DEPRESSIVE SYMPTOMATOLOGY, HIPPOCAMPAL VOLUME, AND CONTEXTUAL MEMORY IN LATE LIFE GENERALIZED ANXIETY DISORDER: AN MRI STUDY

by

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ABSTRACT OF THE THESIS

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Research has shown that reductions in hippocampal volume can result from various forms of stress and are a feature of Major depressive disorder (MDD), with some studies suggesting similar impairments in PTSD. Yet there has been little research on the connection between stress and the hippocampus as it relates to other affective and anxiety disorders. The current study examined hippocampal morphology and functioning and their relationship to depressive symptomatology in older adults with Generalized anxiety disorder (GAD). We hypothesized that GAD patients may show reduced hippocampal volume and functioning; and having GAD might add to or magnify the adverse impact of depression on the hippocampus. Participants were 15 older adults diagnosed with GAD without co-occurring MDD and 15 age- and sex-matched non-anxious controls. Participants completed a diagnostic interview; measures of anxiety and depression; neuropsychological tests; and a structural MRI scan. The Verbal Paired Associates (VPA) test (Wechsler, 1997) was used to assess verbal contextual memory, a common index of hippocampal functioning. Higher levels of depressive symptomatology on the Beck Depression Inventory (Beck & Steer, 1987) were associated with smaller hippocampal

volumes, which were in turn associated with lower scores on VPA recall and on the Stroop color test (StroopC; Trenerry, Crosson, DeBoe, & Leber, 1989). The association between depression and the hippocampus was significantly stronger in the GAD sample. In addition, after controlling for several covariates, reductions in hippocampal volume and in VPA delayed recall and StroopC scores were found in those GAD patients with the highest levels of depression (n = 5). Findings suggest that alterations in hippocampal morphology and functioning may be evident in a subset of GAD patients, specifically those with higher levels of depression. They also support the idea that, in the context of GAD, even moderate levels of depression that fail to meet diagnostic criteria for MDD may have deleterious effects on the brain and mind. Implications for the conceptualization and treatment of GAD are discussed.

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I. Introduction

Generalized anxiety disorder (GAD) is the single most common yet also most poorly understood anxiety disorder among adults ages 60 and over (Flint, 1994; Lenze et al., 2005). The disorder is characterized by excessive, pervasive, and uncontrollable worry about various life circumstances and a range of other symptoms, including muscle tension, restlessness, fatigue, impaired concentration, and sleep disturbances (American Psychiatric Association, 2000, *Diagnostic and Statistical Manual of Mental Disorders*). Both the relatively poor response rates associated with current psychopharmacological and psychosocial treatments and ongoing rapid growth of the older adult population point to a need for improvements in our understanding and conceptualization of this disorder (Mohlman et al., 2009). Most existing studies of GAD have focused almost exclusively on the role of worry in GAD, with relatively little attention given to other symptoms and associated features (Rickels & Rynn, 2001), and almost no research examining the relationship between psychological and neurobiological processes in GAD (Sinha, Mohlman, & Gorman, 2004). It is likely that investigation of these neglected issues will advance our understanding of late life GAD.

One issue that has yet to be examined concerns the relationship of psychosocial stress and stress-related dysphoric mood states to brain structure and functioning in GAD. A large body of research indicates that chronic stress and depression can result in morphological changes in the brain, and damage to the hippocampus in particular (Campbell et al., 2004; McEwen, 2001; Sapolsky, 2001a). Such damage can have important consequences, since the hippocampus is known to play a vital role in certain forms of learning and memory formation. This structure appears to be especially pivotal

to the ability to process and encode information about relationships across contexts and between different environmental stimuli, including spatial, temporal, and contingency relationships (Greene, Gross, Elsinger, & Rao, 2007; Jeffery, 2007). A number of experiments with animals have demonstrated that exposure to chronic stress and sustained exposure to high levels of glucocorticoids (adrenal steroids which are produced in the adrenal cortex in response to various forms of psychosocial stress) can cause cell loss and consequent reductions in size in the rodent and primate hippocampus (McEwen, 2001; Sapolsky, 2001; Sapolsky et al., 1990).

Moreover, research has repeatedly shown reductions in hippocampal volume in long-standing and recurrent major depression in humans, which are thought to be related to cognitive deficits frequently observed in Major Depressive Disorder (MDD) (Bremner et al., 2000; Campbell et al., 2004; Sapolsky, 2001a; Sheline, 2000; Sheline et al., 1996). This volumetric reduction has been found to increase with long-term depression, and tends to be quite enduring, appearing in brain scans years after the depression has remitted (Sapolsky, 2000; Videbech & Ravnkilde, 2004), although several studies suggest that antidepressants may be effective in mitigating these effects (Sapolski, 2001; Warner-Schmidt & Duman, 2006). While earlier research findings were not entirely consistent, recent reviews of the literature and metaanalyses provide overwhelming support for the existence of volumetric reduction in the hippocampus in MDD**.** A metaanalysis of 12 studies of unipolar depression employing sound methodology found bilateral reductions in the hippocampus averaging 8% for the left and 10% for the right side (Videbech & Ravnkilde, 2004). Notably, it appears that older adults may be at elevated risk for these deleterious effects (Sheline, 2000).

There is some evidence to suggest that other forms of stress-related psychopathology, and Post-traumatic stress disorder (PTSD) in particular, may also be characterized by reductions in the hippocampus. However, research in this area is far less extensive and conclusive than the evidence pertaining to major depression. Examining trauma victims**, s**ome studies have identified hippocampal volumetric reductions in severe, unremitting PTSD (e.g., Sapolsky, 2001b**)**, however other studies have failed to find such an effect (Bonne et al., 2001). This issue continues to be debated (Villarreal & King, 2001; Kitayama, Vaccarino, Kutner, Weiss, & Bremner, 2005). Researchers have yet to examine whether similar processes might occur in other mood or anxiety disorders, such as GAD.

To be sure, much of the research on hippocampal volume in human depression has been correlational and cross-sectional, precluding inferences about the direction or nature of causation. It is therefore possible that the observed relationship may be the product of a third variable. It is also possible that the reduction in hippocampal volume might precede the onset of the affective disorder, and might even predispose toward it, rather than emerging as a result of the depression. Indeed, some investigators (e.g., Lyons et al., 2001; Gilbertson et al., 2002) have speculated that divergences in hippocampal volume are heritable and may be a cause, rather than a consequence, of increased stress susceptibility and affective pathology.

Nevertheless, the preclinical and clinical literature provides ample reason to believe that conditions of chronic stress, of the sort that occur during episodes of major depression can in fact result in damage to the hippocampus. (McEwen, 2001; Sheline, 2000). The animal literature, in which experimentally induced stress and increases in

glucocorticoid levels have reliably been shown to cause damage to the hippocampus, provides strong support for a causal relationship, to the extent that findings are generalizable to humans. The possibility of stress-induced neurodegenerative effects is also consistent with relevant knowledge concerning the neurobiology of MDD. Such increases in glucocorticoid levels (cortisol in humans) are frequently, though not universally, seen in major depressive episodes in humans.

It is conceivable, as some models propose (see especially McEwen & Seeman, 1999), that causation may operate in both directions. More concretely, smaller hippocampi, resulting from some interaction of genetic and early environmental factors (e.g., abuse or exposure to high levels of environmental stressors) may operate as a risk factor for the experience of psychological and biological manifestations of negative affect and stress and, in some cases, for the development of stress-related emotional disorders. Conversely, the experience of these disorders and their neurobiological concomitants may in turn affect the hippocampus through a variety of channels that have yet to be fully understood (McEwen & Seeman, 1999; McEwen, 2003).

While the precise biological mechanisms of hippocampal volume loss in MDD remain unclear, research suggests that among some individuals, it may be partly attributable to deregulation of the HPA axis, which results in hypersecretion of cortisol and insensitivity to glucocorticoid feedback, and consequent neuronal loss (Lee, Ogle, & Sapolsky, 2002; Sapolski, 2001; Sheline, 2000). Other possible biological mechanisms include a loss in glial cells, which may increase vulnerability to glutamate neurotoxicity (as excessive levels of glutamate in the synapse cause damage to NMDA receptors); and stress-induced reduction in neurotrophic factors (proteins that support the growth and

differentiation of new neurons and the survival of existing neurons) (McEwen, 2001; Sheline, 2000).

In addition, there is mounting evidence, largely from animal models, that depression and various other types of stress may cause eventual reductions in hippocampal volume by inhibiting neurogenesis, a process which has been found to occur throughout adulthood and is centered primarily in the hippocampus (Mirescu & Gould, 2006). Stress has a direct effect on neurogenesis, causing a protracted decrease in the rate of cell proliferation, and there is increasing consensus that this mechanism may play a large role in producing the hippocampal volume loss and associated cognitive deficits observed in depression (McEwen, 2001; Mirescu & Gould, 2006; Warner-Schmidt & Duman, 2006).

In addition to this direct effect, the proportion of these new cells that survive appears to depend, in large part, on the extent to which the organism engages actively in effortful learning activities, in the absence of which most of the cells fail to survive (Leuner, Gould, & Shors, 2006). Physical activity, in the form of exercise, also appears to have beneficial effects in mitigating the adverse effects of aging (Tong, Shen, Perreau, Balazc, & Cotman, 2001; van Praag, Shubert, Zhao, & Gage, 2005). Clearly, individuals who experience clinical depression or other forms of prolonged psychological distress are not likely to engage in such activities, given the avolition, fatigue, depressed mood, and restricted activity that typically characterize this condition. And the prognosis may be considerably worse for older adults suffering from affective disorders, whose normal lifestyle and environment fail to provide opportunities to engage in the sort of learning required to prevent the loss of new cells.

In this connection, it is notable that many have argued that GAD is tantamount to a state of chronic stress or low level depression, due to the frequent, uncontrollable worry and other impairing symptoms associated with this disorder (Mennin, Heimberg, & Turk, 2004; Sinha, Mohlman, & Gorman, 2004) and the tendency of GAD patients to experience negative emotions with greater frequency and intensity (Mennin et al., 2004; Mennin, Heimberg, Turk, & Fresco, 2005). There is a growing belief that GAD is in many ways similar, and perhaps related, to clinical depression, in terms of its nature, its underlying psychological and pathophysiological mechanisms, and its etiology (see First, 2007). One reason is that the symptom profile of people with GAD is very similar to that of individuals with chronic stress and low levels of depression (Clark, Beck, & Beck, 1994; Donahue, 2005; Mennin, Heimberg, & Turk, 2004). Like depressives, patients with GAD are often prone to irritability, restlessness, fatigue, difficulty concentrating, and sleep disturbances, and many exhibit additional symptoms associated with depression (Kessler et al., 1999). Indeed, overlap in symptom profiles, strikingly high rates of comorbidity between GAD and major depressive disorder (MDD), as well as etiological links brought to light by genetic and developmental research, have prompted many researchers to suggest that the two disorders may be closely related in their nature and etiology, though the precise nature of the relationship remains unclear (Hudson & Rapee, 2004; Kessler, Keller, & Wittchen, 2001; Moffitt et al., 2007).

Given these connections, we might well question whether neurodegenerative processes similar to those evident in major depressive disorder and prolonged stress exposure may also operate in GAD, even in the absence of co-occurring MDD, an issue which has not yet been examined. In fact, there is some reason to believe that some of the

same pathophysiological and psychological mechanisms thought to play a role in hippocampal atrophy in MDD might operate in some individuals with primary GAD. Though research on the neurobiology of GAD is far less advanced and suggests a somewhat different biological profile from that of pure MDD, GAD patients show some signs of HPA axis dysregulation and dysfunction in the body's stress response, such as elevated glucocorticoid release, neuronal loss, and neurotoxicity (Nutt, 2001; Sinha, Mohlman, & Gorman, 2004). Neurobiological abnormalities, which might play a role in the development and maintenance of the disorder and might be further magnified by the cumulative effects of stress over time experienced by many individuals with this disorder. This could make GAD patients vulnerable to the harmful effects of stress and depression on the hippocampus and consequent cognitive impairments.

Adding to the small body of literature on this topic, a recent study by Mantella et al. (2008) found that older adults with GAD showed considerable elevations in basal salivary cortisol levels relative to a non-anxious comparison group (a 40 to 50% increase), with especially large elevations occurring during early morning hours after awakening when cortisol levels normally reach their peak. Such elevations were evident among participants with "pure GAD" as well as those with comorbid anxiety or mood disorders, with no significant differences among groups, and they were positively correlated with measures of GAD symptom severity. As the researchers observe, the pattern shown by GADs in this study strongly resembles that found among individuals with depression, PTSD, or chronic stress, and over time, could conceivably contribute to hippocampal atrophy (Mantella et al., 2008).

Moreover, on the psychological level, individuals with GAD are constantly engaged in worry, which current prevailing models propose functions as means of cognitive avoidance and serves to inhibit emotional processing (Borkovec, Alcaine, & Behar, 2004; Borkovec, Ray, & Stober, 1998). Worry is thought to be maladaptive in the long run, because it has been found to perpetuate anxiety as well as dysphoric mood, in part by interfering with the formation of new associations and learning (Borkovec, Ray, & Stober, 1998). Chronic, excessive pathological worry, which is the hallmark feature of GAD, may interfere with the capacity to downregulate negative emotion and to cope effectively with dysphoric mood and other symptoms of low-level depression. Such worry may also interfere with engagement in other forms of mental activity that might facilitate the potential benefits of adult neurogenesis. Past research has demonstrated that worry involves substantial involvement of the prefrontal cortex and recruits limited executive resources **(**Mohlman et al., 2009; Price & Mohlman, 2007**)**. In GAD, the prefrontal cortex may not be fully available for regulating emotion or for engagement in learning of association among variables and cause and effect relationships, which recruits the hippocampus.

In addition to their overreliance on worry, recent research suggests that individuals with GAD may also experience substantial deficits regulating their emotions (and negative emotions in particular), including difficulties identifying and understanding negative emotions and repairing negative mood states (such as sadness, anxiousness, or anger) (Mennin et al., 2004; Mennin, Heimberg, Turk, & Fresco, 2005). These maladaptive and often ineffective patterns of regulating negative emotions might further increase the vulnerability of GAD patients to the degenerative effects of stress and

depression. In particular, they may increase the likelihood of more prolonged, intense, and frequent periods of stress, in effect, increasing allostatic load (McEwen, 2001).

It is reasonable to expect that older adults with GAD may be particularly at risk for and will be more likely to exhibit such degenerative effects. Several reasons for this are that (1) progressive hippocampal volume loss (averaging about one to two percent annually) has been found to occur even in healthy elderly individuals with no signs of cognitive impairment or dementia (Jack et al., 2000),[1] and (2) on average, older patients are more likely to have experienced a greater amount and longer periods of stress and distress in their lifetime relative to younger counterparts, though individual differences are to be expected. Given these facts, it seems appropriate that initial research on this topic should focus on older adults, both from a pragmatic and a public health perspective.

The current study examined the relationship among psychological distress, and depressive symptomatology, in particular; hippocampal volume and functioning; and late life GAD. The study employed structural magnetic resonance imaging (MRI) to measure regional brain volume and neuropsychological testing to assess neurocognitive abilities. Our predictions were informed by past research on the neurodegenerative effects of stress and clinical depression; the connections between GAD and major depressive disorder; and apparent dysregulation in neurohormonal systems and emotional processing in GAD. In light of this evidence, we hypothesized that older adults with GAD may be particularly vulnerable to stress-related degradation of the hippocampus and accompanying impairments in hippocampal-dependent cognitive processes, such as contextual memory.

The study tested several specific hypotheses. The first hypothesis was that GAD patients (GADs) may show smaller hippocampi, and impaired hippocampal functioning,

relative to psychologically healthy age- and sex-matched controls. Such findings would be consistent with the evidence suggesting a close relationship between GAD and MDD (First, 2007). However, there was also reason to believe such gross inter-group differences might not emerge, given (a) considerable heterogeneity among older adults diagnosed in GAD and (b) the possibility that hippocampal reductions might be linked to more specific symptoms, such as depressive symptomatology, excessive stress, or elevated cortisol levels, which individual patients experience to varying degrees. It is conceivable that the level of stress GAD patients experience, on average, might not be sufficiently intense or enduring to result in the sort of neural damage seen in chronic/recurrent depression.

A second hypothesis tested was that GAD and depression would have additive effects on the hippocampus, such that GAD itself would be associated with compromised hippocampal integrity, but impairment would be greater among individuals who also experience high levels of depression. Alternatively, it is possible that individuals with GAD may be more vulnerable to the adverse effects of dysphoric mood and depressive symptoms on the mind and brain. This seems likely given the evidence of dysfunctions in the neurobiological systems governing the body's stress response, excessive engagement in (and allocation of cognitive resources to) worry, and dysfunctions in emotion regulation and adaptive coping. These factors may make individuals with GAD ineffective at reducing or mitigating the deleterious impact of stress and depressive symptoms on the hippocampus. More concretely, the third hypothesis tested was that, even in non-clinically depressed patients, elevated levels of depressive symptomatology would be associated with smaller hippocampal volumes (along with poorer contextual

memory and other hippocampus-dependent cognitive skills) among GADs, but not among NACs, for whom low to moderate levels of depression are not expected to have nearly the same impact. The study also aimed to test whether the association with the hippocampus is unique to depressive symptoms (as opposed to worry or symptoms more commonly associated with anxiety).

II. Method

Participants

Participants were 30 older adults, ranging from 60 to 77 years of age ($M = 67.87$, $SD = 5.36$), recruited from the Syracuse, NY community via radio and newspaper advertisements, as well as community outreach (e.g., talks at senior centers), as part of clinical trial for late life GAD. Eligibility was determined principally on the basis of a phone screening and subsequent structured interview, the *Structured Clinical Interview Diagnostic for DSM-IV Axis I Disorders* (SCID; First, Spitzer, Gibbon, & Williams, 1995).

The sample comprised 15 individuals who met criteria for GAD in the absence of current comorbid major depression based on the SCID (GADs), along with 15 non-anxious controls (NACs) matched on age and sex who did not meet criteria for any psychiatric disorder. All participants met basic inclusion criteria, specifically: verbal and written fluency in English, intact basic cognitive functioning (operationalized as a Mini-Mental State Exam score ≥ 24; Folstein, Folstein, & McHugh, 1975), and right-handedness. Potential participants were excluded if they exhibited a history of psychotic symptoms, manic or hypomanic episodes, suicidality in the previous year, or metal implants in the body. In addition, they were required to be free of the use of anxiolytic or antidepressant medication at the time of the interview and brain scan and during the year prior.

Slightly more than half of participants (60%; 9 GADs and 9 NACs) were female, and the majority (90%) were Caucasian-American, with African-Americans, Asian Americans, and Latino/Hispanics constituting the remaining 10%. Level of educational

attainment varied. 13.3% were high school graduates, 36.7% had completed one to three years of college or an Associate's degree, and 50% held a four-year college degree or above. Most participants (73.3%) were retired. 40% were married or cohabitating, 30% divorced or annulled, 16.7% widowed, and only 13.3% never married. Information on physical health was also collected from patients during the clinical interview and assessment. The number of major health problems (e.g., emphysema, diabetes, chronic obstructive pulmonary disease) reported ranged from 0 to 4, with a mean of 1.17 (*SD* = 1.12). The number of minor health problems (e.g., joint pains, back pain, allergies, hernias, bunions, prostate problems) ranged from 0 to 7, with an average of 2.33 (*SD* = 1.71). The mean number of prescriptions taken for general medical conditions was 2.10 (*SD* = 1.71; range = 0 – 6). Chi-square and *t*-tests revealed no significant differences between GADs and NACs in educational level, retirement rates, or marital status (all χ^2s < .60, all *p*s > .10), nor in numbers of major or minor health problems, or daily medication used (all *t*s < 1.20, all *p*s > .10).

Procedure

All participants underwent an assessment at the State University of New York Upstate Medical University in Syracuse, NY. Following the consent process, participants completed the *Structured Clinical Interview Diagnostic for DSM-IV* (*SCID*), a packet of psychological questionnaires including the BDI, and neuropsychological tests. In a second session held within two weeks, they underwent a 15-minute structural magnetic resonance imaging (MRI) scan. One of the control participants completed the SCID and MRI, but did not complete the questionnaires or neuropsychological tests due to an unanticipated time conflict.

Interview and self-report measures

Diagnoses were established using the SCID (First et al., 1995), which was administered by masters and doctoral-level assessors who had undergone six months of prior training. 75 percent of patient SCIDs (n = 10) were observed by a supervisor to ensure reliability. In addition, a random sample of 12 audiotaped SCIDs independently rated by a blind assessor produced a Kappa coefficient of .92 for diagnosis of GAD, indicating high inter-rater reliability. However, this estimate might be somewhat inflated since participants had passed a preliminary phone screen designed to ensure a high likelihood of meeting GAD criteria.

Participants in both groups completed a packet of self-report questionnaires assessing symptoms of anxiety and depression. The primary measure of depressive symptomatology was the Beck Depression Inventory (BDI; Beck & Steer, 1987). The BDI is a well validated and widely used 21-item self-report used to assess the severity of depressive symptomatology, spanning the wide range of cognitive, affective, behavioral, motivational, and somatic symptoms associated with depressive mood states. Higher scores on the BDI indicate more severe depressive symptoms, with scores of 10 to 18 indicating mild to moderate depression, scores of 11 to 29 indicating moderate to severe depression, and scores of 30 and above indicate severe depression. Anxious arousal was measured using the Beck Anxiety Inventory (BAI; Beck & Steer, 1987). The Penn State Worry Questionnaire (PSWQ; Meyer et al., 1990) and Spielberger State-Trait Anxiety Inventory – Trait subscale (STAI-T; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983) were used to index the degree of pathological worry and primary (trait) anxiety, respectively. Participants also completed the Geriatric Suicide Ideation Scale (GSIS;

Heisel & Flett, 2006), a measure of late life suicidal risk that assesses suicidal ideation and related constructs (thoughts of death, loss of personal and social worth, and perceived meaning in life). In the current sample, internal consistency (α) coefficients ranged from .86 to .94, with a coefficient of .90 for the BDI.

Additionally, clinician-rated measurements of depression and anxiety were obtained using the Hamilton Rating Scales for depression (HAMD; Hamilton, 1960) and anxiety (HAMA; Hamilton, 1959), which were administered by graduate students trained to a gold standard (a licensed clinical psychologist with 10 years of experience administering these measures). These widely used, interview-based measures were intended to serve as a useful supplement to the self-report measures and provide a means of assessing particular symptom clusters, such as depressed mood; difficulties falling or remaining asleep; psychomotor retardation or agitation; reduction in appetite/eating; somatic symptoms (heaviness in limbs, back, or head, aches, fatigue); and loss of interest or capacity for activities, work, or hobbies. Inter-rater reliability was adequate for the Hamilton scales (κ = .72 for HAMD; .67 for HAMA). All measures have been shown to have sound psychometric properties in older adult samples (e.g., Beck, Stanley, & Zebb, 1995; Gallagher, Nies, & Thompson, 1982; Himmelfarb & Murrell, 1983; Stanley, Beck, & Zebb, 1996).

Neuropsychological measures

All subjects completed a battery of standard neuropsychological tests. Of greatest relevance to the present study, verbal associative/contextual memory was assessed using the Verbal Paired Associates recall and delayed recall (VPA and VPA II) tests from the Wechsler Memory Scale, Third Edition (WMS-III; Wechsler, 1997). These tests involve

a paired word learning task, in which a list of eight word pairs is read aloud to the subject four times. Each reading is followed by a memory trial in which the first item of the pair is presented and the subject is asked to recall the associated word. To preclude positional learning, the order of the word pairs is varied across blocks. Each correctly recalled pairing earns one point and the number of correct responses for the four blocks are summed to obtain a total recall score (termed "Recall" below). Scores on this measure, which assesses cued new learning, have a possible range from 0 to 32. The second part of the task (VPA II) took place following a delay of approximately 15 minutes and comprised an additional recall trial including the previously presented word pairs. Retention was measured by the number of words recalled during this delayed recall trial divided by the number of words the participant correctly recalled previously in the final memory block. Possible retention scores ranged from 0 to 1.0, with higher scores reflecting greater recall on the delayed recall trial. Raw scores on these two measures (VPA I Recall and VPA II Retention) were converted to age-normed standardized scores (*t*-scores) based on normative data provided by Mitrushina, Boone and D'Elia (1999).

Additional tests included the Mini-Mental State Exam (Folstein, Folstein, & McHugh, 1975), the Digit Span test from the Wechsler Adult Intelligence Scale-Third Edition (WAIS-III; Wechsler, 1997), estimated verbal intelligence quotient from the American Nelson Adult Reading Test (AMNART; Grober & Sliwinski, 1991), the Stroop Color and Color-Word Tests (Trenerry, Crosson, DeBoe, & Leber, 1989), and the Similarities Test from the Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999). The Stroop Color Word Test was of particular interest in light of growing evidence that task performance is mediated not only by frontal cortical regions, but also by a broader

network including the hippocampus and various posterior cortical areas (Strauss, Sherman, & Spreen, 2006). Moreoever, performance on this task and on other tests of complex executive skills thought to be mediated largely by the prefrontal cortex may be impaired as a consequence of corticosteroid exposure, where there appears to be some connection with depressive symptoms (Brown et al., 2004).

Brain imaging

Brain images were acquired using a Philips Medical full body MRI scanner, a 1.5 T Intera with Gyroscan version 8.1.3 software. The brain images were acquired using a quadrature radio frequency receive-only head coil and inversion recovery prepped T1 weighted radio frequency pulse sequence covering the brain in transaxial view with a total of 100 1.5mm slice thickness images. Prep time and weighting were optimized to produce best distinction for image post-processing reconstruction and brain structure segmentation. The MRI data was exported to a computer in DICOM format for post processing analysis using MEASURE software (Barta, Dhingra, Royall, & Schwartz, 1997). Non-brain tissue was removed using standard procedures, and the hippocampus was divided into left and right hemispheres using the measurement protocol described by Kates, Abrams, Kaufmann, Breiter, & Reiss (1997). Parcellation was performed by two raters trained to the gold standard suggested by Kates et al. (1997), with an interrater reliability of 96.6% for the hippocampus.

III. Results

Designation of variables of interest

The study employed a correlational, cross-sectional design. The main DVs in this study were hippocampal volume and scores on neuropsychological tests that appear to be associated with hippocampal functioning, including VPA I Recall (designated as "Recall" below), VPA II Delayed Retention (designated as "Retention" below), and Stroop Color (StroopC) and Stroop Color-Word (StroopCW) tests. The primary IVs whose relationships with these DVs were examined were Group (coded 1 for GADs and -1 for NACs) and BDI scores. Two independent measures of depression (the BDI and HAMD) were collected and scores on these two measures were strongly correlated (r = .80, $p <$ .001). However, the BDI was chosen to serve as the primary index in this study due to its wider coverage and range of symptoms and its greater reliability in the sample.

Whole brain volume (WBV) and educational attainment ("Education") were also included as control variables or covariates in several analyses. Education was an ordinal variable with three levels: 1 = high school degree or equivalent, 2 = one to three years of college or a two-year college degree, and 3 = four-year college degree or above), and gender (coded 0 = male, 1 = female). The effects of gender on DVs were considered because the hippocampus tends to be somewhat larger in males, with larger gender differences occurring in whole brain volume (Kandel, Schwartz, & Jessel, 2000) and because gender differences have emerged on many neuropsychological tests (Mitrushina, Boone, & D'Elia, 1999). Education was examined as a control variable due to its utility as a single-measure proxy for socioeconomic status (SES; Duncan, Daly, McDonough, & Williams, 2002) and its reliable association with neuropsychological test performance

(Mitrushina et al., 1999). We also examined the effect of age on hippocampal volume, though age was not considered in analyses of neuropsychological test scores, since the latter were already standardized based on normative data for different age ranges. Prior to analyses, missing values for the one NAC participant who did not complete the clinical questionnaires or neuropsychological tests were addressed through substitution with the group means for NACs.

Descriptive statistics and preliminary analyses

Descriptive statistics for volumetric brain measures, clinical measures, neuropsychological test scores, and additional variables of interest are presented in Table 1. As seen in the table, as a group, GAD patients showed significantly higher scores on measures of depression (BDI, HAMD), anxiety (BAI, STAI-T), and worry (PSWQ) than did NACs. Most NACs tended to report minimal to mild levels of depression. Both higher levels of depression and greater variability were evident among the patient group, who exhibited symptoms ranging from minimal or mild to moderate. No gross differences between GADs and NACs were apparent in neuropsychological test scores or volumetric brain measures. They did not differ in level of education ($p > .10$).

Bivariate correlational analyses were performed to examine the relationship of age, education, BDI, and other clinical measures (BAI, STAI-T, PSWQ) to hippocampal volume, contextual memory (Recall, Retention), and Stroop performance in the full sample ($n = 30$), as well as intercorrelations among IVs and DVs. These associations were also examined through partial correlations controlling for WBV. Results are presented in Tables 2 and 3. Not surprisingly, scores on the two indices of depression (the BDI and HAMD) showed moderate to strong correlations with measures of anxiety,

although the degree of overlap was consistently lower for the BDI (see Table 2). Among neuropsychological measures, there was a moderately strong correlation between VPA Recall and Retention scores (r = .48). There was no evidence to suggest redundancy among neuropsychological measures or the operation of a single, latent construct.

In line with hypotheses, when controlling for WBV, hippocampal volume showed a positive correlation with scores on Retention (one of the measures of contextual memory) and StroopC, with effect sizes suggesting moderately strong relationships (partial rs = .43 and .44, respectively). As hypothesized, BDI scores were inversely correlated with hippocampal volume when shared variance with WBV was partialled out, r = -.46 (a moderately strong association). In contrast, there was no evidence for a relationship between hippocampal volume and BAI, STAIT, or PSWQ scores, even after controlling for WBV (all ps > .10; see Tables 3 and 4).

Thus, initial analyses provided support for the hypothesized unique relationship between depressive symptomatology and the hippocampus. BDI scores were not directly associated with recall or retention scores or with performance on StroopC or StroopCW. Contrary to expectations, the association of age and hippocampal volume controlling for WBV (partial r = .13) was not significant and age was not correlated with Recall or Retention scores in the sample, though age was negatively associated with performance on StroopCW. However, education level (which was an ordinal variable coded with three levels, with 1 = high school degree or equivalent, 2 = one to three years of college or a two-year college degree, and 3 = four-year college degree or above) was positively associated with scores on Recall (partial r = .46) and showed a small but non-significant positive association with Retention (partial r = .24).

A series of independent sample *t*-tests was performed to examine gender differences in brain morphology and functioning and clinical symptoms. As anticipated, there was a highly significant gender differences in WBV, $t(28) = 3.17$, $p = .004$, $r = .51$, as well as a barely-significant difference in hippocampal volume, $t(28) = 2.03$, $p = .05$, $r = .36$. Mean WBV and hippocampal volumes (and corresponding *SD*s) for males were 1235.01 cm^3 (84.26 cm^3) and 6.22 cm^3 (*SD* = .84), respectively, as compared to 1138.12 cm^3 (80.56 cm^3) and 5.56 cm^3 (*SD* = .89) for females. There were no significant gender differences in BDI or anxiety (BAI, STAI-T) or worry (PSWQ) scores (all *p*s > .10) or in neuropsychological test performance, though there was a trend for Recall, with females (*M* = 53.78, *SD* = 7.51) tending to perform better than males (*M* = 47.34, *SD* = 12.03), $t(28) = 1.81$, $p = .08$, $r = .32$.

Main analyses

As a more sensitive test of whether GADs and NACs differ in hippocampal volume and hippocampus-mediated cognitive functions, secondary analyses were performed with selected covariates. Hippocampal volume was analyzed in a one-way Analysis of covariance (ANCOVA) with group designated as a between-subjects factor and WBV and education (a proxy for SES) as covariates.[2] WBV was significantly associated with hippocampal volume, $F(1, 26) = 8.45$, $p < .01$, $r = .48$, whereas the effect of education was not significant, $F(1,26) = .01$, $r = .02$. Contrary to hypotheses, the effect of group on hippocampal volume after controlling for the effects of WBV and education was not significant, $F(1,26) = .16$, $p > .05$, $r = .03$.

Possible between-group differences in neuropsychological performance were also examined in four one-way ANCOVAs of Recall, Retention, StroopC, and StroopCW

scores, with group as the between-subjects factor and education and gender as covariates. Group was not found to have a significant effect on any of these DVs with education and gender covaried (all Fs < .44, all ps > .05). Thus the data provided no support for the hypothesis that GAD, in and of itself, is associated with impairments in hippocampal morphology or hippocampally-dependent cognitive functioning. Yet it is clear that the study did not possess sufficient power to detect any effect that may exist, as the observed power ($1-\beta$) for the detection of potential group effects of small or medium magnitude fell well below the suggested level of .80 (Cohen, 1988) in all four cases.

Next, a hierarchical regression analysis was performed to examine the potential simple, additive, and interactive effects of depression (BDI scores) and GAD on hippocampal volume. The main factors in these analyses were BDI and Group (coded here with a 1 for GADs and a -1 for NACs), while WBV and level of education were included as control variables. In order to avert problems with multicollinearity the continuous variable, BDI, was centered (by subtracting each raw score from the sample mean) and an interaction term, CenteredBDIxGroup (the product of Group and centered BDI scores) was computed prior to analyses. Predictors were entered in three steps, with the control variables entered first, the two main factors entered in the second step, and the interaction term entered in the third step. The analysis was designed to assess several questions, including (1) whether, with WBV and education level controlled, depression (BDI scores) is associated with smaller hippocampal volume, regardless of diagnostic group, and (2) whether having GAD is associated with volumetric reductions when level of depressive symptomatology is held constant. This analysis also enabled us to assess (3) whether the two factors combined may have an additive effect, or whether, as

hypothesized, there might be an interaction such that the relationship between BDI scores (depression) and hippocampal volume differs for GADs vs. NACs.

Visual inspection of a plot of standardized residuals against predicted values, a histogram and normal probability plots of residuals, and partial plots for predictors enabled assessment of the assumptions of linearity, homoscadesticity, and normality. The residuals were dispersed fairly evenly in a random pattern, with no signs of heteroscadisticity, bends, or discernible clusters or trends. The distribution of residuals was unimodal and nearly symmetrical, with no signs of skew, though it was leptokurtic, with a high proportion of residuals clustered about the mean (suggesting a deviation from the normality assumption). A Durbin-Watson statistic value very close to 2 suggested the assumption of independent residuals was met. An examination of tolerance and VIF values provided no indications of multicollinearity, as no tolerance value was below .2 (Cohen, 1988) and the average VIF was not substantially above 1. Moreover, inspection of leverage values, Cook's *d*, and DfFit values revealed no evidence of particular unusual cases that might be exerting too much influence over the model and parameter estimation.

Results of the hierarchical regression analysis are presented in Table 4. As shown in the table, the addition (in step two) of the two main factors (CenteredBDI and Group) added significantly to the prediction of hippocampal volume beyond the control variables, R^2 change = .208, $F(2, 25) = 4.91$, $p = .02$. Thus, acting together, BDI and Group showed a unique association with hippocampal volume above and beyond the effects of WBV and education. Using the f^2 measure of effect size for hierarchical multiple regression and interpretive guidelines provided by Cohen (1988), the linear combination of these two factors had a large effect ($f^2 = .39$). Both variables were

significant in the context of this model. With the effects of WBV and education partialled out, and controlling for diagnostic group, higher BDI scores were associated with reductions in hippocampal volume, $t(25) = -3.1$, $p > .01$, $\beta = -.56$. Similarly, holding levels of depression, WBV, and education constant, Group also had a significant effect, $t(25) = 2.16$, $p = .04$, $\beta = .40$, though the relationship was in the opposite direction from that hypothesized. Specifically, the positive coefficient for Group indicates that, at equivalent levels of WBV, education and BDI scores, GADs tended to have larger hippocampi than NACs, on average (with a mean difference of .025 cm^3).[3] The partial slopes for Centered BDI and Group suggest large and medium sized effects, respectively.

Relative to the second, "additive," model, the interactive model (model three) accounted for significantly more variance in hippocampal volume, with an R^2 increase of .09, $F(1, 24) = 4.38$, $p < .05$, indicating the presence of a significant interaction (see Table 4). The f^2 was .18, suggesting a medium effect size. That the (negative) association of BDI scores with the hippocampus was significantly larger for GADs than for NACs is shown by the negative coefficient for the interaction term, CenteredBDIxGroup ($B = -.31$, $\beta = -.27$). Following up on the regression analysis, analyses of simple slopes were performed to examine the depression relationship separately in the two groups. Among GADs, the slope coefficient ($B = -.05$, $\beta = -.61$) revealed a strong negative relationship of centered BDI with hippocampal volume, $t(24) = -2.73$, $p = .01$. In contrast, for NACs, the slope on centered BDI ($B = .01$, $\beta = .18$) was not significantly different from zero, $t(24) = .18$, $p > .05$ (see Figure 1).

In summary, these analyses revealed that, as a group, GAD participants did not exhibit volumetric reductions in the hippocampus and, contrary to expectations, showed a

tendency to have somewhat larger hippocampi than NACs when WBV, education, and BDI scores were held constant. However, as hypothesized, BDI scores exhibited a robust negative association with hippocampal volume when control variables and Group were held constant; and the strength of this association differed significantly between groups. Specifically, there was a strong relationship between BDI and the hippocampus among GADs, whereas these variables were not significantly related among GADs. These finding are consistent with the hypothesis that the adverse effects of depression on the hippocampus may be larger for GADs than for NACs.

Because item-level data were not available, it was not possible to rule out the possibility that reliability for the DV (hippocampal volume) or IV (BDI) may have differed between the two groups, which could bias results of the hierarchical regression and simple slopes analyses. However, it is seems highly unlikely that reliability differed greatly between the two groups, given the exceptionally high internal consistency coefficient for BDI (.90) and the high interreliability reliability coefficient for hippocampal parcellation (96.6%) observed in the whole sample. (Since sample size was equivalent for GADs and NACs, reliability coefficients for the BDI would have had to be very close to1.00 for GADs for there to have been any difference, which would be nearly unprecedented). On the other hand, lower *variability* in BDI scores in the NAC group as compared to the GAD group (as seen in Table 1), could have conceivably contributed to the failure to find a significant depression/BDI effect among controls.

To assess whether it is depressive symptoms in particular that influence the hippocampus or whether symptoms of anxiety and worry may have similar effects, and whether such effects differ for GADs vs. NACs, a series of three hierarchical regression

analyses was performed. These analyses followed the basic design described above but substituted centered BAI, STAIT, and PSWQ for centered BDI. In all three cases, the additive model failed to add significantly to the prediction of hippocampal volume above and beyond the control variables and the relationship of the anxiety measure and Group to the hippocampus controlling for WBV and education was minimal (for BAI, STAIT, and PSWQ, R^2 change was .01, .05, and .05, respectively, and $F(2,25) = .21$, .92, and .21, $p = .82$, all ps > .10). In no case was there any indication of a Group x anxiety interaction in the absence of significant main effects (all Fs > 2.93, all ps > .10).

Visual inspection of residual plots revealed no signs of violations of normality or linearity (such as a curvilinear relationship) that might indicate the linear regression model was inappropriate for the data. Nor were there signs of unduly influential cases that might be substantially skewing the model (as indicated by the absence of Cook's distances ≥ 1). Thus, findings were consistent with the hypothesis that it is something unique about the relationship between the hippocampus and depressive symptomatology or dysphoric mood (as opposed to other clinical symptoms, such as anxiety symptoms).

Secondary analyses

Additional analyses were conducted to help clarify the main findings. Given the large variability in depressive symptomatology among GADs and the strong association between depression and hippocampal volume found in this group, we examined whether reductions in hippocampal volume might be present among a subset of GAD patients, namely those with the highest levels of depressive symptomatology. For the purposes of this analysis, the GAD sample was divided evenly into three groups based on level of depression, which was measured by a composite scale calculated by averaging

participants' scores on the BDI and the HAMD. While these two measures were very highly correlated (r = .80 in the full sample and .75 in GAD group), a composite measure was used based on the belief that the task of rank-ordering patients and identifying those with the greatest levels of depressive symptomatology might be completed more accurately by utilizing a measure that balanced self-reports with independent, clinician ratings. Such a measure might provide a corrective influence for self-report biases, such as tendencies to underreport or over report symptoms, and cases exhibiting poor insight, which could easily bias a categorical coding scheme. Among GADs, scores on this composite variable ranged from 5 to 30.5, with a mean of 15.17 (SD = 8.03). Participants were classified according to their composite score and group status on a new categorical variable (DepSplit), with a 1 assigned to NACs (N = 15; 9 females), 2 to GADs with composite depression scores below the lower tercile (10.17; N = 5, with 3 females), 3 to GADs with scores below the upper tercile (19.17; N = 5, with 3 females), and 4 to GADs with scores greater than or equal to the upper tercile (N = 5, with 3 females).

Differences among groups in hippocampal volume and neuropsychological test scores (Recall, Retention, StroopC, and StroopCW) were assessed in one-way ANCOVAs with WBV as a covariate in the first analysis and education and gender as covariates in analyses of neuropsychological test scores. Three planned contrasts were designed to test (1) whether GADs with the highest levels of depressive symptomatology differ from NACs (-1, 0, 0, 1; the main question of interest), (2) whether GADs with depression scores in the lower or middle tier show such differences (-2, 1, 1, 0), and (3) whether GADs with the highest levels of depression differ from the less depressed GADs (0, -1, -1, 2).

Results of these ANCOVAs are presented in Tables 5 and 6, with group means displayed in Table 7. As shown in Tables 5 and 6, after adjustment for the covariates, GADs with the highest levels of depression differed significantly from NACs, with the former group showing significantly smaller hippocampi (adjusted *M*s = 4.96 vs. 5.77 cm^3, $\eta 2$ for contrast = .174) and lower Recall scores (adjusted *M*s = 42.20 vs. 52.93, $\eta 2$ for contrast = .166). In comparison to NACs, these individuals also tended to perform worse on StroopC (adjusted *M*s = 50.20 vs. 52.93, $\eta 2$ for contrast = .129), though this difference was significant only at trend levels ($p = .07$). The differences in hippocampal volume and Recall scores represent medium effect sizes. As expected, this group also differed from the other GADs, exhibiting significantly smaller hippocampi and poorer performance on Recall and StroopC (see Tables 5 and 6). As a group, GADs with depression scores in the lowest or middle tiers exhibited significantly larger hippocampus volumes than NACs (adjusted *M*s = 6.34 vs. 5.77 cm^3, η^2 for contrast = .148) though they did not differ from NACs on any of the neuropsychological measures. Thus, reduced hippocampal volume and associated cognitive impairments were evident only among a subset of GAD patients who scored relatively high on measures of depression. In contrast, GADs with depression scores below the top tercile tended to have somewhat larger hippocampi than NACs but showed no differences in neurocognitive performance.

Finally, a series of post-hoc analyses was conducted to determine whether this group of more highly (yet not clinically) depressed GADs might differ from the other GADs, and whether either group might differ from NACs, in other important respects that might relate to the observed differences in hippocampal volume and associated cognitive functioning. Continuous variables were analyzed with ANOVAs with the same series of

pairwise contrasts used in the ANCOVAs described above, while categorical variables were analyzed with chi-square tests along with Fisher's exact tests where appropriate.

No significant between-group differences were detected in gender, level of education, measures related to physical health problems (number of daily medications, number of major health problems, number of minor health problems), or general cognitive functioning (scores on MMSE, Amnart, Boston Naming Task), all *p*s > .10. Nor did the more depressed group differ from the other GAD patients in levels of state anxiety or worry (BAI and PSWQ scores), age of onset or years since onset of GAD, or measures related to general severity of mental distress or impairment (reported number of past psychological treatments, rates of past inpatient treatment, presence or number of psychiatric comorbidities), all *p*s > .10. This group did exhibit significantly higher levels of trait anxiety (STAIT scores), $t(26) = 4.48$, $p < .001$, $r = .66$, and suicidal ideation (GSIS scores), $t(26) = 2.25$, $p = .03$, $r = .40$, than their less depressed counterparts. However, STAIT and GSIS scores were not significantly associated with hippocampal volume in the sample, even controlling for whole brain volume (all *r*s < .10, all *p*s > .10).

IV. Discussion

This study examined hippocampal structure and associated cognitive abilities and their relationship to depressive symptomatology in a sample of older adults with Generalized Anxiety Disorder (GAD) and age- and gender-matched non-anxious controls (NACs). We found that, after controlling for the effects of whole brain volume and level of education, higher levels of depressive symptomatology were associated with smaller hippocampal volumes, which were in turn associated with poorer performance on a test of contextual verbal memory (VPA II Retention scores) and on the Stroop Color task. Moreover, in line with initial hypotheses, the relationship between depression and BDI scores was significantly stronger among GADs than among NACs. It is noteworthy that the relationship between depression and the hippocampus in the GAD group was exceptionally strong, relative to other findings on brain-behavior relationships. Additionally, the likelihood of detecting a relationship size with even a medium effect size in a sample of this size was fairly low. These two facts suggest that the study may be detecting a relationship with real world and possibly clinical significance.

In line with the third of our initial hypotheses, results strongly suggest that a specific association might exist between the depressive, or depression-like, symptoms which characterize many cases of GAD (even among individuals with no recent history of major depressive episodes), and morphological changes in the brain. In particular, they provide support for the idea that, in the context of GAD, even subclinical levels of depressive symptoms, which represent manifestations of psychological stress/distress, may be associated with volumetric reductions in the hippocampus and associated deficits in cognitive abilities such as contextual memory. Findings lend support to the theory that

individuals with GAD may be more sensitive to the adverse effects of depression on the hippocampus than psychologically healthy individuals. It is also noteworthy that the association with hippocampus appears to be specific to the depressive symptoms of GAD, as opposed to symptoms of trait anxiety or worry (for which there was no evidence of a relationship with hippocampal volume).

While it not possible to pinpoint the exact mechanisms responsible for the observed relationship, we posit that several factors may contribute to the increased vulnerability of individuals of GAD. First, in individuals with GAD, mood is more substantially disrupted than among normal individuals and depressive symptoms may be compounded by other potentially harmful symptoms that many GAD patients experience to a greater extent than healthy individuals. Such symptoms might include other forms of psychological distress and mood disturbances (e.g., anger, anxiety, despair), worry, and various physiological symptoms. Moreover, among GADs, depressive symptoms may be more frequent and prolonged or chronic, thereby magnifying their impact on the hippocampus. Second, in GAD patients, there may be a reciprocal reinforcing relationship such that stress and depression are both sustained and reinforced by and further contribute to abnormalities in the body's stress response involving dysregulation of the HPA axis and elevations in cortisol (and perhaps especially peak cortisol) levels (Mantella et al., 2008; Nutt, 2001; Sinha, Mohlman, & Gorman, 2004). Over time, this reciprocal relationship might sustain and magnify the effects of stress and depression on the brain, and the hippocampus in particular.

Fourth, on the psychological level, individuals with GAD appear to have greater difficulty understanding and regulating their emotions (particularly negative emotions)

and repairing negative mood states (including dysphoric or depressive moods) and coping with life stressors through effective behavioral and cognitive strategies (Mennin et al., 2004; Mennin, Heimberg, Turk, & Fresco, 2005). Thus, GADs may fail to implement various forms of adaptive coping responses which, among healthy adults, might mitigate the effects of depressive symptoms on the brain while reducing their endurance, intensity, and frequency. In fact, the constant, pathological worry that is the hallmark feature of GAD may interfere with the capacity to cope effectively with symptoms of low-level depression. Past research has shown that worrying involves the prefrontal cortex and substantial involvement of limited executive resources **(**Mohlman et al., 2009; Price & Mohlman, 2007**)**. Consequently, individuals with GAD, who are constantly worrying, may be in a chronic state of divided attention and their prefrontal cortex may not be fully available for emotion regulation or learning of association among variables especially when there is a delay between the stimulus and response (including cause and effect relationships), which involves the hippocampus. The results may include a failure to downregulate or learn from dysphoric mood and inadequate engagement in the sorts of mental activity that promote the retention and incorporation into neural networks of cells produced in the hippocampus during adult neurogenesis.

The data failed to support the hypothesis that GAD patients, in general, exhibit impairments in hippocampal structure and functioning; on the whole, GADs did not differ from NACs in hippocampal volume or scores on associated neuropsychological tests. Several possible explanations exist for why such differences did not emerge. One possibility is that such differences exist but the study lacked sufficient statistical power to detect them. A second possibility is that, like many other groups defined by DSM-IV

diagnoses, GAD patients might constitute a heterogeneous group who vary on important characteristics, and that only some such individuals evince morphological changes in the brain or impairments in neurocognitive functioning.

Some support for this notion comes from the current study's finding that those GAD patients with the highest levels of depression (but not other GADs) showed reductions in hippocampal volume and hippocampally-mediated cognitive functioning relative to normal controls. These data suggest that hippocampal impairments may be found, perhaps not in all individuals with GAD, but at least among a subset, who seemingly may be distinguished by relatively high levels of depressive symptoms.

There were no systematic differences between this group and other GADs in terms of demographic characteristics; the severity, onset, and duration of their GAD; state anxiety; level of pathological worry; health conditions; or other associated characteristics. However, these GAD patients did show significantly higher levels of trait anxiety as well as elevated levels of ideation really to geriatric suicidal risk (e.g., thoughts of death, signs of hopelessness, diminished sense of self worth)**.** Consistent with these findings, anecdotal observations suggest that these individuals may have been distinguishable from other GAD patients by a sense of purposelessness and a sense that they had not achieved up to their potential in life (Mohlman, personal communication), characteristics associated with depressive thinking. While this evidence is suggestive, further research will be needed to determine more precisely which specific groups of GAD patients are more or less likely to suffer from hippocampal impairments.

While the conclusions drawn from these findings must be regarded as tentative, if replicated, these findings may have important implications for the scientific

understanding of GAD and the connection between emotional problems and the hippocampus. They might also have applications for the treatment of anxious older adults, who account for a growing portion of the population (Lenze et al., 2005). Perhaps most clearly, this study's findings suggest that the link between psychobiological manifestations of stress, such as depression, and the hippocampus may be evident in other forms of psychopathology besides MDD or PTSD, which have been the focus of most of the research in this field. Though our assumption regarding the existence of a causal connection between the variables examined awaits empirical scrutiny, our findings seem to suggest that even subclinical levels of depression, not meeting diagnostic threshold criteria for MDD, may be associated with reductions in hippocampal volume when these symptoms occur in the context of another psychiatric disorder, such as GAD.

Of no less importance, these data suggest a need for greater attention to depressive symptomatology in GAD, which has been largely neglected in the clinical literature. Whether these symptoms, or the physiological processes associated with them, are a cause of reduced hippocampal volume or merely a sign of such reductions remains unclear. So, too, does the question of which particular symptoms are most important to this link. Nevertheless, the strong relationship of these depressive symptoms to the integrity of a vital brain structure among GAD patients suggests that these symptoms should be the subject of greater research and clinical attention.

Ultimately, this line of research may have implications for the treatment of late life GAD. Findings may suggest a need for more careful screening for as well as earlier detection and treatment of relatively subtle, low level depressive symptoms in the context of late life GAD. Clinical researchers may wish to investigate the efficacy of

antidepressant medications as a means of counteracting damage to and preserving the structure and functioning of the hippocampus for at least some GAD patients. Antidepressant medications have already proven effective in accomplishing these aims among patients with MDD (Sapolski, 2001; Warner-Schmidt & Duman, 2006).

In terms of psychosocial treatments, it may prove beneficial to supplement existing treatment packages with modules and strategies designed specifically to reduce dysphoric mood and other depressive symptoms that may be contributing to impairments in the hippocampus, to improve patients' ability to effectively cope with stress and regulate dysphoric mood, as well as to increase patients' engagement in forms of behavior and mental activity that promote neurogenesis and cell survival in the hippocampus (e.g., effortful learning, vigorous exercise). Efforts by Mennin (2004, 2006) to develop and test an intervention that addresses specific emotion regulation deficits that appear to be common in GAD provides but one example of potentially useful strategies. Ultimately, these strategies may prove most beneficial for a subset of GAD patients who are at greatest risk for impairments, though future research would certainly be needed to improve our ability to identify such individuals.

This study had several important limitations. Perhaps most importantly, we must emphasize that we used a correlational design to test hypotheses grounded in a causal model. To be sure, this limitation is by no means unusual in the field, since the vast majority of studies on hippocampal volume in psychological disorders (mostly MDD and PTSD) have relied on non-experimental, cross-sectional designs (see Videbach & Ravnkilde, 2004; Kitayama et al., 2005). The use of this methodology may be seen as an

important first step toward establishing whether there are relationships and differences worthy of further examination.

Nevertheless, because we did not experimentally manipulate stress or depression and did not employ a longitudinal design, we cannot be certain about the direction or nature of causation. While we theorize that depression might play contribute to impairments in the hippocampus within GAD, we cannot rule out the possibility that the smaller hippocampal volume might play a role in the development or maintenance of depressive symptoms in GAD (or possibly, in some individuals, of the disorder itself). Nor can we be certain that other variables are not responsible for the observed relationship. As noted in the introduction, there is some reason to believe the relationship between emotional disorders and the hippocampus may be complex and bidirectional; and animal and clinical research suggest a variety of factors may play a role in the causal pathways that link these variables (e.g., exposure to environmental stressors, individuals' characteristic patterns of emotional response and regulatory styles, and neurophysiological abnormalities; see McEwen, 2001; McEwen & Seeman, 1998). It should be emphasized that a large number of factors appear to affect the hippocampus and neurogenesis within the hippocampus. In fact, scientific research on the relationship between stress, emotional disorders and various other forms of behavior and the hippocampus, and on the hippocampus itself, is still very much in its infancy. We are a long way from understanding even some of the most basic questions in this field.

Another important qualification concerns our findings regarding the relationship between depressive symptoms (BDI scores) and the hippocampus among normal controls. While our findings suggest that the strength of this relationship is moderated by

diagnostic status, it would be unwarranted to conclude that there is *no* relationship between subclinical depression and the hippocampus among individuals with no diagnosable disorder. It is quite possible that some such relationship exists but was obscured by insufficient variability in current depressive symptoms and BDI scores within the control group. It might be that the control group was generally more highly functioning and asymptomatic than the healthy older adult population in general, resulting in artificially deflated variability. On the other hand, when considering questions of representativeness, it should be noted that the control group was heterogeneous in many other respects (including age, level of education, and life experiences). In fact, several participants did have a history of past psychological problems for which they sought treatment (though the details are not currently available to the author). Indeed, it is possible that these factors contributed to the failure to find gross differences between the groups.

One obvious limitation was the relatively small sample size. This factor raises questions about the generalizability of findings and may have also limited our ability to detect small or moderately sized between-group differences in hippocampal volume or associated neuropsychological test scores. The sample size also precluded use of more sophisticated analytic techniques (e.g., structural equation modeling) that might provide opportunities to test and compare competing theoretical models and hypotheses regarding complex causal relationships (including models that suggest a causal role for hippocampal volume).

In terms of the sample composition, it is also necessary to acknowledge the implications of the decision to exclude participants with GAD and comorbid MDD,

which had important benefits as well as drawbacks. Its main advantage was that this strategy allowed us to examine hippocampal morphology and functioning and their relationship to symptoms in relatively pure sample in which findings could not simply be attributed to concurrent major depression. As a consequence, however, the sample is not fully representative of GAD population and it is possible that different findings might emerge were individuals with comorbid GAD and MDD to be included in the study. Research including individuals with GAD and a comparison group with both disorders, as well as healthy controls, might have allowed us to observe overlapping characteristics and elucidate potentially important differences between these two clinical groups. Nevertheless, one would imagine that the relationship between depression levels and the hippocampus would, if anything, be much stronger in such a sample, which would only provide further support for the main findings. In addition, it should be noted that the exclusionary criteria used in the study were in other respects fairly limited. In fact, a number of participants in the sample did have other co-occurring disorders (especially other anxiety disorders) and quite a few (but certainly not all) had undergone previous psychological or psychiatric treatment at some point in their lives. In these respects, they were quite typical of the GAD population (Lenze et al., 2005).

Another limitation was that we not have a good measure of life stressors, past or present. Consequently, it was not possible to control for individual differences in exposure to stressors that could contribute to depressive symptoms and conceivably affect the hippocampus, either directly or in interaction with individuals' psychological, behavioral, and biological responses. Nevertheless, one could argue that having GAD is

tantamount to chronic stress because of the constant worrying, dwelling on the negative, and other forms of self-induced stress.

In light of these limitations, the conclusions based on this study must be regarded as tentative and are in need of replication. Further research is needed to gain a better understanding of the relationship between stress and depressive symptoms and the hippocampus and late life GADs. Future studies are advised to employ larger samples, and may wish to include comparison groups with comorbid GAD and MDD and pure MDD in order to assess the generalizability of findings and isolate commonalities and differences among these groups. They should also incorporate measures of life stressors, as well as biological indices of stress (e.g., cortisol samples), and may wish to assess other factors that could potentially affect the morphology of the hippocampus, such as physical health, diet, exercise, levels of social activity and social support, socioeconomic status, and past treatment history (including past use of anti-depressant medications).

Another question worth exploring is whether the relationships between stress or depression and the hippocampus may be moderated by differences in individual's habitual style of coping with stressors and regulating emotion and emotional states such as dysphoric mood (e.g., the use of such strategies as distraction, help-seeking, emotion suppression, reappraisal, behavioral or experiential avoidance, and worry); and whether such differences may be partly responsible for variation in hippocampal integrity across groups. Though costly, it is also advisable that researchers undertake longitudinal studies which measure exposures to stressors, biological manifestations of stress, psychological symptoms, brain structure and functioning, and diagnostic status repeatedly across multiple time points in order to shed light on the temporal relationships among these

variables and likely causal pathways. Knowledge garnered through this research might eventually lead to improvements in clinical assessment and treatment.

V. Tables

Table 1.
Descriptive Statistics for Volumetric Brain Measures, Neuropsychological Test Scores, and Clinical Measures for Full Sample and by Diagnostic Group.

	Full Sample (n = 30)			NACs (n =15)			GADs (n =15)		
Measure	*M*	*SD*	Range	*M*	*SD*	Range	*M*	*SD*	Range
Volumetric Brain Measures									
WBV	1176.87	93.96	1042.15–1388.87	1173.66	83.09	1051.14–1336.72	1180.09	106.58	1042.15–1388.87
Hipp	5.82	0.92	4.09–7.53	5.75	0.70	4.49–6.83	5.90	1.11	4.09–7.53
Neuropsych Measures (t-scores)									
Recall	51.20	9.91	30–70	52.93	7.13	40–70	49.47	12.09	30–64
Retention	53.07	8.42	34–66	53.00	7.33	37–64	53.13	9.65	34–66
StroopC	52.63	2.77	49–60	53.00	2.27	49–58	52.27	3.24	49–60
StroopCW	44.35	9.67	20–60	45.57	8.92	28–58	43.13	10.53	20–60
Clinical Measures									
BDI	11.93	10.45	2–39	5.93	4.06	2–15	17.93	11.50	4–39**
HAMD	6.81	6.91	0–25	1.21	1.21	0–3	12.40	5.53	6–25**
BAI	11.86	10.84	0–39	4.93	5.96	0–22	18.80	10.23	3–39**
STAIT	41.95	17.79	21–76	28.50	6.23	21–41	55.40	15.14	22–76**
PSWQ	48.11	12.70	22–68	40.43	11.43	22–58	55.80	8.77	35–68**

Note. $^{\dagger}p < .10$. $*p < .05$. $**p < .01$; GADs = Generalized Anxiety Disorder patients. NACs = Non-anxious controls. WBV = whole brain volume (cm^3); Hipp = hippocampal volume (cm^3); BDI = Beck Depression Inventory; HAMD = Hamilton Rating Scale for Depression; BAI = Beck Anxiety Inventory; STAIT = Trait scale of State Trait Anxiety Inventory; PSWQ = Penn State Worry Questionnaire. Volumes measured without cerebrospinal fluid.

Table 2.

Pearson Product-Moment and Point-Biserial Correlations for Full Sample.

	BDI	HAMD	Educ	Gender	Age	BAI	STAIT	PSWQ	WBV	Hipp	Recall	Retention	StroopC	StroopCW
BDI	---													
HAMD	.81**	---												
Educ	-.10	-.07	---											
Gender	.07	-.06	.04	---										
Age	-.12	.04	-.23	-.02	---									
BAI	.54**	.65**	-.13	.02	-.17	---								
STAIT	.82**	.83**	-.01	.01	-.21	.75**	---							
PSWQ	.56**	.64**	.00	.23	.02	.52*	.69**	---						
WBV	.05	.04	.24	-.51**	-.29	.01	.13	-.07	---					
Hipp	-.30	-.18	.13	-.36†	-.04	-.02	-.01	-.06	.51**	---				
Recall	-.19	-.27	.41*	.32†	.08	-.22	-.20	-.20	-.11	.06	---			
Retention	-.26	-.12	.19	.13	.11	-.14	-.26	-.28	-.15	.29	.48**	---		
StroopC	-.15	-.16	.17	-.11	.12	.05	-.12	.01	.24	.49**	.36†	.26	---	
StroopCW	.23	-.07	.03	.21	-.47**	.05	.08	.05	-.06	-.20	.07	-.16	.11	---

Note. $^{\dagger}p < .10$. $*p < .05$. $**p < .0$l. BDI = Beck Depression Inventory; HAMD = Hamilton Rating Scale for Depression; Educ = educational attainment; BAI = Beck Anxiety Inventory; STAIT = Trait scale of State Trait Anxiety Inventory; PSWQ = Penn State Worry Questionnaire; WBV = whole brain volume (cm^3); Hipp = hippocampal volume (cm^3). Volumes measured without cerebrospinal fluid. Gender was coded 0 = male and 1 = female in these analyses.

Table 3.
Partial Correlations Controlling for Whole Brain Volume.

	BDI	HAMD	Educ	Gender	Age	BAI	STAIT	PSWQ	Hipp	Recall	Retention	StroopC	StroopCW
BDI	---												
HAMD	.80**	---											
Educ	-.12	-.09	---										
Gender	.11	-.04	.2	---									
Age	-.11	.05	-.17	-.21	---								
BAI	.54**	.65**	-.14	.03	-.17	---							
STAIT	.82**	.83**	-.04	.09	-.18	.76**	---						
PSWQ	.56**	.64**	.01	.23	.00	.52**	.70**	---					
Hipp	-.38*	-.23	.00	-.13	.13	-.03	-.09	-.03	---				
Recall	-.18	-.27	.46*	.31†	.05	-.22	-.19	-.22	.14	---			
Retention	-.26	-.12	.24	.06	.07	-.14	-.25	-.14	.43*	.47*	---		
StroopC	-.17	-.17	.12	.02	.21	.05	-.16	.05	.44*	.40*	.31	---	
StroopCW	.24	-.07	.05	.2	-.51	.05	.09	.05	-.20	.06	-.17	.13	---

Note. $^{\dagger}p < .10$. $*p < .05$. $**p < .0$l. BDI = Beck Depression Inventory; HAMD = Hamilton Rating Scale for Depression; Educ = educational attainment; BAI = Beck Anxiety Inventory; STAIT = Trait scale of State Trait Anxiety Inventory; PSWQ = Penn State Worry Questionnaire; WBV = whole brain volume (cm^3); Hipp = hippocampal volume (cm^3). Gender was coded 0 = male and 1 = female in these analyses.

Table 4.
Summary of Hierarchical Regression Analysis of Hippocampal Volume.

Variable	β	B	$SE\ B$	R^2	$F\ \Delta\ R^2$
Step 1					
Intercept		-0.063	1.902		
WBV	0.512**	0.005	0.002		
Education	0.003	0.004	0.217	.263	4.82*
Step 2					
Intercept		-0.216	0.017		
WBV	0.516**	0.005	0.001		
Education	0.039	0.050	0.198		
Centered BDI	0.398*	0.359	0.166		
Group	-0.556**	-0.049	0.016	.471	4.91*
Sex					
Intercept		-0.471	1.579		
WBV	0.55**	0.005	0.001		
Education	0.094	0.120	0.189		
Centered BDI	0.150	0.135	0.189		
Group	-0.111	-0.010	0.024		
CenteredBDIxGroup	0.469*	0.051	0.024	.553	4.38*

Note: *$p < .05$. **$p < .01$. ***$p < .001$. WBV = whole brain volume (cm^3); Hipp = hippocampal volume (cm^3); Centered BDI = centered Beck Depression Inventory score. Group was coded 1 = GAD patient and -1 = non-anxious control. β = standardized regression coefficients; B = unstandardized regression coefficients.

Table 5.
Analysis of Covariance of Hippocampal Volume with Planned Contrasts.

Source	Adj. Sum of Squares	*df*	*F*	η^2	Contrast Estimate
WBV	5.60	1	12.18**	.327	
DepSplit	6.44	3	4.67*	.359	
GADs-High vs. NACs	2.42	1	5.26*	.174	-0.80
GADs-Low and GADs-Mid vs. NACs	1.99	1	4.34*	.148	1.16
GADs-High vs. other GADs	6.34	1	13.78**	.355	-2.76
Error	11.50	25			

Note. $^{\dagger}p < .10$. $*p < .05$. $**p < .01$. The IV in this analysis was DepSplit, which was coded 1 = non-anxious control (NACs; $N = 15$), 2 = GAD patient with composite depression score below lower tercile (GADs-Low; $N = 5$), 3 = NACs = GAD patient with score below upper tercile (GADs-Mid; $N = 5$), and 4 = GAD patient with score above upper tercile (GADs-High; $N = 5$). The covariate was WBV (whole brain volume), measured in cm^3.

Table 6.

ANCOVAs of Neuropsychological Test Scores with Planned Contrasts.

Source	Adj. Sum of Squares	*df*	*F*	$\eta 2$	Contrast Estimate
			VPA Recall		
Education	182.18	1	2.63	.099	
Gender	303.27	1	4.38*	.154	
DepSplit	484.44	3	2.33†	.226	
GADs-High vs. NACs	330.16	1	4.77*	.166	-9.52
GADs-Low and GADs-Mid vs. NACs	10.85	1	0.16	.006	2.75
GADs-High vs. Other GADs	396.03	1	5.72*	.193	-21.80
Error	1660.53	24			
			Retention		
Education	30.94	1	0.39	.016	
Gender	35.27	1	0.44	.018	
DepSplit	165.68	3	0.70	.080	
GADs-High vs. NACs	112.86	1	1.42	.056	-5.57
GADs-Low and GADs-Mid vs. NACs	10.16	1	0.13	.005	2.66
GADs-High vs. Other GADs	158.70	1	2.00	.077	-13.80
Error	1906.52	24			
			StroopC		
Education	2.63	1	0.36	.015	
Gender	2.64	1	0.37	.015	
DepSplit	37.54	3	1.73	.178	
GADs-High vs. NACs	25.65	1	3.55†	.129	-2.66
GADs-Low and GADs-Mid vs. NACs	1.14	1	0.16	.007	0.89
GADs-High vs. Other GADs	32.03	1	4.43*	.156	-6.20
Error	173.48	24			
			StroopCW		
Education	6.91	1	0.07	.003	
Gender	114.57	1	1.16	.046	
DepSplit	202.84	3	0.68	.079	
GADs-High vs. NACs	22.107	1	0.22	.009	2.46
GADs-Low and GADs-Mid vs. NACs	117.76	1	1.19	.047	-9.07
GADs-High vs. Other GADs	163.33	1	1.65	.064	14.00
Error	2380.45	24			

Note. $^{\dagger}p < .10$. $*p < .05$. $**p < .01$. NACs = Non-anxious controls; GADs-Low = GAD patients with composite depression scores below lower tercile; GADs-Mid = GAD patients with scores greater than or equal to lower and below upper tercile; GADs-High = GAD patients with scores equal to or above upper tercile.

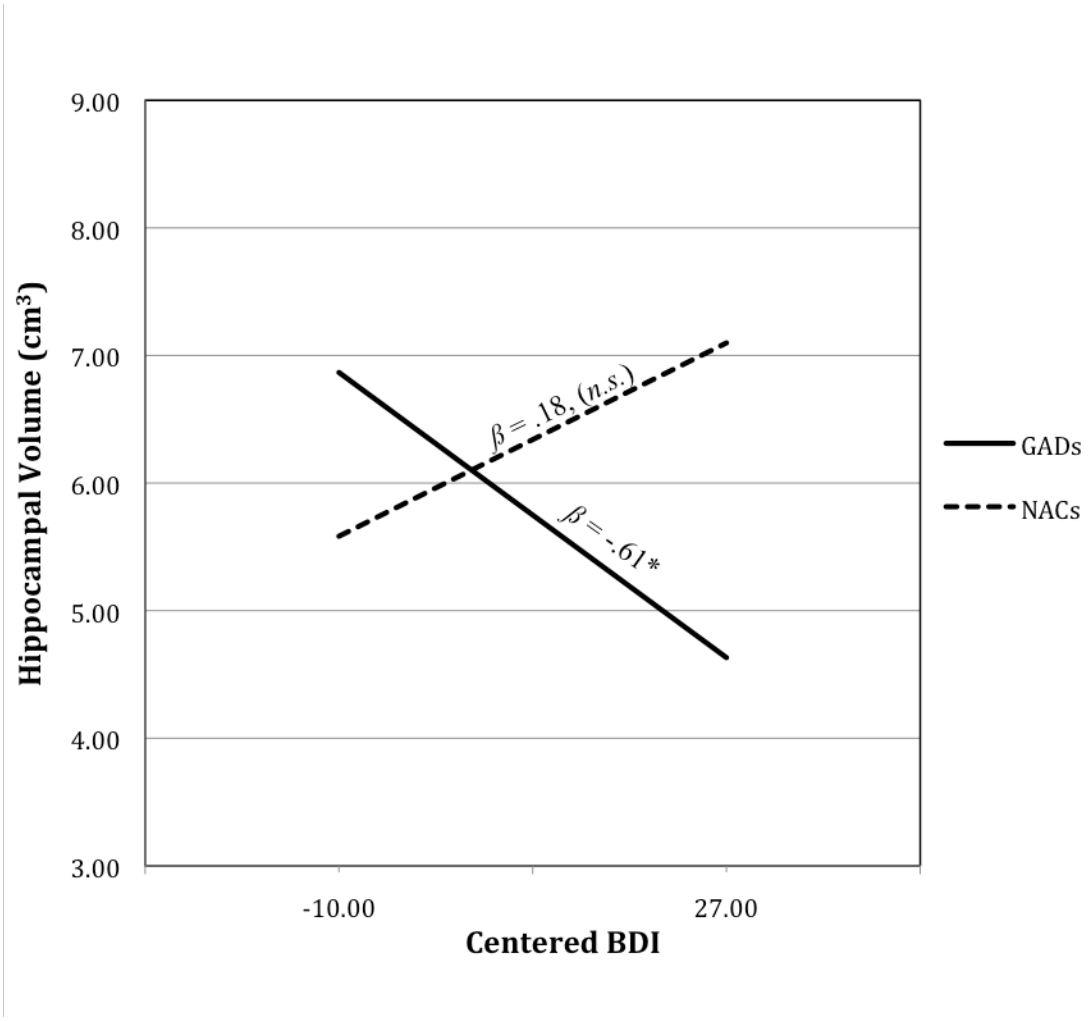
Table 7.
Adjusted and Unadjusted Mean Hippocampal Volumes and Neuropsychological Test Scores for Non-anxious Controls and GAD Patients with Varying Levels of Depression.

Group	Hippocampal Volume		Recall		Retention		StroopC		StroopCW	
	Adj. Mean	Unadj. Mean	Adj. Mean	Unadj. Mean	Adj. Mean	Unadj. Mean	Adj. Mean	Unadj. Mean	Adj. Mean	Unadj. Mean
NACs	5.77[a]	5.75	52.33[b]	52.93	53.22[b]	53.47	52.93[b]	53.00	45.45[v]	45.57
GADs–Low	6.36[a]	6.24	50.98[b]	48.40	54.67[b]	53.60	52.71[b]	52.40	41.50[b]	41.00
GADs–Mid	6.32[a]	6.51	56.43[b]	57.80	54.43[b]	55.00	54.03[b]	54.20	40.33[b]	40.60
GADS–High	4.96[a]	4.94	42.80[b]	42.20	47.65[b]	47.40	50.27[b]	50.20	47.92[b]	47.80

Note. a = means are adjusted for whole brain volume; b = means are adjusted for both education and gender. NACs = Non-anxious controls; GADs-Low = GAD patients with composite depression scores below lower tercile; GADs-Mid = GAD patients with scores greater than or equal to lower and below upper tercile; GADs-High = GAD patients with scores equal to or above upper tercile.

VI. Illustrations

Figure 1.
Simple Slopes for Effects of BDI on Hippocampal Volume by Group Controlling for Whole Brain Volume and Education.



Note. *n.s.* = not significant. *$p < .05$. β = standardized slope coefficient. GADs = GAD patients (n = 15); NACs = Non-anxious controls (n = 15).

VII. References

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VIII. Endnotes

[1] Notably, the degree of loss appears to be moderated by lifestyle factors (such as mental and physical activity), health behaviors (e.g., diet, exercise, sleep, substance use), and individual differences in manner and effectiveness of coping with stressors (McEwen & Seeman, 1999).

[2] Age and gender were not included as covariates in this analysis because their relationship with hippocampal volume in the sample was found to be non-significant when shared variance with WBV was partialled out.

[3] This value was obtained by dividing the unstandardized coefficient for education in the additive model (.050) by the sum of the squares of the contrast coefficients for the two groups (1 + 1 = 2).