries even in young adults, and the variants of the APOE gene have been associated with a number of interesting cognitive outcomes, both in development and late adulthood. The APOE-4 allele may have a beneficial effect in children exposed to dysentery/high levels of intestinal bacteria, suggesting a possibly adaptive evolutionary origin (Oriá et al 2010; Finch 2009). Interestingly, recent evidence also suggests that certain life-long differences in brain anatomy and metabolism exist in APOE-4 carriers, including a thinner entorhinal cortex, which acts as a relay station for information coming into, or out of the hippocampus, and reduced glucose metabolism in the frontal/prefrontal cortices (Donix et al 2010; Suthana et al 2010; Shaw et al 2007; Reiman et al 2004). Though APOE-4 is one of the most robustly associated risk factors for AD, it is an imperfect predictor and slightly less than half of APOE-4 heterozygotes go on to develop AD (Williams et al, 2010). APOE-4 can

nevertheless be used as a factor to separate those at increased risk for AD (Hill et al, 2007).

Though the precise causes of Alzheimer's disease are not fully understood, a uniting principle has emerged from the history of Alzheimer's investigation: by the time cognitive symptoms become apparent in Alzheimer's disease the degree of neuronal loss is already severe enough to impair the chance of recovery. Imaging will play a vital role both in detecting the beginnings of brain changes associated with cognitive decline, and in disentangling the intricate web of lifetime variables, so that treatment and prevention can be optimized.

## II. CHAPTER ONE: PET and MRI Investigations of Risk Factors Associated with Amyloid and Tau Deposition in Alzheimer's disease


### Abstract

Understanding the relationship between risk factors, protein accumulation and cognitive symptoms is fundamental to diagnosis and ultimately prevention. Here we use a position emission tomography (PET) ligand [$^{18}$F]FDDNP to quantify amyloid plaque, and tau neurofibrillary tangle deposition in a large sample of older adults at risk for Alzheimer's disease. Subjects also received APOE genotyping and extensive cognitive assessment. In a multiple linear regression, investigating the relative contribution of genotype, age, gender, BMI, memory performance and anxiety/depression level, age and BMI emerge as significant predictors of plaque and tangle load. [$^{18}$F]FDDNP also shows a significant binding increase in subjects classified as AD as compared to cognitively healthy controls. We were not able to replicate previous results differentiating groups into all three diagnostic groups- control, MCI and AD- and we suspect this is due to a high degree of reference region sensitivity, and low overall signal to noise ratio for this ligand.

## Introduction

Protein Accumulation in Alzheimer's disease, while not necessarily the only cause of cell death, is certainly a contributing factor. ß-amyloid primarily accumulates in extra cellular space as senile plaques (APs) (Price et al. 1999), while tau neurofibrillary tangles (NFTs) accumulate inside the neuron (Braak et al. 1991). Why accumulation follows a relatively stereotyped pattern is not known. Unfortunately the areas of high accumulation in early stages, namely the medial temporal and frontal lobes, are also areas associated with memory, emotional regulation, and complex cognition. Thus disruptions of the 'self' occur very early in disease.

We imaged the accumulation of these proteins in cognitively healthy, MCI and AD subjects using the molecular probe for Positron Emission Tomography (PET) 2-(1-{6-[(2-[F-18]fluoroethl)(methyl)amino]-2-naphthyl}ethylidene)malononitrile or [$^{18}$F]FDDNP (Small et al. 2006). [$^{18}$F]FDDNP functions as an in vivo chemical marker, sometimes called a biomarker (Barrio et al. 1999), and initial studies with this probe have suggested [$^{18}$F]FDDNP can be used to differentiate cognitively normal, MCI and AD subjects (Small et al. 2008). In full cortical with 4D animation [$^{18}$F]FDDNP accumulation pattern over time also tracks with Braak and Braak staging (Braskie et al. 2010).

While several markers for amyloid, such as Pittsburgh compound B (PIB), have been used as diagnostic tools for MCI and AD (Frisoni et al. 2009; Vitali et al. 2008), postmortem [$^{18}$F]FDDNP analysis suggests the added benefit of measuring both amyloid and tau (Smid et al. 2006; Agdeppa et al. 2001). If possible, measuring both protein deposits, which represent the extracellular and intracellular insults to the cell, is the ideal. [$^{18}$F]FDDNP is currently the only PET ligand available that can label both protein deposits in vivo. Several markers for tau are also in development, and ultimately

differential binding patterns of [$^{18}$F]FDDNP, PIB and these tau markers may be used to infer more about the relationship between amyloid and tau.

In addition to differentiating diagnostic groups, Lavretsky et al (2009) demonstrated that increased [$^{18}$F]FDDNP binding in the medial temporal lobe was significantly correlated with increased depression and anxiety traits in cognitively normal older adults without a history of major depression or anxiety disorder. This suggests either that depression may result from very early pathological disruption, or alternatively, that the physiological repercussions of depression contribute to, or create a susceptibility for the pathological processes associated with AD. In addition genetic risk factors known to be associated with increased risk of Alzheimer's disease, specifically the Apolipoprotien E ε-4 allele, may interact with depression. Structural HC differences associated with APOE4 carriers may act synergistically with HC changes seen in depression/anxiety to produce a hugely increased risk of developing AD. In findings published by Irie et al (2008) from the Honolulu-Asia aging study, depressed men who were not carriers of the APOE-4 allele had a 1.6-fold increased risk of developing dementia, while in depressed men who were APOE-4 carriers there was a 7.1-fold increased risk, which is considerably greater than that accounted for by the genetic risk alone. Taken together these findings strongly suggest a convergence of multiple factors.

Body mass index (BMI) also shows convergence with diabetes and hypertension. Overweight and obese people show substantial atrophy in frontal lobes, hippocampus and anterior cingulate, even when they remain cognitively healthy for at least 5 years post-scan (Raji et al 2010). At the same time even moderate physical activity improves gray matter maintenance, and the degree of physical activity predicted gray matter volume in a 9-year follow up longitudinal study (Erickson et al. 2010).

We hypothesized that [$^{18}$F]FDDNP binding in the medial temporal lobe (MTL) would correlate with cognitive status, and memory. Furthermore we expected that

binding would show a significant relationship with both BMI and depression measures, suggesting an increase in AP and NFT deposition in subjects with higher BMI and depression rating, even in cognitively normal subjects.

## Materials and Methods

*Participants*

Our subject population consists of 64 men and women in varying degrees of cognitive health. For this study we recruited cognitively normal, MCI, and Alzheimer's disease subjects through an ongoing collaboration, via an ongoing longitudinal research study. Subjects were recruited from 2002-2009 through the UCLA Center on Aging, directed by Dr. Gary Small. Though the majority of subjects were over the age of 40, we have one 28-year-old and one 38-year-old making our range 28-88 years of age (Mean= 64 +/- 12). Women make up just over half of our sample (34).

All subjects received an extensive clinical assessment and neuropsychiatric testing. Our detailed cognitive battery, lasting approximately 3 hrs, was administered at the UCLA Center on Aging by Dr. Gary Small and colleagues. Positron emission tomography (PET) and magnetic resonance imaging (MRI) scans were always acquired within 3 months of clinical and cognitive assessments to insure that diagnosis was consistent at the time of acquisition. We included tests across a variety of cognitive and psychological domains in order to capture behavioral performance relevant to our research questions. We assessed Symptoms of depression and Anxiety as follows: Geriatric Depression Scale (GDS, self-report)(McGivney et al 1994; Yesavage et al 1982-1983), Profile of Mood States (POMS) (McNair et al 1971), Hamilton Depression and Anxiety Inventory (HamA, HamD)(Hamilton 1959; Hamilton 1960), and State-Trait Anxiety Inventory (STAI) (Spielberger et al 1970). In domains of intelligence and literacy we administered subtests of the Wechsler Adult Intelligence Scale—Third Edition (WAIS-III), and the Wechsler Test of Adult Reading (WTAR) (Wechsler 2001). In some

cases we also tested literacy with the North American Adult Reading Test (NAART) (Uttl, 2002). The Wisconsin Card Sorting Task (Berg 1948), Word Fluency (Cauthern 1978), Boston Naming Test (Goodglass et al. 1972) and Trails (Spreen et al. 1965) provided tests of executive function and language fluency. Digits Forward and Backward (Wechsler 2001), Benton Visual Retention test (Benton 1974), Reye-Osterrith Figure (Rey 1964), California Verbal Learning Test (Delis et al. 1987), WMS-III Logical Memory and Verbal Paired Associates learning test (Wechsler 1997), and Buschke-Fuld Selective Reminding Task (Buschke et al. 1974) gave us measures of working memory, short-term visual and verbal memory and delayed memory. Subtests of the Buschke-Fuld Selective Reminding Task in particular are extremely sensitive even to subtle differences in memory performance.

We administered a number of self-report questionnaires including the memory complaints self-report questionnaire (Gilewski et al. 1990), and we also gave the Neuropsychiatric Inventory (Cummings et al. 1994) and Mini Mental State Exam (Bleecker et al. 1988) as diagnostic inventories. Finally we collected a Family History Questionnaire (Breitner et al. 1984) and a blood sample for APOE genotyping. The laboratory of Dr. Daniel Geschwind performed all genotyping.

*PET Scan Acquisition*

Positron emission tomography (PET) scanning was performed for [$^{18}$F]FDDNP in independent sessions on an ECAT 7 PET Scanner, and patient exposure remained well below the mandated maximum annual. The specific activity of the prepared [$^{18}$F]FDDNP for injection was 37 gigabecquerel (GBq)/ $\mu$ mol or greater. With subjects supine, a 320–550 MBq bolus was injected into intravenous catheter inserted prior to the scan. dynamic scans were subsequently collected for the two-hour period following injection. After the initial scan, the dynamic image time-series is reconstructed correcting for deep-tissue attenuation, including scatter and attenuation of signal (signal drop-off), using the

normalization scan taken prior to the scanning session and a 5.5 mm full-width at half-maximum Hann filtered back-projection. Images with either 47 or 63 contiguous slices resulted, with a plane separation of 2.42mm in both cases (Small et al. 2006; Shoghi-Jadid et al. 2002).

*PET Scan Analysis*

Once scans are acquired, they are converted via Logan graphical analysis into parametric images (DVR) to normalize within-scan and allow for between-scan comparisons (Logan et al. 1996). This was accomplished by manually selecting a portion of cerebellar grey matter as a reference region, as this is a brain structure relatively spared by AD and therefore typically associated with the lowest binding levels. The cerebellum can then be used as a "zero" with which to normalize the within-scan binding values for time points from 60-125 minutes using the clinical application program (CAPP). The Linear slope of this Logan plot then represents the relative distribution volume, or the distribution volume of the tracer in a region of interest divided by the reference region. The relative distribution volume allows us to generate the parametric image (DVR) from which we can collect binding values from selected regions of interest (Small et al. 2006). Some suggestion has been made that the cerebellum is not entirely spared from plaque and tangle deposition in AD. Even if cerebellar gray matter does exhibit some low level of ligand binding in AD, however, this is likely to be insignificant in pre-AD and MCI subjects. Furthermore, in advanced AD this would not disable analysis, but rather slightly decrease binding values in advanced AD subjects making binding estimates more conservative.

Binding values are a direct measure of the amount of ligand, [$^{18}$F]FDDNP, present in the brain. Average binding values were collected from representative regions of interest (ROIs) for 10 brain areas: the posterior cingulate gyrus, the thalamus and striatum, the parietal lobe, the frontal and prefrontal areas of the frontal lobe, the

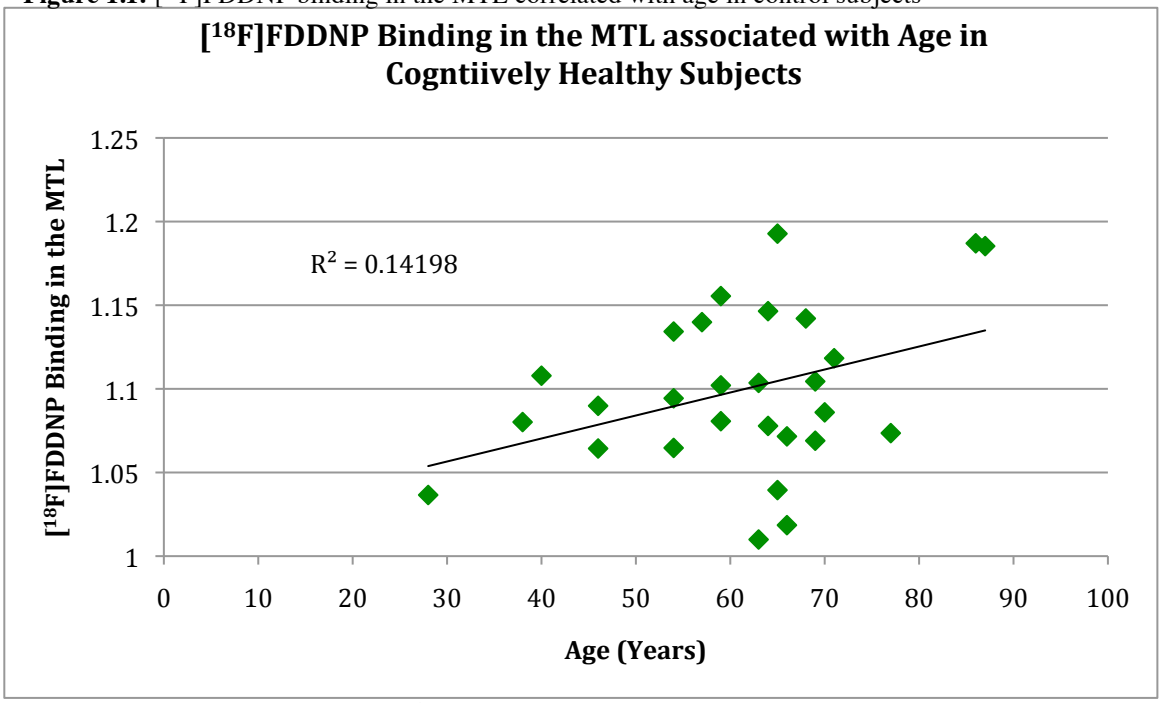
occipital lobe, and the medial and lateral portions of the temporal lobe. ROIs are selected for both the left and right brain. These ROIs are then applied to the normalized brain scans from which binding values can be taken using the clinical application program (CAPP).

Experimenters were blind to diagnosis at all times during analysis. Brain regions were manually selected using anatomical regions of interest as a guide. All scans were analyzed in identical fashion.

## Results

Because of the range in age in our control group, we had a unique opportunity to look at age associated binding in our cognitively healthy population. We found a significant correlation between age and [18F]FDDNP binding in the Medial Temporal Lobe of all subjects ($p < 0.0005$), as well as subgroups (see figures 1 and 2).

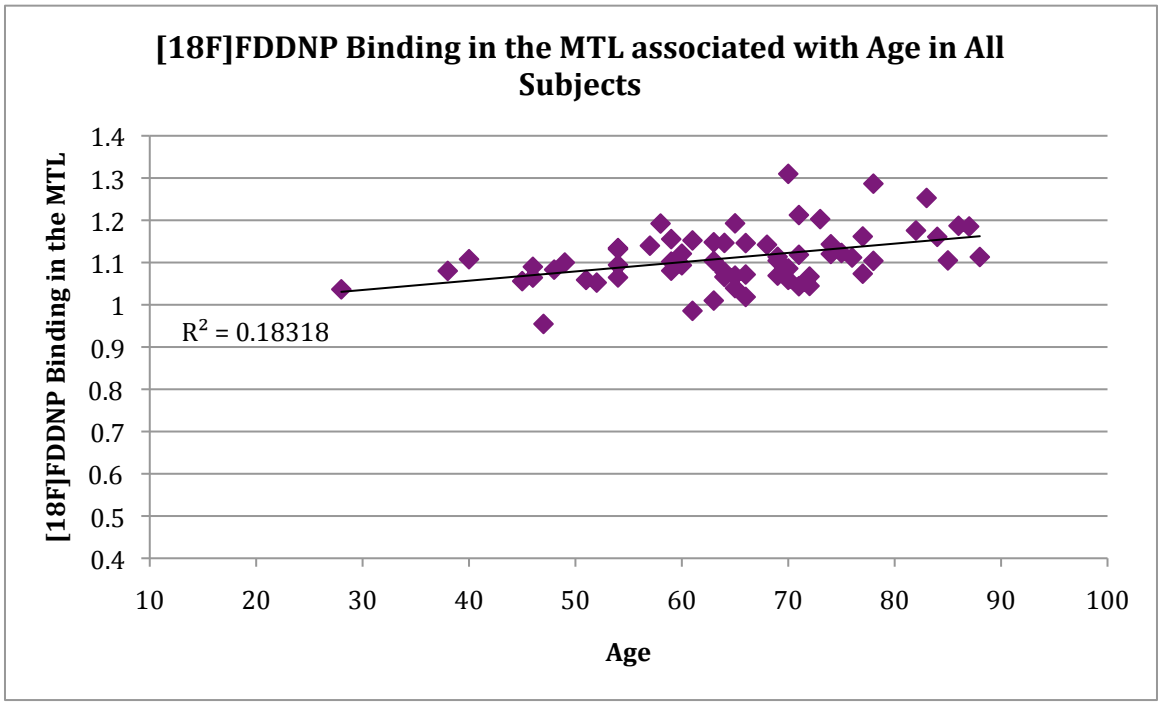
**Figure 1.1:** [18F]FDDNP binding in the MTL correlated with age in control subjects




We modeled the relative contribution of several variables to prediction of MTL [18F]FDDNP binding in a multiple linear regression. Included in the model was clinical diagnosis as a factor (Control, MCI, AD), age, standard score Wechsler Test of Adult

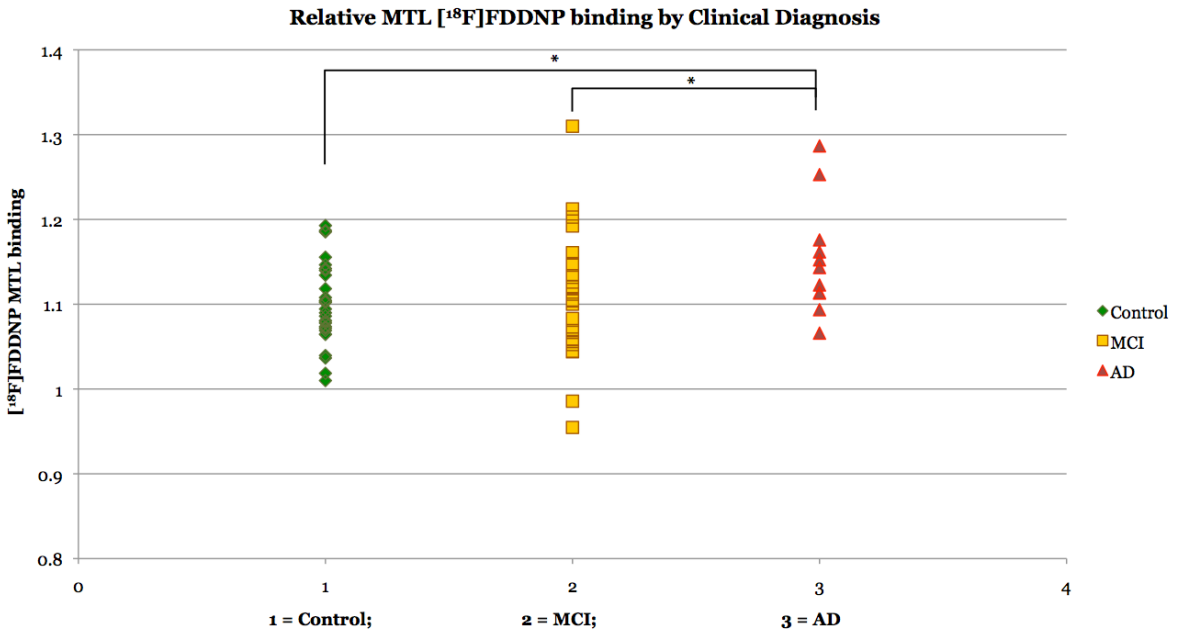
Reading (WTAR) as a verbal IQ proxy for quality/years of education, sex, composite depression Z score (HamD and GDS) and composite anxiety Z score (HamA, Trait Anxiety, and Tension POMS), Weschler Total Logical Memory (LM Total) scores as a proxy for memory, and APOE variant.

**Figure 1.2:** [$^{18}$F]FDDNP binding in the MTL correlated with age in all subjects




Even in the context of multiple variables we saw a highly significant relationship with age ($p < 0.004$), and a significant relationship with performance on the LM total ($p < 0.04$), We also saw a significant increase in binding associated with our AD group ($p < 0.02$), which may in part be attributed to increased age of that diagnostic group (see Table 1.1 and Figure 1.4). The regression model in its entirety was also significant ($p < 0.03$). We did not replicate the significant difference between our MCI and control groups found previously (Small et al 2008), but there was a significantly increased variance in our MCI compared to control group (see Figure 1.3).

**Figure 1.3:** [$^{18}$F]FDDNP by diagnostic group




| Table 1.1 | | | | |
|---|---|---|---|---|
| lm(formula = Medial.Temporal.Lobe.FDDNP ~ as.factor(DX.Short) + Age + SS.WTAR + Sex + DepressionZ + Total.LM + AnxietyZ + APOE, data = data) | | | | |
| | | | | |
| Residuals: | | | | |
| Min | 1Q | Median | 3Q | Max |
| -0.116458 | -0.04301 | 0.003136 | 0.036592 | 0.130049 |
| | | | | |
| Coefficients: | | | | |
| | Estimate | Std. Error | t value | Pr(>\|t\|) |
| (Intercept) | 0.9415597 | 0.1126787 | 8.356 | |
| as.factor(MCI) | 0.0255308 | 0.0208838 | 1.223 | 0.22702 |
| as.factor(AD) | 0.0965419 | 0.0390984 | 2.469 | 0.01686 * |
| Age | 0.0020335 | 0.0006597 | 3.082 | 0.00328 ** |
| SS.WTAR | -0.001186 | 0.0010484 | -1.131 | 0.26317 |
| Sex | 0.009505 | 0.0164045 | 0.579 | 0.56481 |
| DepressionZ | -0.0081402 | 0.013703 | -0.594 | 0.55506 |
| Total.LM | 0.002048 | 0.0009719 | 2.107 | 0.03995 * |
| AnxietyZ | 0.0013334 | 0.0043356 | 0.308 | 0.75965 |
| APOE | 0.0192344 | 0.0131439 | 1.463 | 0.14938 |
| | | | | |
| Residual standard error: 0.05973 on 52 degrees of freedom | | | | |
| Multiple R-squared: 0.2949, Adjusted R-squared: 0.1729 | | | | |
| F-statistic: 2.417 on 9 and 52 DF, p-value: 0.02247 * | | | | |

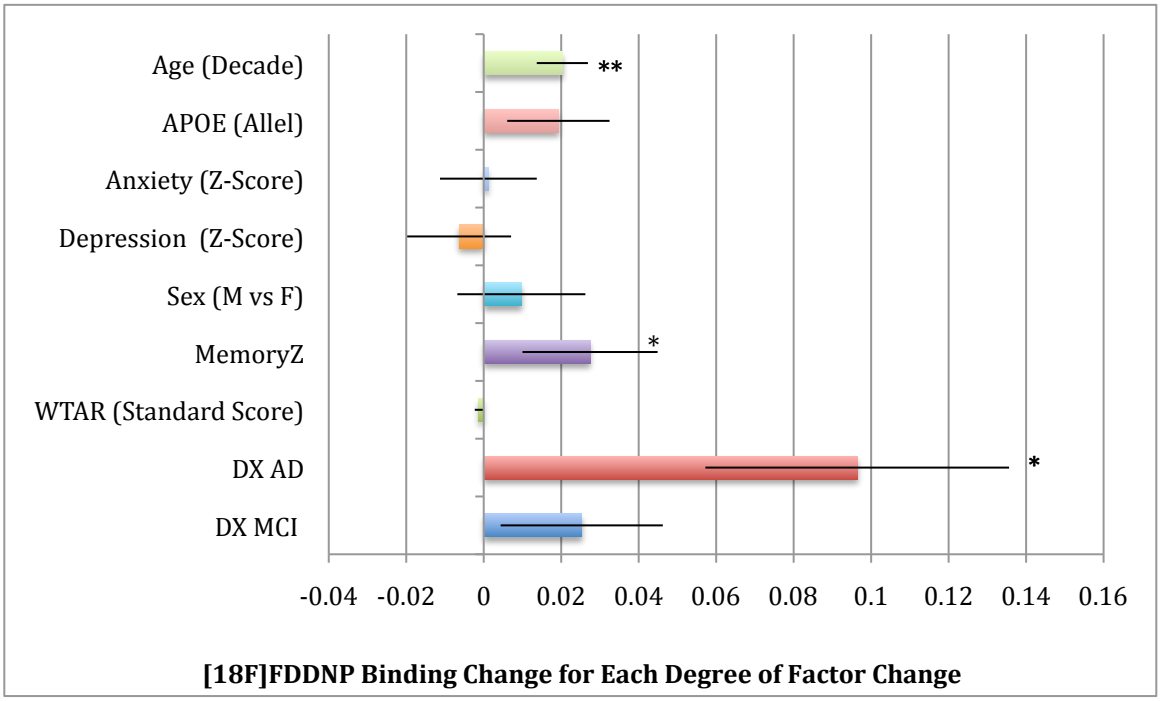



**Figure 1.4:** multiple linear regression model of relative predictive contribution to [$^{18}$F]FDDNP binding in all subjects.

In approximately three fourths of subjects we also had height and weight measurements with which to calculate BMI. In this smaller subset of subjects (48) we were able to add BMI to our regression model. In this case age and BMI are both significant predictors of MTL [$^{18}$F]FDDNP binding. MTL [$^{18}$F]FDDNP binding is significantly correlated with both age and BMI ($p < 0.0005$, and $p < 0.008$ respectively). Overall BMI adds predictive value to the model, and the magnitude of [$^{18}$F]FDDNP signal increase for every 10-point increase in BMI is greater than the increase associated with diagnostic group.

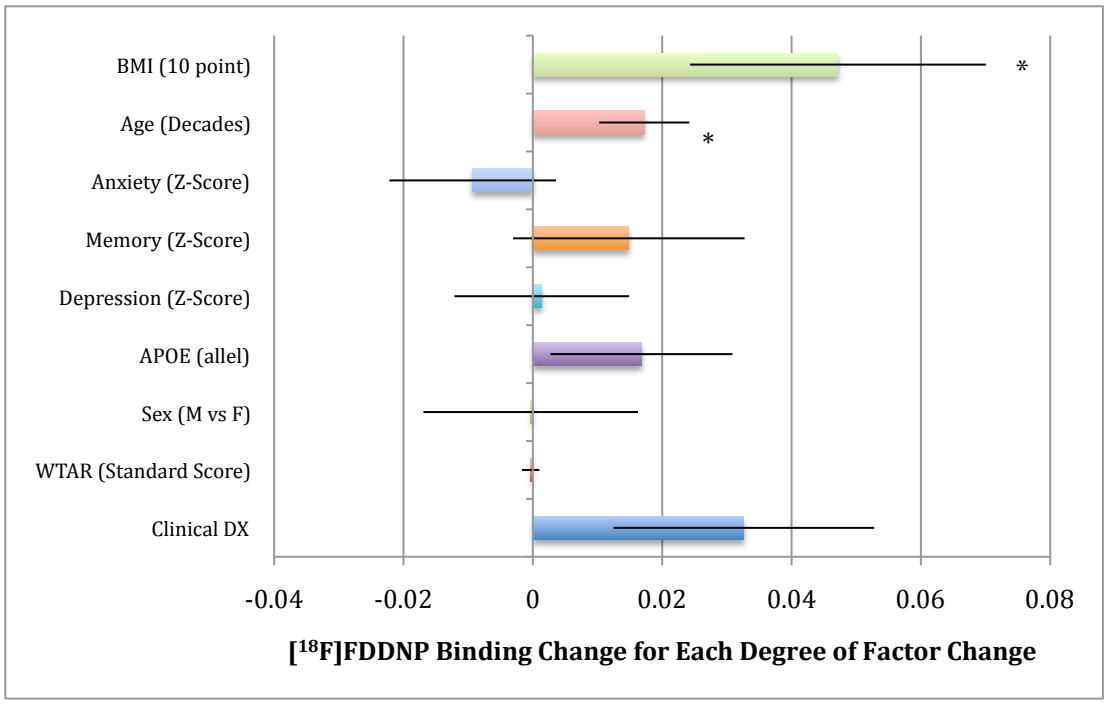



**Figure 1.5**: multiple linear regression model of relative predictive contribution to [$^{18}$F]FDDNP binding in subset with BMI.

## Discussion

Our AD subjects show significantly increased [$^{18}$F]FDDNP binding over both control and MCI subjects. We did not, however see a significant difference between binding on control and MCI groups, and it is unclear why we were not able to replicate the results previously described by our collaborators. Both our MCI and AD groups showed increased variance compared to controls, and the over-all low signal-to-noise ratio for this ligand may be a contributing factor to the discrepancy in findings. Background binding is high relative to signal, making subtle differences in reference region placement likely to produce higher scan-to-scan variance and lower inter-rater reliability. Thus subtle differences in methodology could account for the differences in variance. The overall the magnitude of effect is small enough that reliably overcoming differences in analysis method may not be possible. This burden of individual precision

means the training demands for investigators performing ligand analysis are extremely high, making its application as a diagnostic limited. Regardless, the strongest measurable effects are in the more advanced disease state. We do see a significant relationship with age in our sample in our overall sample as well as in our cognitively healthy controls. [$^{18}$F]FDDNP binding could be used in conjunction with other measures to give a general estimate of overall plaque and tangle load in younger individuals. BMI is also a significant predictor of plaque and tangle load measured with [$^{18}$F]FDDNP. BMI, Diabetes and poor vascular health, is known to negatively impact brain health overall. The upregulated inflammatory response may increase cellular susceptibility to plaque and tangle insults. Focusing on ligands with greater binding specificity is an important next-step to further clarifying these findings.

## III. CHAPTER TWO: Hippocampal Neuronal Loss and Memory, a combined imaging analysis


### Abstract

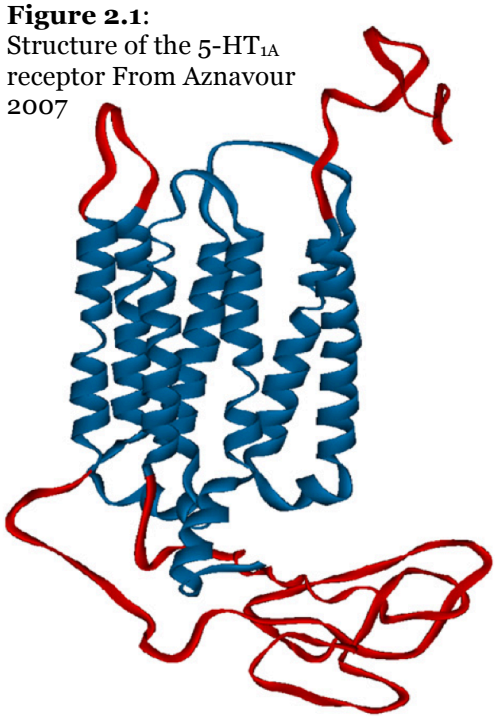
Hippocampal integrity is an extremely sensitive anatomical marker of early age related, and often AD specific, decline. As plaque and tangle load increases cellular function is impaired both inside and outside the cell. Cells first lose dendritic arbors and axonal branches, and eventually die. In addition to these cellular insults there may be fundamental cellular differences in people at risk for Alzheimer's disease, but the precise relationship between risk factors and physiology is not yet known. We used [$^{18}$F]MPPF, a receptor antagonist for the Serotonin (5-HT) 1A receptor, to investigate the relationship between risk factors, hippocampal integrity and memory performance in older adults at risk for AD. Because in the CNS 5-$HT_{1A}$ receptors are expressed heavily and primarily in the hippocampus, quantification of receptor density offers a more sensitive metric of cellular integrity than hippocampal thickness or volume alone. This receptor subtype specifically is also implicated in memory performance. Since loss of dendritic arbors density and reductions in receptor expression likely precede cell death, we hypothesized that this ligand could be used biomarker for very early changes associated with subtle memory decline. We found hippocampal and medial temporal [$^{18}$F]MPPF to be highly associated with performance on a number of memory metrics, and to add predictive value beyond hippocampal volume to a model of memory performance. We also found stronger association with memory performance in left compared to right hippocampus, a finding that is consistent with previous demonstrated functional differences.

## Introduction

5-hydroxytryptamine (5-HT), or serotonin, is well known to be involved in the regulation of many mammalian behaviors. Essential function of serotonin includes modulation of the sleep/wake cycle, appetite, mood, neurogenesis and in particular memory. Although initially disruptions of the cholinergic system, described in the 1970s, were an early focus for Alzheimer's disease research (Fibiger, 1991), the role of serotonin in disease genesis has been increasingly acknowledged as essential in subsequent decades (Rodriguez et al. 2012). The serotonin receptor family is currently divided into seven separate groupings (5-$HT_{1-7}$), each of which contains multiple subtypes. Of these varieties, only one group (5-$HT_3$) contains ligand-gated ion channels, and all the remaining groups are G-protein-coupled receptors (GPCRs). The majority of the metabotropic serotonin receptor families are excitatory, and only the $5HT_1$ and $5HT_5$ groups are primarily coupled to inhibitory G-proteins ($G_i$) (Hoyer et al. 2002).

The 5-HT1A receptor subtype (see Figure 1) was the first to be cloned, and is the most widely distributed of the serotonin receptors, though in the CNS the highest density expression is in the hippocampus, amygdala and raphae nuclei (Pytliak et al, 2011; Passchier et al 2000; Marazziti et al 1994).

**Figure 2.1**:
Structure of the 5-$HT_{1A}$ receptor From Aznavour 2007



Pyramidal neurons in the hippocampus have high levels of 5-$HT_{1A}$ receptor expressed on cell bodies (Van Bogaert et al. 2001). Because the hippocampus is the earliest area affected by Alzheimer's disease, and because this

receptor is so selectively isolated to this region we measured receptor density in older cognitively normal and mildly cognitively impaired adults at risk for AD. We chose to measure receptor density with the 5-$HT_{1A}$ receptor antagonist 4-[F-18]fluoro-N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2pyridinyl) benzamide, also known as [$^{18}$F]MPPF (see figure 2). [$^{18}$F]MPPF is a selective molecular imaging probe that permits the quantification of 5-$HT^{1A}$ receptor densities in the human brain with positron emission tomography (PET) (Passchier et al. 2001). Labeling in animal studies has been found to correspond extremely well to the distribution of 5-$HT_{1A}$ receptors, with highest binding in the hippocampus and dorsal raphe nuclei of the brainstem, moderate binding in other medial temporal and some frontal structures, and lowest binding in the cerebellum (Plenevaux et al. 2000; Le Bars et al. 1998; Shiue et al. 1997) (See figure 2.2). Increased atrophy associated with cognitive decline should follow a similar pattern, and [$^{18}$F]MPPF has been demonstrated as a marker for neuronal loss within the hippocampus in temporal lobe epilepsy patients (Merlet et al. 2004).

We postulated that 5-$HT_{1A}$ receptor density, as a proxy for cell health/density in the hippocampus and medial temporal structures, would correlate highly with memory performance across multiple domains, including immediate, delayed and verbal memory. We also expected that this same decrease in receptor density would be associated with hippocampal volume loss, even after correcting for partial volume effects. Thus we combined structural MRI with PET for the 5-$HT_{1A}$ receptor antagonist [$^{18}$F]MPPF.

[$^{18}$F]MPPF is a 5-$HT_{1A}$ antagonist, and some debate has emerged the efficacy of an antagonist versus an agonist quantifier (Becker et al. 2014). An agonist ligand might provide additional specificity by binding to the 5-$HT_{1A}$ receptor only when in the high affinity (active) state while the antagonist ligand will also bind the low affinity (inactive) state,. Even when ligand binding occurs in the inactive state, however, overall binding

potential will reflect overall receptor density. Ultimately receptor density decreases with pathological changes that occur in the hippocampus, most likely reflecting direct result of dendritic and neuronal loss. In other words, once tissue has died and receptors are no longer present in either active or inactive state overall binding will be lower. Previous studies suggest, then, that decreases in hippocampal pyramidal cell numbers can be selectively measured with PET using [$^{18}$F]MPPF as a marker.

Finally, there are likely early differences in receptor expression that ultimately lead to disease susceptibility or lower overall memory performance even in healthy subjects. The 5-HT 1A receptor is implicated in memory performance in general because this receptor subtype is highly involved in the regulation of several neurotransmitter systems, including serotonin itself (Ögren et al. 2008). We therefore expected to see measurable differential binding patterns in subjects with poor memory performance, and in particular an association with APOE 4 carriers, who are at high risk for AD. Though the spatial resolution of PET prevents an investigation of brain structures at the level of subfields of the hippocampus we can see lateral differences between left and right, and recent findings have suggested that differences in laterality exist in healthy subjects and may also be one of the features associated with APOE-4 carriers. For example, Donix et al (2013) found that cognitively normal APOE-4 carriers showed asymmetry in the entorhinal cortex, with thinner cortex in the left compared to right hemisphere. Non-carriers only showed this asymmetry in the AD patient group, and not in cognitively normal subjects (Donix et al. 2013). Similarly better memory for object-location associations corresponds with higher 5-$HT_{1A}$ receptor density in left compared to right hippocampus (Glikmann et al. 2015). We therefore expected to see laterality differences associated with memory performance in our own subject population.

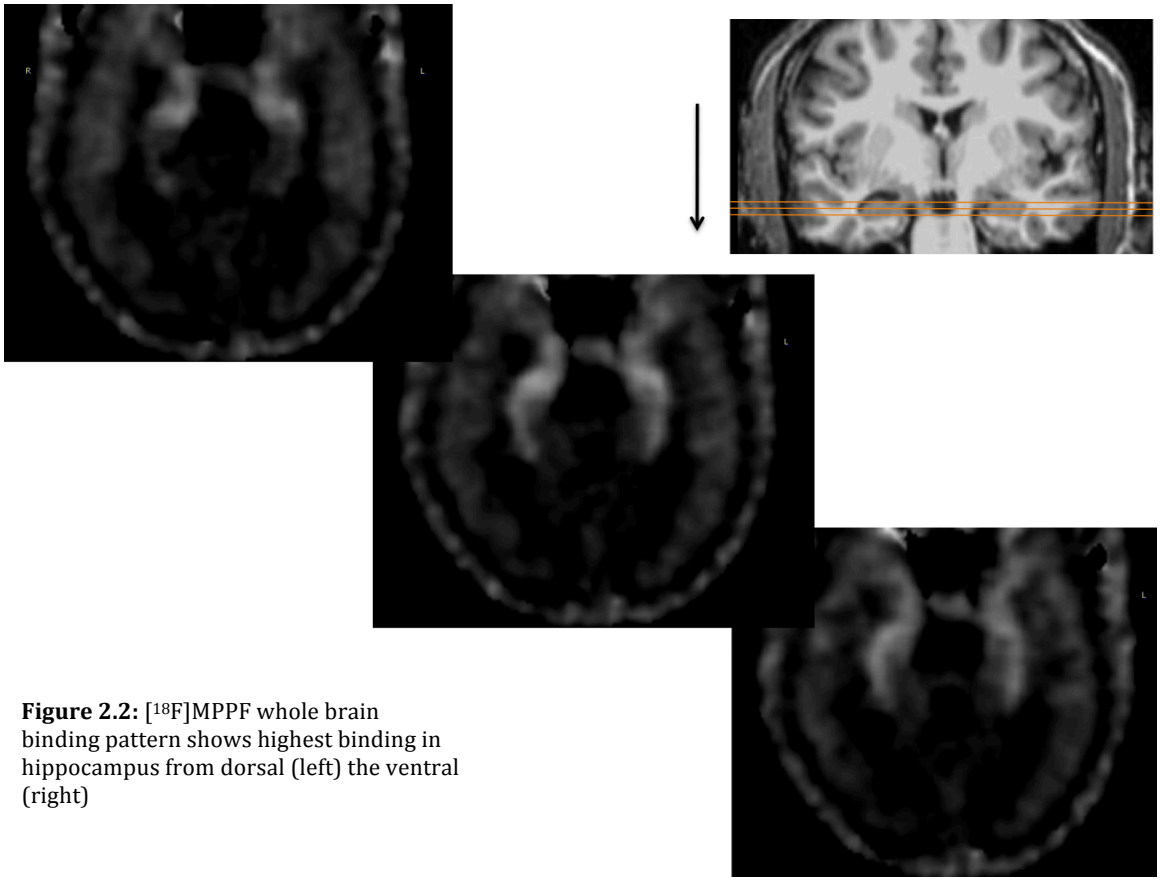


**Figure 2.2:** [$^{18}$F]MPPF whole brain binding pattern shows highest binding in hippocampus from dorsal (left) the ventral (right)

## Materials and Methods

*Participants*

Our subject population consists of healthy older adults, aged 47 and above. Subjects with clinical dementia were excluded. We also excluded subjects with current clinically significant depression and anxiety, though they may have had a history. Active severe depression/anxiety may have confounding cognitive deficits; similarly, demented subjects may appear depressed as a secondary symptom of the dementia itself both neurologically or as a psychological consequence.

Subjects were recruited from 2005-2011 through the UCLA Center on Aging, directed by Dr. Gary Small. Through an ongoing collaboration, subjects were recruited via two ongoing longitudinal research studies.

Inclusion/exclusion criterion: 1) age 50-80 2) no active clinical depression or anxiety disorder (scores above 24 on Hamilton Anxiety/Depression Rating Scale) 3) no treatment for depression (such as SSRIs), as this would confound our PET measure of the 5-$HT_{1A}$ receptor 4) no current dementia (MMSE 25 and above) 5) no other neurological disorders or history of head injury 6) no significant cerebrovascular disease 7) no metallic implants that would prevent magnetic resonance scanning 8) no major psychiatric disorders such as schizophrenia 9) no current or history of drug or alcohol abuse.

All subjects were genotyped for APOE, and subjects also received extensive screening with diagnostics including a neurological exam, neuropsychiatric and neuropsychological evaluation.

The laboratory of Dr. Small oversaw clinical and neuropsychiatric testing. The roughly 3-hour cognitive assessment was always scheduled to occur within three months of PET and MRI scans so that cognitive diagnosis is accurate for the time period in which scans were collected. Symptoms of depression and Anxiety were measured with the following: Hamilton Depression and Anxiety Inventory (HamA, HamD)(Hamilton 1959; Hamilton 1960), Profile of Mood States (POMS) (McNair et al 1971), State-Trait Anxiety Inventory (STAI) (Spielberger et al 1970), and for self report the Geriatric Depression Scale (GDS)(Yesavage et al 1982-1983; McGivney et al 1994). Additional cognitive assessment includes tests in the following domains: Intelligence: Wechsler Adult Intelligence Scale—Third Edition (WAIS-III) (Wechsler 2001); Literacy: Wechsler Test of Adult Reading (WTAR)(Wechsler 2001), and in small number of cases the North American Adult Reading Test (NAART) (Uttl, 2002); Executive Function: Wisconsin Card Sorting Task (Berg 1948), Trails (Spreen et al. 1965), Attention: Digits Forward and Backward (Wechsler 2001); Language: Boston Naming Test (Goodglass et al. 1972), Word Fluency (Cauthern 1978); and Memory: Buschke-Fuld Selective Reminding Task

(Buschke et al. 1974), California Verbal Learning Test (Delis et al. 1987), WMS-III Logical Memory and Verbal Paired Associates learning test (Wechsler 1997), Benton Visual Retention test (Benton 1974), Reye-Osterrith Figure (Rey 1964).

Subjects were also given a Family History Questionnaire (Breitner et al. 1984), memory complaints self-report questionnaire (Gilewski et al. 1990), Neuropsychiatric Inventory (Cummings et al. 1994) and Mini Mental State Exam (Bleecker et al. 1988). These tests are combined into domain scores for executive function, verbal memory (immediate and delayed), non-verbal memory (immediate and delayed), and processing speed, based on age-scaled scores, converted into Z scores which are then averaged to obtain a domain-wide Z score for each participant. This approach minimizes multiple comparisons and attenuates the effects of single outliers on individual tests.

*MRI and PET Scan Acquisition*

In addition to the cognitive and clinical assessments described above, subjects received both and MRI and PET scan. MRI scanning was conducted using a Siemens 3T Trio scanner located at the Staglin Center for Cognitive Neuroscience in the Semel Institute. The scanning protocol is as follows: 1) a 20 second scout scan and a 2 minute sagittal localizing scan for graphic prescription 2) A magnetization-prepared 180 degrees radio-frequency pulses and rapid gradient-echo (MPRAGE) T1 weighted image (Brant-Zawadzki et al, 1992) (TR: 2300; TE 2.93; flip angle 8 degrees; foV 256x256; BW 210 Hz/px; 1mm isotropic voxels) acquired for the purposes of segmentation and coregistration.

PET scanning for [$^{18}$F]MPPF was performed at the Nuclear Medicine Clinic at the UCLA Medical Center, and patient exposure was well below the mandated maximum annual at all times. The radioflourindated ([$^{18}$F]) MPPF probe was prepared, as described in Le Bars et al. (1998), to have high specific activities (>37 GBq/μmol). Subjects were injected with the [$^{18}$F]MPPF bolus (320–550 MBq) through an

intravenous catheter, and dynamic scans were subsequently collect for 2 hours following injection. After scan acquisition, the dynamic image time-series was decay-corrected and reconstructed from the scanner using filtered back-projection (Hann filter, 5.5-mm FWHM) (Kepe et al, 2006). Each scan was also corrected for deep-tissue attenuation and scatter of signal (signal drop-off) using the normalization scan taken prior to the scanning session. The reconstructed scans contained 63 contiguous slices with 2.42 mm plane-to-plane separation (Kepe et al, 2006).

*Subject Demographics*

62 subjects were enrolled and scanned for this study. Of these, 53 subjects produced usable scans for analysis. From the original 62: two subjects were excluded because of refusal to undergo DNA testing, two subjects were found to have contraindications for MRI, one subject was found-through neuropsychological testing after scanning- to have progressed to AD diagnosis at time 1 (see exclusion criteria), and three subjects had technical problems with PET kinetics rendering scans unusable. The remaining subjects were 19 men and 35 women between the ages of 47 and 81 (mean 65.4 +/- 7.8 years). Of these, 5 were Asian American, 3 were African American, one was Native American, one was multi ethnic and the rest (44) were Caucasian. Finally, one subject- the female who self-reported race as Native American- had to be excluded based on biological nonconformity to established norms. Some studies have suggested differences in the serotonin system in different racial populations, which corroborates observations in this study. Outlier testing showed this subject's binding potential to be significantly separate from that of the other subjects in this study. Averaged binding potential for medial temporal lobe regions was 3.8 standard deviations above the mean, and 20% higher than the next closest individual, thus she was excluded from this study.

Based on initial diagnostic criteria (see Appendix 1) 19 of the remaining 53 subjects were considered cognitively normal controls at time 1, while the other 34 exhibited cognitive deficits sufficient to be classified as MCI at time 1.

| Subject Demographics Table | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Sex | Average Age | Average Education | Average WTAR | APOE2 | 3/3 | 3/4 | 4/4 | Control/ MCI |
| 19 M | 70 +/- 7 years | 18 +/- 2 years | 118 +/- 7 | 1 | 9 | 5 | 4 | 4 / 15 |
| 34 F | 62 +/- 6 years | 15 +/- 2 years | 116 +/- 10 | 3 | 22 | 7 | 2 | 14 / 20 |

**Table1:** Subject Demographics by Sex

As an additional wrinkle in this study, subjects were recruited during a time period of transition from an ECAT 7 PET scanner to a newly acquired (2010) PET/CT scanner. This transfer was not accompanied by a balanced recruiting design, nor was an allowance made for multiple within-subject scans across both scanners. Though we have 19 MCI subjects on scanner 1 and 16 on scanner 2, we only have 5 control subjects on scanner 1 while we have 13, all but 1 of whom are female, on scanner 2. See Table 2 below.

| Scanner: 1-ECAT 2-CT | N | M/F | WTAR Average | T-Test WTAR | Age | APOE 2/3/4 |
|---|---|---|---|---|---|---|
| 1CONTROL | 5 | 3/2 | 119 +/- 4 | | 66 +/- 10 | 0/3/2 |
| 1MCI | 19 | 9/10 | 115 +/- 10 | 0.38 | 65 +/- 7 | 1/8/8 |
| | | | | | | |
| 2CONTROL | 13 | 1/12 | 121 +/4 | | 64 +/- 6 | 1/10/2 |
| 2MCI | 16 | 6/10 | 114 +/- 11 | 0.05* | 65 +/- 10 | 2/8/6 |

**Table 2:** Subject Breakdown by Scanner

As noted in tables 1 and 2, we have a larger number of female control subjects. But, despite the fact that verbal IQ scores, measured with the Wechsler Test of Adult

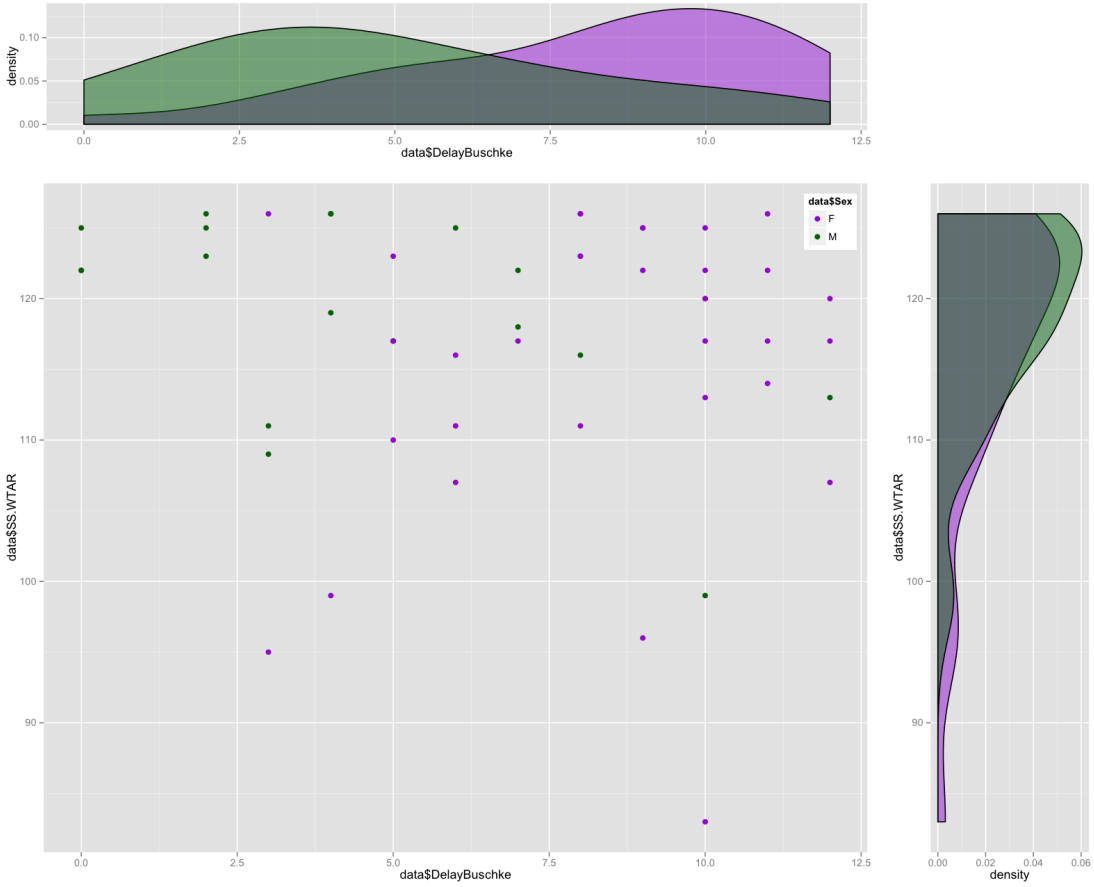
Reading (WTAR) and North American Adult Reading Test (NAART), are similar across all diagnostic categories for males and females, we have a significant performance difference between Male and Female subjects across diagnostic categories on tests of verbal memory, particularly those that are especially sensitive such as the Delayed Buschke-Fuld. This distribution (shown in figure 2.3) is suggestive of an ascertainment problem associated with unbalanced recruiting. Though years of education are slightly higher on average in male subjects, the male subject pool is also slightly older, and it is possible that this is also a contributing factor, nevertheless a lack of male control subjects may not be enough to explain the significantly higher-functioning female subject pool.

Overall literacy has previously been shown to accurately predict years, and more specifically overall quality of education (McFarlane et al 2006). Furthermore literacy or verbal IQ, as a proxy of overall intelligence or education may create a "cognitive reserve" such that those with high verbal literacy experiencing cognitive decline are still high performing compared to cognitively healthy individuals with lower over-all verbal literacy. Some have hypothesized that high education level or quality leads to brain reserve capacity, but there are debates about how this reserve manifests. Education may produce more neuronal connectivity and developed grey matter thus protecting against atrophy and AD (Morbelli & Nobili, 2014). Alternatively, brain reserve capacity could actually cover symptoms because highly educated individuals are high functioning and still perform within accepted norms on standardized neuropsychiatric exams, despite extensive regional atrophy (Perneczky, 2006). We can see a ceiling effect in our own population in scores on the WTAR/NAART, meaning that we are not able to capture the full spectrum literacy/education level in our subject pool (see figure 2.7) It is clear that current methods of cognitive assessment do not capture the full spectrum of cognitive performance. This is a problem that goes well beyond the scope of this research, and the

issues arising from inadequate methodology for cognitive assessment of high performing populations will need to be addressed in future of aging research in general.

Additionally however, there may be differences in the populations of men and women who seek to enroll and participate in a scientific inquiry, especially when considering generational differences associated with the population that is currently in the senior cohort. Many educated women of this generation had neither as many years of formal education nor the professional careers seen in men of the same cohort, but remained very active community participants, and thus might be likely to make the effort to seek out and participate in a study such as ours. If our male cohort was not as active or motivated, this may result in the kind of functioning disparity we see here.

**Figure 2.3:** Scores on the WTAR (Y axis) and Delayed Buschke (X axis). Males are shown in green, females in purple. Above & Right: density plots show estimated population for sample.



*Scanner Correction*

We do see differences in the binding profile for Scanners 1 and 2. For both scanners, MCI subjects show significantly increased variance compared to controls on the same scanner, but in addition mean binding was shifted for scanner 2 compared to scanner 1. Ideally we would create a scaling factor using control subject binding, given the low variance in our control groups, however the uneven distribution of subjects, and a small number of controls scanned on Scanner 1 (5) did not allow this. Importantly, we looked at IQ, education, health and genetics within-group by scanner, to determine what, if any, pattern might be associated with binding differences across scanners, and our regression model showed a significant main scanner effect. Thus we used 2 different correction approaches: First, a z-transform to standardize binding values for each scanner by anatomical region so that scanner 1 and scanner 2 cohorts could be combined into a single analysis. This allowed us to compensate for differences in absolute binding values across scanners, by expressing each individual's regional binding potential relative to their own deviation from that scanner's mean, effectively shifting binding values into the same common "space". Second we used a scaling factor created using only MCI groups, which had roughly equivalent breakdowns of major variable such as age, sex, years of education, VIQNAART/SSWTAR scores, and APOE allele. Because we do not have a signal subject scanned on both scanners we evaluated the overall distribution of subjects. This second approach allowed us to continue expressing binding potential as $BP_{ND}$. Comparisons of the two correction methods yielded similar results.

*MRI and PET Scan Analysis*

Scans were manually checked for motion artifact, both by visual inspection of scan frames/planes and by kinetic modeling of the time-activity curve for each subject. Before a group analysis, each scan was normalized within-scan using a reference region, so that between-scan comparisons could be made. Cerebellar white matter, where

[$^{18}$F]MPPF binding is lowest, was used as the reference region for this study, and all kinetic modeling was performed with the multimodal image software PMOD kinetic-analysis program (version 3.1; PMOD Technologies Ltd, Switzerland), a software packaged designed to incorporated PET and MRI and perform Kinetic Modeling.

With PMOD we applied variations Graphical Analysis (Simplified Reference Tissue Model (STRM) 1 and 2) to evaluate the K2' and generate a binding potential ($BP_{ND}$) map (Logan et al. 1996; Kepe et al 2006; Brown et al 2012). An average binding potential within each volume of interest was generated with the PMOD kinetic module, and a whole-brain voxel-wise binding potential map was generated with the PMOD PX module. $BP_{ND}$ was calculated as $BP_{ND}=R_1*k_2'/k_{2a}$ -1. $R_1$ is the ratio of tracer-delivery parameters for the tissue of interest and reference tissue ($R_1=K_1/K_1'$), and effective rate parameter for transfer of tracer from the tissue of interest to the plasma is expressed as $k_{2a}$ (Brown et al 2012).

Binding potential represents a measure of [$^{18}$F]MPPF binding for each defined region of interest. Volumes of interest (VOIs) were generated anatomically using the automated segmentation and parcellation software Freesurfer 5.1. The T1 weighted (see MRI acquisition protocol above) MPRAGE is segmented into cortical white matter, cortical grey matter, deep brain nuclei (basal ganglia, and thalamus, hippocampus and amygdala), brainstem and Cerebellar white and grey matter. This segmentation is then subparcellated into several different atlases, including the Desikan-Killany (Desikan et al, 2006), which divides the cortex into individual anatomical cortical regions. All segmentations/parcellations must be checked manually for errors, including correct division of regions and exclusion of meninges/non-brain (see Figure 2.4). After manual checks and editing, volumes of interest are extracted and concatenated using a computational feature of the FMRIB Software Library v5.0 (FSL), FSLmaths. Volumes of interest include amygdala, hippocampus, entorhinal cortex, parahippocampal cortex,

Insula, Cingulate (anterior, and posterior), Frontal regions such as orbitofrontal cortex (OFC), dorsolateral prefrontal cortex (DLPFC), brainstem and cerebellar white matter (for full list see Appendix 2). Both right and left volumes were included for all regions. In addition to individual region volumes (in cc) Freesurfer measures intracranial volume and cortical volume, which can be used in measures of overall atrophy.

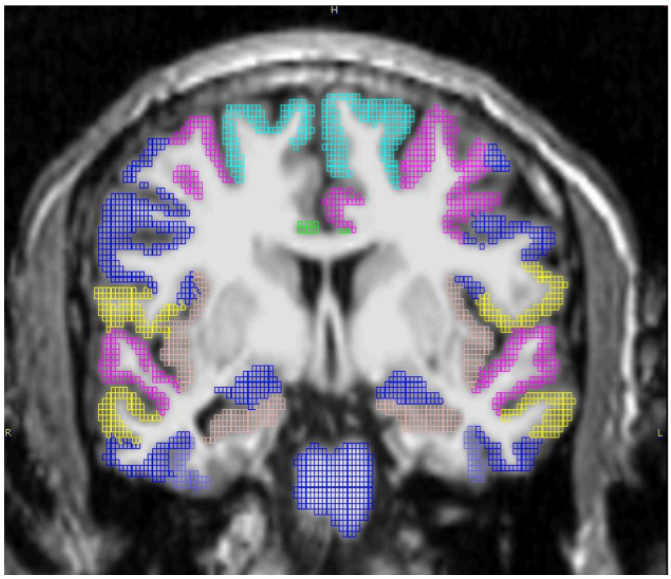


**Figure 2.4:** Freesurfer defined VOIs shown superimposed on T1

We used the PMOD's Fusion module and Freesurfer software to perform coregistration. MPRAGE scans were corrected for field coil intensity differences using the Freesurfer intensity correction optimized for 3 Tesla scanning (Zheng et al. 2009). Then, the raw [$^{18}$F]MPPF PET image for each individual was coregistered to the intensity corrected whole brain structural MRI of the same individual with a 6-paramter rigid-body linear transformation. This allows the precise spatial relationships of brain structures to be maintained across modalities.

*Partial Volume and Eroded Volumes of Interest*

Resolution is an important consideration for PET. The voxel size for PET scanning measures roughly 2.5 x 2.5 x 5mm, which is a dramatic increase from the 1mm isotropic voxel size obtained from the T1 structural MRI scanning. The increased voxel size means a decrease in spatial resolution that cannot be adjusted to match the spatial resolution of structural MRI. Because of this, binding values may be obtained only for whole anatomically defined regions of the hippocampal complex. With whole volumes of interest binding in the hippocampus proper, entorhinal, parahippocampal and

neighboring cortices could be evaluated. We were not, however, able to differentiate between the individual subfields of the hippocampus such as the CA1/2/3 regions.

Some degree of partial volume effect is also a likely possibility. Partial volume effects result where binding values within a voxel are altered because the voxel spans space across multiple tissue types such as grey matter, where signal would be high, and cerebrospinal fluid (CSF) where signal would be low. The PET voxel is large enough to make this configuration likely, particularly in brain regions adjacent to fluid filled spaces or areas of low binding next to areas of high binding. In these cases, the resulting voxel binding reflects the average of the differential signal intensity creating an inaccurate measure of signal at these "edges". Partial volume was a particular concern for this study because we are focusing on a disease sate that is accompanied by considerable atrophy, and because we are focusing on relatively small brain regions in the medial temporal lobe that are surrounded by CSF.

We controlled for this possibility by creating an eroded VOI. The original volumes of interested are algorithmically eroded using an application of FSLMaths in order to shave voxels from the exterior surface of each VOI in 3 dimensions. The resulting VOI is much smaller, and appears significantly inside the boundaries of the anatomical structure. In this way the VOI is not sampling from edge voxels that exhibit an artificially low binding potential because of surrounding white matter or CSF. We found that, though the absolute binding potential value did increase, this increase was roughly equivalent across all subjects, and thus there were no major difference in relationships between binding potential and other factors. The eroded VOI may be a slightly more sensitive measure for accurate binding potential, however there are also drawbacks associated with using the eroded VOI. If there are areas of the hippocampus that show differential 5-HT1A expression, the eroded VOI may remove part or all of these areas, or may remove a different degree of the region from one subject to the next. Visual

inspection does not suggest any outsized isolated areas of binding within the hippocampus, making this risk low but present. In addition, some regions, such as the parahippocampal and entorhinal cortices, are already so thin that after erosion there are not enough voxels remaining to allow accurate kinetic modeling. As a result, in the whole VOI we can look at the combined binding in several MTL structures, while in the eroded VOI we must look only at hippocampal and amygdala binding.

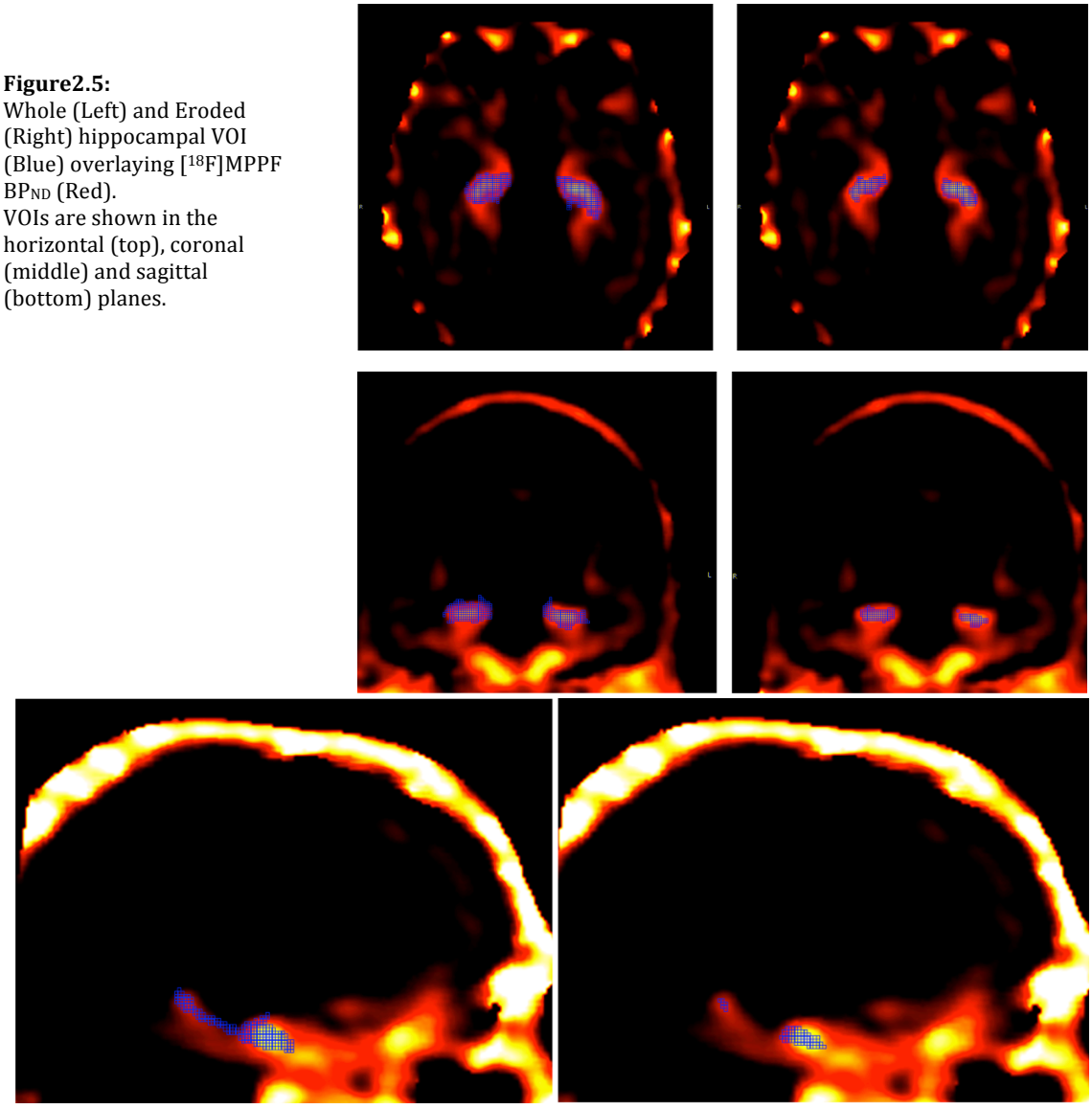
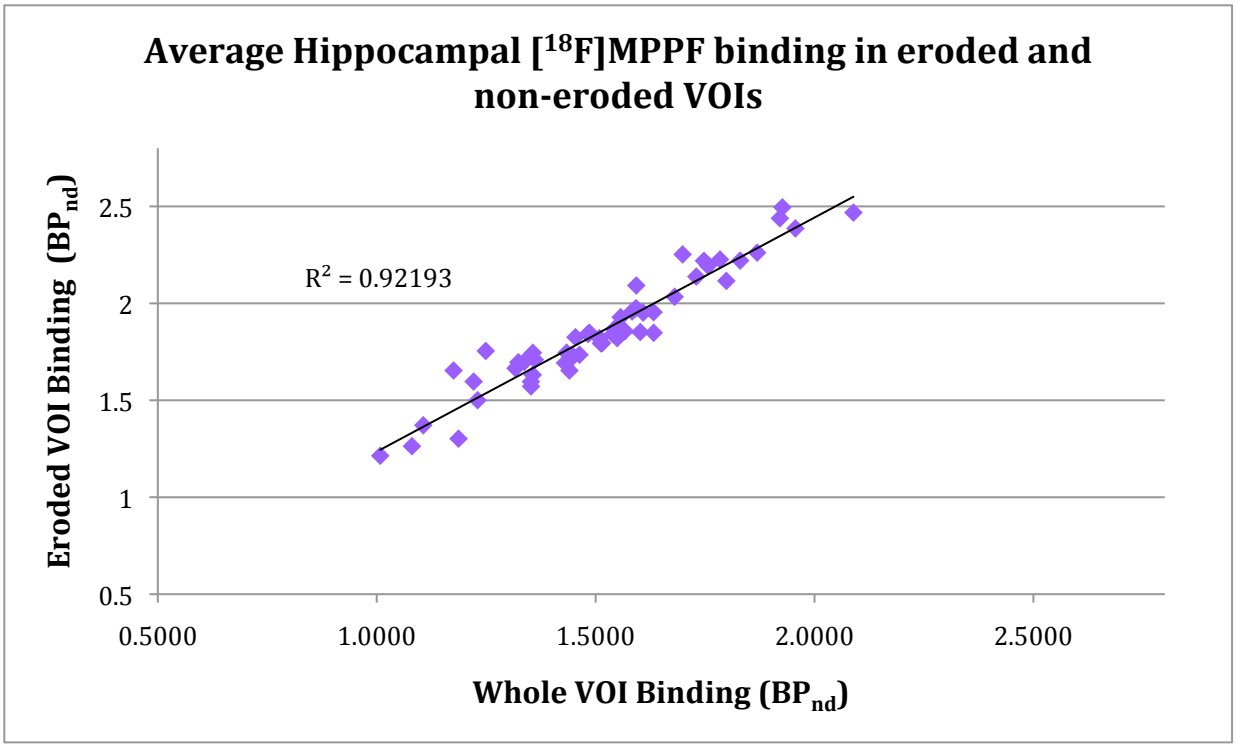


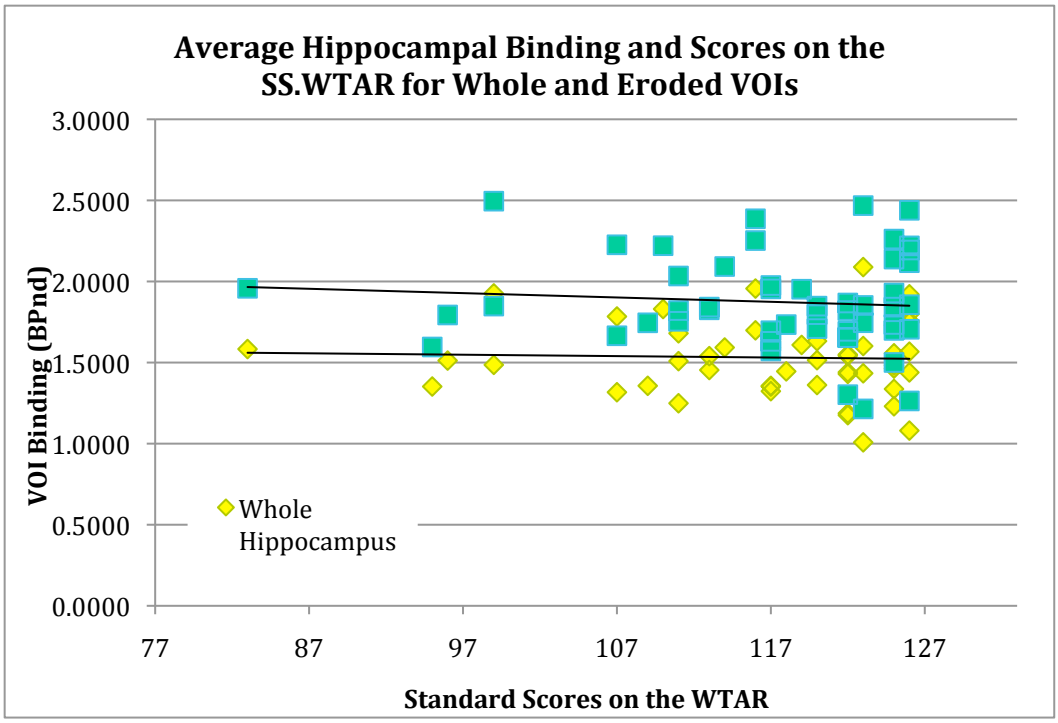
**Figure2.5:**
Whole (Left) and Eroded (Right) hippocampal VOI (Blue) overlaying [$^{18}$F]MPPF $BP_{ND}$ (Red).
VOIs are shown in the horizontal (top), coronal (middle) and sagittal (bottom) planes.

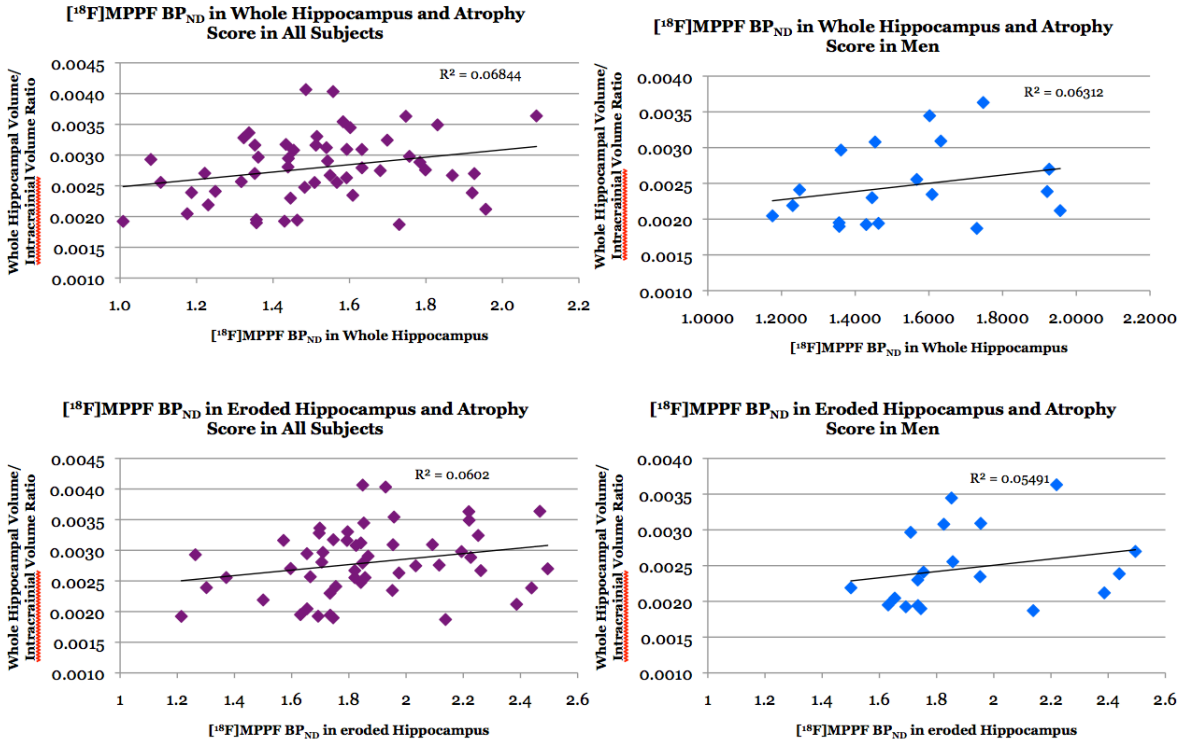
**Figure 2.6:** Correspondence of binding in whole hippocampal VOI, and eroded hippocampal VOI



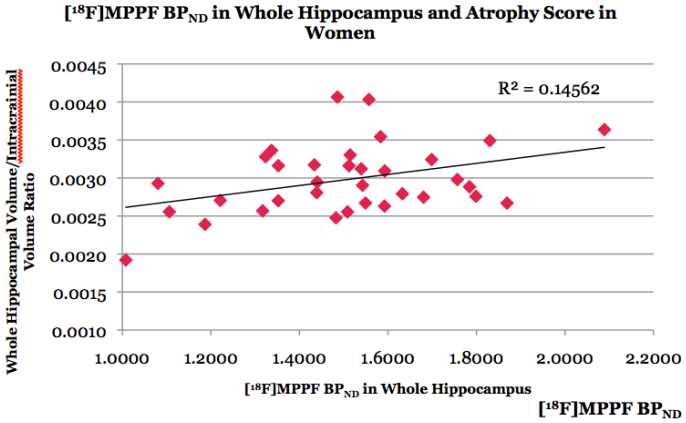

Comparison of the average hippocampal [$^{18}$F]MPPF binding potential for the whole and eroded VOI shows very good correspondence ($R^2$=0.92, see Figure 4). Likewise comparison of the Whole and eroded average hippocampal binding and SS.WTAR scores shows that a very similar relationship is preserved. Finally, comparison of average hippocampal binding potential and atrophy scores reveal that, although there are minor differences in distribution and fit, the overall shape and direction of the trend between these factors remains the same. There is only a slight reduction in least squares fit between the whole and eroded VOIs for the population in total and for male subject (-0.008 and -0.01 respectively).

**Figure 2.7**: Whole (Yellow) vs Eroded (Aqua) hippocampal binding plotted with SS WTAR. Though average eroded values are higher overall, the relationship of binding across individuals is maintained.



Given that the eroded VOI removes edge voxels that might exhibit lower binding potential, we expect the overall $BP_{ND}$ value to increase when calculated with the eroded VOI, as we've seen. We also expect the relationship between atrophy score and binding potential to be preserved only if that relationship is independent of a partial volume effect, and in fact we see an increase in robustness of the correlation between hippocampal $BP_{ND}$ and atrophy score in women when measured with the eroded hippocampal VOI ($R^2$=0.17, Pearson correlation $p<0.01$).



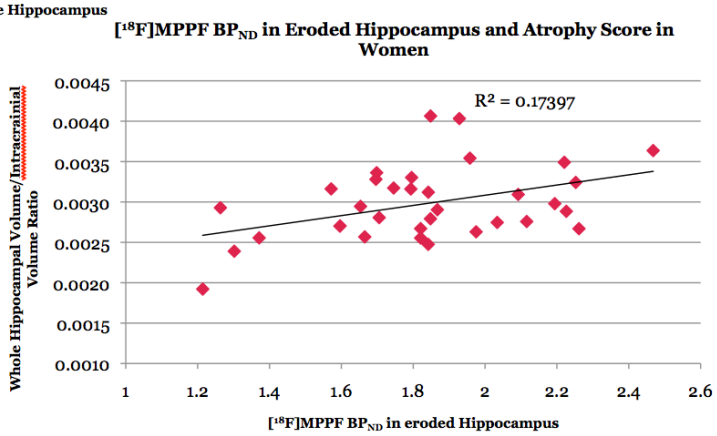




**Figure 2.8 A, B:** Hippocampal [18F]MPPF average binding potential in whole and eroded VOIs correlated with individual subject atrophy score. A: Shown on the previous page in all subjects (purple) Men (blue) B: And on this page in Women (red).

One caveat associated with the use of eroded VOIs is that overall thickness of regions must be high enough to allow voxels to be shaved from the anatomical volume. The entorhinal and parahippocampal cortices, while each spanning approximately half the length of the hippocampus, are extremely thin. Thus these regions cannot be effectively eroded while still preserving the voxel density required for kinetic modeling within region. Fortunately our analysis suggests that the even the whole VOI, because it

is anatomically generated for each individual, is precise enough to minimize partial volume effects and therefore yield valid binding potential.

## Results

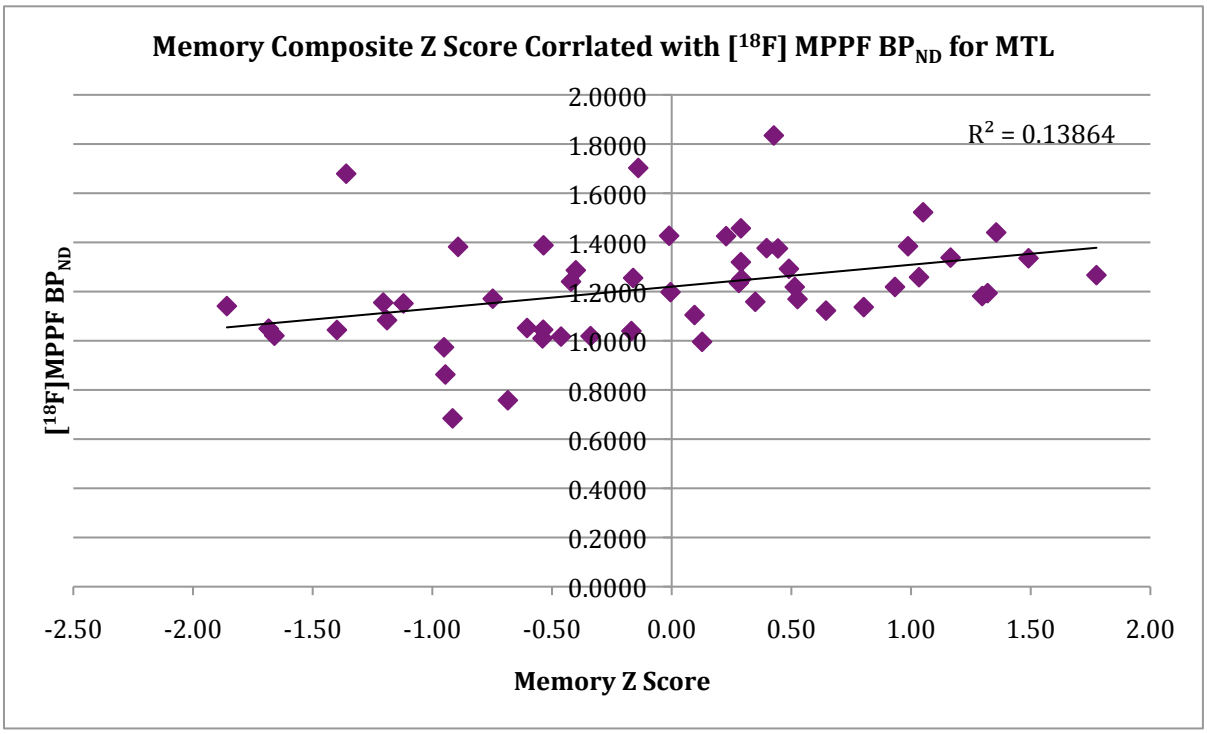


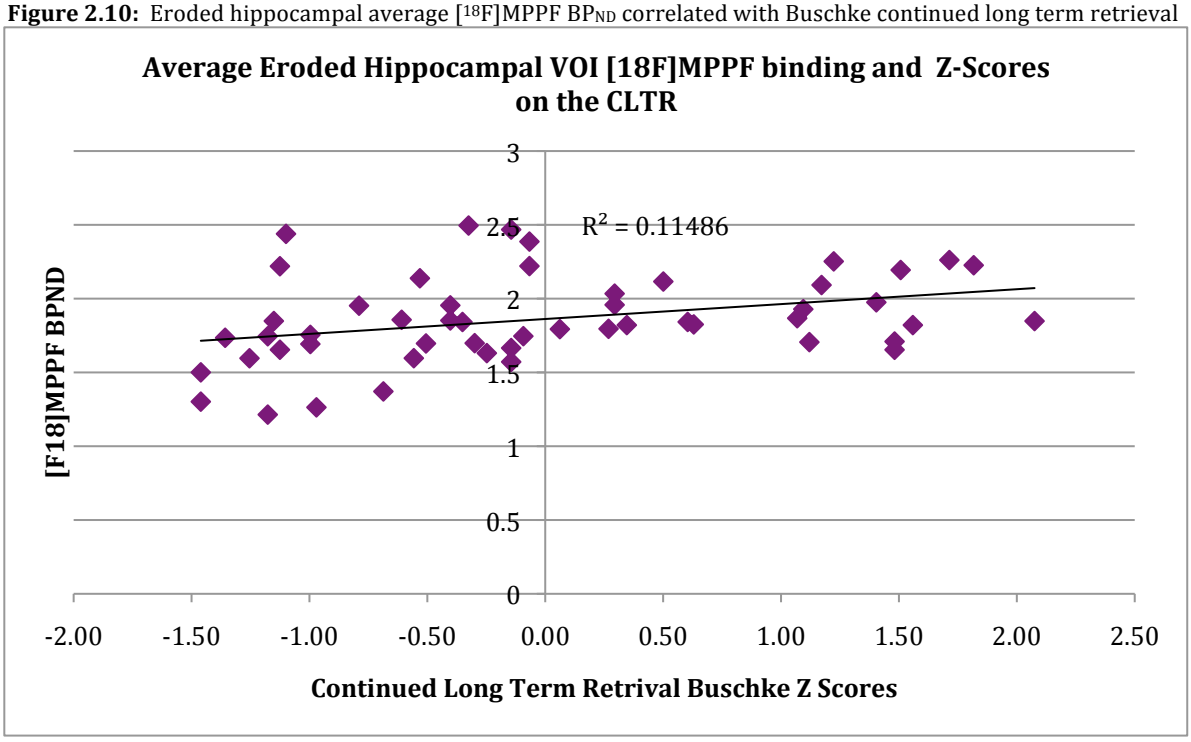

**Figure 2.9:** Medial temporal average [18F]MPPF binding correlated with composite memory Z score

Many memory tests are significantly correlated with [18F]MPPF binding, in the MTL (average of bilateral entorhinal, parahippocampal, amygdala and hippocampus), as well as with whole average hippocampal and eroded average hippocampal binding, including the Wechsler Logical Memory (Total and Delayed) and the Buschke (Total, Delayed) (see Figure 2.9). In particular Buschke Continued Long Term Retrieval, our most sensitive memory measure, was highly correlated with hippocampal binding ($p < 0.006$, see Figure 2.10). We modeled the relative contribution of several variables to prediction of Memory performance (Composite memory Z score) in a multiple linear regression. In a model with Age, Depression Z score, Anxiety Z score, Verbal IQ (SS.WTAR), Age, APOE Allele, Atrophy Ratio, and eroded hippocampal [18F]MPPF $BP_{ND}$.

We found that Atrophy Ratio and $BP_{ND}$ were significant predictors of change in memory performance ($p<0.01$ and 0.04 respectively) (see Figure 2.11). Interestingly, age and atrophy score are highly correlated however atrophy accounts for more of the varience than age and appears to be a significantly more accurate predictor of memory performance.

We confirmed that the relationship between memory performance and hippocampal [$^{18}$F]MPPF $BP_{ND}$ was not an artifact of sex and age differences across scanners in a subset of male and female subjects matched for age, literacy (WTAR/NAART), and APOE status; within our matched sample [$^{18}$F]MPPF $BP_{ND}$ was significantly correlated with memory performance.

**Figure 2.10:** Eroded hippocampal average [$^{18}$F]MPPF $BP_{ND}$ correlated with Buschke continued long term retrieval



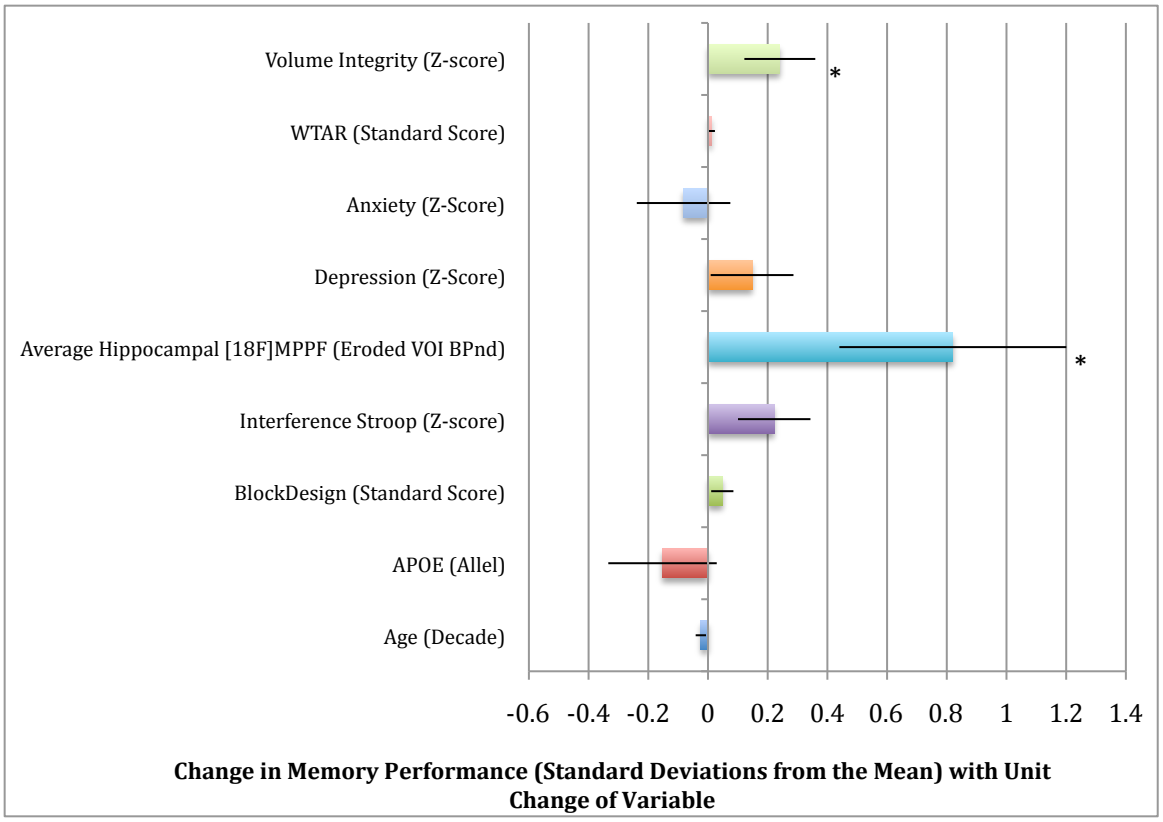
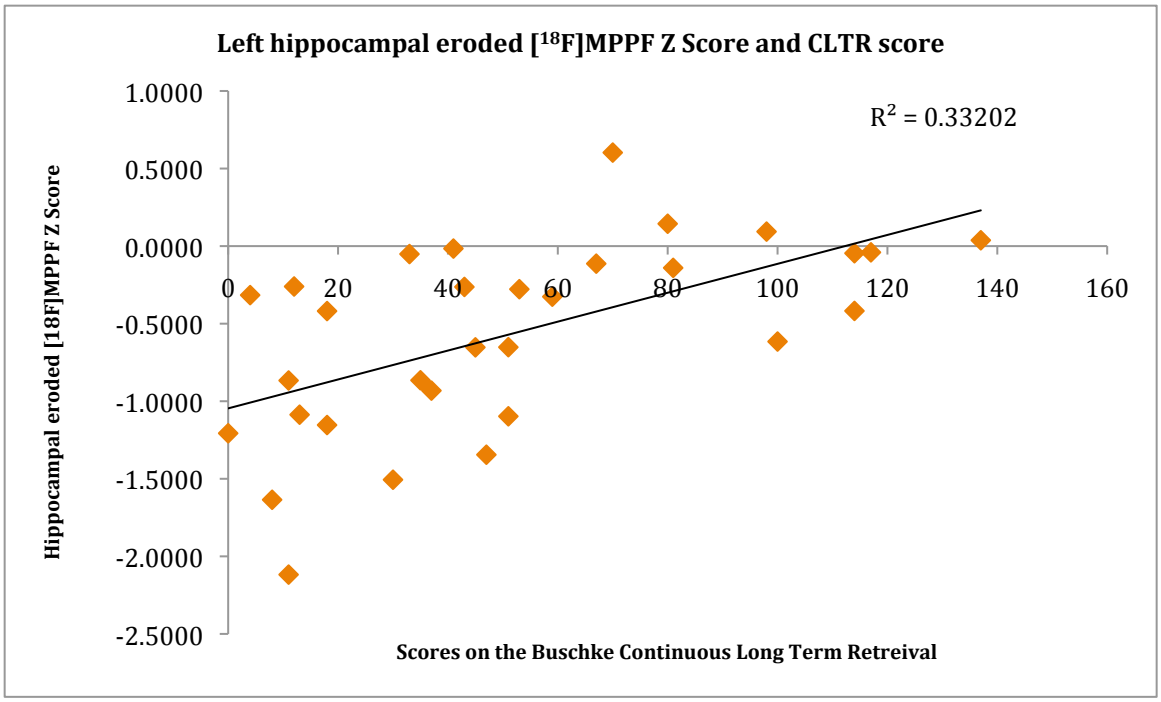



**Figure 2.11:** Regression model predicting change in memory Z score with change in factors including atrophy, literacy (WTAR) Anxiety and Depression Z scores, hippocampal $BP_{ND}$, Stroop, Block design, APOE status and age.

Finally, we also see hemispheric differences in the relationship between hippocampal [$^{18}$F]MPPF $BP_{ND}$ and memory performance, particularly in subjects performing at or below mean memory levels. Group variance increases as memory performance decreases, but left hippocampal [$^{18}$F]MPPF is a highly significant predictor of overall memory performance in this group (see Figure 2.12).



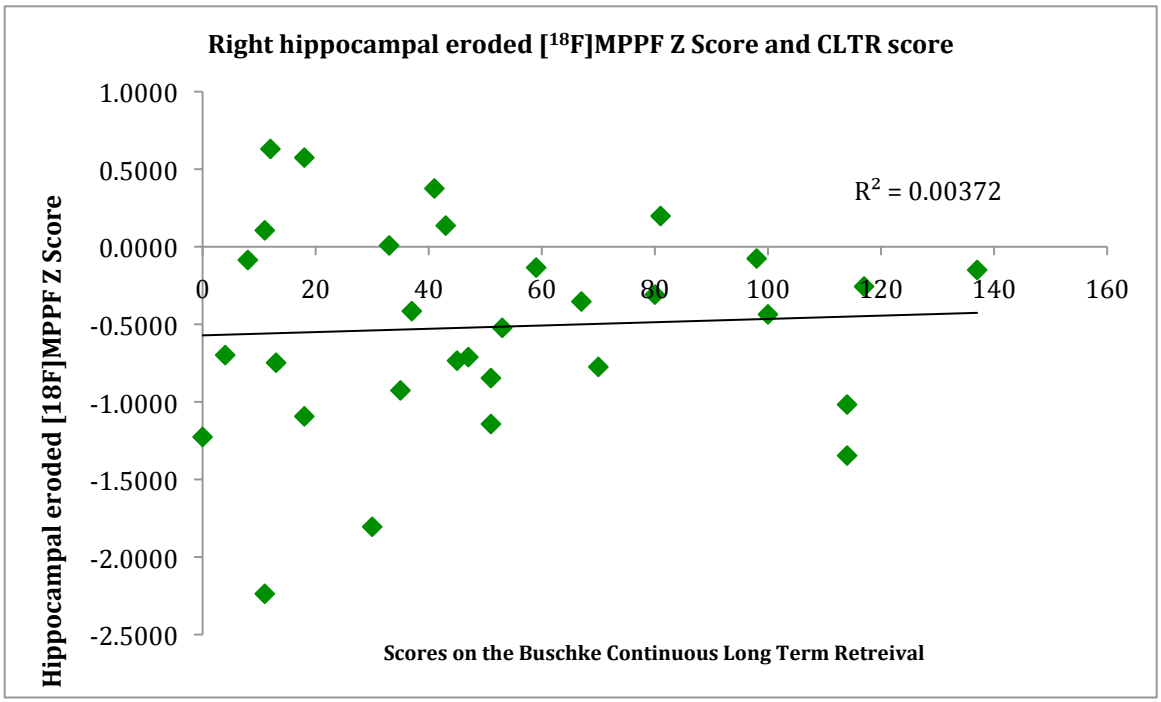




**Figure 2.12:** Differences in correlation with memory performance in left (top) versus right (bottom) hippocampus

## Discussion

Our results confirmed our hypothesis that memory performance is associated with hippocampal receptor density, and that this density corresponds with overall atrophy. Nevertheless [$^{18}$F]MPPF adds predictive value to the model of factors predicting memory performance, and thus may have value as a biomarker.

[$^{18}$F]MPPF is a measure of hippocampal integrity that goes beyond volume alone. First, [$^{18}$F]MPPF adds predictive value to memory performance beyond simple atrophy and thus suggests that hippocampal function is impaired prior to atrophy, at least as we can measure it with MRI. Measuring hippocampal function using this technique may therefore add to our ability to predict decline. Second, the pattern of results suggests that there is more variability in the impaired population. This implies heterogeneity in our subject memory performance indicative of multiple pathways to memory impairment, of which hippocampal dysfunction is just one. It is possible that, using [$^{18}$F]MPPF, we might be able to separate those patients with hippocampal-based memory impairment from those with memory impairment due to other causes. It remains to be seen whether we can make this judgment based on [$^{18}$F]MPPF values alone, but this is an important possibility that should be considered for future study.

It may also be the case that [$^{18}$F]MPPF can differentiate individuals within a range of memory capability. Differences in features like dendritic arbor density and receptor expression may lead to functional differences in memory that are measurable with this tool. We may be measuring functional hippocampal differences with [$^{18}$F]MPPF that represent persistent or life-long capability differences. Future long-term longitudinal studies are needed to investigate this possibility.

Finally we note that the left hippocampus shows a much stronger relationship with overall memory performance than the right. This laterality is in-line with what we know about hippocampal function in memory, which has been previously demonstrated

to show both hemispheric and anterior-posterior differences in activation, volume, and receptor density based on task performance. This gives us a strong indication that binding potential in this study is, in fact, accurately measuring receptor density and providing a hippocampus specific metric of integrity.

## IV. CHAPTER THREE: [$^{18}$F]MPPF Imaging of the Serotonin 1A Receptor in Anxiety and Cognitive Decline


### Abstract

Anxiety and depression have emerged as risk factors for AD, and are clearly related but separate contributors to overall risk. We propose that differences in the serotonergic system that underlie anxiety behaviors may create conditions that increase susceptibility to disease. Beyond its value as a marker for neuronal loss in the hippocampal complex, the serotonin (5-HT) 1A receptor has been implicated in anxiety behaviors of both animals and humans. In particular differences in 5-$HT_{1A}$ receptor densities have been found in a number of brain regions, including the insula, anterior cingulate and amygdala in individuals with anxiety disorders. We find differences in insula, but not amygdala, receptor density predict both anxiety and working memory performance in women. We also note distinct sex differences in receptor-trait associations in women and men.

## Introduction

In addition to its utility as a proxy for neuronal health/cell loss, the serotonin (5-HT) 1A receptor subtype is also highly associated with anxiety in both animals and humans. Blockade of the $5HT_{1A}$ receptor produces increased anxiety behavior in zebra fish (Nowicki et al 2014). Rats raised in overcrowded conditions show increased anxiety behavior and decreased $5\text{-}HT_{1A}$ receptor density, even though overall concentrations of serotonin remain unchanged (Daniels et al 2000). Likewise in mice, disruption of the $5\text{-}HT_{1A}$ receptor in early postnatal development leads to improper synapse formation and causes the animals to exhibit anxiety behavior (Ferreria et al, 2010), while a full $5\text{-}HT_{1A}$ knockout mouse model also shows a significant increase in fear response (Klemenhagen et al, 2006). Furthermore, in a mouse model of low social anxiety, $5\text{-}HT_{1A}$ receptor currents are greatly increased (Proulx et al 2010). In humans, a link between cholesterol disturbances through the long term use of statins and increased depression/anxiety has been established, and changes in the G-coupling of the $5\text{-}HT_{1A}$ receptor may be one of the primary causes (Shrivastava et al 2010). Most anti-depressants, including Lithium, valproate, selective serotonin reuptake inhibitors (SSRIs), and tricyclic antidepressants (TCAs) increase postsynaptic signaling of the $5\text{-}HT_{1A}$ receptor and dysfunction of this receptor has been proposed as one of the possible mechanisms of major depression as well as anxiety (Savitz et al 2009).

Anxiety and depression are common, but still under-diagnosed in older adults, and can often manifest simultaneously or in advance of other illnesses. The relationship between mood disorders and dementia in particular is not well understood, but recently a report released by the NIH surveying 250 primary research articles found depression to be among only a few factors most consistently associated with increased AD risk (Williams et al 2010). The manifestation of depression at the onset of dementia has previously been considered an initial symptom of dementia. Now, however, a number of

longitudinal studies have reported an increased risk for developing Alzheimer's disease in participants who have previously exhibited depression. A new investigation of the Framingham heart study cohort by Saczynski et al. (2010) found that participants for whom depression symptoms at baseline reached 17 or higher on a 60-point Center for Epidemiologic Studies Depression Scale (CES-D) were roughly twice as likely to develop AD compared to participants without these depression symptoms. This risk continued to increase by 40% with each 10-point increase on the depression scale. Furthermore this study was conducted over a 17-year follow-up period in subjects with no cognitive impairment at baseline, and no conversion within 3 years of baseline, strongly suggesting that the depression is not a consequence of subclinical or early dementia.

Similarly, results from the Baltimore Longitudinal Study of Aging showed that subjects with at least one episode of elevated depressive symptoms (EDS) had an 87-92% increased risk of developing dementia, while two or more episodes of EDS doubled this risk (Dotson et al 2010). These results clearly suggest a link between these disease states beyond the presentation of depression as an initial symptom of dementia onset. Supporting this, in mild cognitively impaired (MCI)-amnestic subjects, who have a high conversion to AD, increased apathy rather than depression is the dominant emotional change that predicts conversion (Palmer et al 2010). How depression might increase risk of developing dementia is not currently understood, however certain brain areas, such as the hippocampus, remain highly affected in both diseases.

Shared pathways, or synergistic interactions between multiple biochemical processes could explain the relationship between these disease states. APOE genotype in particular appears to interact synergistically. In findings published by Irie et al (2008) from the Honolulu-Asia aging study, depressed men who were not carriers of the APOE-4 allele had a 1.6-fold increased risk of developing dementia which is consistent with findings from previously mentioned longitudinal studies. Depressed men who were

APOE-4 carriers, on the other hand, showed a 7.1-fold increased risk, which is considerably greater than that accounted for by the depression or genetic risk alone. APOE-4 carriers also appear to show some structural differences that are similar or related to deficits caused by depression (Wolk et al 2010).

Both dementia and mood disorders show reductions in adult neurogenesis and this may be another important mechanism by which these disease states interact. Many of the drugs used to treat mood disorders increase neurogenesis, which is thought to be partially serotonergically mediated, and this is increased neurogenesis may make a significant contribution to the amelioration of symptoms.

In fact, neurogenesis in particular may play a role in both anxiety and depression. Neurogenesis is known to occur throughout adult life, as new neurons are continuously incorporated into the olfactory bulb and dentate gyrus of the hippocampus (Aimone et al 2010). In both cases, pattern separation is an essential function of the brain region, and computational modeling suggests that continual integration of new neurons to the network is required to maintain this capability (O'Reilly et al, 1994; Fenton et al, 2007). Kheirbek and colleagues (2012) postulated that impaired neurogenesis and an associated impairment in pattern discrimination might lead to a greatly increased risk of anxiety disorders and PTSD.

This theory is suggestive of the previously mentioned link between serious depression and later risk of Alzheimer's disease. Mood disorders in general have clear impacts on the hippocampus and the serotonin system that are highly relevant to similar phenomena that occur as dementia develops. Influences of hypothalamo-pituitary-adrenal (HPA) axis activity and stress are also important considerations in the impact on hippocampal function. HPA dysregulation and cortisol levels are clearly related and can affect mood, sleep patterns, memory and a variety of other behaviors (Balbo et al 2010; Aggleton et al 1999). Conditions such as chronic stress can cause depression, and recent

animal models suggest that some antidepressants may act on the HPA system to rescue this phenomenon in the hippocampus (Hu et al 2010). Because the hippocampal complex is among the most sensitive brain structures, and extremely susceptible to anoxia and inflammation, many studies focus on the thickness and volume of HC subregions in disease states, such as Multiple Sclerosis (MS), Epilepsy, head injury and depression. In MS subjects, reduced volume of the CA1 and CA2/3 DG regions correlates with severity of depression symptoms and is also associated with increased cortisol levels (Gold et al 2010); This volume loss is correlated with worsening performance on tasks that require memory encoding, but not tests of functions unrelated to memory such as information processing speed (Sicotte et al 2008). Chronic inflammatory changes also occur in depression and may be a result of elevated stress (Leonard et al 2007).

Depression and anxiety are closely related disorders with high comorbidity rates, especially in geriatric populations (Beattie et al 2010). While some anatomical circuits and systems involved in depression versus anxiety may differ, others are clearly shared and both disorders respond to treatments such as antidepressants. While depression often manifests at the initial onset of quantifiable dementia, it is clearly the case that symptoms of depression experienced throughout life relate to future dementia risk. Recently indications suggest anxiety shows a similar relationship; in a longitudinal follow-up the severity of decline from cognitively healthy to MCI was significantly increased in subjects classified as high anxiety (Pietrzak et al. 2015). Because of high relatedness, shared disease pathways and impact on hippocampal structure and function both depression and anxiety should be considered in the context of dementia risk (Cramer et al 2010). The relationship between anxiety and AD, though not as well-studied, is likely to be related but not equivalent. Just as anxiety and depression have both commonalities and differences, we expect that anxiety should show a separate but important interact with AD. Better understanding of the pathologies common to these

diseases will have important implications for treatment and prevention. Everything from the use of SSRIs and other antidepressants in treatment of AD (Aboukhatwa et al 2010), to the importance and management of lifestyle factors such as stress in treating and preventing anxiety (and potentially also future dementia) should be considered.

We combined PET imaging with structural MRI to investigate differences in 5-$HT_{1A}$ receptor densities within the hippocampus, and other structures associated with anxiety in previous $5HT_{1A}$ investigations, including the insula, anterior cingulate and amygdala.

We predicted that [18F]MPPF binding in both amygdala and insula would be significantly correlated with anxiety across all subjects, and that this would also correspond with memory performance in APOE4 carriers.

## Materials and Methods

*Participants*

The UCLA Center on Aging, directed by Dr. Gary Small, performed subject recruiting as described previously (see Chapter 2). The longitudinal study which provided the cross-sectional time point for this investigation focused only on memory and hippocampus changes over time, thus subjects with clinically significant depression or anxiety at time 1 were excluded from the study on the grounds that active severe depression/anxiety may have confounding cognitive deficits. Nevertheless, because of the high level of relatedness we felt subclinical anxiety within the range of typically aging individuals would still be sensitive, and focusing on these relationships widely applicable to the population at large. Clinically significant depression and anxiety are also typically medicated with drugs that heavily influence serotonin, thus excluding those with clinical symptoms allowed us to also exclude subjects on these medications.

To create a refined measure of depression and anxiety symptoms even at the subclinical level, we combined scores for multiple anxiety and depression tests into an

anxiety Z-score and a depression Z-score for each subject. For anxiety we included the following tests: 1) The Hamilton Anxiety Inventory (HamA; Hamilton 1960), The Trait Anxiety Inventory (TraitAnx; Spielberger et al 1970), and the Profile of Mood States Tension Subtest (POMS; McNair et al 1971). For Depression we included the following tests: 1) Hamilton Depression Inventory (Hamilton 1959), and the Geriatric Depression Scale, which has been demonstrated as a valid diagnostic even in MCI subjects with MMSE score above 15 (GDS; Yesavage et al 1982-1983; McGivney et al 1994).

The laboratory of Dr. Small oversaw clinical and neuropsychiatric testing. The roughly 3-hour cognitive assessment was always scheduled to occur within three months of PET and MRI scans so that cognitive diagnosis is accurate for the time period in which scans were collected. Symptoms of depression and Anxiety were measured with the following: Hamilton Depression and Anxiety Inventory (HamA, HamD)(Hamilton 1959; Hamilton 1960), Profile of Mood States (POMS) (McNair et al 1971), State-Trait Anxiety Inventory (STAI) (Spielberger et al 1970), and for self report the Geriatric Depression Scale (GDS), which has been demonstrated as a valid diagnostic even in MCI subjects with MMSE score above 15 (Yesavage et al 1982-1983; McGivney et al 1994). Additional cognitive assessment includes tests in the following domains: General Intelligence: Subtests of the WAIS-III and literacy with the Wechsler Test of Adult Reading (WTAR), (Wechsler 2001); Executive Function: Wisconsin Card Sorting Task (Berg 1948), Word Fluency (Cauthern 1978), Trails (Spreen et al. 1965), Attention: Digits Forward and Backward (Wechsler 2001); Language: Boston Naming Test (Goodglass et al. 1972), Fluency (Cauthern 1978); and Memory: Buschke-Fuld Selective Reminding Task (Bushke et al. 1974), California Verbal Learning Test (Delis et al. 1987), WMS-III Logical Memory and Verbal Paired Associates learning test (Wechsler 1997), Benton Visual Retention test (Benton 1974), Reye-Osterrith Figure (Rey 1964). In small number of

cases literacy was assessed with the North American Adult Reading Test (NAART) (Uttl, 2002).

Subjects were also given a Family History Questionnaire (Breitner et al. 1984), memory complaints self-report questionnaire (Gilewski et al. 1990), Neuropsychiatric Inventory (Cummings et al. 1994) and Mini Mental State Exam (Bleecker et al. 1988).

*PET and MRI Scan Acquisition*

As before, subjects received both and MRI and PET scan. MRI scanning was conducted using a Siemens 3T Trio scanner located at the Staglin Center for Cognitive Neuroscience in the Semel Institute. The scanning protocol, as previously described, is: 1) a 20 second scout scan and a 2 minute sagittal localizing scan for graphic prescription 2) A magnetization-prepared 180 degrees radio-frequency pulses and rapid gradient-echo (MPRAGE) T1 weighted image (Brant-Zawadzki et al, 1992) (TR: 2300; TE 2.93; flip angle 8 degrees; foV 256x256; BW 210 Hz/px; 1mm isotropic voxels) acquired for the purposes of segmentation and coregistration.

The UCLA Medical Center Nuclear Medicine Clinic acquired all [$^{18}$F]MPPF PET scans. Patient exposure to radioactivity always remained below the required maximum annual exposure limit. The [$^{18}$F]MPPF probe was prepared, as described in Le Bars et al (1998), to have high specific activities (>37 GBq/μmol). An intravenous catheter allowed injection of the [$^{18}$F]MPPF bolus (320–550 MBq), and in the following 2 hours the subject dynamic scan was collected. The dynamic image time-series was then reconstructed, after decay-correction, using filtered back-projection (Hann filter, 5.5-mm FWHM) from the scanner (Kepe et al, 2006). Signal drop-off, or deep-tissue attenuation was also corrected using a normalization scan that is acquired each day prior to scanning sessions. Each reconstructed scan has 2.42 mm plane-to-plane separation between 63 contiguous slices (Kepe et al, 2006).

*PET and MRI Scan Analysis*

We modeled kinetics of the time-activity curve, and visually check for motion artifact to insure that each subject scan was viable. We chose the area of lowest [$^{18}$F]MPPF binding, cerebella white matter, to perform within scan normalization. We normalized scans using the Freesurfer segmented cerebellar white matter as a within-scan reference region. Following this, between-scan comparison could be made for group analysis. We used the multimodal image software PMOD kinetic-analysis program (version 3.1; PMOD Technologies Ltd, Switzerland), a software packaged designed to incorporated PET and MRI for all kinetic modeling.

We used the kinetic module of PMOD to generate an average binding potential within each of our volumes of interest with the Simplified Reference Tissue Model (STRM 1 and 2). This is a variation of the Logan Graphical Analysis which allows us to evaluate the K2' and generate a binding potential ($BP_{ND}$) map (Logan et al. 1996; Kepe et al 2006; Brown et al 2012). We calculated $BP_{ND}$ using $BP_{ND}=R_1*k_2'/k_{2a}$ -1. We defined $R_1$ as the ratio of tracer-delivery parameters for the tissue of interest and reference tissue ($R_1=K_1/K_1'$), and $k_{2a}$ as the effective rate parameter for transfer of tracer from the tissue of interest to the plasma (Brown et al 2012).

As before we used Freesurfer 5.1 to generate volumes of interest (VOIs) anatomically. Automated segmentation and parcellation of the T1 weighted MPRAGE yielded VOIs of cortical white matter, cortical grey matter, deep brain nuclei (basal ganglia, and thalamus, hippocampus and amygdala), brainstem and Cerebellar white and grey matter and also subparcellation of atlases. We chose the Desikan-Killany (Desikan et al, 2006), for our individual anatomical cortical regions of interest. Manual checks are always performed in order to catch segmentation or parcellation errors that are not detected by the software package itself. We extracted and concatenated VOIs using a computational feature of the FMRIB Software Library v5.0 (FSL), FSLmaths. Volumes of interest include amygdala, Insula, Cingulate (anterior) and cerebellar white

matter for reference (for full list see Appendix 2). All volumes are bilaterally included. In addition to individual region volumes (in cc) Freesurfer measures intracranial volume and cortical volume, which can be used in measures of overall atrophy.

Coregistration between PET and MRI was performed with PMOD's Fusion module and Freesurfer. We first used Freesurfer intensity correction optimized for 3 Tesla scanning to correct field coil intensity differences in the MPRAGE scans (Zheng et al. 2009). Next , each individual raw [$^{18}$F]MPPF PET image was coregistered to the same individual's own intensity corrected whole brain structural scan using a 6-paramter rigid-body linear transformation. We were thus able to maintain the spatial relationships of brain structures within each subject's native space.

*Partial Volume and Eroded Volumes of Interest*

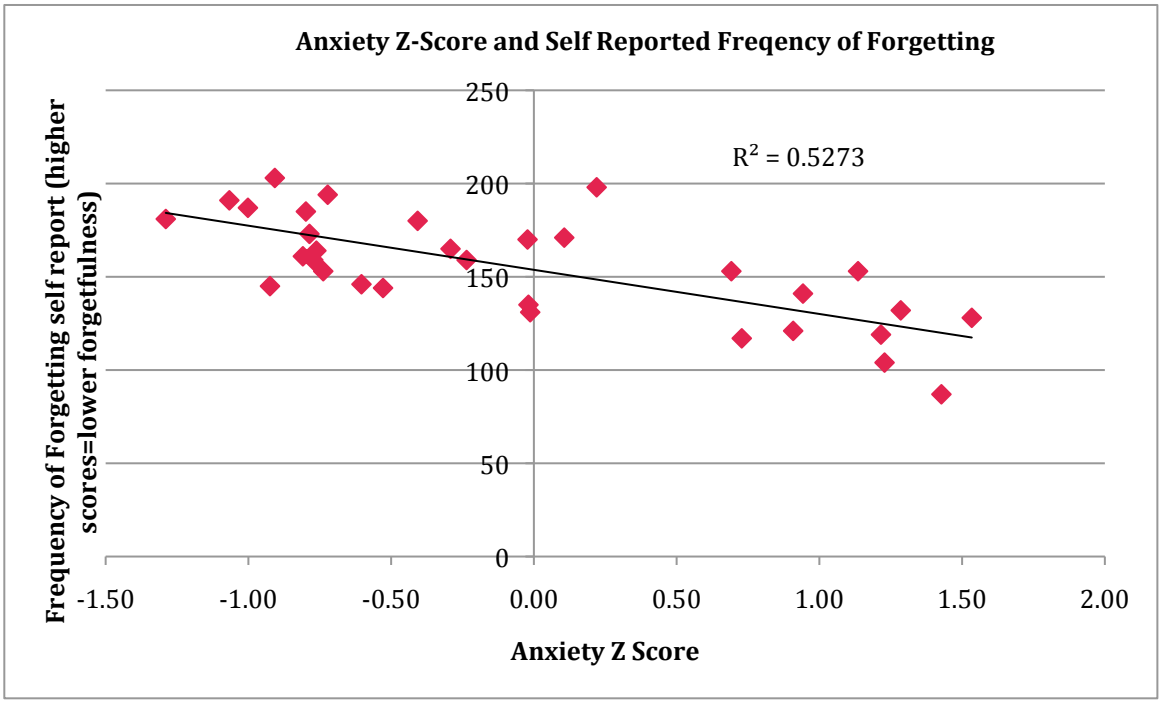
When working with PET, resolution is always a concern. Measuring roughly 2.5 x 2.5 x 5mm, the PET voxel is dramatically larger than the structural MRI voxel. As a result, these two scanning methods will never having matching spatial resolution. This means that a partial volume effect maybe a concern to varying degrees in different brain structures.

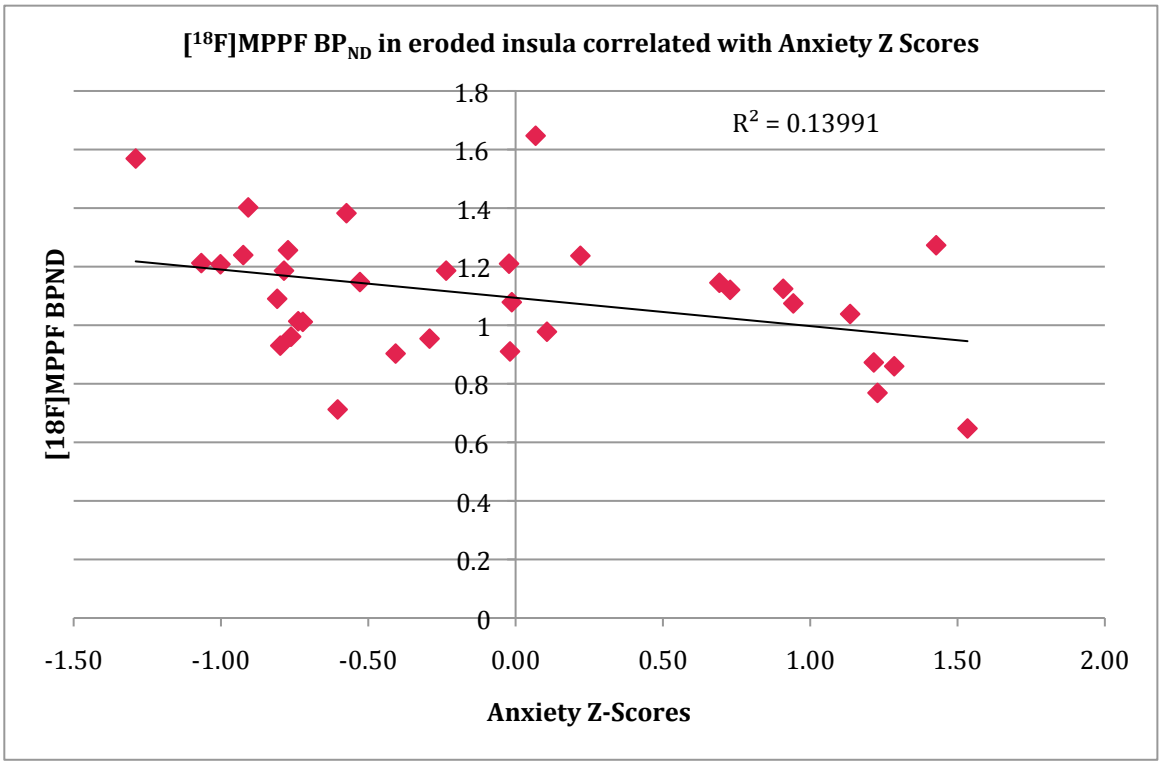
When a brain region contains voxels that span across boarders into other regions or multiple tissue types with differential binding, partial volume effects result. That voxels binding values will then be altered because differential signal values are averaged within the voxel space. This is likely to occur in a number of brain regions, especially near fluid filled spaces or adjacent areas of low and high binding. Ultimately the binding values at these "edges" are inaccurate. Though partial volume was a more prominent concern in the context of the previous study (see chapter 2) because the hippocampus and medial temporal lobe is particularly prone to partial volume effects, atrophy associated with aging means that partial volume should be considered in any PET study of an aging population.

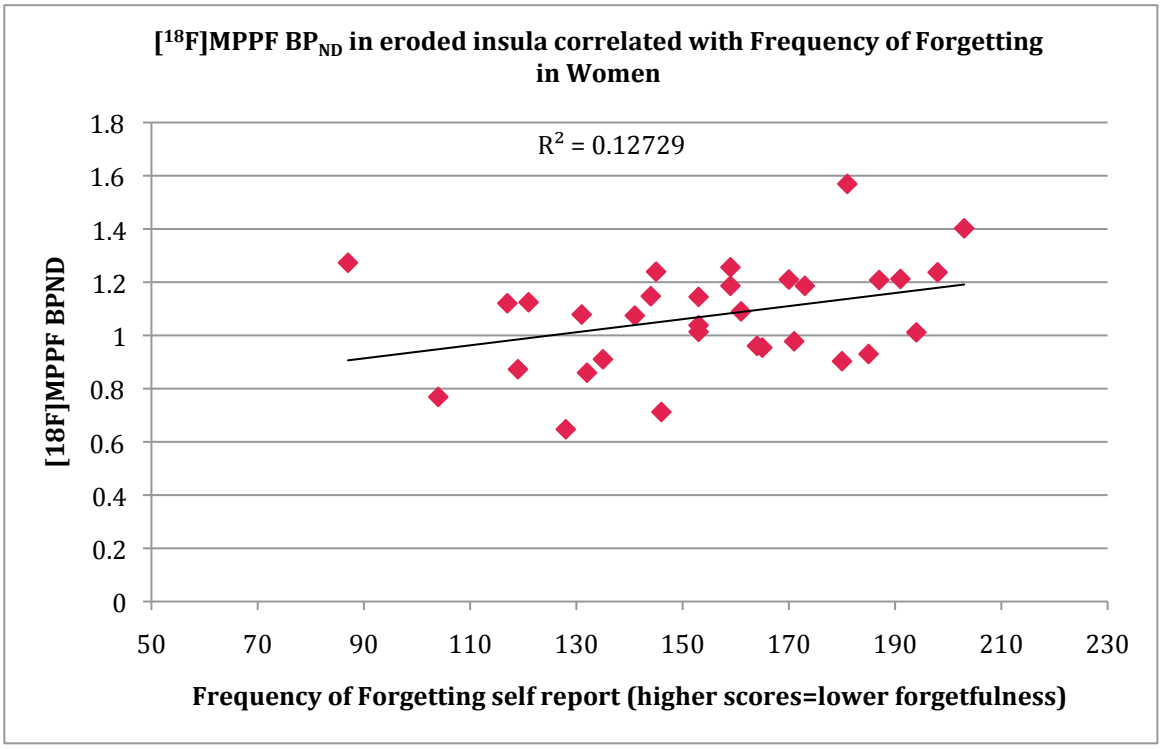
Once again we controlled for partial volume with an eroded VOI. We used FSLMaths to algorithmically erode our VOIs by shaving voxels from the perimeter of each structure in 3 dimensions. Thus, no VOIs voxels spanned boarders across tissues or regions. Once again we found that the absolute binding value for each region showed and increase, but the regional patter of binding across subjects remained the same. We believe that, particularly in the case of areas such as the insula that are surrounded by brain tissue on all sides, the anatomical VOI already does an adequate job of controlling for partial volume effects. The anatomical regions themselves are generated from the higher resolution MRI and provide a very tight mask for the area of binding we wish to capture. The number of voxels, even in the whole VOI, that might be prone to partial volume is therefore already low. The correspondence between binding patterns in whole and eroded VOIs supports this conclusion.

**Results**

[$^{18}$F]MPPF binding in insula (measured with eroded VOI), but not amygdala was significantly correlated with anxiety, as well as with self-reported frequency of forgetting, Clinical Dementia Rating Scale, working memory scores (digit span), and APOE genotype in women (see Figure 3.1). In a linear regression model we sought to predict self-reported frequency of forgetting in women. We included anxiety Z score, Age, Depression Z score, Clinical Dementia Rating, [$^{18}$F]MPPF eroded insula and Amygdala binding, APOE allele and atrophy score. We found that anxiety scores, APOE status, atrophy score, and Insula, but not amygdala binding were all significant predictors of increased self reported frequency of forgetting ($p<0.00001$, see Figure 3.2). Furthermore we found that self reported frequency of forgetting was significantly correlated with scores on the WAIS-digit span, a measure of short-term memory function ($p<0.02$, see Figure 3.3).

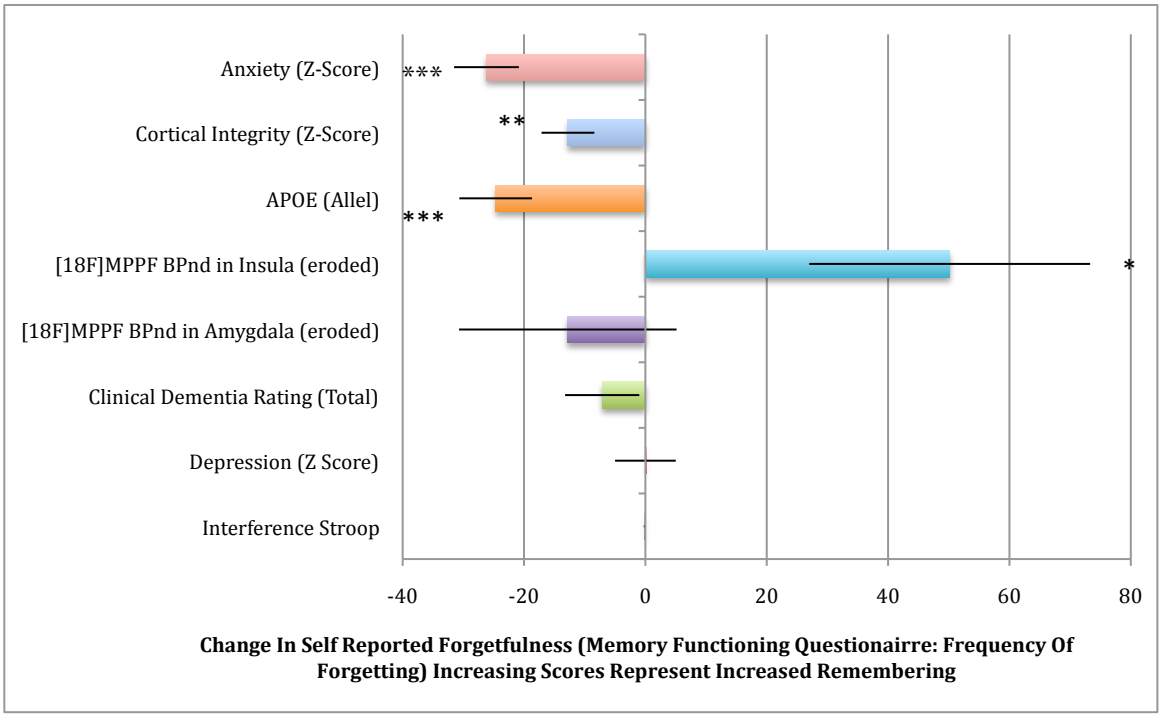

Anxiety Z-Score and Self Reported Freqency of Forgetting
Frequency of Forgetting self report (higher scores=lower forgetfulness)
250
200
150
100
50
0
R² = 0.5273
-1.50
-1.00
-0.50
0.00
0.50
1.00
1.50
2.00
Anxiety Z Score


[18F]MPPF BPND in eroded insula correlated with Anxiety Z Scores
[18F]MPPF BPND
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
R² = 0.13991
-1.50
-1.00
-0.50
0.00
0.50
1.00
1.50
2.00
Anxiety Z-Scores




**Figures 3.1 A, B and C:** A: Anxiety Composite Z-score correlated with self reported frequency of forgetting, where score corresponds to number of things remembered (and there for higher score represents lower forgetting); B: Anxiety Composite Z score correlated with [$^{18}$F] MPPF average eroded binding in insula; C: [$^{18}$F] MPPF average eroded binding in insula correlated with self reported frequency of forgetting, where score corresponds to number of things remembered (and there for higher score represents lower forgetting)

Finally, we see an inverse relationship between [$^{18}$F]MPPF insula binding and anxiety in women versus men. In a subset of men and women matched for age, years of education, and APOE status we found precisely the same trend seen in the total samples of men and women, suggesting that this relationship is robust and not an artifact of ascertainment bias (see Figure 3.4). Our overall sample of men is smaller than our sample of women, thus we do not have a large enough N to model the same predictive linear regression shown for women.



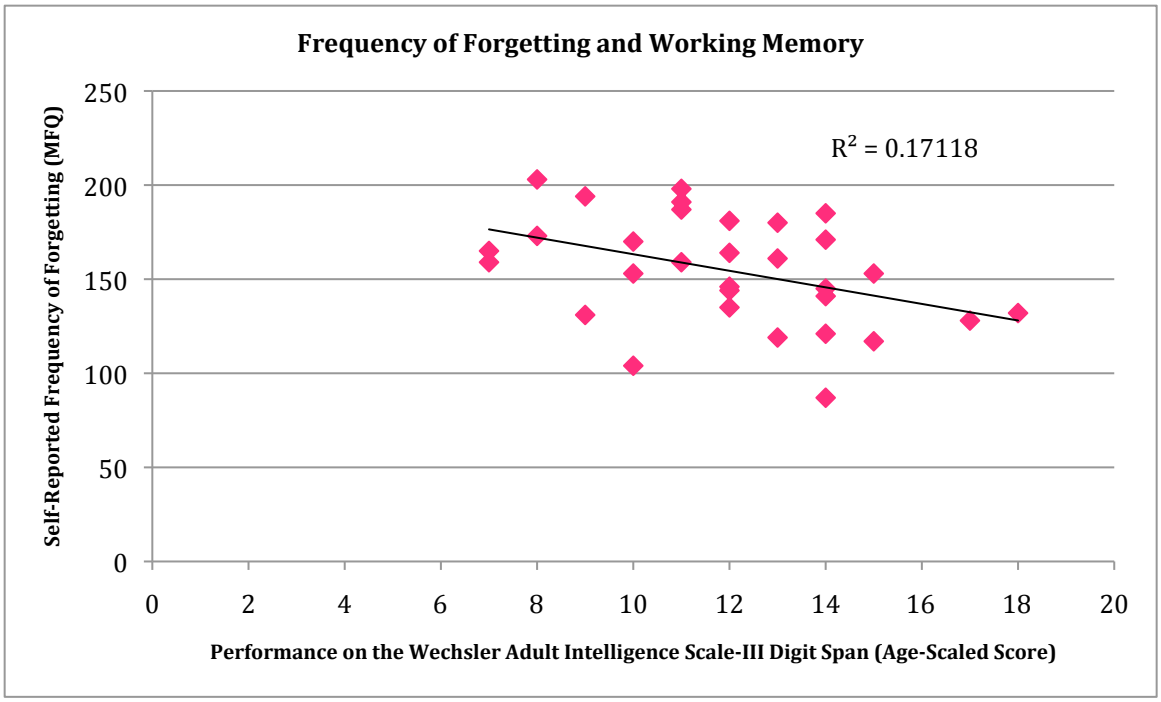
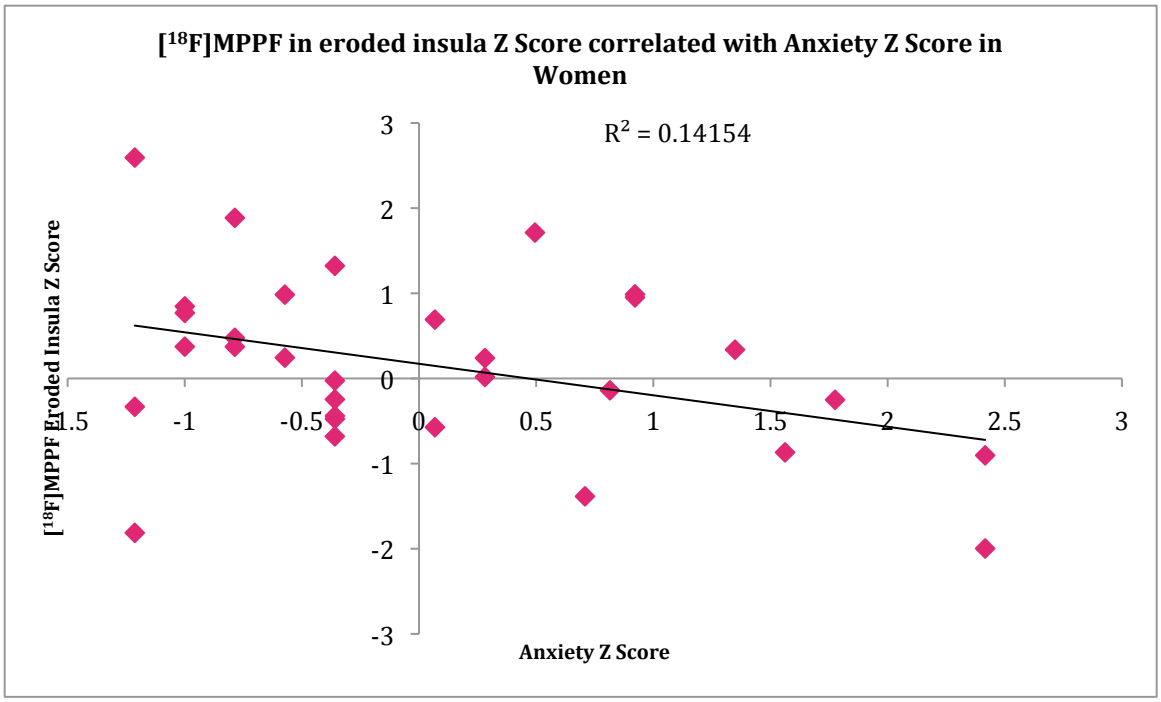




**Figure 3.2(top):** Regression model of Anxiety Composite Z-score, atrophy ratio, self reported frequency of forgetting, where score corresponds to number of things remembered (and there for higher score represents lower forgetting), APOE status, [$^{18}$F] MPPF average eroded binding in insula and amygdala, Clinical Dementia Rating, Depression Composite Z score, and performance on the Interference Stroop task.
**Figure 3.3 (bottom):** Correlation between self-reported frequency of forgetting and scores on the WAIS-digit span test of working memory.



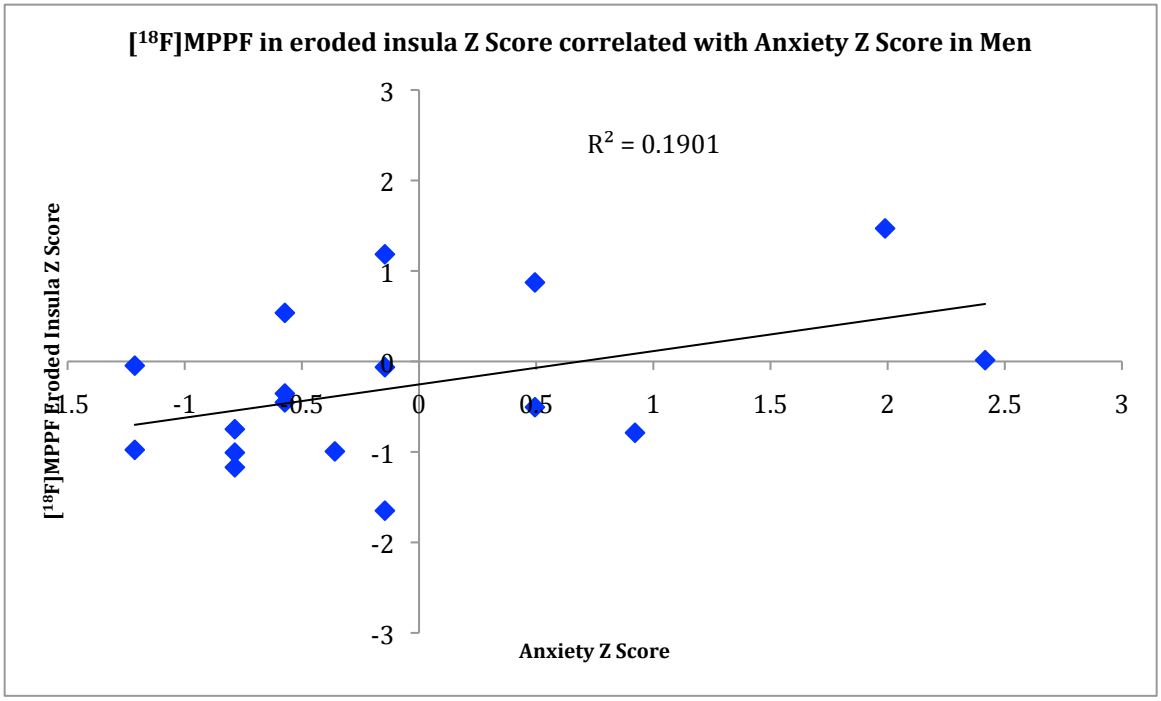




**Figure 3.4:** Anxiety Composite Z-score correlated with [18F]MPPF eroded insula Z score in women (Above in Red) and Men (Below in Blue)

## Discussion

We expected to see higher anxiety correlated with lower binding in areas associated with or previously shown to play a role in anxiety related behaviors and disorders. While, to our surprise, we did not see this relationship in the amygdala, we did see the expected relationship in the insula. Furthermore we saw the expected relationship between anxiety, memory, and insula receptor density in women only. Women with higher 5-$HT_{1A}$ receptor density in insula had lower anxiety and lower forgetting while women with lower binding had higher forgetting and higher anxiety. Frequency of forgetting also correlates with clinical dementia rating (Total), and standard score digit span, suggesting that the perceived frequency of forgetting is not independent of cognitive functioning, and therefore is not solely due to increased general anxiety. Total Clinical Dementia Rating correlates with atrophy score and with average [$^{18}$F]MPPF binding in hippocampus (eroded VOI). This relationship is an important indication of the connection between anxiety and memory performance, particularly in women.

The highly significant relationship between experienced forgetting and measurable anxiety is correlated with non-subjective memory scores as well as Clinical Dementia Rating, suggesting that people's perception of cognitive status is related to actual cognitive status. APOE status and $5HT_{1A}$ binding in the insula are both significant predictors of this relationship, suggested that underling physiology is contributing to both the anxiety and associated memory deficit. As previously mentioned, there is a relationship between early life depression and anxiety and later development of dementia, however the nature of this relationship is still not well understood. It is possible that those particularly susceptible to higher stress and anxiety responses are at greater physiological risk for neurodegeneration in the same way that those who

experience physical head trauma would be. Whether the interaction between anxiety and memory is direct or indirect, the sex differences exhibited here may reflect the same differences that account for differential rates of AD in women and men. Particularly the inverse relationship between insula 5-HT1A receptor density and anxiety in women and men is indicative of fundamental differences in these groups that should be carefully considered in future research

In future, both sex differences and mood disruptions such as stress and anxiety are essential factors that must be included in aging and longitudinal investigations. Attempting to determine overall lifetime burden of stress with diagnostics like the Stressful Life Events Screening Questionnaire (SLESQ), may help to determine how depression and anxiety have impacted HC function and anatomy in older age (Goodman et al 1998).

## V. CONCLUSION

Alzheimer's disease is likely a disease of complex origin in which multiple genetic and environmental pathways can lead to similar outcomes. The challenges of our increasing population age and size will inevitably make cognitive decline in general, and Alzheimer's disease specifically, a focus of great concern. In some sense, living consistently to ages at which cognitive symptoms become prevalent is a privilege, which more and more countries are gaining. This means that Alzheimer's disease will likely become a global concern; especially problematic when the increase in population longevity is accompanied by a decreasing birth rate, a trend common in highly developed and educated countries, because it will put undue burden on a dwindling population of younger caregivers.

Understanding lifestyle factors that increase risk of the disease is a major preventive step in fighting this global challenge. Much like traumatic brain injury, many risk factors may in fact be lifestyle factors that effectively expose the brain to harmful influence. We have shown here that both BMI and age show a significant relationship with plaque and amyloid load, even in cognitively normal and otherwise healthy adults. This corresponds with the link between diabetes and poor vascular health already established, and may be an indication that all of these factors expose brain tissue to a damaging inflammatory response or similar insult. Likewise anxiety and depression, in some cases a form of stress response, may be producing damaging changes in brain chemistry or even harmful cellular gene transcription. Our findings support the notion that anxiety is a predictor of differences in the serotonin system and an associated poor memory performance in women. Whether this anxiety is the result of fundamental life-long differences in biology or very early changes associated with cognitive decline should be the subject of future investigation. It may be the case that something as simple as teaching stress management in youth could hugely reduce risk of dementia later in life.

Finally we see that 5-HT1A receptor density in the hippocampus and medial temporal lobe predicts memory performance. Because this measured density adds predictive value beyond atrophy alone, we propose that the PET ligand [$^{18}$F]MPPF could be used as a biomarker for future studies of both memory and anxiety in healthy adult and longitudinal populations. This ligand may further utility in differentiating lifelong functional differences in hippocampal receptor densities and associated behavioral differences.

Overall the effects of mood, stress and body health on the aging brain should be considered important biological influences worthy of study. For those who wish to know what they can do now to reduce future risk, clearly the same techniques touted for general disease prevention may be applicable here. Successful stress management, and good physical health can only help the aging brain.

# VI. APPENDIX

APPENDIX 1: AAA Memory Rating Scale for Clinical Consensus Diagnostic Criteria

Immediate: LMI, VPI, VRI, Buschke Total, Hopkins Total
Delayed: LMII, VPII, VRII, Buschke Delay, ReyO Delay, Hopkins Delay
Non-Memory: [Digit Symbol, Trails A, Stroop A/B], [FAS, Animals, Boston], [Block Design, ReyO Copy], [Trails B, Stroop C, Similarities, WCST]

✓ **Check One:**

**1.1_____Normal Performance**
- No memory complaints.
- Absence of dementia.
- Memory is above average for peers and is equivalent to young adults.
- Performance is within +/- 1 standard deviation for young adults on all memory tests.
- There is no score below -1 standard deviation on any memory tests.

**1.2_____Age Associated Memory Impairment (AAMI)**
- Complaints of memory loss.
- Absence of dementia.
- Memory may be above average for peer group, but below that of young adults.
- Performances are at least 1 standard deviation below young adults on at least one test.
- At least 50% of memory tests are above 1 standard deviation.

**1.3_____Age Consistent Memory Impairment (ACMI)**
- Memory is consistent, and not better, than age norms.
- Memory performance is within +/- 1 standard deviation for age on 60-75% of memory tests.

**2.1_____Mild Cognitive Impairment—Amnestic Only (MCI-a)**
- Mild to moderate impairments in comparison to age norms, but not frankly demented.
- Performance is 1 SD below age norms on 2 out of 6 delayed memory tests.

**2.2_____Mild Cognitive Impairment—Non-Amnestic Only (MCI-na)**
- Mild to moderate impairments in comparison to age norms, but not frankly demented.
- Performance is 1 SD below age norms on 2 or more of non-memory tests within a domain (psychomotor speed, language, visual spatial, or executive).

**2.3_____Mild Cognitive Impairment—Multiple Domains (MCI-a+)**
- Mild to moderate impairments in comparison to age norms, but not frankly demented.
- Performance is 1 SD below age norms on 2 of 6 delayed memory tests AND on 2 or more of non-memory tests within one or more domains.

**_____Dementia**
- Exhibits signs of frank dementia.
- MMSE score of 24 or lower.
- 2 or more standard deviations below age norms on the majority of memory tests.

APPENDIX 2

| **Cortical and Subcortical Regions Defined in Freesurfer** | | | |
|---|---|---|---|
| 100 | LbanksstsDK | 2001 | RbanksstsDK |
| 1002 | LcaudalanteriorcingulateDK | 2002 | RcaudalanteriorcingulateDK |
| 1003 | LcaudalmiddlefrontalDK | 2003 | RcaudalmiddlefrontalDK |
| 1005 | LcuneusDK | 2005 | RcuneusDK |
| 1006 | LentorhinalDK | 2006 | RentorhinalDK |
| 1007 | LfusiformDK | 2007 | RfusiformDK |
| 1008 | LinferiorparietalDK | 2008 | RinferiorparietalDK |
| 1009 | LinferiortemporalDK | 2009 | RinferiortemporalDK |
| 1010 | ListhmuscingulateDK | 2010 | RisthmuscingulateDK$ |
| 1011 | LlateraloccipitalDK | 2011 | RlateraloccipitalDK |
| 1012 | LlateralorbitofrontalDK | 2012 | RlateralorbitofrontalDK |
| 1013 | LlingualDK | 2013 | RlingualDK |
| 1014 | LmedialorbiofrontalDK | 2014 | RmedialorbiofrontalDK |
| 1015 | LmiddletemporalDK | 2015 | RmiddletemporalDK |
| 1016 | LparahippocampalDK | 2016 | RparahippocampalDK |
| 1017 | LparacentralDK | 2017 | RparacentralDK |
| 1018 | LparsopercularisDK | 2018 | RparsopercularisDK |
| 1019 | LparsorbitalisDK | 2019 | RparsorbitalisDK |
| 1020 | LparstriangularisDK | 2020 | RparstriangularisDK |
| 1021 | LpericalcarineDK | 2021 | RpericalcarineDK |
| 1022 | LpostcentralDK | 2022 | RpostcentralDK |
| 1023 | LposteriorcingulateDK | 2023 | RposteriorcingulateDK |
| 1024 | LprecentralDK | 2024 | RprecentralDK |
| 1025 | LprecuneusDK | 2025 | RprecuneusDK |
| 1026 | LrostralanteriorcingulateDK | 2026 | RrostralanteriorcingulateDK |
| 1027 | LrostralmiddlefrontalDK | 2027 | RrostralmiddlefrontalDK |
| 1028 | LsuperiorfrontalDK | 2028 | RsuperiorfrontalDK |
| 1029 | LsuperiorparietalDK | 2029 | RsuperiorparietalDK |
| 1030 | LsuperiortemporalDK | 2030 | RsuperiortemporalDK |
| 1031 | LsupramarginalDK | 2031 | RsupramarginalDK |
| 1032 | LfrontalpoleDK | 2032 | RfrontalpoleDK |
| 1033 | LtemporalpoleDK | 2033 | RtemporalpoleDK |
| 1034 | LtransversetemporalDK | 2034 | RtransversetemporalDK |
| 1035 | LinsulaDK | 2035 | RinsulaDK |
| 17 | LHipp | 53 | RHipp |
| 18 | LAmyg | 54 | RAmyg |
| 16 | BStem | 7 + 46 | L & R Combined Cereb WM |

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