IDENTIFYING ASSOCIATIVE MEMORY DEFICITS AND NEUROBIOLOGICAL CORRELATES OF ENCODING AND PERFORMANCE IN A NATIONAL SAMPLE OF VETERANS WITH GULF WAR ILLNESS USING MAGNETIC RESONANCE IMAGING

by

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ABSTRACT

IDENTIFYNG ASSOCIATIVE MEMORY DEFICITS AND NEUROBIOLOGICAL CORRELATES OF ENCODING AND PERFORMANCE IN A NATIONAL SAMPLE OF VETERANS WITH GULF WAR ILLNESS USING MAGNETIC RESONANCE IMAGING

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Roughly 26-32% of U.S. veterans who served in the Persian Gulf War of 1991 report suffering from chronic health problems (Golomb, 2008). Memory complaints are regularly reported by ill Gulf War veterans (GWV), but there is scarce data to verify their complaints. Using an associative memory paradigm of faces and names, the present study was conducted to investigate the memory deficits reported by ill GWV in a nationally representative sample comprised of both ill and well GWV. During administration of the memory task, functional magnetic resonance imaging (fMRI) was used to acquire the Blood Oxygenation Level Dependent (BOLD) contrast to serve as a proxy of brain activation evoked by the memory task. Additionally, measures of N-acetylaspartate (NAA) and creatine (Cr) were obtained from the left and right hippocampus of participating veterans using single voxel magnetic resonance spectroscopy (MRS). The ratio of NAA to Cr obtained from MRS serves as a proxy for functional neuronal mass in the hippocampus during a resting state.

It was hypothesized that affected GWV would demonstrate decreased memory performance relative to unaffected GWV on the associative memory test, providing evidence of memory deficits using an objective measure of memory. This was confirmed. In addition, I hypothesized that these memory differences would be related to differences in brain function during the encoding of novel associative memories. Specifically, I predicted that differences would be observed between the ill and well GWV in the amount of brain activation measured during associative memory encoding using BOLD fMRI. Such differences were observed in several brain regions. However, hippocampal differences did not follow predictions. Lastly, I predict that the NAA/Cr ratio level measured in the hippocampus will differ between ill and well GWV. Moreover, I predict that the NAA/Cr ratio measured in the hippocampus will be correlated with performance on the associative memory test. Group differences were observed. However, NAA/Cr concentrations were not consistently correlated to associative memory performance.

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CHAPTER 1

INTRODUCTION

1.1 Gulf War Illness

Between August 1990 and June 1991, roughly 697,000 U.S. military personnel were deployed to the Persian Gulf to serve in operations Desert Shield and Desert Storm. Roughly 1 in 4 of these Gulf War veterans (GWV) report suffering from Gulf War Illness (GWI), a multi-symptom illness thought to have resulted from their military service during the war (for a review, see Golomb, 2008). Gulf War Illness is characterized by chronic fatigue, muscle and joint pain, attention problems, memory deficits, and headaches (Haley, Kurt, & Hom, 1997). Initial research investigating GWI attempted to subtype ill GWV into different syndrome groups with the aim of accommodating the diversity of symptoms reported by these men and women (Haley et al., 1997). Other studies aimed to better understand GWI by identifying neurobiological correlates of the self-reported symptoms associated with service in the first Gulf War in an attempt to provide insight into the possible biological basis of the symptoms reported by ill GWV (Haley & Kurt, 1997; Haley, Marshall, McDonald, Daugherty, Petty, Fleckenstein et al., 2000). In addition, studies have been conducted to investigate changes in brain structure and function associated with neurotoxin exposure in attempt to determine whether or not this exposure is the potential cause of the illness (Haley, Spence, Carmack, Gunst, Schucany, Petty et al., 2009).

While considerable research demonstrates that ill GWV self-report a constellation of symptoms at higher rates than other groups, far less research has objectively verified these complaints (e.g., David et al., 2002; Hom, Haley, & Kurt, 1997; Proctor, Heaton, Heeren, & White, 2006). The primary aim of the current study was to objectively measure one symptom of GWI, deficits in episodic memory, in a national sample of GWV that is demographically representative of those veterans who served in the conflict. The secondary aim was to identify

and offer insight into possible markers of episodic memory deficits present in ill GWV using neuroimaging. Two neurobiological markers were be investigated: (1) functional brain activity measured during memory encoding, using the Blood Oxygenation Level Dependent (BOLD) contrast obtained from functional Magnetic Resonance Imaging (fMRI), and (2) the N-acetylaspartate/creatine ratio (NAA/Cr), measured using single voxel Magnetic Resonance Spectroscopy (MRS). Thus, the study sought to validate the memory symptoms reported by some ill GWV by investigating associative memory and exploring the extent to which these alterations in memory performance are related to brain function.

1.1.1 Background

Epidemiological studies conducted after the conflict demonstrate that roughly 26-32% of GWV suffer from chronic health problems, including chronic fatigue, muscle and joint pain, attention problems, memory deficits, and headaches (Golomb, 2008). A substantial amount of literature demonstrates these self-reported symptoms to be greater among GWV than other veteran groups (e.g., Centers for Disease Control and Prevention, 1995; Fukuda et al., 1998; Haley & Kurt, 1997; Iowa Persian Gulf Study Group, 1997; Steele, 2000). Additionally, the symptoms ill GWV experience, such as fatigue, shortness of breath, muscle pain, joint pain, etc., are found to be higher in GWV than in Vietnam veterans. Such findings bolster the perspective that this illness is not the same as those experienced by veterans of other conflicts, nor is it simply a post-war stress syndrome (National Institute of Health, 1994). Furthermore, when veterans from the current war, which began in 2003, present with symptoms similar to those associated with GWI, their symptoms can usually be explained by diagnosable psychiatric or medical conditions (Hoge et al., 2008; Horn et al., 2006; Hotopf et al., 2003; Hunt et al., 2006; Murphy et al., 2006). The same is not true of ill veterans of operations Desert Shield and Desert Storm, further suggesting that GWI is a unique illness.

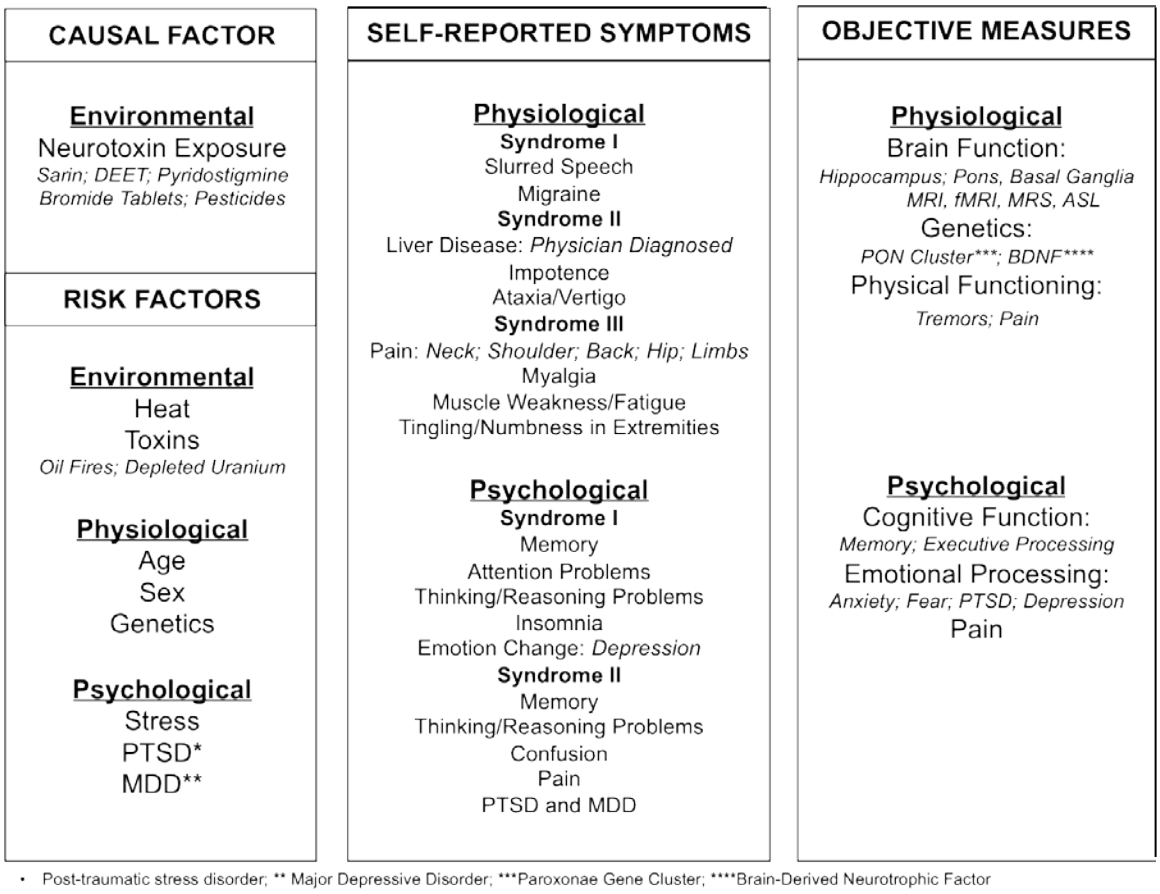
As previously stated, GWI research has demonstrated that GWV self-report a constellation of symptoms at higher rates than other veteran groups, but this research has not

objectively verified these complaints (e.g., David et al., 2002; Hom, Haley, & Kurt, 1997; Proctor, Heaton, Heeren, & White, 2006). For instance, cognitive impairments are chief among the complaints of GWV, with memory deficits being one of the most common complaints. However, GWV identified with GWI (ill GWV) have not reliably demonstrated impaired memory performance relative to appropriate control participants when tested on standardized neuropsychological tests of memory function (David et al., 2002; Hom, Haley, & Kurt, 1997). In the current study, I will investigate the memory deficits reported by ill GWV using an associative memory task, in an attempt to better understand and characterize them. It is predicted that alterations in cognition reported by ill GWV are the result of functional brain differences they experience relative to well GWV. According to the neurotoxin exposure hypothesis, the most prominent account of GWI, the health related issues experienced by ill GWV could have arised from service related exposure to neurotoxins that adversely impacted their central nervous system (Binns et al., 2008). While the current study does not directly measured neurotoxin exposure, the link between potential memory differences and BOLD fMRI activation and NAA/Cr from magnetic resonance spectroscopy (MRS) data of hippocampus was investigated to provide more insight into possible brain and cognitive dysfunction in ill GWV.

1.1.2 Neurotoxin Exposure Hypothesis of Gulf War Illness

GWV are hypothesized to now experience multiple symptoms that are psychological, physical, or both due to exposure to one or more neurotoxins (Haley & Kurt, 1997; Binns et al., 2008). Figure 1.1 is a graphical representation of the neurotoxin exposure hypothesis illustrating the proposed relationship between neurotoxins and GWI. This account is based on the fact that military personnel who served during the Persian Gulf War were exposed to biological and chemical agents. In addition to organophosphate exposure, service men and women were exposed to other environmental hazards such as depleted uranium canisters and large oil fires (see Figure 1.1; Binns et al., 2008). Also as highlighted in Figure 1.1, active duty during the first Gulf War exposed service men and women to psychological stress, which according to the

neurotoxin exposure hypothesis can work in conjunction with neurotoxin exposure to exacerbate the symptoms reported by ill GWV. Furthermore, some of these veterans present with depression and post-traumatic stress disorder (PTSD; see Figure 1.1). According to the neurotoxin exposure hypothesis neither of these psychological conditions are causal factors in GWI, but are related conditions.



• Post-traumatic stress disorder; ** Major Depressive Disorder; ***Paroxonae Gene Cluster; ****Brain-Derived Neurotrophic Factor

Figure 1.1 Conceptual model of Gulf War Illness

With regard to neurotoxin exposure, troops serving in operations Desert Shield and Desert Storm were exposed to acetylcholinesterase inhibitors in the form of insecticides containing N,N-diethyl-meta-toluamide (DEET), organophosphate nerve agents (e.g., sarin), and pyridostigmine bromide. GWV report having used military-supplied DEET and DEET-containing flea collars (Binns et al., 2008). In addition, as the result of the detonation of a

stockpile of sarin and cyclosarin located in Khamisiyah, Iraq, military personnel were exposed to non-lethal low doses of sarin (Binns et al., 2008). The detonation caused these agents to become airborne and dispersed over a large portion of the Persian Gulf. Finally, an estimated 250,000 service men and women were given pyridostigmine bromide tablets as a pretreatment for protecting them against the harmful effects of sarin exposure (Golomb, 2008).

Acetylcholinesterase inhibitors inhibit the action of acetylcholinesterase, an enzyme that breaks down acetylcholine. This inhibition causes a buildup of acetylcholine in the synaptic cleft, leading to the over-excitation and necrosis of cholinergic neurons (Bajgar, 2004; Pohanka, 2011). Cholinergic neurons densely populate the limbic system of the brain. Death of these neurons could lead to impairments in basic cognitive functions and emotional processing due to the role of the limbic system in these functions (e.g., hippocampus for memory and anxiety; amygdala for emotional processing, especially of fear; Packard, 2009). Pyridostigmine bromide tablets were provided to troops as a prophylactic that would prevent the lethal effects of sarin. Specifically, pyridostigmine bromide was used as a pretreatment for sarin exposure. While sarin is longer lived and has irreversible effects, pyridostigmine bromide is temporary and has reversible effects on acetylcholinesterase (Binns et al., 2008). Troops were instructed to use the tables if there was a threat of sarin exposure. While troops were only exposed to low doses of sarin and the effects of pyridostigmine bromide are temporary, long term exposure to low doses of sarin and exposure to pyridostigmine bromide can result in toxic effects with negative health consequences (Binns et al., 2008; Jamal, Hansen, & Julu, 2002). However, research has produced varying results (Brown & Brix, 1998).

At the time of the conflict, the long-term consequences of low-dose nonlethal exposure to sarin, the use of pyridostigmine bromide tablets, and the application of DEET were not well understood. Research conducted after the first Gulf War provided evidence that low-dose exposure to acetylcholinesterase inhibitors has long-term negative impacts on the brain and behavior. Some evidence supporting the neurotoxin exposure account comes from a non-

veteran population exposed to sarin. After the first Gulf War, a civilian population was exposed to an acute low-dose of sarin during a 1995 terror attack on the Tokyo subway system. Of the 641 victims hospitalized and subsequently studied after the subway attack, 83% received an intermediate dose and 17% a high dose of sarin (Okumura, Takasu, Ishimatsu, Miyanoki, Mitsuhashi, Kumada, et al., 1996). Relative to non-exposed controls, victims of the attack presented with delayed and prolonged neurobehavioral and neuropsychological effects of sarin poisoning similar to the symptoms experienced by ill GWV (Okumura, Takasu, Ishimatsu, Miyanoki, Mitsuhashi, Kumada, et al., 1996; Yokoyama, Araki, Murata, Nishikitani, Okumura, Ishimatsu, et al., 1998).

In addition, animal studies demonstrate that exposure to acetylcholinesterase inhibitors results in neuronal death and dysfunction in multiple brain regions, including the hippocampus (Abdel-Rahman, Shetty, & Abou-Donia, 2002; Abdel-Rahman et al., 2004; Abdel-Rahman, Dechkovskaia, et al., 2004; Henderson et al., 2001). More recently, Speed and colleagues (2012) found a delayed, progressive reduction in hippocampal synaptic transmission and spine density following repeated, low-dose organophosphate exposure. Research has also shown low-dose exposure to result in behavioral and cognitive abnormalities in animals (Abdel-Rahman, Dechkovskaia, et al., 2004; Gardner, Ray, Frankenheim, Wallace, Loss, & Robichaud, 1984; Lamproglou et al., 2009). Moreover, exposure to heat and stress exacerbates the negative effects of low-dose organophosphates in animals (Abdel-Rahman, Dechkovskaia, et al., 2004; Lamproglou et al., 2009). These findings support the notion that psychological stress and environmental conditions could exacerbate the negative effects of these agents on GWV and the symptoms associated with GWI.

Research conducted in veteran samples also suggests that low dose exposure to acetylcholinesterase inhibitors has long lasting effects on the central nervous system. With the use of MR spectroscopy, single photon emission computed tomography (SPECT), and arterial spin labeling (ASL), researchers have identified chronic, sustained abnormalities in several

brain regions, including the basal ganglia, pons, and hippocampus associated with GWI (Haley et al., 2000a; Haley et al., 2000b; Haley et al., 2009; Menon et al., 2004; Li et al., 2011; Liu et al., 2011). For example, Haley and colleagues (2000) obtained measures of NAA/Cr from the basal ganglia and brainstem using single voxel MRS. They observed ill GWV to have biochemical evidence of neural damage, as indicated by lower levels of NAA/Cr, relative to well GWV. Similarly, Menon and colleagues (2004) measured NAA/Cr in the left and right hippocampus using single voxel MRS. They observed ill GWV to have lower NAA/Cr ratios than well GWV, suggesting the existence of hippocampal dysfunction. Additionally, recent research has revealed that ill GWV who were exposed to sarin and cyclosarin have decreased gray and white matter volumes, as well as decreased hippocampal volume, compared to unexposed GWV, as measured using structural MRI (Chao et al., 2010; 2011).

Finally, data suggest that GWI is a complex illness, and that ill GWV can be subdivided into 3 distinct but related syndrome types (see Table 1.1). These syndrome types were originally defined by performing a factor analysis on the symptoms reported by a large sample of GWV (Haley, Kurt, & Hom, 1997), and subsequently, the syndrome types were validated using structural equation modeling (Haley, Luck, & Petty, 2001) and recently revalidated in a representative sample of Gulf War veterans (Iannacchione, V.G., Dever, J.A., Bann, C.M., Considine, K.A., Creel, D. et al., 2011). Syndrome 1, “impaired cognition,” is characterized by self-reported problems with executive function. Syndrome 2, “confusion ataxia,” is characterized by confusion and ataxia, problems with declarative memory, and emotional disturbances (Haley et al., 1997). Individuals classified with Syndrome 3, “central neuropathic pain,” report experiencing wide spread bodily pain and abnormalities in bodily sensation (Haley et al., 1997). As highlighted in Table 1.1, each of these syndrome types is associated with neurotoxin exposure, providing additional support to the neurotoxin exposure account of GWI. In line with this, Figure 1.1 further subdivides this wide array of symptoms experienced by ill GWV based

on whether or not the symptoms are physiological or psychological in nature as well as by syndrome type.

Table 1.1 Major Gulf War Syndrome classifications, symptoms, and risk factors

Syndrome	Syndrome Description	Characteristic Symptoms	Risk Factors
I	Impaired Cognition	Mild cognitive deficits; Distractibility; Forgetfulness; Depression; Chronic fatigue	Wore pesticide-containing pet flea collars
II	Confusion/Ataxia	Reduced intellectual functioning; Confusion, Vertigo; Disorientation	Perceived chemical nerve agent exposure; Experienced severe side effects from pyridostigmine tablets
III	Central Neuropathic Pain	Chronic, widespread joint and muscle pain; Other sensory abnormalities such as paresthesias and numbness	Wore insect repellant with concentrations of DEET; Experienced severe side effects from pyridostigmine tablets

Information in table derived from findings by Haley et al. (1997) and Iannacchione et al. (2011).

Collectively, past research demonstrates that ill GWV present with a constellation of behavioral, cognitive, and neurobiological abnormalities that are associated with low-dose exposure to organophosphates. Furthermore, the extant data identify the hippocampus as an area affected by neurotoxin exposure and indicative of physiological variations between ill and well GWV. However, to date, only a scarce number of studies have reported the link between the differences in the brain function of ill and the memory problems reported by ill GWV. Li and colleagues (2011) observed a link between regional cerebral blood flow (rCBF) in the hippocampus and memory performance in ill GWV from the Seabees cohort. Specifically, they found that changes in rCBF in response to physostigmine correlated with successful performance on the face-name associative memory test in the current study. Many studies of deficits in declarative memory in GWV, using neuropsychological tests that investigate verbal

and visual memory, have produced mixed results, with only a few studies observing poorer declarative memory among ill GWV (David et al., 2002; Hom, Haley, & Kurt, 1997; Proctor, Heaton, Heeren, & White, 2006).

Given the importance of the hippocampus for memory function and its observed differences in ill GWV compared to controls, together with the memory complaints of ill GWV, it is remarkable that observed memory performance in ill GWV is equivalent to that of control subjects (Hom, Haley, & Kurt, 1997). A recent study did find differences in GWV's semantic memory performance (Calley et al., 2010). However, semantic memory is facilitated by the middle temporal lobe and is thought to be independent of episodic memory, which relies on the hippocampus, (Sheldon & Moscovitch, 2011; Eichenbaum, Yonelinas, & Ranganath, 2007). To date, only a couple published studies have reported deficits in ill GWV's episodic memory relative to well GWV (Odegard et al. in press; also see Li et al., 2011). However, this initial study was conducted in a small sample of all-male veterans, who were on average older than the majority of the veterans deployed to during the first Gulf War. In addition, the sample was not large enough to adequately account for comorbid conditions that may affect GWI. This lack of adequate sample size limited the ability to investigate the second most prevalent account of GWI, the psychological stress hypothesis (Binns et al., 2008).

1.1.3 Psychological Stress Hypothesis of Gulf War Illness

While the neurotoxin exposure hypothesis posits that self-reported symptoms arise from physiological alterations due to neurotoxin exposure, a competing perspective is that GWI is a sequela resulting from other medical issues related to psychological and physical stressors experienced during the war (Binns et al., 2008). The U.S. Department of Veteran Affairs released a report by the Research Advisory Committee on Gulf War Veterans' Illnesses, which stated that deployed GWV were found to have higher rates of PTSD and other psychiatric disorders relative to GWV who were not deployed (Binns et al., 2008). However, this competing account has been questioned because the rates of psychiatric illnesses observed in GWV are

lower than the rates observed in combat veterans who served in other conflicts (Binns et al., 2008). Moreover, the prevalence rates of these illnesses in GWV are not always significantly different than the prevalence rates observed in civilian populations (for a review see Stimpson, Thomas, Weightman, Dunstan, & Lewis, 2003).

Co-morbid pathologies, such as PTSD and depression, are often accompanied by memory problems, which are under investigation in the current study. Furthermore, memory deficits experienced by individuals suffering from PTSD and depression might be due to underlying neurobiological abnormalities. Past studies of neurobiological abnormalities in PTSD and depression have used measures similar to those of the present study as a means of assessing GWI. Evidence from fMRI in both civilian and veteran populations with PTSD indicates that the hippocampus and other regions involved in the memory network are functionally different in individuals with PTSD than controls (Geuze et al., 2008; , Meindl, Engel, Rosner, Riedel et al., 2009a). Specifically, while associative memory performance might not significantly differ between those with PTSD and those without, those with PTSD show functional alteration such as hyper-activity of the medial temporal lobe (MTL) and hypo-activity of the frontal lobe during encoding of associative items, suggesting possible compensatory mechanisms (Geuze et al., 2008; Werner et al., 2009a). Likewise, Werner and colleagues (2009b) found similar functional dysregulation in patients suffering from depressive disorder.

Similarly, Schuff and colleagues (2008) used the NAA/Cr ratio, measured by MRS, to identify neuronal abnormalities in the hippocampus of patients with PTSD. This marker was investigated as an indicator of hippocampal abnormality in PTSD due to research both finding and failing to find decreases in hippocampal volume in PTSD as well as not hippocampal atrophy compared to controls (Bremner, Randall, Scott, Bronen, Seibyl, Southwick et al., 1995; Gurvits, Shenton, Hokama, Ohta, Lasko et al., 1996; Gilbertson, Shenton, Ciszewski, Kasai, Lasko et al., 2002; Hedges, Allen, Tate, Thatcher, Miller, Rice et al., 2003; Bonne, Brandes, Gilboa, Gomori, Shenton, Pitman et al., 2001; Bonne, Vythillingam, Inagaki, Wood, Neumeister,

Nugent et al., 2008; Schuff, Neylan, Lenoci, Du, Weiss et al., 2001; Fennema-Notestine, Stein, Kennedy, Archibald, Jernigan, 2002; Golier, Yehuda, De Santi, Segal, Dolan et al., 2005; Yehuda, Harvey, Buchsbaum, Tischler, Schmeidler, 2007). In the case of Schuff and colleauges (2008), NAA/Cr was found to be a more sensitive measure of neuronal alterations than brain volume. While there was an absence of hippocampal volumetric reduction, PTSD was associated with reduction of NAA/Cr in the hippocampus (Schuff et al., 2008; Schuff et al., 2001). Several other studies have also observed reduction in hippocampal NAA in PTSD (also see Freeman, Cardwell, Karson, Komoroski, 1998; Mohanakrishan Menon, Nasrallah, Lyons, Scott, Liberto, 2003; Villarreal, Petropoulos, Hamilton, Rowland, Horan et al., 2002) NAA/Cr levels also serve as a marker of treatment response in depression, such that NAA/Cr levels show significant improvement with successful response to treatment and this is related to level of depression (Duan et al., 2011; Kado et al., 2006).

Thus, neurotoxin exposure is not the only possible cause of the brain abnormalities observed in GWV. However, PTSD and depression are accompanied by different types of memory issues than the memory deficits reported by ill GWV. Individuals suffering from PTSD report unwanted intrusive memories of the event that triggered their emotional disturbances. Research on declarative memory in PTSD has been mixed, with some studies showing subtle verbal memory impairments, and others not showing any impairment (Samuelson, 2011). Individuals with depression experience over-general memories, the tendency to report under-specific memories of the past that do not contain source specifying details that link them to specific episodes. Individuals with depression also experience memory sensitivity to negatively valenced stimuli while experiencing some deficits in verbal memory depending on mood severity (Douglas & Porter, 2009; Hamilton & Gotlib, 2008). While a link has been found between depression and memory, this link is not unique to depression but rather a subset of individuals with depression (Burt, Zembar, & Niederehe, 1995). In contrast, given the functional abnormalities that have been observed in ill GWV, these individuals should present with deficits

in episodic memory that differ from the deficits experienced by individuals with depression or PTSD. Specifically, ill GWV should be impaired in their ability to form and retrieve memories of non-threatening episodic events. This stands in contrast to the affective memory impairments exhibited by individuals with PTSD and depression.

To accommodate different perspectives on GWI and account for the potential impact of depression and PTSD in the GWV population, clinical measures of both major depression and PTSD were obtained from all participating veterans in the present study. These were controlled for in several of the analyses. For example, PTSD and depression were covaried when analyzing memory performance, in order to account for any effect these conditions might have on the associative memory deficits predicted in ill GWV relative to well GWV.

1.1.4 Overview of Current Study

In light of these considerations, a national representative sample of GWV, both affected and unaffected, completed a face-name memory paradigm that assessed intermediate states of memory as part of this study (Haley et al. 1997). To address the issue of heterogeneity characterized by individuals with GWI, the ill GWV were subdivided into three syndrome types based on their self-reported symptoms and responses to an extensive questionnaire administered as a computer-assisted telephone interview (CATI) survey: Syndrome 1 (impaired cognition), Syndrome 2 (confusion/ataxia), and Syndrome 3 (central neuropathic pain; Iannacchione et al., 2011).

Figure 1.2 highlights components of Figure 1.1 that will be the focus of the current study. The current study is being conducted to objectively measure the memory difficulties reported by GWV with GWI by using a task that promotes associative memory formation. In addition, the study was being conducted to identify neurobiological correlates of memory performance using neuroimaging. Specifically, the study was conducted to identify potential deficits in associative memory for ill GWV, as well as to identify potential differences in brain activity and integrity using BOLD fMRI and MRS, respectively. Figure 1.2 also highlights how

these physiological measures might directly affect psychological measures. Furthermore, the study also took into account the possible effects that risk factors or psychological disorders (age, PTSD, and major depressive disorder or MDD) have on GWI and the measures used in the study.

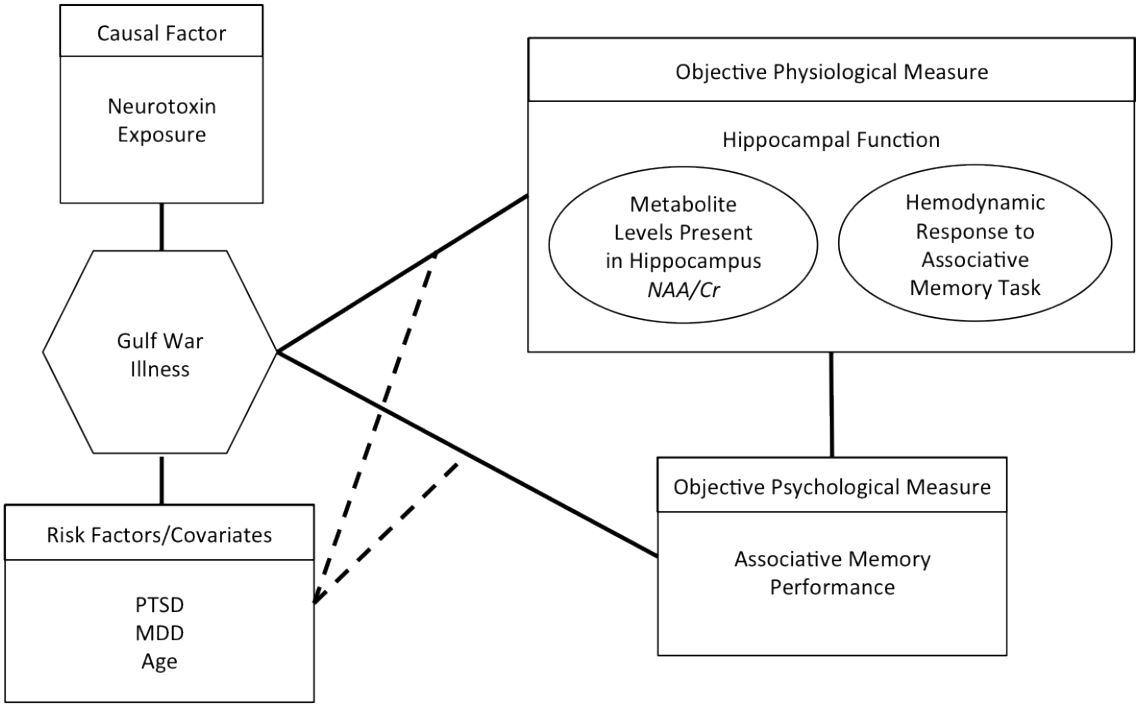


Figure 1.2 Conceptual model of associative memory performance and hippocampal function in veterans with Gulf War Illness

1.1.4.1 Memory

To better characterize the memory problems reported by ill GWV, a face-name associative memory task was administered to participating veterans. An associative memory task was used because of the known reliance of associative memory on hippocampal function, particularly when associated items are from different stimulus domains and processed perceptually in different regions of the cerebral cortex, as is the case with faces and names (Brown & Aggleton, 2001; Diana, Yonelinas, & Ranganath, 2007; Mayes, Montaldi, & Migo, 2007; Piekema, Kessels, Rijpkema, & Fernandez, 2009; Sperling et al., 2001; Sperling et al., 2003). Moreover, the specific paradigm assessed not only successful associative recall of faces'

names, but also an intermediate associate memory state prevalent in real-life situations–that of a person failing to recall a face's name despite knowledge that it had been encountered earlier.

Previous research (Odegard et al. in press) using this face-name associative memory test has demonstrated that ill GWV from the Haley Seabees cohort (24th Reserve Naval Mobile Construction Battalion) are impaired on the intermediate measure of associative memory, *know*ing a name was presented with a face, but not in their overall ability to recall the names that accompanied the faces at study. Moreover, members of all three syndrome types had significantly lower performance on the intermediate form of associative memory relative to controls. These results were surprising, because Odegard and colleagues (in press) predicted that only Syndromes 1 and 2 would show memory deficits relative to controls, based on the self-reported symptoms associated with each of the syndrome types.

The current study intends to replicate and extend the previous study in two critical ways. First, the present study is a replication and extension of the previous study to a larger national sample that is demographically representative of the U.S. veterans who served in the first Gulf War with respect to age, gender, race and ethnicity. The previous study sampled only males from a single construction battalion who were, on average, significantly older than the majority of the individuals who served in the conflict. For the current study, participants were both male and female, were of different races and ethnicities, were of different ranks at the time of the conflict, and were from geographically different parts of the Unites States. While the Seabees sample included some of these demographic varieties, the national sample took them into account with the addition of more variables that are representative of the GWV population that were not included in the Seabees sample. Some of these extensions include gender, race, and ethnicity as stated. Second, the current study includes a larger sample than the previous study, which allows statistical control for the potentially confounding effects of age, MDD and PTSD on memory performance. Third, correlations with MRS measures of NAA/Cr, as investigated in this study, were not performed in the previous study (Odegard et al., in press).

1.1.4.2 Functional Magnetic Resonance Imaging

To better characterize the neurobiological correlates of memory deficits associated with GWI, the BOLD contrast was measured using fMRI during the encoding phase of the associative memory task. fMRI data were collected during encoding because the hippocampus, a known area of dysfunction in ill GWV, is a part of a distributed network of brain regions implicated as important for associative memory encoding (Davachi, 2006; Eichenbaum et al., 1996; Henke et al., 1997; 1999; Mayes et al., 2007; Sperling et al., 2001; 2003; Schacter & Wagner, 1999). Moreover, the hippocampus has been observed to be more active during encoding of associative items than independent items (Achim & Lepage, 2005; Chua et al., 2007; Davachi & Wagner, 2002; Giovanello et al., 2004; Kirwan & Stark, 2004; Stark & Squire, 2001; Yonelinas et al., 2001). For example, Chua and colleagues (2007), using a face-name paradigm in healthy younger adults to probe hippocampal function, observed activity in the posterior hippocampus during encoding of face-name pairs. Furthermore, research using paradigms testing subsequent memory has demonstrated the hippocampus to be more active during the encoding of associations that are successfully remembered at test (Chua et al., 2007; Davachi & Wagner, 2002; Jackson & Schacter, 2004; Sperling et al., 2001, 2003; Staresina & Davachi, 2008; Zeineh et al., 2003). Specifically, the anterior hippocampus has been observed to be active during encoding of successfully remembered associations (Chua et al., 2007). Recently, using a subsequent memory paradigm of face-names, Westerberg and colleagues (2011) observed the hippocampus to be more active during encoding of these associations that were successfully remembered on a later memory test, compared to forgotten associations or remembered individual items.

The participants in the present study underwent fMRI while they were studying face-name pairs. These data were used to identify whether ill GWV have functional brain differences in comparison to well GWV when attempting to learn information for a subsequent associative memory test. Previously published arterial spin labeling (ASL) MRI data have demonstrated a

sample of ill GWV from a construction battalion (Seabees) deployed to the 1991 Iraq-Kuwait theater to have dysfunctional blood flow to the hippocampus (Li et al., 2011). More importantly, hippocampal function, as measured by ASL, was directly related to memory performance on the associative memory task used in the present study. Thus, the present study could replicate and extend these previous findings from the Seabees to an independent measure of hippocampal function (i.e., BOLD fMRI) obtained when a representative national sample of GWV were actually attempting to memorize study items for the subsequent memory test.

Moreover, the previous study by Odegard and colleagues (in press) investigating associative memory with this paradigm in Seabees GWV observed activation in the hippocampus during encoding to be positively correlated with subsequent memory performance. However, the initial study was statistically underpowered. This prohibited meaningful comparisons between the ill and well GWV. The current study replicates and extends the previous Seabees study by directly comparing activation measured during fMRI acquired during encoding between the three syndrome types and control veterans from the larger national sample. Additionally, the previous study did not categorize study items based on subsequent memory performance. Thus, the current study performed subsequent memory analysis of the fMRI data and compared this activation between the three syndrome types and control veterans.

1.1.4.3 Magnetic Resonance Spectroscopy

In addition to fMRI, GWV also had MRS data acquired from the left and right hippocampus during another MR session. The acquisition of this neurochemistry data allows for computation of NAA/Cr ratios, providing a measurement of metabolic function within left and right hippocampus. Among its many roles, NAA is important for brain fluid osmosis and contributes to energy production (Jiang et al., 2009). NAA is found in much higher quantities in neuronal bodies than other cells throughout the brain, such as mature glial cells, and is widely recognized as a marker of neuronal health and viability (Barker, 2001; Friedland et al., 2001;

Traber et al., 2006; Urenjak et al., 1993). Decreases in NAA have been observed in response to neuronal death resulting from incidents such as stroke or neuronal disease (Barker, 2001; Connelly et al., 1994; Graham et al., 1993; Kobayashi et al., 2001; Kwo-On-Yuen et al., 1994; Preul et al., 1996). In addition, NAA has been extensively used to investigate the neuronal integrity of the hippocampus, and it has been demonstrated to differentiate between different pathologies (Traber et al., 2006; Waldman & Rai, 2003). In contrast, Cr is found throughout numerous cell types (e.g., astrocytes, oligodendrocytes) within the brain, and thus is commonly thought to provide a stable and constant signal in the brain and indicates energetic stores and metabolism of neurons and glia (Urenjak et al., 1993). As measured by ^{1}H MRS, the total creatine (tCr) methyl peak is composed of overlapping signals from the methyl groups of "free" creatine (Cr) and phosphocreatine (PCr). Although the amount of tCr is likely constant, Cr and PCr can interconvert, and have different T_2 relaxation times (Ke et al., 2002). Thus, changes in the relative amounts of Cr and PCr can alter the tCr signal intensity, more so in MRS experiments with longer echo times (TE).

Observation of metabolite ratios, such as NAA/Cr, provides an established method for analyzing clinical spectra that have known ranges of normalcy. Past research has used metabolite ratios to investigate neuronal abnormalities in a variety of populations, including older adults, civilian and veteran populations with PTSD, depressed patients, and lead-exposed workers (Caserta et al., 2008; Duan et al., 2011; Jiang et al., 2008; Kado et al., 2006; Schuff, Amend, Knowlton, Norman, Fein, & Weiner, 1999; Schuff et al., 2008; Zimmerman et al., 2008). For example, in a study of normal aging, reductions in hippocampal NAA/Cr were observed as a function of increasing age, which could provide strong support for neuronal loss as reflected in NAA/Cr being more sensitive than volume alone (Schuff et al., 1999). In addition, research has also demonstrated hippocampal NAA/Cr reductions to be more pronounced in mildly impaired (MCI) individuals when compared to their elderly controls, showing this to be a possible marker of future progression to dementia (Caserta et al., 2008).

Additional research has also demonstrated links between these NAA/Cr abnormalities and memory performance. Zimmernan and colleagues (2008) found hippocampal NAA/Cr to be a predictor of performance on a verbal memory task in a population of nondemented older adults. Specifically, lower levels of NAA/Cr predicted poorer memory performance. Additionally, Driscoll and colleagues (2003) used two hippocampal-dependent memory tasks, the virtual Morris water task and the transverse patterning discrimination task, on both younger and older adults, and found age-related decreases in performance as well as reductions in hippocampal NAA/Cr.

With regard to GWI, past research demonstrated that NAA/Cr ratios in the hippocampus are lower in ill GWV than controls and well GWV, further supporting claims of hippocampal dysfunction in ill GWV (Menon et al., 2004). In the present study, both group and correlational analyses were conducted to test for the presence of a relationship between this biomarker of neuronal function and the ability of GWV to remember names that accompanied the faces presented at study. This analysis was conducted to investigate the extent to which individual differences in hippocampal function predict the ability of older adults to engage in episodic retrieval.

1.2 Goals of Current Study

The present study used data from a national sample of veterans, who served in the 1991 Persian Gulf War. This sample was acquired between 2009 and 2010 during a multimodal study of GWI. Specifically, the study aimed to confirm the self-reported memory problems of ill GWV with a hippocampal-dependent memory task and to identify possible neurobiological markers of cognitive and hippocampal function. During associative memory encoding, fMRI was used to acquire the BOLD contrast to serve as a proxy of brain activation evoked by the memory task. Additionally, NAA/Cr levels were obtained from the hippocampus of participating veterans using single voxel MRS taken during another MR session. The NAA/Cr ratio obtained from MRS served as a proxy for functional neuronal mass in the hippocampus during a resting

state. I hypothesized that ill GWV would demonstrate decreased memory performance relative to well GWV on this memory test, providing evidence of memory deficits using an objective measure of memory. In addition, I anticipated that memory differences are due to differences in brain function during the encoding of associative memory formation. As such, I predicted that differences would be observed between the well and ill GWV in the amount of brain activation measured during encoding using BOLD fMRI. Moreover, I predicted that the hippocampus in particular is an area of the brain that would give rise to these anticipated memory differences, and thus would be one of the brain regions observed to be functionally different among the groups. Lastly, the NAA/Cr ratio level measured in the hippocampus were predicted to differ between well and ill GWV. Moreover, the NAA/Cr ratio measured in the hippocampi would be positively correlated with associative memory performance.

CHAPTER 2

METHODS

2.1 Participants

Between 2009 and 2010, a sample of 89 GWV gave written informed consent and participated in this study in exchange for monetary compensation in accordance with a protocol approved by The University of Texas Southwestern Medical Center's institutional review board. Due to missing data or technical error, 9 participants were excluded from analyses. The GWV who participated in the current study were part of a much larger national sample, who completed a phone based interview assessing their health and service history (Iannacchione et al., 2011). The larger sample included veterans from a variety of military branches and positions both deployed and nondeployed during the 1991 Persian Gulf War. Nondeployed veterans of the Persian Gulf War were included to serve as a comparison group of veterans to control for any effect of deployment. From the larger sample, a smaller cross section of these veterans traveled to Dallas, Texas to participate in a week long multi-project study. These participants were housed and monitored at The University of Texas Southwestern Medical Center's Clinical and Translational Research Center (CTRC), supported by the National Institute of Health under the direction of Dr. Milton Packer.

Of the GWV, 19 met the Haley et al. (1997) criteria for GWI Syndrome 1 (GWV-1), 20 met criteria for GWI Syndrome 2 (GWV-2), 18 met criteria for GWI Syndrome 3 (GWV-3), and 24 were age-sex-education-matched GWV who remained well and served as controls (GWV-C; 10 deployed/14 nondeployed). The GWV-1 group ranged in age from 38 to 69 years (M = 49.16; SD = 8.84), the GWV-2 group ranged in age from 38 to 66 years (M = 49.95; SD = 8.39), the GWV-3 group ranged in age from 40 to 67 years (M = 51.44; SD = 8.15), and the control group ranged in age from 39 to 59 years (M = 45.60; SD = 7.41) for deployed and 43 to 66

years (M = 51.50; SD = 7.80) for nondeployed. The control groups did not differ in most demographic information: mean age, sex, race, and ethnicity. Due to the increased prevalence rates of PTSD and depression among veterans, both of these disorders were clinically assessed, and will be used as covariates in some of the analyses (see Table 2.1, Forman-Hoffman et al., 2005; Hom et al., 1997; Iowa Persian Gulf Study Group, 1997; Shalev et al., 1998; Toomey et al., 2007). For a list of demographics and assessment scores, see Table 2.1.

Table 2.1 Demographic data for participating veterans

	Non-deployed	Deployed	GWV-1	GWV-2	GWV-3
No. Participants	14	10	19	20	18
Age, mean years, (SD)	51.50 (7.80)	45.60 (7.41)	49.16 (8.84)	49.95 (8.39)	51.44 (8.15)
No. Left-handed/ ambidextrous (%)	1 (7.14)	0 (0)	2 (10.53)	2 (10)	1 (5.56)
Female (%)	3 (21.43)	1 (10)	6 (31.58)	6 (30)	3 (16.67)
Race/Ethnicity					
Caucasian (%)	12 (85.71)	6 (60)	13 (68.42)	16 (80)	13 (72.22)
Afr. Amer (%)	2 (14.29)	3 (30)	2 (10.53)	3 (15)	3 (16.67)
Hispanic (%)	0 (0)	1 (10)	1 (5.26)	1 (5)	1 (5.56)
Other (%)	0 (0)	0 (0)	3 (15.79)	0 (0)	1 (5.56)
Education scale, mean (SD)[1]	5.21 (2.19)	4.80 (1.90)	5.68 (1.67)	4.74 (1.70)	4.94 (2.13)
MOS SF-12 Mental Component t-Score, mean (SD)	57.83 (3.64)	57.73 (9.21)	36.45 (10.75)	49.45 (9.42)	46.66 (10.71)
MOS SF-12 Physical Component t-Score, mean (SD)	53.44 (5.97)	54.92 (2.26)	36.47 (12.13)	26.45 (7.52)	32.21 (9.66)
MDD Active, SCID (%)	0 (0)	0 (0)	4 (21.05)	1 (5)	3 (16.67)

[1] Education was coded on the following scale (number of GWV at each level provided in parenthesis): 1 = Dropped out of school (0); 2 = GED (3); 3 = HS graduate (6); 4 = Some college, no degree (30); 5 = Associate's degree (10); 6 = Bachelor's degree (15); 7 = Some graduate school, no masters (1); 8 = Graduate/professional degree (9); 9 = Trade school certificate/diploma (5); . = missing (1). Average education was (*M* = 5.13, *SE* = .20) for the GWV. Specifically, GWV-C were (*M* = 5.14, *SE* = .37); GWV-1 were (*M* = 5.60, *SE* = .37); GWV-2 were (*M* = 4.79, *SE* = .38), and GWV-3 were (*M* = 4.94, *SE* = .50) in average education.

Table 2.1 – *Continued*

Antidepressant Use(%)	0 (0)	0 (0)	8 (42.11)	9 (45)	2 (11.11)
Alcohol abuse or dependence, active, SCID (%)	2 (14.29)	0 (0)	5 (26.32)	8 (40)	5 (27.78)
Drug abuse or dependence, active, SCID or admission urine test (%)	1 (6.70)	0 (0)	1 (5.26)	3 (15)	1 (5.56)
PTSD Active, CAPS (%)	0 (0)	0 (0)	8 (42.11)	8 (40)	5 (27.78)
Antianxiety use No. (%)	0 (0)	0 (0)	4 (21.05)	4 (20)	1 (5.56)

SD, standard deviation; CAPS, Clinical Administered PTSD Scale; MDD, Major Depressive Disorder; PTSD, Post-traumatic Stress Disorder; SCID, Structured Clinical Interview for DSM-IV Disorders

2.2 Memory Task: Covert Recall Face-Name Paradigm

The experiment conformed to a 2 (Item Type: Face-Name Items, Face-Only Items) X 4 (Group: GWV-C, GWV-1, GWV-2, GWV-3) design. Item type was manipulated within participants. At study, participants viewed faces studied alone (face-only items) and faces studied with names (face-name items). At test, participants were presented with faces that had been face-name items at study and faces that had been face-only items at study. For each face presented on the test, participants made one of three memory responses. When presented with a face on the memory test, they could respond “recall” in those instances that they thought that a name had been paired with the face and could recall the name, or they could respond “know” if they knew a name had been paired with the face but could not recall the specific name. Both of these responses are considered associative memory judgments. In addition, they could make a non-associative item response when presented with a face on the memory test, by indicating that the face had been studied alone during encoding. The face-only items provided a basis to correct associative memory judgments made to face-name pairs for response bias (i.e., guessing). Group membership was a between subjects quasi-independent variable, such that a sample of GWV with no reported issues (i.e., GWV-C) were sampled to provide a comparison

group for the ill GWV In addition, ill GWV were sampled from the three subtypes of GWI identified by Haley et al (i.e., GWV-1, GWV-2, GWV-3; 1997).

2.2.1 Materials

The paradigm materials consisted of 144 grayscale pictures of Caucasian faces (72 male, 72 female) and 144 names (72 male, 72 female). Names were sampled from the Social Security database of names and were randomly assigned to a face with the restriction that the gender of the name matched the gender of the person depicted in each picture. The face-name pairs were randomly divided into 18 groups of 8 (4 male, 4 female per group) for counterbalancing purposes. There were a total of 6 study lists that each contained 24 items. On each study list, eight study items were faces and the remaining 16 study items were face-name pairs. Examples of the study items are provided in Figure 2.1. Each study list was followed by a memory test. Each memory test consisted of the 24 faces that had been presented on the corresponding study list (see Figure 2.1). Materials were counterbalanced such that all of the faces served as each of the item types on each of the six memory tests an equal number of times across participants. Additionally, a set of 12 faces and names (6 male, 6 female) were used to construct a practice study list and memory test.

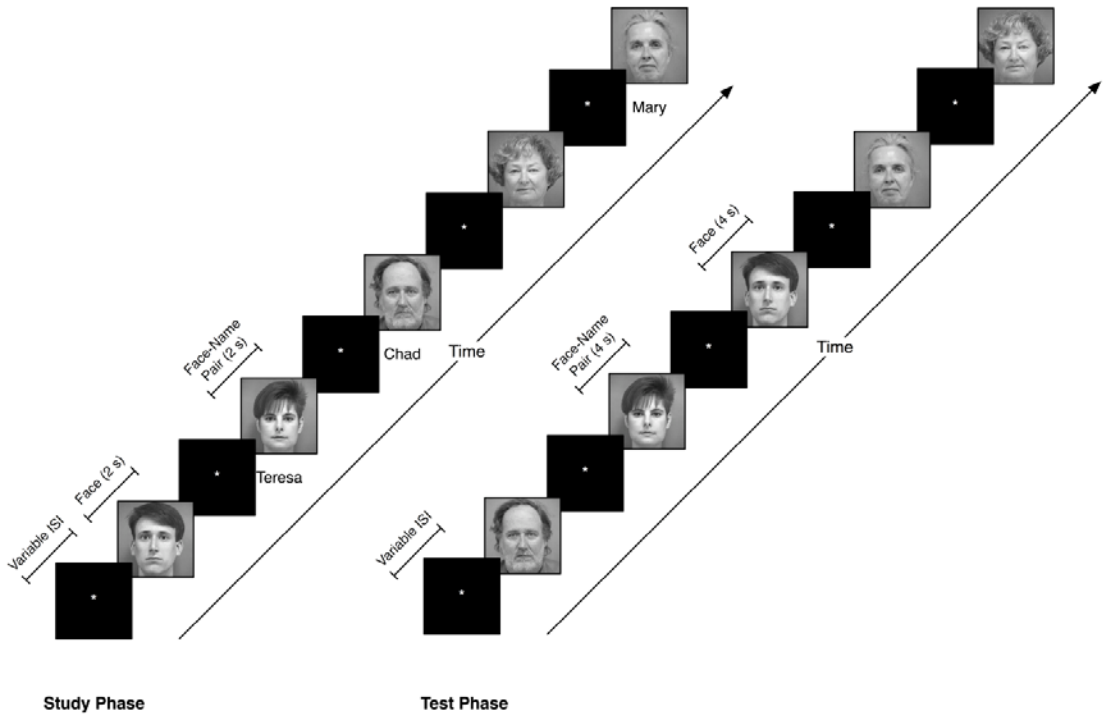


Figure 2.1 Example of study and test items in associative memory task

2.2.2 Procedure

Research personnel, who were blind to whether or not a GWV was ill or well, administered the protocol. Prior to scanning, participants completed two study lists and accompanying memory tests for training purposes outside the MR scanner. Afterwards, participants completed four study lists and memory tests while inside the MR scanner. There was no overlap shared between the practice materials and the materials presented in the scanner. During each study list, participants viewed a series of faces and face-name pairs using a screen connected to a computer running E-Prime (Schneider, Eschman, & Zuccolotto [Psychology Software Tools, Inc], 2002). As depicted in Figure 1.1, each face and face-name pair appeared on the screen for 2 s followed by a variable inter-stimulus interval (ISI) averaging 6 s (range 4-8 s) during study. Immediately after the presentation of each study list, participants

completed a memory test. At test, participants were presented with the same faces that they had seen on the study list (see Figure 2.1). None of the names presented at study appeared at test. Each face was presented for 4 s followed by a variable ISI averaging 6 s (range 4-8 s). Participants were asked to identify which test faces had been presented with a name at study and which had not. To do so, participants made one of three responses. If participants knew that a name had been presented with a face at study and they were able to recall the name, they were to make a "recall" judgment. In contrast, if participants knew that a face had been presented with a name at study but were unable to recall the name, they were to make a "know" judgment. Participants were instructed to respond "face only" if they thought the face had not been accompanied by a name at study. During the memory tasks performed outside of the scanner, participants said the name aloud when making a recall response. While in the scanner, test responses were recorded using a four-button fiber optic response box, and participants were instructed not to actually say aloud the names they recalled. Participants were asked to not state the recalled name to prevent head motion associated with talking and to avoid the difficulty of recording spoken responses in the noisy MR environment.

In a separate clinical interview conducted during the course of the GWV's week-long testing, demographic information and measures of co-morbidity were obtained by the Neuropsychology Tests Sub-Core, Task Order 4.7 of the VA-funded Gulf War Illness project, (John Hart, Munro Cullum, & Melanie Biggs). PTSD was assessed using the clinician-administered PTSD scale (CAPS; Blake et al., 1995; Weathers, Keane, & Davidson, 2001). The CAPS is a semi-structured interview, consisting of a 17-item self-report checklist of life events, used to identify whether or not the individual was exposed to a traumatic event. This was followed by a series of questions to address the presence of 22 symptoms and associated features of PTSD as outlined in the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV; American Psychiatric Association, 1994). The structured clinical interview for DSM-IV Axis 1 disorders (First, Spitzer, Gibbon, & Williams, 1996) includes modules containing question

prompts and scoring for the majority of Axis I and Axis II disorders. This was used to measure major depressive disorder (MDD), alcohol abuse or dependence, and drug abuse.

2.3 Functional Magnetic Resonance Imaging

2.3.1 Materials and Procedure

Participants were prepared for the scanning environment by training in a full-scale mock-up MR scanner to improve compliance, reduce anxiety, and reduce head motion in the MR scanner. Scanning was performed at the University of Texas Southwestern Medical Center on a 3T Siemens Trio Total Imaging Matrix (TIM) scanner with a 12-channel radio frequency head coil. A time of flight Magnetic Resonance Angiography (MRA) sequence (flip angle = 30°; TE = 4 ms; TR = 22 ms; 220 mm field of view (FOV); matrix size = 256 x 256; in-plane resolution = 0.86 x 0.86 mm; slice thickness = 3 mm; 42 axial slices) and a high-resolution T1*-weighted structural 3D MP-RAGE sequence (flip angle = 9°; TE = 3.96 ms; TR = 2250 ms; TI = 900 ms; in-plane resolution = 0.94 x 0.94 mm; slice thickness = 0.90 mm; matrix size = 256 x 240; 176 sagittal slices) were acquired. The acquisition times were 3.50 min and 4.50 min respectfully.

For fMRI, a series of T2*-weighted echo planar image (EPI) sequences with blood oxygenation level dependent (BOLD) contrast (flip angle = 90°; TE = 18 ms; TR = 2000 ms; 220 mm field of view (FOV); matrix size = 74 x 74; in-plane resolution = 2.97 x 2.97 mm; slice thickness = 3 mm; 42 axial slices) were collected from each participant using GRAPPA with an acceleration factor of 2. EPI data were acquired during the four study lists and four memory tests. A total of 105 volumes were collected during the presentation of each study list and 129 volumes were collected during the completion of each memory test. Total acquisition time for all of the fMRI runs was roughly 45 min. This data was collected from each participant to serve as a proxy of neural activation. Specifically, BOLD contrast captures the content of deoxyhemoglobin in the blood throughout the brain. Due to the difference in magnetic

susceptibility of oxygen-free hemoglobin, a greater image intensity can be reflected during the relaxation process of water protons for tissue that expended oxygenated hemoglobin.

2.4 Magnetic Resonance Spectroscopy

2.4.1 Materials and Procedure

In a separate MR session, ^{1}H MRS data were obtained by members of the MRS sub-core group (Sergey Cheshkov, Audrey Chang, Hyeonman Baek, Sandeep Ganji, Evelyn Babcock, & Richard Briggs) of Task Order 4.1 of the VA-funded Gulf War Illness project using a single voxel spectroscopy (SVS) Point RESolved Spectroscopy (PRESS) 1H MR sequence (TR = 2500 ms; TE = 30 ms; NS = 128; spectral width = 2000 Hz, water suppression bandwidth = 50 Hz, data points = 1024) to acquire brain metabolite concentrations and concentration ratios in the left and right hippocampus (see Figure 2.2). Such metabolite concentrations and concentration ratios include, but are not limited to, NAA, creatine (Cr), and NAA/Cr. The voxel volume equaled 3.75 ml (10 mm x 25 mm x 15 mm), and acquisition time was 5.50 min per side. A manual shim current adjustment (5-10 min per side) was utilized to achieve better spectral quality. An unsuppressed water spectrum (8 averages, 0.50 min) was acquired for eddy current compensation and quantification. High-resolution and high-contrast localizer images were used for proper and reproducible voxel positioning (Cheshkov et al., 2007).

As seen in Figure 2.2, voxel placement was in the MTL. The voxel encompassed the extent of the hippocampus, the head and body. It is important to note that due to the geometry of the voxel and shape of the hippocampus, contamination can occur such that data can be collected from other surrounding regions including the parahippocampal gyrus. The left side of the figure shows an example of the data collected by the MRS sub-core group. The horizontal axis represents frequencies normalized to a parts per million (ppm) scale, and each metabolite peak has a specific resonance frequency. After suppressing water in order to make the metabolites visible (the water concentration is about 1000x that of the metabolites) and collecting the data as a free induction decay (FID) signal of amplitude versus time, a Fourier

data transformation was used to separate the combined signals of all the peaks in the voxel into individual peaks at specific frequencies of the spectral plot shown by the white line of Figure 2.2. A simulated spectrum from summed model spectra of individual metabolites is shown by the red line. As can be seen, the data fit tightly with the model. The area under the curve of each peak for each metabolite is proportional to the concentration for that given metabolite. Figure 2.2 was provided by the MRS sub-core group (Sergey Cheshkov, Audrey Chang, Hyeonman Baek, Sandeep Ganji, Evelyn Babcock, & Richard Briggs) of Task Order 4.1 of the VA-funded Gulf War Illness project.

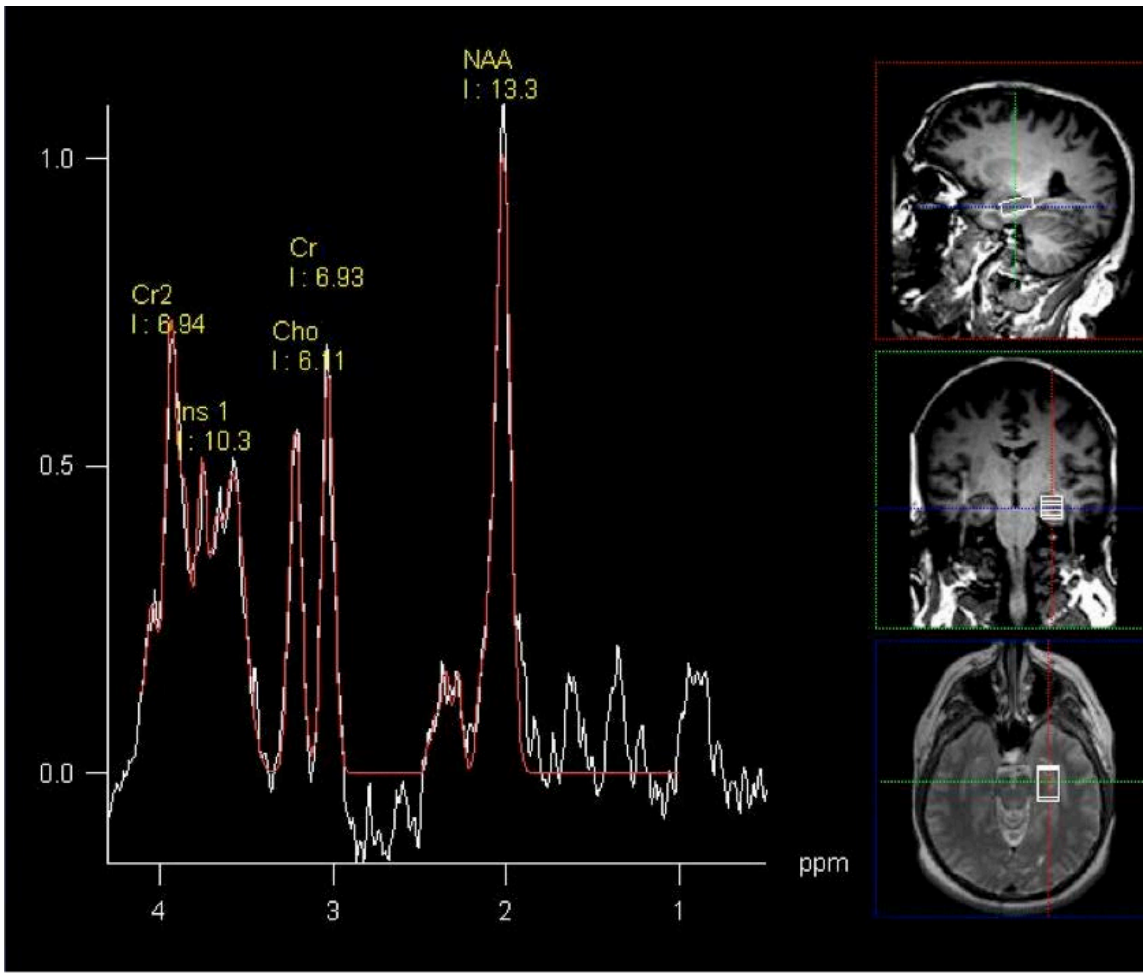


Figure 2.2 Example of magnetic resonance spectroscopy voxel placement and metabolite concentrations in the hippocampus of a participating veteran

CHAPTER 3

DATA ANALYSIS AND RESULTS

The memory paradigm used in this study has previously generated data that allowed for observation of differences between patient populations with sample sizes of 10 per group (Odegard et al., in press). Thus, the sample sizes of 18-29 per group should provide adequate statistical power to perform the analyses. Statistical analyses of behavioral memory performance, and NAA/Cr levels were performed using the SPSS software package (SPSS Inc., 2010). For analysis of fMRI data, a combination of SPSS and Analysis of Functional NeuroImages (AFNI; Cox, 1996) software was used. In the sections that follow, specific data analysis procedures for each aim are described for memory performance, fMRI, and MRS (NAA/Cr). I also included PTSD and MDD diagnoses, as well as age, as covariates where appropriate. Additionally, there are two control groups, deployed and nondeployed controls, which were tested for equivalence on all measures and combined into a single control group (GWV-C) if equivalent, or otherwise kept separate. All subsequent analyses assume equivalence of these control groups. Many studies of GWI do not distinguish GWV into their specific groups, but rather ill versus well GWV. Therefore, in some instances, the GWV were combined based on them being ill or well to increase statistical power and provide general findings where needed (i.e., BOLD fMRI activity).

3.1 Behavioral Memory Performance

3.1.1 Data Processing Prior to Data Analysis

First, the mean proportions of *recall* and *know* responses given to faces studied with names (face-name items) were calculated. Next, response bias was subtracted from the data. To accommodate differences in response bias, these data were corrected using the two-high threshold correction of signal detection theory, which is a simple method used to correct for

response bias by subtracting the proportion of false alarms (i.e., noise) accepted from the proportion of hits (i.e., signal; see Snodgrass & Corwin, 1988). In the case of the present data, the proportion of *recall* responses given to face items was subtracted from the proportion of *recall* responses given to face-name items to correct for response bias in making *recall* responses. To correct for response bias in making *know* responses, the proportion of *know* responses given to faces was subtracted from the proportion of know responses given to face-name pairs. Both the raw and corrected values are reported in Table 3.1. Last, the corrected accurate judgments to test items will then be used for statistical analyses. Specifically, both *recall* and *know* judgments were accurate responses when presented with a face that had been accompanied with a name at study.

Table 3.1. Uncorrected item by response mean proportions and corrected mean proportion of *recall*, *know*, and *recall+know* judgments made to face name pairs as a function of group membership

Uncorrected Item by Response					
	Nondeployed	Deployed	GWV-1	GWV-2	GWV-3
Face-Names					
Recall	.16 (.04)	.20 (.03)	.09 (.03)	.14 (.03)	.17 (.03)
Know	.57 (.04)	.53 (.05)	.54 (.03)	.50 (.03)	.52 (.04)
Faces					
Recall	.02 (.01)	.03 (.01)	.02 (.01)	.05 (.02)	.07 (.03)
Know	.30 (.03)	.36 (.05)	.39 (.03)	.38 (.04)	.37 (.03)
Corrected Face-Name Responses					
	Nondeployed	Deployed	GWV-1	GWV-2	GWV-3
Recall	.14 (.04)	.17 (.03)	.07 (.03)	.09 (.02)	.11 (.02)
Know	.27 (.04)	.16 (.06)	.15 (.03)	.12 (.03)	.15 (.03)
Recall+Know	.41 (.05)	.33 (.06)	.22 (.05)	.21 (.03)	.26 (.03)

Note. *Standard errors are provided in parentheses.*

3.1.2 Data Analysis

All statistical analyses were carried out using a coding system that blinded the researchers to the classification of the GWV during the preparation of the data for analysis. This blinding was not removed until after the data was ready for group analyses. To investigate the impact of possible neurotoxin exposure on the ability of GWV to form and later retrieve long-term episodic memories, statistical analyses were conducted on the mean proportion of corrected accurate judgments provided on the memory test by participants.

In an attempt to validate the memory difficulties that GWV report, the current study focused on associative memory performance during the fMRI scanning session. I conducted a one-way ANOVA and a series of one-way ANCOVAs co-varying the effects of PTSD, depression, and age on the mean proportion of *recall* responses provided to face-name test items corrected for response bias. I also conducted a separate analysis of *know* responses, which served as a measure of intermediate associative memory. Specifically, I conducted a one-way ANOVA and a series of one-way ANCOVAs on the mean proportion of *know* responses provided to face-name test items, corrected for response bias. Lastly, in order to capture associative memory in its entirety, I conducted a one-way ANOVA and a series of one-way ANCOVAs were performed on corrected *recall+know* responses provided to face-name test items. In doing so, I hoped to identify whether GWV classified in the 3 different syndrome types exhibited reliable differences in their rate of these responses.

To confirm whether or not the nondeployed and deployed controls were equivalent on associative memory performance, separate *t*-tests were performed comparing performance between the two control groups on corrected *recall, know*, and *recall+know* judgments given to faces presented at test that had been paired with faces at study. No significant differences were found between the nondelpoyed (M = .14, SE = .04) and deployed (M = .17, SE = .02) controls on *recall* judgments, $t(27) = -0.66$, $p = .55$. Similarly, no differences were found between the nondeployed (M = .25, SE = .04) and deployed (M = .16, SE = .06) controls on *know* judgments,

t(27) = 1.64, *p* = .12. Finally, no differences were found between the nondeployed (*M* = .41, *SE* = .05) and deployed (*M* = .33, *SE* = .05) controls on *recall+know* judgments, *t*(27) = 1.03, *p* = .32. Therefore, all subsequent memory analyses were conducted with the two control groups combined into a collapsed control group, GWV-C.

3.1.3 Group Differences in Recall Performance

First, an ANOVA was conducted to compare the mean proportion of corrected *recall* judgments to face-name pairs between the different groups. As predicted, the ANOVA revealed a main effect of group on *recall* judgments, *F*(3, 80) = 2.60, *MSE* = .01, η_p^2 = .09, *p* = .05 (see Figure 3.1). Post hoc comparisons revealed that GWV-C (*M* = .15, *SE* = .02) provided significantly more *recall* responses than GWV-1 (*M* = .07, *SE* = .03) and GWV-2 (*M* = .09, *SE* = .02), *p* = .01, *p* = .04. There was no significant difference between the GWV-C and GWV-3 (*M* = .11, *SE* = .03), *p* = .17. There were also no significant differences between the GWV-1, GWV-2 and GWV-3, all *p*s > .27. Following these initial analyses, a series of ANCOVAs were conducted to test for any effect of PTSD, MDD, and age on *recall* responses. As hypothesized, none of these variables had a significant effect on *recall* response, all *p*s > .21.

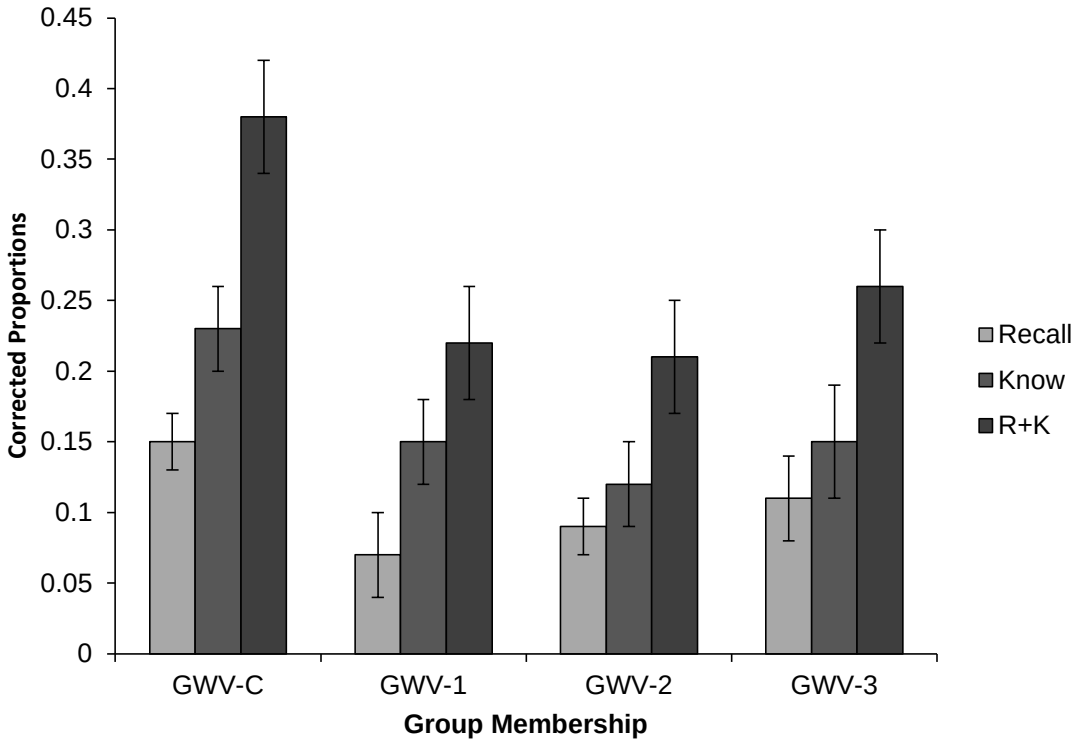


Figure 3.1 Corrected proportions for associative memory judgments, recall, know, and recall+know, given to face-name pairs as a function of Gulf War veteran group membership

3.1.4 Group Differences in Know Performance

Next, an ANOVA was conducted to compare the mean proportion of corrected *know* responses given to face-name pairs. The analysis revealed a marginally significant effect of group, $F(3, 80) = 2.44$, $MSE = .02$, $\eta_p^2 = .09$, $p = .06$ (see Figure 3.1). Planned simple comparisons were conducted to identify whether any of the groups differed in *know* responses given the *a priori* predictions made about how the groups of GWV would differ in face-name *know* memory performance. These comparisons were in line with expectations. GWV-C ($M = .23$, $SE = .03$) provided significantly more *know* responses than GWV-2 ($M = .12$, $SE = .03$), $p = .01$. Marginally significant differences were also observed between GWV-C and GWV-1 ($M = .15$, $SE = .03$) and GWV-3 ($M = .15$, $SE = .04$), $p = .07$, $p = .08$, respectively. Finally, a series of ANCOVAs were conducted to test for the any effect of PTSD, MDD, and age on *know*

responses. As hypothesized, these factors did not significantly impact *know* response, all *p*s > .37.

3.1.5 Group Differences in Recall+Know Performance

Finally, an ANOVA was conducted to compare the mean proportion of corrected *recall+know* responses given to face-name pairs. As predicted, the analysis revealed a significant effect of group, $F(3, 80) = 4.78$, $MSE = .03$, $\eta_p^2 = .16$, $p = .004$ (see Figure 3.1). Pairwise comparisons were in line with predictions. GWV-C ($M = .38$, $M = .04$) provided more *recall+know* responses than GWV-1 ($M = .22$, $SE = .04$), GWV-2 ($M = .21$, $SE = .04$), and GWV-3 ($M = .26$, $SE = .04$), $p = .003$, $p = .001$, $p = .02$. There were no significant differences between the GWV-1, GWV-2 and GWV-3, all *p*s > .40. Finally, a series of ANCOVAs were conducted to test for any effect of PTSD, MDD, and age on *recall+know* responses. As hypothesized, these factors did not have a significant effect on *recall+know* responses, all *p*s > .14.

3.1.6 Summary of Associative Memory Performance

As predicted, I found GWV-1 and GWV-2 to have the poorest associative memory performance, in both *recall* and *know* judgments made to face-name pairs, when compared to GWV-C. GWV-3 were equivalent to GWV-C on these associative memory judgments, suggesting that these veterans do not have a deficit in associative memory that is analogous to the deficits experience by GWV-1 and GWV-2. However, when I combined the associative memory judgments into *recall+know* judgments, differences appeared in all three syndrome types compared to GWV-C, suggesting even GWV-3 to have some associative memory deficits. These findings replicate and extend the findings of Odegard and colleagues (in press) in a national sample of GWV. In this previous study, all of the three syndrome types exhibited deficits in associative memory relative to controls.

3.2 Functional Magnetic Resonance Imaging

3.2.1 Data Processing Prior to Analysis

In preparation for data analysis, the functional data were preprocessed and analyzed with AFNI software (Cox, 1996). The EPI images were corrected for temporal differences in slice acquisition. Next, the EPI data were corrected for head motion by aligning all volumes to the first EPI volume collected using a three-dimensional rigid body registration. The first 3 volumes of each run acquired prior to T1 equilibrium was discarded, and linear drift removed. Finally, the EPI data was spatially smoothed with an isotropic 6 mm full-width half maximum (FWHM) Gaussian filter.

To assess the possible impact of neurotoxin exposure on brain function, the data collected while participants studied faces and face-name pairs was processed in a general linear model for each participant to model hemodynamic response functions (HRF's) evoked at study by faces and face-name pairs presented during the study relative to baseline, resting activation (fixation during the ISI presentation). Furthermore, any face presented at study that received a *recall* response at test, as well as any face or face-name pair presented at study that did not receive any response at test, were modeled but not further analyzed. The HRF's were convolved with a canonical HRF in order to fit a typical response following each stimulus. Specifically, the response function used, GAMA, peaks at the value of 1 at $t = bc$, with Cohen parameters $b = 8.6$, $c = 0.547$. The resulting values from this GLM were spatially standardized to the TT atlas N27 template for Talairach space (Talairach & Tournoux, 1988).

3.2.2 Data Analysis

The current study used a memory paradigm that is assumed to recruit the hippocampus as well as other brain regions in the encoding network to facilitate the formation of associative memory (e.g., Chua, Schachter, Rand-Giovannetti et al., 2007; Odegard et al., in press). First, I conducted whole brain voxel-wise analyses to compare encoding of a face-name pair that later received an associative memory judgment, *recall+know* relative to encoding of a face-name pair

that later received a *face-only* response to investigate differences in brain activation among groups when encoding was successful. Second, I conducted whole brain voxel-wise analyses to compare the amount of activation evoked by the presentation of face-name pairs at study that later received a *know* judgment relative to baseline measured by BOLD fMRI between the three syndrome types and the controls. Lastly, I conducted whole brain voxel-wise comparisons between the three syndrome types and the controls on the BOLD fMRI data obtained while participants studied face-name pairs that later received any associative memory judgment (i.e., *recall+know*) relative to baseline. This helped to identify whether any brain regions showed reliable group differences in activation evoked by the presentation of face-name pairs that later received correct associative memory responses that may be indicative of functional differences based on the GWV being ill or well. For each contrast, pooled analyses were performed in order to be exhaustive and to mimic analyses of other current research in GWI that does not differentiate among the syndrome types or control types (e.g., Chao et al., 2010; 2011).

I conducted a series of voxel-wise t-tests comparing various groups in the activation evoked during encoding. Comparisons are reported for the mean amount of activation evoked by face-name pairs that subsequently received *know* judgments. Following these comparison, I conducted a series of t-tests comparing various groups on the mean amount of activation evoked face name pairs presented at study that subsequently received a *recall* or *know* judgment at test. For these comparisons the critical *t* value was set to a level that resulted in an uncorrected alpha value of .01 for each of the contrasts. To correct for multiple comparisons, a spatial threshold of 35 contiguous voxels (RMM = 1) was set. These parameters resulted in a spatially corrected p value < .05 as determined by a Monte Carlo simulation computed using AlphaSim (Cox, 1996). Clusters of voxels that demonstrated significant differences in the mean activation evoked by face-name pairs presented at study that later received a *know* judgment and those that received an associative memory judgment (*recall+know*) are reported in Table 3.2–3.11. Talairach coordinates are reported for the peak intensity voxel for each of the clusters

in which significant differences were observed. Additional regions were included in the cluster label if a cluster was distributed across multiple brain regions. For all figures the right hemisphere is on the right-hand side conforming to neurologic convention.

3.2.3 Group Differences in Successful Face-Name Encoding

In this section differences are reported in the amount of activation evoked by face-name pairs presented at study that later received a *recall or know* response relative to those that received a *face-only* response between various groups of veterans. First, non-deployed and deployed controls were compared to one another to establish if they should be compared separately to each of the syndrome types prior to pooling the data across the 2 control groups.

No differences were observed between the two control groups, $p > .05$. Therefore, full-brain voxel-wise t-tests were conducted to compare the mean amplitude of the HRF evoked by the successful encoding of face-name pairs for the pooled controls compared to each of the three syndrome types (i.e., GWV-1, GWV-2, GWV-3). No differences were observed between the groups in this comparison, all $ps > .05$. Given the lack of group differences in this comparison, it was important to investigate any differences that might be observed when encoding face-name pairs that were later given a correct associative memory response at test.

3.2.4 Group Differences in Face-Name Know Encoding

In this section differences are reported in the amount of activation evoked by face-name pairs presented at study that later received *know* responses between varying groups of veterans. First, non-deployed and deployed controls were compared to one another to establish if they should be compared separately to each of the syndrome types prior to pooling the data across the two control groups. To foreshadow, differences were observed between the two control groups. Hence, separate full-brain voxel-wise t-tests were conducted to compare the mean amplitude of the HRF evoked by the presentation of face-name pairs during study that received a *know* judgment at test for the non-deployed controls compared to each of the three syndrome types (i.e., GWV-1, GWV-2, GWV-3; see Table 3.2). These analyses were followed

by separate full-brain voxel-wise t-tests to compare the amount of activation evoked by the presentation of face-name pairs during study that received a *know* judgment at test for the deployed controls compared to each of the three syndrome types (i.e., GWV-1, GWV-2, GWV-3; see Table 3.2). Then the two control groups were pooled into a single control group, and separate voxel wise t-tests were conducted to compare the activation they exhibited relative to the three syndrome types separately. Finally, the three syndrome types were combined into a single pooled group of ill GWV and compared to the combined control group. The same analyses were performed on the encoding of face-name pairs that later received a *recall+know* judgment (see Table 3.3).

First, nondeployed and deployed controls exhibited differences in their mean activation evoked by the presentation of face-names pairs that later received a *know* response in seven regions (see Table 3.2). Deployed controls had greater activity in the left culmen (including the fusiform and parahippocampal gyrus), left superior temporal gyrus (STG), left medial frontal gyrus, right inferior frontal gyrus (IFG), right posterior cingulate (including the culmen), as well as the right and left declive compared to nondeployed controls (see Table 3.2 and Figures 3.2 - 3.5 for cluster examples). Therefore, all syndrome types were compared to nondeployed and deployed controls separately.

Table 3.2. Areas in which nondeployed and deployed controls showed differences in activation to faces name pairs presented during study that subsequently received *know* judgments at test.

Region	TLRC coordinates (x, y, z)	Brodmann area (BA)	# of voxels	*t* value
Nondeployed vs. Deployed				
Left Culmen* (Fusiform Gyrus*, Parahippocampal Gyrus)	-37, -43, -19	NA	120	-3.81
Right Declive	14, -86, -18	NA	97	-3.87
Right Inferior Frontal Gyrus* (Insula)	26, 32, -7	47	88	-2.83
Right Posterior Cingulate* (Culmen*)	11, -52, 8	NA	68	-3.55
Left Declive* (Culmen*)	-25, -58, -16	NA	58	-2.96
Left Superior Temporal Gyrus	-55, -46, 17	NA	53	-2.86
Left Medial Frontal Gyrus* (Anterior Cingulate)	-1, 47, 11	10	49	-4.30

*Major regions accounted for in the cluster.

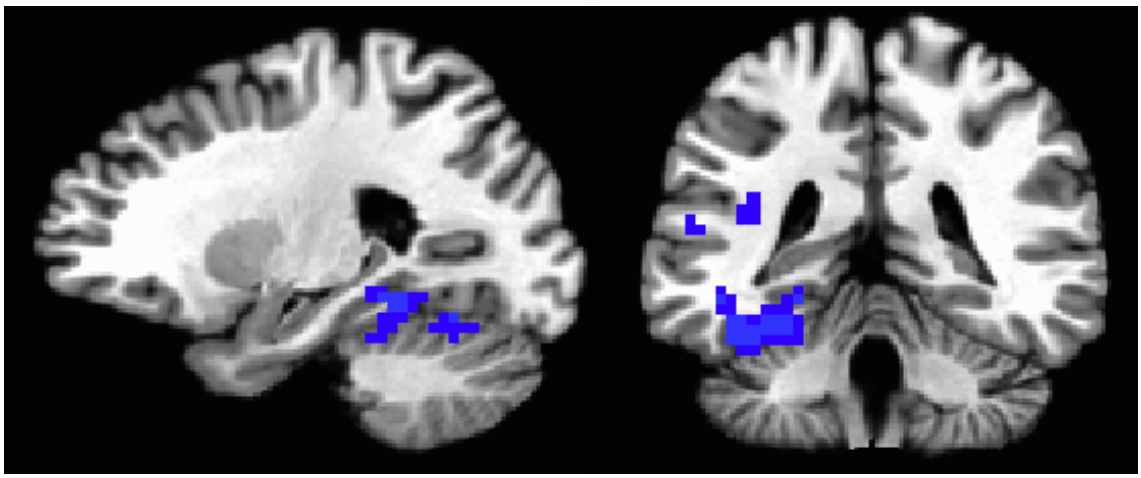


Figure 3.2 Face-name *know* encoding cluster in the left culmen, fusiform and parahippocampal gyri, observed to be greater in deployed relative to nondeployed controls

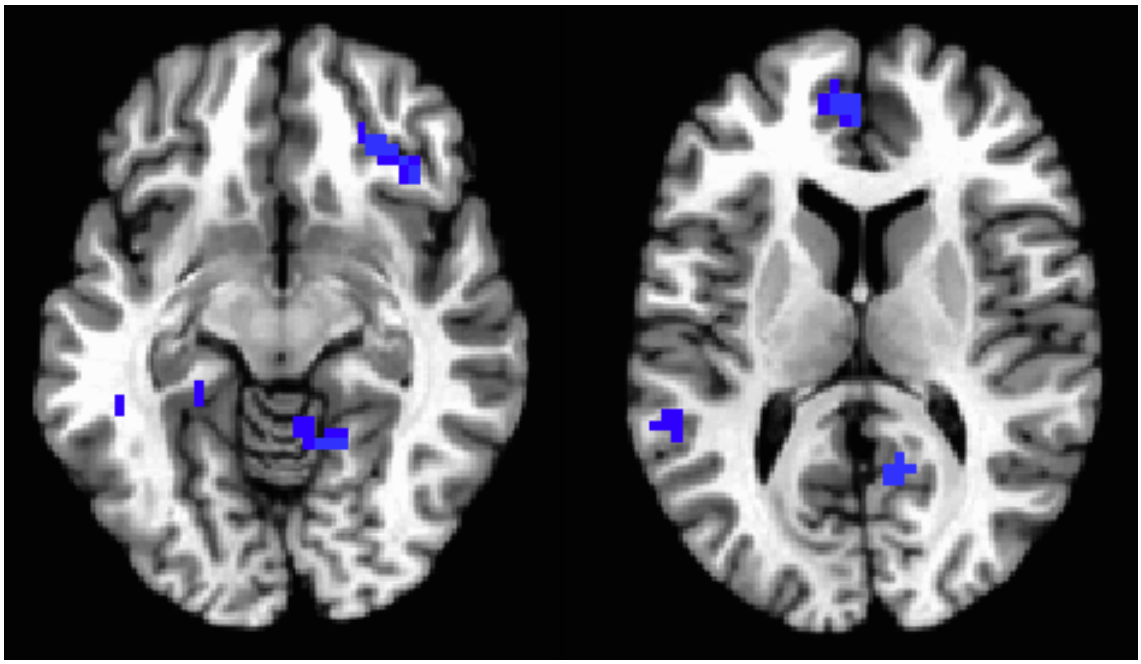


Figure 3.3 Face-name *know* encoding clusters in the medial frontal (right) and inferior frontal (left) gyri observed to be greater in deployed relative to nondeployed controls

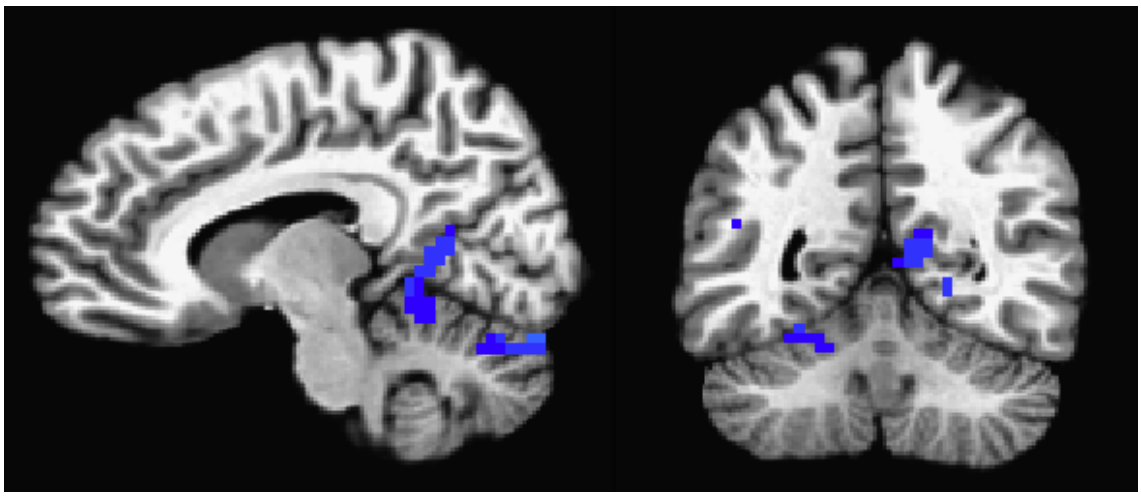


Figure 3.4 Face-name *know* encoding cluster in the right posterior cingulate observed to be greater in deployed relative to nondeployed controls

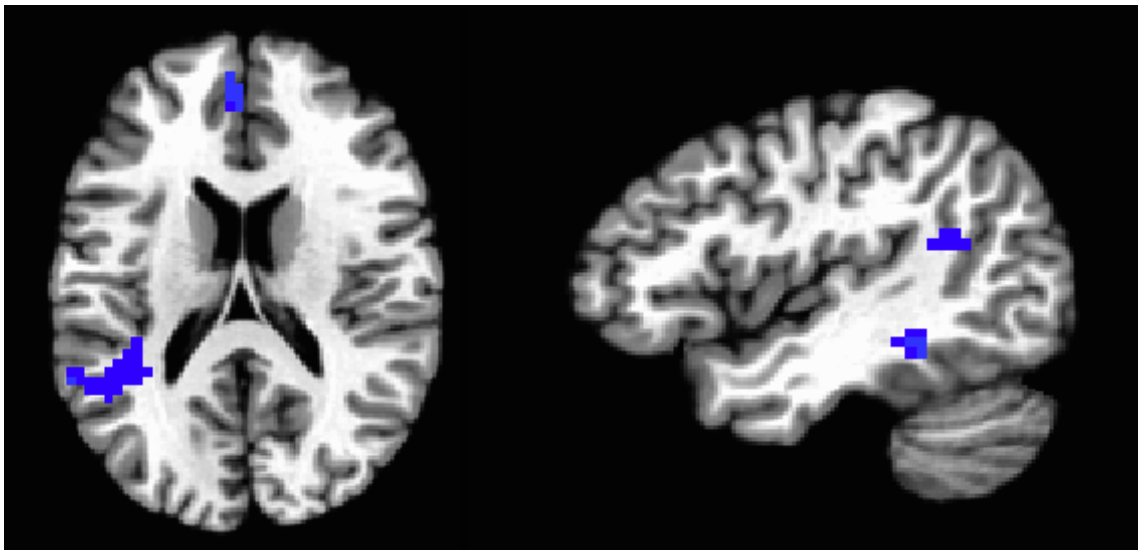


Figure 3.5 Face-name *know* encoding cluster in the left superior temporal gyrus observed to be greater in deployed relative to nondeployed controls

Relative to nondeployed controls, GWV-1 did not differ from these individuals in the amount of functional activity evoked during the encoding face-name pairs that later received a *know* judgment. However, GWV-2 exhibited greater activity in two clusters within bilateral cerebellar regions when compared to nondeployed controls (see Table 3.3). Additionally, GWV-3 had greater activity relative to nondeployed controls in the right cerebellum, and also showed

greater activity in the right parahippocampus, left insula (including the STG), and bilateral declive (including bilateral culmen; see Table 3.3 and Figures 3.6 and 3.7 for cluster examples).

Table 3.3. Areas in which nondeployed controls and GWV of the syndrome types showed differences in activation to faces name pairs presented during study that subsequently received *know* judgments at test.

Region	TLRC coordinates (x, y, z)	Brodmann area (BA)	# of voxels	*t* value
Nondeployed vs. Syndrome 2				
Right Cerebellar Tonsil	38, -43, -37	NA	63	-3.52
Left Cerebellum (*Right* Cerebellum)	-1, -40, -40	NA	51	-4.16
Nondeployed vs. Syndrome 3				
Right Declive* (Culmen*; *Left* Declive, Culmen)	8, -85, -18	NA	600	-3.25
Right Cerbellar Tonsil	32, -43, -37	NA	68	-4.12
Right Cerebellum (Cerebellar Tonsil*)	5, -40, -40	NA	45	-3.17
Right Parahippocampal Gyrus	20, -25, -7	NA	42	-3.85
Left Insula* (BA 13, Superior Temporal Gyrus*, BA 22)	-40, -16, 8	NA	39	-3.45

*Major regions accounted for in the cluster.

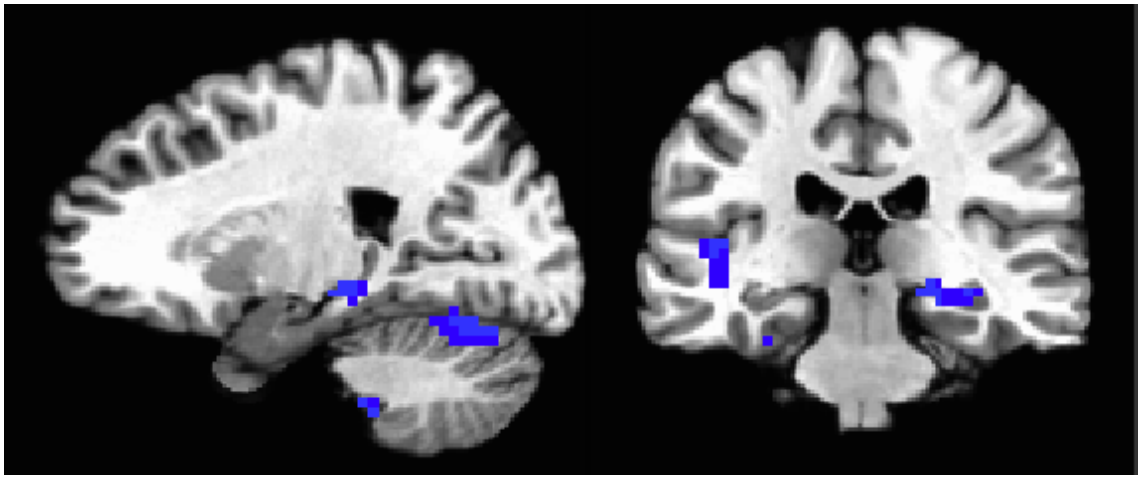


Figure 3.6 Face-name *know* encoding cluster in the right parahippocampal gyrus observed to be greater in GWV-3 relative to nondeployed controls

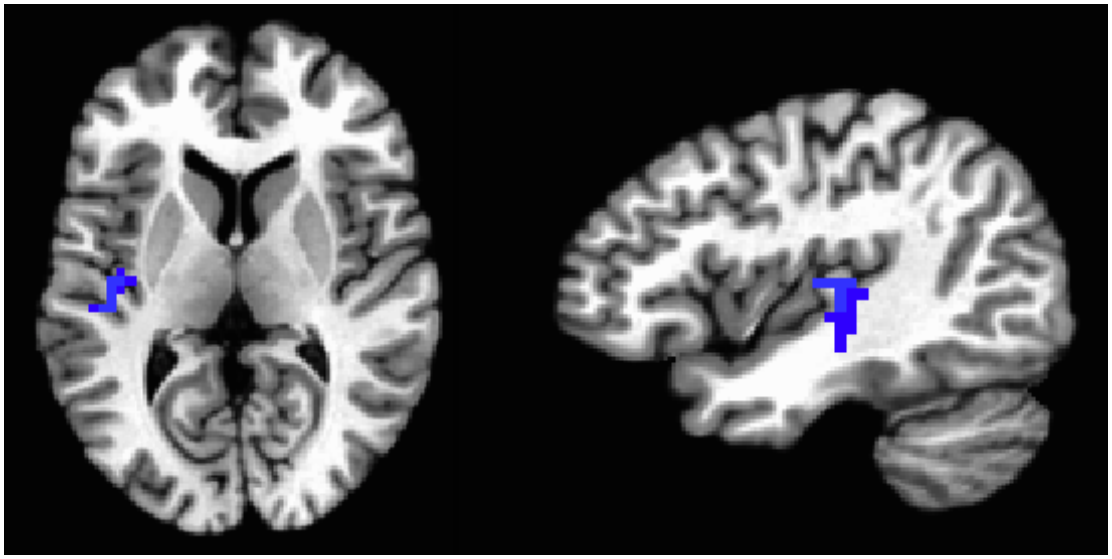


Figure 3.7 Face-name *know* encoding cluster in the left insula (and superior temporal gyrus) observed to be greater in GWV-3 relative to nondeployed controls

Unlike the nondeployed controls, deployed controls showed greater encoding activity for face-name pairs that later received a *know* judgment in several brain regions when compared to the different syndrome types. Relative to GWV-1 deployed controls had greater activity in the left fusiform gyrus, left lentiform nucleus, right STG, right middle occipital gyrus, right lingual gyrus (including the culmen and posterior cingulate), right IFG, and bilateral anterior cingulate when encoding face-name pairs that later received a *know* judgment (see Table 3.4). Relative to GWV-2 deployed controls also had greater activity in the left fusiform gyrus and the left and right posterior cingulate (see Table 3.4). Other clusters showing greater activity in deployed controls relative to GWV-2 included the left declive (including the fusiform gyrus), two clusters in the left inferior parietal lobule (IPL), right precuneus, and the right middle frontal gyrus, which also encompassed the inferior frontal gyrus (see Table 3.4). As for GWV-3, deployed controls again showed greater activity in bilateral posterior cingulate, and the right IFG. Other clusters that were observed to have greater activity for deployed controls compared to GWV-3 included the left cuneus (and middle occipital gyrus) and the right inferior occipital gyrus (see Table 3.4).

Table 3.4. Areas in which deployed controls and GWV of the three syndrome types showed differences in activation to faces name pairs presented during study that subsequently received *know* judgments at test.

Region	TLRC coordinates (x, y, z)	Brodmann area (BA)	# of voxels	*t* value
Deployed vs. Syndrome 1				
Right Inferior Frontal Gyrus	38, 20, -7	NA	166	3.67
Right Lingual gyrus* (Culmen, Posterior Cingulate*, Cuneus)	11, -52, 5	18	153	3.56
Right Anterior Cingulate* (Parahippocampus, Hypothalamus, BA 25, Amygdala)	4, 2, -5	NA	63	3.18
Left Culmen (Fusiform Gyrus*, Parahippocampal Gyrus, BA 20)	-37, -43, -19	NA	53	3.26
Left Lentiform Nucleus	-21, 17, -5	NA	39	3.08
Right Superior Temporal Gyrus	35, 1, -11	NA	38	3.65
Right Middle Occipital Gyrus* (Cuneus, Lingual Gyrus)	35, -85, 8	19	38	3.64
Left Anterior Cingulate	-1, 32, -4	NA	37	2.86
Deployed vs. Syndrome 2				
Right Posterior Cingulate* (Cuneus, BA 30; *Left* Posterior Cingulate Cuneus, BA 30)	11, -58, 11	NA	282	5.12
Left Culmen (Fusiform Gyrus*)	-40, -40, -19	NA	58	3.19
Right Middle Frontal Gyrus* (Inferior Frontal Gyrus*)	47, 14, 26	9	56	5.09
Left Declive* (Fusiform Gyrus*, Lingual Gyrus)	-19, -82, -16	NA	39	3.49
Left Inferior Parietal Lobule (BA 40)	-43, -37, 41	NA	37	3.73
Right Precuneus	20, -70, 50	7	36	3.51
Left Inferior Parietal Lobule	-34, -49, 53	40	35	3.23
Deployed vs. Syndrome 3				
Right Inferior Frontal Gyrus (BA 47)	41, 20, -4	NA	44	3.51
Right Inferior Occipital Gyrus* (Middle Occipital Gyrus, Lingual Gyrus)	23, -91, -10	NA	43	3.24
Left Lingual Gyrus* (Posterior Cingulate*, BA 30, Cuneus,)	-13, 55, 5	NA	42	2.87
Right Posterior Cingulate* (BA 30, 18, Cuneus)	14, -52, 8	NA	40	3.08
Left Cuneus* (Middle Occipital Gyrus*, BA 18)	-22, -91, 11	NA	36	3.92

*Major regions accounted for in the cluster.

Next, the two control groups were pooled together and compared to each of the three syndrome types. As presented in Table 3.5 the controls differed from GWV-2 in the left and right posterior cingulate that also included the cuneus and precuneus. GWV-C had greater activity in

the posterior cingulate relative to GWV-2 when encoding fane-name pairs that later received a *know* judgment (see Figure 3.8). Additionally, GWV-3 were observed to have greater activity in the left and right declive and culmen relative to GWV-C (see Table 3.5). Differences were not observed between GWV-C and GWV-1 in their encoding activity of face-name pairs that received a *know* response.

Table 3.5. Areas in which GWV-C and GWV of the three syndrome types showed differences in activation to faces name pairs presented during study that subsequently received *know* judgments at test.

Region	TLRC coordinates (x, y, z)	Brodmann area (BA)	# of voxels	*t* value
All Controls vs. Syndrome 2				
Right Posterior Cingulate* (BA 30, 31; Cuneus, Precuneus *Left* Posterior Cingulate, BA 31, Cuneus, Precuneus)	11, -58, 11	NA	54	3.60
All Controls vs. Syndrome 3				
Right Declive* (Culmen*; *Left* Declive, Culmen)	23, -61, -16	NA	205	-3.04

*Major regions accounted for in the cluster.

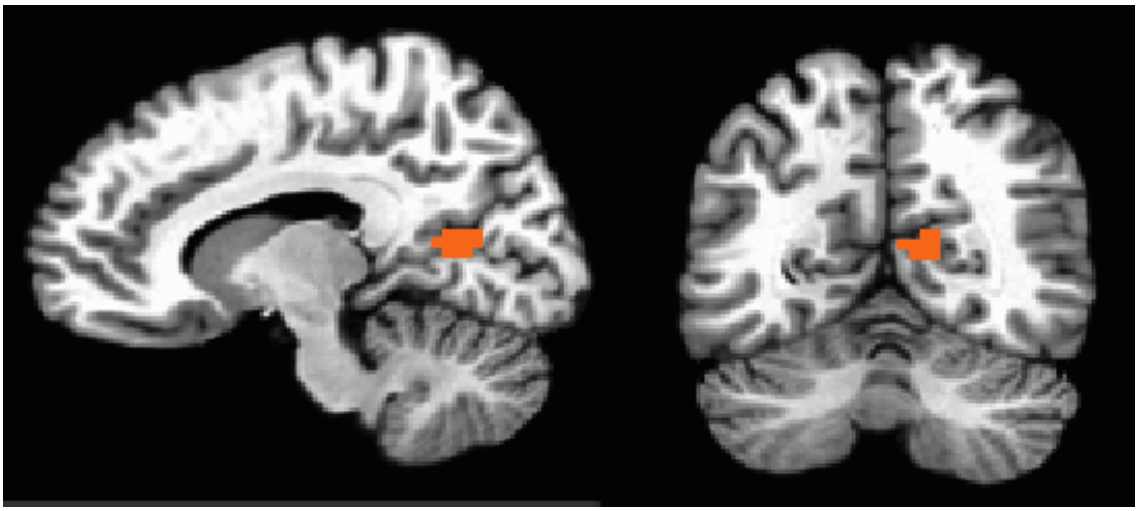


Figure 3.8 Face-name *know* encoding cluster in the right (and left) posterior cingulate, cuneus, and precuneus, observed to be greater in GWV-C relative to GWV-2

As a final step, all syndrome types were pooled and compared to the pooled control group (i.e., deployed and non-deployed controls) in an attempt to identify brain activation during

encoding of face-name pairs that later received a *know* judgment that might be indicative of being an ill GWV. GWV-C were observed to have greater activity in a cluster of the left cuneus that also encompassed the left posterior cingulate, the right cuneus and right precuneus relative to ill GWV (see Table 3.6 and Figure 3.9). On the other hand, ill GWV had greater activity in the right cerebellum relative to GWV-C.

Table 3.6. Areas in which GWV-C and ill GWV showed differences in activation to faces name pairs presented during study that subsequently received *know* judgments at test.

Region	TLRC coordinates (x, y, z)	Brodmann area (BA)	# of voxels	t value
All Controls vs. All Syndrome Types				
Left Cuneus* (BA 18, Posterior Cingulate, *Right* Cuneus, BA 18, Precuneus)	-1, -70, 17	NA	75	3.43
Right Cerebellar Tonsil	44, -52, -34	NA	35	-3.55

*Major regions accounted for in the cluster.

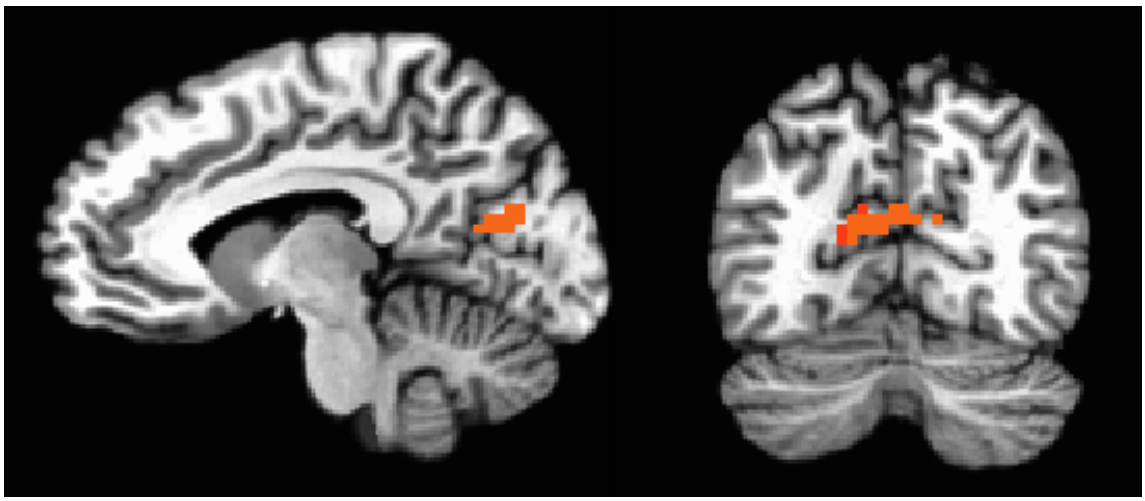


Figure 3.9 Face-name *know* encoding cluster in the left posterior cingulate, cuneus, and precuneus, observed to be greater in GWV-C relative to ill GWV

3.2.5 Group Differences in Face-Name Recall+Know Encoding

Considering know responses are one of two associative memory responses, additional analyses were conducted on those face-name pairs presented at study that received *recall* or *know* judgments at test. Again, nondeployed and deployed controls were first compared on activation evoked during the encoding of face-name pairs that received an associative memory

judgment (*recall+know*). These groups again showed differences. Hence, they were first compared to the three syndrome types separately. Then the two control groups were pooled and compared to the three syndrome types. Finally, the three syndrome types were pooled into a group of ill GWV and compared to the pooled control group.

As with the face-name *know* findings, deployed controls had greater activity in the left culmen (including the fusiform and parahippocampal gyri; see Figure 3.10), right posterior cingulate (and right culmen; see Figure 3.11), and right declive relative to nondeployed controls. An additional cluster including a region of the right parahippocampal gyrus also showed greater activity for deployed relative to nondepoyed controls (see Table 3.7).

Table 3.7. Areas in which nondeployed and deployed controls showed differences in activation to faces name pairs presented during study that subsequently received an associative memory judgment (*recall+know*) at test.

Region	TLRC coordinates (x, y, z)	Brodmann area (BA)	# of voxels	*t* value
Nondeployed vs. Deployed				
Left Culmen* (Fusiform gyrus, Parahippocampal gyrus)	-37, -43, -19	NA	170	-3.45
Right Posterior Cingulate (Culmen*, Lingual Gyrus)	11, -52, 8	NA	97	-3.16
Right Declive	14, -85, -19	NA	56	-3.74
Right Thalamus (Parahippocampus*)	14, -28, -1	NA	45	-3.46

*Major regions accounted for in the cluster.

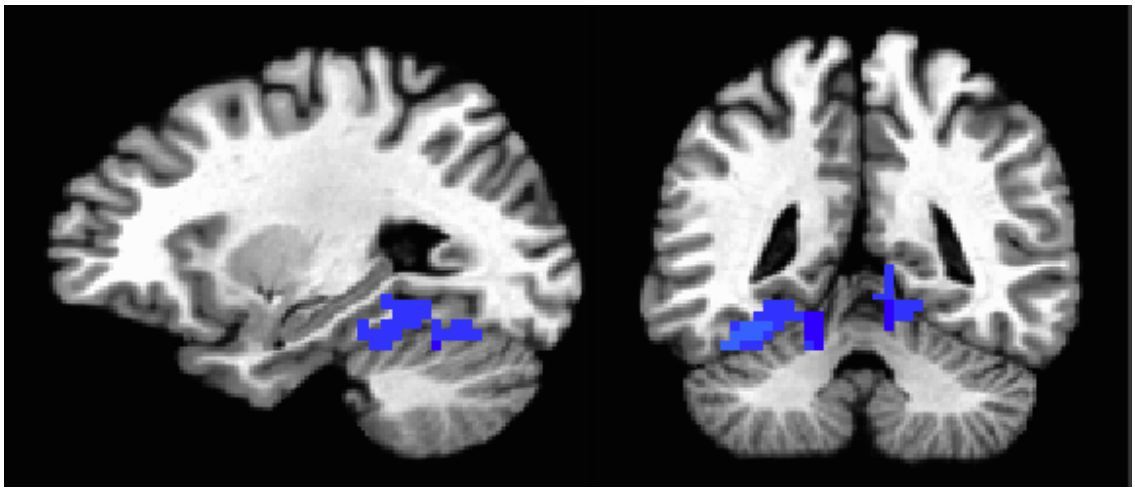


Figure 3.10 Face-name *recall+know* encoding cluster in the left culmen, fusiform and parahippocampal gyri, observed to be greater in deployed relative to nondeployed controls

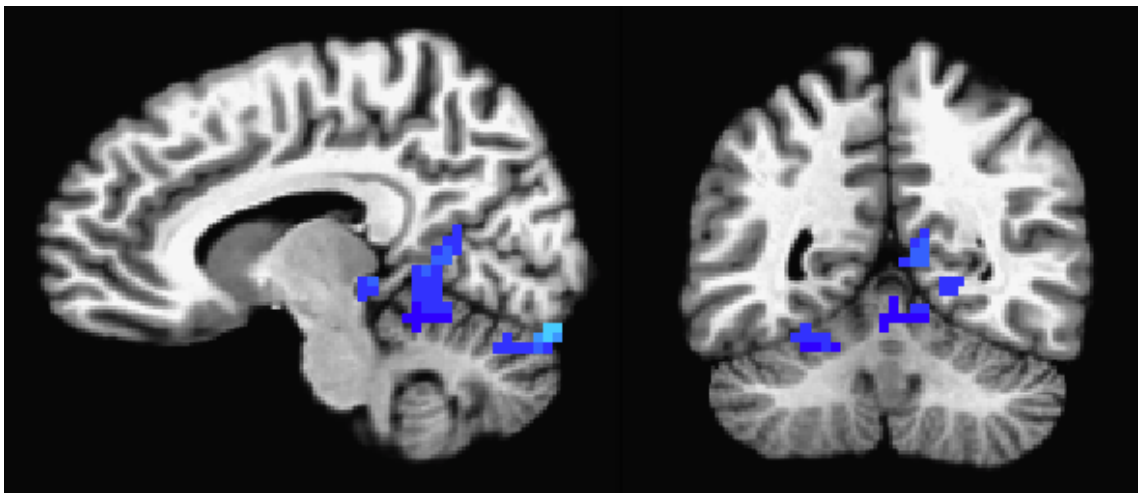


Figure 3.11 Face-name *recall+know* encoding cluster in the right posterior cingulate observed to be greater in deployed relative to nondeployed controls

There were no differences observed between the nondeployed controls and GWV-1 as was seen for face-name *know* encoding. Relative to GWV-2, nondeployed controls showed greater activity in regions of the left posterior cingulate, left middle frontal gyrus, and the left middle temporal gyrus (see Table 3.8 and Figures 3.12–3.14). Whereas, GWV-3 had greater activity in the left declive and right parahippocampus relative to nondeployed controls (see Table 3.8 and Figure 3.15).

Table 3.8. Areas in which nondeployed controls and GWV of the three syndrome types showed differences in activation to faces name pairs presented during study that subsequently received an associative memory judgment (*recall+know*) at test.

Region	TLRC coordinates (x, y, z)	Brodmann area (BA)	# of voxels	*t* value
Nondeployed vs. Syndrome 2				
Left Cuneus (Posterior Cingulate*, Precuneus)	-1, -70, 17	NA	43	3.12
Left Middle Frontal Gyrus	-40, 2, 41	6	42	3.76
Left Middle Temporal Gyrus	-46, -76, 11	39	35	3.26
Nondeployed vs. Syndrome 3				
Left Culmen (Declive*)	-28, -46, -13	NA	57	-2.88
Right Thalamus (Parahippocampal Gyrus*, Hippocampal Body)	17, -25, -7	NA	42	-2.96

*Major regions accounted for in the cluster.

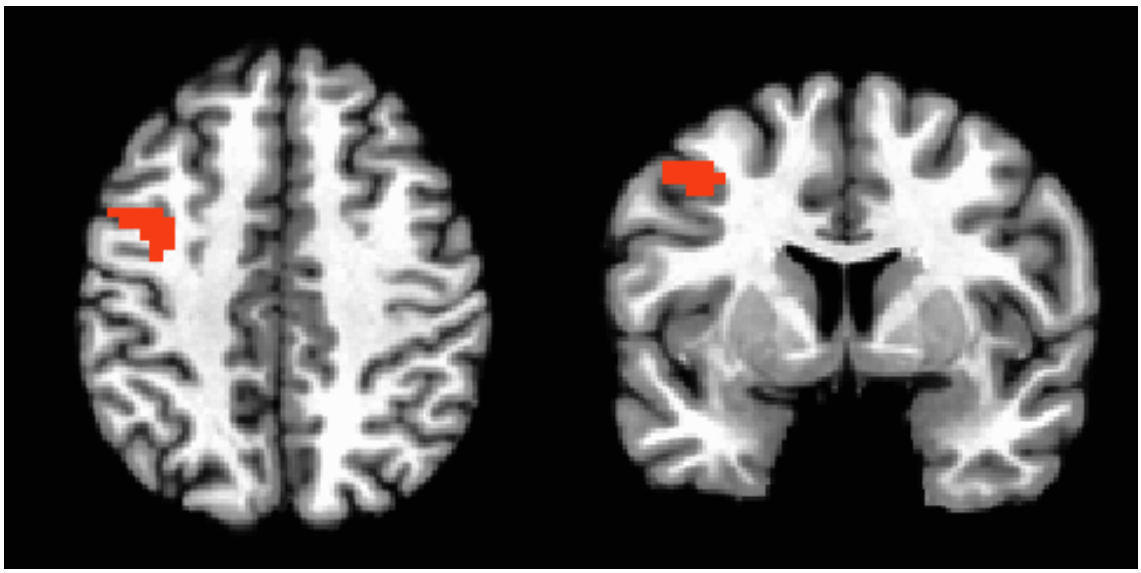


Figure 3.12 Face-name *recall+know* encoding cluster in the left middle frontal gyrus observed to be greater in nondeployed controls relative to GWV-2

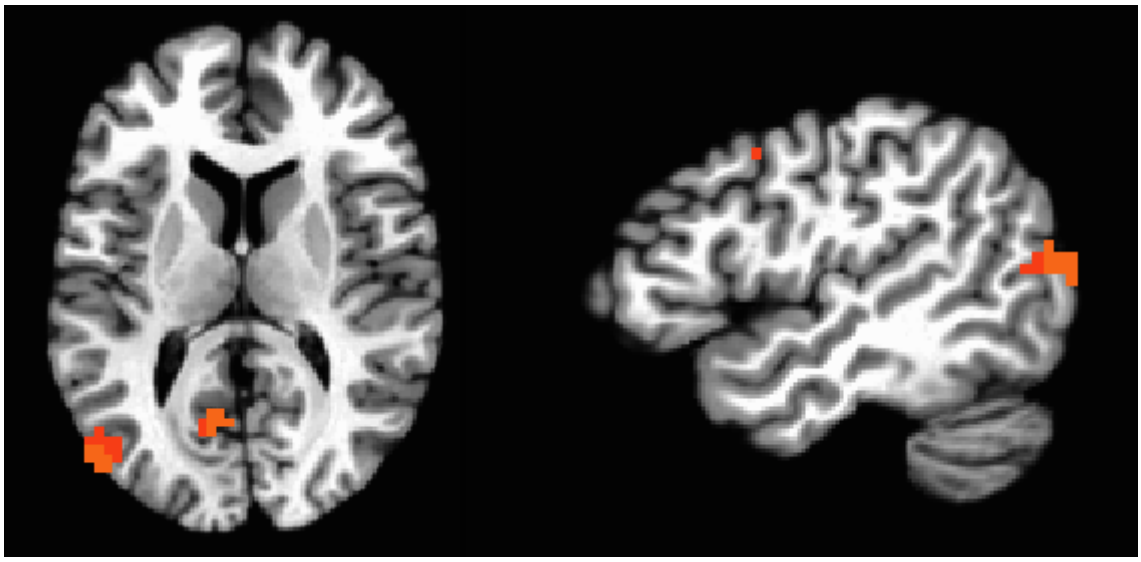


Figure 3.13 Face-name *recall+know* encoding cluster in the left middle temporal observed to be greater in nondeployed controls relative to GWV-2

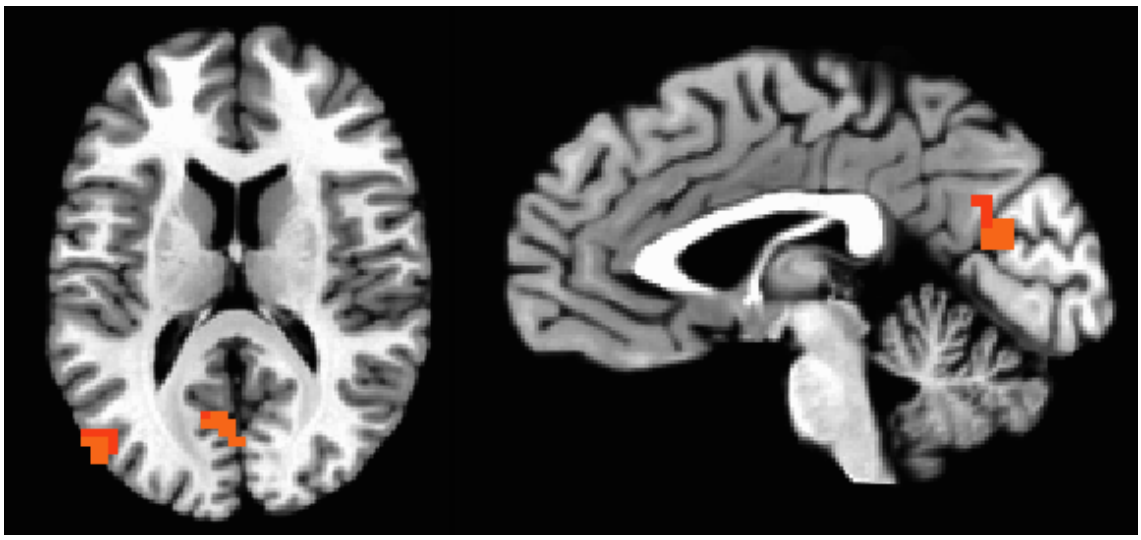


Figure 3.14 Face-name *recall+know* encoding cluster in the left posterior cingulate observed to be greater in nondeployed controls relative to GWV-2

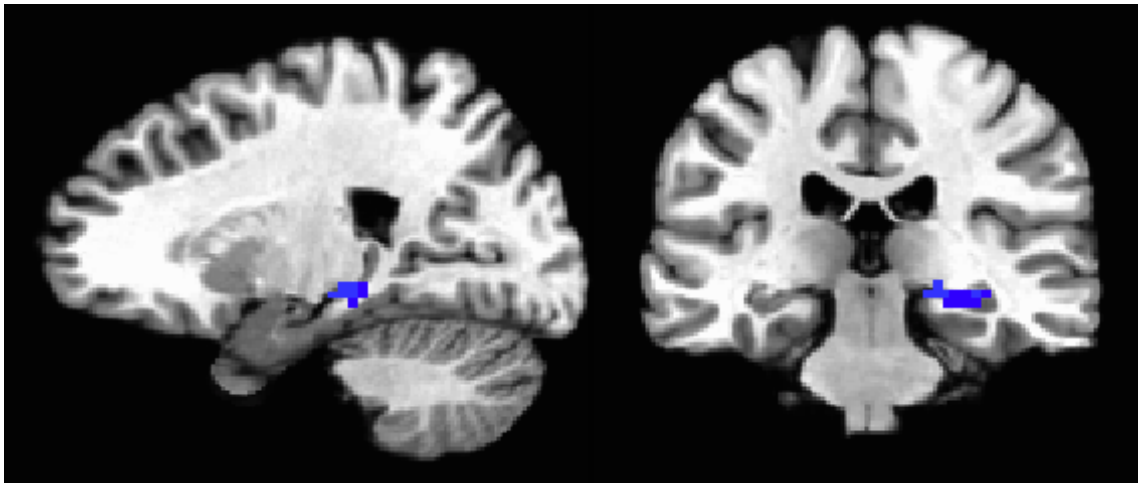


Figure 3.15 Face-name *recall+know* encoding cluster in the right parahippocampal gyrus (and hippocampal body) observed to be greater in GWV-3 relative to nondeployed controls

Next relative to GWV-1, deployed controls had greater activity for face-name *recall+know* encoding in a cluster of the right culmen (and lingual gyrus), right IFG, right anterior cingulate, which extended to the left anterior cingulate, and the right middle occipital gyrus (see Table 3.9). Deployed controls were also observed to have greater activity in several clusters relative to GWV-2. These included the left fusiform gyrus, and right middle frontal gyrus as was seen with face-name *know* encoding (see Table 3.9). The additional clusters with greater

activity for deployed controls compared to GWV-2 included the left precuneus, right thalamus, right IFG, and right posterior cingulate. Relative to GWV-3, deployed controls had greater activity in the right middle occipital gyrus as with face-name *know* responses, along with an additional cluster in the left culmen, which included the fusiform gyrus (see Table 3.9).

Table 3.9. Areas in which deployed controls and GWV of the three syndrome types showed differences in activation to faces name pairs presented during study that subsequently received an associative memory judgment (*recall+know*) at test.

Region	TLRC coordinates (x, y, z)	Brodmann area (BA)	# of voxels	*t* value
Deployed vs. Syndrome 1				
Right Culmen* (Lingual Gyrus)	8, -46, 2	NA	139	3.99
Right Inferior Frontal* (Insula)	41, 14, -7	47	71	3.12
Right Anterior Cingulate (*Left* Anterior Cingulate)	2, 2, -4	25	41	3.50
Right Middle Occipital Gyrus*	35, -85, 8	19	38	4.09
Deployed vs. Syndrome 2				
Right Posterior Cingulate* (Lingual Gyrus, Declive, Precuenus, Cuneus; *Left* Posterior Cingulate, Lingual Gyrus, Precuneus)	12, -57, 9	NA	731	5.36
Right Inferior Frontal Gyrus	26, 35, -10	47	76	3.71
Right Anterior Cingulate (Thalamus*)	2, 2, -4	25	61	2.87
Left Culmen (Fusiform Gyrus*)	-40, -46, -19	NA	57	3.36
Left Declive (Fusiform Gyrus*)	-19, -79, -16	NA	56	4.43
Right Middle Frontal Gyrus* (Inferior Frontal Gyrus)	47, 14, 26	9	45	3.99
Left Superior Parietal Lobule (Precuneus*)	-22, -70, 47	NA	36	3.87
Deployed vs. Syndrome 3				
Right Middle Occipital Gyrus * (Lingual Gyrus, Inferior Occipital Gyrus)	26, -91, 5	NA	45	4.82
Left Culmen (Fusiform Gyrus)	-37, -46, -19	NA	35	4.32

*Major regions accounted for in the cluster.

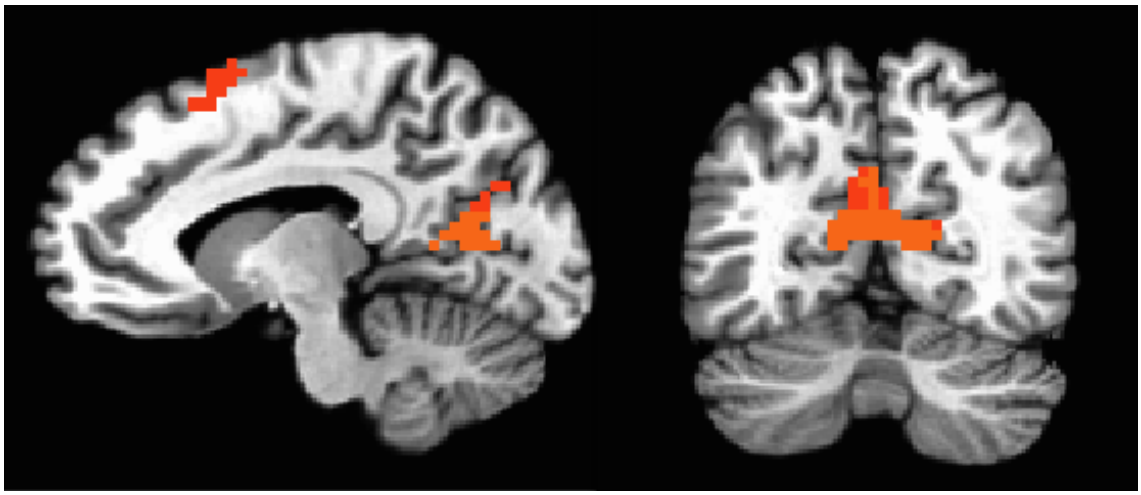
Next, GWV-1, GWV-2, and GWV-3 were compared separately against a pooled control group. Controls did not differ from GWV-1 and GWV-3 on this measure. However, controls exhibited greater activity in the left posterior cingulate when encoding face-name pairs that later received an associative memory judgment relative to GWV-2 as seen with face-name *know*

encoding (see Table 3.10). This cluster also extended to the right posterior cingulate and included bilateral cuneus and precuneus (see Figure 3.16A). Additional regions of greater activity included the left superior frontal gyrus, left middle temporal gyrus, and the right middle frontal gyrus (see Figures 3.17–3.19A for examples).

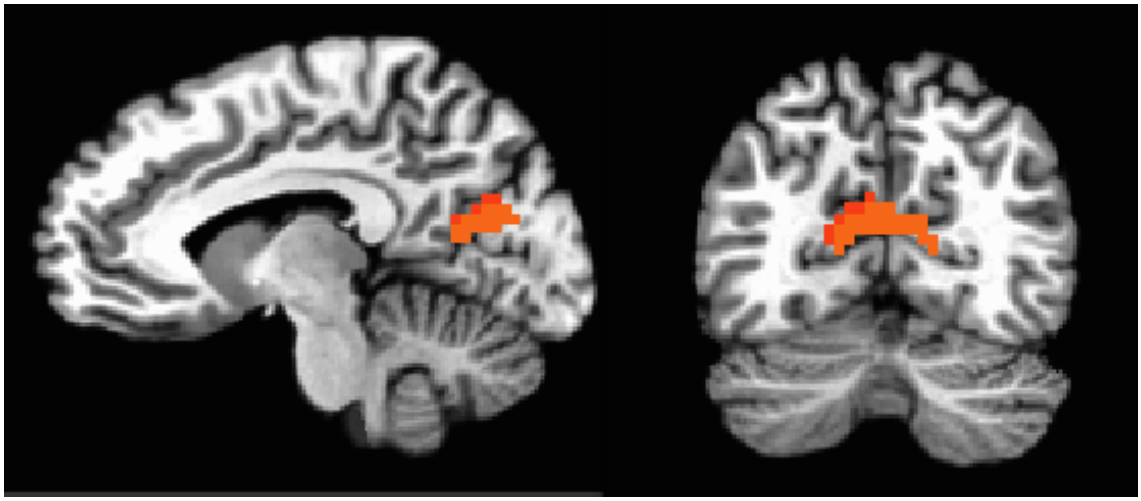
Table 3.10. Areas in which GWV-C and GWV of the three syndrome types showed differences in activation to faces name pairs presented during study that subsequently received an associative memory judgment (*recall+know*) at test.

Region	TLRC coordinates (x, y, z)	Brodmann area (BA)	# of voxels	*t* value
All Controls vs. Syndrome 2				
Left Posterior Cingulate* (Precuneus, Cuneus; *Right* Posterior Cingulate, Precuneus, Cuneus)	-10, -61, 11	NA	278	3.38
Left Superior Frontal Gyrus	-4, 8, 59	6	40	4.99
Left Middle Temporal Gyrus* (Middle Occipital Gyrus)	-46, -76, 11	39	38	3.63
Right Middle Frontal Gyrus* (Inferior Frontal Gyrus)	47, 14, 26	9	37	3.15

*Major regions accounted for in the cluster.



A



B

Figure 3.16 Face-name *recall+know* encoding cluster in the left (and right) posterior cingulate, cuneus, and precuneus observed to be greater in GWV-C relative to GWV-2 (A) and ill GWV (B)

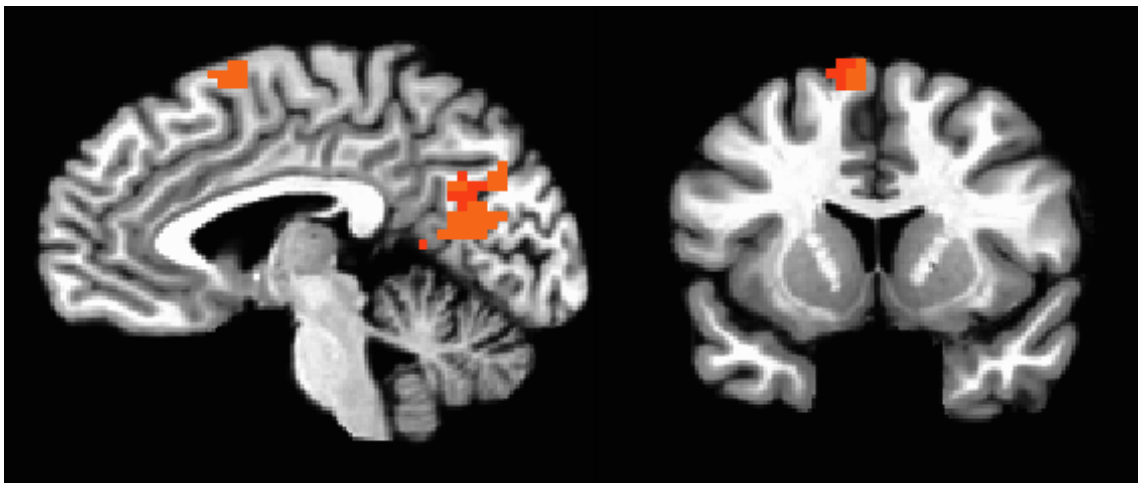


Figure 3.17 Face-name *recall+know* encoding cluster in the left superior frontal gyrus observed to be greater in GWV-C relative to GWV-2

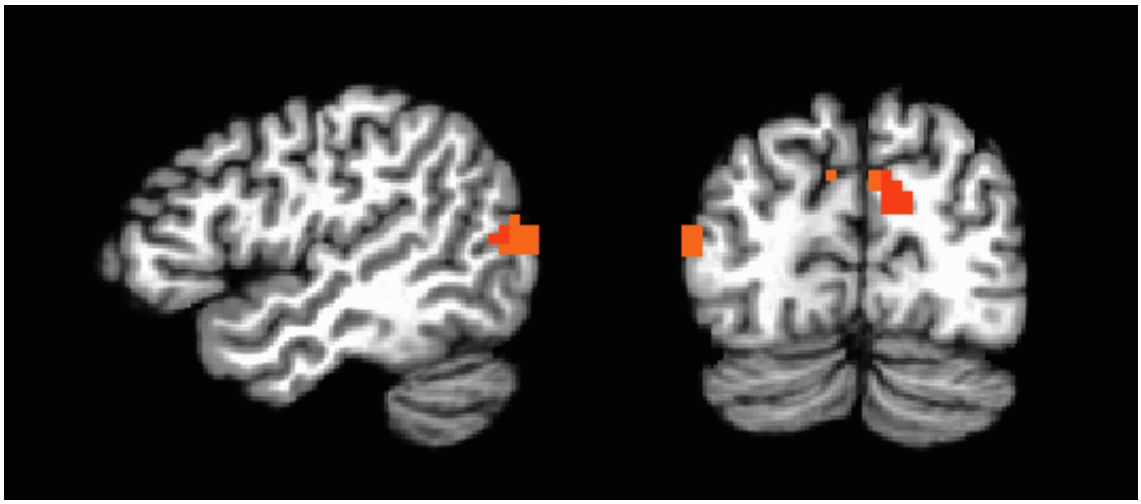


Figure 3.18 Face-name *recall+know* encoding cluster in the left middle temporal gyrus (and middle occipital gyrus) observed to be greater in GWV-C relative to GWV-2

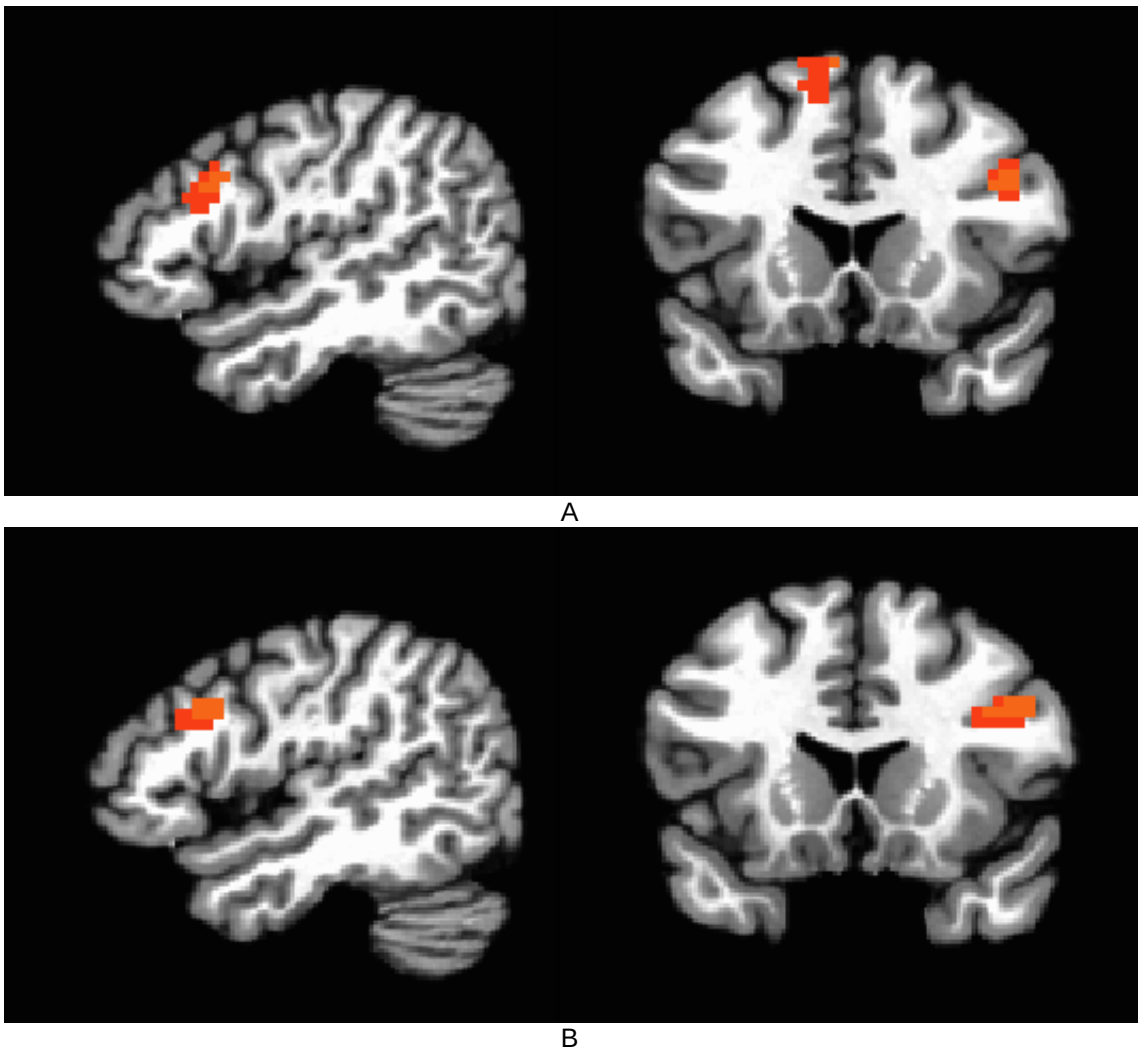


Figure 3.19 Face-name *recall+know* encoding cluster in the right middle frontal gyrus (and inferior frontal gyrus) observed to be greater in GWV-C relative to GWV-2 (A) and ill GWV (B)

Again as a final step, all syndrome types were combined and compared to controls (i.e., non-deployed and deployed). Similar to what was found with face-name *know* encoding, controls exhibited greater activity for face-name *recall+know* encoding in a cluster that included the bilateral posterior cingulate, bilateral cuneus, and right precuneus relative to ill GWV (see Table 3.11 and Figure 3.16B). GWV-C also showed greater activity in the right middle frontal gyrus (including the right IFG) relative to ill GWV (see Figure 3.19B).

Table 3.11. Areas in which GWV-C and ill GWV showed differences in activation to faces name pairs presented during study that subsequently received an associative memory judgment (*recall+know*) at test.

Region	TLRC coordinates (x, y, z)	Brodmann area (BA)	# of voxels	*t* value
All Controls vs. All Syndrome Types				
Left Cuneus (BA 18, Posterior Cingulate*, BA 23, 30, 31, *Right* Cuneus, BA 18, Precuneus, Posterior Cingulate, BA 30, 31)	-1, -70, 17	NA	222	4.05
Right Middle Frontal gyrus (Inferior Frontal gyrus)	47, 14, 26	9	49	3.73

*Major regions accounted for in the cluster.

3.2.6 Summary of Findings for BOLD fMRI Encoding

I predicted that differences between the controls and the different syndrome types would be observed in brain regions implicated in the formation of associative memories, especially regions of the MTL. For example, I expected group differences in hippocampal activity given the role of the hippocampus in the formation of associative memories, especially across different stimulus domains like faces and names (Addis & McAndrews, 2006; Bokde, Tagamets, Friedman, & Horwitz, 2001; Buckner, Kelley, & Petersen, 1999; Giovanello, Schnyer, & Verfaellie, 2004; 2009). Additionally, I predicted that activation in the left amygdala as well as parahippocampal gyrus, could differ between ill and well GWV given their role in processing face information (Sander, Grafman, & Zalla, 2003; Wright & Liu, 2006) and in the encoding of context to support recall and encoding of specific item information to support familiarity (Davachi & Wagner, 2002; Diana et al., 2007; Gonsalves et al., 2005; Ranganath et al., 2004), respectively.

Relative to nondeployed controls, I observed deployed controls and GWV-3 to have greater activity in clusters in the left and right parahippocampus when encoding a face-name pair that would later receive a *know* judgment, respectively. The left parahippocampal cluster seen in deployed controls was part of a larger cluster that also included the fusiform gyrus, which is known to be active with the MTL for face-name encoding (Robinson-Long, Eslinger,

Wang, Meadowcroft, & Yang, 2009). Contrary to predictions, GWV-C and nondeployed controls had lower parahippocampal activity relative to ill GWV groups. Bilateral parahippocampus was also observed to be greater in deployed controls relative to GWV-1 on the same measure. Both of these were within larger clusters, with the right cluster also including the amygdala. In regard to the encoding of face-name pairs that would later receive an associative memory judgment (*recall+know*), deployed controls showed both left and right parahippocampus activity greater than nondeployed controls. Again, this same left cluster also included the fusiform gyrus. As for GWV-3 compared to nondeployed controls, the same right parahippocampal cluster appeared, but also included the hippocampus for face-name *recall+know* encoding.

I did not find the expected hippocampal differences between the groups, despite the hypothesis that GWV-2 might have lower hippocampal activity relative to controls when encoding associative items that would later receive an associative memory judgment. However, other regions implicated in episodic memory formation did show differences between GWV-2 and nondeployed controls. For face-name *know* encoding, GWV-2 had lower activity in the precuneus, inferior and middle frontal gyri, fusiform gyrus, and posterior cingulate when compared to deployed controls. For face-name *recall+know* encoding, these same regions were also found to be lower in GWV-2 compared to deployed controls with the addition of cluster in the middle temporal gyrus (but no fusiform gyrus) when compared to nondeployed controls. I expected that GWV-2 could show the most functional differences due to the severity of their symptoms. This appears to be the case. However, deployed controls also appear to be more functionally different than expected when compared to nondeployed controls.

In regard to the other syndrome types, GWV-1 only showed significantly different encoding activity similar to GWV-2 when compared to the deployed controls. Beside their parahippocampal activity, GWV-3 showed significant differences mostly in the cerebellum and regions involved in visual processing (i.e., lingual and inferior and middle occipital gyri).

3.3 Magnetic Resonance Spectroscopy

3.3.1 Data Processing Prior to Analysis

MRS data for both group and correlational analyses were provided by members of the MRS sub-core group (Sergey Cheshkov, Audrey Chang, Hyeonman Baek, Sandeep Ganji, Evelyn Babcock, & Richard Briggs) of Task Order 4.1 of the VA-funded Gulf War Illness project. Post-processing of the MRS data were performed using LCModel (Cheshkov et al., 2009) software, which utilizes prior spectral knowledge by analyzing the *in vivo* spectrum as a linear combination of *in vitro* acquired metabolite model spectra. Additionally, simulated lipid and macromolecule signals were also included in the modeling. This approach benefits from the inherently higher signal-to-noise ratio with short-TE data, rather than using long-TE data where macromolecule and lipid signals are absent due to their short T_2 values. Metabolite quantities are measured as the total area under the peak. Metabolites modeled include NAA, Cr, Choline (Cho), myo-inositol (Ins), as seen in Figure 2.2. The quantity of specific interest is the NAA/Cr ratio. NAA is widely recognized as a marker of neuronal health and viability, and Cr is a known marker of energy store and metabolism with a methyl peak that is superimposed for creatine and phosphocreatine. Thus the NAA/Cr ratio is thought to be stable and useful as a concentration (Barker, 2001; Friedland et al., 2001; Traber et al., 2006; Urenjak et al., 1993). In previous research, small decreases in NAA/Cr have been detected in pathological conditions such as Alzheimer's disease, MCI, depression, PTSD, and individuals exposed to toxins or consuming alcohol excessively (Caserta et al., 2008; Duan et al., 2011; Friedland et al., 2001; Jiang et al., 2008; Kado et al., 2006; Schuff et al., 2008; Meyerhoff & Durazzo, 2008).

3.3.2 Data Analysis

I conducted a one-way ANOVA and a series of one-way ANCOVAs co-varying the effects of PTSD, depression, and age on NAA/Cr ratios in the left and right hippocampus in order to identify whether GWV classified in the three different syndrome types exhibited reliable differences in these ratios when compared to GWV-C. Additionally, to investigate the

relationship between NAA/Cr concentrations and memory performance in the GWV, I conducted correlational analyses between the NAA/Cr ratios and associative memory performance. Following group analyses, I performed correlations between NAA/Cr and memory performance within each GWV group. Specifically, I ran bivariate correlations were between the left and right NAA/Cr ratio levels and face-name *recall*, *know*, and *recall+know* judgments from the associative memory task. Lastly, I performed partial correlations to determine if PTSD, MDD, and age contribute to any statistically reliable correlations observed between NAA/Cr levels in the hippocampi and memory performance.

To confirm whether or not the nondeployed and deployed controls presented with equivalent levels of NAA/Cr in the left and right hippocampi, *t*-tests were conducted comparing left and right NAA/Cr levels between the two control groups. Contrary to predictions, the nondeployed controls (M = 1.17, SE = .03) presented with greater NAA/Cr levels in the left hippocampus relative to deployed controls (M = 1.07, SE = .02), $t(27) = 2.48$, p = .02. No significant differences were found in the right hippocampal NAA/Cr levels between the nondeployed (M = 1.11, SE = .04) and deployed (M = 1.05, SE = .09) controls, $t(27) = .58$. However, for consistency, all subsequent analyses were conducted with the two control groups divided.

3.3.3 Group Differences in Hippocampal NAA/Cr Concentrations

In regards to the left hippocampus, a one-way ANOVA was conducted to compare NAA/Cr concentrations in the left hippocampus between the nondeployed controls, deployed controls, GWV-1, GWV-2, and the GWV-3. As predicted, differences were observed between the groups in their mean level of NAA/Cr concentrations in the left hippocampus, $F(1, 80) = 4.44$, MSE = .03, η_p^2 = .19, p = .003 (see Figure 3.20). Post hoc comparisons revealed GWV-2 (M = .99, SE = .04) to have had lower NAA/Cr levels than nondeployed controls (M = 1.18, SE = .04), p = 001. Additionally, the GWV-2 had lower levels of NAA/Cr levels relative to GWV-1 (M = 1.15, SE = .04) and GWV-3 (M = 1.16, SE = .04), p = 001, p = .003. Deployed controls (M =

1.05, *SE* = .05) were observed to have marginally lower NAA/Cr levels than GWV-1, *p* = .06. All other pairwise contrasts were not significant, all *p*s > .10. In addition, a series of ANCOVAs were performed on the mean levels of NAA/Cr concentration in the left hippocampus with group (nondeployed control, deployed control, GWV-1, GWV-2, GWV-3) serving as a fixed factor and PTSD, MDD, and age serving as covariates in separate ANCOVAs. PTSD did not have a significant effect on NAA/Cr levels, *F* > 1. Whereas, age had a marginal effect, *F*(1, 80) = 3.18, *MSE* = .03, $p\eta^2$ = .04, *p* = .08, and MDD had a significant effect on levels of NAA/Cr, *F*(1, 80) = 4.67, *MSE* = .03, $p\eta^2$ = .06, *p* = .03.

In regards to the right hippocampus, a one-way ANOVA was conducted to compare NAA/Cr concentrations in the right hippocampus between the different groups. NAA/Cr levels were again observed to differ between the groups, *F*(1, 80) = 3.22, *MSE* = .06, η_p^2 = .15, *p* = .02 (see Figure 3.20). GWV-2 (*M* = .96, *SE* = .05) showed lower NAA/Cr levels than nondeployed controls (*M* = 1.11, *SE* = .06), GWV-1 (*M* = 1.14, *SE* = .06), as well as GWV-3 (*M* = 1.20, *SE* = .06), *p* = 07, *p* = 02, *p* = .003, respectively. Note that the difference between GWV-2 and nondeployed controls was only marginally significant. Deployed controls were observed to have lower NAA/Cr levels than GWV-1 and GWV-3, *p* = .09, *p* = .02. Note that the difference between deployed controls and GWV-1 was only marginally significant. All other pairwise were not significant, all *p*s > .19. Following the initial ANOVA, a series of ANCOVAs were conducted to test for the any effect of PTSD, MDD, and age on NAA/Cr levels in the right hippocampus with group serving as a fixed factor. PTSD and age did not have a significant effect on NAA/Cr levels, *p* > .21. However, MDD did have a marginally significant effect on levels of NAA/Cr, *F*(1, 80) = 3.13, *MSE* = .06, $p\eta^2$ = .04, *p* = .08.

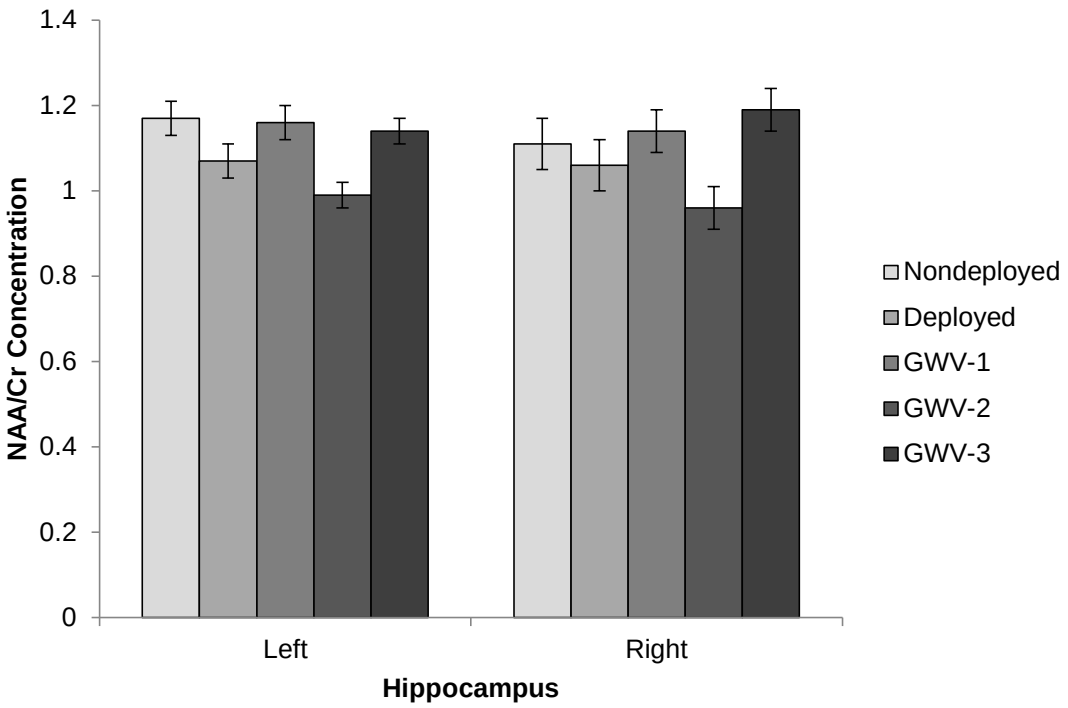


Figure 3.20 Left and right hippocampal NAA/Cr ratio concentrations as a function of Gulf War veteran group membership

3.3.4 Relationship Between Hippocampal NAA/Cr Concentrations and Associative Memory Performance

Correlations between NAA/Cr concentration in the hippocampus and associative memory performance were conducted for the nondeployed controls, deployed controls, GWV-1, GWV-2, and GWV-3 separately. Specifically, I ran bivariate correlations between the left and right NAA/Cr ratio levels and face-name *recall*, *know*, and *recall+know* judgments for each of the groups separately. Then, I performed bivariate correlations between the left and right NAA/Cr ratio levels and face-name recall, know, and *recall+know* judgments for the controls pooled across deployment status. Finally, I conducted bivariate correlations between the left and right NAA/Cr ratio levels and face-name *recall*, *know*, and *recall+know* judgments for ill-GWV pooled across syndrome type.

For nondeployed controls, a significant positive correlation was found between NAA/Cr levels in the right hippocampus and *recall* performance for face-names, $r = .46$, $p = .05$ (see

Figure 3.21). No other correlations were significant, all *p*s > .26. GWV-1 showed a significant negative correlation between NAA/Cr levels in the left hippocampus and *recall+know* judgments to face-name pairs, $r = -.48$, $p = .04$ (see Figure 3.22). No correlations reached significance for GWV-2, all *p*s > .45. As for GWV-3, right hippocampal NAA/Cr levels showed a negative relationship with *recall* responses to face-name pairs, $r = -.43$, $p = .07$ (see Figure 3.23). Note that this effect was marginal. For the last step, partial correlations conducted that controlled for PTSD, MDD, and age revealed these other factors to not significantly impact the results found with the bivariate correlations for NAA/Cr and associate memory performance between the GWV groups.

Additional correlations were conducted on the combined GWV-C group and the combined ill GWV group. These analyses revealed NAA/Cr concentrations in the left hippocampus to be positively correlated with face-name *know* and face-name *recall+know* responses in controls, $r = .39$, $p = .06$; $r = .41$, $p = .05$, respectively (see Figure 3.24). However, the correlation between face-name *know* responses and NAA/Cr concentration levels in the left hippocampus was only marginally significant. No significant correlations were found between NAA/Cr concentrations and memory performance in ill GWV. Partial correlations, controlled for PTSD, MDD, and age, revealed that these other factors did not significantly impact the bivariate correlations for NAA/Cr and associate memory performance for the control participants pooled across deployment status and the ill GWV pooled across syndrome type.

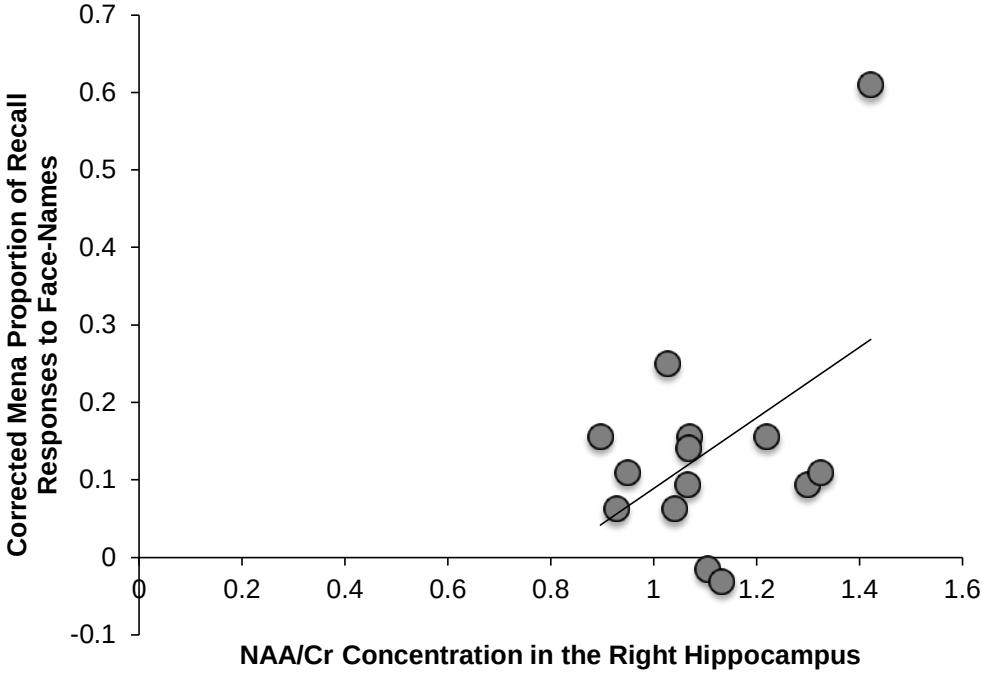


Figure 3.21 Positive correlation between right hippocampal NAA/Cr concentrations and *recall* responses made to face-name pairs in nondeployed controls

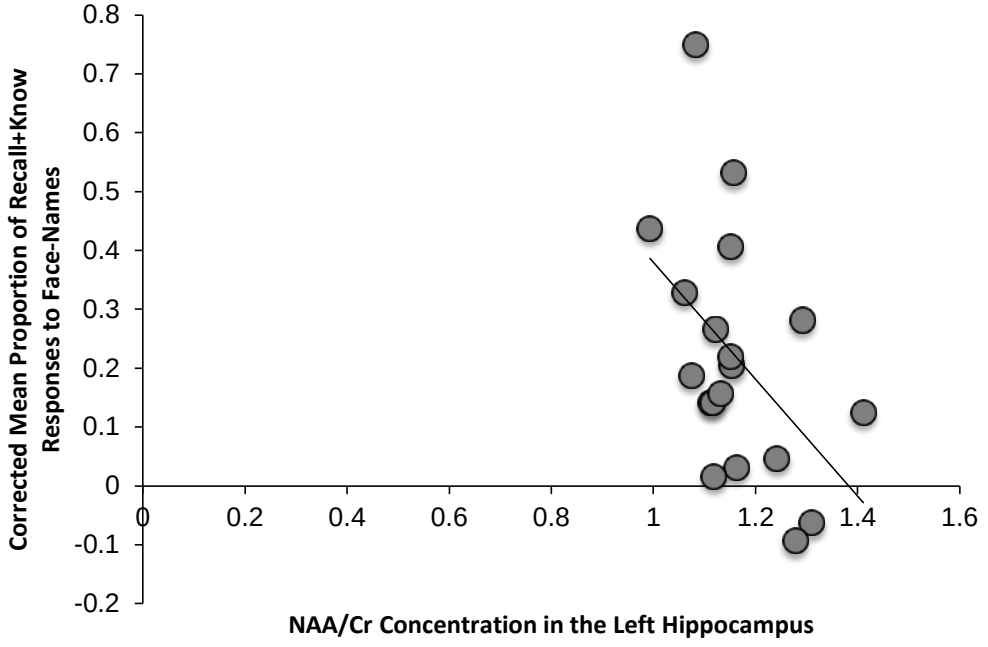


Figure 3.22 Negative correlation between left hippocampal NAA/Cr concentrations and *recall+know* responses made to face-name pairs in GWV-1

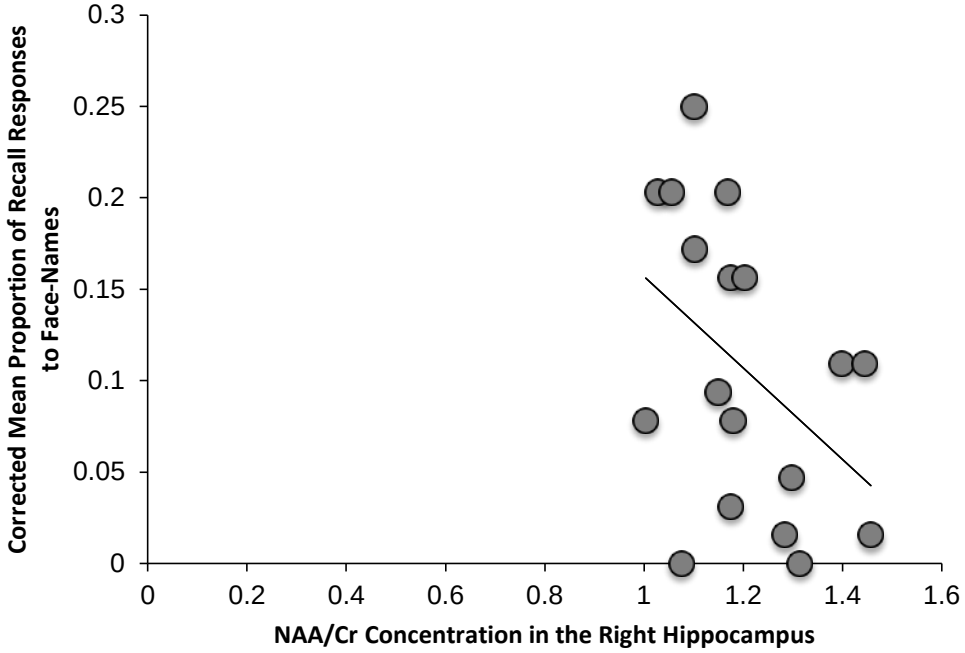


Figure 3.23 Negative correlation between right hippocampal NAA/Cr concentrations and *recall* responses made to face-name pairs in GWV-3

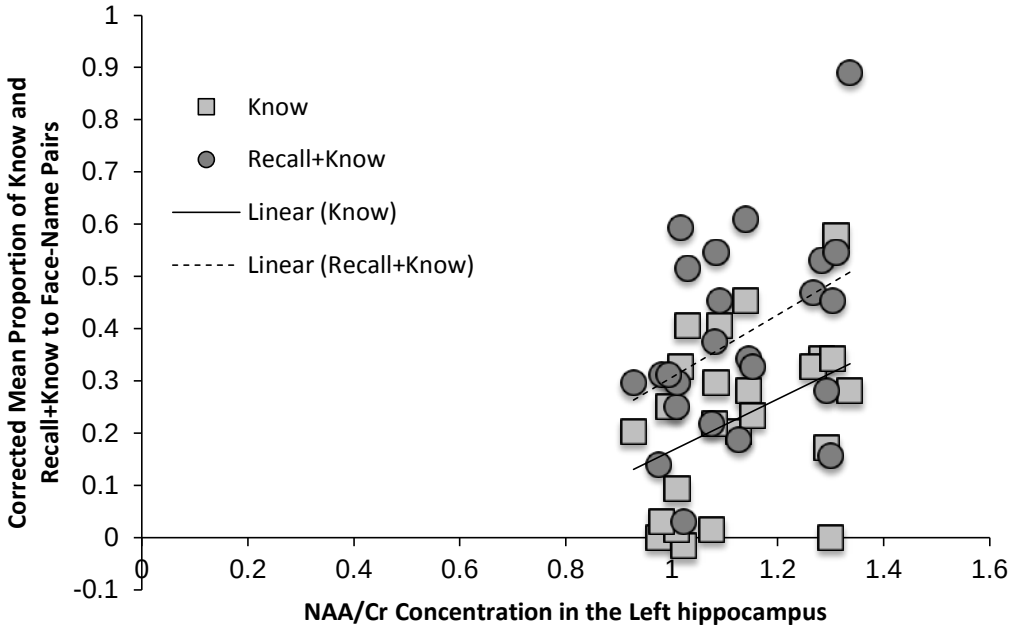


Figure 3.24 Positive correlations between left hippocampal NAA/Cr concentrations and face-name know as well as face-name *recall+know* responses in GWV-C

3.3.5 Summary of Findings for Hippocampal NAA/Cr Concentrations

I predicted the concentration of hippocampal NAA/Cr to differ among the groups, and pair-wise contrasts revealed GWV-2 to have presented with lower concentrations of NAA/Cr levels in the left hippocampus relative to nondeployed controls, GWV-1, and GWV-3. However, unlike predicted, the two control groups differed from another in their NAA/Cr hippocampal concentrations. Therefore, they were kept separate for further analyses revealing the deployed controls to appear quite different than nondeployed controls. Furthermore, I observed deployed controls to be very similar to GWV-2 in their NAA/Cr concentrations. Additionally, while I predicted that all the covariates would reach significance and thus account for the group effect to some degree, the only covariate that appeared to have an effect on NAA/Cr concentration in both the left and right hippocampus was MDD. However, it did not account for the majority of the effect, which does appear to still be driven by GWI.

I predicted positive correlations between NAA/Cr concentration for the left and right hippocampus and associative memory performance. Specifically, as NAA/Cr levels increase, associative memory performance should improve especially in the control groups, but the same may not be true of the three syndrome types. I observed nondeployed controls to have this trend. As NAA/Cr levels increased, their proportion of *recall* responses to face-name pairs also increased. As predicted, when combining the two control groups, I observed a positive correlation in GWV-C between NAA/Cr and memory performance, both for *know* and *recall+know* responses. On the other hand, some GWV groups such as the GWV-1 and GWV-3 exhibited negative correlations with associative memory responses to face-name pairs.

CHAPTER 4

DISCUSSION

Roughly 26-32% of U.S. veterans who served in the Persian Gulf War of 1991 report suffering from chronic health problems that they claim are related to their Gulf War service (Golomb, 2008). A large number of these ill GWV complain of cognitive deficits with memory problems being chief among these complaints. However, only a small number of studies have objectively verified the presence of such deficits (Odegard et al., in press). An inability to reliably demonstrate memory declines in ill GWV relative to well GWV has raised concerns regarding the extent to which the ill GWV have memory impairments. Yet, there was reason to suspect that the self reported memory problems are more substantive. Ill GWV have differences in key brain regions, including the hippocampus, a critical brain region for memory, relative to well GWV, (Menon et al., 2004; Li et al., 2011; Sperling et al., 2001; Sperling et al., 2003). Thus, the aim of the current study was to validate GWI and the memory symptoms these veterans report, and to identify neurobiological correlates of these deficits by investigating associative memory and hippocampal function in a national sample of GWV. To do so, I measured performance on a hippocampal-dependent task and brain activation evoked during encoding of associative memories. Additionally, I obtained levels of NAA/Cr in the hippocampal formation measured during a passive MRI scan.

4.1 Memory

The first purpose of the present study was to objectively measure the memory deficits that many veterans with GWI self-report in a national sample of GWV. The current study also serves as a replication and extension of previous research that observed memory differences between ill and well GWV (Odegard et al., in press; see Li et al., 2011 for memory performance correlated to hippocampal perfusion). To do so, ill and well GWV completed the same face-

name memory paradigm used in the prior research. During the memory test, they were presented with the studied faces and asked to determine whether a face had been accompanied with a name at study, and if so, to indicate whether they could recall the specific name. This face-name associative memory task was used because of the known reliance of associative memory on hippocampal function, which has been observed to be different between ill and well GWV (Menon et al., 2004; Li et al., 2011; Sperling et al., 2001; Sperling et al., 2003). Moreover, the specific paradigm assessed not only successful recall of faces' names, but also an intermediate associative memory state in which one can fail to recall a face's name, but have knowledge that it had been encountered earlier. Similar brain regions support this form of associative recognition and recall (Chua et al., 2007).

4.1.1 Group Differences in Associative Memory Performance

Previous research (Odegard et al., in press) using this face-name associative memory test has demonstrated that ill GWV from a smaller sample of GWV to be impaired on this intermediate measure of associative memory (*know* judgments to face-name pairs). In line with Odegard and colleagues, I predicted that ill GWV in the current study would demonstrate poorer performance in associative memory relative to well GWV. The current study replicated their results and extended the findings to a national sample of GWV. GWV-1 and GWV-2 both showed poorer *recall* and *know* judgments to face-name pairs that were previously studied when compared to controls. Interestingly, when looking at associative memory in its entirety (*recall*+*know* judgments), all three syndrome types, GWV-1, GWV-2, and GWV-3, showed deficits relative to GWV-C.

Overall, these findings are consistent with the symptoms reported by the veterans from the different syndrome types. GWV-1 and GWV-2 report memory and related cognitive problems, while GWV-3 do not. Instead, GWV-3 report problems with chronic pain. I expected that deficits would be seen in GWV-1 and especially GWV-2, based on symptom severity and complaints. Therefore, it is interesting that even GWV-3 (i.e., central neuropathic pain) would

also present with memory deficits relative to controls. It could be the case that the GWV-3 report central pain related to particular neurotoxin exposure, and there may be cognitive deficits related to this neurotoxin exposure that have generally gone undetected.

While both Odegard et al. (in press) and the current study observed similar patterns in ill and well GWV, it is important to better characterize the differences between the two studies and acknowledge potential limitations of the current study. Unlike the prior study, the current study included a group of nondeployed veterans in addition to a group of deployed veterans analogous to the prior study. The current study did not observe differences between deployed and nondeployed controls on the associative memory. However, it is possible that the variance in the deployed controls did not allow for a significant difference to be detected between them and the nondeployed controls. The current study had 10 deployed controls, but it could be the case that deployed controls might look more like ill GWV given a larger sample size.

In addition the current study had a larger sample, which allowed me to better account for potential effects of psychological conditions such as PTSD and MDD as well as aging on memory performance. The main purpose of using scores of PTSD and MDD as covariates was to identify if such conditions explain any memory effects observed in ill GWV. The Psychological Stress Hypothesis takes the perspective that GWI can be explained by psychiatric and medical conditions, which are unrelated to neurotoxin exposure (Binns et al., 2008). With regard to the memory data, PTSD, MDD, and age did not have a significant effect on memory performance. These data help to bolster the Neurotoxin Exposure Hypothesis, which states that GWI is primarily caused by exposure to one or more neurotoxins during the war, at least with regard to memory performance (Binns et al., 2008).

4.2 Functional Magnetic Resonance Imaging

The second aim of the present study was to identify neurobiological correlates of associative memory encoding and performance using magnetic resonance imaging, fMRI and MRS, respectively. For the first neurobiological correlate, the current study investigated the

extent to which ill GWV would exhibit differences in brain function, as measured by BOLD fMRI, when compared to well GWV. Encoding activity was collected and analyzed for the current study to investigate functional differences among the GWV when encoding associative memories. The hippocampus is region of interest due to its role in a distributed network of brain regions implicated as important for associative memory encoding (Davachi, 2006; Eichenbaum et al., 1996; Henke et al., 1997; 1999; Mayes et al., 2007; Sperling et al., 2001; 2003; Schacter & Wagner, 1999) and it is a known area of dysfunction in ill GWV (Li et al., 2011; Menon et al., 2004). Furthermore, when using face-name paradigms like the one in the present study, hippocampal activity during encoding of the face-name pairs has been shown to be greater during the encoding of associations that are remembered at test (Chua et al., 2007; Davachi & Wagner, 2002; Jackson & Schacter, 2004; Sperling et al., 2001, 2003; Staresina & Davachi, 2008; Zeineh et al., 2003).

Associative memory tasks have also been used in fMRI studies of PTSD and depression to provide evidence of neurobiological abnormalities present in these populations (Fairhall, Sharma, Magnusson, & Murphy, 2010; Geuze et al., 2008; Werner et al., 2009a; Werner et al., 2009b). Both PTSD and MDD populations have shown functional differences in BOLD fMRI activity in the encoding network despite a lack of significant deficits in task performance (Fairhall et al., 2010; Geuze et al., 2008; Werner et al., 2009a; Werner et al., 2009b). The participants in the present study underwent fMRI while they were studying face-name pairs. These data were used to identify whether ill GWV have functional brain differences in comparison to well GWV when attempting to learn information for a subsequent associative memory test. Such differences would provide further validation of GWI and the symptoms many ill GWV report.

In pursuit of this aim, the current study could replicate and extend previous research by Odegard and colleagues (in press) to a national sample of GWV showing ill GWV to have functionally different encoding activity for face-name associations than well GWV. Moreover, the

previous study by Odegard and colleagues investigating associative memory with this paradigm in GWV observed activation in the hippocampus during encoding to be positively correlated with subsequent memory performance. However, the initial study was statistically underpowered. This prohibited meaningful comparisons between the ill and well GWV. The current study replicates and extends the previous study by directly comparing activation measured during fMRI acquired during encoding between the three syndrome types and control veterans from the larger national sample. Additionally, the previous study did not categorize study items based on subsequent memory performance. Thus, the current study performed subsequent memory analysis of the fMRI data and compared this activation between the three syndrome types and control veterans. Encoding BOLD fMRI activity during the presentation of face-name pairs that later received a *know* response or a combined associative memory judgment (*recall+know*) was compared between GWV groups (e.g., GWV-C vs GWV-2). This would allow for identification of functional brain differences (including the hippocampus) between the groups.

4.2.1 Group Differences in Associative Memory Encoding

To replicate and extend the findings of Odegard and colleagues (in press) using a larger national sample of GWV and subsequent memory analyses, it was predicted that several clusters would show significant activation during associative memory encoding. Even though Odegard and colleagues did not observe group differences for the encoding of face-name pairs, a cluster in the left MTL, which encompassed the amygdala and parahippocampal gyrus, as well hippocampus, during encoding of face-name pairs was observed to be related to subsequent memory performance. As activation in this cluster increased, associative memory performance improved. A similar cluster could be expected in the current study for subsequent associative memory encoding especially given the role of the amygdala in processing of face information (Sander, Grafman, & Zalla, 2003; Wright & Liu, 2006), the parahippocampus in encoding of isolated items (e.g., words, faces, objects; Davachi & Wagner, 2002; Diana et al., 2007; Gonsalves et al., 2005; Ranganath et al., 2004), and the hippocampus in formation of

associative memories across different stimulus domains (Addis & McAndrews, 2006; Bokde, Tagamets, Friedman, & Horwitz, 2001; Buckner, Kelley, & Petersen, 1999; Giovanello, Schnyer, & Verfaellie, 2004; 2009),. Therefore, the current study predicted significant study activation in the hippocampus for subsequently remembered associations. I also predicted that significant study activation would also be observed in the prefrontal cortex due to its role in top-down processing (Koechlin, Ody, & Kouneiher, 2003; Miller, 1999; Miller & Cohen, 2001), and that this brain activity would be functionally different between ill and well GWV.

With regard to the findings in the current study, deployed controls and GWV-3 exhibited significantly greater activation, when encoding face-name pairs that would later receive *know* judgments, in clusters of voxels located in the left and right parahippocampus relative to nondeployed controls. The left parahippocampal cluster in deployed controls also encompassed the fusiform gyrus possibly suggesting greater activity for the encoding of the face-name pair (Robinson-Long et al., 2009). Even though controls were expected to have greater parahippocampal activity relative to ill GWV groups, this finding was surprising and might actually be compensatory, allowing deployed controls to have comparable performance to the nondeployed controls. With regard to the encoding of face-name pairs that would later receive a composite associative memory judgment (*recall+know*), deployed controls showed both left and right parahippocampus activity greater than nondeployed controls. Again, this same left cluster also included the fusiform gyrus.

Contrary to predictions, no significant differences were found in hippocampal clusters. GWV-2, in particular, were predicted to present with lower levels of hippocampal activation when encoding face name pairs relative to controls. However, these ill GWV classified with syndrome 2 were observed to have lower activity relative to controls in several brain regions believed to be implicated in a compensatory role in memory performance. These included the posterior cingulate, precuneus, middle and inferior frontal gyrus, and middle temporal gyrus for face-name *recall+know* encoding. Earlier studies investigating memory impairment in clinical

populations demonstrated increases in posterior cingulate and precuneus activity in order to maintain accurate memory performance relative to controls (Sperling, Dickerson, Pihlajamaki, Vannini, LaViolette et al., 2010)). These regions are believed to be involved in deep associative encoding in episodic memory and thus can show compensatory activity to maintain performance (Bastin, Feyers, Majerus, Balteau, Degueldre et al., 2012; Hulst, Schoonheim, Roosendaal, Popescu, Schweren et al., 2011). Additionally, the lower frontal and temporal activity (as well as parietal activity) suggests that GWV-2 may not be engaging in enhanced monitoring and control processing as other cognitively impaired clinical populations have been shown to engage when performing memory tasks (Weis, Leube, Erb, Heun, Grodd et al., 2011). Some of these processes are engaged to increase attention, and phonological and visual rehearsal in addition to increasing semantic and contextual processing of the stimuli being encoded through increases in frontal, parietal, and temporal lobe activity, respectively (Weis et al., 2011). GWV-2 do not seem to be activating these important regions implicated in maintaining episodic memory performance.

Although, it was expected that GWV-2 would present with the most differences in brain activation due to the severity of their symptoms, the deployed controls were also drastically different than expected. Unlike GWV-2, deployed controls showed greater activity, relative to nondeployed controls and even GWV-2, in the regions listed above that appear to be activated to assist episodic memory encoding. Furthermore, I observed higher activation in deployed controls for several other important regions shown to play a compensatory role in memory encoding. In addition to greater activity in many of the regions not observed for GWV-2 (e.g., precuneus, posterior cingulate, frontal and temporal regions) compared to nondeployed controls, deployed controls also showed greater activity in the parahippocampal and fusiform gyri relative to nondeployed controls as well as GWV-2. Greater activity in the parahippocampal and fusiform gyri has also been implicated in maintaining memory performance equivalent between cognitively impaired individuals and controls (Guedjj, Bettus, Barbeau, Liégeois-

Chauvel, Confort-Gouny et al., 2011; Hulst et al., 2011). This increase in a perceptive-memory system (parahippocampal and fusiform gyri), a maintenance-control system (frontal-cingulate-parietal regions), as well as a additional semantic-contextual processing might be what allows the deployed controls to have roughly equivalent memory performance to that of nondeployed controls and greater than that of GWV-2 (Gued et al., 2011; Hulst et al., 2011; Weis et al., 2011).

As for the other ill GWV groups, GWV-1 did not show functional differences relative to nondeployed controls, but rather only showed different encoding activity similar to GWV-2 when compared to the deployed controls. This data, in conjunction with their memory performance, might suggest that GWV-1 are less impaired than GWV-2 as predicted, yet do not engage in compensatory processing like nondeployed controls. GWV-3 showed significant differences mostly in the cerebellum and regions involved in visual processing (i.e., lingual and inferior and middle occipital gyri), but also showed a cluster in the insula as well as a cluster in the parahippocampus to be greater than nondeployed controls, with the inclusion of the hippocampus for *recall+know* judgments. Considering GWV-3 only showed memory deficits in the *recall+know* condition, these data could suggest GWV-3 to have less impairment than the other ill GWV groups, but less compensation than that of the deployed controls.

Of the ill GWV groups, GWV-3 was the only ill GWV group to have a cluster that included the hippocampus. Yet, the cluster in the hippocampus showed greater activity than nondeployed controls for face-name *recall+know* encoding. Many studies have sought to observe hippocampal differences between clinical populations and their controls while performing a memory task, but instead they observed functional differences to be driven by other regions (Gued et al., 2011; Hulst et al., 2011; Weis et al., 2011; Werner et al., 2009b). For the current study, BOLD fMRI activity for encoding associative items that are subsequently given an accurate associative memory judgment may not lend itself to observing differences in hippocampal activity. However, it is possible that employing similar analytical methods like that

of Fairhall and colleagues (2010) might provide reveal hippocampal differences between ill and well veterans. In a sample of MDD patients, Fairhall and colleagues (2010) took hippocampal activity and correlated it with memory performance. They observed that even in the absence of a difference in memory performance relative to controls, MDD patients did not show the same relationship as controls such that as hippocampal activity increased, so did memory performance. More importantly, different methods of estimating hemodynamic responses in the hippocampus might reveal alternate findings. Recent data have shown that the hippocampus can have a biphasic hemodynamic response, both positive and negative transients post-stimulus, resulting in mixed fMRI findings across studies (Meltzer, Negishi, & Constable, 2008). Considering the hippocampus does not always fit a typical convolution, different processing methods should be considered for further investigation when there is an absence of hippocampal activity during tasks that probe hippocampal activity (Astur & Constable, 2004).

Overall, these data converge with the memory data and link differences in memory performance to underlying differences in brain function. They suggest that the some ill GWV are experiencing cognitive symptoms that are biologically based. Moreover, the fMRI data suggest that the alterations in brain function differ between the different syndrome types. There appears to be heterogeneity among ill GWV, even when they exhibit similar cognitive deficits. This provides further construct validity for the syndrome classification system.

4.3 Magnetic Resonance Spectroscopy

Past research has used MRS to investigate neuronal abnormalities by observing lower metabolite concentrations relative to controls in a multitude of populations, including but not limited to older adults, civilian and veteran populations with PTSD, depressed patients, and lead-exposed workers (Caserta et al., 2008; Duan et al., 2011; Jiang et al., 2008; Kado et al., 2006; Schuff et al., 1999; 2008; Zimmerman et al., 2008). Furthermore, MRS has also been used in research to demonstrate links between NAA/Cr concentrations and cognition. Specifically, using NAA/Cr to investigate the relationship between neuronal abnormalities in the

hippocampus and memory performance (Zimmerman et al., 2008). Zimmernan and colleagues (2008) found hippocampal NAA/Cr to be a predictor of verbal memory performance in a population of nondemented older adults, such that lower levels of NAA/Cr predicted poorer performance. Additionally, Driscoll and colleagues (2003) used two hippocampal-dependent memory tasks on younger and older adults, and found age-related decreases in NAA/Cr and task performance.

The second goal of the present study was to investigate possible differences in NAA/Cr concentration level measured in the left and right hippocampus across veteran groups. Specifically, the current study conducted group contrasts on left and right hippocampal NAA/Cr concentrations as well as correlational analyses between these hippocampal NAA/Cr concentrations and performance on the associative memory task. First, hippocampal differences between ill and well GWV would replicate and extend the findings by Menon and colleagues (2004) to a national sample of GWV. Second, the correlational analyses would allow for the identification a relationship between NAA/Cr and memory performance in GWI.

4.3.1 Group Differences in Hippocampal NAA/Cr Concentrations

I predicted that NAA/Cr levels would differ among the groups and this prediction was confirmed. Pair-wise contrasts revealed the GWV-2 to have the lowest NAA/Cr levels compared to nondeployed controls and other ill GWV. However, unlike predicted for GWV-C, the two control groups differed from another in their NAA/Cr hippocampal concentrations such that deployed controls had lower NAA/Cr concentrations than nondeployed controls in the left hippocampus. Moreover, deployed controls were observed to be similar to GWV-2 in their NAA/Cr concentrations. Deployed controls showed lower NAA/Cr concentrations relative to GWV-1 and GWV-3. This revealed deployed controls to appear more like GWV-2 than nondeployed controls.

Additionally, it was predicted that the covariates could reach significance accounting for some of the group effect. However, MDD was the only covariate that appeared to account from

some of the differences in NAA/Cr concentration in both the left and right hippocampus. Given the use of MRS in MDD populations to observe change in metabolite concentrations, this result was not surprising, but MDD did not account for the majority of the effect in this population (Duan et al., 2011; Kado et al., 2006). Hippocampal alterations in MDD have been postulated to be a result of increased glucocorticoid levels, which act as a neurotoxin in areas such as the hippocampus (Colla, Kronenberg, Deuschle, Meichel, Hagen et al., 2007; Sapolsky, 2000). Additionally, the marginal effect of age on left hippocampal NAA/Cr was also not surprising. Reductions in hippocampal NAA/Cr have been observed as a function of increasing age, as well as a predictor of dementia (Caserta et al., 2008; Schuff et al., 1999).

It must be noted that the NAA/Cr concentrations in left hippocampus appeared to be more sensitive to the detection of impairment. Specifically, the deployed controls had lower NAA/Cr concentrations than nondeployed controls in the left hippocampus. Both MDD and age effects were also observed in NAA/Cr concentrations in left hippocampus. In regard to these findings, research has shown that hippocampal dysfunction can be lateralized with the left side being more impaired than the right (Mohanakrishan Menon et al., 2003). More importantly, although these analyses were more exhaustive correcting for family-wise error might result in the loss of such differences, especially any that were already marginal.

4.3.2 Relationship Between Hippocampal NAA/Cr Concentrations and Associative Memory Performance

For the correlational analyses between NAA/Cr concentration for the left and right hippocampus and associative memory performance, I predicted that correlations would show a positive relationship. Specifically, as NAA/Cr levels increase, associative memory performance should also increase, and this should be especially true of the control groups. The same may not be the case for GWV of the three syndrome types. Nondeployed controls exhibited the predicted pattern. As their NAA/Cr levels were higher, their proportion of *recall* responses to face-name pairs increased. This finding is in line with past research investigating the link between NAA/Cr concentration in the hippocampus to cognitive performance. For example,

hippocampal NAA/Cr has been observed to predict verbal memory performance in a population of nondemented older adults. Higher levels of NAA/Cr were related to greater memory performance (Zimmernan et al., 2008). Additionally, higher NAA/Cr concentrations in the hippocampus have also been linked to greater performance on hippocampal-dependent memory tasks such as the virtual Morris water task and the transverse patterning discrimination task (Driscoll et al., 2003). However, it must be noted that one nondeployed veteran performed very well on the memory task and thus drove this relationship to some degree. Specifically, while the positive correlation still had a small effect, it was no longer significant when either taking that veteran out of analyses or transforming the data before analyses.

In contrast to the nondeployed controls, the GWV-1 and GWV-3 exhibited negative correlations between hippocampal NAA/Cr concentrations and associative memory performance on the face-name task. For example, greater performance on memory measures that included recall (*recall* or *recall+know*) was related to lower hippocampal NAA/Cr concentrations. Moreover, these correlations were not attenuated after accounting for PTSD, MDD, or age. These findings might indicate that GWV-1 and GWV-3 are impaired relative to the nondeployed controls.

Similarly, the lack of a correlation in deployed controls and GWV-2 indicate they are also different than the nondeployed controls. The lack of a significant correlation for GWV-2 together with their low NAA/Cr concentrations is useful in validating their impairment. The lack a significant correlation for deployed controls together their low NAA/Cr concentrations in the left hippocampus relative to nondeployed controls indicates they are not healthy controls and caution should be taken in merging deployed controls with nondeployed controls.

While many of the correlations discussed above center around significant findings, many of the correlations regardless of significance, produced small effect sizes suggesting a possible link between NAA/Cr and memory performance (Cohen, 1992). For many of the groups, ill GWV and deployed veterans, many of the correlations had a negative trend. These

findings might suggest that, when given a larger sample size, these data investigating at a link between NAA/Cr in the hippocampus to be related to memory performance might produce significant results as well. A larger sample size might show nondeployed controls to have a positive relationship between NAA/Cr and memory performance, with the other groups might show negative relationships. This could possibly suggest some sort of dysfunction among the groups that present with memory deficits and/or functional brain differences.

4.5 Implications and Further Directions

When taken together, the data in the current experiment, demonstrate ill GWV to be different than the deployed and nondeployed controls to varying degrees based on their syndrome type and help to validate Gulf War Illness and the Hayley syndrome classification. GWV-2 were the most severely impaired of the ill GWV groups given their memory deficits and neurobiological abnormalities when compared to nondeployed controls. GWV-1, followed by GWV-3, also appeared to be impaired on these measures relative to nondeployed controls but to a lesser degree than GWV-2. Interestingly, while performing relatively well on the memory task, deployed controls were observed to quite different than nondeployed controls on their neurobiological measures. In most instances, the findings in the current study were not accounted for by PTSD, MDD, or age, suggesting that these conditions may not be driving the impairments seen in GWI, but rather something else it, such as neurotoxin exposure (see Figure 4.1).

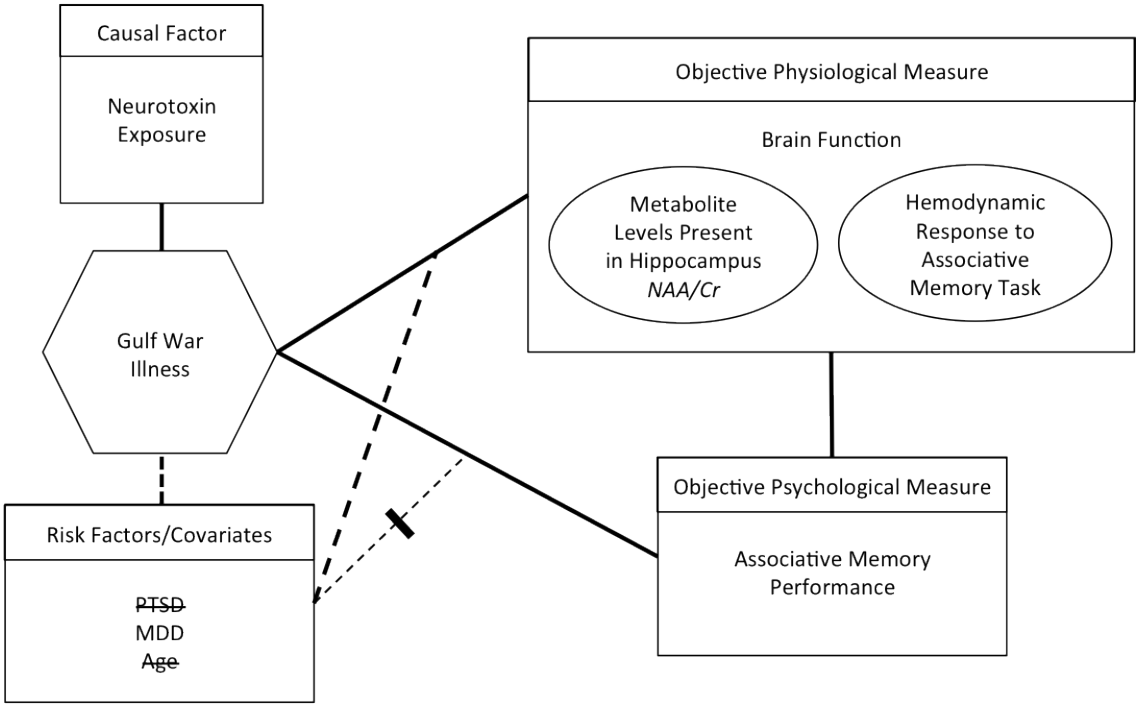


Figure 4.1 Conceptual model of associative memory performance and hippocampal function in veterans with Gulf War Illness

Moving forward, it will be important for future studies to replicate and extend these findings to larger samples of veterans with comparable sized control groups. For the current study, deployed controls had the smallest sample size. Having comparable nondeployed and deployed control groups for comparisons with ill GWV groups would allow for reliability testing. It would be beneficial for future studies to keep these two control groups separate. Although the current study combined them for the associative memory measures due to equivalent performance, follow-up analyses revealed the difference found between GWV-C and GWV-3 on recall+know to be driven by nondeployed controls, and not deployed controls. Additionally, it would be important to investigate specifically what about the deployed controls make them different than nondeployed controls. The sample in the current study did not present with PTSD or MDD, and have not reported similar symptoms as ill GWV, which they attribute to be from neurotoxin exposure. However, it is possible that, due to their deployment, these veterans are experiencing stress-related effects of serving in the war, but not at a level of a diagnosable

condition. Evidence suggests that traumatic stress can alter brain regions involved in memory (Bremner, Krystal, Southwick, & Charney, 1995).

Furthermore, these larger sample sizes would also allow for covariate analyses to be conducted on fMRI data in order to investigate their role in the functional differences observed in the current study. In regard to covariates, future research on GWI should also take into account additional covariates that were outside the scope of the present study. For instance, many ill GWV were diagnosed with alcohol abuse or dependence. This may serve as an important covariate for some of the measures being used in the present study, such as NAA/Cr as measured by MRS. Evidence suggests that individuals with alcohol use disorders (AUD) show neuronal dysfunction by lower brain metabolite concentrations (e.g., NAA and NAA/Cr; for a review see Meyerhoff & Durazzo, 2008). Research has also observed increases in NAA concentrations following one month of abstinence in individuals with alcohol abuse (Durasso, Gazdzinski, Rothlind, Banys, & Meyerhoff, 2006). This trend was also observed with NAA/Cr, and this increase was positively correlated with memory performance (Bendszus, Weijers, Wiesbeck, Warmuth-Metz, Bartsch, Engels, Boning, & Solymosi, 2001).

Similarly, future studies with larger sample sizes would allow self-reported exposure rates to acetylcholinesterase inhibitors and genetic profiles to be included in statistical analyses of memory and neuobiological data (fMRI and MRS). Self-exposure rates may also provide more information that would identify whether deployment status plays a role in the symptoms of GWI. In regard to specific genetic markers, the paraoxonase (PON) cluster and brain-derived neurotrophic factor (BDNF) would be of particular interest. PONs are involved in detoxification of organophosphates and chemical nerve agents like those used during the first Gulf War (Saeed, Siddique, Hung, Usacheva, Liu et al., 2006). Evidence suggests that the PON family is central to a wide array of human conditions from cardiovascular disease to mental disorders (Camps, Marsillach, & Joven, 2009). Some PON polymorphisms are less effective in hydrolyzing toxic organophosphates, and thus increase sensitivity to neurotoxins (Draganov &

La Du, 2004; Saeed et al., 2006). For example, less effective polymorphisms of PON1 has been associated with the neurologic symptom complex of GWI (Saeed et al., 2006).

The second gene of interest, BDNF, is a neurotrophin growth factor that is expressed throughout the central and peripheral nervous system and has been established to be important in cognitive function and neural adaptation (Mattay, Goldberg, Sambataro, & Weinberger, 2008; Pezawas, Verchinski, Mattay, Callicott, Kolachana et al., 2004). BDNF plays a central role in long-term potentiation, the protection of neurons, synaptogenesis and neurogenesis, especially in the hippocampus (Alsina, Vu, & Cohen-Cory, 2001; Hung & Lee, 1996; Lewin & Barde, 1996; Pang & Lu, 2004). Interestingly, low-dose organophosphate exposure has been shown to negatively impact the expression of neurotrophins like BDNF (Slotkin, Seidler, & Fumagalli, 2008). This effect of neurotoxin exposure on BDNF expression in conjunction with variations in BNDF expression based on the polymorphisms present in individuals, could have resulted in different levels of impact for GWV.

Future studies investigating exposure rates and genetic factors could provide additional insight and validation to GWI. More importantly, the knowledge obtained from such studies could help to demonstrate the legitimacy of the veterans' complaints, and may provide a better understanding of their condition and offer knowledge that will aid in formulating a treatment for veterans with GWI and the related memory and cognitive deficits. As for the current study, the memory and neurobiological data help to provide validation that ill GWV are suffering from an illness not explained by PTSD, MDD, or age with some ill GWV groups being more impaired than others.

REFERENCES

Abdel-Rahman, A., et al. (2004). Stress and combined exposure to low doses of pyridostigmine bromide, DEET, and permethrin produce neurochemical and neuropathological alterations in cerebral cortex, hippocampus, and cerebellum. *Journal of Toxicology and Environmental Health*, *67*, 163-92.

Abdel-Rahman A, Dechkovskaia AM, Goldstein LB, Bullman SH, Khan W, El-Masry EM, Abou-Donia MB. (2004). Neurological deficits induced by malathion, DEET, and permethrin, alone or in combination in adult rats. *Journal of Toxicology and Environmental Health*, *67*, 331-56.

Abdel-Rahman, A., Shetty, A.K., & M.B. Abou-Donia (2002). Disruption of the blood-brain barrier and neuronal cell death in cingulate cortex, dentate gyrus, thalamus, and hypothalamus in a rat model of Gulf-War syndrome. *Neurobiology of Disease*, *10*, 306-26.

Achim, A.M., & Lepage, M. (2005). Neural correlates of memory for items and for associations: An event-related functional magnetic resonance imaging study. *Journal of Cognitive Neuroscience*, *17*, 652-67.

Addis, D.R., & McAndrews, M.P. (2006). Prefrontal and hippocampal contributions to the generation and binding of semantic associations during successful encoding. *NeuroImage*, *33*, 1194-1206.

Alsina, B., Vu, T., & Cohen-Cory, S. (2001). Visualizing synapse formation in arborizing optic axons in vivo: dynamics and modulation by BDNF. *Nature Neuroscience*, *4*, 1093-1101.

American Psychiatric Association. (2000). Diagnostic and statistical manual of mental disorders (4th ed., text rev.). Washington, DC: Author.

Asthana, S., Greig, N.H., Hegedus, L., Holloway, H.H., Raffaele, K.C., Schapiro, M.B., & Soncrant, T.T. (1995). Clinical pharmacokinetics of physostigmine in patients with Alzheimer's disease. *Clinical Pharmacology and Therapeutics*, *58*, 299-309.

Astur, R.S., & Constable, R.T. (2004). Hippocampal dampening during a relational memory task. *Behavioral Neuroscience, 118,* 667-675.Bajgar, J. (2004). Organophosphates/nerve agent poisoning: mechanism of action, diagnosis, prophylaxis, and treatment. *Advances in Clinical Chemistry*, *38*, 151-216.

Barker, P.B. (2001): N-acetylaspartate – neuronal marker? *Annals of Neurology*, *4,* 423–424.

Bastin, C., Feyers, D., Majerus, S., Balteau, E., Degueldre, C., Luxen, A., Maquet, P., Salmon, E., Collette, F. (2012). The neural substrates of memory suppression: a FMRI exploration of directed forgetting. *PLoS One*, 7(1):e29905.

Bendszus, M., Weijers, H.G., Wiesbeck, G., Warmuth-Metz, M., Bartsch, A.J., Engels, S., Boning, J., & Solymosi, L. (2001).Sequential MR imaging and proton MR spectroscopy in patients who underwent recent detoxification for chronic alcoholism: correlation with clinical and neuropsychological data. *American Journal of Neuroradiology, 22,* 1926-1932.

Binns, J.H., Barlow, C., Bloom, F.E., Clauw, D.J., Golomb, B.A., Graves, J.C…White, R.F. (2008). Gulf War Illness and the Health of Gulf War Veterans: Scientific Findings and Recommendations. Research Advisory Committee on Gulf War Veterans' Illnesses, U.S. Department of Veteran Affairs. Washington, D.C.: U.S. Government Printing Office.

Blake, D. D., Weathers, F. W., Nagy, L. M., Kaloupek, D. G., Gusman, F. D., Charney, D. S., & Keane, T. M. (1995). The development of a clinician-administered PTSD scale. *Journal of Traumatic Stress*, *8*, 75-90.

Bokde, A. L. W., Tagamets, M., A., Friedman, R.B., & Horwitz, B. (2001). Functional interactions of the inferior frontal cortex during the processing of words and word-like stimuli. *Neuron*, 30, 609-617.

Bonne, O., Brandes, D., Gilboa, A., Gomori, J.M., Shenton,, M.E., Pitman, R.K., & Shalev, A.Y. (2001). Longitudinal MRI study of hippocampal volume in trauma survivors with PTSD. *American Journal of Psychiatry, 15,* 1248-1251.

Bonne, O., Vythillingam, M., Inagaki, M., Wood, S., Neumeister, A., Nugent, A.C…Charney, D.S. (2008). Reduced posterior hippocampal volume in posttraumatic stress disorder. *Journal of Clinical Psychiatry, 69*, 1087-1091.

Bremner, J.D., Krystal, J.H., Southwick, S.M., & Charney, D.S. (1995). Functional neuroanatomical correlates of the effects of stress on memory. *Journal of Traumatic Stress, 8,* 527-553.

Bremner, J.D., Randall, P., Scott, T.M., Bronen, R.A., Seibyl, J.P.Southwick, S.M…Innis, R.B. (1995). MRI-based measurement of hippocampal volume in patients with combat-related posttraumatic stress disorder. *American Journal of Psychiatry, 152,* 973-981.

Brown, M.W., & Aggleton, J.P. (2001). Recognition memory: What are the roles of the perirhinal cortex and hippocampus? *Nature Reviews Neuroscience*, *2*, 51-61.

Brown M.A., & Brix K.A. (1998). Review of health consequences from high-, intermediate- and low-level exposure to organophosphorus nerve agents. *Journal of Applied Toxicology*, *18*, 393–408.

Buckner, R.L., Kelley, W.M., & Petersen, S.E. (1999). Frontal cortex contributes to human memory formation. *Nature Neuroscience*, *2*, 311-314.

Burt, D.B., Zembar, M.J., & Niederehe, G. (1995). Depression and memory impairment: a meta-analysis of the association, its pattern, and specificity. *Psychological Bulletin, 117*, 285-305.

Calley, C.S., Kraut, M.A., Spence, J.S., Briggs, R.W., Haley, R.W., & Hart, J. (2010). The neuroanatomic correlates of semantic memory deficits in patients with Gulf War Illness: a pilot study. *Brain Imaging and Behavior*, *4*, 248-255.

Camps, J., Marsillach, J., Joven, J. (2009). The paraoxonases: role in human diseases and methodological difficulties in measurement. *Critical Reviews in Clinical Laboratory Sciences*, *46*, 83-106.

Caserta, M.T., Ragin, A., Hermida, A.P., Ahrens, R.J., & Wise, L. (2008). Single voxel magnetic resonance spectroscopy at 3 Tesla in a memory disorders clinic: early right hippocampal NAA/Cr loss in mildly impaired subjects. *Psychiatry Research*, *164*, 154-159.

Centers for Disease Control and Prevention (1995). Unexplained illness among Persian Gulf War veterans in an Air National Guard unit: preliminary report—August 1990-March 1995. *MMWR Morb Mortal Wkly Rep, 44,* 443-447.

Chao, L.L., Abadjian, L., Hlavin, J., Meyerhoff, D.J., & Weiner, M.W. (2011). Effects of low-level sarin and cyclosarin exposure and Gulf War Illness on brain structure and function: A study at 4T. *NeuroToxicology*, *32*, 814-822.

Chao, L.L., Rothlind, J.C., Cardenas, V.A., Meyerhoff, D.J., & Weiner, M.W. (2010). Effects of low-level exposure to sarin and cyclosarin during the 1991 Gulf War on brain function and brain structure in US veterans. *NeuroToxicology*, *31*, 493-501.

Cheshkov, S., et al. (2007). Single Voxel Spectroscopy of the Pons: Shimming, Quantization, and Reproducibility Issues at 3T. *Proceedings of the International Society of Magnetic Resonance Medicine, 15,* 1357.

Cheshkov, S., et al. (2009). Basal Ganglia NAA/Cr ratio in Gulf War Syndrome at 3T, *Proceedings of the International Society of Magnetic Resonance Medicine, 17,* 1126.

Chua, E.F., Schachter, D.L., Rand-Giovannetti, E., & Sperling, R.A. (2007). Evidence for a specific role of the anterior hippocampal region in successful associative encoding. *Hippocampus, 17*, 1071-080.

Cohen, J. (1992). A power primer. *Psychological Bulletin: Quantitative Methods in Psychology, 112,* 155-159.

Colla, M., Kronenberg, G., Deuschle, M., Meichel, K., Hagen ,T., Bohrer, M., Heuser, I. (2007). Hippocampal volume reduction and HPA-system activity in major depression. *Journal of Psychiatric Research, 41*, 553-60.

Connelly, A., Jackson, G.D., Duncan, J.S., King, M.D., & Gadian, D.G. (1994). Magnetic resonance spectroscopy in temporal lobe epilepsy. *Neurology*, *44*, 1411-1417.

Cox R. 1996. AFNI: Software for analysis and visualization of functional magnetic resonance neuroimages. Computers and Biomedical Research, 29:162-173.

David, A.S., Farrin, L., Hull, L., Unwin, C., Wessely, S., & Wkyes, T. (2002). Cognitive functioning and disturbances of mood in UK veterans of the Persian Gulf War: a comparative study. *Psychological Medicine, 32*, 1357-1370.

Davachi L. (2006). Item, context and relational episodic encoding in humans. *Current Opinion in Neurobiology, 16,* 693-700.

Davachi, L., & Wagner, A.D. (2002). Hippocampal contributions to episodic encoding: Insights from relational and item-based learning. *Journal of Neurophysiology, 88,* 982-990.

Diana, R.A., Yonelinas, A.P., & Ranganath, C. (2007). Imaging recollection and familiarity in the medial temporal lobe: a three-component model. *Trends in Cognitive Sciences, 11*, 379-386.

Douglas, K.M., & Porter, R.J. (2009). Longitudinal assessment of neuropsychological function in major depression. *Australian and New Zealand Journal of Psychiatry*, *43*, 1105-1117.

Draganov, D.I., La Du, B.N. (2004). Pharmacogenetics of paraoxonases: a brief review. *Naunyn Schmiedebergs Arch Pharmacol*, 369(1):78-88.

Driscoll, I., Hamilton, D.A., Petropoulos, H., Yeo, R.A., Brooks, W.M., Baumgartner, R.N., & Sutherland, R.J. (2003). The aging hippocampus: cognitive, biochemical and structural findings. *Cerebral Cortex*, *13*, 1344-1351.

Duan, D.M., Tu, Y., Jiao, S., & Qin, W. (2011). The relevance between symptoms and magnetic resonance imaging analysis of the hippocampus of depressed patients given electro-acupuncture combined with Fluoxetine intervention - A randomized, controlled trial. *Chinese Journal of Integrative Medicine*, *17*, 190-199.

Durasso, T.C., Gazdzinski, S., Rothlind, J.C., Banys, P., & Meyerhoff, D. (2006). Brain metabolite concentrations and neurocognition during short-term recovery from alcohol dependence: Preliminary evidence of the effects of concurrent chronic cigarette smoking. Alcoholism, *Clinical and Experimental Research, 30,* 539-551.

Eichenbaum, H., Schoenbaum, G., Young, B., & Bunsey, M. (1996). Functional organization of the hippocampal memory system. *Proceedings of the National Academy of Sciences*, *93*, 13500-13507.

Eichenbaum, H., Yonelinas, A.R., & Ranganath, C. (2007). The medial temporal lobe and recognition memory. *Annual Review of Neuroscience*, *30*, 123-152.

Fairhall, S.L., Sharma, S., Magnusson, J., & Murphy, B. (2010). Memory related dysfunction of hippocampal functions in major depressive disorder. *Biological Psychology, 85,* 499-503.

Fennema-Notestine, C., Stein, M.B., Kennedy, C.M., Archibald, S.L., Jernigan, T.L. (2002). Brain morphometry in female victims of intimate partner violence with and without posttraumatic stress disorder. *Biological Psychiatry,* 52(11):1089-101.

Feyers, D., Majerus, S., Balteau, E., Degueldre, C., Luxen, A., Maquet, P., Salmon, E., Collette, F. (2012). The neural substrates of memory suppression: a FMRI exploration of directed forgetting. *PLoS One*, 7(1):e29905.

First, M.B., Spitzer, R.L., Gibbon M., & Williams, J.B.W. (1996). Structured Clinical Interview for DSM-IV Axis I Disorders, Clinician Version (SCID-CV). Washington, D.C.: American Psychiatric Press, Inc.

Forman-Hoffman, V.L., Carney, C.P., Sampson, T.R., Peloso, P.M., Woolson, R.F., Black, D.W., & Doebbeling, B.N. (2005). Mental health comorbidity patterns and impact on quality of life among veterans serving during the first Gulf War. *Quality of Life Research*, *14*, 2303-2314.

Freeman, T.W., Cardwell, D., Karson, C.N., Komoroski, R.A. (1998). In vivo proton magnetic resonance spectroscopy of the medial temporal lobes of subjects with combat-related posttraumatic stress disorder. *Magnetic Resonance in Medicine*, 40(1):66-71.

Friedland, R.P., et al. (2001). Patients with Alzheimer's disease have reduced activities in midlife compared with healthy control-group members. *Proceedings of the National Academy of Sciences USA, 98,* 3440-3445.

Fukuda, K., Nisenbaum, R., Stewart, G., Thompson, W.W., Robin, L., Washko, R.M., Noah, D.L., Barret, D.H., Randall, B., Herwaldt, B.L., Mawle, A.C., Reeves, W.C. (1998). Chronic multsynmptom illness affecting Air Force veterans of the Gulf War. *Journal of the American Medical Association, 280,* 981-988.

Gardner, R., Ray, R., Frankenheim, J., Wallace, K., Loss, M., Robichaud, R., 1984. A possible mechanism for DFP induced memory loss in rats. *Pharmacology Biochemistry and Behavior, 21,* 43-46.

Geuze, E., Vermetten, E., Ruf, M., de Kloet, C.S., & Westenberg, H.G. (2008). Neural correlates of associative learning and memory in veterans with posttraumatic stress disorder. *Journal of Psychiatric Research*, *42*, 659-669.

Gilbertson, M.W., Shenton, M.E., Ciszewski, A., Kasai, K., Lasko, NB., Orr, S.P., Pitman, R.K. (2002). Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma. *Nature Neuroscience, 5*, 1242-7.

Giovanello, K.S., Schnyer, D., Verfaellie, M. (2004). A critical role for the anterior hippocampus in relational memory; Evidence from an fMRI study comparing associative and item recognition. *Hippocampus, 14,* 5-8.

Giovanello, K.S., Schnyer, D., Verfaellie, M. (2009). Distinct hippocampal regions make unique contributions to relational memory. *Hippocampus, 19,* 111-117.

Golier, J.A., Yehuda, R., De Santi, S., Segal, S., Dolan, S., de Leon, M.J. (2005). Absence of hippocampal volume differences in survivors of the Nazi Holocaust with and without posttraumatic stress disorder. *Psychiatry Research*, 139(1):53-64.

Golomb, B.A. (2008). Acetycholinesterase inhibitors and Gulf War illness. *Proceedings of the National Academies of Science, 105,* 4295-4300.

Gonsalves, B.D., Kahn, I., Curran, T., Norman, K.A., & Wagner, A.D. (2005). Memory strength and repetition suppression: Multimodal imaging of medial temporal cortical contributions to recognition. *Neuron, 47,* 751-761.

Graham, G.D., Petroff, O.A., Blamire, A.M., Rajkowska, G., Goldman-Rakic, P., & Prichard, J.W. (1993). Proton magnetic resonance spectroscopy in Creutzfeldt-Jakob disease. *Neurology*, *43*, 2065-2068.

Guedj, E., Bettus, G., Barbeau, E.J., Liégeois-Chauvel, C., Confort-Gouny, S., Bartolomei, F., Chauvel, P., Cozzone, P.J., Ranjeva, J.P., Guye, M. (2011). Hyperactivation of parahippocampal region and fusiform gyrus associated with successful encoding in medial temporal lobe epilepsy. *Epilepsia, 52,* 1100-9.

Gurvits, T.V., Shenton, M.E., Hokama, H., Ohta, H., Lasko, N.B., Gilbertson, M.W., Orr, S.P., Kikinis, R., Jolesz, F.A., McCarley, R.W., Pitman, R.K. (1996). Magnetic resonance imaging study of hippocampal volume in chronic, combat-related posttraumatic stress disorder. *Biological Psychiatry, 40*, 1091-9.

Haley, R.W., Fleckenstein, J.L., Marshall, W., McDonald, G.G., Kramer, G.L., & Petty, F. (2000). Effect of basal ganglia injury on central dopamine activity in Gulf War Syndrome. *Archives Neurology, 57*, 1280-1285.

Haley, R.W., & Kurt, T.L. (1997). Self-reported exposure to neurotoxic chemical combinations in the GulfWar: A cross-sectional epidemiologic study. *Journal of the American Medical Association, 277,* 231-237.

Haley R.W., Kurt T.L., & Hom, J. (1997). Is there a Gulf War syndrome? searching for syndromes by factor analysis of symptoms. *Journal of the American Medical Association, 277*, 215-222.

Haley, R.W., Luck, G.D., & Petty, F. (2001). Use of structural equation modeling to test the construct validity of a case definition of Gulf War Syndrome: Invariance over developmental and validation samples, service branches and publicity. *Psychiatry Research, 102*, 175-200.

Haley, R.W., Marshall, W.W., McDonald, G.G., Daugherty, M.A., Petty, F., & Fleckenstein, J.L. (2000). Brain abnormalities in Gulf War Syndrome: Evaluation with H MR Spectroscopy. *Neuroradiology, 215*, 807-817.

Haley, R.W., Spense, J.S., Carmack, P.S., Gunst, R.F., Schucany, W.R., Pety, F., Devous, M.D., Bonte, F.J., & Trivedi, M.H. (2009). Abnormal brain response to cholinergic challenge in chronic encephalopathy from the 1991 Gulf War. Psychiatry Research *Neuroimaging, 171,* 207-220.

Hamilton, J.P., Gotlib, I.H. (2008). Neural substrates of increased memory sensitivity for negative stimuli in major depression. *Biological Psychiatry*, *63*, 1155-62.

Hedges, D.W., Allen, S., Tate, D.F., Thatcher, G.W., Miller, M.J., Rice, S.A…Bigler, E.D. (2003). Reduced hippocampal volume in alcohol and substance naiive Vietnam combat veterans with posttraumatic stress disorder. *Cognitive and Behavioral Neurology, 16*, 219-224.

Henderson, R.F., Barr, E.B., Blackwell, W.B., Clark, C.R., Conn, C.A., Kalra, R…Langley, R.J. (2001). Response of F344 rats to inhalation of subclinical levels of sarin: exploring potential causes of Gulf War illness. *Toxicology and Industrial Health, 17,* 294-297

Henke, K., Buck, A., Weber, B., & Wieser, H.G. (1997). Human hippocampus establishes associations in memory. *Hippocampus*, *7*, 249-256.

Henke, K., Weber, B., Kneifel, S., Wieser, H.G., & Buck, A. (1999). Human hippocampus associates information in memory. *Proceedings of the National Academy of Sciences*, *96*, 5884-5889.

Hoge, C.W., McGurk D, Thomas, J.L., Cox, A.L., Engel, C.C., Castro, C.A. (2008). Mild traumatic brain injury in U.S. Soldiers returning from Iraq. N*ational England Journal of Medicine, 358,* 453-463.

Hom, J., Haley, R.W., & Kurt, T.L. (1997). Neuropsychological correlates of Gulf War Syndrome. *Archives of Clinical Neuropsychology, 12,* 531-544.

Horn, O., Hull, L., Jones, M., et al. (2006). Is there an Iraq war syndrome? Comparison of the health of UK service personnel after the Gulf and Iraq wars. *Lancet, 367,*1742-1746.

Hotopf, M., David, A., Hull, L., Ismail, K., Unwin, C., Wessely, S. (2003). The health effects of peacekeeping (Bosnia,1992-1996): a cross-sectional study--comparison with nondeployed military personnel. *Military Medicine, 168,* 408-413.

Hulst, H.E., Schoonheim, M.M., Roosendaal, S.D., Popescu, V., Schweren, L.J., van der Werf, Y.D., Visser, L.H., Polman, C.H., Barkhof, F., Geurts, J.J. (2011). Functional adaptive changes within the hippocampal memory system of patients with multiple sclerosis. *Human Brain Mapping*, doi: 10.1002/hbm.21359. [Epub ahead of print]

Hung, H., & Lee, E.H.Y. (1996). The mesolimbic dopaminergic pathway is more resistant than the nigrostriatal dopaminergic pathway to MPTP and MPP+ toxicity: role of BDNF gene expression. *Molecular Brain Research*, *41*, 16-26.

Hunt, S.C., Jakupcak, M., McFall, M., et al. (2006). Re: "Chronic multisymptom illness complex in Gulf War I veterans 10 years later". *American Journal of Epidemiology, 164*, 708-709; author reply 709-710.

Iannacchione, V.G., Dever, J.A., Bann, C.M., Considine, K.A., Creel, D., Carson, C.P. Best, H., Haley, R.W. (2011). Validation of a research case definition of Gulf War Illness in the 1991 U.S. military population. *Neuroepidemiology 37,* 129-140.

Iowa Persian Gulf Study Group (1997). Self-reported illness and health status among Gulf War veterans: a population-based study. *Journal of the American Medical Association, 277,* 238-245.

Jackson, O. 3rd., & Schacter, D.L. (2004). Encoding activity in anterior medial temporal lobe supports subsequent associative recognition. *Neuroimage*, *21*, 456-462.

Jamal, G.A., Hansen, S., & Julu, P.O., (2002). Low level exposure to organophosphorous esters may cause neurotoxicity. *Toxicology, 181-182,* 23-33.

Jiang, Y., Long, L., Zhu, X., Zheng, H., Fu, X., Ou, S…Zheng, W. (2009). Evidence for Altered Hippocampal Volume and Brain Metabolites in Workers Occupationally Exposed to Lead: A Study by Magnetic Resonance Imaging and 1H Magnetic Resonance Spectroscopy. *Toxicology Letters*, *181*, 118-125.

Kado, H., Kimura, H., Murata, T., Nagata, K., & Kanno, I. (2006). Depressive psychosis: clinical usefulness of MR spectroscopy data in predicting prognosis. *Radiology*, *238*, 248-255.

Ke, Y., Cohen, B.M., Lowen, S., Hirashima, F., Nassar, L., Renshaw, P.F. (2002). Biexponential transverse relaxation (T2) of the proton MRS creatine resonance in human brain. *Magnetic Resonance in Medicine, 47,* 232-238.

Kirwan, C.B., & Stark, C.E. (2004). Medial temporal lobe activation during encoding and retrieval of novel face-name pairs. *Hippocampus*, *14*, 919-930.

Kobayashi, M., Takayama, H., Suga, S., & Mihara, B. (2001). Longitudinal changes of metabolites in frontal lobes after hemorrhagic stroke of basal ganglia: a proton magnetic resonance spectroscopy study. *Stroke*, *32*, 2237-2245.

Koechlin, E., Ody, C. l., & Kouneiher, F. d. r. (2003). The Architecture of Cognitive Control in the Human Prefrontal Cortex. *Science, 302*, 1181-1185.

Kwo-On-Yuen, P.F., Newmark, R.D., Budinger, T.F., Kaye, J.A., Ball, M.J., & Jagust, W.J. (1994). Brain N-acetyl-L-aspartic acid in Alzheimer's disease: a proton magnetic resonance spectroscopy study. *Brain Research*, *667*, 167-174.

Lamproglou, I., Barbier, L., Diserbo, M., Fauvelle, F., Fauquette, W., & Amourette, C. (2009). Repeated stress in combination with pyridostigmein Part I: Long-term behavioural consequences. *Behavioural Brain Research, 197,* 301-310.

Lewin, G.R., & Barde, Y.A. (1996). Physiology of the neurotrophins. *Annual Review of Neuroscience*, *19*, 289-317.

Li, X., Spence, J.S., Buhner, D.M., Haley, R.W., & Briggs, R.W. (2012). Dynamic physiostigmine effecys on hippocampus perfusion. *Journal of Magnetic Resonance Imaging*, *35*, 280-286.

Li, X., Spence, J.S., Buhner, D.M., Hart, J., Cullum, C.M., Biggs, M.M., Hester, A., Odegard, T.N., Carmack, P.S., Briggs, R.W., & Haley, R.W. (2011). Hippocampal Dysfunction in Gulf War Veterans: Investigation with ASL Perfusion Imaging and Physostigmine Challenge. *Radiology, 261*, 218-225.

Liu, P.I., Aslan, S., Li, X., Buhner, D.M., Briggs, R.W., Haley, R.W., and Lu, H. (2011). Perfusion deficit to cholinergic challenge in veterans with Gulf War Illness. *Neurotoxicology 32,* 242-246.

Mattay, V.S., Goldberg, T.E., Sambataro, F., & Weinberger, D.R. (2008). Neurobiology of cognitive aging: Insights from imaging genetics. *Biological Psychology*, *79*, 9-22.

Mayes, A., Montaldi, D., & Migo, E. (2007). Associative memory and the medial temporal lobes. *Trends in Cognitive Sciences, 11,* 126-135.

Meltzer, J.A., Negishi, M., & Constable, R.T. (2008). Biphasic hemodynamic responses influence deactivation and may mask activation in block-design fMRI paradigms. *Human Brain Mapping, 29,* 385-399.

Menon, M. et al. (2004). Hippocampal dysfunction in Gulf War Syndrome. A proton MR spectroscopy study. *Brain Research, 1009*, 189-194.

Meyerhoff, D.J. and Durazzo, T.C. (2008). Proton magnetic resonance spectroscopy in alcohol use disorders: a potential new endophenotype? *Alcoholism: Clinical and Experimental Research*, *32*, 1146-1158.

Miller, E. K. (1999). The Prefrontal Cortex: Complex Neural Properties for Complex Behavior. *Neuron, 22*, 15-17.

Miller, E. K., & Cohen, J. D. (2001). An integrative theory of prefrontal cortex function. *Annual Review of Neuroscience, 24*, 167-202.

Mohanakrishnan Menon, P., Nasrallah, H.A., Lyons, J.A., Scott, M.F., Liberto, V. (2003). Single-voxel proton MR spectroscopy of right versus left hippocampi in PTSD. *Psychiatry Research*, *123,* 101-8.

Murphy, D., Hooper, R., French, C., Jones, M., Rona, R., & Wessley, S. (2006). Is the increased reporting of symptomatic ill health in Gulf War veterans related to how one asks the question? *Journal of Psychosomatic Research, 61*, 181-186.

National Institute of Health Technology Assessment Workshop Panel. The Persian Gulf Experience and Health: NIH Technology Assessment Workshop Statement. National Institutes of Health; Apr 27 29, 1994.

Odegard, T.N., Cooper, C.M., Farris, E.A., Arduengo, J., Bartlett, J., Haley, R. (in press). Memory impairment exhibited by veterans with Gulf War Illness. *Neurocase*.

Okumura T, Takasu N, Ishimatsu S, Miyanoki S, Mitsuhashi A, Kumada K, et al. (1996). Report on 640 victims of the Tokyo subway sarin attack. *Annals of Emergency Medicine, 28,* 129–35.

Packard, M.G. (2009). Anxiety, cognition, and habit: A multiple memory systems perspective. *Brain Research*, *1293*, 121-128.

Pang, P.T., & Lu, B. (2004). Regulation of late-phase LTP and long-term memory in normal and aging hippocampus: role of secreted proteins tPA and BDNF. *Ageing Research Reviews*, *3*, 407-430.

Pezawas, L., Verchinski, B. A., Mattay, V. S., Callicott, J. H., Kolachana, B. S., Straub, R. E., Egan, M.F., Meyer-Lindenberg, A., Weinberger, D. R. (2004). The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. *The Journal of Neuroscience*, *24*, 10099-10102.

Piekema, C., Kessels, R.P.C., Rijpkema, M., & Fernandez, G. (2009). The hippocampus supports encoding of between-domain associations within working memory. *Learning & Memory, 16,* 231-234.

Pohanka, M. (2011). Cholinesterases, a target of pharmacology and toxicology. *Biomedical Papers of the Medical Faculty of the University Palacky, Olomouc, Czeckoslovakia*, *155*, 219-230.

Preul, M.C., Caramanos, Z., Collins, D.L., Villemure, J.G., Leblanc, R., Oliver, A…Arnold, D.L. (1996). Accurate, noninvasive diagnosis of human brain tumors by using proton magnetic resonance spectroscopy. *Nature Medicine*, *2*, 323-325.

Proctor, S.P., Heaton, K.J., Heeren, T., White, R.F. (2006). Effects of sarin and cyclosarin exposure during the 1991 Gulf War on neurobehavioral functioning in US army veterans. *Neurotoxicology*, 27(6):931-9.

Ranganath, C., Yonelinas, A.P., Cohen, M.X., Dy, C.J., Tom, S.M., & D'Esposito, M. (2004). Dissociable correlates of recollection and familiarity within the medial temporal lobes. *Neuropsychologia, 42,* 2-13.

Robinson-Long, M., Eslinger, P.J., Wang, J., Meadowcroft, M., & Yang, Q.X. (2009). Functional MRI evidence for distinctive binding and consolidation pathways for face-name associations: analysis of activation maps and BOLD response amplitudes. *Topics in Magnetic Resonance Imaging, 20,* 271-278.

Saeed, M., Siddique, N., Hung, W.Y., Usacheva, E., Liu, E., Sufit, R.L., Heller, S.L., Haines, J.L., Pericak-Vance, M., Siddique, T. (2006). Paraoxonase cluster polymorphisms are associated with sporadic ALS. *Neurology*, *67*, 771-776.

Samuelson, K.W. (2011). Post-traumatic stress disorder and declarative memory functioning: A review. *Dialogues in Clinical Neuroscience*, *13*, 346-351.

Sander, D., Grafman, J., & Zalla, T. (2003). The human amygdala: An evolved system for relevance detection. *Reviews in the Neurosciences, 14,* 303-316.

Sapolsky, R.M. (2000). The possibility of neurotoxicity in the hippocampus in major depression: a primer on neuron death. *Biological Psychiatry*, *48,* 755-65.

Schacter, D.L., & Wagner, A.D. (1999). Medial temporal lobe activations in fMRI and PET studies of episodic encoding and retrieval. *Hippocampus*, *9*, 7-24.

Shalev, A.Y., Freedman, S., Peri, T., Brandes, D., Sahar, T., Orr, S.P., Pitman, R.K. (1998). Prospective study of posttraumatic stress disorder and depression following trauma. *American Journal of Psychiatry*, 155(5):630-7.

Schneider, W., Eschman, A., & Zuccolotto, A. (2002). E-Prime User's Guide. Pittsburgh: Psychology Software Tools, Inc.

Schuff, N., Amend, D.L., Knowlton, R., Norman, D., Fein, G., & Weiner, M.W. (1999). Age-related metabolite changes and volume loss in the hippocampus by magnetic resonance spectroscopy and imaging. *Neurobiology of Aging, 20,* 279-285.

Schuff, N., Neylan, T.C., Lenoci, M.A., Du, A.T., Weiss, D.S., Marmar, C.R., Weiner, M.W. (2001). Decreased hippocampal N-acetylaspartate in the absence of atrophy in posttraumatic stress disorder. *Biological Psychiatry*, *50*, 952-9.

Schuff, N., Neylan, T.C., Fox-Bosetti, S., Lenoci, M., Samuelson, K.W., Studholme, C…Weiner, M.W. (2008). Abnormal N-acetylaspartate in hippocampus and anterior cingulate in posttraumatic stress disorder. *Psychiatry Research*, *162*, 147-157.

Sheldon, S., & Moscovitch, M. (2011). The nature and time-course of medial temporal lobe contributions to semantic retrieval: An fMRI study on verbal fluency. *Hippocampus*. DOI: 10.1002/hipo.20985.

Shiino, A., Watanabe, T., Shirakashi, Y., Kotani, E., Yoshimura, M., Morikawa, S…Akiguchi, I. (2012). The profile of hippocampal metabolites differs between Alzheimer's disease and subcortical ischemic vascular dementia, as measured by proton magnetic resonance spectroscopy. *Journal of Cerebral Blood Flow and Metabolism*. doi: 10.1038/jcbfm.2012.9.

Slotkin, T.A., Seidler, F.J., Fumagalli, F. (2008). Targeting of neurotrophic factors, their receptors, and signaling pathways in the developmental neurotoxicity of organophosphates in vivo and in vitro. *Brain Research Bulletin*, *76*, 424-38.

Snodgrass, J.G. & Corwin, J. (1988) Pragmatics of measuring recognition memory: applications to dementia and amnesia. Journal of Experimental Psychology: General, *117*, 34-50.

Speed, H.E., Blaiss, C.A., Kim, A., Haws, M.E., Melvin, N.R., Jennings, M., Eisch, A.J., & Powell, C.M. (in press). Delayed reduction of hippocampal synaptic transmission and spines following exposure to repeated, subclinical doses of organohosphorous pesticide in adult mice. *Toxicological Sciences*.

Sperling, R.A., Bates, J.F., Cocchiarella, A.J., Schacter, D.L., Rosen, B.R., & Albert, M.S. (2001). Encoding novel face-name associations: A functional MRI study*. Human Brain Mapping, 14,* 129-139.

Sperling, R., Chua, E., Cocchiarella, A., Rand-Giovannetti, E., Poldrack, R., Schacter, D.L. et al. (2003). Putting names to faces: Successful encoding of associative memories activates the anterior hippocampal formation. *NeuroImage, 20*, 1400-1410.

Sperling, R.A., Dickerson, B.C., Pihlajamaki, M., Vannini, P., LaViolette, P.S., Vitolo, O.V., Hedden, T., Becker, J.A., Rentz, D.M., Selkoe, D.J., Johnson, K.A. (2010). Functional alterations in memory networks in early Alzheimer's disease. *Neuromolecular Med*, 12(1):27-43.

SPSS Inc. (2010). SPSS Base 19.0 for Mac User's Guide. SPSS Inc., Chicago IL.

Staresina, B.P. & Davachi, L. (2008). Selective and shared contributions of the hippocampus and perirhinal cortex to episodic item and associative encoding. *Journal of Cognitive Neuroscience, 17,* 112-118.

Stark, C.E., & Squire, L.R. (2001). Simple and associative recognition memory in the hippocampal region. *Learning & Memory*, *8*, 190-197.

Steele L. (2000). Prevalence and patterns of Gulf War illness in Kansas veterans: Association of symptoms with characteristics of person, place, and time of military service. *American Journal of Epidemiology, 152,* 992–1002.

Stimpson, N.J., Thomas, H.V., Weightman, A.L., Dunstan, F., & Lewis, G. (2003). Psychiatric disorders in veterans of the Persian Gulf War of 1991. Systematic Review. *British Journal of Psychiatry, 182,* 391-403.

Talairach J, Tournoux P. 1988. Co-planar stereotaxic atlas of the human brain. Stuttgart, New York: Thieme Medical Publishers, Inc.

Toomey, R., Kang, H.K., Karlinsky, J., Baker, D.G., Vasterling, J.J., Alpem, R…Eisen, S.A. (2007). Mental health of US Gulf War veterans 10 years after the war. *British Journal of Psychiatry*, *190*, 385-393.

Träber, F., Block, W., Freymann, N., Gür, O., Kucinski, T., Hammen, T., Ende, G., Pilatus, U., Hampel, H., Schild, H.H., Heun, R., Jessen, F. (2006). A multicenter reproducibility

study of single-voxel 1H-MRS of the medial temporal lobe. *European Radiology*, 16(5):1096-103.

Urenjak, J., Williams, S. R., Gadian, D. G. & Noble, M. 1993 Proton nuclear magnetic resonance spectroscopy unam- biguously identi¢es di¡erent neural cell types. *Journal of Neuroscience*, *13*, 981-989.

Villarreal, G., Petropoulos, H., Hamilton, D.A., Rowland, L.M., Horan, W.P., Griego, J.A., Moreshead, M., Hart, B.L., Brooks, W.M. (2002). Proton magnetic resonance spectroscopy of the hippocampus and occipital white matter in PTSD: preliminary results. *Canada Journal of Psychiatry*, *47*, 666-70.

Waldman, A.D., Rai, G.S. (2003) The relationship between cognitive impairment and in vivo metabolite ratios in patients with clinical Alzheimer's disease and vascular dementia: a proton magneticresonance spectroscopy study. *Neuroradiology, 45*, 507-12.

Weathers, F.W., Keane, T.M., & Davidson, J.R. (2001). Clinician-administered PTSD scale: A review of the first ten years of research. *Depression & Anxiety*, *13*, 132-156.

Weis, S., Leube, D., Erb, M., Heun, R., Grodd, W., Kircher, T. (2011). Functional neuroanatomy of sustained memory encoding performance in healthy aging and in Alzheimer's disease. *International Journal of Neuroscience*, *121*, 384-92.

Werner, N.S., Meindl, T., Engel, R.R., Rosner, R., Riedel, M., Reiser, M., & Fast, K. (2009a). Hippocampal function during associative learning in patients with posttraumatic stress disorder. *Journal of Psychiatric Research*, *43*, 309-318.

Werner, Meindl, T., Materne, J., Engel, R.R., Huber, D., Riedel, M…Hennig-Fast, K. (2009b). Functional MRI study of memory-related brain regions in patients with depressive disorder. *Journal of Affective Disorders*, *119*, 124-131.

Westerberg, C.E., Voss, J.L., Reber, P.J., & Paller, K.A. (2011). Medial temporal contributions to successful face-name learning. *Human Brain Mapping*. DOI: 10.1002/hbm.21316.

White RF, Proctor SP, Heeren T, Wolfe J, Krengel M, Vasterling J, et al. (2001). Neuropsychological function in Gulf War veterans: relationships to self-reported toxicant exposures. *American Journal of Industrial Medicine, 40,* 42–54.

Wright, P., & Liu, Y. (2006). Neutral faces activate the amygdala during identity matching. *NeuroImage, 29,* 628-636.

Yehuda, R., Harvey, P.D., Buchsbaum, M., Tischler, L., Schmeidler, J. (2007). Enhanced effects of cortisol administration on episodic and working memory in aging veterans with PTSD. *Neuropsychopharmacology*, *32*, 2581-91.

Yokoyama K, Araki S, Murata K, Nishikitani M, Okumura T, Ishimatsu S, et al. (1998). Chronic neurobehavioral and central and autonomic nervous system effects of Tokyo subway sarin poisoning. *Jounral of Physiology - Paris, 92,* 317–323.

Yonelinas, A.P., Hopfinger, J.B., Buonocore, M.H., Kroll, N.E., & Baynes, K. (2001). Hippocampal, parahippocampal and occipital-temporal contributions to associative and item recognition memory: An fMRI study. *Neuroreport*, *12*, 359-363.

Zeineh, M.M., Engel, S.A., Thompson, P.M., & Bookhelmer, S.Y. (2003). Dynamics of the hippocampus during encoding and retrieval of face-name pairs. *Science, 299,* 577-580.

Zimmerman, M.E., Pan, J.W., Hetherington, H.P., Katz, M.J., Verghese, J., Buschke, H…Lipton, R.B. (2008). Hippocampal neurchemistsry, neuromorphometry, and verbal memory in nondemented older adults. *Neurology*, *70*, 1594-1600.

BIOGRAPHICAL INFORMATION

Crystal joined Dr. Odegard's lab at UT-Arlington in Fall 2007, shortly after receiving her BS in Psychology from UT-Arlington. She conducted her Master's thesis on strategies that highlight both the positive and negative aspects of normative age-related patterns in human episodic memory. She received her MS in Health Psychology and Neuroscience in Spring 2009. In April 2012, Crystal plans to defend her dissertation on memory deficits and neurobiological correlates of encoding and performance in Gulf War Illness. During her graduate career at UT-Arlington, Crystal conducted research ranging from behavioral memory paradigms to longitudinal interventions in the aging population as well as brain imaging in younger adults to veterans with Gulf War Illness.

Crystal's research interests include lifespan human learning and memory as well as cognitive and neural plasticity. While in her graduate training, her research emphasis was in aging, as well as the neurobiological mechanisms that underlie normal and abnormal changes in aging cognition using brain imaging techniques (magnetic resonance imaging and electroencephography) and measures of metabolic biomarkers. Moving forward, Crystal would like to apply what she has learned to clinical population conducting research on identifying biomarkers that predict treatment outcomes in order to help personalize treatment. Crystal will be joining Dr. Madhukar Trivedi's Mood Disorders Research Program and Clinic in the Department of Psychiatry at UT-Southwestern Medical Center as a T32 postdoctoral fellow.