THE WAR WITHIN

Elbert Geuze

THE WAR WITHIN

Neurobiological Alterations in Posttraumatic Stress Disorder
Neurobiologische veranderingen bij posttraumatische stress stoornis

(met een samenvatting in het Nederlands)

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de rector magnificus, prof.dr. W.H. Gispen, ingevolge
het besluit van het college voor promoties in het openbaar te
verdedigen op dinsdag 24 oktober 2006 des ochtends te 10.30 uur

door

Samuel Gijsbert Geuze
geboren op 13 augustus 1979, te Barneveld

Printing of this dissertation was financially supported by the Central Military Hospital,
the Dutch Ministry of Defense, and the Muntz Marketing Communication Group

ISBN-10: 90-393-4323-3
ISBN-13: 978-90-393-4323-4

Design and lay-out by: Muntz Marketing Communication Group
Printed by Koninklijke Drukkerij C.C. Callenbach BV

Promotor: Prof. Dr. H.G.M.Westenberg

Co-promotor: Dr. E. Vermetten

TABLE OF CONTENTS

INTRO
DUCTION

Introduction

and outline

of the studies

1

Introduction and outline of the studies

Introduction

Throughout the course of life, from conception to death, the human body in general and the brain in particular are shaped by experience. Every event and occurrence that upsets homeostasis will lead to adaptation and subsequent formation of a new equilibrium. The brain's response to stress is inevitable, swift and usually adequate. However, when the immediate response to stress and subsequent adaptive mechanisms are inadequate, pathology may develop.

Posttraumatic Stress Disorder

Strife, conflict, human violence, and natural disasters are an unavoidable part of life. Human reaction to traumatic events has not changed much throughout the years. Early Greek and Roman writings mention a wide array of human reactions that occur in the aftermath of traumatic stress; reactions that are very similar to those witnessed today. Medicine devoted little interest to pathological human responses to stress; it was not until the American Civil War that a syndrome involving symptoms of exhaustion and an increased physiological response, known as *'soldier's heart'* was first described.[1] World War I and World War II sparked renewed interest in extreme human responses to combat stress culminating in a treatise titled 'Traumatic Neuroses of War' by Kardiner in 1941. Early versions of the Diagnostic and Statistical Manual of Mental Disorders (DSM) acknowledged the existence of human adjustment problems after stress, but it was not until after the Vietnam War, that the persistent pathological effect of traumatic stress was first recognized. DSM-III and IV and the International Classification of Diseases, Injuries, and Causes of Death (ICD) 9 and 10 first contained the diagnosis of posttraumatic stress disorder (PTSD).

Psychiatrists using the DSM-IV may diagnose PTSD if the person has been exposed to a traumatic event in which "the person experienced, witnessed, or was confronted with an event or events that involved actual or threatened death or serious injury, or a threat to the physical integrity of self or others **and** the person's response involved intense fear, helplessness, or horror (APA,1994; see appendix). Besides the presence of these criteria (the A1 and A2 criterion respectively), the person must reexperience the event and display persistent avoidance of stimuli associated with the trauma and numbing of general responsiveness (not present before the trauma). Finally, symptoms of increased arousal should also be present. If all these criteria are met, symptoms persist for a period greater than one month and cause significant clinical distress, a person is given the diagnosis of PTSD.

Dutch Army

Since World War II, the concept of war has changed considerably (see van Creveld[2]). Modern forms of armed conflict are characterized by an increase in intra-national (as opposed to inter-national) conflicts. These conflicts are led by individual leaders (as opposed to governments) and are motivated by religious, nationalistic, or ethnic factors (as opposed to territorial expansion). International organizations, such as the United Nations, North Atlantic Treaty Organization, European Union, and

the Organization for Security and Co-operation in Europe, have played an important role in observing, monitoring and resolving these armed conflicts. The Dutch Army has participated in a large number of these observational, peacekeeping and peace enforcement operations (see Klep and van Gils[3]). The "war on terrorism" has sparked a new series of military operations with (predominantly) the USA and the UK in which the Dutch play an increasingly antagonistic role. Although these armed conflicts in which the Dutch Army were involved caused few direct casualties, the psychological impact of war and trauma on veterans was slowly becoming evident. Veterans 'relived' their traumatic experiences, avoided stimuli associated with their deployment, and suffered from disordered sleep. Their family members noticed a numbing and general disinterest with those things formerly deemed of great value and emotional valence, as well as increased aggression and heightened irritability. For these individuals the war was not over, but waged on inside. They experienced a "war within"; they were at war with themselves. However, throughout the first nine decades of the 20th century, politicians, media, the general public, and the Department of Defense had little or no attention for the 'adjustment' problems experienced by returning veterans.

Although at first military psychiatry was reluctant to invest time and energy into the new concept of PTSD, this apparent lack of interest changed. This changing interest was due in part to a number of individuals who were important motivators and the driving force behind the start of a broad experimental 'multi-modular' treatment program for PTSD. Throughout the department of Defense, there was also growing support and realization that the problems experienced by returning veterans needed to be addressed. A growing team of therapists, psychologists, psychiatric nurses, and psychiatrists became involved and the first group therapy session for veterans from Lebanon started in September of 1994.

Research

While the treatment program was slowly establishing its roots at the Central Military Hospital and financial and political hurdles were overcome, there was still little empirical basis for the program. Good research lies at the basis of good treatment and is part of the drive to professionalize. The Department of Military Psychiatry of the Central Military Hospital joined forces with the Department of Psychiatry of Utrecht University Medical Center to develop a neurobiological research program. Some tactical political maneuvering led to the announcement in November 2002 by Secretary of Defense H.A.L. van Hoof that the Ministry of Defense was willing to invest in a five year project.

The first main project, titled "Neurobiological Parameters of Posttraumatic Stress Symptoms of Veterans after Deployment" commenced in September 2002. In November of that year, a new project was anticipated which bore the wonderfully ambiguous title "Neuroimaging and PTSD". Thankfully, the ambiguous nature of the title opened up a realm of possibilities. Over the course of three years three state of the art neuroimaging techniques were employed at three separate facilities. Structural magnetic resonance imaging (MRI) of the brain was a part of the original project and was performed at Utrecht University Medical Center. Positron Emission Tomography (PET) was a new project, which was carried out in association with the Free University of Amsterdam. Functional MRI (fMRI) scanning of the brain was realized in collaboration with the Central Institute of Mental Health of the University of Heidelberg in Mannheim, Germany.

Outline of the Studies

The primary objective of this dissertation is to examine neurobiological correlates of PTSD, using neuroimaging techniques and neuropsychology. These tools are used to provide more insight into the etiology, course and nature of PTSD. This dissertation begins by exploring structural changes in the brain after traumatic stress. The chapters immediately following this introductory chapter contain two companion papers about a review of hippocampal volumetry in neuropsychiatric disorders. Hippocampal volumetry is a widely employed technique in neuropsychiatric disorders. Researchers are notorious for a streak of (sometimes healthy) stubbornness. However, the wide range of protocols employed to determine hippocampal volume makes it hard to compare results across studies. In **chapter 2** various aspects of manual hippocampal segmentation protocols are discussed in relation to optimal determination of hippocampal volume. **Chapter 3** provides an overview of hippocampal volumetric findings in neuropsychiatric disorders.

Structural neuroimaging studies in PTSD have focused primarily on structural alterations in the medial temporal lobe, and few have examined gray matter reductions in the cortex. Recent advances in computational analysis provide new opportunities to use semi-automatic techniques to determine cortical thickness, but these techniques have not yet been applied in PTSD. In **chapter 4** preliminary structural neuroimaging data is reported from twenty-five male veterans with PTSD and twenty-five male veterans without PTSD matched for age, year and region of deployment. Individual cortical thickness maps were calculated from structural MR images. All the subjects' brains were aligned using cortex-based alignment in a region of interest based approach. Regions of interest examined included the bilateral superior frontal gyri, bilateral middle frontal gyri, bilateral inferior frontal gyri, bilateral superior temporal gyri, and bilateral middle temporal gyri.

In the next three chapters of this dissertation the results of three functional neuroimaging studies in PTSD are reported. Gamma-amino-butyric acid (GABA) is the principal inhibitory neurotransmitter in the brain, and as such plays an important role in orchestrating neural activity throughout the cerebrum. $GABA_A$ receptors are thought to play an important role in the modulation of the central nervous system response to stress. Animal data have shown alterations in the $GABA_A$ receptor complex by uncontrollable stressors. Single photon emission computed tomography (SPECT) imaging with benzodiazepine ligands revealed lower distribution volumes of the benzodiazepine-$GABA_A$ receptor in the prefrontal cortex of patients with PTSD in one, but not in another study.[4;5] In **chapter 5**, nine veterans with PTSD and seven veterans without PTSD were examined using [^{11}C]-flumazenil PET. [^{11}C]-flumazenil is a benzodiazepine antagonist which binds to the benzodiazepine binding site on

the $GABA_A$ receptor. The binding potential of [^{11}C]-flumazenil provides important information on the function of the GABA system in veterans with PTSD.

Pain is a complex multi-faceted yet basic entity with a profound impact on human experience. In ancient times, a lack of understanding of the nature of pain led to methods of pain relief such as trepanation to release the demons confined within the human skull. The steady progression of science and human knowledge has led to a better understanding of pain and (thankfully) a demise of the traditional methods. Within the last century tremendous progress has been made in expanding our knowledge of pain. However, it is not till recently that the advent of neuroimaging techniques enables us to visualize pain processing within the human brain. Pain experience in PTSD has been reported to be significantly increased compared to controls[6;7] However, previous research has also reported that patients with PTSD experience less pain after being exposed to stress, such as traumatic reminders.[8] In **chapter 6** the neural correlates of pain processing were explored in twelve Dutch veterans with PTSD compared to twelve veterans without PTSD using fMRI in combination with painful tonic heat stimuli. Both fixed temperature heat stimuli which was the same for all subjects, and individual temperature heat stimuli which were adjusted for equal subjective pain in all subjects, were used.

Besides the previously mentioned triad of core symptoms of PTSD (recurrent and intrusive distressing recollection of the event, persistent avoidance of stimuli associated with the traumatic experience, and increased symptoms of arousal), patients with PTSD also report increased difficulties with new learning, memory, and attention.[9-11] Several studies performed in patients with PTSD have shown that PTSD is associated with deficits in memory performance.[12;13] These deficits in memory performance are usually proposed to be related to decreased frontal cortex function and smaller hippocampal volume in PTSD. [14] However, no study has provided strong support for these suppositions. In **chapter 7** data from an fMRI study on the neural correlates of associative learning and memory is reported. The objective of this study was to find out whether brain areas purportedly dysfunctional in PTSD would also display altered activity during associative learning and memory.

Although several studies have examined memory performance in PTSD, nearly all of these studies had unresolved methodological issues. One of the most important of these methodological issues is that the patients in previous studies used psychotropic medication. In the studies described in this dissertation a unique population of veterans with PTSD and control veterans which were optimally matched were examined. The patients included in these studies were free of psychotropic medication and the majority were medication naïve. In **chapter 8**, memory performance was compared in twenty-five veterans with PTSD and twenty-five veterans without PTSD. The objective of this study was to provide support for a structural memory deficit in PTSD that could not be attributed to medication use, intelligence, years of education, or traumatic experience. In addition, memory performance was also related to social and occupational functioning. Although this relation is frequently assumed to exist, no study has yet provided support that memory difficulties have an effect on social and occupational functioning. In chapter 8, this relationship is also examined. **Chapter 9** contains a summary of the results of these studies and concluding remarks.

References

1. DaCosta, J.M. On irritable heart: a clinical study of a form of functional cardiac disorder and its consequences. Am J Med Sci 161, 17-52 (1871).
2. Van Creveld, M. The Transformation of War. The Free Press, New York (1991).
3. Klep, C. and van Gils, R. Van Korea tot Kabul. Sdu Uitgevers, The Hague (2005).
4. Bremner, J.D. et al. Decreased benzodiazepine receptor binding in prefrontal cortex in combat-related posttraumatic stress disorder. Am J Psychiatry 157, 1120-6 (2000).
5. Fujita, M. et al. Central type benzodiazepine receptors in Gulf War veterans with posttraumatic stress disorder. Biol Psychiatry 56, 95-100 (2004).
6. Beckham, J.C. et al. Chronic posttraumatic stress disorder and chronic pain in Vietnam combat veterans. J Psychosom Res 43, 379-89 (1997).
7. Asmundson, G.J., Coons, M.J., Taylor, S., and Katz, J. PTSD and the experience of pain: research and clinical implications of shared vulnerability and mutual maintenance models. Can J Psychiatry 47, 930-7 (2002).
8. Pitman, R.K., van der Kolk, B.A., Orr, S.P., and Greenberg, M.S. Naloxone-reversible analgesic response to combat-related stimuli in posttraumatic stress disorder. A pilot study. Arch Gen Psychiatry 47, 541-4 (1990).
9. Thygesen, P., Hermann, K., and Willanger, R. Concentration camp survivors in Denmark persecution, disease, disability, compensation. A 23-year follow-up. A survey of the long-term effects of severe environmental stress. Dan Med Bull 17, 65-108 (1970).
10. Pitman, R.K. Post-traumatic stress disorder, hormones, and memory. Biol Psychiatry 26, 221-3 (1989).
11. White, N.S. Posttraumatic stress disorder. Hosp Community Psychiatry 34, 1061-2 (1983).
12. Horner, M.D. and Hamner, M.B. Neurocognitive functioning in posttraumatic stress disorder. Neuropsychol Rev 12, 15-30 (2002).
13. Buckley, T.C., Blanchard, E.B., and Neill, W.T. Information processing and PTSD: a review of the empirical literature. Clin Psychol Rev 20, 1041-65 (2000).
14. Isaac, C.L., Cushway, D., and Jones, G.V. Is posttraumatic stress disorder associated with specific deficits in episodic memory? Clin Psychol Rev (2006).

Appendix: Diagnostic Criteria for Posttraumatic Stress Disorder

A. The person has been exposed to a traumatic event in which both of the following were present:

(1) the person experienced, witnessed, or was confronted with an event or events that involved actual or threatened death or serious injury, or a threat to the physical integrity of self or others
(2) the person's response involved intense fear, helplessness, or horror.
Note: in children, this may be expressed instead by disorganized or agitated behavior

B. The traumatic event is persistently reexperienced in at least one of the following ways:

(1) recurrent and intrusive distressing recollections of the event, including images, thoughts, or perceptions.
Note: in young children, repetitive play may occur in which themes or aspects of the trauma are expressed
(2) recurrent distressing dreams of the event.
Note: In children there may be frightening dreams without recognizable content
(3) acting or feeling as if the traumatic event were recurring (includes a sense of reliving the experience, illusions, hallucinations, and dissociative flashback episodes, including those that occur on awakening or when intoxicated).
Note: In young children, trauma-specific reenactment may occur
(4) intense psychological distress at exposure to internal or external cues that symbolize or resemble and aspect of the traumatic event
(5) physiological reactivity on exposure to internal or external cues that resemble an aspect of the traumatic event

C. Persistent avoidance of stimuli associated with the trauma and numbing of general responsiveness (not present before the trauma), as indicated by three (or more) of the following:
(1) efforts to avoid thoughts, feelings, or conversations associated with the trauma
(2) efforts to avoid activities, places, or people that arouse recollections of the trauma
(3) inability to recall an important aspect of the trauma
(4) markedly diminished interest or participation in significant activities
(5) feeling of detachment or estrangement from others
(6) restricted range of affect (e.g. unable to have loving feelings)
(7) sense of a foreshortened future (e.g. does not expect to have a career, marriage, children, or a normal life span)

D. Persistent symptoms of increased arousal (not present before the trauma), as indicated by two (or more) of the following:
(1) difficulty falling or staying asleep
(2) irritability or outbursts or anger
(3) difficulty concentrating
(4) hypervigilance
(5) exaggerated startle response

E. Duration of the disturbance (symptoms in Criteria B, C and D) is more than 1 month.

F. The disturbance causes clinically significant distress or impairment in social, occupational, or other important areas of functioning

Specify if: **Acute**: if duration of symptoms is less than 3 months
Chronic: if duration of symptoms is 3 months or more
Specify if: **With Delayed Onset**: if onset of symptoms is at least 6 months after the stressor

From DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, 4th ed. Copyright American Psychiatric Association, 1994.

SECTION

Structural

Neuroimaging

2

MR Based *in vivo* Hippocampal Volumetrics: 1. Review of Methodologies Currently Employed

E. Geuze, E. Vermetten, and J.D. Bremner.
2005 **Molecular Psychiatry** *10(2): 147-159*

Introduction

The advance of neuroimaging techniques has resulted in a burgeoning of studies reporting abnormalities in brain structure and function in a number of neuropsychiatric disorders. One of the brain structures which has been a focus of research is the hippocampal formation. Magnetic resonance (MR) based in vivo measurement of hippocampal volume is an accepted technique which has been performed in the aged, [1] and in healthy subjects, [2] and has revealed a number of structural abnormalities in a variety of neurological and psychiatric disorders, such as temporal lobe epilepsy, [3] Huntington's disease, [4] Turner's syndrome, [5] Cushing's disease, [6] Down's syndrome, [7] Alzheimer's disease (AD) , [8] mild cognitive impairment, [9] schizophrenia, [10] major depression (MD) , [11] bipolar disorder, [12] PTSD, [13] borderline personality disorder, [14] chronic alcoholism, [15] obsessive-compulsive disorder, [16] and panic disorder. [17]

The MR derived hippocampal volumetric technique has demonstrated good validity and reproducibility, [18-20] and accuracy of the measurements has been shown by MRI volumetric measurement of phantoms with a known volume. [18;21] However studies on hippocampal volume in neuropsychiatric disorders are inconclusive and do not always provide consistent results. There are differences in laterality (right or left), direction (increase or decrease), and degree of the hippocampal volumetric changes. For example, smaller bilateral hippocampi in patients with schizophrenia have been found by a large number of research groups, [22-27] but not by others. [28-31] Similarly, several groups found smaller bilateral hippocampi in patients with PTSD, [32;33]whereas others where unable to find significantly smaller hippocampi in PTSD. [34-36] In major depression, significantly smaller bilateral hippocampal volumes have been reported by some, [11;37;38] but not by others. [39;40]

Part of the discrepancy among research findings may be attributed to the use of different methods for establishing hippocampal volume. The accuracy and reproducibility of MRI based in vivo hippocampal volume measurements depends on three broad factors, namely image acquisition, post-acquisition processing, and volumetric assessment. [19] This paper provides a discussion of the various methods that studies of hippocampal volume use. The technical aspects of image acquisition and post-acquisition processing depend on the technical characteristics and type of scanner available. It is not the purpose of this review to present researchers with another optimal protocol. Rather, this is intended as a review of some of the important factors in which these protocols diverge, and then to present recommendations for optimizing hippocampal volume analysis.

Methods

We performed a Medline Indexed search with the keywords "hippocampus," "volume," and "MRI." From this database all English-language, human subject, data-driven papers were selected yielding a database of 423 records (only papers published before December 31, 2003 were included). Reviews, case studies, and volumetry studies using CT were all excluded. We assessed the methodology sections of all these papers, to determine if the paper refers to methods used by other

studies, in order to come up with the original protocols. This yielded a database of approximately 115 "original" protocols. Only protocols in which the manual tracing method (with or without the simultaneous use of region-growing or thresholding) was used were included in this database. Manual tracing protocols constitute the vast majority of the protocols and are used by 90% of the studies on hippocampal volume in our database. For this reason, and because both point counting methods (e.g. MacFall *et al.* [41] and Mackay *et al.* [42]) and voxel-based morphometric methods (e.g. Wright *et al.* [31]) are different analysis techniques which are judged by a different set of criteria, they are difficult to compare to the manual tracing protocols. Therefore they are not included in this review. Nevertheless, although the methodological differences in these protocols are not mentioned in this paper, the results from these studies are discussed in the companion paper "MR based in vivo hippocampal volumetrics II: Volumetric estimates in neuropsychiatric disorders" (see chapter 3).

Results

One of the first general findings which emerges from this analysis is that there is a wide range in the amount of reported detail about methodology. Whereas some protocols provide clear data acquisition and data processing parameters, as well as detailed anatomical criteria, a larger number of publications do not provide a great amount of detail making it difficult to compare studies. The protocols may differ in a number of factors related to image acquisition, image processing, and anatomical guidelines which are important for accurate hippocampal volume determination, namely: image acquisition parameters, magnetic field strength, the number of slices assessed and the thickness of slices, hippocampal orientation correction, volumetric correction, software used, interrater reliability, and anatomical boundaries of the hippocampus. [43-45] These differences are discussed in greater detail below.

Image Acquisition

The protocols employ a wide array of acquisition sequences. In all, 35% of the protocols use a three dimensional (3D) spoiled gradient echo recalled sequence (3D SPGR), 15% use a 3D magnetization prepared rapid acquisition gradient echo (3D-MPRAGE) sequence, 11% use a spin echo (SE) sequence, 7% use an inversion recovery (IR) sequence, 7% use some other type of gradient recalled echo (GRE) sequence, 6% use a fast low-angle shot (FLASH) sequence, 4% use some other type of fast field echo sequence, 4% use a fast spin echo (FSE) sequence, 3% use some other type of echo sequence, 2% use some other type of acquisition sequence, and 6% do not mention the acquisition sequence used. In addition parameters affecting signal to noise ratio and contrast, such as repetition time (TR), echo time (TE), flip angle, field of view, matrix size, and slice thickness vary greatly from study to study. The protocols make use of General Electric (52%), Siemens/CTI (26%), Philips (12%), Picker (3%), Toshiba (1%), and Ansaldo (1%) scanners. Of the protocols, 5% do not mention the manufacturer of their scanner.

Most, (88%), of the protocols used a 1.5 T scanner, 4% of the protocols mention using a 1 T scanner, 3% scanned at 0.5 T , and 3% used a scanner with a magnetic field strength below 0.5 T. Several protocols (2%) used a scanner operating at a magnetic field strength greater than 1.5 T. Bartzokis ***et al.*** [46] have compared volumetry of different brain structures at 0.5 and 1.5 T and demonstrated good interscanner reliability. Although images acquired on the 0.5 T scanner were acquired using a similar sequence they differed in quality and tissue T_2 relaxation times. [47] Similarly, although measurement error is lower and measurement reliability is improved at 3 T due to increased tissue contrast, this is not significantly different from that at 1.5 T and does not dramatically increase at 3 T; the increased field strength does not significantly affect the volume measurement. [48] However, there has also been one report which compared images of the hippocampus at 1.5 T to images acquired at 4 T. [49] Using a slightly different imaging sequence at 1.5T and 4T, they found that high resolution imaging provided superior volumetry as well as an ability to visualize sub-regions of the hippocampus. [49;50] Optimization of image acquisition parameters in combination with increased field strength may thus provide superior contrast and improved hippocampal volumetry.

Not all of the studies report exactly how many slices they have assessed, but they do mention whether they assessed the whole hippocampus, part of the hippocampus (body or head) or the whole amygdala-hippocampal complex. In the past a number of researchers [13;51] have used the body of the hippocampus to evaluate its volume, as this correlates with total hippocampal size. [52] Lower resolution in early studies also made it difficult to see the amygdala-hippocampal boundary. Currently, however, measurements of the body of the hippocampus only are not acceptable, as this seriously affects the face validity of the volumetric measurements. Others measured the tail and body of the hippocampus, but did not include the head, [53] Jack *et al.* [54] measured the head and the body of the hippocampus but excluded the tail. Some researchers, such as Shenton *et al.* [55] measured the amygdala-hippocampal complex, whereas others reliably differentiate between amygdala and hippocampus. [20;21;56] Of the protocols in this database, 80% attempted to measure as much of the hippocampus as they could, and included the head and the body in their measurements. Only a small minority of these studies excluded the tail. In all, 16% of the protocols measured the whole amygdala-hippocampal complex, and 4% measured the body of the hippocampus only.

Image acquisition protocols change rapidly, as technology advances. At one time state of the art MRI incorporated contiguous 5 mm thick slices [57] however, lately contiguous of 1.5 mm or less are commonly used. [58;59] Thus although Watson *et al.* [20] used 3 mm thick slices, later studies performed by this research group [60;61] report using slice thicknesses of 1.5 mm.

The number of slices assessed during hippocampal volumetry is a variable that is not reported very often, although a good inference of this variable can be made from the slice thickness that is used, a variable that is always reported. The number of slices assessed during a typical session will vary inversely with the thickness of the slice. Thus using thicker slices implies that fewer slices have been assessed, unless the images have been reformatted and resliced using computer software. Using thicker (and thus fewer) slices is less time consuming, and may in some cases be preferable to using thinner slices.

Image Processing

The hippocampi are variably tilted; thus ideal image collection involves perpendicular acquisition of MR images. [57;62] Although such an acquisition is fairly straightforward with 2D acquisition sequences, 3D acquisition sequences perpendicular to the hippocampal axis are impossible to perform on a substantial number of MR units. [63] Alternatively, this type of acquisition may also be attained by tilting the patient's head, at the expense of increasing patient discomfort. It is also possible to reformat the acquired images perpendicular to the axis of the hippocampal formation using computer software.

Of the 115 protocols, 39% use various acquisitions but reformat the slices at an angle perpendicular to the long axis of the hippocampal formation. A total of 32% do not mention which acquisition orientation they used, or if they used reformatted images, 22% report acquisitions perpendicular to the AC-PC line without reformatting of images, 5% report acquisitions perpendicular to the Sylvian fissure, and 3% reported using a head tilt acquisition. Although there is no proof that these different acquisition protocols result in systematic over- or underestimation of absolute hippocampal volume, [43] these protocols achieve statistically significantly different results. [63]

There are a number of different software packages available for manual tracing.

Almost all of the software that is used employs a combination of thresholding, manual tracing and sometimes region growing. The diversity of software packages that is used is so large that it would be too much to dwell on the differences between them in this paper. However, if we look at those software packages that have been used in more than three protocols, we see that Analyze is by far the most popular software package that is used (20.0% of the protocols). Other software packages which are commonly used are MIDAS (6.1%), MEASURE (3.1%), NIH Image (2.6%), BRAINS (2.6%), and DISPLAY (2.6%). In all 27 protocols (23.5%) report using custom or native scanner software for analyzing their data. Again a considerable portion of the protocols (14.8%) do not report which computer program they have used. The other 22.6% of the researchers use various other computer programs both commercial and freely distributed. In some programs (such as BRAINS, MEASURE, and Display) researchers are able to view the brain in three orthogonal (saggital, coronal, and horizontal) planes simultaneously, thus allowing identification of anatomical boundaries with greater accuracy. All software packages employ some method of thresholding and/or region growing in combination with manual tracing.

People with large intracranial volumes tend to have larger brain structures, such as larger ventricles and larger hippocampi. [64;65] Hippocampal volumes should thus be corrected for intersubject variation in head size. Correcting for head size or whole brain volume introduces two separate sources of error and thus produces measures with lower reliability. [66] However, as Mathalon *et al.* [67] showed, head-size correction also improves criterion validity and thus produces higher correlations with age and with diagnostic status than absolute values do.

In order to control for these factors, Jack *et al.* [68] introduced a region of interest normalization by dividing the region of interest by total intracranial volume an approach which they borrowed from the CT literature (see Huckman *et al.*[69]). The majority (34%) of the protocols follows Jack *et al.*'s [68] example and uses total intracranial volume to correct for intersubject variation in head size. Another method which has been used quite often (21% of the protocols) is to use division by whole brain volume for normalization. [1;56;70] Surprisingly, a substantial number of protocols (34%) do not use a correction factor at all. Although, in some cases, absolute volumes are needed (in epilepsy research, or when comparing automatic and manual volumetrics, for example) and thus controlling for head size is not warranted. A small number of studies (4%) uses the correlational method [71;72] introduced by Jack *et al.* [54], where the corrected hippocampal volume (HV_n) is derived by taking the original hippocampal volume (HV_o) and subtracting the product of the regression line between the hippocampal volume and the intracranial volume, and the difference between the individual intracranial volume (TIV_i) and the mean intracranial volume (TIV_{mean}). $HV_n = HV_o - GRAD(TIV_i – TIV_{mean})$. Other cerebral measures, such as whole brain volume or another cerebral control area may be substituted for the intracranial volume in this formula as well. [73]

The main factor determining accuracy of volumetric measurements by manual tracing and thresholding seems to be the reliability of the within rater measurements. [18;74] Apparently, individual reproduction of the hippocampal boundaries is consistent, but reliability between observers is difficult to obtain even if they are using the same anatomical criteria. [43;75] One study on intra- and interobserver variability provides an interesting illustration of this, and showed that one of the observers consistently overestimated hippocampal volume in comparison to the other observer, thus intraobserver variability was fairly consistent with correlation values of 0.88 and 0.97 as opposed to the interobserver correlation values which ranged from 0.62-0.73. [76]

The inter-rater or intrarater reliability that researchers achieve varies greatly across studies and ranges from 0.64 to 0.99. Of the 115 'original' protocols, 60 (52%) report ICCs, [77] inter-rater or intrarater reliability values greater than 0.90. In all, 25 protocols (21%) report values of 0.80 to 0.89, and 6% of the protocols report values lower than 0.80. Still the importance of reporting reliability values has not been taken to heart by all researchers. A substantial portion of the protocols (21%) do not report any reliability values at all.

Anatomical Guidelines

The anatomical guidelines that researchers use vary greatly as well. In our database of 423 studies we have approximately 60 different anatomical guidelines. It would not be practical to report all the anatomical guidelines or variations of them that these protocols use; however it is interesting to look at the variations among the most widely used protocols. The most widely used protocols were defined as those protocols that were used in five or more studies in the original database of 423 records. These protocols have varying anatomical guidelines, which are summarized in Table 1. The protocol of Jack *et al* [54], which has been revised in 1994, [57] and that of Watson *et al.* [20] are the most popular protocols and are reported to have been used in 31 studies each. The protocols of Soininen

et al. [56] and Cook *et al.* [21] are two other important and popular ones, which are also used frequently. Together, these 14 protocols account for 46% of the hippocampal volumetric studies performed to this date. The anatomical criteria of these major protocols are used in a few other protocols as well, and are employed in 51% of the research studies.

Discussion

Research groups use a variety of different methods, such as manual volumetrics, voxel-based morphometry, and stereology (or point-counting), to assess hippocampal volumes in various neuropsychiatric populations. Manual volumetric assessment of the hippocampus has been denoted the "gold standard", but considerable variation exists among research studies, and there is no standard protocol or methodology to which all researchers adhere. The differences in these protocols have been attributed to various disparities in acquisition, post-acquisition processing, and anatomical guidelines.

Image acquisition protocols should maximize image quality and resolution, and should minimize error such as partial volume effects, image quality, head tilt, plane of view, and movement artefacts. Image acquisitions should also maximize grey matter-white contrast, as this has been shown to affect hippocampal volumetry. [78] The contrast between different brain tissue types is dependent on the image acquisition sequences used and may thus influence the hippocampal measurement.

Studies of hippocampal volumetry use a variety of different image acquisitions. Gradient recalled echo (GRE) sequences (such as 3D SPGR and FLASH, [79-81]) are popular in the field of hippocampal volumetry, and have been developed to reduce scanning time. Although eliminating the 180° refocusing pulse allows for a significantly shorter TE and TR, GRE sequences are sensitive to susceptibility effects and do not compensate for the chemical shift between water and fat. [82] Thus a number of techniques such as the frequency selective prepulse, and inversion recovery are needed to increase contrast. [81;83] An inversion recovery GRE variant, the MPRAGE acquisition sequence, is also popular in the field of hippocampal volumetry. Optimized 3D MPRAGE sequences yield higher white matter to grey matter signal to noise ratios than do optimized 3D FLASH sequences. [84] Inversion recovery sequences,[85] provide high contrast images of the brain, and more consistent hippocampal measurements.[78] Spin echo sequences are commonly used in neuroradiology, and provide excellent anatomic detail at the expense of longer scan times. [83] Fast spin echo acquisitions, based on RARE or HASTE sequences employ more than one spin echo, and allow faster imaging than the regular spin echo sequence, without loss of contrast. [83] Speed-accuracy tradeoff is an important issue in research as well. Researchers should ideally employ image acquisition sequences which provide high signal-to-noise ratios, and maximum anatomic detail such as fast spin echo sequences. Magnetization-prepared GRE techniques such as MPRAGE and fast-spoiled-GRASS-prepared sequences also provide good signal-to-noise ratio and are preferred to conventional GRE sequences.

Field strengths of 0.5 T or lower, place severe limits on resolution. Researchers should use scanners with field strengths of 1.5T or more, to ensure accurate hippocampal boundary delineation. Volumetry at 4 T is more sensitive in detecting hippocampal atrophy than at 1.5 T. [49] Images of the human hippocampus at 7 T even allow researchers to make some distinction between hippocampal layers. [86] In the future, increasing magnetic field strengths with superior spatial and temporal resolution and increased signal to noise ratio, will allow better delineation of the anatomical boundaries of the hippocampus, with resultant improvements in accuracy and reliability.

Future research studies on hippocampal volume should also make use of thin contiguous slices, because they are less likely to be affected by a single false estimate. [58] In 1997, Laakso *et al.* [58], examined the effect of slice thickness. They studied 10 normal subjects and acquired 3D contiguous coronal images with a slice thickness of 1.5-2mm, which they reformatted into 1, 3, and 5 mm slices oriented perpendicular to the hippocampal axis. The hippocampal volumes acquired did not differ significantly between the different slice thicknesses used. Currently, researchers should no longer make use of 5 mm or 3 mm slices. As Laakso et al. [58] have recommended earlier, thinner slices should be used, since they are less affected by a single false estimate.

Visualization of the hippocampus perpendicular to its long axis improves the reliability and reproducibility of measurements. [18;43;46;58;62;63;87] The majority of the studies do not properly report which acquisition orientation they used. Of those who do provide sufficient detail, the majority report using images reformatted perpendicular to the long axis of the hippocampal formation. [57] A substantial number of protocols use different acquisition sequences (either perpendicular to the AC-PC line [25;88;89], or the Sylvian fissure[3;20]), but do not reformat their images, and a very small number of studies employ head tilt protocols. [90-92] Three dimensional imaging techniques allow researchers to save valuable scan time, by eliminating the need for pilot scans needed for consistent positioning of images based on internal landmarks, and accomplishing this after the scan using multiplanar image reconstruction capabilities. [87]

Although Sullivan *et al.* [93] were unable to find an effect of slice orientation, this is probably because they only assessed the effect of the APC-hippocampus angle (defined as the angle variation of the longitudinal axis of the hippocampus relative to the AC-PC line) on hippocampal volume. Hasboun *et al.* [63] have reliably demonstrated that using reformatted images as opposed to non reformatted images or acquisitions using a head-tilt result in statistically different hippocampal volumetric estimates, thus it must be emphasized that researchers should provide sufficient detail in their study design mentioning which method they used. In a study addressing various aspects of amygdala and hippocampal volumetric measurement, Kates *et al.* [44] revealed that rotating images perpendicular to the long axis of the hippocampal formation resulted in a significantly higher intrarater reliability in measuring the hippocampus. In contrast to Hasboun *et al.* [63], Kates *et al.* [44] did not find any significant difference in hippocampal volumes obtained with images oriented perpendicular to the long axis of the hippocampus, or images oriented perpendicular to the AC-PC line. Bartzokis *et al.* [87] compared scan-rescan reliability as well as intra-rater reliability and found that reformatted 3D images showed

Table 1: Anatomical boundaries of the human hippocampus in representative studies

Reference	*Image acquisition sequence / Scanner*	*Hippocampal measurement*	*Most anterior slice*	*Most posterior slice*	*Medial border*	*Lateral border*	*Inferior border*	*Additional notes*	*Normative hippocampal volume (cm^3)*	
									Left	*Right*
Bartzokis et al, 1993/1998 [46;87]	3D spoiled GRASS TR/TE/FA 25/5/35 GE 1.5T	Whole hippocampus	Level at which the alveus distinguishes amygdala from hippocampus	Level where the inferior and superior colliculi are jointly visualized	Gray matter of the subiculum is included in the measurement	Gray/white matter interface	Gray/white matter interface	Subiculum and alveus included in the measurement	—	—
Bigler et al, 1997 [105]	FSE TR/TE 500/11 GE 1.5T	Whole hippocampus	Anterior aspect of the hippocampus, or the uncal recess separating amygdala from the hippocampus	Two of four criteria: presence of superior colliculi, presence of the medial pulvinar nucleus, visibility of the oblong position of the hippocampus at the crura of the fornices, presence of a distinct separation of the temporal horn from the atria	Anterior choroidal artery, or the point at which the boundaries of the ambient cistern/choriodal fissure most readily identified	Medial wall of the temporal horn	Not mentioned	Hippocampal volume of controls from Bigler et al, 1995	2.350	2.470
Bogerts et al, 1990 [136]	3D FLASH TR/TE 40/15 Siemens 1T	Amygdala hippocampal complex	Level at which amygdala acquires oval shape	Level at which ascending fornix surrounding the pulvinar becomes distinct	Border between the subiculum and the parahippocampal gyrus	Not mentioned	Not mentioned		—	—
Bremner et al, 1995 [13]	3D spoiled GRASS TR/TE/FA 25/5/45 GE 1.5T	Body of the hippocampus	First slice anterior to the superior colliculus	Proceed 5 contiguous 3 mm slices	Mesial edge of the temporal lobe	Temporal horn of the lateral ventricle	Include the subicular complex and the uncal cleft		—	—
Convit et al, 1995 [137]	SE TR/TE 630/20 Philips 1.5T	Neck to tail measurement	Level of the anterior margin of the lateral geniculate body	Level at which the posterior pulvinar becomes visible	CSF of the choroidal, hippocampal and transverse tissues	Medial wall of the temporal horn	White matter of the parahippocampal gyrus, subiculum was included		—	—
Cook et al, 1992 [21]	GRE 3D TR/TE/FA 35/5/35 GE 1.5T	Whole hippocampus	Level at which the alveus distinguishes amygdala from hippocampus	Slice at which the greatest length of fornix becomes visible	Hippocampal and uncal fissures	Not mentioned	Not mentioned		3.229	3.185
Giedd et al, 1996 [102]	3D spoiled GRASS TR/TE/FA 24/5/4 GE 1.5T	Whole hippocampus	Coronal slice containing the most anterior portions of the mammillary bodies	Slice in which the fibers of the fornix are still visible	Not mentioned	Not mentioned	Not mentioned	Cornu ammonis, dentate gyrus, and subiculum included in the measurement	—	—

Honeycutt et al, 1995/98 [2;138]	3D MPRAGE TR/ TE/FA 11/4/15 Siemens 1.5T	Whole hippocampus	Level at which the alveus distinguishes amygdala from hippocampus	Slice where the fornix is visible	Mesial edge of the temporal lobe	Temporal horn of the lateral ventricle	White matter of the parahippocampal gyrus	Alveus included in the measurement	—	—
Jack et al, 1989/94 [54;57]	3D SPGR TR/TE/ FA min/min/45 GE 1.5T	Whole hippocampus	Level at which the uncal recess of the temporal horn, or the alveus is visible	Slice where the crura of the fornices are seen in full profile	CSF in the uncal and ambient cistern	CSF in the temporal horn	Gray/white matter junction between the subiculum and the white matter in the parahippocampal gyrus	Adopted Watson et al's (1992) posterior boundary definition of the hippocampus in 1994	2.400	2.800
Shenton et al, 1992 [55]	3D FT-SPGR TR 35 GE 1.5T	Amygdala hippocampal complex	White matter tract linking temporal lobe with rest of brain	Slice in which the fibers of the fornix are still visible	Not mentioned	Not mentioned	Not mentioned	Mamillary bodies used to separate amygdala and hippocampus	2.400 (anterior hippocampus)	—
Soininen et al, 1994 [56]	3D MP-RAGE TR/ TE/FA 10/4/12 Siemens 1.5T	Whole hippocampus	Level at which the head of the hippocampus first appears below the amygdala	Slice in which the crura of the fornices depart from the lateral wall of the lateral ventricles / fornices not included	Medial wall of the lateral ventricle / subiculum and dentate gyrus included	Not mentioned	Uncal portion of the dorsal hippocampus included		3.353	3.714
Van Paesschen et al, 1997 [139]	3D MP-RAGE TR/ TE/FA 10/4/12 Siemens 1.5T	Whole hippocampus	Where the mamillary bodies are present / alveus used as a boundary	First slice where the fornix is visible	Mesial edge of the temporal lobe	Temporal horn of the lateral ventricle	White matter of the parahippocampal gyrus	From the first three slices one was chosen at random and from that slice every third slice was measured systematically	3.320	3.330
Watson et al 1992 [20]	3D GRE TR/TE/FA 75/16/60 Philips 1.5T	Whole hippocampus	The CSF in the uncal recess of the temporal horn, when visible, is the most reliable boundary between the hippocampal head and the amygdala, if not visible, the alveus may be used, if neither is visible, then a straight line is drawn connecting the plane of the inferior horn of the lateral ventricle with the surface of the uncus	Slice where the crura of the fornices are seen in full profile	Mesial edge of the temporal lobe	Temporal horn of the lateral ventricle	Include the subicular complex and the uncal cleft with the border separating the subicular complex from the parahippocampal gyrus	Subicular complex, dentate gyrus, alveus, and fimbria included in measurement. These are the most popular anatomical criteria which are used by 15% of the studies	4.903	5.264
Zipursky et al, 1994 [89]	MEFCCG TR/TE 2800/40,80 GE 1.5T	Whole hippocampus	Slice where hippocampus was clearly distinguished from the amygdala	One slice (3mm) anterior to the image where the vertical fissures of the Sylvian fissure are no longer present	The regional outline at the choroidal fissure	Not mentioned	The interface of the hippocampal tissue and parahippocampal gyrus white matter	This method excludes the most posterior region of the hippocampal body and tail	1.990	2.070

GRASS = gradient radiofrequency at steady state, FSE = fast spin echo, FLASH = fast low-angle shots, SE = spin echo,
MP-RAGE = magnetization prepared rapid gradient echo, FT-SPGR = fourier transform – spoiled gradient-recalled
SPGR = spoiled gradient-recalled echo, MEFCCG = multi-echo flow compensated cardiac gated,
TR = repetition time (ms) TE = echo time (ms), FA = flip angle, min = minimal

significantly less scan-rescan variability than non-reformatted images, without sacrificing intra-rater reliability. Using reformatted images decreases the sample size needed to detect volumetric changes by a factor of two. Researchers should thus ideally employ images reformatted perpendicular to the long axis of the hippocampal formation in order to increase scan-rescan reliability and improve visualization of the hippocampus.

Custom software remains popular in the research world. Technological changes occur very rapidly and it is easier to implement these changes if you employ custom software. All software packages utilize some method of thresholding and/or region growing in combination with manual tracing. Identification of anatomical boundaries is more accurate if researchers are able to view the brain in three orthogonal planes simultaneously. Small differences in the algorithms used to calculate or derive hippocampal volume may account for some of the variation in the volumes derived in the studies, although there is no reason to assume that these differences are significant.

People with large intracranial volumes tend to have larger brain structures, such as larger ventricles and larger hippocampi. [64;65] The two major methods to control for intersubject variation in head size are dividing the region of interest by total intracranial volume [68] or division by whole brain volume. [1] Free *et al.* [73] investigated several control regions for their relationship to hippocampal volume, including the corpus callosum, the cranial area, parenchymal area on midsagittal sections, the area of the brain stem on an axial section, and cranial volume and cerebral volume taken from nine coronal sections throughout the cerebrum. The strongest correlation was between cerebral volume and hippocampal volume. However correction via the covariance method introduced by Jack *et al.* [54] was superior to correction by division, resulted in a greater reduction in variance, and increased identification of hippocampal sclerosis in patients with TLE. Correction through division by whole brain volume is more effective than division by total intracranial volume, as the total intracranial volume remains constant with age, whereas total brain volume decreases. [94] Several studies have also shown that total brain volume is a significant predictor of subcortical volumes. [73;95;96] An important study by Bigler ***et al.*** [97] revealed that hippocampal volumes corrected with whole brain volume rather than total intracranial volume provide greater specificity and sensitivity.

The reliability of measurements and scan-rescan reproducibility of hippocampal volume measurement research is a source of major interstudy measurement variability. [43] The reductions found in the various disorders are usually small and change little over time, thus careful measurements that are reproducible should be made at all times. [87] In all studies, regardless of whether one or more raters are used, interrater and intrarater reliability values equal to or greater than 0.9 should be attained for the hippocampus. Prospective studies should also employ a similar protocol at all times even though better criteria exist several years after the original study was performed. Research has demonstrated that MRI derived hippocampal volumes may be reliably acquired in different research centers. [98]

As becomes evident from Table 1, hippocampal boundaries differ quite a lot among the major protocols. There is also considerable variation among the way researchers describe the hippocampal borders. Whereas some provide accurate descriptions supported with pictures and diagrams, others are very meagre in their account of what they consider to be the hippocampus. Although, previously, a reliable distinction between amygdala and the hippocampus was difficult, due to technical limitations, currently there is no empirical reason to warrant not measuring the structures separately. Measurements of the hippocampus should include the hippocampus only and should not be done on the hippocampal-amygdala complex as a whole. Studies of fear conditioning have shown that the amygdala plays a critical role in linking external stimuli to defense responses, especially those associated with fear. [99] In addition, the amygdala is a site for some aspects of emotional memory and modulates memory-related processes in the hippocampus. [100] In many of the manual tracing protocols, the amygdala is measured in addition to the hippocampus. The variability in volumetric studies of the amygdala is less than in the field of hippocampal volumetry, but striving for more consistency in that field should also be encouraged. Illness affects the hippocampus and amygdala differently, and researchers should measure the structures separately to obtain a more accurate picture of the morphological changes underlying neuropsychiatric disorders.

Whereas some protocols include the alveus in their conception of the hippocampus, [20;46;101] others choose to ignore the alveus. [102] Strictly speaking, the alveus is a white matter tract containing axons from hippocampal, subicular, and septal neurons.[103] To avoid confusion, it may be best to include it in its entirety. A large number of the protocols use the alveus to separate the hippocampus from the amygdala. Others use the mamillary bodies to separate amygdaloid and hippocampal tissue. [55] Similarly, the protocols differ in their inclusion of the subiculum and the uncal cleft, which seriously affects the comparability of these protocols. Inclusion of the subiculum may increase the volume of the hippocampus by as much as 15%. Measuring the tail of the hippocampus is the most difficult part, but using the coronal section on which the crux of the fornices is seen in full profile, allows measurement of the head, body, and most of the tail of the hippocampus (90-95%). [20;57] Several authors define the posterior border of the hippocampus as the crura of the fornices, [20;21;54;57;104] but others use the presence of the inferior and superior colliculli, [46;105] or the absence of the vertical fissures of the Sylvian fissure instead. [89] All of these differing anatomical boundary definitions are a source of major variation among the protocols and constitute the largest source of discrepancy in normative hippocampal volumes found by the various research studies. Proper referencing to a detailed description of the anatomical criteria used, or complete descriptions of the criteria used should be included by all researchers.

Besides the issues mentioned above, other factors such as developmental and gender aspects also affect hippocampal volume. Hippocampal volumetric studies should use proper control groups, which are matched for handedness, IQ, gender, and age. Szabo *et al.* [106] showed that right-to-left volume ratios differed significantly between right- and left-handed participants for both amygdala and hippocampus. Full scale IQ and explicit memory are significantly related to hippocampal volume. [107-109] Hippocampal volumes are also subject to gender differences.

Several studies have shown [110]that the volume of the hippocampal formation is larger in men than in women. [1;73;111] In developing children aged 4-18, the hippocampus increases with age. [112] In men, the hippocampus declines with age, starting in the third life decade. [113] From the age of 54 years hippocampal volume starts to decline at an increased rate (compared to total brain atrophy) in both men and women. [114] These factors should also be taken into account when comparing the results found in different studies.

Although manual volumetry is still one of the most popular methods to determine hippocampal volumes, automated methods are coming into vogue as well. One of the most troubling aspects of manual tracing is the subjective interpretation of anatomic variations. As early as 1993, Colombo *et al.* [28] introduced an automated method for determining the volume of the amygdala-hippocampal complex. Voxel-based morphometry is an automatic-whole-brain method which is gaining popularity and has been used to determine hippocampal morphometric changes, [31;115-124] however, these do not provide absolute hippocampal volumes.

Other automated methods that do provide absolute hippocampal volumes are being developed at a rapid pace. The Knowledge-Guided MRI analysis program is one such program, which uses a combination of pixel intensity and spatial relationship of atomic structures to derive hippocampal volume. [125] A similar method introduced by Ashton *et al.* [126] makes use of gray-scale and edge-detection algorithms as well as some *a priori* knowledge in determining hippocampal volume. The method proposed by Webb *et al.* [127] involves warping an atlas (obtained by manual volumetrics of 30 individuals) to the individual MR image. Another important automated method is the method used by Haller and colleagues, [128-131] which uses a high-dimensional fluid transformation to warp a template of the hippocampus and surrounding anatomical structures to an individual MR image. This method has also been validated, and was found to have less variability than manual tracing. [132] Regional fluid registration of serial MRI to investigate brain change has also been shown to have superior scan-rescan volumetric consistency; the mean absolute volume difference between manual and automatic methods was 0.7% .[133] However, not all, of these deformable shape methods take normal hippocampal shape variation into account. Using a deformable shape method which combines geometric properties of hippocampal boundaries, statistical characterization of normal shape variation, and manually defined boundary points, Shen *et al.* [134] demonstrated excellent agreement between automatic and manual volumetrics of the hippocampus. Shenton *et al.* [135] has used an active, flexible deformable shape model for the automatic volumetrics of the amygdala-hippocampal complex to investigate volumetric changes in schizophrenia. These automated methods mark the onset of a new era in structural neuroimaging. It will not be long before manual volumetrics is replaced by automatic volumetric methods which produce similar but more consistent results. This will also make it easier to implement a common methodology, although the various opinions on automated volumetric methods that exist today will very likely continue their existence far into the future.

Future Directions

An appreciation of the differences in research methodology helps to understand discrepancies in research findings. Ideally, researchers would adopt a universal methodology. This would lead to more consistent results in neuropsychiatric studies of hippocampal volume, and allow researchers to compare results of different studies. However, diversity, fuelled by healthy scepticism is inevitably part of the advancement of science. Automated volumetrics, which has already found widespread use in various other brain structures, may also play an important role in the field of hippocampal volumetry. Until then, manual tracing remains the gold standard.

References

1. Bhatia, S., Bookheimer, S.Y., Gaillard, W.D., Theodore, W.H. Measurement of whole temporal lobe and hippocampus for MR volumetry: normative data. Neurology 43, 2006-10 (1993).

2. Honeycutt, N.A. and Smith, C.D. Hippocampal volume measurements using magnetic resonance imaging in normal young adults. J Neuroimaging 5, 95-100 (1995).

3. Cendes, F., Andermann, F., Gloor, P., Evans, A., Jones-Gotman, M., Watson, C. et al. MRI volumetric measurement of amygdala and hippocampus in temporal lobe epilepsy. Neurology 43, 719-25 (1993).

4. Rosas, H.D., Koroshetz, W.J., Chen, Y.I., Skeuse, C., Vangel, M., Cudkowicz, M.E. et al. Evidence for more widespread cerebral pathology in early HD: An MRI-based morphometric analysis. Neurology 60, 1615-20 (2003).

5. Murphy, D.G., DeCarli, C., Daly, E., Haxby, J.V., Allen, G., White, B.J. et al. X-chromosome effects on female brain: a magnetic resonance imaging study of Turner's syndrome. Lancet 342, 1197-200 (1993).

6. Starkman, M.N., Gebarski, S.S., Berent, S., Schteingart, D.E. Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with Cushing's syndrome. Biol Psychiatry 32, 756-65 (1992).

7. Raz, N., Torres, I.J., Briggs, S.D., Spencer, W.D., Thornton, A.E., Loken, W.J. et al. Selective neuroanatomic abnormalities in Down's syndrome and their cognitive correlates: evidence from MRI morphometry. Neurology 45, 356-66 (1995).

8. Chetelat, G. and Baron, J.C. Early diagnosis of Alzheimer's disease: contribution of structural neuroimaging. Neuroimage 18, 525-41 (2003).

9. Jack, C.R. Jr, Petersen, R.C., Xu, Y.C., O'Brien, P.C., Smith, G.E., Ivnik, R.J. et al. Prediction of AD with MRI-based hippocampal volume in mild cognitive impairment. Neurology 52, 1397-403 (1999).

10. Heckers, S. Neuroimaging studies of the hippocampus in schizophrenia. Hippocampus 11, 520-8 (2001).

11. Sheline, Y.I., Wang, P.W., Gado, M.H., Csernansky, J.G., Vannier, M.W. Hippocampal atrophy in recurrent major depression. Proc Natl Acad Sci U S A 93, 3908-13 (1996).

12. Strakowski, S.M., DelBello, M.P., Sax, K.W., Zimmerman, M.E., Shear, P.K., Hawkins, J.M. et al. Brain magnetic resonance imaging of structural abnormalities in bipolar disorder. Arch Gen Psychiatry 56, 254-60 (1999).

13. Bremner, J.D., Randall, P., Scott, T.M., Bronen, R.A., Seibyl, J.P., Southwick, S.M. et al. MRI-based measurement of hippocampal volume in patients with combat-related posttraumatic stress disorder. Am J Psychiatry 152, 973-81 (1995).

14. Driessen, M., Herrmann, J., Stahl, K., Zwaan, M., Meier, S., Hill, A. et al. Magnetic resonance imaging volumes of the hippocampus and the amygdala in women with borderline personality disorder and early traumatization. Arch Gen Psychiatry 57, 1115-22 (2000).

15. Sullivan, E.V., Marsh, L., Mathalon, D.H., Lim, K.O., Pfefferbaum, A. Anterior hippocampal volume deficits in nonamnesic, aging chronic alcoholics. Alcohol Clin Exp Res 19, 110-22 (1995).

16. Kwon, J.S., Shin, Y.W., Kim, C.W., Kim, Y.I., Youn, T., Han, M.H. et al. Similarity and disparity of obsessive-compulsive disorder and schizophrenia in MR volumetric abnormalities of the hippocampus-amygdala complex. J Neurol Neurosurg Psychiatry 74, 962-4 (2003).

17. Vythilingam, M., Anderson, E.R., Goddard, A., Woods, S.W., Staib, L.H., Charney, D.S. et al. Temporal lobe volume in panic disorder--a quantitative magnetic resonance imaging study. Psychiatry Res 99, 75-82 (2000).

18. Jack, C.R. Jr, Bentley, M.D., Twomey, C.K., Zinsmeister, A.R. MR imaging-based volume measurements of the hippocampal formation and anterior temporal lobe: validation studies. Radiology 176, 205-9 (1990).

19. Pantel, J., O'Leary, D.S., Cretsinger, K., Bockholt, H.J., Keefe, H., Magnotta, V.A. et al. A new method for the in vivo volumetric measurement of the human hippocampus with high neuroanatomical accuracy. Hippocampus 10, 752-8 (2000).

20. Watson, C., Andermann, F., Gloor, P., Jones-Gotman, M., Peters, T., Evans, A. et al. Anatomic basis of amygdaloid and hippocampal volume measurement by magnetic resonance imaging. Neurology 42, 1743-50 (1992).

21. Cook, M.J., Fish, D.R., Shorvon, S.D., Straughan, K., Stevens, J.M. Hippocampal volumetric and morphometric studies in frontal and temporal lobe epilepsy. Brain 115 (Pt 4) , 1001-15 (1992).

22. Bogerts, B., Lieberman, J.A., Ashtari, M., Bilder, R.M., Degreef, G., Lerner, G. et al. Hippocampus-amygdala volumes and psychopathology in chronic schizophrenia. Biol Psychiatry 33, 236-46 (1993).

23. Breier, A., Buchanan, R.W., Elkashef, A., Munson, R.C., Kirkpatrick, B., Gellad, F. Brain morphology and schizophrenia. A magnetic resonance imaging study of limbic, prefrontal cortex, and caudate structures. Arch Gen Psychiatry 49, 921-6 (1992).

24. Gur, R.E., Turetsky, B.I., Cowell, P.E., Finkelman, C., Maany, V., Grossman, R.I. et al. Temporolimbic volume reductions in schizophrenia. Arch Gen Psychiatry 57, 769-75 (2000).

25. Rossi, A., Stratta, P., Mancini, F., Gallucci, M., Mattei, P., Core, L. et al. Magnetic resonance imaging findings of amygdala-anterior hippocampus shrinkage in male patients with schizophrenia. Psychiatry Res 52, 43-53 (1994).

26. Suddath, R.L., Christison, G.W., Torrey, E.F., Casanova, M.F., Weinberger, D.R. Anatomical abnormalities in the brains of monozygotic twins discordant for schizophrenia. N Engl J Med 322, 789-94 (1990).

27. Velakoulis, D., Stuart, G.W., Wood, S.J., Smith, D.J., Brewer, W.J., Desmond, P. et al. Selective bilateral hippocampal volume loss in chronic schizophrenia. Biol Psychiatry 50, 531-9 (2001).

28. Colombo, C., Abbruzzese, M., Livian, S., Scotti, G., Locatelli, M., Bonfanti, A. et al. Memory functions and temporal-limbic morphology in schizophrenia. Psychiatry Res 50, 45-56 (1993).

29. Deicken, R.F., Pegues, M., Amend, D. Reduced hippocampal N-acetylaspartate without volume loss in schizophrenia. Schizophr Res 37, 217-23 (1999).

30. Torres, I.J., Flashman, L.A., O'Leary, D.S., Swayze, V. 2nd, Andreasen, N.C. Lack of an association between delayed memory and hippocampal and temporal lobe size in patients with schizophrenia and healthy controls. Biol Psychiatry 42, 1087-96 (1997).

31. Wright, I.C., McGuire, P.K., Poline, J.B., Travere, J.M., Murray, R.M., Frith, C.D. et al. A voxel-based method for the statistical analysis of gray and white matter density applied to schizophrenia. Neuroimage 2, 244-52 (1995).

32. Gurvits, T.V., Shenton, M.E., Hokama, H., Ohta, H., Lasko, N.B., Gilbertson, M.W. et al. Magnetic resonance imaging study of hippocampal volume in chronic, combat-related posttraumatic stress disorder. Biol Psychiatry 40, 1091-9 (1996).

33. Villarreal, G., Hamilton, D.A., Petropoulos, H., Driscoll, I., Rowland, L.M., Griego, J.A. et al. Reduced hippocampal volume and total white matter volume in posttraumatic stress disorder. Biol Psychiatry 52, 119-25 (2002).

34. Bonne, O., Brandes, D., Gilboa, A., Gomori, J.M., Shenton, M.E., Pitman, R.K. et al. Longitudinal MRI study of hippocampal volume in trauma survivors with PTSD. Am J Psychiatry 158, 1248-51 (2001).

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

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

37. Frodl, T., Meisenzahl, E.M., Zetzsche, T., Born, C., Groll, C., Jager, M. et al. Hippocampal changes in patients with a first episode of major depression. Am J Psychiatry 159, 1112-8 (2002).

38. Sheline, Y.I., Sanghavi, M., Mintun, M.A., Gado, M.H. Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. J Neurosci 19, 5034-43 (1999).

39. Vakili, K., Pillay, S.S., Lafer, B., Fava, M., Renshaw, P.F., Bonello-Cintron, C.M. et al. Hippocampal volume in primary unipolar major depression: a magnetic resonance imaging study. Biol Psychiatry 47, 1087-90 (2000).

40. von Gunten, A., Fox, N.C., Cipolotti, L., Ron, M.A. A volumetric study of hippocampus and amygdala in depressed patients with subjective memory problems. J Neuropsychiatry Clin Neurosci 12, 493-8 (2000).

41. MacFall, J.R., Byrum, C.E., Parashos, I., Early, B., Charles, H.C., Chittilla, V. et al. Relative accuracy and reproducibility of regional MRI brain volumes for point-counting methods. Psychiatry Res 55, 167-77 (1994).

42. Mackay, C.E., Webb, J.A., Eldridge, P.R., Chadwick, D.W., Whitehouse, G.H., Roberts, N. Quantitative magnetic resonance imaging in consecutive patients evaluated for surgical treatment of temporal lobe epilepsy. Magn Reson Imaging 18, 1187-99 (2000).

43. Jack, C.R. Jr, Theodore, W.H., Cook, M., McCarthy, G. MRI-based hippocampal volumetrics: data acquisition, normal ranges, and optimal protocol. Magn Reson Imaging 13, 1057-64 (1995).

44. Kates, W.R., Abrams, M.T., Kaufmann, W.E., Breiter, S.N., Reiss, A.L. Reliability and validity of MRI measurement of the amygdala and hippocampus in children with fragile X syndrome. Psychiatry Res 75, 31-48 (1997).

45. Pruessner, J.C., Li, L.M., Serles, W., Pruessner, M., Collins, D.L., Kabani, N. et al. Volumetry of hippocampus and amygdala with high-resolution MRI and three-dimensional analysis software: minimizing the discrepancies between laboratories. Cereb Cortex 10, 433-42 (2000).

46. Bartzokis, G., Mintz, J., Marx, P., Osborn, D., Gutkind, D., Chiang, F. et al. Reliability of in vivo volume measures of hippocampus and other brain structures using MRI. Magn Reson Imaging 11, 993-1006 (1993).

47. Bartzokis, G., Aravagiri, M., Oldendorf, W.H., Mintz, J., Marder, S.R. Field dependent transverse relaxation rate increase may be a specific measure of tissue iron stores. Magn Reson Med 29, 459-64 (1993).

48. Briellmann, R.S., Syngeniotis, A., Jackson, G.D. Comparison of hippocampal volumetry at 1.5 tesla and at 3 tesla. Epilepsia 42, 1021-4 (2001).

49. Levy-Reiss, I., Gonzalez-Atavales, J., King-Stephens, D., French, J. A., Baltuch, G., Bagley, L.,Detre, J. A. High Resolution Imaging at 4 Tesla for Hippocampal Volumetry in Temporal Lobe Epilepsy. Proc Intl Soc Magn Reson Med 8, 15. 2000.

50. Detre, J. A. Personal Communication . 2003.

51. Kaye, J.A., Swihart, T., Howieson, D., Dame, A., Moore, M.M., Karnos, T. et al. Volume loss of the hippocampus and temporal lobe in healthy elderly persons destined to develop dementia. Neurology 48, 1297-304 (1997).

52. Kim, J.H., Tien, R.D., Felsberg, G.J., Osumi, A.K., Lee, N. MR measurements of the hippocampus for lateralization of temporal lobe epilepsy: value of measurements of the body vs the whole structure. AJR Am J Roentgenol 163, 1453-7 (1994).

53. Ashtari, M., Barr, W.B., Schaul, N., Bogerts, B. Three-dimensional fast low-angle shot imaging and computerized volume measurement of the hippocampus in patients with chronic epilepsy of the temporal lobe. AJNR Am J Neuroradiol 12, 941-7 (1991).

54. Jack, C.R. Jr, Twomey, C.K., Zinsmeister, A.R., Sharbrough, F.W., Petersen, R.C., Cascino, G.D. Anterior temporal lobes and hippocampal formations: normative volumetric measurements from MR images in young adults. Radiology 172, 549-54 (1989).

55. Shenton, M.E., Kikinis, R., Jolesz, F.A., Pollak, S.D., LeMay, M., Wible, C.G. et al. Abnormalities of the left temporal lobe and thought disorder in schizophrenia. A quantitative magnetic resonance imaging study. N Engl J Med 327, 604-12 (1992).

56. Soininen, H.S., Partanen, K., Pitkanen, A., Vainio, P., Hanninen, T., Hallikainen, M. et al. Volumetric MRI analysis of the amygdala and the hippocampus in subjects with age-associated memory impairment: correlation to visual and verbal memory. Neurology 44, 1660-8 (1994).

57. Jack, C.R. Jr. MRI-based hippocampal volume measurements in epilepsy. Epilepsia 35 Suppl 6, S21-9 (1994).

58. Laakso, M.P., Juottonen, K., Partanen, K., Vainio, P., Soininen, H. MRI volumetry of the hippocampus: the effect of slice thickness on volume formation. Magn Reson Imaging 15, 263-5 (1997).

59. MacQueen, G.M., Campbell, S., McEwen, B.S., Macdonald, K., Amano, S., Joffe, R.T. et al. Course of illness, hippocampal function, and hippocampal volume in major depression. Proc Natl Acad Sci U S A 100, 1387-92 (2003).

60. Fuerst, D., Shah, J., Shah, A., Watson, C. Hippocampal sclerosis is a progressive disorder: a longitudinal volumetric MRI study. Ann Neurol 53, 413-6 (2003).

61. Watson, C., Cendes, F., Fuerst, D., Dubeau, F., Williamson, B., Evans, A. et al. Specificity of volumetric magnetic resonance imaging in detecting hippocampal sclerosis. Arch Neurol 54, 67-73 (1997).

62. Press, G.A., Amaral, D.G., Squire, L.R. Hippocampal abnormalities in amnesic patients revealed by high-resolution magnetic resonance imaging. Nature 341, 54-7 (1989).

63. Hasboun, D., Chantome, M., Zouaoui, A., Sahel, M., Deladoeuille, M., Sourour, N. et al. MR determination of hippocampal volume: comparison of three methods. AJNR Am J Neuroradiol 17, 1091-8 (1996).

64. Synek, V. and Reuben, J.R. The ventricular-brain ratio using planimetric measurement of EMI scans. Br J Radiol 49, 233-7 (1976).

65. Zatz, L.M. and Jernigan, T.L. The ventricular-brain ratio on computed tomography scans: validity and proper use. Psychiatry Res 8, 207-14 (1983).

66. Arndt, S., Cohen, G., Alliger, R.J., Swayze, V.W. 2nd, Andreasen, N.C. Problems with ratio and proportion measures of imaged cerebral structures. Psychiatry Res 40, 79-89 (1991).

67. Mathalon, D.H., Sullivan, E.V., Rawles, J.M., Pfefferbaum, A. Correction for head size in brain-imaging measurements. Psychiatry Res 50, 121-39 (1993).

68. Jack, C.R. Jr, Petersen, R.C., O'Brien, P.C., Tangalos, E.G. MR-based hippocampal volumetry in the diagnosis of Alzheimer's disease. Neurology 42, 183-8 (1992).

69. Huckman, M.S., Fox, J., Topel, J. The validity of criteria for the evaluation of cerebral atrophy by computed tomography. Radiology 116, 85-92 (1975).

70. Pearlson, G.D., Harris, G.J., Powers, R.E., Barta, P.E., Camargo, E.E., Chase, G.A. et al. Quantitative changes in mesial temporal volume, regional cerebral blood flow, and cognition in Alzheimer's disease. Arch Gen Psychiatry 49, 402-8 (1992).

71. Colchester, A., Kingsley, D., Lasserson, D., Kendall, B., Bello, F., Rush, C. et al. Structural MRI volumetric analysis in patients with organic amnesia, 1: methods and comparative findings across diagnostic groups. J Neurol Neurosurg Psychiatry 71, 13-22 (2001).

72. Kopelman, M.D., Lasserson, D., Kingsley, D., Bello, F., Rush, C., Stanhope, N. et al. Structural MRI volumetric analysis in patients with organic amnesia, 2: correlations with anterograde memory and executive tests in 40 patients. J Neurol Neurosurg Psychiatry 71, 23-8 (2001).

73. Free, S.L., Bergin, P.S., Fish, D.R., Cook, M.J., Shorvon, S.D., Stevens, J.M. Methods for normalization of hippocampal volumes measured with MR. AJNR Am J Neuroradiol 16, 637-43 (1995).

74. Cascino, G.D., Jack, C.R. Jr, Parisi, J.E., Marsh, W.R., Kelly, P.J., Sharbrough, F.W. et al. MRI in the presurgical evaluation of patients with frontal lobe epilepsy and children with temporal lobe epilepsy: pathologic correlation and prognostic importance. Epilepsy Res 11, 51-9 (1992).

75. Cendes, F., Leproux, F., Melanson, D., Ethier, R., Evans, A., Peters, T. et al. MRI of amygdala and hippocampus in temporal lobe epilepsy. J Comput Assist Tomogr 17, 206-10 (1993).

76. Achten, E., Deblaere, K., De Wagter, C., Van Damme, F., Boon, P., De Reuck, J. et al. Intra- and interobserver variability of MRI-based volume measurements of the hippocampus and amygdala using the manual ray-tracing method. Neuroradiology 40, 558-66 (1998).

77. Shrout, P. E. and Fleiss, J. L. Psychological Bulletin 86, 420-428. 79.

78. Wieshmann, U.C., Free, S.L., Stevens, J.M., Shorvon, S.D. Image contrast and hippocampal volumetric measurements. Magn Reson Imaging 16, 13-7 (1998).

79. Haase, A. Snapshot FLASH MRI. Applications to T1, T2, and chemical-shift imaging. Magn Reson Med 13, 77-89 (1990).

80. Haase, A., Frahm, J., Matthaei, W., Haenicke, W.,Merboldt, K. D. FLASH Imaging, Rapid NMR imaging using low flip angle pulses. J Magn Reson 67, 256-266. 86 .

81. Reiser, M. and Faber, S.C. Recent and future advances in high-speed imaging. Eur Radiol 7 Suppl 5, 166-73 (1997).

82. Mugler, J.P. 3rd. Overview of MR imaging pulse sequences. Magn Reson Imaging Clin N Am 7, 661-97 (1999).

83. Jackson, E.F., Ginsberg, L.E., Schomer, D.F., Leeds, N.E. A review of MRI pulse sequences and techniques in neuroimaging. Surg Neurol 47, 185-99 (1997).

84. Deichmann, R., Good, C.D., Josephs, O., Ashburner, J., Turner, R. Optimization of 3-D MP-RAGE sequences for structural brain imaging. Neuroimage 12, 112-27 (2000).

85. Bydder, G.M., Hajnal, J.V., Young, I.R. MRI: use of the inversion recovery pulse sequence. Clin Radiol 53, 159-76 (1998).

86. Wieshmann, U.C., Symms, M.R., Mottershead, J.P., MacManus, D.G., Barker, G.J., Tofts, P.S. et al. Hippocampal layers on high resolution magnetic resonance images: real or imaginary? J Anat 195 (Pt 1), 131-5 (1999).

87. Bartzokis, G., Altshuler, L.L., Greider, T., Curran, J., Keen, B., Dixon, W.J. Reliability of medial temporal lobe volume measurements using reformatted 3D images. Psychiatry Res 82, 11-24 (1998).

88. Jacobsen, L.K., Giedd, J.N., Vaituzis, A.C., Hamburger, S.D., Rajapakse, J.C., Frazier, J.A. et al. Temporal lobe morphology in childhood-onset schizophrenia. Am J Psychiatry 153, 355-61 (1996).

89. Zipursky, R.B., Marsh, L., Lim, K.O., DeMent, S., Shear, P.K., Sullivan, E.V. et al. Volumetric MRI assessment of temporal lobe structures in schizophrenia. Biol Psychiatry 35, 501-16 (1994).

90. Buchanan, R.W., Breier, A., Kirkpatrick, B., Elkashef, A., Munson, R.C., Gellad, F. et al. Structural abnormalities in deficit and nondeficit schizophrenia. Am J Psychiatry 150, 59-65 (1993).

91. Coffey, C.E., Wilkinson, W.E., Weiner, R.D., Parashos, I.A., Djang, W.T., Webb, M.C. et al. Quantitative cerebral anatomy in depression. A controlled magnetic resonance imaging study. Arch Gen Psychiatry 50, 7-16 (1993).

92. DeLisi, L.E., Tew, W., Xie, S., Hoff, A.L., Sakuma, M., Kushner, M. et al. A prospective follow-up study of brain morphology and cognition in first-episode schizophrenic patients: preliminary findings. Biol Psychiatry 38, 349-60 (1995).

93. Sullivan, E.V., Marsh, L., Mathalon, D.H., Lim, K.O., Pfefferbaum, A. Age-related decline in MRI volumes of temporal lobe gray matter but not hippocampus. Neurobiol Aging 16, 591-606 (1995).

94. Mori, E., Hirono, N., Yamashita, H., Imamura, T., Ikejiri, Y., Ikeda, M. et al. Premorbid brain size as a determinant of reserve capacity against intellectual decline in Alzheimer's disease. Am J Psychiatry 154, 18-24 (1997).

95. Finlay, B.L. and Darlington, R.B. Linked regularities in the development and evolution of mammalian brains. Science 268, 1578-84 (1995).

96. Mueller, E.A., Moore, M.M., Kerr, D.C., Sexton, G., Camicioli, R.M., Howieson, D.B. et al. Brain volume preserved in healthy elderly through the eleventh decade. Neurology 51, 1555-62 (1998).

97. Bigler, E.D. and Tate, D.F. Brain volume, intracranial volume, and dementia. Invest Radiol 36, 539-46 (2001).

98. Jack, C.R. Jr, Slomkowski, M., Gracon, S., Hoover, T.M., Felmlee, J.P., Stewart, K. et al. MRI as a biomarker of disease progression in a therapeutic trial of milameline for AD. Neurology 60, 253-60 (2003).

99. LeDoux, J. The emotional brain, fear, and the amygdala. Cell Mol Neurobiol 23, 727-38 (2003).

100. Richter-Levin, G. The amygdala, the hippocampus, and emotional modulation of memory. Neuroscientist 10, 31-9 (2004).

101. Spencer, S.S., McCarthy, G., Spencer, D.D. Diagnosis of medial temporal lobe seizure onset: relative specificity and sensitivity of quantitative MRI. Neurology 43, 2117-24 (1993).

102. Giedd, J.N., Vaituzis, A.C., Hamburger, S.D., Lange, N., Rajapakse, J.C., Kaysen, D. et al. Quantitative MRI of the temporal lobe, amygdala, and hippocampus in normal human development: ages 4-18 years. J Comp Neurol 366, 223-30 (1996).

103. Duvernoy, H. M. The Human Hippocampus: Functional Anatomy, Vascularization, and Serial Sections with MRI. 98.

104. Steciuk, M., Kram, M., Kramer, G.L., Petty, F. Acute stress does not alter 5-HT1A receptor density. Prog Neuropsychopharmacol Biol Psychiatry 24, 155-61 (2000).

105. Bigler, E.D., Blatter, D.D., Anderson, C.V., Johnson, S.C., Gale, S.D., Hopkins, R.O. et al. Hippocampal volume in normal aging and traumatic brain injury. AJNR Am J Neuroradiol 18, 11-23 (1997).

106. Szabo, C.A., Xiong, J., Lancaster, J.L., Rainey, L., Fox, P. Amygdalar and hippocampal volumetry in control participants: differences regarding handedness. AJNR Am J Neuroradiol 22, 1342-5 (2001).

107. Andreasen, N.C., Flaum, M., Swayze, V. 2nd, O'Leary, D.S., Alliger, R., Cohen, G. et al. Intelligence and brain structure in normal individuals. Am J Psychiatry 150, 130-4 (1993).

108. Foster, J.K., Meikle, A., Goodson, G., Mayes, A.R., Howard, M., Sunram, S.I. et al. The hippocampus and delayed recall: bigger is not necessarily better? Memory 7, 715-32 (1999).

109. Chantome, M., Perruchet, P., Hasboun, D., Dormont, D., Sahel, M., Sourour, N. et al. Is there a negative correlation between explicit memory and hippocampal volume? Neuroimage 10, 589-95 (1999).

110. Salmenpera, T., Kalviainen, R., Partanen, K., Mervaala, E., Pitkanen, A. MRI volumetry of the hippocampus, amygdala, entorhinal cortex, and perirhinal cortex after status epilepticus. Epilepsy Res 40, 155-70 (2000).

111. Raz, N., Gunning, F.M., Head, D., Dupuis, J.H., McQuain, J., Briggs, S.D. et al. Selective aging of the human cerebral cortex observed in vivo: differential vulnerability of the prefrontal gray matter. Cereb Cortex 7, 268-82 (1997).

112. Giedd, J.N., Castellanos, F.X., Rajapakse, J.C., Vaituzis, A.C., Rapoport, J.L. Sexual dimorphism of the developing human brain. Prog Neuropsychopharmacol Biol Psychiatry 21, 1185-201 (1997).

113. Pruessner, J.C., Collins, D.L., Pruessner, M., Evans, A.C. Age and gender predict volume decline in the anterior and posterior hippocampus in early adulthood. J Neurosci 21, 194-200 (2001).

114. Liu, R.S., Lemieux, L., Bell, G.S., Sisodiya, S.M., Shorvon, S.D., Sander, J.W. et al. A longitudinal study of brain morphometrics using quantitative magnetic resonance imaging and difference image analysis. Neuroimage 20, 22-33 (2003).

115. Burton, E.J., Karas, G., Paling, S.M., Barber, R., Williams, E.D., Ballard, C.G. et al. Patterns of cerebral atrophy in dementia with Lewy bodies using voxel-based morphometry. Neuroimage 17, 618-30 (2002).

116. Job, D.E., Whalley, H.C., McConnell, S., Glabus, M., Johnstone, E.C., Lawrie, S.M. Structural gray matter differences between first-episode schizophrenics and normal controls using voxel-based morphometry. Neuroimage 17, 880-9 (2002).

117. Karas, G.B., Burton, E.J., Rombouts, S.A., van Schijndel, R.A., O'Brien, J.T., Scheltens, P. et al. A comprehensive study of gray matter loss in patients with Alzheimer's disease using optimized voxel-based morphometry. Neuroimage 18, 895-907 (2003).

118. Keller, S.S., Wieshmann, U.C., Mackay, C.E., Denby, C.E., Webb, J., Roberts, N. Voxel based morphometry of grey matter abnormalities in patients with medically intractable temporal lobe epilepsy: effects of side of seizure onset and epilepsy duration. J Neurol Neurosurg Psychiatry 73, 648-55 (2002).

119. Kubicki, M., Shenton, M.E., Salisbury, D.F., Hirayasu, Y., Kasai, K., Kikinis, R. et al. Voxel-based morphometric analysis of gray matter in first episode schizophrenia. Neuroimage 17, 1711-9 (2002).

120. Maguire, E.A., Spiers, H.J., Good, C.D., Hartley, T., Frackowiak, R.S., Burgess, N. Navigation expertise and the human hippocampus: a structural brain imaging analysis. Hippocampus 13, 250-9 (2003).

121. Mummery, C.J., Patterson, K., Price, C.J., Ashburner, J., Frackowiak, R.S., Hodges, J.R. A voxel-based morphometry study of semantic dementia: relationship between temporal lobe atrophy and semantic memory. Ann Neurol 47, 36-45 (2000).

122. Shah, P.J., Ebmeier, K.P., Glabus, M.F., Goodwin, G.M. Cortical grey matter reductions associated with treatment-resistant chronic unipolar depression. Controlled magnetic resonance imaging study. Br J Psychiatry 172, 527-32 (1998).

123. Woermann, F.G., Free, S.L., Koepp, M.J., Ashburner, J., Duncan, J.S. Voxel-by-voxel comparison of automatically segmented cerebral gray matter--A rater-independent comparison of structural MRI in patients with epilepsy. Neuroimage 10, 373-84 (1999).

124. Yamasue, H., Kasai, K., Iwanami, A., Ohtani, T., Yamada, H., Abe, O. et al. Voxel-based analysis of MRI reveals anterior cingulate gray-matter volume reduction in posttraumatic stress disorder due to terrorism. Proc Natl Acad Sci U S A 100, 9039-43 (2003).

125. Gosche, K.M., Mortimer, J.A., Smith, C.D., Markesbery, W.R., Snowdon, D.A. An automated technique for measuring hippocampal volumes from MR imaging studies. AJNR Am J Neuroradiol 22, 1686-9 (2001).

126. Ashton, E.A., Berg, M.J., Parker, K.J., Weisberg, J., Chen, C.W., Ketonen, L. Segmentation and feature extraction techniques, with applications to MRI head studies. Magn Reson Med 33, 670-7 (1995).

127. Webb, J., Guimond, A., Eldridge, P., Chadwick, D., Meunier, J., Thirion, J.P. et al. Automatic detection of hippocampal atrophy on magnetic resonance images. Magn Reson Imaging 17, 1149-61 (1999).

128. Cardenas, V.A., Du, A.T., Hardin, D., Ezekiel, F., Weber, P., Jagust, W.J. et al. Comparison of methods for measuring longitudinal brain change in cognitive impairment and dementia. Neurobiol Aging 24, 537-44 (2003).

129. Csernansky, J.G., Joshi, S., Wang, L., Haller, J.W., Gado, M., Miller, J.P. et al. Hippocampal morphometry in schizophrenia by high dimensional brain mapping. Proc Natl Acad Sci U S A 95, 11406-11 (1998).

130. Haller, J.W., Banerjee, A., Christensen, G.E., Gado, M., Joshi, S., Miller, M.I. et al. Three-dimensional hippocampal MR morphometry with high-dimensional transformation of a neuroanatomic atlas. Radiology 202, 504-10 (1997).

131. Wang, L., Joshi, S.C., Miller, M.I., Csernansky, J.G. Statistical analysis of hippocampal asymmetry in schizophrenia. Neuroimage 14, 531-45 (2001).

132. Hogan, R.E., Mark, K.E., Wang, L., Joshi, S., Miller, M.I., Bucholz, R.D. Mesial temporal sclerosis and temporal lobe epilepsy: MR imaging deformation-based segmentation of the hippocampus in five patients. Radiology 216, 291-7 (2000).

133. Crum, W.R., Scahill, R.I., Fox, N.C. Automated hippocampal segmentation by regional fluid registration of serial MRI: validation and application in Alzheimer's disease. Neuroimage 13, 847-55 (2001).

134. Shen, D., Moffat, S., Resnick, S.M., Davatzikos, C. Measuring size and shape of the hippocampus in MR images using a deformable shape model. Neuroimage 15, 422-34 (2002).

135. Shenton, M.E., Gerig, G., McCarley, R.W., Szekely, G., Kikinis, R. Amygdala-hippocampal shape differences in schizophrenia: the application of 3D shape models to volumetric MR data. Psychiatry Res 115, 15-35 (2002).

136. Bogerts, B., Ashtari, M., Degreef, G., Alvir, J.M., Bilder, R.M., Lieberman, J.A. Reduced temporal limbic structure volumes on magnetic resonance images in first episode schizophrenia. Psychiatry Res 35, 1-13 (1990).

137. Convit, A., de Leon, M.J., Tarshish, C., De Santi, S., Kluger, A., Rusinek, H. et al. Hippocampal volume losses in minimally impaired elderly. Lancet 345, 266 (1995).

138. Honeycutt, N.A., Smith, P.D., Aylward, E., Li, Q., Chan, M., Barta, P.E. et al. Mesial temporal lobe measurements on magnetic resonance imaging scans. Psychiatry Res 83, 85-94 (1998).

139. Van Paesschen, W., Connelly, A., King, M.D., Jackson, G.D., Duncan, J.S. The spectrum of hippocampal sclerosis: a quantitative magnetic resonance imaging study. Ann Neurol 41, 41-51 (1997).

3

MR based in vivo Hippocampal Volumetrics: 2. Findings in Neuropsychiatric Disorders

E. Geuze, E. Vermetten, and J.D. Bremner.

2005 **Molecular Psychiatry** *10(2): 160-184*

Introduction

MR based in vivo hippocampal volumetric assessment of the hippocampus has been a widely employed neuroimaging technique in various neuropsychiatric disorders. The hippocampus plays a vital role in processes of memory formation and stress and emotional regulation. Although the functions of the hippocampus are still somewhat elusive, in humans, the hippocampus has been directly implemented in spatial and episodic memory (see Burgess et al. [1] for a review). Lately the role of the hippocampus in semantic memory has been elucidated as well. [2;3] In addition, the hippocampus is also involved in novelty processing. [4;5] Within the hippocampus, functional segregation exists, with the left anterior hippocampus processing both behaviourally relevant and behaviourally irrelevant novelty as well as register mismatches between expectation and experience, and the posterior hippocampi processing familiarity. [4;6;7] Regulation of the hypothalamo-pituitary-adrenal (HPA) axis is another important function of the hippocampus. [8]

Glucocorticoid receptors in the hippocampus are activated by rising glucocorticoid levels during stress, in order to mediate fast feedback inhibition of the HPA axis. Stress, hypoxia, and increased glutamate have been associated with damage to the hippocampus, which has increased interest in this area in neuropsychiatric disorders. The hippocampus has been implicated in several neuropsychiatric disorders. Sullivan et al. [9] examined the extent to which genes and the environment exert differential contributions to hippocampal structural integrity in humans, and showed that the volume of the hippocampus, as measured on MRI, is subject to substantially less genetic control than comparison brain regions. Environmental factors thus play a large role in determining hippocampal morphometry.

The advent of MRI in the last few decades has witnessed an escalation of hippocampal volumetric studies in various neuropsychiatric disorders. The medial temporal limbic area is specifically affected in Alzheimer's disease (AD) and temporal lobe epilepsy (TLE), and hippocampal volumetric assessment has aided in diagnosis and etiology of these disorders. [10;11] Similarly, the psychotic features of schizophrenia have been attributed to abnormal hippocampal activity and a disturbance of hippocampal-cortical connections. [12] Work by Sapolsky et al. [13;14] and others on the effect of glucocorticoids and stress exposure on the hippocampus in rats provided the theoretical framework for hippocampal volumetric studies in stress- and anxiety-related disorders such as depression and PTSD. The noninvasive nature of MR based volumetric assessment has enabled researchers to assess the nature and longitudinal course of hippocampal volume in numerous other neuropsychiatric disorders as well.

However, studies have used a variety of different research designs and methodologies, and have also come up with (sometimes) inconsistent results. The companion paper (see chapter 2) has focused on the differences in segmentation protocols used. This paper will focus on findings in hippocampal volume in studies across the spectrum of neuropsychiatric disorders, from temporal lobe epilepsy and Huntington's disease, to schizophrenia and PTSD, thus establishing a global overview

of hippocampal volumetric findings which may be used to make theoretical assumptions as to what these hippocampal volume reductions actually mean, and how they relate to the etiology and course of these disorders.

Methods

We performed a Medline Indexed search with the keywords "hippocampus," "volume," and "MRI." All the abstracts were carefully scrutinized, and from this database all English-language, human subject, data-driven papers were selected yielding a database of 423 records (only papers published before December 31, 2003 were included). Major advances in MRI hardware and software were implemented from 1988, [15] and thus studies prior to 1988 were not included. In cases, where MRI studies reported data from the same subjects, but used different analyses, both references were included.

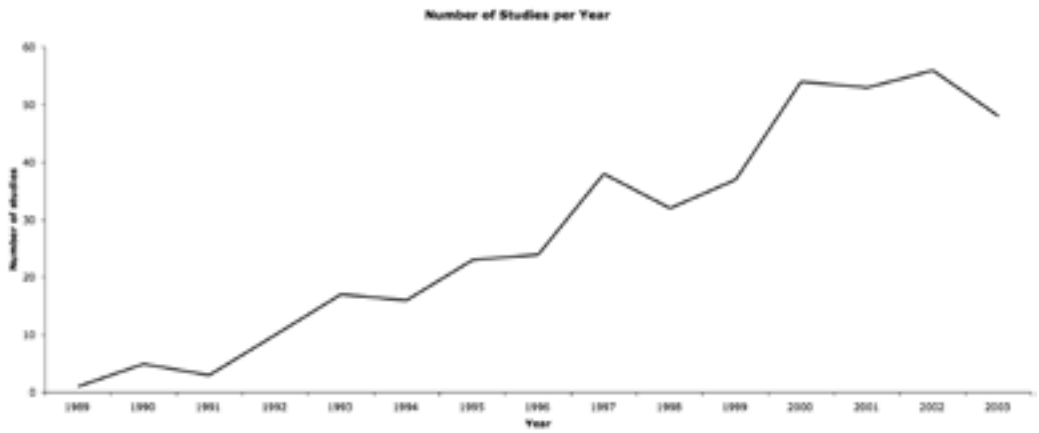


Figure 1: The number of MR-based hippocampal volumetric studies per year from 1989 to 2003.

Results

The number of MRI hippocampal volumetric studies performed has steadily increased over the last decades (see Figure 1). From 1992 onwards, the number of studies on hippocampal volume increases linearly. This increase stabilizes at approximately 50 studies per year by the year 2000. The increase in studies since 1992 was fuelled by several researchers who have published volumetric protocols and neuroanatomical guidelines which have been adopted by others. [16-21]

Hippocampal volumetric studies have been performed in more than forty different populations, and are especially popular in disorders such as TLE, schizophrenia, and AD. In our database, these populations have been re-grouped into thirty-four diagnostic categories (see Table 1). In the majority of these studies a decrease in hippocampal volume was expected, and subsequently found. However in a large number of neuropsychiatric disorders the data is not always as consistent as in studies with temporal lobe epileptic or AD patients. Although within disorders there is some consistency in the type of protocols that researchers have used, slight variations in each of these protocols may amount to significant differences in their findings (for a review see chapter 2).

Temporal Lobe Epilepsy

In temporal lobe epilepsy hippocampal volumetry has been played an important role in the determination of hippocampal sclerosis (HS) or hippocampal atrophy. Significant reduction in hippocampal volumes is used as a specific marker for HS, and right-side minus left-side hippocampal formation volume (DHF) is used to quantify unilateral HF atrophy. [22-31] These methods are superior to visual inspection of MR images. [32] Hippocampal volumetric analysis with MRI is not always able to detect hippocampal sclerosis accurately, [33] however, in those cases the additional analysis of entorhinal cortex volume or volume ratio analysis may be able to provide accurate lateralization of seizure focus (see Bernasconi et al. [34] and Vossler et al. [35] respectively). These methods have demonstrated considerable efficacy, especially with the addition of T2 relaxation time data. [36-41]

Patients with mesial temporal lobe epilepsy exhibit smaller hippocampal volumes. [16;42-47] This hippocampal volume reduction is highly concordant with the side of the epileptogenic focus, and hippocampal deficits are most pronounced ipsilateral to the epileptic focus. [48-52] If amygdala volume reductions are also documented, an additional gain in specificity of seizure lateratalization is achieved. [53;54] Quigg et al. [46] showed that hippocampi contralateral to the epileptic focus are also smaller in TLE than in controls, but larger than hippocampi ipsilateral to the epileptic focus (see also Lambert et al. [55]). Unilateral hippocampal volume loss and increased T2 value were found in 71% of patients with HS, and bilaterally normal hippocampal volume and T2 value were found in 67% of patients without HS. [36] Within the hippocampus, volume reduction is usually not uniform; the hippocampal head is more atrophic than the hippocampal body and hippocampal tail. [56] Lately several studies have also determined progressive volume loss in mesial TLE. [57;58] Hippocampal volume is correlated with entorhinal cortex volume in TLE, [59] and with flumazenil binding. [60]

A longer epilepsy duration, [61-64] a high number of seizures, [44;65-67] an earlier age of onset, [61;65;66;68] the presence of early aberrant neurological insults such as febrile convulsions, [65;66;68-70] and even gender (men have increased risk of seizure damage) , [71] have all been associated with smaller hippocampal volume in TLE. Some discrepancies exist here as well, as some studies have been unable to find a relation between seizure frequency or longer epilepsy duration and hippocampal volume. [72;73] In some studies satisfactory surgical outcome seems to be related to hippocampal atrophy prior to surgery, [50;74;75] but not in others. [47] Prompt treatment after a status epilepticus may prevent progressive hippocampal volume reduction. [76;77]

The volume reduction witnessed in TLE is the result of neuronal cell death. Lee et al. [78] compared MRI hippocampal volumes prior to anterior temporal lobectomy with quantitative neuronal density measurements in resected hippocampal specimens and found evidence for a significant correlation

Table 1: The number of studies in various neuropsychiatric disorders which have examined hippocampal volumes with MRI with some general findings.

Disorder	*Number of studies*	*General findings*
Temporal Lobe Epilepsy	84	↓ hippocampi, most pronounced ipsilateral to epileptic focus
Schizophrenia	76	↓ / ⟷hippocampi bilaterally
Alzheimer's Disease	56	↓ hippocampi bilaterally; marker for temporal lobe degeneration
Normal Controls	44	hippocampal volume is dependent on gender, handedness, and age
Other Epilepsy	23	↓ hippocampi bilaterally
Major Depression	20	⟷/ recently ↓ hippocampi bilaterally have been demonstrated
Aged	15	smaller hippocampi are associated with normal aging
PTSD	14	↓ /⟷smaller hippocampi bilaterally
Other Dementia	11	↓ hippocampi
Alcoholism	9	↓ / ⟷hippocampi bilaterally
Bipolar Disorder	7	↓ / ↑ hippocampal volume
Mild Cognitive Impairment	7	hippocampal volume loss predictive of conversion to AD
TBI	6	↓ hippocampi bilaterally
Autism	5	↓ / ↑ hippocampal volume
Down's Syndrome	5	↓ hippocampal volume bilaterally
APOE -epsilon 4 allele positive	3	additionally ↓ hippocampi compared to controls
Borderline Personality Disorder	3	↓ hippocampi bilaterally
Febrile Seizures	3	↓ / ⟷hippocampi
Herpes Simplex	3	↓ hippocampi
Korsakoff's Syndrome	3	↓ / ⟷ hippocampi
OCD	3	↓ / ⟷hippocampi bilaterally
Amnesia	2	↓ hippocampi bilaterally which correlates with impaired memory
Cardiac Arrest	2	↓ hippocampi
Cushing's Disease	2	↓ hippocampi bilaterally; volume increases after treatment
Fragile X Syndrome	2	↑ hippocampi bilaterally
Low Birth Weight	2	↓ hippocampi
Panic Disorder	2	⟷ hippocampi compared to controls
Parkinson's Disease	2	↓ hippocampi bilaterally
ADHD	1	⟷ hippocampi compared to controls
Anorexia Nervosa	1	⟷ hippocampi compared to controls
Antisocial Personality Disorder	1	volume of posterior hippocampi negatively correlated with psychopathy
Breast Cancer Surgery	1	↓ left hippocampi in women with distressing recollections
Congenital Adrenal Hyperplasia	1	⟷ hippocampi compared to controls
Fetal Alcohol Syndrome	1	⟷ hippocampi compared to controls
Huntington	1	↓ hippocampi bilaterally
Sleep Apnea	1	↓ gray matter concentration in hippocampi
Turner's Syndrome	1	↓ hippocampi bilaterally

↓ = smaller ↑ = larger ↓/↑ = both smaller and larger hippocampal volumes haven been reported ⟷no significant changes ↓/⟷= both smaller and no significant studies have been reported

of MR derived hippocampal volume with neuronal density in the CA1, CA2, and CA3 subfields of the hippocampus. This finding has been confirmed by Luby et al. [75] and Briellmann et al. [79] who found that the ipsilateral hippocampal volume best predicted the neuron cell count in the dentate gyrus, whereas the T2 relaxation time, on the other hand, best predicted the glial cell count in the dentate gyrus (see also Diehl et al. [80], Kuzniecky et al.[81], and Van Paesschen et al.[82]). It is not clear whether the neuronal cell death also constitutes functionally relevant tissue, as hippocampal volume loss is not a major determinant of regional hypometabolism in TLE. [83] Although a later study by Theodore et al. [84] was able to find a significant relation between hippocampal volume and glucose metabolism.

Studies in TLE have also correlated the left hippocampus with verbal memory. [85;86] Trenerry and colleagues found that the ratio of the right versus left hippocampal volume is significantly correlated with postoperative verbal memory change. [87] Later they demonstrated that left anterior temporal lobectomy (ATL) patients revealed an expected decrease in verbal memory postoperatively regardless of whether the volumetrically symmetric hippocampi were atrophic. [88] Left temporal lobectomy patients with bilaterally atrophic hippocampi have the poorest verbal memory before and after operation, a finding that has been corroborated by Martin et al. [89] who showed that patients with left TLE and the presence of bilateral hippocampal atrophy had worse verbal memory before and after ATL compared to patients with unilateral hippocampal atrophy or patients with right TLE and bilateral hippocampal atrophy. Baxendale et al. [90] demonstrated that patients with smaller remnant hippocampal volumes demonstrated more postoperative memory decline than those with larger remnant hippocampal volume, and that extensive shrinkage of the remnant volume was associated with postoperative memory decline in both right and left ATL patient groups.

Right temporal lobectomy patients tend to have improved verbal memory postoperatively independent of bilateral hippocampal atrophy. Although a relation of hippocampal volume with visual memory has been much harder to find, [85] Baxendale et al. [91] did show that right hippocampal volume was significantly correlated with delayed recall of a complex figure. Hippocampal asymmetry (right minus left hippocampal volume) is significantly correlated with right minus left intracarotid amobarbital memory scores. [92]

Hippocampal volumetry has also been used to determine region of interest, [93-95] or partial volume correction [96] for PET in temporal lobe epilepsy. A number of studies have also examined methodological issues in hippocampal volumetry in epilepsy such as, optimizing hippocampal volume determination, [17;97] the necessity of hippocampal volume normalization, [98-100] the comparability and reliability of manual and digitizer measurements, [49] the correlation of hippocampal body with total hippocampal volume, [101] the intra- and interobserver variability, [102] and the utility of automated methods. [31;103]

In summary, hippocampal volumetry with MRI is primarily utilized in the determination of hippocampal atrophy and hippocampal sclerosis. Pre- and postoperative hippocampal volumes are correlated with neurophysiological, neuropathological, neuropsychological, and clinical findings, as well as surgical outcome. [30] The presence of decreased hippocampal volume in TLE has been correlated with

decreased verbal memory pre- and postoperatively. Several studies have also evaluated the link between hippocampal volume and other predictors with outcome measures of ATL.

Other Epilepsy

In patients with porencephaly-related seizures, bilateral amygdala-hippocampal atrophy exists in the presence of unilateral cysts. [104] Reduced hippocampal volume, or loss of volume asymmetry has also been found in partial epilepsy, [105;106] and childhood epilepsy. [107;108] Voxel-by-voxel comparison of brain regions in juvenile myoclonic epilepsy and TLE failed to show hippocampal atrophy in either disorder. [109] Hippocampal volumetry data in temporal lobe epilepsy should be corrected for total brain volume, as this is the largest predictor of hippocampal volume. [110]

Traumatic Brain Injury

Arciniegas and colleagues reported significantly smaller hippocampal volume bilaterally in traumatic brain injury (TBI) patients compared to matched normal control subjects. [111] In two large samples of 94 and 118 patients with TBI, Bigler et al. [112;113] showed that TBI patients had bilaterally smaller hippocampi compared to normal controls. In three cases of TBI acquired at birth, at age 4, and at age 9, 3D volumetric MRI revealed bilateral hippocampal volume reduction 13-15 years after the occurrence of TBI. [114] This volume reduction is not always related to the severity of the injury. No significant volume differences were found in mild versus severe TBI. [115] In a morphometric study before and after anterior cingulotomy significantly smaller bilateral hippocampi were not found.[116]

Alzheimer's Disease

In Alzheimer's disease (AD) hippocampal volume loss is a hallmark of the disorder. [117] [118-130] Smaller hippocampal volume is also present in mild AD, [131;132] in African Americans with AD, [133] and is more pronounced in those AD patients who carry the epsilon 4 allele [134-136] (for an exception see Bigler et al. [137]). A study comparing mild AD patients with nondemented controls using large deformation high-dimensional brain mapping found significant volume loss over time and different patterns of hippocampal shape change over time, that distinguished mild AD from healthy aging.[138] Although hippocampal volume loss is not specific to AD, volume loss is more severely manifested in AD than in other dementias. [139-141] There is one study, however, where hippocampal volume loss present in demented Parkinson's disease (PD) patients, was significantly worse than the volume loss exhibited in AD patients. [142] The hippocampal volume loss in AD has been shown to be related to the degree of neurophysiological activity as measured by magnetoencephalography.[143]

Researchers have found that hippocampal volume loss is able to discriminate patients and controls accurately, and that age- and gender- adjusted, normalized MRI-based hippocampal volumetric measurements provide a sensitive marker of the mesial temporal lobe neuroanatomic degeneration in AD. [121;144-146] However, use of hippocampal volume exclusively is not advocated by all authors, [147-149] and other structures such as the amygdala and the entorhinal cortex may also need to be measured,[150-153] or hippocampal N-acetyl aspartate measurements may need to be performed to improve diagnosis. [154] Karas et al. [155] performed voxel based morphometric analysis in AD and found volume loss of other structures to be equally predictive of AD. Others have provided evidence that assessment of delayed recall with the Visual Reproduction Test is of high diagnostic accuracy, even surpassing hippocampal volumetry. [156] Despite the theoretical rationale for the superiority of entorhinal measurements in early AD, Xu et al. [157], present evidence that measurements of the hippocampus and entorhinal cortex were approximately equivalent at intergroup discrimination. Because of the ambiguity surrounding entorhinal cortex measurement, measurements of the hippocampus may actually be preferable due to superior reproducibility of the measurements. Age transformation may provide an easily applicable method to increase the clinical diagnostic accuracy of hippocampal measurements by considering the effect of aging on hippocampus volume. [158] Progressive measurements of hippocampal volume loss provide some additional information, but do not increase the discriminating power significantly. [159] Very accurate volumetric measurements of the whole hippocampal formation can be obtained by MRI, which strongly correlates with neuronal numbers, and suggest a high anatomical validity of magnetic resonance imaging volume measurements. [160]

In AD patients the volumes of the left hippocampus correlated significantly with the Mini Mental State Examination score and with immediate and delayed verbal memory; the smaller the volume the more impaired the memory performance. [124] Other researchers have found a similar correlation between memory performance and hippocampal volume decline. [161-164] Kohler et al. [165] also examined this relation and found that hippocampal volume correlated positively with delayed, but not immediate recall of a verbal auditory list learning task. In normal controls there was a trend towards a negative association between hippocampal volumes and delayed verbal recall. De Toledo-Morrell et al. [166] showed that left hippocampal volume was the best predictor of free recall and delayed free recall of verbal information, and that recall and delayed recall of the spatial location of verbal items were best predicted by right hippocampal volume. They also showed a differential effect, as this relation between hippocampal volume and memory function observed in cases with AD did not hold for healthy aged control subjects. Some research groups have not been able to link hippocampal volume loss with either severity of memory impairment, [167] or general or emotional memory performance. [168]

In several studies, decreased hippocampal volume has been shown to be a risk factor for AD. [169-173] Individuals carrying the apolipoprotein E epsilon 4 allele (APOE-epsilon 4 allele) are at high risk for developing AD. The presence of a single APOE-epsilon 4 allele is associated with an increased rate of hippocampal volume loss in healthy women in their sixth decade of life that is not related to any detectable memory changes. [174] Similarly, non-demented elderly subjects carrying the APOE-epsilon 4 allele display decreased hippocampal volume symmetry on MRIs. [175] MRI measurements of hippocampal volume begin to decrease in conjunction with memory decline in cognitively normal

persons at risk for Alzheimer's disease, [176] and the rate of hippocampal volume loss correlates with change in clinical status.[177]

The determination of hippocampal volume in AD may be reliably and consistently assessed across different research centers. [178] Crum et al. [179] and Gosche et al. [180] have examined automated methods of deriving hippocampal volumetry and found them to be equally reliable to manual segmentation methods in AD. The finding of a strong relationship between left hippocampal volume and performance on odor identification tasks is compatible with left-hemisphere superiority for verbally mediated olfactory tasks, suggesting a neural substrate for the breakdown in functional performance on verbally mediated odor identification tasks in Alzheimer's disease. [181]

Dementia

Studies of hippocampal volume have also been performed in dementias other than AD. In a study comparing demented patients with cognitive impairment subjects and elderly controls, demented patients showed the greatest annual rates of volume loss in the hippocampus and cortex.[182] This volume loss was also significantly greater in demented patients compared with both cognitive impaired and elderly control subjects. Similarly, Grunwald et al. [183] found hippocampal volume loss in dementia, and Barber et al. [184] found a loss of hippocampal asymmetry in patients dementia with Lewy bodies (DLB) (as well as AD patients) compared to normal controls. Volumetric MRI of the brain in elderly subjects with lacunes, mild cognitive impairment, a group of patients with dementia, and a group with probable AD revealed hippocampal volume loss in all three patient groups .[185] Du et al. [186] assessed hippocampal volume loss in cognitively normal subjects, patients with subcortical ischemic vascular dementia, and patients with AD. Patients with subcortical ischemic vascular dementia had smaller hippocampi than cognitively normal subjects, but larger hippocampi than patients with AD. Voxel-based morphometric analysis of patients with semantic dementia and a group of age-matched normal controls did not find evidence of significantly smaller hippocampi. [187] In a study comparing global and regional atrophy on MRI in subjects with DLB, AD, vascular dementia, and normal aging, subjects with DLB had significantly larger temporal lobe, hippocampal, and amygdala volumes than those with AD. [188] No significant volumetric difference between subjects with DLB and vascular dementia was observed. The first study to use voxel-based morphometry to assess hippocampal volume in DLB showed preservation of hippocampal volume relative to AD.[189] Bigler et al. [190] found a significant relationship between hippocampal volume loss and performance on the Mini-Mental-State-Examination Questionnaire. In patients with semantic dementia (the temporal variant of frontotemporal dementia, there was no significant positive correlation between recollection and volume of the hippocampus. [191] For temporal horn and hippocampal volume determination, correction with total brain volume rather than total intracranial volume may provide a more clinically meaningful correction. [192]

Mild Cognitive Impairment

In line with investigations in AD, our database also includes studies which have specifically examined hippocampal volume in mild cognitive impairment (MCI). MCI is a transitional state between the cognitive changes of normal aging and AD, in which persons experience unacceptable memory loss, without meeting criteria for AD. [193] Heterogeneity in the use of the term MCI is significant, so it is important to recognize diagnostic criteria that studies use. One of the first studies measured volumes of the hippocampus in age-associated cognitive impairment subjects (as defined by criteria from Crook et al. [194])and age- and sex-matched controls, and did not find evidence of smaller hippocampal volume, [20] although the volumetric asymmetry between the right and left hippocampi was reduced in age-associated cognitive impairment subjects. Another earlier study investigated hippocampal atrophy in normals, patients with AD, and minimally impaired individuals (with a MMSE > 23, Global Deterioration Scale (GDS) of 3), Clinical Dementia Rating (CDR) of 0.5).[195] Significantly smaller hippocampi differentiated the minimally impaired individuals from the control group. People with mild cognitive impairment are at a higher risk for developing AD. An investigation by Jack et al. [196] revealed that hippocampal volume loss determined by premorbid MRI volumetric analysis is predictive of subsequent conversion to AD, a finding that was corroborated by others. [130;197;198] Convit et al. [199] also assessed the ability of medial temporal lobe volume loss to predict decline of MCI to AD and found that addition of baseline medial occipitotemporal, and the combined middle and inferior temporal gyri as predictors increased overall classification accuracy and sensitivity. Encoding and retrieval memory deficits in patients with amnestic mild cognitive impairment, as defined by criteria from Petersen et al. [193], are correlated with declines in hippocampal grey matter density.[200]

Aged

Smaller hippocampi have been associated with normal aging [201-209] (in contrast to Sullivan et al. [210]), and may even constitute a risk factor for the development of dementia. [211;212] In a sample of elderly persons, MR derived hippocampal volume was correlated with delayed memory performance. [213] In another sample of elderly people with suspected normal pressure hydrocephalus, the volume of the hippocampus was correlated with MMSE scores.[214] Elderly women experience greater hippocampal volume loss than aged men. [215] In a large sample study, den Heijer et al. [216] found that higher plasma homocysteine levels, which are associated with AD, are correlated with smaller hippocampi in the elderly. Sullivan et al. [9] examined the balance of environmental and genetic effects on hippocampal size in a large sample of elderly twin men and provide evidence that only 40% of the hippocampal volume variance was attributable to genetic influences. In nondemented elderly subjects, hippocampal head size has been related to verbal memory performance. [217]

Estrogen seems to have a neuroprotective effect. [218;219] A recent study by Eberling et al. [220] compared hippocampal volume in women taking estrogen replacement therapy (ERT) with matched controls. Women taking ERT had larger right hippocampal volumes and bilateral anterior hippocam-

pal volumes than women not taking ERT. However, another recent study investigating the relation between endogenous estradiol levels found that aged women with higher total estradiol levels had smaller hippocampal volumes and poorer memory performance.[221]

Autism

The first volumetric MRI studies in autism did not reveal a significant hippocampal volume reduction in autistic individuals when compared to normal control subjects. [222;223] However, when corrected for whole brain volume, Aylward et al. [224] were able to find evidence of significant hippocampal volume loss. Similarly, a study comparing high-functioning autistic and normal school-age boys, all with normal intelligence, found that the hippocampus-amygdala complex appeared to be relatively smaller in the autistic than in the typically developing brain. [225] In contrast to all these reports, Sparks et al. [226] reported significantly increased hippocampal volumes in young children with autism spectrum disorder bilaterally when compared to age-matched control groups of typically developing and developmentally delayed children.

Down's Syndrome

Raz et al. [227] examined neuroanatomic abnormalities in adults with Down's syndrome (DS) and revealed that DS subjects had substantially smaller hippocampal formations compared to sex-matched healthy control subjects, a finding that was corroborated by others. [228-230] A similar study with a larger number of subjects revealed decreased left hippocampal volume in adults with DS compared to healthy controls. [231] In a study examining both demented and nondemented DS subjects, all DS subjects revealed significantly smaller hippocampi than controls. [232] Non-demented Down's syndrome adults have an age-related decrease of hippocampus volume, which is not found in age-matched healthy comparison subjects.[230] Children with Down's syndrome also display smaller hippocampi bilaterally. [229]

Schizophrenia

Volumetric studies of the hippocampus constitute the second largest diagnostic category in the database with a total of 76 hippocampal volumetric MRI studies in patients with schizophrenia, patients with first-episode schizophrenia, and in relatives of patients with schizophrenia. Smaller bilateral hippocampi in schizophrenia have been found by a large number of research groups. [233-247] This reduction in volume is related to symptom severity. [248] Luchins et al. [249] was only able to provide evidence of smaller bilateral hippocampi in patients with schizophrenia and hypo-osmolemia. A twin study by Baare et al. [250] revealed that twins discordant for schizophrenia had smaller hippocampal volumes compared to healthy twin pairs, irrespective of zygosity. Becker et al. [251] and Narr et al. [252] reported smaller bilateral posterior hippocampi in patients with schizophrenia. Others found evidence for a smaller anterior amygdala-hippocampal complex and anterior hippocampus bilaterally in schizophrenia respectively. [253;254]

Some studies were only able to find evidence for significantly smaller left hippocampal volume. [255-257] Stefanis et al. [258] found evidence for smaller left hippocampi only in patients with schizophrenia and birth complications. Others have failed to find any evidence of smaller hippocampi in patients with schizophrenia, compared to controls. [52;259-270] Meta analysis of hippocampal volumetric studies in schizophrenia concluded that schizophrenia was associated with bilateral hippocampal volume loss.[271]

Lately new techniques, such as hippocampal shape analysis in schizophrenia patients are providing some interesting results. [252] Csernansky et al. [272] shows that shape analysis reveal differences between patients with schizophrenia and controls in the absence of volumetric changes. Similarly, in another study they were not able to find significant hippocampal volume changes in patients with schizophrenia and comparison subjects, but did provide evidence for abnormal hippocampal shape and asymmetry in schizophrenia. [261] Shenton et al. [273] also showed that shape analysis may provide group discrimination in schizophrenia. Velakoulis et al. [246] provided evidence that the volume loss behind the head of the hippocampus is discriminating for schizophrenia. Wang et al. [274] also found that the hippocampal asymmetry was different in schizophrenia.

Other hippocampal volumetric studies in schizophrenia have also been performed. De Lisi et al. [275] performed a longitudinal study in chronic schizophrenia and found a progressive decrease in size of the amygdala-hippocampal complex over time. In a treatment study, Arango et al. [276] found that there was no significant difference in hippocampal volume between schizophrenia patients treated with haloperidol versus patients treated with clozapine.

There are now several studies investigating hippocampal volumetry in first-episode (FE) schizophrenia. Studying FE-schizophrenia is important because confounds such as chronic illness and chronic medication are absent. Bogerts et al. [277] and Kubicki et al. [278] found evidence of a smaller left hippocampus in FE patients compared to controls. Hirayasu et al. [279] found smaller left posterior amygdala hippocampal complex volumes, and Velakoulis et al. [247] found an additional left hippocampal volume reduction in FE-schizophrenia compared to chronic schizophrenia. Others found smaller hippocampal volume bilaterally, [280;281] or smaller bilateral anterior hippocampi. [282-284] However other studies did not find any significant hippocampal volume reduction in FE-schizophrenia. [264;285-289] Both Wood et al. [290] and Lieberman et al. [283] performed longitudinal studies in FE-schizophrenia. They did not find progressive hippocampal volume loss over time. Szeszko et al. [291] investigated neuropsychological correlates of smaller hippocampi in FE-schizophrenia. Among men, worse executive and motor functioning correlated significantly with smaller anterior hippocampal volume. Among women no relationship between neuropsychological variables and either posterior or anterior hippocampal volumes was found.

Several studies have also assessed hippocampal volumes in childhood-onset schizophrenia. However, whereas some studies have shown reduction of the left hippocampus after a 2 year follow-up in comparison to controls, [292] or bilateral hippocampal volume loss over time, [293] others did not find smaller hippocampi in early onset schizophrenia, [294;295] although it seems that normal hippocampal

asymmetry (right greater than left) is lacking in childhood-onset schizophrenia. [294;296] Barta et al. [297] examined hippocampal volumes in patients with late-onset schizophrenia, AD, and normal elderly controls. They found that patients with late-onset schizophrenia had significantly smaller left hippocampi in comparison to healthy controls.

In individuals at high risk for developing schizophrenia, researchers have found smaller bilateral hippocampi, [298;299] as well as no significant hippocampal volumetric changes. [300] A study comparing schizophrenia patients with subjects at high risk for developing schizophrenia and controls, found that the left amygdala-hippocampal complex was smaller in FE-schizophrenia than in the high-risk group, which had a smaller left amygdala-hippocampal complex than controls. [301] Hippocampal volume and shape analysis showed that the hippocampi of unaffected siblings of schizophrenia subjects are smaller and that the head of the hippocampi are deformed compared to controls. [302] The unaffected siblings' hippocampi were indistinguishable from schizophrenic subjects.

Major Depression

Several studies have examined hippocampal volumetry with MRI in MD. An early MRI volumetric study was unable to find evidence of a significantly smaller amygdala-hippocampal complex in depressed patients. [303] Comorbid hypercortisolemia does not significantly influence hippocampal volume either. [304] Lately, studies have found smaller bilateral hippocampal volume in patients with a first episode of depression, and a past history (multiple episodes) of depression respectively, compared to controls. [305-307] These last findings have been corroborated by MacQueen et al. [308] who compared hippocampal volumes in depressed subjects experiencing a post pubertal onset of depression with matched healthy control subjects, and found that only depressed subjects with multiple depressive episodes had hippocampal volume reductions.

Statistically significant smaller left hippocampal volumes were found in patients with multiple episodes of depression currently treated with antidepressant medication, [309] and in patients with treatment-resistant depression. [310] Voxel based morphometry in chronic depressed patients revealed reduced grey matter density in the left hippocampus, which was correlated with measures of verbal memory. [311] Others did not observe any significant differences in hippocampal volumes of patients with major depression and control subjects. [312;313] In an effort to explain the inconsistencies in hippocampal volume findings in prior morphometric studies of MD, Vythilingam et al. [314] assessed hippocampal volume in depressed subjects with and without childhood abuse, as well as in control subjects. Depressed subjects with childhood abuse had an 18% smaller mean left hippocampal volume than the nonabused depressed subjects and a 15% smaller mean left hippocampal volume than the healthy subjects.

Posener and colleagues used high-dimensional mapping of the hippocampus to quantitatively characterize size and shape of the hippocampus in patients with MD and controls. [315] While the depressed patients and comparison subjects did not differ in hippocampal volume, there were highly significant group differences in hippocampal shape. In a treatment study, Sheline et al. [316] investigated the effect of antidepressant treatment on hippocampal volume in MD, and found that longer durations during which depressive episodes went untreated with antidepressant medication were associated with reductions in hippocampal volume, suggesting that antidepressants may have a neuroprotective effect in MD.

Kim et al. [317] found no amygdala-hippocampal complex volumetric differences in deluded depressed geriatric patients versus non-deluded depressed geriatric patients. In other studies on geriatric depression, Steffens et al. [318] found that patients tended to have smaller bilateral hippocampal volumes compared to controls, whereas Bell-McGinty et al. [319] demonstrated smaller right hippocampal volumes in geriatric depression. Hsieh and colleagues expanded this finding and showed that subjects with small right hippocampal volumes were less likely to achieve remission. [320] Smaller left hippocampal volumes in geriatric depression seem to be a risk factor for developing dementia. [321] Although significantly smaller hippocampi were not found in one study of pediatric patients with MD, volumetric MRI has revealed significantly increased amygdala: hippocampal volume ratios in pediatric MD. [322] A very recent study in a small sample of pediatric patients with MD did reveal decreased hippocampal volumes bilaterally. [323] However, in this study a slightly older population of patients was used.

Bipolar Disorder

Swayze et al. [267] compared bipolar patients with controls and found a significantly smaller right hippocampus in bipolar patients. Later hippocampal volumetric studies conducted in bipolar patients did not find significantly smaller hippocampal volumes in bipolar patients versus controls. [324-326] Later studies were also unable to find significant hippocampal volume reductions between bipolar patients and normal controls regardless of the number of episodes. [327;328] Increased right hippocampal volumes associated with poorer neuropsychological functioning in bipolar patients have been reported in two studies which did not include a control group. [329;330]

Posttraumatic Stress Disorder

The first study of hippocampal volume in PTSD by Bremner et al. [331] provided evidence that combat-related PTSD patients had statistically significantly smaller right hippocampal volumes relative to that of comparison subjects. Other studies found evidence of significant bilateral hippocampal volume loss in combat-related PTSD [332], or in PTSD patients with various traumas. [333] In childhood physical and sexual abuse related PTSD, Bremner et al. [334] reported a decrease in left hippocampal volume in comparison with matched controls. Stein et al. [335] who examined hippocampal volume in women with sexual abuse, and matched controls without abuse, also found significantly smaller left hippocampi. Bilateral hippocampal volume was significantly smaller in a small sample study of substance and alcohol naïve subjects with combat related PTSD compared to controls. [336] In monozygotic twins discordant for trauma exposure, Gilbertson et al. [337] revealed that the identi-

cal non-exposed twins of PTSD combat veterans had comparable hippocampi to their PTSD twin, but significantly smaller hippocampi than combat veterans without PTSD and their non-combat exposed twins, showing that smaller hippocampi may constitute a risk factor for the development of stress-related psychopathology.

Contrary to all these positive findings of hippocampal volume loss in PTSD, a study assessing hippocampal volume in recent trauma victims did not find evidence of hippocampal volume loss in recent survivors of trauma who later developed PTSD, both within two weeks of the trauma, and six months after the event compared to other trauma survivors. [338] Although six months might be too short a time in which to see hippocampal volumetric changes. Another small sample study examining female victims of intimate partner violence with and without posttraumatic stress disorder was unable to find evidence of smaller hippocampal volume. [339] Schuff et al. [340] and Neylan et al. [341] were also unable to find significantly smaller hippocampal volume in patients with PTSD compared to controls, although patients with PTSD did display a significant reduction in N-acetylaspartate in the hippocampus bilaterally. In chronic alcoholics with PTSD hippocampal volume was not additionally reduced. [342]

Recently, it has also been shown that women with childhood sexual abuse and PTSD have smaller hippocampi than women with PTSD but without childhood sexual abuse, or than women without PTSD but with childhood sexual abuse. [343] Long-term treatment with paroxetine is associated with increased hippocampal volumes and improvement of verbal declarative memory in PTSD.[344] In a recent study with voxel-based morphometry, Yamasue et al. [345] did not find evidence of hippocampal volume loss in PTSD. In contrast to the findings in adult PTSD, children with PTSD do not exhibit smaller hippocampi in comparison with matched controls. [346-349] (see Table 2)

Table 2: Hippocampal volumetric findings in pediatric and adult manifestations of various neuropsychiatric disorders

Population	*Pediatric*	*Adult*
Epilepsy	↓ hippocampi bilaterally	↓ hippocampi bilaterally
Schizophrenia	⟷ in hippocampal volume	↓ hippocampi bilaterally
Depression	⟷ in hippocampal volume; larger amydala: hippocampus ratios in depressed subjects	↓ hippocampi bilaterally
PTSD	⟷ in hippocampal volume	↓ hippocampi bilaterally
TBI	↓ hippocampi bilaterally	↓ hippocampi bilaterally
Autism	↓ / ↑ hippocampi bilaterally	↓ hippocampi bilaterally
Down's Syndrome	↓ hippocampi bilaterally	↓ hippocampi bilaterally

↓ = smaller ↑ = larger ↓ / ↑ = both smaller and larger hippocampal volumes haven been reported ⟷ no significant changes ↓ / ⟷ = both smaller and no significant studies have been reported

Chronic Alcoholism

A study by Sullivan et al. [350] revealed bilateral anterior hippocampal volume loss in men with chronic alcoholism compared to healthy male control subjects. Agartz et al. [342] examined hippocampal volume in chronic alcoholics and compared this to overall brain volume. They found that in chronic alcoholism, the reduction of hippocampal volume is proportional to the reduction of whole brain volume. Another study also provided evidence of significantly reduced hippocampal volumes in chronic alcoholics compared to controls. [351] Laakso et al. [352] compared hippocampal volume in late- onset type 1 alcoholics to early-onset type 2 alcoholics, as well as in normal volunteers. Compared to the controls, the right, but not left, hippocampi were significantly smaller in both alcoholic groups, even after controlling for intracranial volume. De Bellis and colleagues found significantly smaller bilateral hippocampi in subjects with alcohol abuse disorders compared to comparison subjects. [353]

Recently, pathologically raised levels of plasma homocysteine have been shown to be significantly correlated to smaller hippocampi. [354] In addition the presence of an association between hippocampal volume reduction and first-onset alcohol withdrawal seizure was examined. They found the average hippocampal volumes measured by high resolution MRI to be significantly reduced in alcoholics compared with healthy controls, but found no correlation with seizures [355] confirming results of an earlier study by Sullivan et al. [356]. A study by Di Sclafani et al. [357] investigated hippocampal volumes in crack-cocaine, crack-cocaine/alcohol-dependent subjects, and age-matched controls, but did not find any hippocampal differences between the three groups.

Other Disorders

There are a number of studies which have investigated hippocampal volumes in other neuropsychiatric disorders. The results of these studies are summarized in Table 3. Decreased hippocampal volumes have been reported in borderline personality disorder, in obsessive-compulsive disorder, in cardiac arrest, in Cushing's disease, in herpes simplex encephalitis, in Parkinson's disease, in Huntington's disease, in Turner's syndrome, and in survivors of low birth weight. Children with fragile X syndrome display significantly increased hippocampal volumes. In panic disorder, in anorexia nervosa, in congenital hyperplasia, in children with fetal alcohol syndrome, and in attention-deficit and hyperactivity disorder hippocampal volume is preserved.

Normal Controls

In several studies with normal control subjects, the right hippocampus has been found to be larger than the left hippocampus, [19;358;359] although this difference may not always reach significance. [360] This asymmetry is also present in children. [361] Szabo et al. [362] compared amygdala and hippocampal volume measurements bilaterally between right- and left-handed participants. Right-to-left volume ratios differed significantly between right- and left-handed participants for both amygdala and hippocampus.

Table 3: Hippocampal volumetric findings in various neuropsychiatric disorders.

Population	*Study*	*Subjects*	*Finding*
Borderline Personality Disorder	Driessen et al. [418]	21 female patients with BPD, and 21 healthy controls	Bilateral hippocampal volume reduction
	Schmahl et al. [412]	10 patients with BPD, and 23 control subjects	Bilateral hippocampal volume reduction
	Tebartz van Elst et al. [419]	8 unmedicated female patients with BPD, and 8 matched healthy controls	Bilateral hippocampal volume reduction
Febrile Seizures	Szabo et al. [420]	5 children 22-68 months old, and 11 controls, 15-83 months old	Reduced hippocampal volume in children with CFS, and right to left ratios greater than 1 in all 5 children with CFS compared to controls
	Tarkka et al. [421]	24 patients with a prolonged first febrile seizure, 8 with an unprovoked seizure after the first febrile seizure, and 32 age-, sex-, and handedness- matched control subjects	Mean total volumes of the right and left hippocampal formations did not differ significantly between any of the three groups
	Scott et al. [325]	14 patient with prolonged febrile seizures	Hippocampal volume reduction, and significant increase in hippocampal volume asymmetry
Herpes Simplex	Yoneda et al. [422]	5 post- herpes simplex encephalitic (post-HSE) patients with temporal lobe damage and memory impairment, and 10 age-matched control subjects	Two patients had a marked atrophy of the hippocampal formation, 3 patients had larger hippocampi
	Caparros-Lefebvre et al. [423]	11 patients with clinically presumed HSVE, and 5 matched controls	Hippocampal volume reduction
	Colchester et al. [424]	11 Korsakoff's syndrome, 9 herpes encephalitis, 6 focal frontal lesion patients, and 10 healthy controls	Hippocampal volume reduction present in herpes encephalitis
Korsakoff's Syndrome	Visser et al. [425]	13 subjects with Korsakoff's syndrome, 13 subjects with chronic alcoholism without Korsakoff's syndrome, and 13 control subjects	Reduced hippocampal volume in Korsakoff's syndrome compared to subjects with chronic alcoholism and healthy controls
	Colchester et al. [424]	11 Korsakoff's syndrome, 9 herpes encephalitis, 6 focal frontal lesion patients, and 10 healthy controls	No reduction in hippocampal volume in Korsakoff's syndrome.
	Sullivan et al. [426]	5 Korsakoff's syndrome, 20 AD, 36 healthy controls	Bilateral hippocampal volume deficits in Korsakoff's syndrome and AD compared to controls
OCD	Jenike et al. [427]	10 female patients with OCD, and 10 matched female control subjects	No significant differences
	Szeszko et al. [428]	26 patients with OCD, and 26 healthy comparison subjects	OCD patients lacked the normal hemispheric asymmetry of the hippocampus-amygdala complex.
	Kwon et al. [242]	22 patients with OCD, 22 patients with schizophrenia, and 22 normal subjects	Hippocampal volume was bilaterally reduced in both OCD and schizophrenic patients versus the normal controls
Amnesia	Kopelman et al. [429]	40 patients with organic amnesia, and 10 healthy controls	Loss of hippocampal volume correlates significantly with impaired memory performance
	Isaacs et al. [430]	10 adolescents with a diagnosis of developmental amnesia (DA), 11 adolescents born preterm (PT), and 8 age-matched normal controls	Bilateral reduction in hippocampal volume in the two patient groups with DA significantly < PT significantly < controls
Cardiac Arrest	Fujioka et al. [416]	11 vegetative patients after cardiac arrest, and 22 healthy matched controls	Bilateral hippocampal volume reduction
	Grubb et al. [431]	17 out-of-hospital cardiac arrest survivors, and 12 patients with uncomplicated myocardial infarction	Left amygdala-hippocampal volume was reduced in memory-impaired OHCA victims compared with control subjects
Cushing's Disease	Starkman et al. [432]	12 patients with Cushing's disease	Reduced hippocampal formation volume
	Starkman et al. [433]	22 patients with Cushing's disease	Increased hippocampal formation volume after treatment
Fragile X Syndrome	Reiss et al. [415]	15 fragile X subjects and 26 age- and IQ-matched control subjects.	Hippocampal volumes in children with fragile X were significantly increased bilaterally
	Kates et al. [434]	6 fragile X subjects and 7 normal controls	Hippocampal volumes in children with fragile X were significantly increased
Low Birth Weight	Peterson et al. [435]	25 eight-year-old preterm children, and 39 matched term control children	Bilateral hippocampal volume reduction in preterm children compared to controls
	Abernethy et al. [413]	87 children (aged 15-16 years) with a history of very low birth weight (<1500 g), and 8 age matched full term controls	Children with a low IQ had smaller left hippocampi, and a smaller hippocampal ratio (left volume:right volume) than those with normal IQ
Panic Disorder	Vythilingam et al. [436]	13 patients with panic disorder, and 14 healthy subjects	No hippocampal volume reduction
	Uchida et al. [437]	11 patients with panic disorder, and 11 matched controls	No significant hippocampal volume reduction

Parkinson's Disease	Camicioli et al. [438]	10 patients with PD, 10 with PD and dementia or mild cognitive impairment, 11 with Alzheimer's Disease, 12 control subjects	Bilateral hippocampal volume reduction in all patient groups compared to controls
	Laakso et al. [142]	50 patients with AD, 9 patients with vascular dementia, 12 patients with PD without dementia, 8 patients with PD and dementia, and 34 elderly control subjects.	Significant reduction of hippocampal volume in all patient groups compared to controls
ADHD	Castellanos et al. [439]	57 boys with ADHD, and 55 healthy matched controls	No hippocampal volume reduction
Antisocial Personality Disorder	Laakso et al. [440]	18 male violent offenders with antisocial personality disorder	Volume of the bilateral posterior hippocampus was negatively correlated with scores on the Psychopathy Checklist-Revised (which measures the degree of psychopathy).
Anorexia Nervosa	Giordano et al. [441]	Twenty AN females, and age-matched healthy female controls	No significant difference was found between right and left HAF in both patients and CG
Breast Cancer Surgery	Nakano et al. [442]	67 women who had had breast cancer surgery 3 or more years earlier and had no history of PTSD or major depression before the cancer	The volume of the left hippocampus was significantly smaller in the subjects with a history of distressing cancer-related recollections (N=28) than in those without any such history (N=39). There was no significant difference in right hippocampal volume or whole brain volume measured as a control
Congenital Adrenal Hyperplasia	Merke et al. [443]	27 children with CAH, and 47 sex- and age-matched controls	No hippocampal volume reduction
Fetal Alcohol Syndrome	Archibald et al. [444]	14 FAS, 12 patients with prenatal exposure to alcohol, and 41 healthy controls	No hippocampal volume reduction
Huntington's Disease	Rosas et al. [445]	18 patients with HD, and 18 age-matched healthy controls	Bilateral hippocampal volume reduction in HD compared to controls
Sleep Apnea	Morrell et al. [446]	7 male patients with obstructive sleep apnea, 7 age and handedness matched male controls	Significantly lower gray matter concentration within the left hippocampus
Turner's Syndrome	Murphy et al. [414]	18 women with TS, and nineteen healthy age-matched women	Bilateral hippocampal volume reduction in TS compared to controls

In children hippocampi may also be measured reliably (see Obenaus et al. [363] for a detailed protocol). Developmental aspects of the hippocampus in children have been examined. [364;365] In developing children aged 4-18, the hippocampus increases with age.[364] Pfluger et al. [366] developed normative volumetric data of the developing hippocampus in children.

Hippocampal volumes are also subject to gender differences. Bhatia et al. [201] found evidence for smaller left hippocampi in women. Others also reported that the volume of the hippocampal formation was larger in men than in women. [99;367] Contrary to this, a study by Filipek et al. [368] reported that women have larger hippocampi than men. Two other studies were not able to find gender differences in hippocampal volume. [369;370] Similarly, gender did not affect right-to-left amygdala and hippocampal volume ratios in right- or left-handed participants. [362] In men, the hippocampus declines with age, starting in the third life decade. [371] From the age of 54 hippocampal volume starts to decline at an increased rate (compared to total brain atrophy) in both men and women. [372]

Several studies performed in healthy subjects have examined the relation of hippocampal volume to IQ and memory. Full scale IQ is significantly related to hippocampal volume, [373] and left hippocampal volume is negatively associated with the level of delayed verbal recall performance. [374] Bilateral hippocampal volume corrected for whole brain volume is negatively correlated with explicit memory, [375] but not with motor performance. [376] In related work, Maguire et al. [377] showed that the posterior hippocampi of London taxi drivers were significantly larger relative to those of control subjects, and that this volume correlated with the amount of time spent as a taxi driver, but was not related with innate navigational expertise. [378] These data provided evidence for the theory that the posterior hippocampus stores a spatial representation of the environment and has the ability to expand regionally in order to accommodate elaboration of this representation in people with a high dependence on navigational skills.

Methodological issues related to hippocampal volumetry have been ironed out with healthy controls. Several studies have used healthy controls to assess the reliability of new manual tracing protocols, [18;21;363;379-383] point-counting methods, [384] or automated segmentation techniques. [385-387] Other studies have looked at specific methodological issues, such as magnetic field strength, [379;388;389] hippocampal orientation, [390] the use of reformatted 3D images, [391] the effect of slice thickness, [392] handedness, [362] and economical means of acquiring hippocampal volumes. [393]

Discussion

In epilepsy research and in temporal lobe epilepsy in particular, hippocampal volumetry with MRI is primarily utilized in the determination of hippocampal atrophy and hippocampal sclerosis. In addition, researchers have correlated pre- and postoperative hippocampal volumes with neurophysiological, neuropathological, neuropsychological, and clinical findings, as well as surgical outcome. [30] The hippocampal sclerosis and hippocampal atrophy present in mesial TLE is indicative of the epileptogenic focus and is related to neuronal cell death. A large number of predisposing, maintaining, and

exacerbating factors of hippocampal atrophy in TLE have also been established. The presence of decreased hippocampal volume in TLE has been correlated with decreased verbal memory pre- and postoperatively. In addition the ratio between right and left hippocampal volume, as well as gender, is correlated with postoperative verbal memory. [394] Several studies have also evaluated the link between hippocampal volume and other predictors with outcome measures of ATL.

An important issue in TLE is whether seizures are the cause or the result of hippocampal sclerosis. Kalviainen and Salmenpera,[65] who sought to answer this question by using MRI to investigate the appearance of medial temporal lobe damage during the course of partial epilepsy, and, particularly, to determine whether recurrent or prolonged seizures contribute to the atrophy, provided evidence that hippocampal damage may indeed be both cause and consequence of TLE. This debate is by no means resolved, although longitudinal studies which allow determination of cerebral damage when it occurs, as well as new MRI techniques such as diffusion tensor imaging [395] may provide answers. Longitudinal studies are ongoing in patients with newly diagnosed and chronic epilepsy, with an inter-scan interval of 3.5 years, using complementary voxel-based and region-of-interest-based methods that can detect changes in hippocampal and cerebellar volumes of 3%.

In AD hippocampal volume loss is a manifested morphological abnormality of the disease. Some studies have also shown that decreased hippocampal volume may also be a risk factor for developing AD. Generally it is assumed that hippocampal volume loss is able to discriminate patients and controls, especially when combined with entorhinal cortex and temporal neocortical volume. [10] The reduced hippocampal volume present in these patients is related to MMSE scores and memory performance. Hippocampal volume declines with age, and hippocampal volume loss is generally present in demented patients, and in mild cognitive impairment. Traumatic brain injury is also associated with bilateral hippocampal volume loss. In mild cognitive impairment the hippocampal volume loss has been shown to be an early marker for developing AD later. [196;197]

In schizophrenia, abundant evidence exists which points to smaller bilateral hippocampal volume that is associated with both chronic and first-episode schizophrenia, [12;271] although the exact nature of the smaller hippocampi is still a contested issue. Whether these hippocampal volume losses are progressive or developmental are issues which longitudinal MRI studies will address.[275;396] Some recent studies have emphasized the need for future research to pay more attention to the issue of shape analysis, as this has provided more consistent results and may provide group discrimination in schizophrenia. [246;272;273] In individuals at high risk for developing schizophrenia and first-degree relatives of patients with schizophrenia, smaller hippocampi are also present.

Proton magnetic resonance spectroscopy studies in schizophrenia have reported low N-acetyl-aspartate levels of the hippocampus, [262;397;398] which is also present in the unaffected relatives of patients with schizophrenia. [399] The subtle volume reductions found in schizophrenia and the presence of smaller hippocampi early in the course of the disease seems to argue against a neurodegenerative mechanism in schizophrenia. The presence of hippocampal pathology in relatives of schizophrenic probands may point to a genetic risk factor instead. [12;299] Research with both monozygotic and dizygotic twins has shown that smaller hippocampal volumes are present in both the healthy twin and the twin with schizophrenia providing additional evidence that smaller hippocampal volumes are a genetic risk factor for schizophrenia, [250;302] although additional decreases in hippocampal volume following onset of psychosis may augment the developmental impairment.[400;401] In a review article of studies which have assessed hippocampal pathology with different modalities, Weinberger [402] postulates that genes involved in the formation and maintenance of hippocampal circuitry play a role in susceptibility. In rats, it has been shown that not only do neonatal excitotoxic lesions disrupt development of the prefrontal cortex, but transient inactivation of the ventral hippocampus during a critical period of development may also produce subtle anatomical changes in the hippocampus, sufficient to disrupt normal maturation of the prefrontal cortex (and perhaps, other interconnected late maturing regions). [403] Recently, it was demonstrated that schizophrenia (as well as bipolar disorder) was associated with a reduction of key oligodendrocyte-related and myelin-related genes, showing that connectivity issues will play an important role in unravelling the mystery of schizophrenia and other psychosis related disorders. [404]

In animal research, an extensive literature abounds, which has shown that prolonged exposure to stress or glucocorticoids, has adverse effects on the rodent hippocampus. [405] Hippocampal volume loss in Cushing's disease, which is characterized by a pathologic oversecretion of glucocorticoids; major depression, often associated with hypersecretion of glucocorticoids; and PTSD, have been theorized to be the result of glucocorticoid excess. [405;406] Although stress is not always associated with elevated cortisol levels,[407] this does not preclude the possibility that elevated levels of cortisol at the time of trauma (which we are unable to measure) are associated with hippocampal damage. [408] PTSD patients exhibit significantly higher cortisol levels during and shortly after traumatic script exposure compared to controls, which is consistent with elevated cortisol levels at time of initial trauma exposure.[409] Heightened sensitivity of the glucocorticoid receptor, associated with PTSD, has also been shown to lead to hippocampal volume loss, and this may also explain the volume loss present in PTSD. [8;407] Another possible explanation is that smaller hippocampi may constitute a risk factor for the development of stress-related psychopathology. [337] However, long-term treatment with paroxetine is associated with increased hippocampal volumes and improvement of verbal declarative memory in PTSD, and this makes it unlikely that genetic factors are exclusively responsible for smaller hippocampal volume in PTSD.[344]

Failure of adult neurogenesis in patients with MD has been proposed to constitute the biological and cellular basis of this disorder. [410;411] In patients with depression and childhood abuse, smaller hippocampi could also be explained by elevated cortisol levels at time of trauma. Patients with Cushing's disease exhibit reduced hippocampal volumes which are associated with the pathological oversecretion of cortisol. In patients with borderline personality disorder and childhood abuse, the reduction in hippocampal volume has been theorized to be the result of increased glucocorticoid

levels, reduced levels of brain-derived neurotrophic factors, and inhibition of neurogenesis, due to early life stress exposure. [412] Increased levels of glucocorticoids have also been thought to be accountable for smaller hippocampal volume in individuals who survived very low birth weight without major disability. [413] Cardiac arrest and herpes simplex encephalitis have also been associated with smaller hippocampi. In a study with patients who had undergone breast cancer surgery, the volume of the left hippocampus was significantly smaller in the subjects with a history of distressing cancer-related recollections than in those without such a history.

In alcoholism hippocampal volume loss may reflect general brain atrophy present in chronic alcoholism as the hippocampal volume loss is proportional to general reduction of brain volume. [342] Increased packing density of small immature neurons with truncated dendritic development indicative of curtailment in the development of the neurons and neuropil are proposed to be responsible for the hippocampal volume decrease in autism. [224] In Down and Turner's syndrome hippocampal volume loss has been related to developmental abnormalities, but the exact mechanisms are still unclear. [229;414] Increased hippocampal volume in individuals with fragile X syndrome, may result from neurotoxins, subclinical seizures or kindling, denervation of afferent pathways, abnormalities of the cellular-neurochemical-receptor interaction, or a combination of these factors. [415]

Brief cardiac arrest is typically followed by transient global ischemia, which leads to delayed neuronal cell death and has been suggested to underlie the hippocampal volume loss witnessed in humans with cardiac arrest. [416] In Parkinson's disease it has been proposed that demise of the entorhinal cortex in PD (through the presence of neurofibrillary tangles isolates the hippocampus from its isocortical inputs and thus causes volume loss. [142] In Huntington's disease a similar explanation may hold, as the entorhinal region is atrophied in HD as well. [417]

In studies specifically performed in healthy controls, it has been shown that the right hippocampus is larger than the left. Hippocampal volumes are also subject to right- and left-handedness, to gender, and to age. The hippocampus has been directly implemented in spatial, [1] episodic, [1] and even semantic memory in humans. [2;3] In addition, the hippocampus is also involved in novelty processing, [4;5] and stress regulation. [8] A lot of the methodological ground work for reliably measuring hippocampal volumes has been performed in healthy subjects, and has helped straighten out several methodological issues.

Future Directions

Although there are still obvious discrepancies in the research findings in a large number of these disorders, conflicting results and methodological issues are being resolved. Greater consistency may be achieved in the future with the introduction of reliable automated methods of hippocampal volume determination. The use of MRI derived hippocampal volume is a proven method with diagnostic value, which is also used in the determination of etiology and course of neuropsychiatric diseases. As such it is an indispensable technique and further studies are needed to focus research on unraveling the mechanisms of hippocampal volume loss in these disorders. Additional neuroimaging techniques such as diffusion tensor imaging, magnetization transfer imaging, magnetic resonance spectroscopy, shape analysis, functional magnetic resonance imaging, receptor imaging with PET, and functional connectivity analysis are vital instruments in achieving these goals.

References

1. Burgess, N., Maguire, E.A., O'Keefe, J. The human hippocampus and spatial and episodic memory. Neuron 35, 625-41 (2002).

2. Bayley, P.J., Hopkins, R.O., Squire, L.R. Successful recollection of remote autobiographical memories by amnesic patients with medial temporal lobe lesions. Neuron 38, 135-44 (2003).

3. Manns, J.R., Hopkins, R.O., Squire, L.R. Semantic memory and the human hippocampus. Neuron 38 , 127-33 (2003).

4. Strange, B. and Dolan, R. Functional segregation within the human hippocampus. Mol Psychiatry 4, 508-11 (1999).

5. Vinogradova, O.S. Hippocampus as comparator: role of the two input and two output systems of the hippocampus in selection and registration of information. Hippocampus 11, 578-98 (2001).

6. Strange, B.A., Fletcher, P.C., Henson, R.N., Friston, K.J., Dolan, R.J. Segregating the functions of human hippocampus. Proc Natl Acad Sci U S A 96, 4034-9 (1999).

7. Strange, B.A. and Dolan, R.J. Adaptive anterior hippocampal responses to oddball stimuli. Hippocampus 11, 690-8 (2001).

8. Hoschl, C. and Hajek, T. Hippocampal damage mediated by corticosteroids--a neuropsychiatric research challenge. Eur Arch Psychiatry Clin Neurosci 251 Suppl 2, II81-8 (2001).

9. Sullivan, E.V., Pfefferbaum, A., Swan, G.E., Carmelli, D. Heritability of hippocampal size in elderly twin men: equivalent influence from genes and environment. Hippocampus 11, 754-62 (2001).

10. Chetelat, G. and Baron, J.C. Early diagnosis of Alzheimer's disease: contribution of structural neuroimaging. Neuroimage 18, 525-41 (2003).

11. Jack, C.R. Jr. Medial temporal lobe volumetrics in traumatic brain injury. AJNR Am J Neuroradiol 18, 25-8 (1997).

12. Heckers, S. Neuroimaging studies of the hippocampus in schizophrenia. Hippocampus 11, 520-8 (2001).

13. Sapolsky, R.M. A mechanism for glucocorticoid toxicity in the hippocampus: increased neuronal vulnerability to metabolic insults. J Neurosci 5, 1228-32 (1985).

14. Sapolsky, R.M., Krey, L.C., McEwen, B.S. Prolonged glucocorticoid exposure reduces hippocampal neuron number: implications for aging. J Neurosci 5, 1222-7 (1985).

15. Shenton, M.E., Dickey, C.C., Frumin, M., McCarley, R.W. A review of MRI findings in schizophrenia. Schizophr Res 49, 1-52 (2001).

16. Cook, M.J., Fish, D.R., Shorvon, S.D., Straughan, K., Stevens, J.M. Hippocampal volumetric and morphometric studies in frontal and temporal lobe epilepsy. Brain 115 (Pt 4) , 1001-15 (1992).

17. Jack, C.R. Jr. MRI-based hippocampal volume measurements in epilepsy. Epilepsia 35 Suppl 6, S21-9 (1994).

18. Jack, C.R. Jr, Bentley, M.D., Twomey, C.K., Zinsmeister, A.R. MR imaging-based volume measurements of the hippocampal formation and anterior temporal lobe: validation studies. Radiology 176, 205-9 (1990).

19. Jack, C.R. Jr, Twomey, C.K., Zinsmeister, A.R., Sharbrough, F.W., Petersen, R.C., Cascino, G.D. Anterior temporal lobes and hippocampal formations: normative volumetric measurements from MR images in young adults. Radiology 172, 549-54 (1989).

20. Soininen, H.S., Partanen, K., Pitkanen, A., Vainio, P., Hanninen, T., Hallikainen, M. et al. Volumetric MRI analysis of the amygdala and the hippocampus in subjects with age-associated memory impairment: correlation to visual and verbal memory. Neurology 44, 1660-8 (1994).

21. Watson, C., Andermann, F., Gloor, P., Jones-Gotman, M., Peters, T., Evans, A. et al. Anatomic basis of amygdaloid and hippocampal volume measurement by magnetic resonance imaging. Neurology 42, 1743-50 (1992).

22. Adam, C., Baulac, M., Saint-Hilaire, J.M., Landau, J., Granat, O., Laplane, D. Value of magnetic resonance imaging-based measurements of hippocampal formations in patients with partial epilepsy. Arch Neurol 51, 130-8 (1994).

23. Ashtari, M., Barr, W.B., Schaul, N., Bogerts, B. Three-dimensional fast low-angle shot imaging and computerized volume measurement of the hippocampus in patients with chronic epilepsy of the temporal lobe. AJNR Am J Neuroradiol 12, 941-7 (1991).

24. Bernasconi, N., Bernasconi, A., Caramanos, Z., Andermann, F., Dubeau, F., Arnold, D.L. Morphometric MRI analysis of the parahippocampal region in temporal lobe epilepsy. Ann N Y Acad Sci 911, 495-500 (2000).

25. Bernasconi, A., Cendes, F., Lee, J., Reutens, D.C., Gotman, J. EEG background delta activity in temporal lobe epilepsy: correlation with volumetric and spectroscopic imaging. Epilepsia 40, 1580-6 (1999).

26. Cascino, G.D., Jack, C.R. Jr, Parisi, J.E., Marsh, W.R., Kelly, P.J., Sharbrough, F.W. et al. MRI in the presurgical evaluation of patients with frontal lobe epilepsy and children with temporal lobe epilepsy: pathologic correlation and prognostic importance. Epilepsy Res 11, 51-9 (1992).

27. Jack, C.R. Jr, Sharbrough, F.W., Twomey, C.K., Cascino, G.D., Hirschorn, K.A., Marsh, W.R. et al. Temporal lobe seizures: lateralization with MR volume measurements of the hippocampal formation. Radiology 175, 423-9 (1990).

28. Paterson, A., Winder, J., Bell, K.E., McKinstry, C.S. An evaluation of how MRI is used as a pre-operative screening investigation in patients with temporal lobe epilepsy. Clin Radiol 53, 353-6 (1998).

29. Tien, R.D., Felsberg, G.J., Campi de Castro, C., Osumi, A.K., Lewis, D.V., Friedman, A.H. et al. Complex partial seizures and mesial temporal sclerosis: evaluation with fast spin-echo MR imaging. Radiology 189, 835-42 (1993).

30. Watson, C., Jack, C.R. Jr, Cendes, F. Volumetric magnetic resonance imaging. Clinical applications and contributions to the understanding of temporal lobe epilepsy. Arch Neurol 54, 1521-31 (1997).

31. Webb, J., Guimond, A., Eldridge, P., Chadwick, D., Meunier, J., Thirion, J.P. et al. Automatic detection of hippocampal atrophy on magnetic resonance images. Magn Reson Imaging 17, 1149-61 (1999).

32. Reutens, D.C., Stevens, J.M., Kingsley, D., Kendall, B., Moseley, I., Cook, M.J. et al. Reliability of visual inspection for detection of volumetric hippocampal asymmetry. Neuroradiology 38, 221-5 (1996).

33. Jackson, G.D., Kuzniecky, R.I., Cascino, G.D. Hippocampal sclerosis without detectable hippocampal atrophy. Neurology 44, 42-6 (1994).

34. Bernasconi, N., Bernasconi, A., Caramanos, Z., Dubeau, F., Richardson, J., Andermann, F. et al. Entorhinal cortex atrophy in epilepsy patients exhibiting normal hippocampal volumes. Neurology 56, 1335-9 (2001).

35. Vossler, D.G., Kraemer, D.L., Knowlton, R.C., Kjos, B.O., Rostad, S.W., Wyler, A.R. et al. Temporal ictal electroencephalographic frequency correlates with hippocampal atrophy and sclerosis. Ann Neurol 43, 756-62 (1998).

36. Briellmann, R.S., Jackson, G.D., Kalnins, R., Berkovic, S.F. Hemicranial volume deficits in patients with temporal lobe epilepsy with and without hippocampal sclerosis. Epilepsia 39, 1174-81 (1998).

37. Mackay, C.E., Webb, J.A., Eldridge, P.R., Chadwick, D.W., Whitehouse, G.H., Roberts, N. Quantitative magnetic resonance imaging in consecutive patients evaluated for surgical treatment of temporal lobe epilepsy. Magn Reson Imaging 18, 1187-99 (2000).

38. Van Paesschen, W., Connelly, A., King, M.D., Jackson, G.D., Duncan, J.S. The spectrum of hippocampal sclerosis: a quantitative magnetic resonance imaging study. Ann Neurol 41, 41-51 (1997).

39. Van Paesschen, W., Duncan, J.S., Stevens, J.M., Connelly, A. Longitudinal quantitative hippocampal magnetic resonance imaging study of adults with newly diagnosed partial seizures: one-year follow-up results. Epilepsia 39, 633-9 (1998).

40. Van Paesschen, W., Sisodiya, S., Connelly, A., Duncan, J.S., Free, S.L., Raymond, A.A. et al. Quantitative hippocampal MRI and intractable temporal lobe epilepsy. Neurology 45, 2233-40 (1995).

41. Woermann, F.G., Barker, G.J., Birnie, K.D., Meencke, H.J., Duncan, J.S. Regional changes in hippocampal T2 relaxation and volume: a quantitative magnetic resonance imaging study of hippocampal sclerosis. J Neurol Neurosurg Psychiatry 65, 656-64 (1998).

42. DeCarli, C., Hatta, J., Fazilat, S., Fazilat, S., Gaillard, W.D., Theodore, W.H. Extratemporal atrophy in patients with complex partial seizures of left temporal origin. Ann Neurol 43, 41-5 (1998).

43. Jutila, L., Ylinen, A., Partanen, K., Alafuzoff, I., Mervaala, E., Partanen, J. et al. MR volumetry of the entorhinal, perirhinal, and temporopolar cortices in drug-refractory temporal lobe epilepsy. AJNR Am J Neuroradiol 22, 1490-501 (2001).

44. Kalviainen, R., Salmenpera, T., Partanen, K., Vainio, P., Riekkinen, P., Pitkanen, A. Recurrent seizures may cause hippocampal damage in temporal lobe epilepsy. Neurology 50, 1377-82 (1998).

45. Lawson, J.A., Vogrin, S., Bleasel, A.F., Cook, M.J., Bye, A.M. Cerebral and cerebellar volume reduction in children with intractable epilepsy. Epilepsia 41, 1456-62 (2000).

46. Quigg, M., Bertram, E.H., Jackson, T. Longitudinal distribution of hippocampal atrophy in mesial temporal lobe epilepsy. Epilepsy Res 27, 101-10 (1997).

47. Quigg, M., Bertram, E.H., Jackson, T., Laws, E. Volumetric magnetic resonance imaging evidence of bilateral hippocampal atrophy in mesial temporal lobe epilepsy. Epilepsia 38, 588-94 (1997).

48. Bernasconi, N., Bernasconi, A., Andermann, F., Dubeau, F., Feindel, W., Reutens, D.C. Entorhinal cortex in temporal lobe epilepsy: a quantitative MRI study. Neurology 52, 1870-6 (1999).

49. Hoshida, T., Sakaki, T., Morimoto, T., Hashimoto, H., Kurokawa, S., Nakase, H. et al. Manual and digitizer measurements of amygdalohippocampal volume: reliability in comparison to computer-based measurement. Psychiatry Clin Neurosci 49, S223-5 (1995).

50. Jack, C.R. Jr, Sharbrough, F.W., Cascino, G.D., Hirschorn, K.A., O'Brien, P.C., Marsh, W.R. Magnetic resonance image-based hippocampal volumetry: correlation with outcome after temporal lobectomy. Ann Neurol 31, 138-46 (1992).

51. Marsh, L., Morrell, M.J., Shear, P.K., Sullivan, E.V., Freeman, H., Marie, A. et al. Cortical and hippocampal volume deficits in temporal lobe epilepsy. Epilepsia 38, 576-87 (1997).

52. Marsh, L., Sullivan, E.V., Morrell, M., Lim, K.O., Pfefferbaum, A. Structural brain abnormalities in patients with schizophrenia, epilepsy, and epilepsy with chronic interictal psychosis. Psychiatry Res 108, 1-15 (2001).

53. Cendes, F., Andermann, F., Gloor, P., Evans, A., Jones-Gotman, M., Watson, C. et al. MRI volumetric measurement of amygdala and hippocampus in temporal lobe epilepsy. Neurology 43, 719-25 (1993).

54. Cendes, F., Leproux, F., Melanson, D., Ethier, R., Evans, A., Peters, T. et al. MRI of amygdala and hippocampus in temporal lobe epilepsy. J Comput Assist Tomogr 17, 206-10 (1993).

55. Lambert, M.V., Brierley, B., Al-Sarraj, S., Shaw, P., Polkey, C.E., Chandler, C. et al. Quantitative magnetic resonance imaging of the amygdala in temporal lobe epilepsy-clinico-pathological correlations (a pilot study). Epilepsy Res 53, 39-46 (2003).

56. Bernasconi, N., Bernasconi, A., Caramanos, Z., Antel, S.B., Andermann, F., Arnold, D.L. Mesial temporal damage in temporal lobe epilepsy: a volumetric MRI study of the hippocampus, amygdala and parahippocampal region. Brain 126, 462-9 (2003).

57. Briellmann, R.S., Berkovic, S.F., Syngeniotis, A., King, M.A., Jackson, G.D. Seizure-associated hippocampal volume loss: a longitudinal magnetic resonance study of temporal lobe epilepsy. Ann Neurol 51, 641-4 (2002).

58. Fuerst, D., Shah, J., Shah, A., Watson, C. Hippocampal sclerosis is a progressive disorder: a longitudinal volumetric MRI study. Ann Neurol 53, 413-6 (2003).

59. Salmenpera, T., Kalviainen, R., Partanen, K., Pitkanen, A. Quantitative MRI volumetry of the entorhinal cortex in temporal lobe epilepsy. Seizure 9, 208-15 (2000).

60. Lamusuo, S., Pitkanen, A., Jutila, L., Ylinen, A., Partanen, K., Kalviainen, R. et al. [11 C]Flumazenil binding in the medial temporal lobe in patients with temporal lobe epilepsy: correlation with hippocampal MR volumetry, T2 relaxometry, and neuropathology. Neurology 54, 2252-60 (2000).

61. Fuerst, D., Shah, J., Kupsky, W.J., Johnson, R., Shah, A., Hayman-Abello, B. et al. Volumetric MRI, pathological, and neuropsychological progression in hippocampal sclerosis. Neurology 57, 184-8 (2001).

62. Jokeit, H., Ebner, A., Arnold, S., Schuller, M., Antke, C., Huang, Y. et al. Bilateral reductions of hippocampal volume, glucose metabolism, and wada hemispheric memory performance are related to the duration of mesial temporal lobe epilepsy. J Neurol 246, 926-33 (1999).

63. Seeck, M., Lazeyras, F., Murphy, K., Naimi, A., Pizzolatto, G.P., de Tribolet, N. et al. Psychosocial functioning in chronic epilepsy: relation to hippocampal volume and histopathological findings. Epileptic Disord 1, 179-85 (1999).

64. Spencer, S.S., McCarthy, G., Spencer, D.D. Diagnosis of medial temporal lobe seizure onset: relative specificity and sensitivity of quantitative MRI. Neurology 43, 2117-24 (1993).

65. Kalviainen, R. and Salmenpera, T. Do recurrent seizures cause neuronal damage? A series of studies with MRI volumetry in adults with partial epilepsy. Prog Brain Res 135, 279-95 (2002).

66. Salmenpera, T., Kalviainen, R., Partanen, K., Pitkanen, A. Hippocampal and amygdaloid damage in partial epilepsy: a cross-sectional MRI study of 241 patients. Epilepsy Res 46, 69-82 (2001).

67. Saukkonen, A., Kalviainen, R., Partanen, K., Vainio, P., Riekkinen, P., Pitkanen, A. Do seizures cause neuronal damage? A MRI study in newly diagnosed and chronic epilepsy. Neuroreport 6, 219-23 (1994).

68. Trenerry, M.R., Jack, C.R. Jr, Sharbrough, F.W., Cascino, G.D., Hirschorn, K.A., Marsh, W.R. et al. Quantitative MRI hippocampal volumes: association with onset and duration of epilepsy, and febrile convulsions in temporal lobectomy patients. Epilepsy Res 15, 247-52 (1993).

69. Barr, W.B., Ashtari, M., Schaul, N. Bilateral reductions in hippocampal volume in adults with epilepsy and a history of febrile seizures. J Neurol Neurosurg Psychiatry 63, 461-7 (1997).

70. Keller, S.S., Wieshmann, U.C., Mackay, C.E., Denby, C.E., Webb, J., Roberts, N. Voxel based morphometry of grey matter abnormalities in patients with medically intractable temporal lobe epilepsy: effects of side of seizure onset and epilepsy duration. J Neurol Neurosurg Psychiatry 73, 648-55 (2002).

71. Briellmann, R.S., Berkovic, S.F., Jackson, G.D. Men may be more vulnerable to seizure-associated brain damage. Neurology 55, 1479-85 (2000).

72. Cendes, F., Andermann, F., Gloor, P., Lopes-Cendes, I., Andermann, E., Melanson, D. et al. Atrophy of mesial structures in patients with temporal lobe epilepsy: cause or consequence of repeated seizures? Ann Neurol 34, 795-801 (1993).

73. Spanaki, M.V., Kopylev, L., Liow, K., DeCarli, C., Fazilat, S., Gaillard, W.D. et al. Relationship of seizure frequency to hippocampus volume and metabolism in temporal lobe epilepsy. Epilepsia 41, 1227-9 (2000).

74. Jack, C.R. Jr, Trenerry, M.R., Cascino, G.D., Sharbrough, F.W., So, E.L., O'Brien, P.C. Bilaterally symmetric hippocampi and surgical outcome. Neurology 45, 1353-8 (1995).

75. Luby, M., Spencer, D.D., Kim, J.H., deLanerolle, N., McCarthy, G. Hippocampal MRI volumetrics and temporal lobe substrates in medial temporal lobe epilepsy. Magn Reson Imaging 13, 1065-71 (1995).

76. Liu, R.S., Lemieux, L., Bell, G.S., Sisodiya, S.M., Bartlett, P.A., Shorvon, S.D. et al. The structural consequences of newly diagnosed seizures. Ann Neurol 52, 573-80 (2002).

77. Salmenpera, T., Kalviainen, R., Partanen, K., Mervaala, E., Pitkanen, A. MRI volumetry of the hippocampus, amygdala, entorhinal cortex, and perirhinal cortex after status epilepticus. Epilepsy Res 40, 155-70 (2000).

78. Lee, N., Tien, R.D., Lewis, D.V., Friedman, A.H., Felsberg, G.J., Crain, B. et al. Fast spin-echo, magnetic resonance imaging-measured hippocampal volume: correlation with neuronal density in anterior temporal lobectomy patients. Epilepsia 36, 899-904 (1995).

79. Briellmann, R.S., Kalnins, R.M., Berkovic, S.F., Jackson, G.D. Hippocampal pathology in refractory temporal lobe epilepsy: T2-weighted signal change reflects dentate gliosis. Neurology 58, 265-71 (2002).

80. Diehl, B., Najm, I., Mohamed, A., Babb, T., Ying, Z., Bingaman, W. Interictal EEG, hippocampal atrophy, and cell densities in hippocampal sclerosis and hippocampal sclerosis associated with microscopic cortical dysplasia. J Clin Neurophysiol 19, 157-62 (2002).

81. Kuzniecky, R., Palmer, C., Hugg, J., Martin, R., Sawrie, S., Morawetz, R. et al. Magnetic resonance spectroscopic imaging in temporal lobe epilepsy: neuronal dysfunction or cell loss? Arch Neurol 58, 2048-53 (2001).

82. Van Paesschen, W., Revesz, T., Duncan, J.S., King, M.D., Connelly, A. Quantitative neuropathology and quantitative magnetic resonance imaging of the hippocampus in temporal lobe epilepsy. Ann Neurol 42, 756-66 (1997).

83. O'Brien, T.J., Newton, M.R., Cook, M.J., Berlangieri, S.U., Kilpatrick, C., Morris, K. et al. Hippocampal atrophy is not a major determinant of regional hypometabolism in temporal lobe epilepsy. Epilepsia 38, 74-80 (1997).

84. Theodore, W.H., Gaillard, W.D., De Carli, C., Bhatia, S., Hatta, J. Hippocampal volume and glucose metabolism in temporal lobe epileptic foci. Epilepsia 42, 130-2 (2001).

85. Martin, R.C., Hugg, J.W., Roth, D.L., Bilir, E., Gilliam, F.G., Faught, E. et al. MRI extrahippocampal volumes and visual memory: correlations independent of MRI hippocampal volumes in temporal lobe epilepsy patients. J Int Neuropsychol Soc 5, 540-8 (1999).

86. Pegna, A.J., Caldara-Schnetzer, A.S., Perrig, S.H., Lazeyras, F., Khateb, A., Mayer, E. et al. Is the right amygdala involved in visuospatial memory? Evidence from MRI volumetric measures. Eur Neurol 47, 148-55 (2002).

87. Trenerry, M.R., Jack, C.R. Jr, Ivnik, R.J., Sharbrough, F.W., Cascino, G.D., Hirschorn, K.A. et al. MRI hippocampal volumes and memory function before and after temporal lobectomy. Neurology 43, 1800-5 (1993).

88. Trenerry, M.R., Jack, C.R. Jr, Cascino, G.D., Sharbrough, F.W., So, E.L. Bilateral magnetic resonance imaging-determined hippocampal atrophy and verbal memory before and after temporal lobectomy. Epilepsia 37, 526-33 (1996).

89. Martin, R.C., Sawrie, S.M., Knowlton, R.C., Bilir, E., Gilliam, F.G., Faught, E. et al. Bilateral hippocampal atrophy: consequences to verbal memory following temporal lobectomy. Neurology 57, 597-604 (2001).

90. Baxendale, S.A., Thompson, P.J., Kitchen, N.D. Postoperative hippocampal remnant shrinkage and memory decline: a dynamic process. Neurology 55, 243-9 (2000).

91. Baxendale, S.A., van Paesschen, W., Thompson, P.J., Connelly, A., Duncan, J.S., Harkness, W.F. et al. The relationship between quantitative MRI and neuropsychological functioning in temporal lobe epilepsy. Epilepsia 39, 158-66 (1998).

92. Baxendale, S.A., Van Paesschen, W., Thompson, P.J., Duncan, J.S., Shorvon, S.D., Connelly, A. The relation between quantitative MRI measures of hippocampal structure and the intracarotid amobarbital test. Epilepsia 38, 998-1007 (1997).

93. Koepp, M.J., Hammers, A., Labbe, C., Woermann, F.G., Brooks, D.J., Duncan, J.S. 11C-flumazenil PET in patients with refractory temporal lobe epilepsy and normal MRI. Neurology 54, 332-9 (2000).

94. Koepp, M.J., Richardson, M.P., Brooks, D.J., Poline, J.B., Van Paesschen, W., Friston, K.J. et al. Cerebral benzodiazepine receptors in hippocampal sclerosis. An objective in vivo analysis. Brain 119 (Pt 5), 1677-87 (1996).

95. Szelies, B., Weber-Luxenburger, G., Mielke, R., Pawlik, G., Kessler, J., Pietrzyk, U. et al. Interictal hippocampal benzodiazepine receptors in temporal lobe epilepsy: comparison with coregistered hippocampal metabolism and volumetry. Eur J Neurol 7, 393-400 (2000).

96. Koepp, M.J., Richardson, M.P., Labbe, C., Brooks, D.J., Cunningham, V.J., Ashburner, J. et al. 11C-flumazenil PET, volumetric MRI, and quantitative pathology in mesial temporal lobe epilepsy. Neurology 49, 764-73 (1997).

97. Liu, R.S., Lemieux, L., Bell, G.S., Bartlett, P.A., Sander, J.W., Sisodiya, S.M. et al. A longitudinal quantitative MRI study of community-based patients with chronic epilepsy and newly diagnosed seizures: methodology and preliminary findings. Neuroimage 14, 231-43 (2001).

98. Free, S.L., Li, L.M., Fish, D.R., Shorvon, S.D., Stevens, J.M. Bilateral hippocampal volume loss in patients with a history of encephalitis or meningitis. Epilepsia 37, 400-5 (1996).

99. Free, S.L., Bergin, P.S., Fish, D.R., Cook, M.J., Shorvon, S.D., Stevens, J.M. Methods for normalization of hippocampal volumes measured with MR. AJNR Am J Neuroradiol 16, 637-43 (1995).

100. King, D., Spencer, S.S., McCarthy, G., Luby, M., Spencer, D.D. Bilateral hippocampal atrophy in medial temporal lobe epilepsy. Epilepsia 36, 905-10 (1995).

101. Kim, J.H., Tien, R.D., Felsberg, G.J., Osumi, A.K., Lee, N. MR measurements of the hippocampus for lateralization of temporal lobe epilepsy: value of measurements of the body vs the whole structure. AJR Am J Roentgenol 163, 1453-7 (1994).

102. Achten, E., Deblaere, K., De Wagter, C., Van Damme, F., Boon, P., De Reuck, J. et al. Intra- and interobserver variability of MRI-based volume measurements of the hippocampus and amygdala using the manual ray-tracing method. Neuroradiology 40, 558-66 (1998).

103. Hogan, R.E., Mark, K.E., Wang, L., Joshi, S., Miller, M.I., Bucholz, R.D. Mesial temporal sclerosis and temporal lobe epilepsy: MR imaging deformation-based segmentation of the hippocampus in five patients. Radiology 216, 291-7 (2000).

104. Ho, S.S., Kuzniecky, R.I., Gilliam, F., Faught, E., Bebin, M., Morawetz, R. Congenital porencephaly: MR features and relationship to hippocampal sclerosis. AJNR Am J Neuroradiol 19, 135-41 (1998).

105. Baulac, M., De Grissac, N., Hasboun, D., Oppenheim, C., Adam, C., Arzimanoglou, A. et al. Hippocampal developmental changes in patients with partial epilepsy: magnetic resonance imaging and clinical aspects. Ann Neurol 44, 223-33 (1998).

106. Martinez, M., Santamaria, J., Mercader, J.M., Catafau, A., Cardenal, C., Lomena, F. Correlation of MRI hippocampal volume analysis, video/EEG monitoring and inter- and postictal single photon emission tomography in refractory focal epilepsy. Neuroradiology 36, 11-6 (1994).

107. Grunewald, R.A., Farrow, T., Vaughan, P., Rittey, C.D., Mundy, J. A magnetic resonance study of complicated early childhood convulsion. J Neurol Neurosurg Psychiatry 71, 638-42 (2001).

108. Lawson, J.A., Cook, M.J., Bleasel, A.F., Nayanar, V., Morris, K.F., Bye, A.M. Quantitative MRI in outpatient childhood epilepsy. Epilepsia 38, 1289-93 (1997).

109. Woermann, F.G., Free, S.L., Koepp, M.J., Ashburner, J., Duncan, J.S. Voxel-by-voxel comparison of automatically segmented cerebral gray matter--A rater-independent comparison of structural MRI in patients with epilepsy. Neuroimage 10, 373-84 (1999).

110. Lawson, J.A., Vogrin, S., Bleasel, A.F., Cook, M.J., Burns, L., McAnally, L. et al. Predictors of hippocampal, cerebral, and cerebellar volume reduction in childhood epilepsy. Epilepsia 41, 1540-5 (2000).

111. Arciniegas, D.B., Topkoff, J.L., Rojas, D.C., Sheeder, J., Teale, P., Young, D.A. et al. Reduced hippocampal volume in association with p50 nonsuppression following traumatic brain injury. J Neuropsychiatry Clin Neurosci 13, 213-21 (2001).

112. Bigler, E.D., Anderson, C.V., Blatter, D.D., Andersob, C.V. Temporal lobe morphology in normal aging and traumatic brain injury. AJNR Am J Neuroradiol 23, 255-66 (2002).

113. Bigler, E.D., Blatter, D.D., Anderson, C.V., Johnson, S.C., Gale, S.D., Hopkins, R.O. et al. Hippocampal volume in normal aging and traumatic brain injury. AJNR Am J Neuroradiol 18, 11-23 (1997).

114. Vargha-Khadem, F., Gadian, D.G., Watkins, K.E., Connelly, A., Van Paesschen, W., Mishkin, M. Differential effects of early hippocampal pathology on episodic and semantic memory. Science 277, 376-80 (1997).

115. Di Stefano, G., Bachevalier, J., Levin, H.S., Song, J.X., Scheibel, R.S., Fletcher, J.M. Volume of focal brain lesions and hippocampal formation in relation to memory function after closed head injury in children. J Neurol Neurosurg Psychiatry 69, 210-6 (2000).

116. Rauch, S.L., Kim, H., Makris, N., Cosgrove, G.R., Cassem, E.H., Savage, C.R. et al. Volume reduction in the caudate nucleus following stereotactic placement of lesions in the anterior cingulate cortex in humans: a morphometric magnetic resonance imaging study. J Neurosurg 93, 1019-25 (2000).

117. Convit, A., De Leon, M.J., Tarshish, C., De Santi, S., Tsui, W., Rusinek, H. et al. Specific hippocampal volume reductions in individuals at risk for Alzheimer's disease. Neurobiol Aging 18, 131-8 (1997).

118. de Leon, M.J., Convit, A., George, A.E., Golomb, J., de Santi, S., Tarshish, C. et al. In vivo structural studies of the hippocampus in normal aging and in incipient Alzheimer's disease. Ann N Y Acad Sci 777, 1-13 (1996).

119. Fama, R., Sullivan, E.V., Shear, P.K., Cahn-Weiner, D.A., Marsh, L., Lim, K.O. et al. Structural brain correlates of verbal and nonverbal fluency measures in Alzheimer's disease. Neuropsychology 14, 29-40 (2000).

120. Freeborough, P.A., Fox, N.C., Kitney, R.I. Interactive algorithms for the segmentation and quantitation of 3-D MRI brain scans. Comput Methods Programs Biomed 53, 15-25 (1997).

121. Jack, C.R. Jr, Petersen, R.C., O'Brien, P.C., Tangalos, E.G. MR-based hippocampal volumetry in the diagnosis of Alzheimer's disease. Neurology 42, 183-8 (1992).

122. Jack, C.R. Jr, Petersen, R.C., Xu, Y., O'Brien, P.C., Smith, G.E., Ivnik, R.J. et al. Rate of medial temporal lobe atrophy in typical aging and Alzheimer's disease. Neurology 51, 993-9 (1998).

123. Kidron, D., Black, S.E., Stanchev, P., Buck, B., Szalai, J.P., Parker, J. et al. Quantitative MR volumetry in Alzheimer's disease. Topographic markers and the effects of sex and education. Neurology 49, 1504-12 (1997).

124. Laakso, M.P., Soininen, H., Partanen, K., Helkala, E.L., Hartikainen, P., Vainio, P. et al. Volumes of hippocampus, amygdala and frontal lobes in the MRI-based diagnosis of early Alzheimer's disease: correlation with memory functions. J Neural Transm Park Dis Dement Sect 9, 73-86 (1995).

125. O' Brien, T.J., Ames, D., Desmond, P., Lichtenstein, M. , Binns, D., Schweitzer, I. et al. Combined magnetic resonance imaging and single-photon emission tomography scanning in the discrimination of Alzheimer's disease from age-matched controls. Int Psychogeriatr 13, 149-61 (2001).

126. Pantel, J., Schroder, J., Essig, M., Popp, D., Dech, H., Knopp, M.V. et al. Quantitative magnetic resonance imaging in geriatric depression and primary degenerative dementia. J Affect Disord 42, 69-83 (1997).

127. Pitkanen, A., Laakso, M., Kalviainen, R., Partanen, K., Vainio, P., Lehtovirta, M. et al. Severity of hippocampal atrophy correlates with the prolongation of MRI T2 relaxation time in temporal lobe epilepsy but not in Alzheimer's disease. Neurology 46, 1724-30 (1996).

128. Smith, C.D., Malcein, M., Meurer, K., Schmitt, F.A., Markesbery, W.R., Pettigrew, L.C. MRI temporal lobe volume measures and neuropsychologic function in Alzheimer's disease. J Neuroimaging 9, 2-9 (1999).

129. Teipel, S.J., Bayer, W., Alexander, G.E., Bokde, A.L., Zebuhr, Y., Teichberg, D. et al. Regional pattern of hippocampus and corpus callosum atrophy in Alzheimer's disease in relation to dementia severity: evidence for early neocortical degeneration. Neurobiol Aging 24, 85-94 (2003).

130. Visser, P.J., Scheltens, P., Verhey, F.R., Schmand, B., Launer, L.J., Jolles, J. et al. Medial temporal lobe atrophy and memory dysfunction as predictors for dementia in subjects with mild cognitive impairment. J Neurol 246, 477-85 (1999).

131. de Toledo-Morrell, L., Sullivan, M.P., Morrell, F., Wilson, R.S., Bennett, D.A., Spencer, S. Alzheimer's disease: in vivo detection of differential vulnerability of brain regions. Neurobiol Aging 18, 463-8 (1997).

132. Lehericy, S., Baulac, M., Chiras, J., Pierot, L., Martin, N., Pillon, B. et al. Amygdalohippocampal MR volume measurements in the early stages of Alzheimer disease. AJNR Am J Neuroradiol 15, 929-37 (1994).

133. Sencakova, D., Graff-Radford, N.R., Willis, F.B., Lucas, J.A., Parfitt, F., Cha, R.H. et al. Hippocampal atrophy correlates with clinical features of Alzheimer disease in African Americans. Arch Neurol 58, 1593-7 (2001).

134. Geroldi, C., Laakso, M.P., DeCarli, C., Beltramello, A., Bianchetti, A., Soininen, H. et al. Apolipoprotein E genotype and hippocampal asymmetry in Alzheimer's disease: a volumetric MRI study. J Neurol Neurosurg Psychiatry 68, 93-6 (2000).

135. Hashimoto, M., Yasuda, M., Tanimukai, S., Matsui, M., Hirono, N., Kazui, H. et al. Apolipoprotein E epsilon 4 and the pattern of regional brain atrophy in Alzheimer's disease. Neurology 57, 1461-6 (2001).

136. Lehtovirta, M., Soininen, H., Laakso, M.P., Partanen, K., Helisalmi, S., Mannermaa, A. et al. SPECT and MRI analysis in Alzheimer's disease: relation to apolipoprotein E epsilon 4 allele. J Neurol Neurosurg Psychiatry 60, 644-9 (1996).

137. Bigler, E.D., Tate, D.F., Miller, M.J., Rice, S.A., Hessel, C.D., Earl, H.D. et al. Dementia, asymmetry of temporal lobe structures, and apolipoprotein E genotype: relationships to cerebral atrophy and neuropsychological impairment. J Int Neuropsychol Soc 8, 925-33 (2002).

138. Wang, L., Swank, J.S., Glick, I.E., Gado, M.H., Miller, M.I., Morris, J.C. et al. Changes in hippocampal volume and shape across time distinguish dementia of the Alzheimer type from healthy aging. Neuroimage 20, 667-82 (2003).

139. Frisoni, G.B., Laakso, M.P., Beltramello, A., Geroldi, C., Bianchetti, A., Soininen, H. et al. Hippocampal and entorhinal cortex atrophy in frontotemporal dementia and Alzheimer's disease. Neurology 52, 91-100 (1999).

140. Galton, C.J., Patterson, K., Graham, K., Lambon-Ralph, M.A., Williams, G., Antoun, N. et al. Differing patterns of temporal atrophy in Alzheimer's disease and semantic dementia. Neurology 57, 216-25 (2001).

141. Hashimoto, M., Kitagaki, H., Imamura, T., Hirono, N., Shimomura, T., Kazui, H. et al. Medial temporal and whole-brain atrophy in dementia with Lewy bodies: a volumetric MRI study. Neurology 51, 357-62 (1998).

142. Laakso, M.P., Partanen, K., Riekkinen, P., Lehtovirta, M., Helkala, E.L., Hallikainen, M. et al. Hippocampal volumes in Alzheimer's disease, Parkinson's disease with and without dementia, and in vascular dementia: An MRI study. Neurology 46, 678-81 (1996).

143. Maestu, F., Arrazola, J., Fernandez, A., Simos, P.G., Amo, C., Gil-Gregorio, P. et al. Do cognitive patterns of brain magnetic activity correlate with hippocampal atrophy in Alzheimer's disease? J Neurol Neurosurg Psychiatry 74, 208-12 (2003).

144. Jack, C.R. Jr, Petersen, R.C., Xu, Y.C., Waring, S.C., O'Brien, P.C., Tangalos, E.G. et al. Medial temporal atrophy on MRI in normal aging and very mild Alzheimer's disease. Neurology 49, 786-94 (1997).

145. Laakso, M.P., Soininen, H., Partanen, K., Lehtovirta, M., Hallikainen, M., Hanninen, T. et al. MRI of the hippocampus in Alzheimer's disease: sensitivity, specificity, and analysis of the incorrectly classified subjects. Neurobiol Aging 19, 23-31 (1998).

146. Wolf, H., Grunwald, M., Kruggel, F., Riedel-Heller, S.G., Angerhofer, S., Hojjatoleslami, A. et al. Hippocampal volume discriminates between normal cognition; questionable and mild dementia in the elderly. Neurobiol Aging 22, 177-86 (2001).

147. De Santi, S., de Leon, M.J., Rusinek, H., Convit, A., Tarshish, C.Y., Roche, A. et al. Hippocampal formation glucose metabolism and volume losses in MCI and AD. Neurobiol Aging 22, 529-39 (2001).

148. El Fakhri, G., Kijewski, M.F., Johnson, K.A., Syrkin, G., Killiany, R.J., Becker, J.A. et al. MRI-guided SPECT perfusion measures and volumetric MRI in prodromal Alzheimer disease. Arch Neurol 60, 1066-72 (2003).

149. Morys, J., Bobek-Billewicz, B., Dziewiatkowski, J., Bidzan, L., Ussorowska, D., Narklewicz, O. Changes in the volume of temporal lobe structures related to Alzheimer's type dementia. Folia Neuropathol 40, 47-56 (2002).

150. Dickerson, B.C., Goncharova, I., Sullivan, M.P., Forchetti, C., Wilson, R.S., Bennett, D.A. et al. MRI-derived entorhinal and hippocampal atrophy in incipient and very mild Alzheimer's disease. Neurobiol Aging 22, 747-54 (2001).

151. Du, A.T., Schuff, N., Amend, D., Laakso, M.P., Hsu, Y.Y., Jagust, W.J. et al. Magnetic resonance imaging of the entorhinal cortex and hippocampus in mild cognitive impairment and Alzheimer's disease. J Neurol Neurosurg Psychiatry 71, 441-7 (2001).

152. Killiany, R.J., Hyman, B.T., Gomez-Isla, T., Moss, M.B., Kikinis, R., Jolesz, F. et al. MRI measures of entorhinal cortex vs hippocampus in preclinical AD. Neurology 58, 1188-96 (2002).

153. Pearlson, G.D., Harris, G.J., Powers, R.E., Barta, P.E., Camargo, E.E., Chase, G.A. et al. Quantitative changes in mesial temporal volume, regional cerebral blood flow, and cognition in Alzheimer's disease. Arch Gen Psychiatry 49, 402-8 (1992).

154. Schuff, N., Amend, D., Ezekiel, F., Steinman, S.K., Tanabe, J., Norman, D. et al. Changes of hippocampal N-acetyl aspartate and volume in Alzheimer's disease. A proton MR spectroscopic imaging and MRI study. Neurology 49, 1513-21 (1997).

155. Karas, G.B., Burton, E.J., Rombouts, S.A., van Schijndel, R.A., O'Brien, J.T., Scheltens, P. et al. A comprehensive study of gray matter loss in patients with Alzheimer's disease using optimized voxel-based morphometry. Neuroimage 18, 895-907 (2003).

156. Laakso, M.P., Hallikainen, M., Hanninen, T., Partanen, K., Soininen, H. Diagnosis of Alzheimer's disease: MRI of the hippocampus vs delayed recall. Neuropsychologia 38, 579-84 (2000).

157. Xu, Y., Jack, C.R. Jr, O'Brien, P.C., Kokmen, E., Smith, G.E., Ivnik, R.J. et al. Usefulness of MRI measures of entorhinal cortex versus hippocampus in AD. Neurology 54, 1760-7 (2000).

158. Hampel, H., Teipel, S.J., Bayer, W., Alexander, G.E., Schwarz, R., Schapiro, M.B. et al. Age transformation of combined hippocampus and amygdala volume improves diagnostic accuracy in Alzheimer's disease. J Neurol Sci 194, 15-9 (2002).

159. Laakso, M.P., Lehtovirta, M., Partanen, K., Riekkinen, P.J., Soininen, H. Hippocampus in Alzheimer's disease: a 3-year follow-up MRI study. Biol Psychiatry 47, 557-61 (2000).

160. Bobinski, M., de Leon, M.J., Wegiel, J., Desanti, S., Convit, A., Saint Louis, L.A. et al. The histological validation of post mortem magnetic resonance imaging-determined hippocampal volume in Alzheimer's disease. Neuroscience 95, 721-5 (2000).

161. Deweer, B., Lehericy, S., Pillon, B., Baulac, M., Chiras, J., Marsault, C. et al. Memory disorders in probable Alzheimer's disease: the role of hippocampal atrophy as shown with MRI. J Neurol Neurosurg Psychiatry 58, 590-7 (1995).

162. Heun, R., Mazanek, M., Atzor, K.R., Tintera, J., Gawehn, J., Burkart, M. et al. Amygdala-hippocampal atrophy and memory performance in dementia of Alzheimer type. Dement Geriatr Cogn Disord 8, 329-36 (1997).

163. Petersen, R.C., Jack, C.R. Jr, Xu, Y.C., Waring, S.C., O'Brien, P.C., Smith, G.E. et al. Memory and MRI-based hippocampal volumes in aging and AD. Neurology 54, 581-7 (2000).

164. Wilson, R. S., Sullivan, M., de Toledo-Morrell, L., Stebbins, G. T., Bennett, D. A.,Morrell, F. Association of Memory and Cognition in Alzheimer's Disease With Volumetric Estimates of Temporal Lobe Structures. Neuropsychology 10(4), 459-463. 96.

165. Kohler, S., Black, S.E., Sinden, M., Szekely, C., Kidron, D., Parker, J.L. et al. Memory impairments associated with hippocampal versus parahippocampal-gyrus atrophy: an MR volumetry study in Alzheimer's disease. Neuropsychologia 36, 901-14 (1998).

166. de Toledo-Morrell, L., Dickerson, B., Sullivan, M.P., Spanovic, C., Wilson, R., Bennett, D.A. Hemispheric differences in hippocampal volume predict verbal and spatial memory performance in patients with Alzheimer's disease. Hippocampus 10, 136-42 (2000).

167. Mori, E., Yoneda, Y., Yamashita, H., Hirono, N., Ikeda, M., Yamadori, A. Medial temporal structures relate to memory impairment in Alzheimer's disease: an MRI volumetric study. J Neurol Neurosurg Psychiatry 63, 214-21 (1997).

168. Mori, E., Ikeda, M., Hirono, N., Kitagaki, H., Imamura, T., Shimomura, T. Amygdalar volume and emotional memory in Alzheimer's disease. Am J Psychiatry 156, 216-22 (1999).

169. Fox, N.C., Warrington, E.K., Freeborough, P.A., Hartikainen, P., Kennedy, A.M., Stevens, J.M. et al. Presymptomatic hippocampal atrophy in Alzheimer's disease. A longitudinal MRI study. Brain 119 (Pt 6), 2001-7 (1996).

170. Fox, N.C., Warrington, E.K., Stevens, J.M., Rossor, M.N. Atrophy of the hippocampal formation in early familial Alzheimer's disease. A longitudinal MRI study of at-risk members of a family with an amyloid precursor protein 717Val-Gly mutation. Ann N Y Acad Sci 777, 226-32 (1996).

171. Marquis, S., Moore, M.M., Howieson, D.B., Sexton, G., Payami, H., Kaye, J.A. et al. Independent predictors of cognitive decline in healthy elderly persons. Arch Neurol 59, 601-6 (2002).

172. Mungas, D., Reed, B.R., Jagust, W.J., DeCarli, C., Mack, W.J., Kramer, J.H. et al. Volumetric MRI predicts rate of cognitive decline related to AD and cerebrovascular disease. Neurology 59, 867-73 (2002).

173. Schott, J.M., Fox, N.C., Frost, C., Scahill, R.I., Janssen, J.C., Chan, D. et al. Assessing the onset of structural change in familial Alzheimer's disease. Ann Neurol 53, 181-8 (2003).

174. Cohen, R.M., Small, C., Lalonde, F., Friz, J., Sunderland, T. Effect of apolipoprotein E genotype on hippocampal volume loss in aging healthy women. Neurology 57, 2223-8 (2001).

175. Soininen, H., Partanen, K., Pitkanen, A., Hallikainen, M., Hanninen, T., Helisalmi, S. et al. Decreased hippocampal volume asymmetry on MRIs in nondemented elderly subjects carrying the apolipoprotein E epsilon 4 allele. Neurology 45, 391-2 (1995).

176. Reiman, E.M., Uecker, A., Caselli, R.J., Lewis, S., Bandy, D., de Leon, M.J. et al. Hippocampal volumes in cognitively normal persons at genetic risk for Alzheimer's disease. Ann Neurol 44, 288-91 (1998).

177. Jack, C.R. Jr, Petersen, R.C., Xu, Y., O'Brien, P.C., Smith, G.E., Ivnik, R.J. et al. Rates of hippocampal atrophy correlate with change in clinical status in aging and AD. Neurology 55, 484-89 (2000).

178. Jack, C.R. Jr, Slomkowski, M., Gracon, S., Hoover, T.M., Felmlee, J.P., Stewart, K. et al. MRI as a biomarker of disease progression in a therapeutic trial of milameline for AD. Neurology 60, 253-60 (2003).

179. Crum, W.R., Scahill, R.I., Fox, N.C. Automated hippocampal segmentation by regional fluid registration of serial MRI: validation and application in Alzheimer's disease. Neuroimage 13, 847-55 (2001).

180. Gosche, K.M., Mortimer, J.A., Smith, C.D., Markesbery, W.R., Snowdon, D.A. An automated technique for measuring hippocampal volumes from MR imaging studies. AJNR Am J Neuroradiol 22, 1686-9 (2001).

181. Murphy, C., Jernigan, T.L., Fennema-Notestine, C. Left hippocampal volume loss in Alzheimer's disease is reflected in performance on odor identification: a structural MRI study. J Int Neuropsychol Soc 9, 459-71 (2003).

182. Cardenas, V.A., Du, A.T., Hardin, D., Ezekiel, F., Weber, P., Jagust, W.J. et al. Comparison of methods for measuring longitudinal brain change in cognitive impairment and dementia. Neurobiol Aging 24, 537-44 (2003).

183. Grunwald, M., Busse, F., Hensel, A., Kruggel, F., Riedel-Heller, S., Wolf, H. et al. Correlation between cortical theta activity and hippocampal volumes in health, mild cognitive impairment, and mild dementia. J Clin Neurophysiol 18, 178-84 (2001).

184. Barber, R., McKeith, I.G., Ballard, C., Gholkar, A., O'Brien, J.T. A comparison of medial and lateral temporal lobe atrophy in dementia with Lewy bodies and Alzheimer's disease: magnetic resonance imaging volumetric study. Dement Geriatr Cogn Disord 12, 198-205 (2001).

185. Fein, G., Di Sclafani, V., Tanabe, J., Cardenas, V., Weiner, M.W., Jagust, W.J. et al. Hippocampal and cortical atrophy predict dementia in subcortical ischemic vascular disease. Neurology 55, 1626-35 (2000).

186. Du, A.T., Schuff, N., Laakso, M.P., Zhu, X.P., Jagust, W.J., Yaffe, K. et al. Effects of subcortical ischemic vascular dementia and AD on entorhinal cortex and hippocampus. Neurology 58, 1635-41 (2002).

187. Mummery, C.J., Patterson, K., Price, C.J., Ashburner, J., Frackowiak, R.S., Hodges, J.R. A voxel-based morphometry study of semantic dementia: relationship between temporal lobe atrophy and semantic memory. Ann Neurol 47, 36-45 (2000).

188. Barber, R., Ballard, C., McKeith, I.G., Gholkar, A., O'Brien, J.T. MRI volumetric study of dementia with Lewy bodies: a comparison with AD and vascular dementia. Neurology 54, 1304-9 (2000).

189. Burton, E.J., Karas, G., Paling, S.M., Barber, R., Williams, E.D., Ballard, C.G. et al. Patterns of cerebral atrophy in dementia with Lewy bodies using voxel-based morphometry. Neuroimage 17, 618-30 (2002).

190. Bigler, E.D., Kerr, B., Victoroff, J., Tate, D.F., Breitner, J.C. White matter lesions, quantitative magnetic resonance imaging, and dementia. Alzheimer Dis Assoc Disord 16, 161-70 (2002).

191. Simons, J.S., Verfaellie, M., Galton, C.J., Miller, B.L., Hodges, J.R., Graham, K.S. Recollection-based memory in frontotemporal dementia: implications for theories of long-term memory. Brain 125, 2523-36 (2002).

192. Bigler, E.D. and Tate, D.F. Brain volume, intracranial volume, and dementia. Invest Radiol 36, 539-46 (2001).

193. Petersen, R.C., Doody, R., Kurz, A., Mohs, R.C., Morris, J.C., Rabins, P.V. et al. Current concepts in mild cognitive impairment. Arch Neurol 58, 1985-92 (2001).

194. Crook, T., Bahar, H., Sudilovsky, A. Age-associated memory impairment: diagnostic criteria and treatment strategies. Int J Neurol 21-22, 73-82 (1987-1988).

195. Convit, A., de Leon, M.J., Golomb, J., George, A.E., Tarshish, C.Y., Bobinski, M. et al. Hippocampal atrophy in early Alzheimer's disease: anatomic specificity and validation. Psychiatr Q 64, 371-87 (1993).

196. Jack, C.R. Jr, Petersen, R.C., Xu, Y.C., O'Brien, P.C., Smith, G.E., Ivnik, R.J. et al. Prediction of AD with MRI-based hippocampal volume in mild cognitive impairment. Neurology 52, 1397-403 (1999).

197. Grundman, M., Sencakova, D., Jack, C.R. Jr, Petersen, R.C., Kim, H.T., Schultz, A. et al. Brain MRI hippocampal volume and prediction of clinical status in a mild cognitive impairment trial. J Mol Neurosci 19, 23-7 (2002).
Notes: CORPORATE NAME: Alzheimer's Disease Cooperative Study.

198. Visser, P.J., Verhey, F.R., Hofman, P.A., Scheltens, P., Jolles, J. Medial temporal lobe atrophy predicts Alzheimer's disease in patients with minor cognitive impairment. J Neurol Neurosurg Psychiatry 72, 491-7 (2002).

199. Convit, A., de Asis, J., de Leon, M.J., Tarshish, C.Y., De Santi, S., Rusinek, H. Atrophy of the medial occipitotemporal, inferior, and middle temporal gyri in non-demented elderly predict decline to Alzheimer's disease. Neurobiol Aging 21, 19-26 (2000).

200. Chetelat, G., Desgranges, B., de la Sayette, V., Viader, F., Berkouk, K., Landeau, B. et al. Dissociating atrophy and hypometabolism impact on episodic memory in mild cognitive impairment. Brain 126, 1955-67 (2003).

201. Bhatia, S., Bookheimer, S.Y., Gaillard, W.D., Theodore, W.H. Measurement of whole temporal lobe and hippocampus for MR volumetry: normative data. Neurology 43, 2006-10 (1993).

202. Convit, A., de Leon, M.J., Tarshish, C., De Santi, S., Kluger, A., Rusinek, H. et al. Hippocampal volume losses in minimally impaired elderly. Lancet 345, 266 (1995).

203. Convit, A., de Leon, M.J., Hoptman, M.J., Tarshish, C., De Santi, S., Rusinek, H. Age-related changes in brain: I. Magnetic resonance imaging measures of temporal lobe volumes in normal subjects. Psychiatr Q 66, 343-55 (1995).

204. Jernigan, T.L., Archibald, S.L., Fennema-Notestine, C., Gamst, A.C., Stout, J.C., Bonner, J. et al. Effects of age on tissues and regions of the cerebrum and cerebellum. Neurobiol Aging 22, 581-94 (2001).

205. Mu, Q., Xie, J., Wen, Z., Weng, Y., Shuyun, Z. A quantitative MR study of the hippocampal formation, the amygdala, and the temporal horn of the lateral ventricle in healthy subjects 40 to 90 years of age. AJNR Am J Neuroradiol 20, 207-11 (1999).

206. Mueller, E.A., Moore, M.M., Kerr, D.C., Sexton, G., Camicioli, R.M., Howieson, D.B. et al. Brain volume preserved in healthy elderly through the eleventh decade. Neurology 51, 1555-62 (1998).

207. Raz, N., Gunning-Dixon, F.M., Head, D., Dupuis, J.H., Acker, J.D. Neuroanatomical correlates of cognitive aging: evidence from structural magnetic resonance imaging. Neuropsychology 12, 95-114 (1998).

208. Tisserand, D.J., Visser, P.J., van Boxtel, M.P., Jolles, J. The relation between global and limbic brain volumes on MRI and cognitive performance in healthy individuals across the age range. Neurobiol Aging 21, 569-76 (2000).

209. Wolf, O.T., Convit, A., de Leon, M.J., Caraos, C., Qadri, S.F. Basal hypothalamo-pituitary-adrenal axis activity and corticotropin feedback in young and older men: relationships to magnetic resonance imaging-derived hippocampus and cingulate gyrus volumes. Neuroendocrinology 75, 241-9 (2002).

210. Sullivan, E.V., Marsh, L., Mathalon, D.H., Lim, K.O., Pfefferbaum, A. Age-related decline in MRI volumes of temporal lobe gray matter but not hippocampus. Neurobiol Aging 16, 591-606 (1995).

211. Kaye, J.A., Swihart, T., Howieson, D., Dame, A., Moore, M.M., Karnos, T. et al. Volume loss of the hippocampus and temporal lobe in healthy elderly persons destined to develop dementia. Neurology 48, 1297-304 (1997).

212. Wu, C.C., Mungas, D., Petkov, C.I., Eberling, J.L., Zrelak, P.A., Buonocore, M.H. et al. Brain structure and cognition in a community sample of elderly Latinos. Neurology 59, 383-91 (2002).

213. Golomb, J., Kluger, A., de Leon, M.J., Ferris, S.H., Convit, A., Mittelman, M.S. et al. Hippocampal formation size in normal human aging: a correlate of delayed secondary memory performance. Learn Mem 1, 45-54 (1994).

214. Golomb, J., de Leon, M.J., George, A.E., Kluger, A., Convit, A., Rusinek, H. et al. Hippocampal atrophy correlates with severe cognitive impairment in elderly patients with suspected normal pressure hydrocephalus. J Neurol Neurosurg Psychiatry 57, 590-3 (1994).

215. Murphy, D.G., DeCarli, C., McIntosh, A.R., Daly, E., Mentis, M.J., Pietrini, P. et al. Sex differences in human brain morphometry and metabolism: an in vivo quantitative magnetic resonance imaging and positron emission tomography study on the effect of aging. Arch Gen Psychiatry 53, 585-94 (1996).

216. den Heijer, T., Vermeer, S.E., Clarke, R., Oudkerk, M., Koudstaal, P.J., Hofman, A. et al. Homocysteine and brain atrophy on MRI of non-demented elderly. Brain 126, 170-5 (2003).

217. Hackert, V.H., den Heijer, T., Oudkerk, M., Koudstaal, P.J., Hofman, A., Breteler, M.M. Hippocampal head size associated with verbal memory performance in nondemented elderly. Neuroimage 17, 1365-72 (2002).

218. Kawas, C., Resnick, S., Morrison, A., Brookmeyer, R., Corrada, M., Zonderman, A. et al. A prospective study of estrogen replacement therapy and the risk of developing Alzheimer's disease: the Baltimore Longitudinal Study of Aging. Neurology 48, 1517-21 (1997).

219. Tang, M.X., Jacobs, D., Stern, Y., Marder, K., Schofield, P., Gurland, B. et al. Effect of oestrogen during menopause on risk and age at onset of Alzheimer's disease. Lancet 348, 429-32 (1996).

220. Eberling, J.L., Wu, C., Haan, M.N., Mungas, D., Buonocore, M., Jagust, W.J. Preliminary evidence that estrogen protects against age-related hippocampal atrophy. Neurobiol Aging 24, 725-32 (2003).

221. den Heijer, T., Geerlings, M.I., Hofman, A., de Jong, F.H., Launer, L.J., Pols, H.A. et al. Higher estrogen levels are not associated with larger hippocampi and better memory performance. Arch Neurol 60, 213-20 (2003).

222. Haznedar, M.M., Buchsbaum, M.S., Wei, T.C., Hof, P.R., Cartwright, C., Bienstock, C.A. et al. Limbic circuitry in patients with autism spectrum disorders studied with positron emission tomography and magnetic resonance imaging. Am J Psychiatry 157, 1994-2001 (2000).

223. Piven, J., Bailey, J., Ranson, B.J., Arndt, S. No difference in hippocampus volume detected on magnetic resonance imaging in autistic individuals. J Autism Dev Disord 28, 105-10 (1998).

224. Aylward, E.H., Minshew, N.J., Goldstein, G., Honeycutt, N.A., Augustine, A.M., Yates, K.O. et al. MRI volumes of amygdala and hippocampus in non-mentally retarded autistic adolescents and adults. Neurology 53 , 2145-50 (1999).

225. Herbert, M.R., Ziegler, D.A., Deutsch, C.K., O'Brien, L.M., Lange, N., Bakardjiev, A. et al. Dissociations of cerebral cortex, subcortical and cerebral white matter volumes in autistic boys. Brain 126, 1182-92 (2003).

226. Sparks, B.F., Friedman, S.D., Shaw, D.W., Aylward, E.H., Echelard, D., Artru, A.A. et al. Brain structural abnormalities in young children with autism spectrum disorder. Neurology 59, 184-92 (2002).

227. Raz, N., Torres, I.J., Briggs, S.D., Spencer, W.D., Thornton, A.E., Loken, W.J. et al. Selective neuroanatomic abnormalities in Down's syndrome and their cognitive correlates: evidence from MRI morphometry. Neurology 45, 356-66 (1995).

228. Krasuski, J.S., Alexander, G.E., Horwitz, B., Rapoport, S.I., Schapiro, M.B. Relation of medial temporal lobe volumes to age and memory function in nondemented adults with Down's syndrome: implications for the prodromal phase of Alzheimer's disease. Am J Psychiatry 159, 74-81 (2002).

229. Pinter, J.D., Brown, W.E., Eliez, S., Schmitt, J.E., Capone, G.T., Reiss, A.L. Amygdala and hippocampal volumes in children with Down syndrome: a high-resolution MRI study. Neurology 56, 972-4 (2001).

230. Teipel, S.J., Schapiro, M.B., Alexander, G.E., Krasuski, J.S., Horwitz, B., Hoehne, C. et al. Relation of corpus callosum and hippocampal size to age in nondemented adults with Down's syndrome. Am J Psychiatry 160, 1870-8 (2003).

231. Pearlson, G.D., Breiter, S.N., Aylward, E.H., Warren, A.C., Grygorcewicz, M., Frangou, S. et al. MRI brain changes in subjects with Down syndrome with and without dementia. Dev Med Child Neurol 40, 326-34 (1998).

232. Aylward, E.H., Li, Q., Honeycutt, N.A., Warren, A.C., Pulsifer, M.B., Barta, P.E. et al. MRI volumes of the hippocampus and amygdala in adults with Down's syndrome with and without dementia. Am J Psychiatry 156, 564-8 (1999).

233. Bogerts, B., Lieberman, J.A., Ashtari, M., Bilder, R.M., Degreef, G., Lerner, G. et al. Hippocampus-amygdala volumes and psychopathology in chronic schizophrenia. Biol Psychiatry 33, 236-46 (1993).

234. Breier, A., Buchanan, R.W., Elkashef, A., Munson, R.C., Kirkpatrick, B., Gellad, F. Brain morphology and schizophrenia. A magnetic resonance imaging study of limbic, prefrontal cortex, and caudate structures. Arch Gen Psychiatry 49, 921-6 (1992).

235. Buchanan, R.W., Breier, A., Kirkpatrick, B., Elkashef, A., Munson, R.C., Gellad, F. et al. Structural abnormalities in deficit and nondeficit schizophrenia. Am J Psychiatry 150, 59-65 (1993).

236. Falkai, P., Honer, W.G., Alfter, D., Schneider-Axmann, T., Bussfeld, P., Cordes, J. et al. The temporal lobe in schizophrenia from uni- and multiply affected families. Neurosci Lett 325, 25-8 (2002).

237. Flaum, M., Swayze, V.W. 2nd, O'Leary, D.S., Yuh, W.T., Ehrhardt, J.C., Arndt, S.V. et al. Effects of diagnosis, laterality, and gender on brain morphology in schizophrenia. Am J Psychiatry 152, 704-14 (1995).

238. Fukuzako, H., Fukazako, T., Hashiguchi, T., Hokazono, Y., Takeuchi, K., Hirakawa, K. et al. Reduction in hippocampal formation volume is caused mainly by its shortening in chronic schizophrenia: assessment by MRI. Biol Psychiatry 39, 938-45 (1996).

239. Fukuzako, H., Yamada, K., Kodama, S., Yonezawa, T., Fukuzako, T., Takenouchi, K. et al. Hippocampal volume asymmetry and age at illness onset in males with schizophrenia. Eur Arch Psychiatry Clin Neurosci 247, 248-51 (1997).

240. Gur, R.E., Turetsky, B.I., Cowell, P.E., Finkelman, C., Maany, V., Grossman, R.I. et al. Temporolimbic volume reductions in schizophrenia. Arch Gen Psychiatry 57, 769-75 (2000).

241. Haller, J.W., Banerjee, A., Christensen, G.E., Gado, M., Joshi, S., Miller, M.I. et al. Three-dimensional hippocampal MR morphometry with high-dimensional transformation of a neuroanatomic atlas. Radiology 202, 504-10 (1997).

242. Kwon, J.S., Shin, Y.W., Kim, C.W., Kim, Y.I., Youn, T., Han, M.H. et al. Similarity and disparity of obsessive-compulsive disorder and schizophrenia in MR volumetric abnormalities of the hippocampus-amygdala complex. J Neurol Neurosurg Psychiatry 74, 962-4 (2003).

243. Rossi, A., Stratta, P., Mancini, F., Gallucci, M., Mattei, P., Core, L. et al. Magnetic resonance imaging findings of amygdala-anterior hippocampus shrinkage in male patients with schizophrenia. Psychiatry Res 52, 43-53 (1994).

244. Savas, H.A., Unal, B., Erbagci, H., Inaloz, S., Herken, H., Canan, S. et al. Hippocampal volume in schizophrenia and its relationship with risperidone treatment: a stereological study. Neuropsychobiology 46, 61-6 (2002).

245. Suddath, R.L., Christison, G.W., Torrey, E.F., Casanova, M.F., Weinberger, D.R. Anatomical abnormalities in the brains of monozygotic twins discordant for schizophrenia. N Engl J Med 322, 789-94 (1990).

246. Velakoulis, D., Stuart, G.W., Wood, S.J., Smith, D.J., Brewer, W.J., Desmond, P. et al. Selective bilateral hippocampal volume loss in chronic schizophrenia. Biol Psychiatry 50, 531-9 (2001).

247. Velakoulis, D., Pantelis, C., McGorry, P.D., Dudgeon, P., Brewer, W., Cook, M. et al. Hippocampal volume in first-episode psychoses and chronic schizophrenia: a high-resolution magnetic resonance imaging study. Arch Gen Psychiatry 56, 133-41 (1999).

248. Flaum, M., O'Leary, D.S., Swayze, V.W. 2nd, Miller, D.D., Arndt, S., Andreasen, N.C. Symptom dimensions and brain morphology in schizophrenia and related psychotic disorders. J Psychiatr Res 29, 261-76 (1995).

249. Luchins, D.J., Nettles, K.W., Goldman, M.B. Anterior medial temporal lobe volumes in polydipsic schizophrenic patients with and without hypo-osmolemia: a pilot study. Biol Psychiatry 42, 767-70 (1997).

250. Baare, W.F., van Oel, C.J., Hulshoff Pol, H.E., Schnack, H.G., Durston, S., Sitskoorn, M.M. et al. Volumes of brain structures in twins discordant for schizophrenia. Arch Gen Psychiatry 58, 33-40 (2001).

251. Becker, T., Elmer, K., Schneider, F., Schneider, M., Grodd, W., Bartels, M. et al. Confirmation of reduced temporal limbic structure volume on magnetic resonance imaging in male patients with schizophrenia. Psychiatry Res 67, 135-43 (1996).

252. Narr, K., Thompson, P., Sharma, T., Moussai, J., Zoumalan, C., Rayman, J. et al. Three-dimensional mapping of gyral shape and cortical surface asymmetries in schizophrenia: gender effects. Am J Psychiatry 158, 244-55 (2001).

253. Anderson, J.E., Wible, C.G., McCarley, R.W., Jakab, M., Kasai, K., Shenton, M.E. An MRI study of temporal lobe abnormalities and negative symptoms in chronic schizophrenia. Schizophr Res 58, 123-34 (2002).

254. Pegues, M.P., Rogers, L.J., Amend, D., Vinogradov, S., Deicken, R.F. Anterior hippocampal volume reduction in male patients with schizophrenia. Schizophr Res 60, 105-15 (2003).

255. Hulshoff Pol, H.E., Schnack, H.G., Mandl, R.C., van Haren, N.E., Koning, H., Collins, D.L. et al. Focal gray matter density changes in schizophrenia. Arch Gen Psychiatry 58, 1118-25 (2001).

256. Shenton, M.E., Kikinis, R., Jolesz, F.A., Pollak, S.D., LeMay, M., Wible, C.G. et al. Abnormalities of the left temporal lobe and thought disorder in schizophrenia. A quantitative magnetic resonance imaging study. N Engl J Med 327, 604-12 (1992).

257. Woodruff, P.W., Wright, I.C., Shuriquie, N., Russouw, H., Rushe, T., Howard, R.J. et al. Structural brain abnormalities in male schizophrenics reflect fronto-temporal dissociation. Psychol Med 27, 1257-66 (1997).

258. Stefanis, N., Frangou, S., Yakeley, J., Sharma, T., O'Connell, P., Morgan, K. et al. Hippocampal volume reduction in schizophrenia: effects of genetic risk and pregnancy and birth complications. Biol Psychiatry 46 , 697-702 (1999).

259. Bryant, N.L., Buchanan, R.W., Vladar, K., Breier, A., Rothman, M. Gender differences in temporal lobe structures of patients with schizophrenia: a volumetric MRI study. Am J Psychiatry 156, 603-9 (1999).

260. Colombo, C., Abbruzzese, M., Livian, S., Scotti, G., Locatelli, M., Bonfanti, A. et al. Memory functions and temporal-limbic morphology in schizophrenia. Psychiatry Res 50, 45-56 (1993).

261. Csernansky, J.G., Wang, L., Jones, D., Rastogi-Cruz, D., Posener, J.A., Heydebrand, G. et al. Hippocampal deformities in schizophrenia characterized by high dimensional brain mapping. Am J Psychiatry 159, 2000-6 (2002).

262. Deicken, R.F., Pegues, M., Amend, D. Reduced hippocampal N-acetylaspartate without volume loss in schizophrenia. Schizophr Res 37, 217-23 (1999).

263. Kegeles, L.S., Shungu, D.C., Anjilvel, S., Chan, S., Ellis, S.P., Xanthopoulos, E. et al. Hippocampal pathology in schizophrenia: magnetic resonance imaging and spectroscopy studies. Psychiatry Res 98, 163-75 (2000).

264. Kelsoe, J.R. Jr, Cadet, J.L., Pickar, D., Weinberger, D.R. Quantitative neuroanatomy in schizophrenia. A controlled magnetic resonance imaging study. Arch Gen Psychiatry 45, 533-41 (1988).

265. Rajarethinam, R., DeQuardo, J.R., Miedler, J., Arndt, S., Kirbat, R., Brunberg, J.A. et al. Hippocampus and amygdala in schizophrenia: assessment of the relationship of neuroanatomy to psychopathology. Psychiatry Res 108, 79-87 (2001).

266. Sanfilipo, M., Lafargue, T., Rusinek, H., Arena, L., Loneragan, C., Lautin, A. et al. Volumetric measure of the frontal and temporal lobe regions in schizophrenia: relationship to negative symptoms. Arch Gen Psychiatry 57, 471-80 (2000).

267. Swayze, V.W. 2nd, Andreasen, N.C., Alliger, R.J., Yuh, W.T., Ehrhardt, J.C. Subcortical and temporal structures in affective disorder and schizophrenia: a magnetic resonance imaging study. Biol Psychiatry 31, 221-40 (1992).

268. Torres, I.J., Flashman, L.A., O'Leary, D.S., Swayze, V. 2nd, Andreasen, N.C. Lack of an association between delayed memory and hippocampal and temporal lobe size in patients with schizophrenia and healthy controls. Biol Psychiatry 42, 1087-96 (1997).

269. Wright, I.C., McGuire, P.K., Poline, J.B., Travere, J.M., Murray, R.M., Frith, C.D. et al. A voxel-based method for the statistical analysis of gray and white matter density applied to schizophrenia. Neuroimage 2, 244-52 (1995).

270. Zipursky, R.B., Marsh, L., Lim, K.O., DeMent, S., Shear, P.K., Sullivan, E.V. et al. Volumetric MRI assessment of temporal lobe structures in schizophrenia. Biol Psychiatry 35, 501-16 (1994).

271. Nelson, M.D., Saykin, A.J., Flashman, L.A., Riordan, H.J. Hippocampal volume reduction in schizophrenia as assessed by magnetic resonance imaging: a meta-analytic study. Arch Gen Psychiatry 55, 433-40 (1998).

272. Csernansky, J.G., Joshi, S., Wang, L., Haller, J.W., Gado, M., Miller, J.P. et al. Hippocampal morphometry in schizophrenia by high dimensional brain mapping. Proc Natl Acad Sci U S A 95, 11406-11 (1998).

273. Shenton, M.E., Gerig, G., McCarley, R.W., Szekely, G., Kikinis, R. Amygdala-hippocampal shape differences in schizophrenia: the application of 3D shape models to volumetric MR data. Psychiatry Res 115, 15-35 (2002).

274. Wang, L., Joshi, S.C., Miller, M.I., Csernansky, J.G. Statistical analysis of hippocampal asymmetry in schizophrenia. Neuroimage 14, 531-45 (2001).

275. DeLisi, L.E., Tew, W., Xie, S., Hoff, A.L., Sakuma, M., Kushner, M. et al. A prospective follow-up study of brain morphology and cognition in first-episode schizophrenic patients: preliminary findings. Biol Psychiatry 38, 349-60 (1995).

276. Arango, C., Breier, A., McMahon, R., Carpenter, W.T. Jr, Buchanan, R.W. The relationship of clozapine and haloperidol treatment response to prefrontal, hippocampal, and caudate brain volumes. Am J Psychiatry 160, 1421-7 (2003).

277. Bogerts, B., Ashtari, M., Degreef, G., Alvir, J.M., Bilder, R.M., Lieberman, J.A. Reduced temporal limbic structure volumes on magnetic resonance images in first episode schizophrenia. Psychiatry Res 35, 1-13 (1990).

278. Kubicki, M., Shenton, M.E., Salisbury, D.F., Hirayasu, Y., Kasai, K., Kikinis, R. et al. Voxel-based morphometric analysis of gray matter in first episode schizophrenia. Neuroimage 17, 1711-9 (2002).

279. Hirayasu, Y., Shenton, M.E., Salisbury, D.F., Dickey, C.C., Fischer, I.A., Mazzoni, P. et al. Lower left temporal lobe MRI volumes in patients with first-episode schizophrenia compared with psychotic patients with first-episode affective disorder and normal subjects. Am J Psychiatry 155, 1384-91 (1998).

280. Sumich, A., Chitnis, X.A., Fannon, D.G., O'Ceallaigh, S., Doku, V.C., Falrowicz, A. et al. Temporal lobe abnormalities in first-episode psychosis. Am J Psychiatry 159, 1232-5 (2002).

281. Whitworth, A.B., Honeder, M., Kremser, C., Kemmler, G., Felber, S., Hausmann, A. et al. Hippocampal volume reduction in male schizophrenic patients. Schizophr Res 31, 73-81 (1998).

282. Bilder, R.M., Bogerts, B., Ashtari, M., Wu, H., Alvir, J.M., Jody, D. et al. Anterior hippocampal volume reductions predict frontal lobe dysfunction in first episode schizophrenia. Schizophr Res 17, 47-58 (1995).

283. Lieberman, J., Chakos, M., Wu, H., Alvir, J., Hoffman, E., Robinson, D. et al. Longitudinal study of brain morphology in first episode schizophrenia. Biol Psychiatry 49, 487-99 (2001).

284. Szeszko, P.R., Goldberg, E., Gunduz-Bruce, H., Ashtari, M., Robinson, D., Malhotra, A.K. et al. Smaller anterior hippocampal formation volume in antipsychotic-naive patients with first-episode schizophrenia. Am J Psychiatry 160, 2190-7 (2003).

285. Cahn, W., Pol, H.E., Bongers, M., Schnack, H.G., Mandl, R.C., Van Haren, N.E. et al. Brain morphology in antipsychotic-naive schizophrenia: a study of multiple brain structures. Br J Psychiatry Suppl 43, s66-72 (2002).

286. Job, D.E., Whalley, H.C., McConnell, S., Glabus, M., Johnstone, E.C., Lawrie, S.M. Structural gray matter differences between first-episode schizophrenics and normal controls using voxel-based morphometry. Neuroimage 17, 880-9 (2002).

287. Kasai, K., Shenton, M.E., Salisbury, D.F., Hirayasu, Y., Lee, C.U., Ciszewski, A.A. et al. Progressive decrease of left superior temporal gyrus gray matter volume in patients with first-episode schizophrenia. Am J Psychiatry 160, 156-64 (2003).

288. Laakso, M.P., Tiihonen, J., Syvalahti, E., Vilkman, H., Laakso, A., Alakare, B. et al. A morphometric MRI study of the hippocampus in first-episode, neuroleptic-naive schizophrenia. Schizophr Res 50, 3-7 (2001).

289. Niemann, K., Hammers, A., Coenen, V.A., Thron, A., Klosterkotter, J. Evidence of a smaller left hippocampus and left temporal horn in both patients with first episode schizophrenia and normal control subjects. Psychiatry Res 99, 93-110 (2000).

290. Wood, S.J., Velakoulis, D., Smith, D.J., Bond, D., Stuart, G.W., McGorry, P.D. et al. A longitudinal study of hippocampal volume in first episode psychosis and chronic schizophrenia. Schizophr Res 52, 37-46 (2001).

291. Szeszko, P.R., Strous, R.D., Goldman, R.S., Ashtari, M., Knuth, K.H., Lieberman, J.A. et al. Neuropsychological correlates of hippocampal volumes in patients experiencing a first episode of schizophrenia. Am J Psychiatry 159, 217-26 (2002).

292. Jacobsen, L.K., Giedd, J.N., Castellanos, F.X., Vaituzis, A.C., Hamburger, S.D., Kumra, S. et al. Progressive reduction of temporal lobe structures in childhood-onset schizophrenia. Am J Psychiatry 155, 678-85 (1998).

293. Giedd, J.N., Jeffries, N.O., Blumenthal, J., Castellanos, F.X., Vaituzis, A.C., Fernandez, T. et al. Childhood-onset schizophrenia: progressive brain changes during adolescence. Biol Psychiatry 46, 892-8 (1999).

294. Levitt, J.G., Blanton, R.E., Caplan, R., Asarnow, R., Guthrie, D., Toga, A.W. et al. Medial temporal lobe in childhood-onset schizophrenia. Psychiatry Res 108, 17-27 (2001).

295. Matsumoto, H., Simmons, A., Williams, S., Pipe, R., Murray, R., Frangou, S. Structural magnetic imaging of the hippocampus in early onset schizophrenia. Biol Psychiatry 49 , 824-31 (2001).

296. Jacobsen, L.K., Giedd, J.N., Vaituzis, A.C., Hamburger, S.D., Rajapakse, J.C., Frazier, J.A. et al. Temporal lobe morphology in childhood-onset schizophrenia. Am J Psychiatry 153, 355-61 (1996).

297. Barta, P.E., Powers, R.E., Aylward, E.H., Chase, G.A., Harris, G.J., Rabins, P.V. et al. Quantitative MRI volume changes in late onset schizophrenia and Alzheimer's disease compared to normal controls. Psychiatry Res 68, 65-75 (1997).

298. Keshavan, M.S., Dick, E., Mankowski, I., Harenski, K., Montrose, D.M., Diwadkar, V. et al. Decreased left amygdala and hippocampal volumes in young offspring at risk for schizophrenia. Schizophr Res 58, 173-83 (2002).

299. Phillips, L.J., Velakoulis, D., Pantelis, C., Wood, S., Yuen, H.P., Yung, A.R. et al. Non-reduction in hippocampal volume is associated with higher risk of psychosis. Schizophr Res 58, 145-58 (2002).

300. Keshavan, M.S., Montrose, D.M., Pierri, J.N., Dick, E.L., Rosenberg, D., Talagala, L. et al. Magnetic resonance imaging and spectroscopy in offspring at risk for schizophrenia: preliminary studies. Prog Neuropsychopharmacol Biol Psychiatry 21, 1285-95 (1997).

301. Lawrie, S.M., Whalley, H., Kestelman, J.N., Abukmeil, S.S., Byrne, M., Hodges, A. et al. Magnetic resonance imaging of brain in people at high risk of developing schizophrenia. Lancet 353, 30-3 (1999).

302. Tepest, R., Wang, L., Miller, M.I., Falkai, P., Csernansky, J.G. Hippocampal deformities in the unaffected siblings of schizophrenia subjects. Biol Psychiatry 54, 1234-40 (2003).

303. Coffey, C.E., Wilkinson, W.E., Weiner, R.D., Parashos, I.A., Djang, W.T., Webb, M.C. et al. Quantitative cerebral anatomy in depression. A controlled magnetic resonance imaging study. Arch Gen Psychiatry 50, 7-16 (1993).

304. Axelson, D.A., Doraiswamy, P.M., McDonald, W.M., Boyko, O.B., Tupler, L.A., Patterson, L.J. et al. Hypercortisolemia and hippocampal changes in depression. Psychiatry Res 47, 163-73 (1993).

305. Frodl, T., Meisenzahl, E.M., Zetzsche, T., Born, C., Groll, C., Jager, M. et al. Hippocampal changes in patients with a first episode of major depression. Am J Psychiatry 159, 1112-8 (2002).

306. Sheline, Y.I., Sanghavi, M., Mintun, M.A., Gado, M.H. Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. J Neurosci 19, 5034-43 (1999).

307. Sheline, Y.I., Wang, P.W., Gado, M.H., Csernansky, J.G., Vannier, M.W. Hippocampal atrophy in recurrent major depression. Proc Natl Acad Sci U S A 93, 3908-13 (1996).

308. MacQueen, G.M., Campbell, S., McEwen, B.S., Macdonald, K., Amano, S., Joffe, R.T. et al. Course of illness, hippocampal function, and hippocampal volume in major depression. Proc Natl Acad Sci U S A 100, 1387-92 (2003).

309. Bremner, J.D., Narayan, M., Anderson, E.R., Staib, L.H., Miller, H.L., Charney, D.S. Hippocampal volume reduction in major depression. Am J Psychiatry 157, 115-8 (2000).

310. Mervaala, E., Fohr, J., Kononen, M., Valkonen-Korhonen, M., Vainio, P., Partanen, K. et al. Quantitative MRI of the hippocampus and amygdala in severe depression. Psychol Med 30, 117-25 (2000).

311. Shah, P.J., Ebmeier, K.P., Glabus, M.F., Goodwin, G.M. Cortical grey matter reductions associated with treatment-resistant chronic unipolar depression. Controlled magnetic resonance imaging study. Br J Psychiatry 172, 527-32 (1998).

312. Vakili, K., Pillay, S.S., Lafer, B., Fava, M., Renshaw, P.F., Bonello-Cintron, C.M. et al. Hippocampal volume in primary unipolar major depression: a magnetic resonance imaging study. Biol Psychiatry 47, 1087-90 (2000).

313. von Gunten, A., Fox, N.C., Cipolotti, L., Ron, M.A. A volumetric study of hippocampus and amygdala in depressed patients with subjective memory problems. J Neuropsychiatry Clin Neurosci 12, 493-8 (2000).

314. Vythilingam, M., Heim, C., Newport, J., Miller, A.H., Anderson, E., Bronen, R. et al. Childhood trauma associated with smaller hippocampal volume in women with major depression. Am J Psychiatry 159, 2072-80 (2002).

315. Posener, J.A., Wang, L., Price, J.L., Gado, M.H., Province, M.A., Miller, M.I. et al. High-dimensional mapping of the hippocampus in depression. Am J Psychiatry 160, 83-9 (2003).

316. Sheline, Y.I., Gado, M.H., Kraemer, H.C. Untreated depression and hippocampal volume loss. Am J Psychiatry 160, 1516-8 (2003).

317. Kim, D.K., Kim, B.L., Sohn, S.E., Lim, S.W., Na, D.G., Paik, C.H. et al. Candidate neuroanatomic substrates of psychosis in old-aged depression. Prog Neuropsychopharmacol Biol Psychiatry 23, 793-807 (1999).

318. Steffens, D.C., Byrum, C.E., McQuoid, D.R., Greenberg, D.L., Payne, M.E., Blitchington, T.F. et al. Hippocampal volume in geriatric depression. Biol Psychiatry 48, 301-9 (2000).

319. Bell-McGinty, S., Butters, M.A., Meltzer, C.C., Greer, P.J., Reynolds, C.F. 3rd, Becker, J.T. Brain morphometric abnormalities in geriatric depression: long-term neurobiological effects of illness duration. Am J Psychiatry 159, 1424-7 (2002).

320. Hsieh, M.H., McQuoid, D.R., Levy, R.M., Payne, M.E., MacFall, J.R., Steffens, D.C. Hippocampal volume and antidepressant response in geriatric depression. Int J Geriatr Psychiatry 17, 519-25 (2002).

321. Steffens, D.C., Payne, M.E., Greenberg, D.L., Byrum, C.E., Welsh-Bohmer, K.A., Wagner, H.R. et al. Hippocampal volume and incident dementia in geriatric depression. Am J Geriatr Psychiatry 10, 62-71 (2002).

322. MacMillan, S., Szeszko, P.R., Moore, G.J., Madden, R., Lorch, E., Ivey, J. et al. Increased amygdala: hippocampal volume ratios associated with severity of anxiety in pediatric major depression. J Child Adolesc Psychopharmacol 13, 65-73 (2003).

323. MacMaster, F.P. and Kusumakar, V. Hippocampal volume in early onset depression. BMC Med 2, 2 (2004).

324. Altshuler, L.L., Bartzokis, G., Grieder, T., Curran, J., Mintz, J. Amygdala enlargement in bipolar disorder and hippocampal reduction in schizophrenia: an MRI study demonstrating neuroanatomic specificity. Arch Gen Psychiatry 55, 663-4 (1998).

325. Blumberg, H.P., Kaufman, J., Martin, A., Whiteman, R., Zhang, J.H., Gore, J.C. et al. Amygdala and hippocampal volumes in adolescents and adults with bipolar disorder. Arch Gen Psychiatry 60, 1201-8 (2003).

326. Strakowski, S.M., DelBello, M.P., Sax, K.W., Zimmerman, M.E., Shear, P.K., Hawkins, J.M. et al. Brain magnetic resonance imaging of structural abnormalities in bipolar disorder. Arch Gen Psychiatry 56, 254-60 (1999).

327. Hauser, P., Matochik, J., Altshuler, L.L., Denicoff, K.D., Conrad, A., Li, X. et al. MRI-based measurements of temporal lobe and ventricular structures in patients with bipolar I and bipolar II disorders. J Affect Disord 60, 25-32 (2000).

328. Strakowski, S.M., DelBello, M.P., Zimmerman, M.E., Getz, G.E., Mills, N.P., Ret, J. et al. Ventricular and periventricular structural volumes in first- versus multiple-episode bipolar disorder. Am J Psychiatry 159, 1841-7 (2002).

329. Ali, S.O., Denicoff, K.D., Altshuler, L.L., Hauser, P., Li, X., Conrad, A.J. et al. Relationship between prior course of illness and neuroanatomic structures in bipolar disorder: a preliminary study. Neuropsychiatry Neuropsychol Behav Neurol 14, 227-32 (2001).

330. Ali, S.O., Denicoff, K.D., Altshuler, L.L., Hauser, P., Li, X., Conrad, A.J. et al. A preliminary study of the relation of neuropsychological performance to neuroanatomic structures in bipolar disorder. Neuropsychiatry Neuropsychol Behav Neurol 13, 20-8 (2000).

331. Bremner, J.D., Randall, P., Scott, T.M., Bronen, R.A., Seibyl, J.P., Southwick, S.M. et al. MRI-based measurement of hippocampal volume in patients with combat-related posttraumatic stress disorder. Am J Psychiatry 152, 973-81 (1995).

332. Gurvits, T.V., Shenton, M.E., Hokama, H., Ohta, H., Lasko, N.B., Gilbertson, M.W. et al. Magnetic resonance imaging study of hippocampal volume in chronic, combat-related posttraumatic stress disorder. Biol Psychiatry 40, 1091-9 (1996).

333. Villarreal, G., Hamilton, D.A., Petropoulos, H., Driscoll, I., Rowland, L.M., Griego, J.A. et al. Reduced hippocampal volume and total white matter volume in posttraumatic stress disorder. Biol Psychiatry 52, 119-25 (2002).

334. Bremner, J.D., Randall, P., Vermetten, E., Staib, L., Bronen, R.A., Mazure, C. et al. Magnetic resonance imaging-based measurement of hippocampal volume in posttraumatic stress disorder related to childhood physical and sexual abuse--a preliminary report. Biol Psychiatry 41, 23-32 (1997).

335. Stein, M.B., Koverola, C., Hanna, C., Torchia, M.G., McClarty, B. Hippocampal volume in women victimized by childhood sexual abuse. Psychol Med 27, 951-9 (1997).

336. Hedges, D.W., Allen, S., Tate, D.F., Thatcher, G.W., Miller, M.J., Rice, S.A. et al. Reduced hippocampal volume in alcohol and substance naive Vietnam combat veterans with posttraumatic stress disorder. Cogn Behav Neurol 16, 219-24 (2003).

337. Gilbertson, M.W., Shenton, M.E., Ciszewski, A., Kasai, K., Lasko, N.B., Orr, S.P. et al. Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma. Nat Neurosci 5, 1242-7 (2002).

338. Bonne, O., Brandes, D., Gilboa, A., Gomori, J.M., Shenton, M.E., Pitman, R.K. et al. Longitudinal MRI study of hippocampal volume in trauma survivors with PTSD. Am J Psychiatry 158, 1248-51 (2001).

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

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

341. Neylan, T.C., Schuff, N., Lenoci, M., Yehuda, R., Weiner, M.W., Marmar, C.R. Cortisol levels are positively correlated with hippocampal N-acetylaspartate. Biol Psychiatry 54, 1118-21 (2003).

342. Agartz, I., Momenan, R., Rawlings, R.R., Kerich, M.J., Hommer, D.W. Hippocampal volume in patients with alcohol dependence. Arch Gen Psychiatry 56, 356-63 (1999).

343. Bremner, J.D., Vythilingam, M., Vermetten, E., Southwick, S.M., McGlashan, T., Nazeer, A. et al. MRI and PET study of deficits in hippocampal structure and function in women with childhood sexual abuse and posttraumatic stress disorder. Am J Psychiatry 160, 924-32 (2003).

344. Vermetten, E., Vythilingam, M., Southwick, S.M., Charney, D.S., Bremner, J.D. Long-term treatment with paroxetine increases verbal declarative memory and hippocampal volume in posttraumatic stress disorder. Biol Psychiatry 54, 693-702 (2003).

345. Yamasue, H., Kasai, K., Iwanami, A., Ohtani, T., Yamada, H., Abe, O. et al. Voxel-based analysis of MRI reveals anterior cingulate gray-matter volume reduction in posttraumatic stress disorder due to terrorism. Proc Natl Acad Sci U S A 100, 9039-43 (2003).

346. Carrion, V.G., Weems, C.F., Eliez, S., Patwardhan, A., Brown, W., Ray, R.D. et al. Attenuation of frontal asymmetry in pediatric posttraumatic stress disorder. Biol Psychiatry 50, 943-51 (2001).

347. De Bellis, M.D., Keshavan, M.S., Shifflett, H., Iyengar, S., Beers, S.R., Hall, J. et al. Brain structures in pediatric maltreatment-related posttraumatic stress disorder: a sociodemographically matched study. Biol Psychiatry 52, 1066-78 (2002).

348. De Bellis, M.D., Hall, J., Boring, A.M., Frustaci, K., Moritz, G. A pilot longitudinal study of hippocampal volumes in pediatric maltreatment-related posttraumatic stress disorder. Biol Psychiatry 50, 305-9 (2001).

349. De Bellis, M.D., Keshavan, M.S., Clark, D.B., Casey, B.J., Giedd, J.N., Boring, A.M. et al. A.E. Bennett Research Award. Developmental traumatology. Part II: Brain development. Biol Psychiatry 45, 1271-84 (1999).

350. Sullivan, E.V., Marsh, L., Mathalon, D.H., Lim, K.O., Pfefferbaum, A. Anterior hippocampal volume deficits in nonamnesic, aging chronic alcoholics. Alcohol Clin Exp Res 19 , 110-22 (1995).

351. Beresford, T., Arciniegas, D., Rojas, D., Sheeder, J., Teale, P., Aasal, R. et al. Hippocampal to pituitary volume ratio: a specific measure of reciprocal neuroendocrine alterations in alcohol dependence. J Stud Alcohol 60, 586-8 (1999).

352. Laakso, M.P., Vaurio, O., Savolainen, L., Repo, E., Soininen, H., Aronen, H.J. et al. A volumetric MRI study of the hippocampus in type 1 and 2 alcoholism. Behav Brain Res 109, 177-86 (2000).

353. De Bellis, M.D., Clark, D.B., Beers, S.R., Soloff, P.H., Boring, A.M., Hall, J. et al. Hippocampal volume in adolescent-onset alcohol use disorders. Am J Psychiatry 157, 737-44 (2000).

354. Bleich, S., Bandelow, B., Javaheripour, K., Muller, A., Degner, D., Wilhelm, J. et al. Hyperhomocysteinemia as a new risk factor for brain shrinkage in patients with alcoholism. Neurosci Lett 335, 179-82 (2003).

355. Bleich, S., Sperling, W., Degner, D., Graesel, E., Bleich, K., Wilhelm, J. et al. Lack of association between hippocampal volume reduction and first-onset alcohol withdrawal seizure. A volumetric MRI study. Alcohol Alcohol 38, 40-4 (2003).

356. Sullivan, E.V., Marsh, L., Mathalon, D.H., Lim, K.O., Pfefferbaum, A. Relationship between alcohol withdrawal seizures and temporal lobe white matter volume deficits. Alcohol Clin Exp Res 20, 348-54 (1996).

357. Di Sclafani, V. and Truran, D. L. Abstinent Chronic Crack-Cocaine and Crack-Cocaine/Alcohol Abusers Evidence Normal Hippocampal Volumes on MRI Despite Persistent Cognitive Impairments. Addiction Biology 3(3), 261-71. 98.

358. Honeycutt, N.A. and Smith, C.D. Hippocampal volume measurements using magnetic resonance imaging in normal young adults. J Neuroimaging 5, 95-100 (1995).

359. Pruessner, J.C., Li, L.M., Serles, W., Pruessner, M., Collins, D.L., Kabani, N. et al. Volumetry of hippocampus and amygdala with high-resolution MRI and three-dimensional analysis software: minimizing the discrepancies between laboratories. Cereb Cortex 10, 433-42 (2000).

360. Mackay, C.E., Roberts, N., Mayes, A.R., Downes, J.J., Foster, J.K., Mann, D. An exploratory study of the relationship between face recognition memory and the volume of medial temporal lobe structures in healthy young males. Behav Neurol 11, 3-20 (1998).

361. Giedd, J.N., Vaituzis, A.C., Hamburger, S.D., Lange, N., Rajapakse, J.C., Kaysen, D. et al. Quantitative MRI of the temporal lobe, amygdala, and hippocampus in normal human development: ages 4-18 years. J Comp Neurol 366, 223-30 (1996).

362. Szabo, C.A., Xiong, J., Lancaster, J.L., Rainey, L., Fox, P. Amygdalar and hippocampal volumetry in control participants: differences regarding handedness. AJNR Am J Neuroradiol 22, 1342-5 (2001).

363. Obenaus, A., Yong-Hing, C.J., Tong, K.A., Sarty, G.E. A reliable method for measurement and normalization of pediatric hippocampal volumes. Pediatr Res 50, 124-32 (2001).

364. Giedd, J.N., Castellanos, F.X., Rajapakse, J.C., Vaituzis, A.C., Rapoport, J.L. Sexual dimorphism of the developing human brain. Prog Neuropsychopharmacol Biol Psychiatry 21, 1185-201 (1997).

365. Utsunomiya, H., Takano, K., Okazaki, M., Mitsudome, A. Development of the temporal lobe in infants and children: analysis by MR-based volumetry. AJNR Am J Neuroradiol 20, 717-23 (1999).

366. Pfluger, T., Weil, S., Weis, S., Vollmar, C., Heiss, D., Egger, J. et al. Normative volumetric data of the developing hippocampus in children based on magnetic resonance imaging. Epilepsia 40, 414-23 (1999).

367. Raz, N., Gunning, F.M., Head, D., Dupuis, J.H., McQuain, J., Briggs, S.D. et al. Selective aging of the human cerebral cortex observed in vivo: differential vulnerability of the prefrontal gray matter. Cereb Cortex 7, 268-82 (1997).

368. Filipek, P.A., Richelme, C., Kennedy, D.N., Caviness, V.S. Jr. The young adult human brain: an MRI-based morphometric analysis. Cereb Cortex 4, 344-60 (1994).

369. Caviness, V.S. Jr, Kennedy, D.N., Richelme, C., Rademacher, J., Filipek, P.A. The human brain age 7-11 years: a volumetric analysis based on magnetic resonance images. Cereb Cortex 6, 726-36 (1996).

370. Gur, R.C., Gunning-Dixon, F., Bilker, W.B., Gur, R.E. Sex differences in temporo-limbic and frontal brain volumes of healthy adults. Cereb Cortex 12, 998-1003 (2002).

371. Pruessner, J.C., Collins, D.L., Pruessner, M., Evans, A.C. Age and gender predict volume decline in the anterior and posterior hippocampus in early adulthood. J Neurosci 21, 194-200 (2001).

372. Liu, R.S., Lemieux, L., Bell, G.S., Sisodiya, S.M., Shorvon, S.D., Sander, J.W. et al. A longitudinal study of brain morphometrics using quantitative magnetic resonance imaging and difference image analysis. Neuroimage 20, 22-33 (2003).

373. Andreasen, N.C., Flaum, M., Swayze, V. 2nd, O'Leary, D.S., Alliger, R., Cohen, G. et al. Intelligence and brain structure in normal individuals. Am J Psychiatry 150, 130-4 (1993).

374. Foster, J.K., Meikle, A., Goodson, G., Mayes, A.R., Howard, M., Sunram, S.I. et al. The hippocampus and delayed recall: bigger is not necessarily better? Memory 7, 715-32 (1999).

375. Chantome, M., Perruchet, P., Hasboun, D., Dormont, D., Sahel, M., Sourour, N. et al. Is there a negative correlation between explicit memory and hippocampal volume? Neuroimage 10, 589-95 (1999).

376. Raz, N., Williamson, A., Gunning-Dixon, F., Head, D., Acker, J.D. Neuroanatomical and cognitive correlates of adult age differences in acquisition of a perceptual-motor skill. Microsc Res Tech 51, 85-93 (2000).

377. Maguire, E.A., Gadian, D.G., Johnsrude, I.S., Good, C.D., Ashburner, J., Frackowiak, R.S. et al. Navigation-related structural change in the hippocampi of taxi drivers. Proc Natl Acad Sci U S A 97, 4398-403 (2000).

378. Maguire, E.A., Spiers, H.J., Good, C.D., Hartley, T., Frackowiak, R.S., Burgess, N. Navigation expertise and the human hippocampus: a structural brain imaging analysis. Hippocampus 13, 250-9 (2003).

379. Bartzokis, G., Mintz, J., Marx, P., Osborn, D., Gutkind, D., Chiang, F. et al. Reliability of in vivo volume measures of hippocampus and other brain structures using MRI. Magn Reson Imaging 11, 993-1006 (1993).

380. Bilir, E., Craven, W., Hugg, J., Gilliam, F., Martin, R., Faught, E. et al. Volumetric MRI of the limbic system: anatomic determinants. Neuroradiology 40, 138-44 (1998).

381. Honeycutt, N.A., Smith, P.D., Aylward, E., Li, Q., Chan, M., Barta, P.E. et al. Mesial temporal lobe measurements on magnetic resonance imaging scans. Psychiatry Res 83, 85-94 (1998).

382. Lemieux, L., Liu, R.S., Duncan, J.S. Hippocampal and cerebellar volumetry in serially acquired MRI volume scans. Magn Reson Imaging 18, 1027-33 (2000).

383. Pantel, J., O'Leary, D.S., Cretsinger, K., Bockholt, H.J., Keefe, H., Magnotta, V.A. et al. A new method for the in vivo volumetric measurement of the human hippocampus with high neuroanatomical accuracy. Hippocampus 10, 752-8 (2000).

384. MacFall, J.R., Byrum, C.E., Parashos, I., Early, B., Charles, H.C., Chittilla, V. et al. Relative accuracy and reproducibility of regional MRI brain volumes for point-counting methods. Psychiatry Res 55, 167-77 (1994).

385. Ashton, E.A., Berg, M.J., Parker, K.J., Weisberg, J., Chen, C.W., Ketonen, L. Segmentation and feature extraction techniques, with applications to MRI head studies. Magn Reson Med 33, 670-7 (1995).

386. Hsu, Y.Y., Schuff, N., Du, A.T., Mark, K., Zhu, X., Hardin, D. et al. Comparison of automated and manual MRI volumetry of hippocampus in normal aging and dementia. J Magn Reson Imaging 16, 305-10 (2002).

387. Shen, D., Moffat, S., Resnick, S.M., Davatzikos, C. Measuring size and shape of the hippocampus in MR images using a deformable shape model. Neuroimage 15, 422-34 (2002).

388. Briellmann, R.S., Syngeniotis, A., Jackson, G.D. Comparison of hippocampal volumetry at 1.5 tesla and at 3 tesla. Epilepsia 42, 1021-4 (2001).

389. Levy-Reiss, I., Gonzalez-Atavales, J., King-Stephens, D., French, J. A., Baltuch, G., Bagley, L.,Detre, J. A. High Resolution Imaging at 4 Tesla for Hippocampal Volumetry in Temporal Lobe Epilepsy. Proc Intl Soc Magn Reson Med 8, 15. 2000.

390. Hasboun, D., Chantome, M., Zouaoui, A., Sahel, M., Deladoeuille, M., Sourour, N. et al. MR determination of hippocampal volume: comparison of three methods. AJNR Am J Neuroradiol 17, 1091-8 (1996).

391. Bartzokis, G., Altshuler, L.L., Greider, T., Curran, J., Keen, B., Dixon, W.J. Reliability of medial temporal lobe volume measurements using reformatted 3D images. Psychiatry Res 82, 11-24 (1998).

392. Laakso, M.P., Juottonen, K., Partanen, K., Vainio, P., Soininen, H. MRI volumetry of the hippocampus: the effect of slice thickness on volume formation. Magn Reson Imaging 15, 263-5 (1997).

393. Chee, M.W., Low, S., Tan, J.S., Lim, W., Wong, J. Hippocampal volumetry with magnetic resonance imaging: a cost-effective validated solution. Epilepsia 38, 461-5 (1997).

394. Trenerry, M.R., Westerveld, M., Meador, K.J. MRI hippocampal volume and neuropsychology in epilepsy surgery. Magn Reson Imaging 13, 1125-32 (1995).

395. Duncan, J.S. MRI studies. Do seizures damage the brain? Prog Brain Res 135, 253-61 (2002).

396. Whalley, H.C., Kestelman, J.N., Rimmington, J.E., Kelso, A., Abukmeil, S.S., Best, J.J. et al. Methodological issues in volumetric magnetic resonance imaging of the brain in the Edinburgh High Risk Project. Psychiatry Res 91, 31-44 (1999).

397. Bertolino, A., Nawroz, S., Mattay, V.S., Barnett, A.S., Duyn, J.H., Moonen, C.T. et al. Regionally specific pattern of neurochemical pathology in schizophrenia as assessed by multislice proton magnetic resonance spectroscopic imaging. Am J Psychiatry 153, 1554-63 (1996).

398. Nasrallah, H.A., Skinner, T.E., Schmalbrock, P., Robitaille, P.M. Proton magnetic resonance spectroscopy (1H MRS) of the hippocampal formation in schizophrenia: a pilot study. Br J Psychiatry 165, 481-5 (1994).

399. Callicott, J.H., Egan, M.F., Bertolino, A., Mattay, V.S., Langheim, F.J., Frank, J.A. et al. Hippocampal N-acetyl aspartate in unaffected siblings of patients with schizophrenia: a possible intermediate neurobiological phenotype. Biol Psychiatry 44, 941-50 (1998).

400. Church, S.M., Cotter, D., Bramon, E., Murray, R.M. Does schizophrenia result from developmental or degenerative processes? J Neural Transm Suppl 129-47 (2002).

401. Lieberman, J.A. Is schizophrenia a neurodegenerative disorder? A clinical and neurobiological perspective. Biol Psychiatry 46, 729-39 (1999).

402. Weinberger, D.R. Cell biology of the hippocampal formation in schizophrenia. Biol Psychiatry 45, 395-402 (1999).

403. Lipska, B.K. and Weinberger, D.R. A neurodevelopmental model of schizophrenia: neonatal disconnection of the hippocampus. Neurotox Res 4, 469-475 (2002).

404. Tkachev, D., Mimmack, M.L., Ryan, M.M., Wayland, M., Freeman, T., Jones, P.B. et al. Oligodendrocyte dysfunction in schizophrenia and bipolar disorder. Lancet 362, 798-805 (2003).

405. Sapolsky, R.M. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiatry 57, 925-35 (2000).

406. Sapolsky, R.M. Why stress is bad for your brain. Science 273, 749-50 (1996).

407. Yehuda, R. Are glucocortoids responsible for putative hippocampal damage in PTSD? How and when to decide. Hippocampus 11, 85-9; discussion 82-4 (2001).

408. Bremner, J.D. Hypotheses and controversies related to effects of stress on the hippocampus: an argument for stress-induced damage to the hippocampus in patients with posttraumatic stress disorder. Hippocampus 11, 75-81; discussion 82-4 (2001).

409. Elzinga, B.M., Schmahl, C.G., Vermetten, E., van Dyck, R., Bremner, J.D. Higher cortisol levels following exposure to traumatic reminders in abuse-related PTSD. Neuropsychopharmacology 28, 1656-65 (2003).

410. Jacobs, B.L., Praag, H., Gage, F.H. Adult brain neurogenesis and psychiatry: a novel theory of depression. Mol Psychiatry 5, 262-9 (2000).

411. Kempermann, G. and Kronenberg, G. Depressed new neurons--adult hippocampal neurogenesis and a cellular plasticity hypothesis of major depression. Biol Psychiatry 54, 499-503 (2003).

412. Schmahl, C.G., Vermetten, E., Elzinga, B.M., Douglas Bremner, J. Magnetic resonance imaging of hippocampal and amygdala volume in women with childhood abuse and borderline personality disorder. Psychiatry Res 122, 193-8 (2003).

413. Abernethy, L.J., Palaniappan, M., Cooke, R.W. Quantitative magnetic resonance imaging of the brain in survivors of very low birth weight. Arch Dis Child 87, 279-83 (2002).

414. Murphy, D.G., DeCarli, C., Daly, E., Haxby, J.V., Allen, G., White, B.J. et al. X-chromosome effects on female brain: a magnetic resonance imaging study of Turner's syndrome. Lancet 342, 1197-200 (1993).

415. Reiss, A.L., Lee, J., Freund, L. Neuroanatomy of fragile X syndrome: the temporal lobe. Neurology 44, 1317-24 (1994).

416. Fujioka, M., Nishio, K., Miyamoto, S., Hiramatsu, K.I., Sakaki, T., Okuchi, K. et al. Hippocampal damage in the human brain after cardiac arrest. Cerebrovasc Dis 10, 2-7 (2000).

417. Braak, H., Del Tredici, K., Bohl, J., Bratzke, H., Braak, E. Pathological changes in the parahippocampal region in select non-Alzheimer's dementias. Ann N Y Acad Sci 911, 221-39 (2000).

418. Driessen, M., Herrmann, J., Stahl, K., Zwaan, M., Meier, S., Hill, A. et al. Magnetic resonance imaging volumes of the hippocampus and the amygdala in women with borderline personality disorder and early traumatization. Arch Gen Psychiatry 57, 1115-22 (2000).

419. Tebartz van Elst, L., Hesslinger, B., Thiel, T., Geiger, E., Haegele, K., Lemieux, L. et al. Frontolimbic brain abnormalities in patients with borderline personality disorder: a volumetric magnetic resonance imaging study. Biol Psychiatry 54, 163-71 (2003).

420. Szabo, C.A., Wyllie, E., Siavalas, E.L., Najm, I., Ruggieri, P., Kotagal, P. et al. Hippocampal volumetry in children 6 years or younger: assessment of children with and without complex febrile seizures. Epilepsy Res 33, 1-9 (1999).

421. Tarkka, R., Paakko, E., Pyhtinen, J., Uhari, M., Rantala, H. Febrile seizures and mesial temporal sclerosis: No association in a long-term follow-up study. Neurology 60, 215-8 (2003).

422. Yoneda, Y., Mori, E., Yamashita, H., Yamadori, A. MRI volumetry of medial temporal lobe structures in amnesia following herpes simplex encephalitis. Eur Neurol 34, 243-52 (1994).

423. Caparros-Lefebvre, D., Girard-Buttaz, I., Reboul, S., Lebert, F., Cabaret, M., Verier, A. et al. Cognitive and psychiatric impairment in herpes simplex virus encephalitis suggest involvement of the amygdalo-frontal pathways. J Neurol 243, 248-56 (1996).

424. Colchester, A., Kingsley, D., Lasserson, D., Kendall, B., Bello, F., Rush, C. et al. Structural MRI volumetric analysis in patients with organic amnesia, 1: methods and comparative findings across diagnostic groups. J Neurol Neurosurg Psychiatry 71, 13-22 (2001).

425. Visser, P.J., Krabbendam, L., Verhey, F.R., Hofman, P.A., Verhoeven, W.M., Tuinier, S. et al. Brain correlates of memory dysfunction in alcoholic Korsakoff's syndrome. J Neurol Neurosurg Psychiatry 67, 774-8 (1999).

426. Sullivan, E.V. and Marsh, L. Hippocampal volume deficits in alcoholic Korsakoff's syndrome. Neurology 61, 1716-9 (2003).

427. Jenike, M.A., Breiter, H.C., Baer, L., Kennedy, D.N., Savage, C.R., Olivares, M.J. et al. Cerebral structural abnormalities in obsessive-compulsive disorder. A quantitative morphometric magnetic resonance imaging study. Arch Gen Psychiatry 53, 625-32 (1996).

428. Szeszko, P.R., Robinson, D., Alvir, J.M., Bilder, R.M., Lencz, T., Ashtari, M. et al. Orbital frontal and amygdala volume reductions in obsessive-compulsive disorder. Arch Gen Psychiatry 56, 913-9 (1999).

429. Kopelman, M.D., Lasserson, D., Kingsley, D., Bello, F., Rush, C., Stanhope, N. et al. Structural MRI volumetric analysis in patients with organic amnesia, 2: correlations with anterograde memory and executive tests in 40 patients. J Neurol Neurosurg Psychiatry 71, 23-8 (2001).

430. Isaacs, E.B., Vargha-Khadem, F., Watkins, K.E., Lucas, A., Mishkin, M., Gadian, D.G. Developmental amnesia and its relationship to degree of hippocampal atrophy. Proc Natl Acad Sci U S A 100, 13060-3 (2003).

431. Grubb, N.R., Fox, K.A., Smith, K., Best, J., Blane, A., Ebmeier, K.P. et al. Memory impairment in out-of-hospital cardiac arrest survivors is associated with global reduction in brain volume, not focal hippocampal injury. Stroke 31, 1509-14 (2000).

432. Starkman, M.N., Gebarski, S.S., Berent, S., Schteingart, D.E. Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with Cushing's syndrome. Biol Psychiatry 32, 756-65 (1992).

433. Starkman, M.N., Giordani, B., Gebarski, S.S., Berent, S., Schork, M.A., Schteingart, D.E. Decrease in cortisol reverses human hippocampal atrophy following treatment of Cushing's disease. Biol Psychiatry 46, 1595-602 (1999).

434. Kates, W.R., Abrams, M.T., Kaufmann, W.E., Breiter, S.N., Reiss, A.L. Reliability and validity of MRI measurement of the amygdala and hippocampus in children with fragile X syndrome. Psychiatry Res 75, 31-48 (1997).

435. Peterson, B.S., Vohr, B., Staib, L.H., Cannistraci, C.J., Dolberg, A., Schneider, K.C. et al. Regional brain volume abnormalities and long-term cognitive outcome in preterm infants. JAMA 284, 1939-47 (2000).

436. Vythilingam, M., Anderson, E.R., Goddard, A., Woods, S.W., Staib, L.H., Charney, D.S. et al. Temporal lobe volume in panic disorder--a quantitative magnetic resonance imaging study. Psychiatry Res 99, 75-82 (2000).

437. Uchida, R.R., Del-Ben, C.M., Santos, A.C., Araujo, D., Crippa, J.A., Guimaraes, F.S. et al. Decreased left temporal lobe volume of panic patients measured by magnetic resonance imaging. Braz J Med Biol Res 36, 925-9 (2003).

438. Camicioli, R., Moore, M.M., Kinney, A., Corbridge, E., Glassberg, K., Kaye, J.A. Parkinson's disease is associated with hippocampal atrophy. Mov Disord 18, 784-90 (2003).

439. Castellanos, F.X., Giedd, J.N., Marsh, W.L., Hamburger, S.D., Vaituzis, A.C., Dickstein, D.P. et al. Quantitative brain magnetic resonance imaging in attention-deficit hyperactivity disorder. Arch Gen Psychiatry 53, 607-16 (1996).

440. Laakso, M.P., Vaurio, O., Koivisto, E., Savolainen, L., Eronen, M., Aronen, H.J. et al. Psychopathy and the posterior hippocampus. Behav Brain Res 118, 187-93 (2001).

441. Giordano, G.D., Renzetti, P., Parodi, R.C., Foppiani, L., Zandrino, F., Giordano, G. et al. Volume measurement with magnetic resonance imaging of hippocampus-amygdala formation in patients with anorexia nervosa. J Endocrinol Invest 24, 510-4 (2001).

442. Nakano, T., Wenner, M., Inagaki, M., Kugaya, A., Akechi, T., Matsuoka, Y. et al. Relationship between distressing cancer-related recollections and hippocampal volume in cancer survivors. Am J Psychiatry 159, 2087-93 (2002).

443. Merke, D.P., Fields, J.D., Keil, M.F., Vaituzis, A.C., Chrousos, G.P., Giedd, J.N. Children with classic congenital adrenal hyperplasia have decreased amygdala volume: potential prenatal and postnatal hormonal effects. J Clin Endocrinol Metab 88, 1760-5 (2003).

444. Archibald, S.L., Fennema-Notestine, C., Gamst, A., Riley, E.P., Mattson, S.N., Jernigan, T.L. Brain dysmorphology in individuals with severe prenatal alcohol exposure. Dev Med Child Neurol 43, 148-54 (2001).

445. Rosas, H.D., Koroshetz, W.J., Chen, Y.I., Skeuse, C., Vangel, M., Cudkowicz, M.E. et al. Evidence for more widespread cerebral pathology in early HD: An MRI-based morphometric analysis. Neurology 60, 1615-20 (2003).

446. Morrell, M.J., McRobbie, D.W., Quest, R.A., Cummin, A.R., Ghiassi, R., Corfield, D.R. Changes in brain morphology associated with obstructive sleep apnea. Sleep Med 4, 451-4 (2003).

4

Cortical Thinning in the Prefrontal Cortex of Veterans with Posttraumatic Stress Disorder

Elbert Geuze, Eric Vermetten, Armin Heinecke, Carien S. de Kloet, Rainer Goebel, and Herman G. M. Westenberg. **Submitted.**

Introduction

Neuroimaging studies in PTSD have identified a number of functional and structural alterations that are associated with this disorder. Structural neuroimaging studies in PTSD have focused primarily on manual based hippocampal volumetry (see chapters 2 and 3), and have revealed that PTSD is associated with bilateral smaller hippocampal volume. [1;2] However, while considerable attention has been placed on hippocampal volume changes, very little attention has been directed to gray matter reductions in the cortex. Several voxel based morphometry studies, a whole-brain technique, have been applied to PTSD and have shown reduced gray matter density in the anterior cingulate gyrus, hippocampus, and insula. [3-5] Functional neuroimaging studies have identified a number of brain areas with altered activity in patients with PTSD, including the prefrontal cortex, temporal cortex, insula, amygdala, and hippocampus. [6]

Cortical thickness measurements and volumetric analysis receive increasing interest in the imaging community because they allow relation of cognitive abilities, effect of aging, and effect of diseases to (subtle) structural changes in the brain. Practical constraints, however, related to intensive measurement techniques and the use of postmortem samples, make it difficult to compare differences in cytoarchitecture across the cortex. Whole brain volumetric techniques are also compromised by large inter-individual differences in neuroanatomy making it difficult to match the same macroscopic regions between subjects. Recent advances in computational analysis provide new opportunities to use imaging data to derive more knowledge on cortical thickness. In this study, we have applied the new cortical thickness analysis tools in BrainVoyager QX to compare cortical thickness in frontal and temporal cortex in veterans with PTSD to veterans without PTSD. To maximize the spatial correspondence mapping between cortical macrostructures, we also made use of the new technique of cortex-based alignment, which aligns reconstructed cortices using curvature information reflecting the gyral/sulcal folding pattern. It has been shown that a cortical matching approach substantially improves statistical analysis across subjects by reducing anatomical variability.[7-9] Based on previous structural and functional neuroimaging studies which identified alterations in frontal and temporal cortex, we hypothesized that veterans with PTSD would reveal reduced cortical thickness in these brain areas.

Methods

Subjects

The sample consisted of twenty-five male Dutch veterans with PTSD, and twenty-five age matched control veterans without PTSD. All patients were recruited from the department of Military Psychiatry at the Central Military Hospital in Utrecht. Control subjects were recruited via direct mail to veterans who were registered at the Veterans Institute in Doorn, the Netherlands. All participants had served in UN peacekeeping missions in Lebanon, Cambodia, or Bosnia. Control veterans were matched to the patient group with respect to age, year of deployment, and country of deployment. All control veterans fulfilled DSM-IV criterion A1 (i.e. they had all experienced a traumatic event). None of the

veterans included were physically injured at the time of deployment. At study entry, twenty-one of the twenty-five patients were drug naïve. The other four subjects had not taken any psychotropic medications or abused substances for at least six months. Subjects with a history of neurological illness and psychiatric illness, other than mood and anxiety disorders, were excluded. This study was approved by the Institutional Review Board of the University Medical Centre of Utrecht, the Netherlands. Written informed consent was obtained from all subjects who participated in the study after a complete written and verbal description of the study. The study was performed between August 2002 and September 2005.

Clinical Assessments

All subjects were assessed using the Structured Clinical Interview for DSM-IV [10] and the Clinician Administered PTSD Scale (CAPS). [11] Diagnosis of PTSD was confirmed by the CAPS and through consensus by three clinicians (EG, EV, CdK). Only subjects with a CAPS score greater than 50 were included in the patient group. Subjects with a CAPS score greater than 20 were excluded from the trauma control group. Hamilton Depression Scale (HAM-D), and the Hamilton Anxiety Scale (HAM-A) scores were also obtained for all subjects.

MRI

Magnetic resonance imaging (MRI) of all subjects was performed at the Department of Radiology of the University Medical Centre of Utrecht. MRIs were acquired using a 1.5T scanner (Philips Gyroscan; Philips Medical Systems, Best, the Netherlands). T1-weighted, 3D, fast field echo scans with 160 to 180 1.2 mm contiguous coronal slices (echo time, 4.6 ms; repetition time, 30 ms; flip angle 30°; field of view 256 mm) of the whole head were used for cortical thickness analysis.

Preprocessing and Advanced Segmentation Analysis

All image data preparation and preprocessing, cortical thickness analysis, and cortex-based alignment, were carried out using BrainVoyager QX 1.7 (Brain Innovation, Maastricht, the Netherlands). The anatomical data (DICOM format) of each subject was loaded and converted into BrainVoyager's internal data format, resampled into 1mm resolution, and transformed into ACPC and Talairach standard space. The spatial transformations were combined and applied in one step to avoid quality loss due to successive data sampling.[12] Prior to cortical thickness analysis, several preparatory advanced segmentation steps were performed. Data were converted to 0.5 mm iso-voxels using sinc interpolation. Then the brain was segmented from surrounding head tissue using an automatic "brain peeling" tool.[12] Subcortical structures and the cerebellum were removed using a mask. Tissue contrast and homogeneity were enhanced using a sigma filter (which smoothes intensity values around each voxel while taking care that only voxels included in this process contain intensity values close to the intensity of a considered voxel). Next, the white matter–gray matter border was segmented automatically using an adaptive region growing step which uses two sources of information to separate white from gray matter voxels (i.e. locally computed histograms and gradient information). Then the gray matter–cerebrospinal fluid (CSF) border was segmented using a dilation process which begins at the white matter-gray matter boundary and labels gray matter voxels by moving towards the CSF boundary. This process was controlled by computed gradient fields and histogram analysis of gray matter–CSF threshold values. Both the white matter–gray matter and gray matter–CSF borders were 'polished' by calculating a magnitude map based on the computed gradient maps of the binary segmentation results.

Cortical Thickness Analysis

Cortical thickness maps for each subject were calculated. Since cortical thickness varies substantially across space, a simple orthogonal measurement technique - going in a fixed, orthogonal direction from one side of gray matter to the other side - may lead to erroneous thickness estimates. To avoid these problems, the cortical thickness measurements in BrainVoyager QX are based on the method proposed by Jones et al [13]. This method makes use of Laplace's equation, which is a partial differential equation, frequently used in many fields of science such as electromagnetism, astronomy, and fluid dynamics. In three dimensions, the problem is to find twice-differentiable real-valued functions Φ of real variables x, y, and z such that

$$\nabla^2\Phi = \frac{\partial^2\Phi}{\partial x^2} + \frac{\partial^2\Phi}{\partial y^2} + \frac{\partial^2\Phi}{\partial z^2} = 0$$

Solutions of this partial differential equation have to fulfill the constraint that the sum of the (unmixed) second partial derivatives is zero. This is fulfilled if the gradient slopes do not change along each dimension. For cortical thickness measures, BrainVoyager defines two different "potential" (intensity) values, one at the white-gray matter (WM-GM) boundary and one at the gray matter-CSF (GM-CSF) boundary. A solution of Laplace's equation then results in a smooth transition of voltages (intensities) from one boundary to the other ($\nabla^2\Phi = 0$). Such a solution can be found simply by keeping the values at the boundaries fixed and by smoothing the "voltage" values in between (gray matter voxels). From the obtained smooth field, a gradient value ($\nabla\Phi$) can be calculated at each voxel. Integrating along these gradient values results in "field lines" or "streamlines". To calculate a cortical thickness value, the program starts at any boundary voxel, checks the gradient and performs a small step along the gradient direction. Then the gradient is re-evaluated at the new point and the next step along the gradient direction is performed. This procedure is repeated until the other boundary is reached. The sum of the performed small step sizes provides the cortical thickness value. By going in both gradient directions ("up" and "down"), thickness values for any voxel between the boundary voxels may be calculated by simply adding up two partial streamlines.

Cortical Alignment

To improve the spatial correspondence mapping between subjects' brains beyond Talairach space matching, reconstructed cortices were aligned using curvature information reflecting the gyral and sulcal folding pattern. While functional areas do not precisely follow cortical landmarks, it has been shown that a cortical matching approach substantially improves statistical analysis across subjects by reducing anatomical variability.[9] First, the reconstructed folded cortical representations of each subject and hemisphere were morphed into a spherical representation, which provides a parameterizable surface well-suited for across-subject non-rigid alignment. Each vertex on the sphere (spherical coordinate system) corresponded to a vertex of the folded cortex (Cartesian coordinate system) and vice versa. The curvature information computed in the folded representation was preserved as a curvature map on the spherical representation. The curvature information (folding pattern) was smoothed along the surface to provide spatially extended gradient information driving intercortex alignment by minimizing the mean squared differences between the curvature of the source and the target sphere. The alignment proceeded iteratively following a coarse-to-fine matching strategy, which starts with highly smoothed curvature maps and progresses to only slightly smoothed representations. Starting with a coarse alignment as provided by Talairach space, this method ensures that the smoothed curvature of the two cortices possess enough overlap for a locally operating gradient-descent procedure to converge without user intervention.[12;14]

Table 1: Subject Demographics and Psychometric Data

	PTSD	*Controls*
N	25	25
Age	35.08 (4.44)	34.01 (5.61) ns
Year of deployment (range 1980-2002)	1993 (1.23)	1993 (1.67) ns
Country of deployment	Bosnia (n = 16)	Bosnia (n = 16)
	Lebanon (n = 5)	Lebanon (n = 4)
	Cambodia (n = 3)	Cambodia (n = 4)
	Afghanistan (n = 1)	Afghanistan (n = 1)
CAPS total score	74.88 (11.95)	6.67 (6.38) *
Hamilton A	19.92 (1.57)	1.32 (1.57) *
Hamilton D	15.84 (4.58)	0.92 (1.44) *
Years of education	11.88 (1.05)	11.76 (0.97) ns

Hamilton A, Hamilton Anxiety Scale; Hamilton D, Hamilton Depression Scale; Means for both groups are given. Standard deviations are reported in between brackets. ns this difference was not significant, * this difference was significant $p<0.0001$.

Although tests have shown that alignment results are very similar when using different target spheres, selection of a specific target brain might lead to suboptimal results in brain regions where the selected brain has an idiosyncratic folding pattern, therefore all brains were aligned using a moving target approach. In the moving target group averaging approach, the selection of a target sphere is not required. In this approach, the goal function is specified as a moving target computed repeatedly during the alignment process as the average curvature across all hemispheres at a given alignment stage.

Region of Interest Analysis

In order to increase statistical power, a region of interest (ROI) approach was applied. After the target (group) brain was aligned to the atlas brain provided by Brainvoyager QX, several ROI were selected. Conform to our hypothesis the ROI selected included the bilateral superior frontal gyri, bilateral middle frontal gyri, bilateral inferior frontal gyri, bilateral superior temporal gyri, and bilateral middle temporal gyri (see Figure 1, pg 229). Individual cortical thickness values from these ROI were exported to SPSS 12.0 and compared using multivariate analysis of variance (MANOVA). Post hoc one-way analysis of variance (ANOVA) was performed to examine group differences in average thickness values from the ROI.

Results

Psychometric Data

PTSD patients and control veterans were matched with respect to age, gender, year, and region of deployment. There were also no significant differences in years of education between the two groups. Patients with PTSD had significantly greater CAPS, Hamilton A, and Hamilton D scores, (see Table 1). The PTSD group met lifetime (past) DSM-IV (American Psychiatric Association, 1994) diagnostic criteria for the following disorders: major depressive disorder (n = 11), bipolar disorder (n = 3), alcohol abuse (n = 4), substance abuse (n=2), and panic disorder with agoraphobia (n = 2). Eight subjects with PTSD met current diagnostic criteria for major depression. One subject with PTSD met current diagnostic criteria for panic disorder with agoraphobia. Four control subjects met lifetime (past) DSM-IV diagnostic criteria for major depressive disorder, and one subject met lifetime diagnostic criteria for panic disorder with agoraphobia. The SCID did not reveal any current psychiatric disorders among our control subjects.

Cortical Thickness Analysis

The MANOVA revealed a significant main effect of cortical thickness ($F_{10,35} = 2.556$; $p<0.05$). Mean cortical thickness values and standard deviations for the selected ROI are shown in Table 2. Post hoc ANOVAs revealed significantly lower cortical thickness values in the bilateral superior and middle frontal gyri, left inferior frontal gyrus, and the left superior temporal gyrus in veterans with PTSD compared to control veterans (see Table 2).

Discussion

This is the first study in the field of PTSD that provides data on the cortical thickness of veterans with PTSD compared to healthy control veterans. Cortical thickness is a relatively new semi-automatic technique that has recently been implemented in the neuroimaging program Brainvoyager QX 1.7. In this ROI based analysis, patients revealed reduced cortical thickness in the bilateral prefrontal cortex and the left superior temporal gyrus compared to controls.

Table 2: Cortical thickness in veterans with and without PTSD

	PTSD			*Controls*				
ROI	*n*	*M**	*SD*	*n*	*M**	*SD*	*ANOVA*	*p*
Left hemisphere								
Superior Frontal Gyrus	25	2.19	0.52	25	2.64	0.41	F(1,48) = 11.54	0.001
Middle Frontal Gyrus	25	2.12	0.50	25	2.52	0.42	F(1,48) = 9.36	0.004
Inferior Frontal Gyrus	25	2.57	0.52	25	2.89	0.31	F(1,48) = 6.86	0.012
Superior Temporal Gyrus	25	2.74	0.45	25	2.97	0.25	F(1,48) = 4.87	0.032
Middle Temporal Gyrus	25	3.21	0.58	25	3.26	0.25	F(1,48) = 0.20	0.659
Right hemisphere								
Superior Frontal Gyrus	24	2.34	0.56	23	2.72	0.50	F(1,45) = 6.00	0.018
Middle Frontal Gyrus	24	2.35	0.52	23	2.68	0.47	F(1,45) = 5.30	0.026
Inferior Frontal Gyrus	24	2.71	0.47	23	2.91	0.35	F(1,45) = 2.58	0.115
Superior Temporal Gyrus	23	2.80	0.38	23	2.87	0.27	F(1,44) = 0.446	0.507
Middle Temporal Gyrus	24	3.03	0.51	23	3.00	0.28	F(1,45) = 0.050	0.825

Multivariate analysis (MANOVA) was used to compare the two groups on these measures; post hoc one way ANOVA results are presented in the table. * in mm.

In PTSD several different areas of the prefrontal cortex including the dorsolateral prefrontal cortex, the ventrolateral prefrontal cortex, the medial prefrontal cortex, the anterior cingulate cortex, and the orbital frontal cortex have revealed altered function compared to controls. [15-22] In several functional imaging studies, we have also shown that veterans with PTSD show altered function of the prefrontal cortex in pain processing,[23] and during encoding and retrieval of paired-associates. [24] PTSD has long been thought to be associated with a “hypofrontality”, or reduced blood flow and neural activation in frontal areas. [25] The results from this study of cortical thickness support the involvement of the prefrontal cortex in PTSD.

Deficits in the temporal lobe (particularly the medial temporal lobe) are frequently witnessed in patients with PTSD. [1;23-25] PTSD subjects show less activation in the superior temporal gyrus in response to traumatic scripts. [22] In a study of associative memory processing, however, veterans with PTSD revealed increased left superior temporal gyrus activation.[24] Children with PTSD have significantly greater right superior temporal gyrus gray matter volume. [26]

Cortical thickness is a reflection of the size, density, and arrangement of neurons, glial cell and nerve fibers. Cortical thinning in these areas likely reflects changes in the gray-white matter boundary related to a loss of dendrites and dendritic spines or changes in myelination within specific brain systems. [27;28] Alternatively, cortical thinning may also indicate changes in the cortical mantle related to the size and density of neurons.[29] The deleterious effect of stress on neuronal growth (predominately in the medial temporal lobe, but also in other brain areas) has been shown in a wide range of animal studies.[30-32] Proposed mechanisms for these findings include high levels of glucocorticoids released during stress,[33;34] increased production of corticotrophin releasing factor,[35] inhibition of neurogenesis,[36] or stress related inhibition of brain derived neurotrophic factor.[37;38]

This study compared two groups of veterans well-matched on age, years of education, region of deployment, and year of deployment. In addition, the majority of the patient group was medication naïve (for psychotropic medication) and the rest were free of psychotropic medication for six months. However, there are also several limitations of this study. The cortical-thickness method described in this chapter is a new method and although valid, it has not been applied much in psychiatric research. Conclusions drawn from this study should therefore be modest. In this ROI based analysis, we defined several gyri a priori; however, this type of analysis precludes us from making conclusions about other gyri in the brain. We preferred the ROI analysis to a whole-brain method of analysis, because at this time, a proper correction for multiple comparisons has not yet been implemented. Future research should also attempt to relate cortical thickness values to known functions or psychopathology associated with these gyri. This would provide valuable information, not only for PTSD and the psychiatric field, but perhaps also for the whole field of neuroscience.

References

1. Geuze, E., Vermetten, E., and Bremner, J.D. MR-based in vivo hippocampal volumetrics: 2. Findings in neuropsychiatric disorders. Mol Psychiatry 10, 160-84 (2005).

2. Kitayama, N., Vaccarino, V., Kutner, M., Weiss, P., and Bremner, J.D. Magnetic resonance imaging (MRI) measurement of hippocampal volume in posttraumatic stress disorder: a meta-analysis. J Affect Disord 88, 79-86 (2005).

3. Chen, S. et al. Gray matter density reduction in the insula in fire survivors with posttraumatic stress disorder: a voxel-based morphometric study. Psychiatry Res 146, 65-72 (2006).

4. Corbo, V., Clement, M.H., Armony, J.L., Pruessner, J.C., and Brunet, A. Size versus shape differences: contrasting voxel-based and volumetric analyses of the anterior cingulate cortex in individuals with acute posttraumatic stress disorder. Biol Psychiatry 58, 119-24 (2005).

5. Yamasue, H. et al. Voxel-based analysis of MRI reveals anterior cingulate gray-matter volume reduction in posttraumatic stress disorder due to terrorism. Proc Natl Acad Sci U S A 100, 9039-43 (2003).

6. Francati, V., Vermetten, E., and Bremner, J. Functional Neuroimaging Studies in Posttraumatic Stress Disorder: Methods & Findings. Depr Anx (in press).

7. Fischl, B., Sereno, M.I., and Dale, A.M. Cortical surface-based analysis. II: Inflation, flattening, and a surface-based coordinate system. Neuroimage 9, 195-207 (1999).

8. Dale, A.M., Fischl, B., and Sereno, M.I. Cortical surface-based analysis. I. Segmentation and surface reconstruction. Neuroimage 9, 179-94 (1999).

9. Fischl, B., Sereno, M.I., Tootell, R.B., and Dale, A.M. High-resolution intersubject averaging and a coordinate system for the cortical surface. Hum Brain Mapp 8, 272-84 (1999).

10. First, M. B., Spitzer, R. L., Gibbon, M., and Williams, J. B. W. Structured Clinical Interview for DSM-IV Axis I Disorders. SCID-I/P . 97.

11. Blake, D.D. et al. The development of a Clinician-Administered PTSD Scale. J Trauma Stress 8, 75-90 (1995).

12. Goebel, R., Esposito, F., and Formisano, E. Analysis of functional image analysis contest (FIAC) data with brainvoyager QX: From single-subject to cortically aligned group general linear model analysis and self-organizing group independent component analysis. Hum Brain Mapp 27, 392-401 (2006).

13. Jones, S.E., Buchbinder, B.R., and Aharon, I. Three-dimensional mapping of cortical thickness using Laplace's equation. Hum Brain Mapp 11, 12-32 (2000).

14. Formisano, E., Esposito, F., Di Salle, F., and Goebel, R. Cortex-based independent component analysis of fMRI time series. Magn Reson Imaging 22, 1493-504 (2004).

15. Bremner, J.D. et al. Neural correlates of the classic color and emotional stroop in women with abuse-related posttraumatic stress disorder. Biol Psychiatry 55, 612-20 (2004).

16. Bremner, J.D., Vythilingam, M., Vermetten, E., Vaccarino, V., and Charney, D.S. Deficits in hippocampal and anterior cingulate functioning during verbal declarative memory encoding in midlife major depression. Am J Psychiatry 161, 637-45 (2004).

17. Bremner, J.D. et al. Decreased benzodiazepine receptor binding in prefrontal cortex in combat-related posttraumatic stress disorder. Am J Psychiatry 157, 1120-6 (2000).

18. Bremner, J.D. et al. Neural correlates of exposure to traumatic pictures and sound in Vietnam combat veterans with and without posttraumatic stress disorder: a positron emission tomography study. Biol Psychiatry 45, 806-16 (1999).

19. Bremner, J.D. et al. Positron emission tomography measurement of cerebral metabolic correlates of yohimbine administration in combat-related posttraumatic stress disorder. Arch Gen Psychiatry 54, 246-54 (1997).

20. Britton, J.C., Phan, K.L., Taylor, S.F., Fig, L.M., and Liberzon, I. Corticolimbic blood flow in posttraumatic stress disorder during script-driven imagery. Biol Psychiatry 57 , 832-40 (2005).

21. Shin, L.M. et al. A functional magnetic resonance imaging study of amygdala and medial prefrontal cortex responses to overtly presented fearful faces in posttraumatic stress disorder. Arch Gen Psychiatry 62, 273-81 (2005).

22. Lindauer, R.J. et al. Cerebral blood flow changes during script-driven imagery in police officers with posttraumatic stress disorder. Biol Psychiatry 56, 853-61 (2004).

23. Geuze, E. et al. Altered Pain Processing in Veterans with Posttraumatic Stress Disorder. Arch Gen Psychiatry (in press).

24. Geuze, E., Vermetten, E., Ruf, M., de Kloet, C.S., and Westenberg, H.G.M. Neural Correlates of Associative Memory Processing in Veterans with Posttraumatic Stress Disorder. In Geuze, E. (ed.) The War Within. Utrecht (2006).

25. Nemeroff, C.B. et al. Posttraumatic stress disorder: A state-of-the-science review. J Psychiatr Res 40, 1-21 (2006).

26. De Bellis, M.D. et al. Superior temporal gyrus volumes in maltreated children and adolescents with PTSD. Biol Psychiatry 51, 544-52 (2002).

27. Narr, K.L. et al. Cortical thinning in cingulate and occipital cortices in first episode schizophrenia. Biol Psychiatry 58, 32-40 (2005).

28. Harrison, P.J. The neuropathology of schizophrenia. A critical review of the data and their interpretation. Brain 122 (Pt 4), 593-624 (1999).

29. Shaw, P. et al. Longitudinal mapping of cortical thickness and clinical outcome in children and adolescents with attention-deficit/hyperactivity disorder. Arch Gen Psychiatry 63, 540-9 (2006).

30. Sapolsky, R.M. Chickens, eggs and hippocampal atrophy. Nat Neurosci 5, 1111-3 (2002).

31. Sapolsky, R.M. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiatry 57, 925-35 (2000).

32. Vermetten, E. and Bremner, J.D. Circuits and systems in stress. I. Preclinical studies. Depress Anxiety 15, 126-47 (2002).

33. McEwen, B.S. et al. Paradoxical effects of adrenal steroids on the brain: protection versus degeneration. Biol Psychiatry 31, 177-99 (1992).

34. Sapolsky, R.M., Uno, H., Rebert, C.S., and Finch, C.E. Hippocampal damage associated with prolonged glucocorticoid exposure in primates. J Neurosci 10, 2897-902 (1990).

35. Brunson, K.L., Eghbal-Ahmadi, M., Bender, R., Chen, Y., and Baram, T.Z. Long-term, progressive hippocampal cell loss and dysfunction induced by early-life administration of corticotropin-releasing hormone reproduce the effects of early-life stress. Proc Natl Acad Sci U S A 98, 8856-61 (2001).

36. Gould, E., McEwen, B.S., Tanapat, P., Galea, L.A., and Fuchs, E. Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. J Neurosci 17, 2492-8 (1997).

37. Nibuya, M., Morinobu, S., and Duman, R.S. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. J Neurosci 15, 7539-47 (1995).

38. Smith, M.A., Makino, S., Kvetnansky, R., and Post, R.M. Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. J Neurosci 15, 1768-77 (1995).

SECTION 2

Functional

Neuroimaging

5

Reduced $GABA_A$ Benzodiazepine Binding in Veterans with Posttraumatic Stress Disorder

Elbert Geuze, Bart N. M. van Berckel, Adriaan A. Lammertsma, Ronald Boellaard, Carien S. de Kloet, Eric Vermetten, and Herman G. M. Westenberg. **Submitted.**

Introduction

PTSD is associated with alterations in a number of neuroendocrine and neurotransmission systems, including the hypothalamic-pituitary-adrenal axis, and serotonin, noradrenalin, and gamma-amino-butyric acid (GABA) systems. GABA is the principal inhibitory neurotransmitter in the brain exerting control over excitability in most brain areas. GABA plays an important role in homeostasis during stress, and alterations in GABAergic systems have been implicated in the pathogenesis of anxiety disorders, including PTSD, and depression. Potential involvement of the GABA system in PTSD has been examined using a variety of different paradigms, including preclinical studies, pharmacologic studies, and neuroimaging techniques. In the stress-restress paradigm, an animal model that shows resemblance to PTSD, stress evoked a sustained decrease in hippocampal GABA levels.[1] Exposure of rodents to inescapable foot shock, a model for stress-related depression characterized by deficits in learning and memory, resulted in decreased $GABA_A$ function and binding in the cerebral cortex and hippocampus.[2-4] Benzodiazepines, which modulate the $GABA_A$ receptor function, inhibit the startle response induced by predator stress, which models aspects of hyperarousal seen in patients with PTSD.[5]

Pharmacological studies have also provided support for the involvement of the GABA system in PTSD. Benzodiazepines have fast-acting anxiety reducing properties, which have led to their widespread use as treatment for anxiety disorders including PTSD. Although benzodiazepines are effective in reducing anxiety in PTSD, they have no effect on the core symptoms of this disorder, such as intrusive thoughts, numbing, and hyperarousal.[6-8] A putative role of GABA in PTSD is also supported by treatment studies with other GABAergic compounds, such as tiagabine, a selective GABA reuptake inhibitor. [9,10] Low plasma GABA levels after a traumatic event are predictive of subsequent development of PTSD [11], further suggesting that the GABA system is involved in the pathophysiology of PTSD.

Neuroimaging techniques provide a unique opportunity to investigate receptor binding and neurotransmitter release in vivo. Only a few neuroimaging studies of the benzodiazepine-$GABA_A$ receptor in PTSD have been performed and all these studies used single photon emission computed tomography (SPECT) and [^{123}I]-iomazenil. Using this technique, it has been reported that Vietnam veterans with PTSD had lower volumes of distribution of [^{123}I]-iomazenil in the prefrontal cortex (Brodmann's area 9) when compared to healthy controls.[12] A more recent [^{123}I]-iomazenil SPECT study, however, was unable to confirm these results in Gulf War veterans compared to undeployed military controls.[13] Positron emission tomography (PET) using [^{11}C]-flumazenil is a more accurate technique for quantifying benzodiazepine- $GABA_A$ receptor binding. Apart from being fully quantitative, PET also has a superior spatial resolution compared to SPECT. Based on preclinical studies and clinical findings, the working hypothesis of the present study was that veterans with PTSD have reduced [^{11}C]-flumazenil binding potential in both cortical areas and the hippocampus compared to veterans without PTSD.

Methods

Subjects

Nine male Dutch veterans with PTSD and seven male Dutch veterans without PTSD were recruited for this study. PTSD patients were recruited from the Department of Military Psychiatry at the Central Military Hospital in Utrecht. Control subjects were recruited via direct mail to veterans who were registered at the Veterans Institute in the Netherlands. All participants had served in UN peacekeeping missions in Lebanon and Bosnia. Control veterans were matched to the patient group with respect to age, year of deployment, and country of deployment. None of the veterans included were physically injured during the time of deployment. At study entry, all subjects were free of any psychotropic medication for at least four weeks. In addition, none of the participants had a history of benzodiazepine usage within six months prior to the study. Urinary drug screening was performed in all subjects, and subjects in whom the presence of benzodiazepines or other drugs was detected were excluded from the study. In addition, subjects were excluded when they had received an investigational medication within 30 days prior to the start of this study, or when they had a history of neurological illness or psychiatric illness other than mood or anxiety disorders. In addition, for all participants, no clinically significant abnormalities were visible on the MRI scan.

All subjects were evaluated with the Structured Clinical Interview for DSM-IV (SCID), Clinician-Administered PTSD Scale for DSM-IV (CAPS)[14], Hamilton Depression Scale (HAM-D), and Hamilton Anxiety Scale (HAM-A). In all patients with PTSD, PTSD was the primary diagnosis. PTSD was confirmed by the CAPS and through consensus by three clinicians (EG, EV, CdK). Control subjects were only included if their CAPS score was less than 20. Written informed consent was obtained from all participants after a complete written and verbal description of the study.

Table 1: Subject Demographic and Psychometric Data

	PTSD	*Controls*
N	9	7
Age	35.3 (6.3)	36.4 (4.7) ns
Year of deployment (range 1980-1999)	1991 (2.1)	1992 (2.8) ns
Country of deployment	Bosnia (n = 7)	Bosnia (n = 5)
	Lebanon (n = 2)	Lebanon (n = 2)
CAPS total	76.0 (15.5)	3.6 (6.1) *
Hamilton A	18.6 (5.3)	0.4 (0.5) *
Hamilton D	16.2 (4.2)	0.3 (0.5) *

Means for both groups are given. Standard deviations are reported between brackets. ns this difference was not significant, * this difference was significant at $p<0.0001$. CAPS, Clinician Administered PTSD Scale; Hamilton A, Hamilton Anxiety Scale; Hamilton D, Hamilton Depression Scale

The study was performed between August 2003 and July 2004. This study was approved by the Ethical Review Boards of the University Medical Centre of Utrecht, the Netherlands and the VU University Medical Centre, Amsterdam, the Netherlands.

Production of [^{11}C]-flumazenil

[^{11}C]-flumazenil was produced according to Good Medical Practice (GMP) guidelines. Briefly, $^{11}CO_2$ was produced by irradiation of $^{14}N/O_2$ (95.5%/0.5%) with 18 MeV protons using an "18/9 cyclone" cyclotron (IBA, Louvain-la Neuve, Belgium) from the VU Cyclotron BV in Amsterdam. Subsequently collected $^{11}CO_2$ was reacted with lithiumaluminium-hydride in tetrahydrofurane. Hydrogeniodide was added to yield [^{11}C]-methyliodide. Next, [^{11}C]-methyliodide was distilled into a mixture of RO15-5528 (desmethylflumazenil) and tetrabutylammoniumhydroxide in dimethylformamide and heated for two minutes at 40 °C. [^{11}C]-flumazenil was purified by semipreparative HPLC. After quality control analyses and approval by the hospital pharmacist the solution was ready for injection.

PET

PET scans were performed on an ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, USA), which is located at the department of Nuclear Medicine & PET Research of the VU University Medical Centre in Amsterdam, the Netherlands. This scanner enables the acquisition of 63 transaxial planes over a 15.5 cm axial field of view, thus allowing the whole brain to be imaged. Characteristics of this scanner have been described elsewhere.[15;16] All subjects received an indwelling radial artery cannula, which was used for blood sampling in order to generate a metabolite corrected input curve. In addition, a venous cannula was inserted for tracer injection.

First a 10-minute transmission scan was performed in 2D acquisition mode using three retractable rotating line sources. This scan was used to correct the subsequent emission scan for photon attenuation. Then a dynamic emission scan in 3D acquisition mode was started simultaneously with the intravenous injection of 371 ± 56 MBq (mean ± SD) of [^{11}C]-flumazenil using an infusion pump at 0.8 ml/sec after which the line was flushed with 42 ml saline at 2.0 ml/sec (Med-Rad, Beek, the Netherlands). This dynamic emission scan consisted of 16 frames with progressive increase in frame duration (4×15, 4×60, 2×150, 2×300, 4×600 seconds) and a total duration of 60 minutes. Arterial blood was withdrawn continuously at a rate of 5ml/min for the first 10 minutes and 2.5 ml/min thereafter, using an on line detection system (Veenstra Instruments, Joure, the Netherlands), which was cross-calibrated against the PET scanner.[17] At 2.5, 5, 10, 20, 30, 40, and 60 minutes post-injection, continuous withdrawal was briefly interrupted for manual collection of blood samples.

Image Reconstruction

All PET sinograms were corrected for dead time, tissue attenuation, decay, scatter and randoms, and reconstructed using a standard filtered back projection algorithm with a Hanning filter cutoff

at 0.5 times the Nyquist frequency. A zoom factor of 2 and a matrix size of 256 × 256 × 63 were used resulting in a voxel size of 1.2 × 1.2 × 2.4 mm and a spatial resolution of approximately 7 mm full-width at half-maximum at the centre of the field of view. Images were then transferred to Sun Microsystems workstations for further analysis.

MRI

Magnetic resonance imaging (MRI) of all subjects was performed at the Department of Radiology of the University Medical Centre of Utrecht. MRI scans were acquired using a 1.5T scanner (Philips Gyroscan; Philips Medical Systems, Best, the Netherlands). These scans were used for segmentation of gray and white matter and for delineation of regions of interest (ROI). T1-weighted, 3D, fast field echo scans with 160 to 180 1.2 mm contiguous coronal slices (echo time, 4.6 ms; repetition time, 30 ms; flip angle 30°; field of view 256 mm) of the whole head were used for coregistration with PET.

Region of Interest Definition

MRI images were aligned to corresponding PET images using a mutual information algorithm included in MIRIT (Multimodality Image Registration using Information Theory[18;19]). A template including 35 regions of interest (ROI) was projected on the fused MRI image using automatic delineation based on a probability map. This method has previously been described and validated.[20] In addition, manual ROIs for the bilateral hippocampi, the bilateral amygdala, and the pons were defined on the fused MRI scan using DISPLAY (http://www.bic.mni.mcgill.ca/) according to standard anatomical criteria.[21;22] Left and right hippocampus and amygdala were also summed and used as additional ROIs.

Kinetic Analysis

Kinetic analyses were performed using dedicated software developed within Matlab 5.3 (The Mathworks, Natick, MA, USA). Manual samples collected during scanning were used to calibrate the online blood curve, to determine plasma to whole blood ratios of radioactivity, and to measure plasma metabolite fractions. Metabolite fractions were determined using HPLC, as previously described.[23] The online blood curve was calibrated using the manual whole-blood samples. Plasma to whole blood ratios were fitted to an exponential function and multiplied with the whole blood (online sampler) curve to obtain the corresponding plasma curve. Finally, this plasma-curve was multiplied with the parent fraction using a Hill function to obtain a metabolite-corrected plasma input function. [^{11}C]-flumazenil time-activity curves were generated by projecting all ROI onto all frames of the dynamic [^{11}C]-flumazenil scan. Metabolite-corrected plasma input curves were available for seven patients and seven controls due to technical reasons. These data were analyzed using a single tissue compartment model yielding the outcome parameter volume of distribution (V_d). This was used to confirm the use of the pons as reference tissue. The single tissue compartment includes free, non-specific, and specific compartments, and assumes that all tissue pools equilibrate rapidly with respect to blood-brain barrier transport rates.[24] Data from all nine patients and seven controls were then analysed using the simplified reference tissue model (SRTM) resulting in the outcome parameter Binding Potential (BP[25]). The use of the SRTM (with pons as reference tissue) for quantification of [^{11}C]-flumazenil BP has recently been validated.[26] Due to the small sample size and because data were not normally distributed, the Mann-Whitney U test was used for all ROI data. All statistical analyses were performed with SPSS 12.0 for Windows (SPSS, Chicago, Illinois). The statistical threshold for significance for all measures was set at $p < 0.05$.

Table 2: [^{11}C]-flumazenil binding potential in veterans with PTSD and control veterans

	Binding Potential (SRTM)					
	PTSD		*Controls*			
Template Based ROIs	*Mean*	*SD*	*Mean*	*SD*	*Mann-Whitney U*	*p*
Orbital Frontal Cortex	4.92	0.37	5.72	0.70	9.00	0.016
Medial Inferior Frontal Cortex	5.10	0.44	5.93	0.63	7.00	0.008
ACC	5.12	0.54	5.96	0.81	13.00	0.055
Thalamus	1.95	0.19	2.36	0.35	9.00	0.016
Insula	5.06	0.50	5.78	0.63	9.00	0.016
Caudate	1.33	0.18	1.58	0.26	15.50	0.091
Putamen	2.16	0.31	2.48	0.34	17.00	0.142
Superior Temporal Cortex	5.20	0.43	6.01	0.72	9.00	0.016
Parietal Cortex	4.57	0.36	5.40	0.63	7.00	0.008
Medial Inferior Temporal Cortex	5.05	0.37	5.90	0.66	8.00	0.012
Superior Frontal Cortex	4.61	0.41	5.40	0.54	8.00	0.012
Occipital Cortex	5.36	0.42	6.22	0.80	9.00	0.016
Sensory Motor Cortex	4.11	0.41	4.73	0.51	12.00	0.042
Posterior Cingulate Cortex	4.94	0.67	5.81	0.88	13.00	0.055
Enthorinal Cortex	4.01	0.37	4.52	0.56	13.00	0.055
Striatum	1.78	0.27	2.49	1.16	17.00	0.142
Midbrain	0.36	0.15	0.44	0.20	19.00	0.210
Cerebellum	3.72	0.30	4.30	0.55	11.00	0.031
Total Brain	3.55	0.41	4.22	0.49	8.00	0.012
Manual ROIs						
L Hippocampus	2.97	0.36	3.59	0.44	7.00	0.008
R Hippocampus	2.93	0.34	3.47	0.59	11.00	0.031
Hippocampus	2.95	0.31	3.52	0.51	8.50	0.012
L Amygdala	3.22	0.30	3.87	0.64	10.00	0.023
R Amygdala	3.23	0.40	3.65	0.60	13.00	0.055
Amygdala	3.21	0.30	3.75	0.60	18.00	0.174
Prefrontal Cortex	4.64	0.30	5.40	0.53	7.00	0.008

SPM analysis

Data were also analysed on a voxel by voxel level. For this analysis, BP images were generated using Ichise plots of dynamic data from 10 to 60 min post injection.[27] Prior to Ichise plot analysis dynamic scans were smoothed using an additional 10 mm FWHM Gaussian filter to reduce noise, thereby avoiding noise-induced bias during Ichise analysis. Next, these plots were used in a voxel-based comparison between the two groups using Statistical Parametric Mapping (SPM 2; http://www.fil.ion.ucl.ac.uk/spm). As images were already smoothed prior to Ichise analysis, the usual smoothing within SPM was omitted. SPM was performed without proportional scaling. Proportional scaling may be omitted because, following smoothing, Ichise plots are quantitatively accurate even at lower noise levels. The images were thresholded at $p < 0.001$, uncorrected for multiple comparisons, using a cluster size $k > 50$ voxels.

Results

Psychometric Data

PTSD patients and control veterans were matched with respect to age, gender, year, and region of deployment. Patients with PTSD had significantly greater CAPS, Hamilton A, and Hamilton D scores, (see Table 1). According to the SCID, the PTSD group met lifetime (past) DSM-IV (American Psychiatric Association 1994) diagnostic criteria for the following disorders: major depressive disorder (n = 4), bipolar disorder (n = 1), alcohol abuse (n = 2), manic episode (n = 1), and panic disorder with agoraphobia (n = 1). Only one subject with PTSD met current diagnostic criteria for panic disorder with agoraphobia. Among the control subjects, the SCID did not reveal any psychiatric disorders.

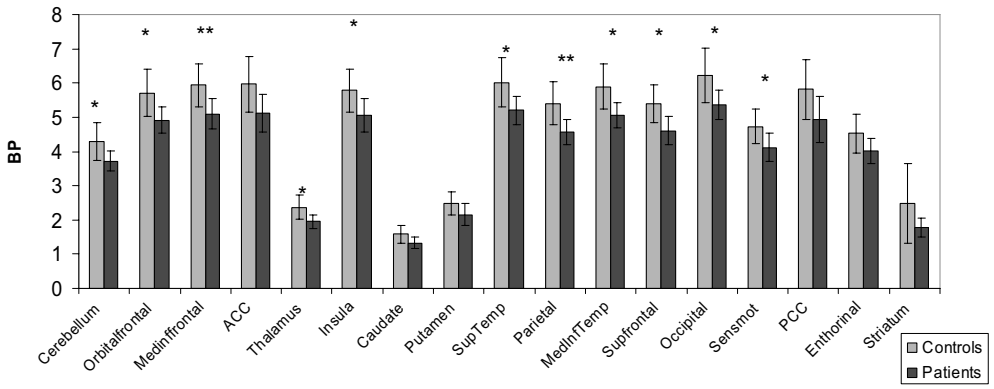


Figure 1: Binding potential (BP) values derived from the simplified reference tissue model (SRTM) for the template based ROI. Veterans with PTSD show a significant decrease in [11C]-flumazenil binding potential in most of the ROI compared to control veterans without PTSD. (* $p < 0.05$, and ** $p < 0.01$, Mann Whitney U Test). All values are given as mean ± standard deviation (SD).

ROI Analysis

There was no statistically significant difference between the volume of distribution of [^{11}C]-flumazenil in the pons of PTSD patients and controls ($V_{d(patients)} = 0.94 \pm 0.08$ [mean ± SD], $V_{d(controls)} = 0.89 \pm 0.13$ [mean ± SD]; Mann-Whitney U = 19.00, p=0.535). For this reason, and because an additional two subjects could be included, SRTM with pons as reference tissue was used to compare the two groups. Results from SRTM revealed significantly decreased BP in a large number of the template based ROIs in veterans with PTSD compared to veterans without PTSD (see Figure 1 and Table 2). Patients with PTSD showed decreased BP throughout the brain, including bilateral orbital frontal cortex, superior frontal cortex, medial inferior frontal cortex, superior temporal cortex, medial inferior temporal cortex, parietal cortex, occipital cortex, cerebellum, thalamus, insula, right anterior cingulate cortex (ACC), right posterior cingulate cortex, left enthorinal cortex, and left striatum (see Table 2). In addition, patients also showed decreased BP in several manually delineated ROIs including the left hippocampus and left amygdala (see Figure 2).

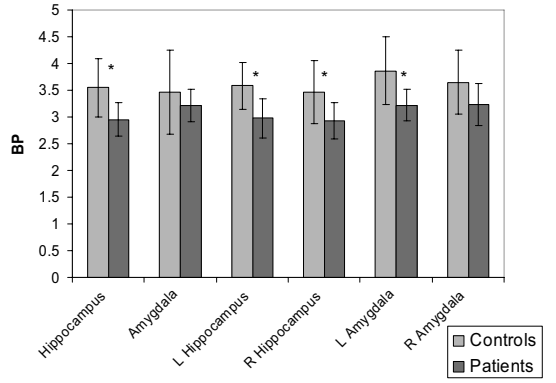


Figure 2: Binding potential (BP) values derived from the simplified reference tissue model (SRTM) for the manually delineated ROIs. Veterans with PTSD show a significant decrease in [11C]-flumazenil binding potential in most of the ROI compared to control veterans without PTSD. (* $p < 0.05$, Mann Whitney U Test). All values are given as mean ± standard deviation (SD).

SPM Analysis

SPM analysis of Ichise derived BP images showed statistically significant decreased [^{11}C]-flumazenil binding in PTSD subjects compared to controls throughout the occipital cortex, temporal cortex, parietal cortex, prefrontal cortex, insular cortex, thalamus, and the hippocampus (see Figure 3, page 228).

Discussion

This is the first fully quantitative PET study demonstrating reduced binding to the benzodiazepine-$GABA_A$ receptor complex in patients with PTSD. Widespread reduced BP was found throughout the cortex, hippocampus, and thalamus of patients with PTSD in comparison to control veterans without PTSD. Both SRTM and Ichise plots showed similar results. As the control subjects in this study had also experienced traumatic events, the observed difference in [^{11}C]- flumazenil binding in this study is disorder-related and not due to trauma. However, this does not preclude the possibility that stress or trauma, in trauma-exposed individuals who do not develop PTSD, can alter GABA function. In the absence of a healthy control group of subjects who have never experienced psychological trauma, it cannot be determined whether the observed difference in binding between veterans with and without PTSD is unique to PTSD or represents a quantitative difference in binding density. Animal models support the notion that stress and trauma can alter $GABA_A$-benzodiazepine binding density.[1;5;28-30]

Benzodiazepine binding sites in the brain are located predominantly on the $GABA_A$ receptors, and therefore their distribution in the brain reflects the distribution of $GABA_A$ receptors. Benzodiazepines potentiate the function of GABA through conformational changes in the receptor, thereby increasing the effectiveness of GABA for opening the ion channel. The present finding of reduced [^{11}C]-flumazenil binding throughout most of the cortical areas may be indicative of an a priori difference in subunit composition of $GABA_A$ - benzodiazepine receptors, a lower expression of the $GABA_A$ receptor in PTSD patients, or a disease- or trauma-induced modulation or downregulation of the $GABA_A$ receptor complex. These explanations are consistent with other clinical studies that have suggested altered GABAergic function in PTSD.[6;9;11] A reduced binding density can also be explained by the presence of endogenous ligands or increased levels of GABA. Interestingly, Spivak et al [31] reported elevated levels of the neurosteroids dehydroepiandrosterone and dehydorepiandrosterone sulphate, which have antagonistic properties at the $GABA_A$ receptor, in patients with PTSD.

Decreased binding to benzodiazepine-$GABA_A$ receptors is consistent with an [^{123}I]-iomazenil SPECT study in Vietnam veterans with PTSD. [12] In this study, Vietnam veterans with PTSD had a decreased V_d of [^{123}I] -iomazenil in the prefrontal cortex (one of the two a priori defined regions) compared to age-matched healthy controls. Another [^{123}I]-iomazenil SPECT study in Gulf War veterans with PTSD, however, was unable to find any significant differences in distribution volume of [^{123}I]-iomazenil compared to age-matched undeployed military personnel.[13] Possible factors that could account for the discrepancy in findings of these studies are, as mentioned by Fujita et al [13], related to type of controls used and the interval between traumatic experiences and the time of study. In the first study healthy subjects were used as controls, and the interval was about 25 years. In the second study controls used were military personnel who had served in the army at the same time, but who were not deployed. In addition, the interval between deployment and time of study was about 10 years. These differences, however, cannot explain differences in findings with the present data. In the present study control subjects were veterans who were matched to the patient group, who had been deployed to the same countries at the same time as the patients, and the interval between traumatic experiences and the PET study was also about 10-12 years.

SPECT is a semi-quantitative method that requires global normalization prior to SPM analysis. This may explain why these studies did not report a global reduction in distribution volume of [^{123}I]-iomazenil in PTSD. Interestingly, in panic disorder, which is related to PTSD, a global reduction of [^{11}C]-flumazenil binding was found throughout the brain [32], suggesting that the GABAergic system is indeed involved in these anxiety disorders.

Several studies have shown that the prefrontal cortex is dysfunctional in PTSD.[33-37] Across these studies, however, dysfunctional alterations occurred in different parts of the prefrontal cortex, indicating that the exact nature of the failure of prefrontal inhibition in PTSD needs to be clarified in the future. In the present study, a reduction of $GABA_A$-benzodiazepine BP throughout the frontal cortex was found. This may underlie the working memory deficit in patients with PTSD.[38] Decreases in $GABA_A$ receptor binding are also associated with alterations in working memory performance.[29;30] In addition, appropriate GABA neurotransmission in the frontal cortex is required for a normal working memory function.[39]

The hippocampus plays an important role in managing the stress response, novelty detection, and memory processing. The hippocampus is also an important site for GABA and serotonin interaction, which is thought to modulate emotional behavior.[40] In patients with PTSD, structural and functional alterations of the hippocampus have consistently been demonstrated. [41-44] Benzodiazepine agonists enhance $GABA_A$ receptor function in the CA1 region of the hippocampus and can thus disrupt memory formation and hippocampal synaptic plasticity.[45] It is also possible that in patients with PTSD increased sensitivity to stress causes the alterations in $GABA_A$ receptors. In a previous SPECT study, patients with panic disorder also revealed decreased $GABA_A$-benzodiazepine receptor binding in the left hippocampus.[46]

In the present study, SRTM with pons as reference tissue was used to analyze the data. Apart from enabling inclusion of data from all subjects (due to technical reasons the arterial plasma curve could not be used in two subjects), an important advantage of this method is that it measures binding potential for specific binding only. In contrast, the generally accepted single tissue compartment model with arterial input measures the volume of distribution of total binding (i.e. specific and non-specific binding). Noninvasive models such as SRTM require more assumptions than invasive models, but they exhibit less variance than invasive models, making them more reproducible.[47] Recently the use of SRTM with pons as reference tissue in the quantification of [^{11}C]-flumazenil PET has been validated in a large sample of normal controls and patients with depression.[26]

Other potential confounders of this study are related to the sample size and the study population. As in most PET studies the number of subjects was relatively small. One of the main strengths of this study is the use of matched trauma controls. Therefore the results can be ascribed to the ef-

fect of PTSD itself, not the effect of having witnessed traumatic events or the stress of deployment. The drawback of this approach is, however, that only male veterans with PTSD were studied. Care should be taken in extrapolating these findings to females with PTSD, and to PTSD related to other types of trauma, and further studies are warranted. Future research should also consider whether the observed effects are basal or adaptive changes, as the present study cannot distinguish between predisposing, mediating, or resultant factors.

In conclusion, this study has shown decreased [^{11}C]-flumazenil binding throughout the brain of veterans with PTSD. This provides evidence for the involvement of $GABA_A$ benzodiazepine receptors in PTSD and warrants further investigation of the GABAergic regulation of anxiety in PTSD.

References

1. Harvey, B.H., Oosthuizen, F., Brand, L., Wegener, G., and Stein, D.J. Stress-restress evokes sustained iNOS activity and altered GABA levels and NMDA receptors in rat hippocampus. Psychopharmacology (Berl) 175, 494-502 (2004).

2. Lippa, A.S. et al. Relationship between benzodiazepine receptors and experimental anxiety in rats. Pharmacol Biochem Behav 9, 853-6 (1978).

3. Medina, J.H., Novas, M.L., Wolfman, C.N., Levi de Stein, M., and De Robertis, E. Benzodiazepine receptors in rat cerebral cortex and hippocampus undergo rapid and reversible changes after acute stress. Neuroscience 9, 331-5 (1983).

4. Drugan, R.C., Basile, A.S., Crawley, J.N., Paul, S.M., and Skolnick, P. Inescapable shock reduces. Pharmacol Biochem Behav 24, 1673-7 (1986).

5. Adamec, R., Strasser, K., Blundell, J., Burton, P., and McKay, D.W. Protein synthesis and the mechanisms of lasting change in anxiety induced by severe stress. Behav Brain Res 167, 270-86 (2006).

6. Davidson, J.R. Use of benzodiazepines in social anxiety disorder, generalized anxiety disorder, and posttraumatic stress disorder. J Clin Psychiatry 65 Suppl 5, 29-33 (2004).

7. Gelpin, E., Bonne, O., Peri, T., Brandes, D., and Shalev, A.Y. Treatment of recent trauma survivors with benzodiazepines: a prospective study. J Clin Psychiatry 57, 390-4 (1996).

8. Viola, J. et al. Pharmacological management of post-traumatic stress disorder: clinical summary of a five-year retrospective study, 1990-1995. Mil Med 162, 616-9 (1997).

9. Connor, K.M., Davidson, J.R., Weisler, R.H., Zhang, W., and Abraham, K. Tiagabine for posttraumatic stress disorder: effects of open-label and double-blind discontinuation treatment. Psychopharmacology (Berl) 184, 21-5 (2006).

10. Taylor, F.B. Tiagabine for posttraumatic stress disorder: a case series of 7 women. J Clin Psychiatry 64, 1421-5 (2003).

11. Vaiva, G. et al. Low posttrauma GABA plasma levels as a predictive factor in the development of acute posttraumatic stress disorder. Biol Psychiatry 55, 250-4 (2004).

12. Bremner, J.D. et al. Decreased benzodiazepine receptor binding in prefrontal cortex in combat-related posttraumatic stress disorder. Am J Psychiatry 157, 1120-6 (2000).

13. Fujita, M. et al. Central type benzodiazepine receptors in Gulf War veterans with posttraumatic stress disorder. Biol Psychiatry 56, 95-100 (2004).

14. Blake, D. et al. A Clinican Rating Scale for Assessing Current and Lifetime PTSD: The CAPS-1. Behav Ther 13, 187-188 (1990).

15. Brix, G. et al. Performance evaluation of a whole-body PET scanner using the NEMA protocol. National Electrical Manufacturers Association. J Nucl Med 38, 1614-23 (1997).

16. Adam L.E. et al. Performance of the whole-body PET scanner ECAT EXACT HR + following the IEC standard. IEEE Trans Nucl Sci 44, 1172-1179 (1997).

17. Boellaard, R., van Lingen, A., van Balen, S.C., Hoving, B.G., and Lammertsma, A.A. Characteristics of a new fully programmable blood sampling device for monitoring blood radioactivity during PET. Eur J Nucl Med 28, 81-9 (2001).

18. Maes, F., Collignon, A., Vandermeulen, D., Marchal, G., and Suetens, P. Multimodality image registration by maximization of mutual information. IEEE Trans Med Imaging 16, 187-98 (1997).

19. West, J. et al. Comparison and evaluation of retrospective intermodality brain image registration techniques. J Comput Assist Tomogr 21, 554-66 (1997).

20. Svarer, C. et al. MR-based automatic delineation of volumes of interest in human brain PET images using probability maps. Neuroimage 24, 969-79 (2005).

21. Watson, C. et al. Anatomic basis of amygdaloid and hippocampal volume measurement by magnetic resonance imaging. Neurology 42, 1743-50 (1992).

22. Duvernoy, H.M. and Bourgouin, P. The human brain: surface, three-dimensional sectional anatomy and MRI, and blood supply. Springer Verlag, Vienna (1999).

23. Greuter, H.N. et al. Validation of a multiwell gamma-counter for measuring high-pressure liquid chromatography metabolite profiles. J Nucl Med Technol 32, 28-32 (2004).

24. Koeppe, R.A., Holthoff, V.A., Frey, K.A., Kilbourn, M.R., and Kuhl, D.E. Compartmental analysis of. J Cereb Blood Flow Metab 11, 735-44 (1991).

25. Lammertsma, A.A. and Hume, S.P. Simplified reference tissue model for PET receptor studies. Neuroimage 4, 153-8 (1996).

26. Klumpers, U.M.H. et al. Comparison of various models for analysing [11C] flumazenil studies. Journal of Cerebral Blood Flow & Metabolism 25, S660 (2005).

27. Ichise, M. et al. Graphical analysis and simplified quantification of striatal and extrastriatal dopamine D2 receptor binding with. J Nucl Med 40, 1902-12 (1999).

28. Caldji, C., Francis, D., Sharma, S., Plotsky, P.M., and Meaney, M.J. The effects of early rearing environment on the development of GABAA and central benzodiazepine receptor levels and novelty-induced fearfulness in the rat. Neuropsychopharmacology 22, 219-29 (2000).

29. Weizman, R. et al. Repeated swim stress alters brain benzodiazepine receptors measured in vivo. J Pharmacol Exp Ther 249, 701-7 (1989).

30. Drugan, R.C. et al. Stress-induced behavioral depression in the rat is associated with a decrease in GABA receptor-mediated chloride ion flux and brain benzodiazepine receptor occupancy. Brain Res 487, 45-51 (1989).

31. Spivak, B. et al. Elevated circulatory level of GABA(A)--antagonistic neurosteroids in patients with combat-related post-traumatic stress disorder. Psychol Med 30, 1227-31 (2000).

32. Malizia, A.L. et al. Decreased brain GABA(A)-benzodiazepine receptor binding in panic disorder: preliminary results from a quantitative PET study. Arch Gen Psychiatry 55, 715-20 (1998).

33. Bremner, J.D. et al. Neural correlates of the classic color and emotional stroop in women with abuse-related posttraumatic stress disorder. Biol Psychiatry 55, 612-20 (2004).

34. Bremner, J.D. et al. Neural correlates of exposure to traumatic pictures and sound in Vietnam combat veterans with and without posttraumatic stress disorder: a positron emission tomography study. Biol Psychiatry 45, 806-16 (1999).

35. Bremner, J.D. et al. Positron emission tomography measurement of cerebral metabolic correlates of yohimbine administration in combat-related posttraumatic stress disorder. Arch Gen Psychiatry 54, 246-54 (1997).

36. Britton, J.C., Phan, K.L., Taylor, S.F., Fig, L.M., and Liberzon, I. Corticolimbic blood flow in posttraumatic stress disorder during script-driven imagery. Biol Psychiatry 57 , 832-40 (2005).

37. Shin, L.M. et al. A functional magnetic resonance imaging study of amygdala and medial prefrontal cortex responses to overtly presented fearful faces in posttraumatic stress disorder. Arch Gen Psychiatry 62, 273-81 (2005).

38. Weber, D.L. et al. Abnormal frontal and parietal activity during working memory updating in post-traumatic stress disorder. Psychiatry Res 140, 27-44 (2005).

39. Lewis, D.A., Volk, D.W., and Hashimoto, T. Selective alterations in prefrontal cortical GABA neurotransmission in schizophrenia: a novel target for the treatment of working memory dysfunction. Psychopharmacology (Berl) 174, 143-50 (2004).

40. Nazar, M., Siemiatkowski, M., Czlonkowska, A., Sienkiewicz-Jarosz, H., and Plaznik, A. The role of the hippocampus and 5-HT/GABA interaction in the central effects of benzodiazepine receptor ligands. J Neural Transm 106, 369-81 (1999).

41. Geuze, E., Vermetten, E., and Bremner, J.D. MR-based in vivo hippocampal volumetrics: 2. Findings in neuropsychiatric disorders. Mol Psychiatry 10, 160-84 (2005).

42. Kitayama, N., Vaccarino, V., Kutner, M., Weiss, P., and Bremner, J.D. Magnetic resonance imaging (MRI) measurement of hippocampal volume in posttraumatic stress disorder: a meta-analysis. J Affect Disord 88, 79-86 (2005).

43. Nemeroff, C.B. et al. Posttraumatic stress disorder: A state-of-the-science review. J Psychiatr Res 40, 1-21 (2006).

44. Bremner, J.D. et al. Neural correlates of declarative memory for emotionally valenced words in women with posttraumatic stress disorder related to early childhood sexual abuse. Biol Psychiatry 53, 879-89 (2003).

45. Seabrook, G.R., Easter, A., Dawson, G.R., and Bowery, B.J. Modulation of long-term potentiation in CA1 region of mouse hippocampal brain slices by GABAA receptor benzodiazepine site ligands. Neuropharmacology 36, 823-30 (1997).

46. Bremner, J.D. et al. SPECT. Biol Psychiatry 47, 96-106 (2000).

47. Ichise, M., Meyer, J.H., and Yonekura, Y. An introduction to PET and SPECT neuroreceptor quantification models. J Nucl Med 42, 755-63 (2001).

6

Altered Pain Processing in Veterans with Posttraumatic Stress Disorder

Elbert Geuze, Herman G. M. Westenberg, Anja Jochims, Carien S. de Kloet, Martin Bohus, Eric Vermetten, and Christian Schmahl.

2006 **Archives of General Psychiatry**, *in press*

Introduction

Pain experience consists of a sensory-discriminative, an affective, and a cognitive component, which are mediated by different parts of the central nervous system. From a neuroanatomical point of view, the sensory-discriminative pathway of pain has been localized in the lateral nociceptive system (lateral thalamic nuclei, primary and secondary somatosensory cortex). The affective component of pain can anatomically be connected with the medial nociceptive system (medial thalamic nuclei, insula, and anterior cingulate cortex (ACC), whereas the cognitive component can be localized in the prefrontal cortex.[1] Imaging studies using functional magnetic resonance or positron emission tomography have confirmed the somatosensory cortex, ACC, limbic cortex, insular cortex, and prefrontal cortex as part of the neural circuitry of pain. [2-8]

PTSD is a chronic anxiety disorder which may occur in individuals exposed to a traumatic event, and is characterized by re-experiencing of the event, avoidance of stimuli related to the event, and chronic arousal. Clinical studies have reported that pain experience in persons with PTSD is significantly increased compared to controls, and that chronic pain is a commonly reported symptom of patients with PTSD.[9] [10;11]However, previous empirical research has also reported that patients with PTSD report a decrease in pain intensity ratings after being exposed to traumatic reminders. [12] This has been purported to be related to opioid mediated stress induced analgesia.[13] Activation of the μ-opioid receptor system by endogenous opioid peptides has indeed been associated with reductions in sensory and affective ratings of pain experience. [14;15]

Despite the increase in the numbers of neuroimaging studies of pain, as of yet, no functional imaging study has explored neural correlates of pain processing in patients with PTSD. Recently, a functional neuroimaging study in borderline personality disorder (BPD), a related psychiatric condition, revealed an antinociceptive neural network including right amygdala, rostral ACC, and prefrontal cortex. [16] The current study used the same design (fMRI in combination with painful tonic phasic heat stimuli) to compare the brain activity in patients with PTSD and controls. Both fixed temperature heat stimuli, which were the same for all subjects, and individual temperature heat stimuli, which were adjusted for equal subjective pain in all subjects, were used. We predicted that patients with PTSD would display altered activity in brain areas related to pain processing.

Methods

Subjects

Twelve male Dutch veterans with PTSD, and twelve male Dutch veterans without PTSD, were recruited. PTSD patients were recruited from the Department of Military Psychiatry at the Central Military Hospital in Utrecht. Control subjects were recruited via direct mail to veterans who were registered at the Veterans Institute in the Netherlands. All participants were veterans who had served in UN peacekeeping missions in Lebanon, Cambodia, and Bosnia. Control veterans were matched to the patient group with respect to age, handedness, year of deployment, and country of deployment.

PTSD was diagnosed using DSM-IV criteria, and confirmed using the Clinician Administered PTSD Scale (CAPS)[17] and through consensus with three clinicians (EG, CdK, EV). Only patients with CAPS scores > 50 were included in the study. Comorbid disorders were examined utilizing the Structured Clinical Interview for DSM-IV (SCID).[18] Control subjects were also assessed with both the SCID and the CAPS. All control subjects fulfilled DSM-IV criterion A1 (i.e. they had all experienced a traumatic event). All subjects received a physical examination by a physician. Subjects were excluded if they had any clinical significant abnormality of a clinical laboratory test, a history of psychiatric illness (controls only) or neurological dysfunction (all subjects), a history of alcohol and/or drug abuse (DSM-IV criteria) within six months prior to the study, or claustrophobia. None of the veterans included were physically injured at the time of deployment. All of the participants were free of psychotropic and analgesic medication for a period of four weeks prior to entering the study. Written informed consent of all subjects was obtained from all subjects who participated in the study after a complete written and verbal description of the study. This study was approved by the Institutional Review Boards of the University Medical Centre of Utrecht, the Netherlands, and the Central Institute of Mental Health, Mannheim, Germany.

Table 1: Subject Demographics and Psychometric Data

	PTSD	*Controls*
N	12	12
Age	34.50 (6.02)	33.26 (4.24) ^{ns}
Year of deployment (range 1980-2002)	1994 (5.90)	1994 (6.90) ^{ns}
Country of deployment	Bosnia (n = 8)	Bosnia (n = 8)
	Lebanon (n = 2)	Lebanon (n =2)
	Cambodia (n = 1)	Cambodia (n = 1)
	Afghanistan (n = 1)	Afghanistan (n = 1)
Edinburgh Handedness Inventory	+85 (29)	+88 (26) ^{ns}
DSS pre fMRI	10.58 (11.58)	1.25 (2.98)*
DSS post fMRI	14.08 (14.59)	0.58 (1.44)**
Aversive inner tension pre fMRI	2.42 (1.92)	0.67 (0.88)*
Aversive inner tension post fMRI	1.92 (2.60)	0.42 (1.16) ^{ns}

Means for both groups are given. Standard deviations are reported in between brackets. Edinburgh Handedness scores range from -100 (extreme left handedness) to +100 (extreme right handedness). DSS and aversive inner tension scores are given immediately prior to and after fMRI. ^{ns} not significant, * this difference was significant $p<0.05$, two tailed t-test; **this difference was significant $p<0.001$, two tailed t-test

Experimental Procedure

The experimental procedure followed a procedure first used by Schmahl et al [16] and consisted of a psychophysical assessment, performed outside the scanner prior to the scanning procedure, and a neuroimaging session.

Part I: Psychophysical Assessment

After a verbal introductory instruction phase, the subjective pain intensity of various painful stimuli was assessed using the Thermal Sensory Analyzer II (TSA from Medoc, Israel) as a device to induce thermal stimuli. The TSA-II NeuroSensory Analyzer is a precise, computer-controlled device capable of generating and recording a response to highly repeatable thermal stimulus, i.e., warmth, cold, heat-induced pain or cold-induced pain. The TSA-II utilizes a device called a thermode (3 × 3 cm) which is placed on the patient's skin, and is capable of heating or cooling the skin as needed (at a rate of 4 °C/s). The baseline temperature is approximately 35 °C. The thermal sensory analyzer is widely used in pain research.[19-22]

Before fMRI, the temperature which was estimated by all participants to have equal pain intensity was assessed. Temperatures between 40 and 48 °C were applied in blocks of 30 seconds in length with one-minute intervals on the dorsal right hand of each subject. These temperatures oscillated with an amplitude of 2 °C to avoid adaptation. Five stimulus temperatures (40, 42, 44, 46, and 48 °C) were applied consecutively in increasing and decreasing order. The full protocol consisted of 20 heat stimuli (5 stimuli, 4 repetitions). Subjective pain estimates were given by the participants immediately after the temperature blocks, using a numeric rating scale (NRS) ranging from 0 (no pain at all) to 100 (worst imaginable pain). These values were used to calculate the temperature equalling a subjective pain intensity of 40, using regression analysis.

Part II: Neuroimaging Using fMRI

Part II of the experiment was performed on a 1.5 Tesla Scanner (Magnetom Vision, Siemens, Erlangen, Germany). Functional data was assessed using a functional echoplanar series (EPI; 25 contiguous transversal slices; thickness, 5 mm; field of view, 220 x 220 mm^2; matrix, 64 x 64 pixels; slice acquisition time, 107 ms; volume acquisition time, 2675 ms; repetition time, 4175 ms). During acquisition of the functional data sets, 10 blocks 30 seconds in length were applied either with the temperature equalling the subjective pain intensity of 40 on the NRS (individual temperature) or a fixed temperature of 43 °C (see Figure 1, page 229). Interspersed between these blocks were one minute intervals with a baseline temperature of 35 °C. Subjects were asked for a pain rating on a NRS of 0 to 100 after the first post stimulation scan and prior to the baseline scan. Thermal stimuli were applied to the dorsal right hand using the TSA-II with a thermode designed for use in the MR imaging room. After the functional data was acquired, a high-resolution anatomical series using MPRAGE (3D magnetization prepared rapid acquisition gradient echo) with a voxel size of 1 x 1 x 1

mm^3 was performed. This series was used as an individual template for coregistration of functional and anatomical data and for spatial standardization into the stereotactic system of Talairach and Tournoux (1988). Immediately prior to and after the fMRI data acquisition, a dissociative state questionnaire (DSS)[23] was given to all participants. This questionnaire consists of twenty items measuring dissociation, and one item measuring aversive inner tension.

Data Analysis

All the image data preparation and preprocessing steps as well as statistical analyses and the map volumetric projection were performed in Brain Voyager QX 1.6 (Brain Innovation, Maastricht, the Netherlands). The first two scans were excluded from data analysis. Three dimensional data preprocessing included slicescan time correction (using sinc interpolation), linear trend removal, temporal high-pass filtering to remove low-frequency non-linear drifts of 3 or fewer cycles per time course, and 3D motion correction to detect and correct for small head movements by spatial alignment of all volumes to the first volume by rigid body transformations. Estimated translation and rotation parameters were inspected and never exceeded 3 mm. Functional data were smoothed with a 4 mm full width half maximum (FWHM) Gaussian kernel. Co-registration of functional and 3-D structural measurements was computed by relating T2*-weighted images and the T1-weighted 3-D MP RAGE measurement, which yields a 4-D functional data set. Structural 3-D and functional 4-D data sets were transformed into the standard space corresponding to the atlas of Talairach and Tournoux. [24]
The stimulation protocol was convoluted with a hemodynamic response function [25] to account for the expected delay and generic shape of the BOLD signal. In order to correct for multiple comparisons, the false discovery rate (FDR) controlling procedure was applied on the resulting p values for all voxels. The value of q specifying the maximum FDR tolerated on average was set to 0.01. With this value, a single-voxel threshold is chosen by the FDR procedure, which ensures that from all voxels shown as active, only 1% or less are false-positives. [26;27] Brain areas responding to the fixed and the individual temperature conditions were identified by the temperature-specific main effects in the general linear model (heat vs. baseline) and were corrected for serial correlations.[16] Voxel level and region of interest (ROI) level inter-group linear contrasts were computed using two-tailed t-tests. Three-dimensional statistical maps were overlaid on the Talairach-transformed Montreal Neurological Institute T1-weighted brain template (http://www.bic.mni.mcgill.ca).
Due to the sample size and the relatively high variability of the population compared to the signal-to-noise ratio of the measurements, inter-individual variance was not accounted for in the voxel-level group analysis, and all contrast maps were computed using a fixed-effects model.[28] In order to extend our inferences and results to the clinical population, significantly activated clusters of 200 voxels in the first level inter-group voxel-level analysis were selected for a more sensitive second level ROI analysis using a random effects model in the two sample t-tests.

Results

Psychometric Data

PTSD patients and control veterans were matched with respect to age (34.50 ± 6.02 vs 33.26 ± 4.24, ns). Patients with PTSD had significantly greater CAPS, Hamilton Anxiety, and Hamilton Depression scores (see Table 1). According to the SCID, the PTSD group met lifetime (past) DSM-IV (APA 1994) diagnostic criteria for major depressive disorder (n = 2), bipolar disorder (n = 2), alcohol abuse (n = 4), alcohol dependence (n = 2), substance abuse (n = 2), substance dependence (n = 1), and panic disorder without agoraphobia (n = 2). Only one subject with PTSD met current diagnostic criteria for panic disorder. Among our control subjects, the SCID did not reveal any current or lifetime psychiatric disorders. None of the subjects had a pain disorder, or somatization disorder as determined by the SCID. In addition, none of the subjects reported the presence of current or chronic pain.

Psychophysics

When plotted in linear coordinates (see Figure 1a), the mean ratings given were positively accelerating functions of heat temperature. Patients with PTSD rated the objectively equal temperatures as subjectively less painful (see Figure 2a, page 230). The fixed temperature of 43°C was rated as a mean of 10.5 ± 7.0 (mean ± SD) by patients, compared to 23.9 ± 12.7 by controls (T = 3.20, df = 22, $p < 0.005$, two tailed t-test). Consequently, the temperature that corresponded to a NRS rating of 40 was on average 46.82 ± 1.03 °C for patients compared to an average of 45.35 ± 1.73 °C for controls (T = 2.53, df = 22, $p < 0.05$, two tailed t-test). This temperature weakly correlated with the CAPS total score ($r=0.438$, $p<0.05$) and the Hamilton Anxiety scores ($r = 0.383$, $p < 0.05$), but not with the Hamilton Depression scores ($r = 0.333$, $p > 0.05$). All subjects were able to discriminate between different pain intensities (range of individual Pearson's r, 0.707 to 0.993, $p < 0.05$). Patients with PTSD showed a significantly reduced offset of the stimulus-response function (i.e. the stimulus response function of the patients is shifted to the right with respect to the controls; y-intercept at the mean stimulus temperature of 44°C, patients: 16.77 ± 10.07, controls 32.06 ± 15.42; T = 2.87, df = 22, $p < 0.01$, two tailed t-test). When plotted in log-log coordinates (see Figure 2b, page 231), the functions were fit by linear regression lines. Correlation coefficients (Pearson r) computed for these regressions of mean ratings against stimulus temperatures revealed a highly significant effect of temperature on pain ratings in both groups (patients: $r = 0.832$, $p < 0.05$ and controls: $r = 0.978$, $p < 0.01$). The linearity of these functions in double logarithmic coordinates indicates that the relationships between the scaling responses and stimulus temperature intensity (minus baseline temperature of 34°C) can be described by the power function ($f(x) = k(t-34)^x$; where k is a constant, t is the stimulus temperature in °C, and x is the heat pain exponent). [29;30] Pain heat exponents were obtained by computing the slope of the regression lines (in double logarithmic coordinates) and were not significantly different (patients: 4.03 ± 1.37 and controls: 3.07 ± 0.92; T = 1.40, df = 22, $p > 0.05$).

Table 2: Main effects of the fixed temperature (43 °C) and the individual temperature (NRS 40) condition in veterans with PTSD, and control veterans without PTSD.

	Fixed temperature condition (43 °C)								*Individual temperature condition*							
	Controls				*PTSD*				*Controls*				*PTSD*			
Region	*x*	*y*	*z*	*Z-score*	*x*	*y*	*z*	*Z-score*	*x*	*y*	*z*	*Z-score*	*x*	*y*	*z*	*Z-score*
Right Anterior Cingulate Gyrus (peri)	11	35	8	8.02	13	35	8	10.22								
Right Anterior Cingulate Gyrus (mid)	8	19	42	8.70	4	24	42	7.56	8	20	43	10.53	6	18	42	10.38
Left Anterior Cingulate Gyrus (mid)	-3	19	41	8.73	-6	24	43	8.12	-2	20	42	9.93	-6	14	42	10.26
Left Anterior Cingulate Gyrus (peri)	-11	34	8	6.27	-11	36	9	10.26								
Left Posterior Cingulate Gyrus	-6	-46	17	-7.13	-1	-54	23	-7.72	-5	-52	17	-6.93	-2	-51	18	-7.15
Left Cingulate Gyrus	-3	-47	27	-5.17	-1	-44	27	-6.90	-6	-44	28	-5.10	-5	-44	28	-7.49
Right Precuneus					2	-48	31	-6.46					4	-48	31	-7.43
Left Precuneus	-3	-47	32	-5.72	-3	-47	31	-6.73	-1	-53	31	-6.95	-9	-47	31	-7.52
Right Posterior Cingulate Gyrus	3	-47	17	-7.02	4	-42	25	-6.90	7	-50	17	-5.25	1	-50	18	-7.15
Right Posterior Parietal Cortex	38	-54	35	7.27	41	-56	36	6.45	36	-53	35	8.41	38	-54	35	11.37
Left Posterior Parietal Cortex	-42	-48	40	5.93	-42	-50	39	5.78	-44	-47	36	6.63	-40	-55	35	9.19
Right Precentral Gyrus	45	-15	41	-8.92					47	-14	41	-6.31				
Right Postcentral Gyrus	47	-16	44	-8.92	48	-17	44	-5.58	38	-21	47	-5.59	38	-23	47	-5.02
Right Middle Frontal Gyrus BA9	46	13	28	7.49	39	35	19	8.91	42	14	34	8.48	47	13	28	12.52
Right Middle Frontal Gyrus BA6									29	46	0	6.33				
Left Middle Frontal Gyrus	-29	28	28	6.61	-35	23	28	7.06	-30	27	28	5.83	-29	30	28	6.95
Right Inferior Frontal Gyrus	41	43	3	9.11	43	45	4	9.63	42	43	3	10.94	41	43	1	10.82
Right Inferior Frontal Gyrus	49	15	3	8.73	34	26	1	7.10	49	13	3	10.03	49	14	4	11.25
Right Superior Temporal Gyrus	53	-1	5	6.07	52	-3	4	4.23	53	-3	5	7.35	53	-3	6	10.97
Left Superior Temporal Gyrus					-51	-6	5	6.08	-49	-2	6	7.06	-49	0	7	12.66
Left Middle Temporal Gyrus	-48	-57	11	-7.47	-48	-66	11	-4.57	-47	-55	12	-8.11				
Left Insula	-40	15	1	8.68	-30	21	9	5.65	-40	15	1	12.14	-40	13	1	10.90
Right Insula	33	17	10	7.87	37	16	2	5.99	38	15	1	12.03	32	16	1	13.25
Left Medial Thalamus													-6	-15	0	6.63
Right Medial Thalamus									2	-15	-1	5.57	6	-15	1	5.04
Left Claustrum	-29	16	4	7.90	-29	16	3	6.09					-28	15	3	10.73
Right Amygdala	23	-8	-11	5.24	25	-11	-11	-3.80					25	-10	-15	-4.50
Left Hippocampus	-29	-11	-19	-4.47	-25	-12	-19	7.41					-27	-16	-20	4.70
Left Putamen									-17	4	4	5.70	-17	4	5	7.45
Right Putamen					18	2	6	5.48					30	-8	-2	9.44

These exponents are similar to those found in other heat-pain studies and indicate that the rate of change in the pain rating is not significantly different between the groups. [29;31] During scanning, the subjects were also asked to give pain ratings after each 30s block. Again the fixed temperature of 43°C during scanning was rated as a mean of 5.0 ± 5.5 on the NRS by patients, compared to a mean of 30.8 ± 20.9 by control subjects, which was significantly different (T = 4.13, df = 22, p < 0.001, two tailed t-test). During fMRI scanning the mean pain intensity for the individual temperature was rated as 39.9 ± 19.6 by patients and 49.9 ± 18.1 by controls (T = 1.31, df = 22, p > 0.05, two tailed t-test).

fMRI Psychometrics

Patients with PTSD experienced significantly more aversive inner tension 2.4 ± 1.9 compared to 0.7 ± 0.9 prior to scanning (T = 2.86, df = 22, p < 0.05) (scores range from 0 to 9). After scanning, aversive inner tension was not significantly different. Patients experienced significantly more dissociative symptoms prior to and after fMRI (10.6 ± 11.6 and 14.1 ± 14.6 in patients compared to 1.3 ± 3.0 and 0.6 ± 1.4 in controls; T = 2.70, df = 22, p < 0.05; and T = 3.19, df = 22 p < 0.01). However, the difference between levels of dissociative symptoms after fMRI scanning compared to levels of dissociative symptoms before scanning was not significantly different between patients (3.5 ± 16.9) and controls (-0.7 ± 1.6; T = 0.85, df = 22, p > 0.05).

fMRI Main Effects

In a first analysis step, activations for the main effects of the two conditions were investigated. Activations were thresholded at [q(FDR) ≤ 0.01] (see Table 2). In the patient group, solid activations were seen in the bilateral ventrolateral prefrontal cortex (vlPFC), dorsolateral prefrontal cortex (dlPFC), medial prefrontal cortex (mPFC), parietal cortex, ACC, left claustrum, left hippocampus, and right putamen in the fixed temperature condition, as well as increased activity in the left and right superior temporal gyri, bilateral insular cortex, and a region of reduced activity in the bilateral precuneus, posterior cingulate cortex (PCC), right somatosensory cortex, and right amygdala. A similar pattern of activity was seen in the individual temperature condition. In the control group in the fixed temperature condition, significant activations were seen in the bilateral PFC, parietal cortex, ACC, insular cortex, left claustrum, and right amygdala, as well as regions of signal decrease in the left precuneus, bilateral PCC, right pre- and post-central gyri, and left amygdala. In the individual temperature condition this pattern of activity was more pronounced. In this condition the right medial thalamus and the left putamen were also significantly activated in controls.

fMRI Group Analysis

In the group comparison patients displayed altered activity in the vlPFC, insula, precentral gyrus, putamen, amygdala, and the hippocampus (see Table 3). In the fixed temperature condition, patients

with PTSD revealed more activity in the left hippocampus (t < 0 in 2 patients and 9 controls; t > 0 in 10 patients and 3 controls; $t_{patients}$, 2.08 ± (2.21) [mean ± SD], $t_{controls}$, -0.91 ± 1.78; randomeffects analysis, p <0.001; see Figure 3a, page 232). In addition, patients with PTSD displayed less activity in the bilateral ventrolateral prefrontal cortex (left vlPFC: t < 0 in 8 patients and 2 controls; t > 0 in 4 patients and 10 controls; $t_{patients}$, -0.96 ± 2.25, $t_{controls}$, 1.76 ± 2.30; random effects analysis, p < 0.01; and right vlPFC: t < 0 in 8 patients and 2 controls; t > 0 in 4 patients and 10 controls; $t_{patients}$, -0.71 ± 2.63, $t_{controls}$, 1.95 ± 1.99; random effects analysis, p < 0.01; see Figure 3b, page 233). Compared to controls, patients with PTSD also displayed a signal decrease in the right amygdala (t < 0 in 9 patients and 2 controls; t > 0 in 3 patients and 10 controls; $t_{patients}$, -1.18 ± 1.81, $t_{controls}$, 0.83 ± 1.54; random effects analysis, p < 0.01; see Figure 3a, page 232). In the individual temperature condition, patients with PTSD revealed more activity in the right putamen (t < 0 in 1 patient and 5 controls; t > 0 in 11 patients and 7 controls; $t_{patients}$, 2.33 ± 1.80, $t_{controls}$, -0.00 ± 2.29; random effects analysis, p < 0.01). A similar pattern of more activity in patients was found in the bilateral anterior insula (left anterior insula: t < 0 in 1 patient and 4 controls; t > 0 in 11 patients and 8 controls; $t_{patients}$, 2.77 ± 2.15, $t_{controls}$, 0.72 ± 1.17; random effects analysis, p < 0.01; and right anterior insula: t < 0 in 2 controls; t > 0 in all patients and 10 controls; $t_{patients}$, 3.80 ± 2.04, $t_{controls}$, 1.62 ± 1.76; random effects analysis, p < 0.01; see Figure 4, page 234). In the right precentral gyrus patients with PTSD also displayed more activity (t < 0 in 2 patients and 7 controls; t > 0 in 10 patients and 5 controls; $t_{patients}$, 1.40 ± 1.47, $t_{controls}$, -0.60 ± 1.61; random effects analysis, p < 0.01). Patients with PTSD continued to display significantly less right amygdala activity in the individual temperature condition (t < 0 in 9 patients and 4 controls; t > 0 in 3 patients and 8 controls; $t_{patients}$, -1.37 ± 1.65, $t_{controls}$, 0.51 ± 1.42; random effects analysis, p < 0.01; see Figure 4, page 234).

Table 3: Regions of interest with significant group differences for the two conditions

	Fixed temperature condition (43 °C)				*Individual temperature condition*			
Region	*x*	*y*	*z*	*Z-score*	*x*	*y*	*z*	*Z-score*
Right vlPFC	31	40	4	-6.47				
Left vlPFC	-36	30	9	-6.69				
Right Precentral Gyrus					52	-6	38	4.80
Right Anterior Insula					30	16	13	5.37
Left Anterior Insula					-35	19	13	5.07
Right Putamen					30	-9	-2	5.74
Right Amygdala	27	-8	-13	-4.91	23	-8	-14	-4.57
Left Hippocampus	-28	-12	-19	7.39				

Discussion

To our knowledge this is the first functional MRI study which investigates pain processing in patients with PTSD. Patients with PTSD showed a shifted stimulus response function to heat stimuli. Prior to fMRI patients with PTSD already showed a significant reduction in pain sensitivity. While in the scanner patients with PTSD rated a fixed temperature as significantly less painful than control veterans. This contrasts with previous reports of increased pain sensitivity and more subjective complaints in patients with PTSD. [9-11]In the fixed temperature condition, patients with PTSD revealed increased activation in the left hippocampus, and decreased activation in the bilateral vlPFC, and the right amygdala compared to controls. During stimulation with an individually adjusted temperature, patients with PTSD showed increased activation in the right putamen and bilateral insula, as well as decreased activity in right precentral gyrus, and the right amygdala.

The reduced right amygdala activation is a prominent finding that was displayed by veterans with PTSD in both the fixed temperature and the individual temperature condition. The amygdala integrates nociceptive information and plays a dual facilitatory and inhibitory role in the modulation of emotional pain behaviour.[32] The amygdala is an important brain structure involved in the processing of analgesia information and has been hypothesized to play a role in opioid antinociception. [33] Prolonging the anticipated duration of a nociceptive stimulus also results in deactivation of the amygdala, and may reflect cognitive modulation of the aversiveness of the heat stimulus. [34;35] Reduced right amygdala activity was also displayed by patients with borderline personality disorder (BPD) in an earlier study on neural correlates of pain processing in BPD by Schmahl et al.[16] PTSD and BPD may be seen along a trauma spectrum perspective, since both show similar underlying etiology and several overlapping symptoms. [36] Both BPD and PTSD symptoms tap into neural circuitry involved in emotional regulation such as the hippocampus, orbitofrontal cortex, and the amygdala. [37;38] Though traumatic experience is involved in both disorders, BPD involves developmental or acquired brain dysfunction possibly associated with early traumatic experience in a majority of patients, whereas in PTSD patients (especially in veterans) trauma usually occurs later in life.[39]

Negative blood oxygenation level dependent (BOLD) responses should be interpreted with caution, as this may reflect either a reduction or suppression of neuronal activity resulting in decreased cerebral blood flow,[40;41] or a hemodynamic effect in which blood is diverted or allocated to the most active areas while adjacent areas reveal reduced blood flow.[42;43] However in our case it was not likely that this negative BOLD response was induced by a reallocation of blood flow from the PCC or the amygdala to adjacent areas (there were no adjacent areas that exhibited a significantly increased cerebral blood flow) thus a reduction of neuronal activity in these areas (when compared to baseline activity) is a more plausible explanation. Other studies have also found a decrease in these areas after application of noxious stimuli, and these signal decreases were not unexpected.[16;44-46]

In the individual temperature condition each subject received a stimulus which corresponded with a score of 40 on the NRS. In this condition the affective value given to the stimulus was similar in

both groups. Pain ratings that were taken during the scanning session for the individual condition were slightly higher than prior to fMRI for both patients and controls. However, there were no significant differences between the affective ratings given by patients compared to controls during fMRI, showing that both groups attributed the same affective label to stimuli in this condition. In the individual temperature condition, patients with PTSD revealed increased activity in the bilateral insula compared to controls. Activity of the insula in pain research is common and usually related to discrimination of stimulus intensity and emotional processing.[47-51] Bilateral anterior insular cortex activation is also related to cognitive evaluation of pain intensity.[52] Perhaps the observed increase in activity in patients is related to a similar mechanism.

We had also expected that the rostral ACC which plays an important role in affective pain processing [6;53] would be less active in patients because patients consistently rated the fixed temperature as less painful. However it may be that we did not have sufficient power to differentiate between patients and controls in this area, as in both groups the ACC was highly active compared to baseline activity. Cognitive attention given to other stimuli (e.g. other sounds in the MR room) by patients could lead to reduced pain perception, while ACC activity would still be present.[6]

In the fixed temperature condition (43 °C), veterans with PTSD displayed reduced activation in the bilateral vlPFC and the right amygdala as well as increased activation in the hippocampus. Reduced activation of the vlPFC is consistent with previous functional imaging research which has also found decreased neural activity in the prefrontal cortex of patients with PTSD.[54-60] Activation of prefrontal regions is usually attributed to mediation of the cognitive dimension of pain processing associated with localization and encoding of the attended stimulus.[47;53] Previous research has indicated that the vlPFC is activated during cutaneous painful heat stimulation. [61] The reduced activity in the vLPFC found in patients with PTSD may be related to the reduced cognitive pain processing during the fixed temperature condition. However, the reduced activity of the vlPFC is observed in the context of lower pain intensity reports by veterans with PTSD, which may also explain this particular finding.

In patients with PTSD the hippocampus, which plays an important role in the regulation of the hypothalamo-pituitary-adrenal (HPA) axis, is both structurally and functionally abnormal. Patients have shown reduction of hippocampal volume and displayed altered activity in the hippocampal area in a variety of paradigms. [58;62-65] The hippocampal role in pain processing is usually attributed to the intensity of nociceptive stimulation.[6] That patients show a net signal increase in this area during the fixed temperature condition cannot be related to stimulus intensity, as this was the same in both groups. Other hippocampal functions such as memory encoding, memory retrieval, novelty detection, or contextual conditioning are a more probable explanation for the increased activity observed in patients. Glucocorticoid receptors in the hippocampus are activated by rising glucocorticoid levels during stress, in order to mediate fast feedback inhibition of the HPA axis. It is also plausible that the increased BOLD response in the left hippocampus (which extended into the parahippocampal gyrus) witnessed in our patients reflects this activity. The net signal decrease in the left hippocampus displayed by controls has been previously reported, and is thought to pertain to the affective response to pain.[46;53]

The fMRI results need to be interpreted in the light of an altered stimulus response to heat stimuli. A mechanism which may be involved in this shift is stress induced analgesia. Earlier studies have demonstrated that patients with PTSD reveal opioid mediated stress induced analgesia after watching a stressful combat video.[12;13] Activation of the μ-opioid receptor system by endogenous opioid peptides is associated with reductions in sensory and affective ratings of pain experience. [14;15] High levels of opioid receptor binding are seen in the ACC, prefrontal cortex, caudate nucleus, putamen, amygdala, and the insular cortex.[66-69] Several pain regions which have shown an altered response in PTSD in our study, most notably the vlPFC, amygdala, and insular cortex, are also involved in the endogenous opioid modulation of sensory and affective pain elements. [14;15] In our study patients with PTSD experienced significantly more aversive inner tension during the fMRI scanning session than controls. However, whether the stress of the psychophysical examination prior to fMRI and the stress experienced during fMRI scanning was enough to induce opioid-mediated analgesia needs to be investigated.

The finding of a signal decrease in the precentral gyrus ipsilateral to stimulus presentation in both groups is more difficult to interpret, although this phenomenon has been shown before.[53] It may be speculated that it is related to an inhibition of movement, or a contrast-enhancing mechanism such as anticipation or stimulus repetition.[53] In addition both groups showed reduced activity in the precuneus and PCC compared to baseline in both conditions.

The pain model utilized in this study is phasic thermal heat pain and may not necessarily be related to clinical pain conditions. Nevertheless, it is a useful probe for the examination of pain regulation mechanisms in PTSD. Future research should extend these findings to patients with PTSD related to civilian traumas. The use of trauma controls in this study allows us to differentiate between these groups based on the absence and presence of PTSD only. The addition of a third control group consisting of healthy subjects who have not been subjected to trauma would allow us to observe differences in pain processing related to the effect of trauma or (former) occupation (all subjects were veterans). Although the number of subjects in this study was sufficient to permit random effects analysis and allow generalization of these results to the clinical population, future research should include more subjects to enable sub-group analyses.To our knowledge, this is the first study that used fMRI to differentiate pain processing in patients with PTSD compared to trauma controls. Compared to controls, veterans with PTSD revealed an analgesic response when subjected to heat stimuli. Patients with PTSD showed altered pain processing in brain areas associated with affective and cognitive pain processing, such as the ventrolateral prefrontal cortex, the insula, the hippocampus, and the amygdala. It is proposed that the neural pattern with decreased activity in the right amygdala and the bilateral vlPFC reflects altered pain regulation mechanisms in patients with PTSD.

References

1. Treede, R.D., Kenshalo, D.R., Gracely, R.H., and Jones, A.K. The cortical representation of pain. Pain 79, 105-11 (1999).
2. Brooks, J.C., Zambreanu, L., Godinez, A., Craig, A.D., and Tracey, I. Somatotopic organisation of the human insula to painful heat studied with high resolution functional imaging. Neuroimage (2005).
3. Casey K.L. and Minoshima S. Can pain be imaged? . *Proceedings of the 8th World Congress on Pain, Progress in Pain Research and Management* 8, 855-866. 97.
4. Davis, K.D. The neural circuitry of pain as explored with functional MRI. Neurol Res 22, 313-7 (2000).
5. Ingvar, M. Pain and functional imaging. Philos Trans R Soc Lond B Biol Sci 354, 1347-58 (1999).
6. Bantick, S.J. *et al.* Imaging how attention modulates pain in humans using functional MRI. Brain 125, 310-9 (2002).
7. Singer, T. *et al.* Empathy for pain involves the affective but not sensory components of pain. Science 303, 1157-62 (2004).
8. Talbot, J.D. *et al.* Multiple representations of pain in human cerebral cortex. Science 251, 1355-8 (1991).
9. Smith, M.Y., Egert, J., Winkel, G., and Jacobson, J. The impact of PTSD on pain experience in persons with HIV/AIDS. Pain 98, 9-17 (2002).
10. Asmundson, G.J., Coons, M.J., Taylor, S., and Katz, J. PTSD and the experience of pain: research and clinical implications of shared vulnerability and mutual maintenance models. Can J Psychiatry 47, 930-7 (2002).
11. Beckham, J.C. *et al.* Chronic posttraumatic stress disorder and chronic pain in Vietnam combat veterans. J Psychosom Res 43, 379-89 (1997).
12. Pitman, R.K., van der Kolk, B.A., Orr, S.P., and Greenberg, M.S. Naloxone-reversible analgesic response to combat-related stimuli in posttraumatic stress disorder. A pilot study. Arch Gen Psychiatry 47, 541-4 (1990).
13. van der Kolk, B.A., Greenberg, M.S., Orr, S.P., and Pitman, R.K. Endogenous opioids, stress induced analgesia, and posttraumatic stress disorder. Psychopharmacol Bull 25, 417-21 (1989).
14. Zubieta, J.K. *et al.* Regulation of human affective responses by anterior cingulate and limbic mu-opioid neurotransmission. Arch Gen Psychiatry 60, 1145-53 (2003).
15. Zubieta, J.K. *et al.* Regional mu opioid receptor regulation of sensory and affective dimensions of pain. Science 293, 311-5 (2001).
16. Schmahl, C.S. *et al.* Neural Correlates of Antinociception in Borderline Personality Disorder. Archives of General Psychiatry in press, (2006).
17. Blake, D.D. *et al.* The development of a Clinician-Administered PTSD Scale. J Trauma Stress 8, 75-90 (1995).
18. First, M. B., Spitzer, R. L., Gibbon, M., and Williams, J. B. W. Structured Clinical Interview for DSM-IV Axis I Disorders. SCID-I/P. 97.
19. Davis, K.D., Pope, G.E., Crawley, A.P., and Mikulis, D.J. Neural correlates of prickle sensation: a percept-related fMRI study. Nat Neurosci 5, 1121-2 (2002).
20. Koyama, Y., Koyama, T., Kroncke, A.P., and Coghill, R.C. Effects of stimulus duration on heat induced pain: the relationship between real-time and post-stimulus pain ratings. Pain 107, 256-66 (2004).
21. Koyama, T., McHaffie, J.G., Laurienti, P.J., and Coghill, R.C. The single-epoch fMRI design: validation of a simplified paradigm for the collection of subjective ratings. Neuroimage 19, 976-87 (2003).
22. Ness, T.J., Fillingim, R.B., Randich, A., Backensto, E.M., and Faught, E. Low intensity vagal nerve stimulation lowers human thermal pain thresholds. Pain 86, 81-5 (2000).
23. Stiglmayr, C., Braakmann, D., Haaf, B., Stieglitz, R., and Bohus, M. Entwicklung und psychometrische Charakterisierung der Dissozaitions-Spannungs-Skala akut (DSS-akut). Psychotheraphie, Psychiatrie und Medizinische Psychologie 53, 287-94 (2003).
24. Talairach J. and P. Tournoux. Coplanar Stereotaxic Atlas of the Human Brain. 3-Dimensional Proportional System: An Approach to Cerebral Imaging. Thieme Medical, New York (1988).
25. Boynton, G.M., Engel, S.A., Glover, G.H., and Heeger, D.J. Linear systems analysis of functional magnetic resonance imaging in human V1. J Neurosci 16, 4207-21 (1996).
26. Benjamini, Y. and Hochberg, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc., B 57, 289-300 (1995).
27. Genovese, C.R., Lazar, N.A., and Nichols, T. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. Neuroimage 15, 870-8 (2002).
28. Friston, K.J., Holmes, A.P., and Worsley, K.J. How many subjects constitute a study? Neuroimage 10, 1-5 (1999).
29. Price, D.D., Bush, F.M., Long, S., and Harkins, S.W. A comparison of pain measurement characteristics of mechanical visual analogue and simple numerical rating scales. Pain 56, 217-26 (1994).
30. Price, D.D., McGrath, P.A., Rafii, A., and Buckingham, B. The validation of visual analogue scales as ratio scale measures for chronic and experimental pain. Pain 17, 45-56 (1983).
31. Coghill, R.C., Mayer, D.J., and Price, D.D. Wide dynamic range but not nociceptive-specific neurons encode multidimensional features of prolonged repetitive heat pain. J Neurophysiol 69, 703-16 (1993).
32. Neugebauer, V., Li, W., Bird, G.C., and Han, J.S. The amygdala and persistent pain. Neuroscientist 10, 221-34 (2004).

33. Manning, B.H. A lateralized deficit in morphine antinociception after unilateral inactivation of the central amygdala. J Neurosci 18, 9453-70 (1998).

34. Simpson, J.R. Jr, Drevets, W.C., Snyder, A.Z., Gusnard, D.A., and Raichle, M.E. Emotion-induced changes in human medial prefrontal cortex: II. During anticipatory anxiety. Proc Natl Acad Sci U S A 98, 688-93 (2001).

35. Petrovic, P., Carlsson, K., Petersson, K.M., Hansson, P., and Ingvar, M. Context-dependent deactivation of the amygdala during pain. J Cogn Neurosci 16, 1289-301 (2004).

36. Bremner, J.D. Neurological Consequences of Stress as a Biological Basis for a Common Spectrum of Psychiatric Disorders. In Bremner, J.D. (ed.) Does Stress Damage The Brain. Norton, New York (2002).

37. Schmahl, C. and Bremner, J.D. Neuroimaging in borderline personality disorder. J Psychiatr Res (2005).

38. Nemeroff, C.B. *et al.* Posttraumatic stress disorder: A state-of-the-science review. J Psychiatr Res 40, 1-21 (2006).

39. Golier, J.A. *et al.* The relationship of borderline personality disorder to posttraumatic stress disorder and traumatic events. Am J Psychiatry 160, 2018-24 (2003).

40. Hutchinson, M. *et al.* Task-specific deactivation patterns in functional magnetic resonance imaging. Magn Reson Imaging 17, 1427-36 (1999).

41. Buckner, R.L., Koutstaal, W., Schacter, D.L., and Rosen, B.R. Functional MRI evidence for a role of frontal and inferior temporal cortex in amodal components of priming. Brain 123 Pt 3, 620-40 (2000).

42. Harel, N., Lee, S.P., Nagaoka, T., Kim, D.S., and Kim, S.G. Origin of negative blood oxygenation level-dependent fMRI signals. J Cereb Blood Flow Metab 22, 908-17 (2002).

43. Woolsey, T.A. *et al.* Neuronal units linked to microvascular modules in cerebral cortex: response elements for imaging the brain. Cereb Cortex 6, 647-60 (1996).

44. Coghill, R.C. *et al.* Distributed processing of pain and vibration by the human brain. J Neurosci 14, 4095-108 (1994).

45. Becerra, L.R. *et al.* Human brain activation under controlled thermal stimulation and habituation to noxious heat: an fMRI study. Magn Reson Med 41, 1044-57 (1999).

46. Derbyshire, S.W. *et al.* Pain processing during three levels of noxious stimulation produces differential patterns of central activity. Pain 73, 431-45 (1997).

47. Peyron, R., Laurent, B., and Garcia-Larrea, L. Functional imaging of brain responses to pain. A review and meta-analysis (2000). Neurophysiol Clin 30, 263-88 (2000).

48. Phillips, M.L. *et al.* A specific neural substrate for perceiving facial expressions of disgust. Nature 389, 495-8 (1997).

49. Craig, A.D., Chen, K., Bandy, D., and Reiman, E.M. Thermosensory activation of insular cortex. Nat Neurosci 3, 184-90 (2000).

50. Craig, A.D. How do you feel? Interoception: the sense of the physiological condition of the body. Nat Rev Neurosci 3, 655-66 (2002).

51. Phan, K.L., Wager, T.D., Taylor, S.F., and Liberzon, I. Functional neuroimaging studies of human emotions. CNS Spectr 9, 258-66 (2004).

52. Kong, J. *et al.* Using fMRI to dissociate sensory encoding from cognitive evaluation of heat pain intensity. Hum Brain Mapp (2005).

53. Peyron, R. *et al.* Haemodynamic brain responses to acute pain in humans: sensory and attentional networks. Brain 122 (Pt 9), 1765-80 (1999).

54. Liberzon, I., Britton, J.C., and Phan, K.L. Neural correlates of traumatic recall in posttraumatic stress disorder. Stress 6, 151-6 (2003).

55. Williams, L.M. *et al.* Trauma modulates amygdala and medial prefrontal responses to consciously attended fear. Neuroimage (2005).

56. Lanius, R.A. *et al.* Recall of emotional states in posttraumatic stress disorder: an fMRI investigation. Biol Psychiatry 53, 204-10 (2003).

57. Bremner, J.D. *et al.* Positron emission tomographic imaging of neural correlates of a fear acquisition and extinction paradigm in women with childhood sexual-abuse-related post-traumatic stress disorder. Psychol Med 35, 791-806 (2005).

58. Bremner, J.D. *et al.* Neural correlates of declarative memory for emotionally valenced words in women with posttraumatic stress disorder related to early childhood sexual abuse. Biol Psychiatry 53, 879-89 (2003).

59. Shin, L.M. *et al.* A functional magnetic resonance imaging study of amygdala and medial prefrontal cortex responses to overtly presented fearful faces in posttraumatic stress disorder. Arch Gen Psychiatry 62, 273-81 (2005).

60. Farrow, T.F. *et al.* Quantifiable change in functional brain response to empathic and forgivability judgments with resolution of posttraumatic stress disorder. Psychiatry Res (2005).

61. Strigo, I.A., Duncan, G.H., Boivin, M., and Bushnell, M.C. Differentiation of visceral and cutaneous pain in the human brain. J Neurophysiol 89, 3294-303 (2003).

62. Geuze, E., Vermetten, E., and Bremner, J.D. MR-based in vivo hippocampal volumetrics: 2. Findings in neuropsychiatric disorders. Mol Psychiatry 10, 160-84 (2005).

63. Neylan, T.C. *et al.* Attention, learning, and memory in posttraumatic stress disorder. J Trauma Stress 17, 41-6 (2004).

64. Schuff, N. *et al.* Decreased hippocampal N-acetylaspartate in the absence of atrophy in posttraumatic stress disorder. Biol Psychiatry 50, 952-9 (2001).

65. Sakamoto, H. *et al.* Parahippocampal activation evoked by masked traumatic images in posttraumatic stress disorder: a functional MRI study. Neuroimage 26, 813-21 (2005).

66. Delay-Goyet, P., Zajac, J.M., Javoy-Agid, F., Agid, Y., and Roques, B.P. Regional distribution of mu, delta and kappa opioid receptors in human brains from controls and parkinsonian subjects. Brain Res 414, 8-14 (1987).

67. Jones, A.K. *et al.* In vivo distribution of opioid receptors in man in relation to the cortical projections of the medial and lateral pain systems measured with positron emission tomography. Neurosci Lett 126, 25-8 (1991).

68. Jones, A.K., Watabe, H., Cunningham, V.J., and Jones, T. Cerebral decreases in opioid receptor binding in patients with central neuropathic pain measured by. Eur J Pain 8, 479-85 (2004). Notes: diprenorphine binding and PET.

69. Pilapil, C., Welner, S., Magnan, J., Zamir, N., and Quirion, R. Mu opioid receptor binding sites in human brain. NIDA Res Monogr 75, 319-22 (1986).

7

Neural Correlates of Associative Learning and Memory in Veterans with Posttraumatic Stress Disorder

Elbert Geuze, Eric Vermetten, Matthias Ruf, Carien S. de Kloet, and Herman G. M. Westenberg. **Submitted.**

Introduction

Exposure to traumatic events may leave deep invisible scars on individuals that can lead to the development of psychopathology, such as PTSD. Patients with PTSD do not only experience recurrent intrusive thoughts and (sometimes vivid) memories of the traumatic event, but also symptoms of hyperarousal, avoidance and numbing, and difficulties of attention and memory.[1] Over the last decades, several empirical studies have reported alterations in learning and memory in patients with PTSD, which are consistent with both deficits in encoding on explicit memory tasks and deficits in retrieval, as well as enhanced encoding or retrieval for specific trauma-related material. [2-8] The majority of these studies examined verbal memory, with a relative absence of studies in attention or visual memory. [9]

Neuroimaging has become an important technique for understanding brain function and is widely used to examine both functional and morphological changes in neuropsychiatric disorders, including PTSD. Several structural MRI studies have found smaller hippocampi in patients with PTSD, however, this has never been consistently related to decreased memory performance.[10] In a recent positron emission tomography (PET) and MRI study in women with PTSD related to childhood sexual abuse, Bremner et al [11] found decreased hippocampal blood flow in patients with PTSD compared to controls during paragraph encoding. However, hippocampal volume was unrelated to hippocampal blood flow during activation tasks. In another paradigm, women with PTSD showed greater decreases in blood flow in frontal cortex and left hippocampus, and increases in visual association and motor cortex during recall of emotionally valenced word pairs. [12] Patients with PTSD revealed significantly less activation of the thalamus, the anterior cingulate gyrus, and the medial frontal gyrus than controls in an fMRI study on episodic traumatic memories.[13] In a verbal working memory task, patients with PTSD also had decreased frontal cortex activity.[14;15]

Although patients with PTSD frequently report memory difficulties and empirical research provides support for a memory deficit in PTSD, as of yet, no fMRI study has adequately investigated the neural correlates of associative learning and memory of neutral (i.e. not trauma related) material in patients with PTSD compared to controls. In studies with healthy subjects, memory processing has been investigated using various fMRI designs. These studies indicate that a fronto-temporal network (including the prefrontal cortex, entorhinal cortex, parahippocampal gyrus, and the hippocampus) constitute a neural substrate for the encoding and retrieval of memory.[16-18] Hippocampal activation in memory tasks is most likely in tasks of associative memory.[19-21] This study was designed to investigate associative memory processing in PTSD with fMRI using the encoding and retrieval of 12 word-pair associates as a neurocognitive task in Dutch veterans with PTSD and without PTSD.[22] Based on previous research in PTSD which has identified hippocampal and frontal lobe deficits, we hypothesized that patients with PTSD would reveal decreased activation patterns in fronto-temporal regions during encoding and retrieval of word-pair associates.

Methods

Subjects

Twelve male Dutch veterans with PTSD, and twelve male Dutch veterans without PTSD, were recruited. PTSD patients were recruited from the Department of Military Psychiatry at the Central Military Hospital in Utrecht. Control subjects were recruited via direct mail to veterans who were registered at the Veterans Institute in the Netherlands. All participants were male Dutch veterans who had served in UN peacekeeping missions in Lebanon, Cambodia, and Bosnia. None of the included veterans were physically injured at the time of deployment. Control veterans were matched to the patient group with respect to age, handedness, year of deployment, and country of deployment.

PTSD was diagnosed using DSM-IV criteria, and confirmed using the Clinician Administered PTSD Scale (CAPS)[23], and by consensus with three clinicians (EG, EV, CdK). Only patients with CAPS scores > 50 were included in the study. Comorbid disorders were examined utilizing the Structured Clinical Interview for DSM-IV (SCID).[24] Control subjects were also assessed with both the SCID and the CAPS. Control subjects met the A1 criterion for PTSD (i.e. they had all experienced a traumatic event). All subjects received a physical examination by a physician. Subjects were excluded if they had any clinical significant abnormality of a clinical laboratory test, a history of psychiatric illness (controls only) or neurological dysfunction (all subjects), a history of alcohol and/or drug abuse (DSM-IV criteria) within six months prior to the study, or claustrophobia. None of the participants were taking psychotropic drugs at the time of the study. Two patients had used a SSRI for several months prior to the start of the study. Written informed consent was obtained from all subjects who participated in the study after a complete written and verbal description of the study. The study was performed between August 2005 and February 2006. This study was approved by the Ethical Review Boards of the University Medical Centre of Utrecht, the Netherlands, and the Central Institute of Mental Health, Mannheim, Germany.

Table 1: Subject Demographics and Psychometric Data

	PTSD	*Controls*
N	12	12
Age	34.82 (5.78)	34.69 (3.70) ^{ns}
CAPS total	67.11 (19.39)	4.50 (5.27)*
Hamilton A	14.56 (4.28)	1.42 (1.78)*
Hamilton D	13.22 (4.60)	1.00 (1.28)*
Edinburgh Handedness Inventory	+88 (37)	+87 (34) ^{ns}

Means for both groups are given. Standard deviations are reported in between brackets. Edinburgh Handedness scores range from -100 (extreme left handedness) to +100 (extreme right handedness). ^{ns} not significant, * this difference was significant p<0.001, two tailed t-test

Experimental Procedure

FMRI was carried out on a 1.5 Tesla Scanner (Magnetom Vision, Siemens, Erlangen, Germany) at the Central Institute of Mental Health in Mannheim, Germany. Comfortable transportation and lodging was arranged for all subjects. Functional data was assessed using a functional echoplanar series (EPI) using 25 contiguous transverse slices (thickness, 5 mm; 1 mm gap; field of view, 220 x 220 mm^2; matrix, 64 x 64 voxels; slice acquisition time, 104 ms; volume acquisition time, 2600 ms; repetition time, 3089 ms).

The fMRI protocol consisted of two separate tasks: encoding and retrieval of 12 word-pairs (a slightly adapted form of the paradigm used by Ino et al [22]; see Figure 1, page 235). Stimuli were back-projected onto a screen that was visible from inside the scanner via a mirror. To minimize head motion, no output was required of subjects during the scans. The first task consisted of twelve 21 s blocks (6 stimuli per block, 3.5 s ISI) in which encoding blocks (presentations of word-pairs, e.g. rose-flower) were alternated with control blocks (presentations of successive numbers of two figures, e.g. 31–32), starting with the latter. The subjects were required to memorize word-pairs in the encoding block and to silently repeat the two figures in the control block. The number of word-pairs (12 pairs), was presented in the same order three times for the encoding condition. To avoid differences in encoding strategies among subjects and to minimize the strategic difference between the first, second, and third encoding conditions, the subjects were instructed to rote memorize as much as they could. They were prohibited from using a specific strategy except for making visual images, because this process often occurs automatically and restraining it would be very difficult.

The anatomical 3D magnetization prepared rapid acquisition gradient echo (MPRAGE) scan with a voxel size of 1 x 1 x 1 mm^3 and a field of view of 256 x 256 mm^2 was acquired after encoding, prior to the retrieval run. This series was used as an individual template for coregistration of functional and anatomical data and for spatial standardization into the stereotactic system of Talairach and Tournoux (1988). To prevent a rehearsal effect from occurring, subjects were instructed to mentally count numbers starting from one for about two minutes between the end of the last encoding block and the beginning of the MPRAGE.

Immediately after the MPRAGE, approximately twenty minutes after the encoding run, the retrieval commenced. The same scanning parameters were used as in the encoding run. This run consisted of four 21 s blocks (6 stimuli per block, 3.5 s ISI) in which retrieval blocks (presentations of the first word of a pair, e.g. rose) were alternated with control blocks (presentations of one number of two figures, e.g. 28), starting with the latter. The subjects were required to silently recall the second word in response to the first word for the retrieval block and to silently repeat the number during the control block. Repeating numerals during the control block also helped to prevent rehearsal. The material for the control task (numbers) was different from that of the memory task (words) and therefore the proactive/retroactive interference of memory formation by the control task was restrained.[22] In addition, it has been suggested that memory-related regions are activated less by a simple task involving

numerals than by no task at all.[25] Immediately after the second run, the subjects were taken out of the scanner, and were required to recall the second word of the word-pairs in response to the first word. This time, they recalled it by voice and their responses were recorded for later analysis.

Data Analysis

All the image data preparation and preprocessing steps as well as statistical analyses and the map volumetric projection were performed in Brain Voyager QX 1.6 (Brain Innovation, Maastricht, the Netherlands). The first four scans were excluded from data analysis to minimize T1 effects. Three-dimensional data preprocessing included slicescan time correction (using sinc interpolation), linear trend removal, and temporal high-pass filtering to remove low-frequency non-linear drifts of three or fewer cycles per time course. In addition, 3D motion correction was performed, to detect and correct for small head movements, by spatial alignment of all volumes to the first volume by rigid body transformations. Translation and rotation parameters were inspected and never exceeded 1 mm or 1 degree respectively. All functional imaging data were smoothed with a 4 mm FWHM Gaussian kernel. Co-registration of functional EPI and 3D structural measurements was computed by relating T2*-weighted images and the T1-weighted 3D MP RAGE measurement. This yields a transformation matrix for each individual subject and enables the creation of a 4D functional data set. Structural 3D and functional 4D data sets were transformed into the standard space corresponding to the atlas of Talairach and Tournoux. [26]

The stimulation protocol was convoluted with a hemodynamic response function [27] to account for the expected delay and generic shape of the BOLD signal. In order to correct for multiple comparisons, the false discovery rate (FDR) controlling procedure was applied on the resulting p values for all voxels. The value of q specifying the maximum FDR tolerated on average was set to 0.05. With this value, a single-voxel threshold is chosen by the FDR procedure which ensures that from all voxels shown as active, only 5% or less are false-positives. [28;29] Voxel level and region of interest (ROI) level inter-group linear contrasts were computed using two-tailed t-tests. Three-dimensional statistical maps were overlaid on the Talairach-transformed Montreal Neurological Institute T1-weighted brain template (http://www.bic.mni.mcgill.ca).

Due to the sample size and the relatively high variability of the population compared to the signal-to-noise ratio of the measurements, inter-individual variance was not accounted for in the voxel-level group analysis, and all contrast maps were computed using a fixed-effects model.[30] In order to extend our inferences and results, significantly activated clusters of greater than 50 voxels in the first level inter-group voxel-level analysis were selected for a more sensitive second level ROI analysis using a random effects model in the two sample t-tests, using a threshold of [$p_{\text{random effects}} \leq 0.01$].

Psychometric data and performance data were analyzed using two-tailed t-tests. Correlation analyses using Pearson's R were also performed with performance data (number of correctly recalled words), total CAPS score, and individual t-values for each of the ROIs. The correlations were computed within each of the groups. These statistical analyses were performed with SPSS 12.0 for Windows (SPSS, Chicago, Illinois). The statistical threshold of significance for these measures was set at $p < 0.05$.

Results

Psychometric Data

PTSD patients and control veterans were optimally matched with respect to age (34.8 (5.8) vs 34.7 (3.7), p>0.05). Patients with PTSD had significantly greater CAPS, Hamilton A, and Hamilton D scores (see Table 1). According to the SCID, the PTSD group met lifetime (past) DSM-IV diagnostic criteria for major depressive disorder (n = 2), alcohol abuse (n = 2), alcohol dependence (n = 1), substance abuse (n = 1), substance dependence (n = 1), and panic disorder without agoraphobia (n = 1). None of the patients with PTSD had any current comorbid disorder. Among the control subjects, the SCID did not reveal any current or lifetime psychiatric disorders.

Task Performance

Patients with PTSD displayed a trend to reduced performance on the retrieval of the paired associates (3.7 ± 1.6 vs. 6.2 ± 4.2); p= 0.071; two-tailed t-test, unequal variances assumed).

Main Effects Encoding

In all subjects solid activations were seen in the bilateral dorsolateral prefrontal cortex (DLPFC), medial prefrontal cortex (mPFC), anterior cingulate cortex (ACC), parietal lobe, lingual gyri, left temporal lobe, left parahippocampal gyrus, left hippocampus, and the bilateral globus pallidus. In the patient group, additional significant activation was seen in the bilateral orbitofrontal cortex (OFC).

Main Effects Retrieval

In all subjects, solid activations were seen in the left mPFC, bilateral caudate, bilateral lingual gyrus, bilateral precuneus, and left inferior parietal lobe. In the patient group additional activations were seen in the right prefrontal cortex, left ACC and left insula. In the control group additional significant activations were seen in the bilateral OFC, bilateral medial temporal lobe, left parahippocampal gyrus, and left inferior parietal lobe.

fMRI Group Analysis Encoding

During the encoding condition, patients with PTSD showed altered activity in fronto-temporal areas (see Table 2 and Figure 2, page 236). Patients revealed decreased activation in the right inferior frontal gyrus BA46 (t_{patients}, -1.40 ± 1.22 [mean ± SD], t_{controls}, 0.78 ± 1.46) and left inferior frontal gyrus BA10 (t_{patients}, -0.67 ± 1.53, t_{controls}, 1.37 ± 1.23). In addition, patients with PTSD displayed reduced activation in the left DLPFC (left middle frontal gyrus BA6: t_{patients}, 0.13 ± 1.71, t_{controls}, 2.70 ± 1.46;

and left superior frontal gyrus BA6: $t_{patients}$, -0.61 ± 2.02, $t_{controls}$, 1.63 ± 1.30). In the right DLPFC, patients with PTSD revealed increased activation during encoding (right middle frontal gyrus BA9: $t_{patients}$, 1.48 ± 1.72, $t_{controls}$, -0.63 ± 1.69). Compared to controls, patients with PTSD also displayed increased activation in the bilateral superior temporal gyri (right superior temporal gyrus BA22: $t_{patients}$, -0.84 ± 1.43, $t_{controls}$, -1.41 ± 1.87; left superior temporal gyrus BA22 (-46,-22,4): $t_{patients}$, 1.17 ± 0.97, $t_{controls}$, -0.97 ± 1.90, and left superior temporal gyrus BA22 (-52,-2,2) $t_{patients}$, 1.49 ± 1.47, $t_{controls}$, -0.67 ± 2.13). Patients also showed increased activation in the right parahippocampal gyrus BA30 ($t_{patients}$, 2.26 ± 1.06, $t_{controls}$, 0.00 ± 1.15), the right middle temporal gyrus BA37 ($t_{patients}$, 1.08 ± 0.97, $t_{controls}$, -1.01 ± 1.16), and the left inferior temporal gyrus BA37 ($t_{patients}$, 1.54 ± 1.65, $t_{controls}$, -0.56 ± 1.01). Decreased activation in PTSD was observed in the left middle temporal gyrus BA39 ($t_{patients}$, -0.19 ± 1.69, $t_{controls}$, 1.76 ± 1.20), and the left precuneus ($t_{patients}$, -0.04 ± 1.46, $t_{controls}$, 1.78 ± 1.45). In the control veterans group, task performance correlated significantly with activity in the left middle temporal gyrus BA39 (Pearson's r = 0.617; p<0.05), but not with any of the other ROI.

Table 2: Regions of interest with significant group differences for the encoding condition

Region of Activation	*left/ right*	*Talairach coordinates*				$T_{(random\ effects)}$	$p_{(random\ effects)}$
		x	*y*	*z*	*BA*		
Increases during encoding in PTSD compared to controls							
DLPFC	R	45	7	36	9	3.17	0.004415
Parahippocampal Gyrus	R	11	-45	0	30	5.22	0.000031
Inferior Temporal Gyrus	L	-44	-67	2	37	3.93	0.000722
Middle Temporal Gyrus	R	42	-66	11	37	5.00	0.000052
Superior Temporal Gyrus	L	-46	-22	4	22	3.64	0.001452
Superior Temporal Gyrus	L	-52	-2	2	22	3.02	0.006329
Superior Temporal Gyrus	R	56	2	3	22	3.45	0.002306
Decreases during encoding in PTSD compared to controls							
Inferior Frontal Gyrus	L	-38	46	-1	10	3.75	0.001109
Inferior Frontal Gyrus	R	50	33	9	46	4.15	0.000421
DLPFC	L	-33	1	52	6	4.12	0.000447
DLPFC	L	-9	22	56	6	3.38	0.002699
Posterior Middle Temporal Gyrus	L	-37	-67	27	39	3.42	0.002449
Precuneus	L	-10	-65	34	7	3.19	0.004215

BA = Brodmann Area; DLPFC = dorsolateral prefrontal cortex

In the PTSD group, task performance was not correlated with activity in the ROI during encoding. CAPS scores in the control group were not correlated with activity in any of the ROI during encoding, however, in the PTSD group, CAPS scores were significantly correlated with activity in the right middle temporal gyrus BA37 (Pearson's r = -0.833; p<0.005) and the left precuneus BA7 (Pearson's r = -0.791; p<0.005).

fMRI Group Analysis Retrieval

In the retrieval condition, patients with PTSD displayed less activation in fronto-temporal areas (see Table 3 and Figure 3, page 238). Compared to controls, patients showed less activity in the right inferior frontal gyrus BA 45 ($t_{patients}$, -0.34 ± 0.46, $t_{controls}$, 0.38 ± 0.53), right DLPFC BA6 ($t_{patients}$, -0.60 ± 0.33, $t_{controls}$, 0.11 ± 0.48), left postcentral gyrus BA43 ($t_{patients}$, -0.32 ± 0.32, $t_{controls}$, 0.40 ± 0.56), bilateral middle temporal gyrus (right middle temporal gyrus BA39: $t_{patients}$, -0.19 ± 0.37, $t_{controls}$, 0.48 ± 0.47; left middle temporal gyrus BA22: $t_{patients}$, 0.01 ± 0.67, $t_{controls}$, 0.85 ± 0.55), left superior temporal gyrus BA21 ($t_{patients}$, -0.20 ± 0.39, $t_{controls}$, 0.54 ± 0.51), left hippocampus/parahippocampal gyrus BA30 ($t_{patients}$, -0.16 ± 0.51, $t_{controls}$, 0.49 ± 0.35), and right lingual gyrus BA17 ($t_{patients}$, -0.16 ± 0.64, $t_{controls}$, 0.50 ± 0.55). In the control veterans, neither task performance nor CAPS score correlated significantly with activity in the ROI. In patients with PTSD task performance correlated significantly with activity in the left middle temporal gyrus BA22 (Pearson's R = 0.639; p < 0.05), but not with any other ROI. CAPS score correlated significantly with activity in the right precentral gyrus BA6 (Pearson's R = 0.630; p < 0.05) and the left superior temporal gyrus BA 21 (Pearson's R = -0.668; p < 0.05).

Table 3: Regions of interest with significant group differences for the retrieval condition

Region of Activation	*left/ right*	*Talairach coordinates*				$T_{(random\ effects)}$	$p_{(random\ effects)}$
		x	*y*	*z*	*BA*		
Decreases during retrieval in PTSD compared to controls							
Inferior Frontal Gyrus	R	61	11	20	45	3.73	0.001178
DLPFC	R	59	-1	8	6	4.13	0.000437
Postcentral Gyrus	L	-50	-13	19	43	4.05	0.000538
Hippocampus/Parahippocampal Gyrus	L	-11	-47	5	30	4.11	0.000464
Middle Temporal Gyrus	L	-57	-35	4	22	3.65	0.001409
Middle Temporal Gyrus	R	44	-54	8	39	4.05	0.000536
Superior Temporal Gyrus	L	-62	-21	-3	21	4.22	0.000352
Lingual Gyrus	R	9	-90	-4	17	2.76	0.011539

BA = Brodmann Area; DLPFC = dorsolateral prefrontal cortex

Discussion

During encoding of the word pairs, both patients and controls revealed solid activations in the bilateral DLPFC, mPFC, OFC, ACC, parietal lobe, lingual gyri, left temporal lobe, left parahippocampal gyrus, and the bilateral globus pallidus. These activations are consistent with previous neuroimaging studies which have consistently revealed involvement of fronto-temporal regions during encoding of paired-associates. [17;22;31] To our knowledge, this is the first functional MRI study which investigates associative memory processing in patients with PTSD. Patients with PTSD showed less activity in the bilateral inferior frontal gyri, and left prefrontal cortex than healthy controls. The role of the frontal cortex in the formation of memories has been an important subject area for neuroimaging studies. [32] The left DLPFC appears to be activated when memorizing verbal stimuli, whereas the right DLPFC is more active when memorizing visual stimuli, and both left and right DLPFC appear to be activated when memorizing objects. [33;34] In functional neuroimaging studies of long-term episodic memory it has also been postulated that there is a functional asymmetry with the left prefrontal cortex being more active during encoding, whereas the right prefrontal cortex is more active during retrieval (hemispheric encoding and retrieval asymmetry, HERA). [35-37] It is not clear why patients with PTSD showed increased right middle frontal gyrus activation during the encoding phase compared to controls. It may reflect a difference in strategy use (i.e. patients with PTSD might employ more visual imagery during encoding than controls). [38] These findings of decreased mPFC activation are consistent with previous neuroimaging studies of traumatic reminders in patients with PTSD. [38;39] In patients with bipolar disorder and schizophrenia, impaired recruitment of the DLPFC during encoding has also been a frequent finding. [40;41] In the right DLPFC, patients with PTSD displayed increased activity. This pattern of activity is similar to a study on verbal working memory in PTSD, where patients with PTSD showed decreased activity in the left DLPFC, but increased activity in the right DLPFC.[14] These findings are congruent with the proposal that patients with PTSD have increased dependence on nonverbal working memory areas as a strategy for coping with decreased verbal memory abilities.[38]

The involvement of the medial temporal lobe in the formation and retrieval of memory has been established in a variety of paradigms, including neuroimaging. The medial temporal lobe receives input from the association cortices and is largely responsible for the formation of the initial memory trace. [42-44] Encoding activity in the medial temporal lobe is especially increased when subjects learn pairs of new items, such as during a paired-associates task. [45] Patients with PTSD showed increased activation in the bilateral temporal lobes during the encoding of verbal paired associates. This was unexpected, as increased activity in the temporal lobes during encoding is usually associated with increased task performance, not decreased performance as witnessed in the patient group. [20;22;46] Correlation analysis revealed that indeed activity in these areas was not related to task performance, while activity in the right middle temporal gyrus was significantly correlated with CAPS score in patients with PTSD. Patients with schizophrenia display a similar pattern of increased temporal activity and underactivation of the frontal cortex, suggesting that in patients with PTSD, as in patients with schizophrenia, the normal functional connectivity of prefrontal and temporo-limbic structures is disrupted. [41;47;48] It may also be that patients with PTSD have recruited these additional areas during the encoding task to compensate for reduced activation of the frontal network. [49] Patients also showed increased right posterior parahippocampal activity during encoding, again consistent with a greater dependence of patients with PTSD on nonverbal encoding strategies. [38] The right posterior parahippocampal gyrus is activated predominately by visual stimuli. [50] Alternatively, the right parahippocampal activation in patients may reflect attentional orienting to the presented stimuli or novelty detection. [46;51]

Precuneus activity is usually associated with source memory processing, or spatial location encoding.[52;53] In addition, the left precuneus is also proposed to be involved in mental imagery and buffering of working memory.[17] Ineffectual activation of the precuneus in PTSD during encoding could possibly contribute to the retrieval deficit in PTSD. The precuneus and surrounding posteromedial areas are amongst the brain structures displaying the highest resting metabolic rates and are characterized by transient decreases in the tonic activity during engagement in non-self-referential goal-directed actions (default mode of brain function). Therefore, it has recently been proposed that the precuneus is involved in the interwoven network of the neural correlates of self-consciousness, engaged in self-related mental representations during rest. [54] Perhaps patients are also less self-conscious when engaged in a mental activity. Activity in the precuneus was significantly correlated with severity of PTSD symptoms.

During the retrieval condition, both groups showed significant activity in the left mPFC, right prefrontal cortex, left ACC, left insula, bilateral caudate, bilateral lingual gyrus, bilateral precuneus, left temporal lobe, and left inferior parietal lobe. Patients with PTSD revealed less activity in the right inferior frontal gyrus and right precentral gyrus compared to controls during retrieval. Several neuroimaging studies have provided support for a role of the right inferior frontal gyrus and DLPFC in successful retrieval of memory. [55-60] Recent work suggests that the DLPFC actively promotes the formation of long-term memory through its role in the organization of information during working-memory related processes. [61] This pattern of activity could explain the poorer performance of the patients with PTSD on the retrieval task. In other neuroimaging paradigms patients with PTSD have also shown reduced activation of right frontal cortex during traumatic reminders. [13;62;63] During retrieval of emotional-valenced word pairs, patients with PTSD have also revealed reduced activity of OFC, mPFC, and ACC. [12] Decreased right lingual activity in patients with PTSD during the retrieval phase is proposed to be related to reduced processing of semantic information related to retrieval. [64] Improved recall of verbal material has been associated with increased activity in the right lingual gyrus that is strategy related. [58]

The importance of the medial and lateral temporal lobe in the retrieval of memory has been well established. [65;66] Retrieval of the paired associates resulted in less activity in these regions in vet-

erans with PTSD compared to control veterans without PTSD. Correlational analysis showed that this decrease in activity is not related to reduced task performance. Activity in the left superior temporal gyrus correlated significantly with PTSD symptom severity (as indicated by the CAPS score). In other neuropsychiatric disorders, such as schizophrenia, retrieval deficits are also related to reductions in temporal lobe activity.[67] Patients with PTSD have shown reduction of hippocampal volume and displayed altered activity in the hippocampus in a variety of neuroimaging paradigms.[68-72] In the retrieval condition, patients showed less activity in an area that corresponds to the left posterior hippocampus and left parahippocampal gyrus. This is nearly the same area where Bremner et al [11] found less activity in PTSD during verbal memory encoding. Activation of the hippocampal formation during encoding and retrieval is known to reflect successful retrieval of paired associates. [20]
Future research should extend these findings to patients with PTSD related to civilian traumas. The use of trauma controls in this study is very powerful, and allows us to differentiate between these groups based on the absence and presence of PTSD only. However some of the differences we observed may be the effect of trauma or (former) occupation (all subjects were veterans). The addition of a third control group consisting of healthy subjects who have not been subjected to trauma could have filtered out these effects. Although the number of subjects in this study was sufficient to permit random effects analysis and allow generalization of these results to the clinical population, future research should include more subjects to enable sub-group analysis. The trend to decreased performance on the memory task in veterans with PTSD would probably have reached significance in a larger sample. In this study, we chose to use repetition of numerals as a control task to minimize rehearsal and strategic processing during the task, however, it is still possible that both verbal and visual elaboration may have taken place. One of the mechanisms which may play a role in reduced memory performance in patients with PTSD is acute glucocorticoid release, which has a profound impact on memory consolidation. [73] Unfortunately we did not assess glucocorticoid release in our population; future research should address this.
Findings of altered activity in both the frontal lobe and temporal lobe reflect the strong connections between the hippocampus and the frontal cortex.[17;74;75] The results of our study provide support for a role of both the hippocampus and the prefrontal cortex in associative memory in general and in PTSD in particular. [76] Among individuals with PTSD, however, these processes may be qualitatively different and reflect differences in how these structures function during learning and memory.

References

1. Thygesen, P., Hermann, K., and Willanger, R. Concentration camp survivors in Denmark persecution, disease, disability, compensation. A 23-year follow-up. A survey of the long-term effects of severe environmental stress. Dan Med Bull 17, 65-108 (1970).

2. Andrews, B. et al. The timing, triggers and qualities of recovered memories in therapy. Br J Clin Psychol 39 (Pt 1), 11-26 (2000).

3. Buckley, T.C., Blanchard, E.B., and Neill, W.T. Information processing and PTSD: a review of the empirical literature. Clin Psychol Rev 20, 1041-65 (2000).

4. Gilbertson, M.W., Gurvits, T.V., Lasko, N.B., Orr, S.P., and Pitman, R.K. Multivariate assessment of explicit memory function in combat veterans with posttraumatic stress disorder. J Trauma Stress 14, 413-32 (2001).

5. Pitman, R.K. Post-traumatic stress disorder, hormones, and memory. Biol Psychiatry 26, 221-3 (1989).

6. Roca, V. and Freeman, T.W. Complaints of impaired memory in veterans with PTSD. Am J Psychiatry 158, 1738-9 (2001).

7. Vasterling, J.J., Brailey, K., Constans, J.I., and Sutker, P.B. Attention and memory dysfunction in posttraumatic stress disorder. Neuropsychology 12, 125-33 (1998).

8. Wolfe, J. and Schlesinger, L.K. Performance of PTSD patients on standard tests of memory. Implications for trauma. Ann N Y Acad Sci 821, 208-18 (1997).

9. Horner, M.D. and Hamner, M.B. Neurocognitive functioning in posttraumatic stress disorder. Neuropsychol Rev 12, 15-30 (2002).

10. Isaac, C.L., Cushway, D., and Jones, G.V. Is posttraumatic stress disorder associated with specific deficits in episodic memory? Clin Psychol Rev (2006).

11. Bremner, J.D. et al. MRI and PET study of deficits in hippocampal structure and function in women with childhood sexual abuse and posttraumatic stress disorder. Am J Psychiatry 160, 924-32 (2003).

12. Bremner, J.D. et al. Neural correlates of declarative memory for emotionally valenced words in women with posttraumatic stress disorder related to early childhood sexual abuse. Biol Psychiatry 53, 879-89 (2003).

13. Lanius, R.A. et al. Neural correlates of traumatic memories in posttraumatic stress disorder: a functional MRI investigation. Am J Psychiatry 158, 1920-2 (2001).

14. Clark, C.R. et al. Cerebral function in posttraumatic stress disorder during verbal working memory updating: a positron emission tomography study. Biol Psychiatry 53, 474-81 (2003).

15. Weber, D.L. et al. Abnormal frontal and parietal activity during working memory updating in post-traumatic stress disorder. Psychiatry Res 140, 27-44 (2005).

16. Eichenbaum, H. A cortical-hippocampal system for declarative memory. Nat Rev Neurosci 1, 41-50 (2000).

17. Taylor, J.G. et al. Decomposing memory: functional assignments and brain traffic in paired word associate learning. Neural Netw 13, 923-40 (2000).

18. Squire, L.R., Stark, C.E., and Clark, R.E. The medial temporal lobe. Annu Rev Neurosci 27, 279-306 (2004).

19. Eichenbaum, H. Hippocampus: cognitive processes and neural representations that underlie declarative memory. Neuron 44, 109-20 (2004).

20. Meltzer, J.A. and Constable, R.T. Activation of human hippocampal formation reflects success in both encoding and cued recall of paired associates. Neuroimage 24, 384-97 (2005).

21. Cohen, N.J. et al. Hippocampal system and declarative (relational) memory: summarizing the data from functional neuroimaging studies. Hippocampus 9, 83-98 (1999).

22. Ino, T., Doi, T., Kimura, T., Ito, J., and Fukuyama, H. Neural substrates of the performance of an auditory verbal memory: between-subjects analysis by fMRI. Brain Res Bull 64 , 115-26 (2004).

23. Blake, D.D. et al. The development of a Clinician-Administered PTSD Scale. J Trauma Stress 8, 75-90 (1995).

24. First, M. B., Spitzer, R. L., Gibbon, M., and Williams, J. B. W. Structured Clinical Interview for DSM-IV Axis I Disorders. SCID-I/P . 97.

25. Stark, C.E. and Squire, L.R. When zero is not zero: the problem of ambiguous baseline conditions in fMRI. Proc Natl Acad Sci U S A 98, 12760-6 (2001).

26. Talairach J. and P. Tournoux. Coplanar Stereotaxic Atlas of the Human Brain. 3-Dimensional Proportional System: An Approach to Cerebral Imaging. Thieme Medical, New York (1988).

27. Boynton, G.M., Engel, S.A., Glover, G.H., and Heeger, D.J. Linear systems analysis of functional magnetic resonance imaging in human V1. J Neurosci 16, 4207-21 (1996).

28. Benjamini, Y. and Hochberg, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc., B 57, 289-300 (1995).

29. Genovese, C.R., Lazar, N.A., and Nichols, T. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. Neuroimage 15, 870-8 (2002).

30. Friston, K.J., Holmes, A.P., and Worsley, K.J. How many subjects constitute a study? Neuroimage 10, 1-5 (1999).

31. Law, J.R. et al. Functional magnetic resonance imaging activity during the gradual acquisition and expression of paired-associate memory. J Neurosci 25, 5720-9 (2005).

32. Fletcher, P.C. and Henson, R.N. Frontal lobes and human memory: insights from functional neuroimaging. Brain 124, 849-81 (2001).

33. Buckner, R.L., Kelley, W.M., and Petersen, S.E. Frontal cortex contributes to human memory formation. Nat Neurosci 2, 311-4 (1999).

34. Iidaka, T., Sadato, N., Yamada, H., and Yonekura, Y. Functional asymmetry of human prefrontal cortex in verbal and non-verbal episodic memory as revealed by fMRI. Brain Res Cogn Brain Res 9, 73-83 (2000).

35. Habib, R., Nyberg, L., and Tulving, E. Hemispheric asymmetries of memory: the HERA model revisited. Trends Cogn Sci 7, 241-245 (2003).

36. McDermott, K.B. et al. Direct comparison of episodic encoding and retrieval of words: an event-related fMRI study. Memory 7, 661-78 (1999).

37. Babiloni, C. et al. Functional frontoparietal connectivity during encoding and retrieval processes follows HERA model. A high-resolution study. Brain Res Bull 68, 203-12 (2006).

38. Bremner, J.D. et al. Neural correlates of exposure to traumatic pictures and sound in Vietnam combat veterans with and without posttraumatic stress disorder: a positron emission tomography study. Biol Psychiatry 45, 806-16 (1999).

39. Bremner, J.D. et al. Neural correlates of memories of childhood sexual abuse in women with and without posttraumatic stress disorder. Am J Psychiatry 156, 1787-95 (1999).

40. Deckersbach, T. et al. Impaired recruitment of the dorsolateral prefrontal cortex and hippocampus during encoding in bipolar disorder. Biol Psychiatry 59, 138-46 (2006).

41. Ragland, J.D. et al. Event-related fMRI of frontotemporal activity during word encoding and recognition in schizophrenia. Am J Psychiatry 161, 1004-15 (2004).

42. Hoffman, K.L. and McNaughton, B.L. Coordinated reactivation of distributed memory traces in primate neocortex. Science 297, 2070-3 (2002).

43. Lavenex, P. and Amaral, D.G. Hippocampal-neocortical interaction: a hierarchy of associativity. Hippocampus 10, 420-30 (2000).

44. Suzuki, W.A. and Amaral, D.G. Functional neuroanatomy of the medial temporal lobe memory system. Cortex 40, 220-2 (2004).

45. Sakai, K. and Passingham, R.E. Prefrontal selection and medial temporal lobe reactivation in retrieval of short-term verbal information. Cereb Cortex 14, 914-21 (2004).

46. Fernandez, G. et al. Successful verbal encoding into episodic memory engages the posterior hippocampus: a parametrically analyzed functional magnetic resonance imaging study. J Neurosci 18, 1841-7 (1998).

47. Weinberger, D.R., Berman, K.F., Suddath, R., and Torrey, E.F. Evidence of dysfunction of a prefrontal-limbic network in schizophrenia: a magnetic resonance imaging and regional cerebral blood flow study of discordant monozygotic twins. Am J Psychiatry 149 , 890-7 (1992).

48. Friston, K.J. and Frith, C.D. Schizophrenia: a disconnection syndrome? Clin Neurosci 3, 89-97 (1995).

49. Tisserand, D.J., McIntosh, A.R., van der Veen, F.M., Backes, W.H., and Jolles, J. Age-related reorganization of encoding networks directly influences subsequent recognition memory. Brain Res Cogn Brain Res 25, 8-18 (2005).

50. Leube, D.T., Erb, M., Grodd, W., Bartels, M., and Kircher, T.T. Differential activation in parahippocampal and prefrontal cortex during word and face encoding tasks. Neuroreport 12, 2773-7 (2001).

51. Gabrieli, J.D., Brewer, J.B., Desmond, J.E., and Glover, G.H. Separate neural bases of two fundamental memory processes in the human medial temporal lobe. Science 276, 264-6 (1997).

52. Lundstrom, B.N., Ingvar, M., and Petersson, K.M. The role of precuneus and left inferior frontal cortex during source memory episodic retrieval. Neuroimage 27, 824-34 (2005).

53. Frings, L. et al. Precuneus is involved in allocentric spatial location encoding and recognition. Exp Brain Res (2006).

54. Cavanna, A.E. and Trimble, M.R. The precuneus: a review of its functional anatomy and behavioural correlates. Brain 129, 564-83 (2006).

55. Greenberg, D.L. et al. Co-activation of the amygdala, hippocampus and inferior frontal gyrus during autobiographical memory retrieval. Neuropsychologia 43, 659-74 (2005).

56. Karlsgodt, K.H., Shirinyan, D., van Erp, T.G., Cohen, M.S., and Cannon, T.D. Hippocampal activations during encoding and retrieval in a verbal working memory paradigm. Neuroimage 25, 1224-31 (2005).

57. Henson, R.N., Shallice, T., and Dolan, R.J. Right prefrontal cortex and episodic memory retrieval: a functional MRI test of the monitoring hypothesis. Brain 122 (Pt 7), 1367-81 (1999).

58. Kondo, Y. et al. Changes in brain activation associated with use of a memory strategy: a functional MRI study. Neuroimage 24, 1154-63 (2005).

59. Sandrini, M., Cappa, S.F., Rossi, S., Rossini, P.M., and Miniussi, C. The role of prefrontal cortex in verbal episodic memory: rTMS evidence. J Cogn Neurosci 15, 855-61 (2003).

60. Tsukiura, T. et al. Medial temporal lobe activation during context-dependent relational processes in episodic retrieval: an fMRI study. Functional magnetic resonance imaging. Hum Brain Mapp 17, 203-13 (2002).

61. Blumenfeld, R.S. and Ranganath, C. Dorsolateral prefrontal cortex promotes long-term memory formation through its role in working memory organization. J Neurosci 26, 916-25 (2006).

62. Shin, L.M. et al. Hippocampal function in posttraumatic stress disorder. Hippocampus 14, 292-300 (2004).

63. Shin, L.M. et al. Regional cerebral blood flow during script-driven imagery in childhood sexual abuse-related PTSD: A PET investigation. Am J Psychiatry 156, 575-84 (1999).

64. Kuchinke, L. et al. Incidental effects of emotional valence in single word processing: an fMRI study. Neuroimage 28, 1022-32 (2005).

65. Miyashita, Y. Cognitive memory: cellular and network machineries and their top-down control. Science 306, 435-40 (2004).

66. Sakai, K. Reactivation of memory: role of medial temporal lobe and prefrontal cortex. Rev Neurosci 14, 241-52 (2003).

67. Achim, A.M. and Lepage, M. Episodic memory-related activation in schizophrenia: meta-analysis. Br J Psychiatry 187, 500-9 (2005).

68. Geuze, E., Vermetten, E., and Bremner, J.D. MR-based in vivo hippocampal volumetrics: 2. Findings in neuropsychiatric disorders. Mol Psychiatry 10, 160-84 (2005).

69. Bremner, J.D. et al. Neural correlates of declarative memory for emotionally valenced words in women with posttraumatic stress disorder related to early childhood sexual abuse. Biol Psychiatry 53, 879-89 (2003).

70. Neylan, T.C. et al. Attention, learning, and memory in posttraumatic stress disorder. J Trauma Stress 17, 41-6 (2004).

71. Schuff, N. et al. Decreased hippocampal N-acetylaspartate in the absence of atrophy in posttraumatic stress disorder. Biol Psychiatry 50 , 952-9 (2001).

72. Sakamoto, H. et al. Parahippocampal activation evoked by masked traumatic images in posttraumatic stress disorder: a functional MRI study. Neuroimage 26, 813-21 (2005).

73. Roozendaal, B. Systems mediating acute glucocorticoid effects on memory consolidation and retrieval. Prog Neuropsychopharmacol Biol Psychiatry 27, 1213-23 (2003).

74. Squire, L.R., Knowlton, B., and Musen, G. The structure and organization of memory. Annu Rev Psychol 44, 453-95 (1993).

75. Rolls, E.T. Memory systems in the brain. Annu Rev Psychol 51, 599-630 (2000).

76. Sweatt, J.D. Hippocampal function in cognition. Psychopharmacology (Berl) 174, 99-110 (2004).

SECTION 3

Neuropsychology

8

Neuropsychological Performance in Relation to Current Social and Occupational Functioning in Veterans with Posttraumatic Stress Disorder

Elbert Geuze, Eric Vermetten, Carien S. de Kloet, Ron Hijman, and Herman G. M. Westenberg. **Submitted.**

Introduction

Each day hundreds of thousands of the world's inhabitants are exposed to severe life-threatening trauma and may experience an emotional response such as fear, feelings of helplessness, or horror. Some of these individuals (7-15%) will develop PTSD.[1;2] Core symptoms of PTSD include recurrent and intrusive distressing recollection of the event, persistent avoidance of stimuli associated with the traumatic experience, and increased symptoms of arousal. In addition to these symptoms, patients with PTSD also report increased difficulties with new learning, memory, and attention.[3-5]

In addition to the extensive literature which exists about the phenomenology and neurobiology of PTSD, an emerging body of literature has examined neuropsychological functioning in PTSD. Patients with PTSD display poorer performance on several tests of learning and memory, including the Wechsler Memory Scale - Revised (WMS-R) [6-8], California Verbal Learning Test (CVLT) [9-13], Rey Auditory Verbal Learning Test (RAVLT) [14-16], Selective Reminding Test (SRT) [17], and the Rivermead Behavioural Memory Test (RBMT).[18;19] The majority of these studies were performed in patients with PTSD related to combat experience,[20-23] whereas only some have examined cognitive performance in PTSD related to civilian trauma, [24-26] or in children with PTSD.[18,27] Most of the studies were confined to single tests of memory performance and were not corrected for important variables such as age, age at the time of trauma, and intelligence. Other confounding factors were also often present in these studies, such as high measures of comorbidity, substance abuse, and the use of psychotropic medication. In addition, although clinical experience and epidemiological studies indicate that PTSD has an impact on social and occupational functioning, few studies have examined functional impairments.[28;29] To date, no study has been published relating memory performance in patients with PTSD to occupational and social functioning.

This study compared the performance of Dutch veterans with PTSD to age matched control veterans without PTSD on a large number of measures related to memory performance and intellectual function. It was hypothesized that patients with PTSD would display significantly poorer performance on neuropsychological tests measuring memory performance in the absence of differences in intellectual performance. In addition, we hypothesized that performance on memory tests would predict current social and occupational functioning.

Methods

Subjects

The sample consisted of twenty-five male Dutch veterans with PTSD, and twenty-five age matched control veterans without PTSD. All patients were recruited from the department of Military Psychiatry at the Central Military Hospital in Utrecht. Control subjects were recruited via direct mail to veterans who were registered at the Veterans Institute in Doorn, the Netherlands. All participants had served in UN peacekeeping missions in Lebanon, Cambodia, or Bosnia. Control veterans were matched to the patient group with respect to age, year of deployment, and country of deployment. None of

the veterans included were physically injured at the time of deployment. At study entry, twenty of the twenty-five patients were drug naïve. The other five subjects had not taken any psychotropic medications or abused substances for at least six months. Subjects with a history of neurological illness, psychiatric illness other than mood and anxiety disorders, were excluded. This study was approved by the Institutional Review Board of the University Medical Centre of Utrecht, the Netherlands. Written informed consent was obtained from all subjects who participated in the study after a complete written and verbal description of the study. The study was performed between August 2002 and September 2005.

Table 1 Subject Demographics and Psychometric Data

	PTSD	*Controls*
N	25	25
Age	33.32 (4.76)	34.80 (4.60) ns
Year of deployment (range 1980-2002)	1992 (1.12)	1992 (1.28) ns
Country of deployment	Bosnia (n = 15)	Bosnia (n = 15)
	Lebanon (n = 6)	Lebanon (n =5)
	Cambodia (n = 3)	Cambodia (n = 4)
	Afghanistan (n = 1)	Afghanistan (n = 1)
CAPS total	73.64 (13.40)	8.09 (7.09) *
CAPS C3	3.63 (2.31)	0.36 (0.21) *
CAPS D3	5.33 (1.58)	0.76 (1.36) *
Hamilton A	20.09 (7.63)	1.60 (1.50) *
Hamilton A item 5	2.00 (1.17)	0.28 (0.61) *
Hamilton D	15.09 (4.77)	1.04 (1.43) *
Years of education	11.76 (1.01)	11.76 (1.05) ns
Estimated total IQ**	97.84 (10.68)	102.56 (11.30) ns

Hamilton A, Hamilton Anxiety Scale; Hamilton D, Hamilton Depression Scale; IQ, intelligence quotient as measured with four subtests from the Wechsler Adult Intelligence Scale; CAPS C3, CAPS D3, and means for both groups are given. Standard deviations are reported in between brackets. ns this difference was not significant, * this difference was significant p<0.0001. **Estimated total IQ was based on prorated score including the WAIS III Vocabulary, Similarities, Picture Arrangement, and Block Design subtests.

Clinical Assessments

All subjects were assessed using the Structured Clinical Interview for DSM-IV [30] and the Clinician Administered PTSD Scale (CAPS).[31] Diagnosis of PTSD was confirmed by the CAPS and through consensus by three clinicians (EG, EV, CdK). Only subjects with a CAPS score greater than 50 were included in the patient group. Subjects with a CAPS score greater than 20 were excluded from the trauma control group. Hamilton Depression Scale (HAM-D), and the Hamilton Anxiety Scale (HAM-A) scores were also obtained for all subjects.

Neuropsychological Testing

Total IQ of all subjects was estimated based upon a prorated score including the following WAIS-III subtests: Similarities, Vocabulary, Block Design, and Picture Arrangement subtests to provide a measure of intelligence. Tests of memory performance included the Dutch version of the Rey Auditory Verbal Learning Task AVLT,[32;33] , the Dutch version of the CVLT, [34;35] and the Dutch version of the WMS-R logical memory I and II and the WMS-R Visual Reproduction I and II.[36] Neuropsychological assessment was performed by a trained neuropsychological graduate student, under direct supervision of a qualified neuropsychologist (RH). The AVLT consists of five presentations of a 15-word list, followed by a delayed free recall and recognition testing after a period of 30 minutes. Variables of interest for the AVLT included the sum of correctly recalled words from the list for trial 1-5, the number of correctly recalled words after a delay of 30 minutes, the total number of correct words from the recognition, and the total false positives recognized. The CVLT consists of five presentations of a 16-word list. The following variables for the CVLT were used: CVLT sum of correctly recalled words from list A trials 1-5, immediate recall of list A, immediate cued recall of list A, delayed recall of list A, delayed cued recall of list A, and total number of correctly recognized words from list A. The CVLT and the AVLT were not administered on the same day, and there was always one week between the two tests. Additional variables of interest from the WMS-R included logical memory immediate recall (correctly recalled elements from story A and story B), logical memory delayed recall, figural memory immediate reproduction (using the WMS-R scoring criteria [36]), figural memory delayed recall, and figural memory recognition. In addition to these objective measures, we also collected three measures of subjective memory and attention performance: CAPS criteria C3, which measures the inability to recall an important aspect of the traumatic event, and criteria D3, which rates attention difficulties, as well as item 5 from the Hamilton Anxiety Scale, which rates subjectively experienced difficulties in attention and memory.

Current Occupational and Social Functioning

Several measures related to current occupational and social functioning were also collected. From the CAPS, item 21 measuring impairment in social functioning, and item 22 measuring impairment in occupational or (if currently not working) other important area of functioning were used. In addition we collected employment status (currently working, currently not working), and three scores from the SF-36[37;38]: sum of items 17-19 measuring problems with work or regular daily activities as a result of an emotional problem, item 20 which inquires to what extent physical health or emotional problems interfered with normal social activities, and item 32 which measures how much of the time physical health or emotional problems interfered with social activities.

Table 2: Memory performance in veterans with PTSD and control veterans without PTSD

	PTSD			Controls				
Test	*n*	*M*	*SD*	*n*	*M*	*SD*	*ANOVA*	*p*
AVLT								
Total Correct Trial 1-5	25	42.00	6.93	25	50.68	6.81	F(1,48) = 20.25	0.000
Total Correctly Recalled after Delay	25	8.68	3.11	25	10.88	2.62	F(1,48) = 7.35	0.009
Total Correct Recognition	25	13.76	1.96	25	14.68	0.63	F(1,48) = 4.54	0.038
Total False Positives	25	1.04	1.97	25	0.24	0.83	F(1,48) = 2.19	0.146
CVLT								
Total Correct Trial 1-5	22	53.14	11.35	25	57.52	8.00	F(1,45) = 2.39	0.129
Immediate Recall	22	11.86	3.37	25	12.12	2.42	F(1,45) = 0.09	0.764
Immediate Cued Recall	22	11.86	2.59	25	12.56	2.48	F(1,45) = 0.88	0.352
Delayed Recall	22	11.77	3.35	25	12.40	2.47	F(1,45) = 0.54	0.465
Delayed Cued Recall	22	12.45	2.72	25	12.76	2.28	F(1,45) = 0.18	0.677
Total Correct Recognition	22	15.05	1.13	25	15.24	1.01	F(1,45) = 0.39	0.537
WMS-R								
Figural Memory - Reproduction	24	36.21	3.15	25	37.16	2.48	F(1,47) = 2.91	0.095
Figural Memory - Delayed Recall	24	31.75	7.40	25	36.20	3.34	F(1,47) = 7.07	0.011
Figural Memory - Recognition	24	3.54	0.66	25	3.96	0.20	F(1,47) = 6.98	0.011
Logical Memory - Immediate Recall	25	25.48	7.08	25	30.76	6.35	F(1,48) = 8.44	0.006
Logical Memory - Delayed Recall	25	23.04	8.84	25	27.64	7.08	F(1,48) = 4.50	0.039

PTSD, posttraumatic stress disorder; AVLT, Rey Auditory Verbal Learning Test; CVLT, California Verbal Learning Test (Dutch version VLGT); WMS-R, Wechsler Memory Scale-Revised. Multivariate analysis (MANOVA) was used to compare the two groups on these measures, and post hoc one way ANOVA results are presented in the table.

Data Analysis

Group demographical variables and psychometric variables were examined using two-tailed independent t-tests. A multivariate analysis (MANOVA) was used to evaluate group differences in overall memory performance. Memory performance and general intellectual function were taken as separate domains, thus one MANOVA was used to compare the results of the various memory tasks. Post hoc one-way analysis of variance (ANOVA) was performed to examine group differences in individual items and contrast measures. Current occupational and social functioning measures were examined using the Mann-Whitney U test for ordinal variables, and Fisher's Exact Test for categorical variables. In addition, five individual backward regression analyses were performed, using a model involving all memory measures as predictors, and current occupational and social functioning measures as dependent variables. All statistical analyses were performed with SPSS 12.0 for Windows (SPSS, Chicago, Illinois). The statistical threshold for significance for all measures was set at $p < 0.05$.

Results

Psychometric Data

PTSD patients and control veterans were matched with respect to age, gender, year of deployment, region of deployment, and IQ. Patients with PTSD had significantly greater CAPS, Hamilton A, and Hamilton D scores, (see table 1). All of the control veterans met the DSM-IV A1 criterion for PTSD (i.e. exposure to a traumatic event). According to the SCID, the PTSD group met lifetime (past) DSM-IV (American Psychiatric Association, 1994) diagnostic criteria for the following disorders: major depressive disorder (n = 12), bipolar disorder (n = 3), alcohol abuse (n = 3), substance abuse (n=2), and panic disorder with agoraphobia (n = 1). Seven subjects with PTSD met current diagnostic criteria for major depression. One subject with PTSD met current diagnostic criteria for panic disorder with agoraphobia. Four control subjects met lifetime (past) DSM-IV diagnostic criteria for major depressive disorder, and one subject met lifetime diagnostic criteria for panic disorder with agoraphobia. The SCID did not reveal any current psychiatric disorders among our control subjects.

Neuropsychological Assessment

The performance of all subjects on tests of learning, verbal memory, and visual memory are shown in Table 2. The MANOVA for neuropsychological tests of learning and memory included all measures of the AVLT, CVLT, WMS-R logical memory, and WMS-R figural memory, and revealed a significant main effect of memory performance ($F_{15,31} = 2.039$; p=0.046). Post hoc ANOVAs revealed significantly poorer performance by patients with PTSD for nearly all measures (see table 2): total correct (trial 1-5) on the AVLT, delayed recall score, and the total number of correctly recognized items. In addition, patients with PTSD also revealed significantly reduced performance on the WMS-R figural memory delayed recall score, the WMS-R figural memory recognition score, the WMS-R logical

memory immediate recall score, and the WMS-R delayed recall score. Patients with PTSD did not perform significantly worse on any of the measures of the CVLT.

Veterans with PTSD also scored significantly higher on subjective measures related to attention and memory difficulties such as the CAPS C3 and D3 criteria and Hamilton Anxiety Item 5 (see Table 1). These subjective measures were negatively correlated with a number of objective measures of learning, and immediate and delayed recall of both verbal and visual memory (Pearson's R ranged from -0.337 to -0.602; $p<0.05$).

Table 3: Social and occupational functioning in veterans with PTSD and control veterans without PTSD

Test Variable	*PTSD*	*Controls*	*Mann-Whitney U*	*p*
Employment status				
Currently working	n = 13	n = 24		0.001
Currently not working	n = 12	n = 1		
SF-36				
Sum of items 17-19 (range 0-3)	1.52 (0.81)	0.18 (0.50)	37.5	0.000
Item 20 (range 1-5)	3.19 (1.08)	1.14 (0.35)	10.5	0.000
Item 32 (range 1-5)	2.57 (0.81)	4.50 (0.74)	27.0	0.000
CAPS				
CAPS social functioning (range 0 - 4)	2.21 (0.88)	0.04 (0.20)	14.5	0.000
CAPS occupational functioning (range 0 - 4)	2.25 (0.53)	0.00 (0.00)	0.0	0.000

SF-36, Short Form General Health Survey, scores on item 20 range from 1 (not at all) to 5 (extremely), scores on item 32 range from 1 (all of the time) to 5 (none of the time); Means for both groups are given. Standard deviations are reported in between brackets.

Current Occupational and Social Functioning

Patients with PTSD reported greater impairment in social and occupational (or if currently not working, other important area) of functioning ($p<0.0001$). Patients also reported more problems with work or regular activities as a result of an emotional problem ($p<0.0001$). Physical health and emotional problems interfered more with normal social activities in both intensity ($p<0.0001$) and duration ($p<0.0001$). As was expected, veterans with PTSD were less likely to be currently at work (n=13) than control veterans (n=24) (Fisher's Exact Test, $p<0.001$). Individual regression analyses revealed that memory performance accurately predicted current social and occupational functioning (see table 4). Memory performance accounted for as much as 41% of model variance.

Discussion

This study revealed reduced performance by patients with PTSD on measures of immediate and delayed recall of verbal and visual explicit memory material compared to controls. These findings could not be explained by differences in IQ, years of education, medication use, or substance abuse, providing support for a structural disorder-related memory deficit in patients with PTSD. In addition, we examined several measures related to current social and occupational functioning. Objective memory performance was an accurate predictor of current occupational and social functioning. In this study, veterans with PTSD displayed significantly poorer performance on measures of learning, and immediate and delayed recall of both structured and non-structured verbal material. In the list learning tests, veterans with PTSD acquired fewer words compared to control veterans, and were able to recall fewer words immediately after acquisition. After a delay period, significantly fewer words were recalled by patients with PTSD. The scores on the WMS-R Logical Memory test also indicate impairment of both immediate and delayed recall. These findings are consistent with previous research.[7;15;39;40] Several studies which have examined verbal memory deficits in PTSD with the CVLT were unable to reveal significant differences between patients and controls.[11;22] Our study enables a direct comparison between these two list-learning tests, and shows that although veterans with PTSD perform poorly on both tests, the AVLT is the only test on which their performance was significantly different from veterans without PTSD. This may indicate that the CVLT is not sensitive enough to discriminate reliably between the two groups, and may explain why several of the other studies were unable to confirm a memory deficit. Studies using the CVLT report normal initial acquisition, whereas studies using the AVLT report impairment of initial acquisition.[41] Stuss et al. [42] have argued that patients with impaired frontal lobe function may be less impaired when material is semantically organized (as is the case with the CVLT) than when recalling semantically unrelated material (such as the AVLT).

Table 4: Backward regression for individual model containing memory measures as predictors and current occupational and social functioning as dependent variables

Predictors	*Dependent Variable*	R^2	*Adjusted* R^2	*F*	*df*	*p*
AVTotCor, AVDelay, WMSLA, CVDel, CVDelCu	CAPS Occupational Functioning	0.438	0.368	6.230	5,40	<0.001
AVTotCor, AVDelay, WMSRec, WMSLA, WMSLB, CVIm, CVDel, CVDelCu	CAPS Social Functioning	0.442	0.321	3.661	8,37	0.003
AVTotCor, AVFPos, WMSRec, WMSFA, WMSLA, WMSLB, CVDelCu	SF-36 sum of items 17-19	0.517	0.417	5.192	7,34	<0.001
AVTotCor, WMSFA, WMSLA	SF-36 item 20	0.329	0.276	6.218	3,38	0.002
AVTotCor, CVIm, CVImCu	SF-36 item 32	0.208	0.145	3.322	3,38	0.030

AVTotCor, Rey Auditory Verbal Learning Test (AVLT) total correct trial 1-5; AVDelay, AVLT total correctly recalled after delay; AVFPos, AVLT total false positives; WMSFA, Wechsler Memory Scale Revised (WMS-R) figural memory reproduction; WMSRec, WMS-R recognition; WMSLA, WMS-R logical memory immediate recall; WMSLB, WMS-R logical memory delayed recall; CVTot, California Verbal Learning Test (CVLT) total correct trial 1-5; CVIm, CVLT immediate recall; CVImCu, CVLT immediate cued recall; CVDel, CVLT delayed recall; CVDelCu, CVLT delayed cued recall.

Memory deficits in PTSD have often been linked to the consistent finding of reduced hippocampal volume in patients with PTSD (for review see [40;43;44]). The hippocampus plays an important role in stress regulation, novelty seeking, contextual binding, as well as memory encoding and retrieval. The involvement of the hippocampus in memory consolidation has been firmly established empirically.[45;46] Although the relationship between poorer memory performance and smaller hippocampal volume is often postulated, it is difficult to find a relationship between structural MRI findings and neuropsychological data. Several studies provide evidence that smaller hippocampal volume in patients with PTSD are correlated with memory performance [17;47], but others were unable to find a significant relationship between hippocampal volume and memory deficits.[13;48] In a PET study using word stem completion, patients with PTSD revealed abnormal blood flow in the hippocampus.[49] It has also been shown that women with PTSD have reduced hippocampal activation during memory encoding.[50] Verbal memory impairment in patients with epilepsy correlates significantly with hippocampal pyramidal cell density in the CA3 region and hillar area of the hippocampus.[51]

The underlying neurobiology of the delayed explicit memory deficit found in patients with PTSD in our study could be related to the hippocampal pathology that is often present. The deficits in immediate memory, on the other hand, are more likely to be related to prefrontal cortex and anterior cingulate dysfunction in PTSD. The prefrontal cortex and the anterior cingulate play an important role in attention and working memory performance. Functional imaging studies in patients with PTSD have revealed abnormalities in the dorsolateral prefrontal cortex, medial prefrontal cortex, and anterior cingulate cortex.[50;52-56] As has been addressed, earlier patients with PTSD revealed deficits in recall of semantically unrelated material, but not when explicit memory material was semantically organized. This pattern of performance may reflect frontal lobe dysfunction in PTSD.[41] In a verbal working memory task, patients with PTSD also revealed decreased frontal cortex activity.[57;58] In an fMRI study we also found reduced activation of the prefrontal cortex in patients with PTSD compared to controls during encoding of verbal paired associates.[59]

Visual memory was only assessed in a small number of previously performed studies in PTSD.[7;12-14;24;39;60] Only a few studies were able to demonstrate significant differences between patients with PTSD and controls.[14;60] In our study patients with PTSD did not reveal any differences in immediate recall and reproduction of the four geometrical figures, but did reveal impairment of delayed recall and recognition of these figures. Like Bremner et al [7] we also found that a lower score for the delayed recall Figural Memory subtest of the WMS-R was related to greater PTSD symptom severity as measured with the CAPS. These visual memory deficits may reflect abnormal working memory updating in PTSD.[57;58]

Veterans with PTSD scored significantly lower on measures related to social and occupational functioning. This is in line with epidemiological research, which has also shown that PTSD is associated with substantial levels of disability and loss of quality of life.[29] Impaired social and occupational functioning in PTSD are largely responsible for the enormous societal costs of this disorder (Kessler et al 1995; Kessler et al 2000). In a study of Israeli war veterans with PTSD, occupational and interpersonal functioning in PTSD were also impaired, and positively correlated with PTSD symptoms.[28] In this study, we showed that objective memory performance is an accurate predictor of current social and occupational functioning, providing support for a consideration of cognitive deficits as targets for psychopharmacological intervention in PTSD, as is currently proposed by the MATRICS program in patients with schizophrenia.[61;62]

Findings of a structural memory deficit in PTSD were also consistent with subjective complaints of new learning, memory, and attention deficits in patients with PTSD.[3-5;63;64] Subjective measures of memory and attention difficulties were examined in this study and as expected patients reported more problems with both attention and memory. These subjective measures were negatively correlated with most of the objective memory measures indicating that the subjective reports of memory and attention deficits of patients with PTSD reflect the presence of an actual memory deficit which may be objectively verified.

In this study veterans with PTSD were accurately matched to healthy control veterans with respect to age, year of deployment, and region of deployment. The studied population was of a relatively young age and was in good physical health. Although a large number of studies of neuropsychological function in patients with PTSD have found some type of cognitive dysfunction including memory deficits, specific results differ markedly across studies. Most studies have concentrated on merely one memory test, and did not comprehensively examine memory deficits. Nearly all of these studies also included patients using psychotropic medication, although we know that these drugs have profound effects on cognitive function, particularly during testing. In a comprehensive review of the neuropsychological effects of psychotropic drugs, Stein and Strickland [65] identified several agents commonly prescribed in PTSD, such as benzodiazepines, TCAs and SRRIs, as having persisting anti-cholinergic associated memory impairment and attention decrements. In our study the majority of patients were drug naïve and all patients had not used psychotropic medication or abused substances for a period of at least 6 months prior to inclusion in this study. Thus the demonstrated memory deficit in this study could not be attributed to the effects of medication, substance abuse, or trauma. Several studies were also marred by the added presence of numerous comorbid disorders, as well as differences in IQ. In our study there were no significant differences in IQ between veterans with PTSD and controls. Comorbidity in this study was kept to a minimum. Our patients did have fairly high Hamilton Depression scores, but considering that both PTSD and depression share ten of seventeen symptoms on the Hamilton Depression Scale, this is not unexpected.[66] Findings of memory and attention deficits are also not specific for PTSD, but are found in various other psychiatric disorders such as schizophrenia [67] and major depression.[68] Future research should focus on using memory and attention as outcome parameters for pharmacotherapeutic intervention.[69] Little is known of the neural circuitry underlying the memory and attentional dysfunction in PTSD. Research using PET, fMRI and diffusion tensor imaging may elucidate the neural mechanisms responsible for the observed disorder of learning and memory in PTSD.

References

1. Hoge, C.W. et al. Combat duty in Iraq and Afghanistan, mental health problems, and barriers to care. N Engl J Med 351, 13-22 (2004).

2. Kang, H.K., Natelson, B.H., Mahan, C.M., Lee, K.Y., and Murphy, F.M. Post-traumatic stress disorder and chronic fatigue syndrome-like illness among Gulf War veterans: a population-based survey of 30,000 veterans. Am J Epidemiol 157, 141-8 (2003).

3. White, N.S. Posttraumatic stress disorder. Hosp Community Psychiatry 34, 1061-2 (1983).

4. Thygesen, P., Hermann, K., and Willanger, R. Concentration camp survivors in Denmark persecution, disease, disability, compensation. A 23-year follow-up. A survey of the long-term effects of severe environmental stress. Dan Med Bull 17, 65-108 (1970).

5. Pitman, R.K. Post-traumatic stress disorder, hormones, and memory. Biol Psychiatry 26, 221-3 (1989).

6. Golier, J.A. et al. Memory performance in Holocaust survivors with posttraumatic stress disorder. Am J Psychiatry 159, 1682-8 (2002).

7. Bremner, J.D. et al. Deficits in short-term memory in posttraumatic stress disorder. Am J Psychiatry 150, 1015-9 (1993).

8. Nixon, R.D., Nishith, P., and Resick, P.A. The accumulative effect of trauma exposure on short-term and delayed verbal memory in a treatment-seeking sample of female rape victims. J Trauma Stress 17, 31-5 (2004).

9. Yehuda, R., Golier, J.A., Halligan, S.L., and Harvey, P.D. Learning and memory in Holocaust survivors with posttraumatic stress disorder. Biol Psychiatry 55, 291-5 (2004).

10. Yehuda, R., Golier, J.A., Tischler, L., Stavitsky, K., and Harvey, P.D. Learning and memory in aging combat veterans with PTSD. J Clin Exp Neuropsychol 27, 504-15 (2005).

11. Stein, M.B., Kennedy, C.M., and Twamley, E.W. Neuropsychological function in female victims of intimate partner violence with and without posttraumatic stress disorder. Biol Psychiatry 52, 1079-88 (2002).

12. Stein, M.B., Hanna, C., Vaerum, V., and Koverola, C. Memory functioning in adult women traumatized by childhood sexual abuse. J Trauma Stress 12, 527-34 (1999).

13. Lindauer, R.J., Olff, M., Meijel, E.P., Carlier, I.V., and Gersons, B.P. Cortisol, Learning, Memory, and Attention in Relation to Smaller Hippocampal Volume in Police Officers with Posttraumatic Stress Disorder. Biol Psychiatry (2005).

14. Uddo, M., Vasterling, J.J., Brailey, K., and Sutker, P.B. Memory and Attention in Combat-Related Posttraumatic Stress Disorder (PTSD). Journal of Psychopathology and Behavioural Assessment 15, 43-52 (1993).

15. Vasterling, J.J. et al. Attention, learning, and memory performances and intellectual resources in Vietnam veterans: PTSD and no disorder comparisons. Neuropsychology 16, 5-14 (2002).

16. Brandes, D. et al. PTSD symptoms and cognitive performance in recent trauma survivors. Psychiatry Res 110, 231-8 (2002).

17. Bremner, J.D. et al. Deficits in short-term memory in adult survivors of childhood abuse. Psychiatry Res 59, 97-107 (1995).

18. Moradi, A.R., Doost, H.T., Taghavi, M.R., Yule, W., and Dalgleish, T. Everyday memory deficits in children and adolescents with PTSD: performance on the Rivermead Behavioural Memory Test. J Child Psychol Psychiatry 40, 357-61 (1999).

19. Koso, M. and Hansen, S. Executive function and memory in posttraumatic stress disorder: a study of Bosnian war veterans. Eur Psychiatry (2005).

20. Gilbertson, M.W., Gurvits, T.V., Lasko, N.B., Orr, S.P., and Pitman, R.K. Multivariate assessment of explicit memory function in combat veterans with posttraumatic stress disorder. J Trauma Stress 14, 413-32 (2001).

21. Vythilingam, M. et al. Smaller head of the hippocampus in Gulf War-related posttraumatic stress disorder. Psychiatry Res 139, 89-99 (2005).

22. Neylan, T.C. et al. Attention, learning, and memory in posttraumatic stress disorder. J Trauma Stress 17, 41-6 (2004).

23. Yehuda, R. et al. Learning and memory in combat veterans with posttraumatic stress disorder. Am J Psychiatry 152, 137-9 (1995).

24. Bremner, J.D., Vermetten, E., Afzal, N., and Vythilingam, M. Deficits in verbal declarative memory function in women with childhood sexual abuse-related posttraumatic stress disorder. J Nerv Ment Dis 192, 643-9 (2004).

25. Pederson, C.L. et al. Hippocampal volume and memory performance in a community-based sample of women with posttraumatic stress disorder secondary to child abuse. J Trauma Stress 17, 37-40 (2004).

26. Yehuda, R. et al. Relationship between cortisol and age-related memory impairments in Holocaust survivors with PTSD. Psychoneuroendocrinology 30, 678-87 (2005).

27. Beers, S.R. and De Bellis, M.D. Neuropsychological function in children with maltreatment-related posttraumatic stress disorder. Am J Psychiatry 159, 483-6 (2002).

28. Bleich, A. and Solomon, Z. Evaluation of psychiatric disability in PTSD of military origin. Isr J Psychiatry Relat Sci 41, 268-76 (2004).

29. Alonso, J. et al. Disability and quality of life impact of mental disorders in Europe: results from the European Study of the Epidemiology of Mental Disorders (ESEMeD) project. Acta Psychiatr Scand Suppl 38-46 (2004).

30. First, M. B., Spitzer, R. L., Gibbon, M., and Williams, J. B. W. Structured Clinical Interview for DSM-IV Axis I Disorders. SCID-I/P . 97.

31. Blake, D.D. et al. The development of a Clinician-Administered PTSD Scale. J Trauma Stress 8, 75-90 (1995).

32. Rey, A. L'examen clinique en psychologie. Presses Universitaires de France, Paris (1964).

33. Brand, N. and Jolles, J. Learning and retrieval rate of words presented auditorily and visually. J Gen Psychol 112, 201-10 (1985).

34. Delis, D.C., Freeland, J., Kramer, J.H., and Kaplan, E. Integrating clinical assessment with cognitive neuroscience: construct validation of the California Verbal Learning Test. J Consult Clin Psychol 56, 123-30 (1988).

35. Dekker, P.H., Mulder, J.C., and Dekker R. Scoreprogramma voor de Verbale Leer en Geheugen Test. Swets & Zeitlinger, Lisse, the Netherlands (1996).

36. Wechsler, D. The Wechsler Memory Scale-Revised. Pscychological Corp., San Antonio, TX (1987).

37. McHorney, C.A., Ware, J.E. Jr, and Raczek, A.E. The MOS 36-Item Short-Form Health Survey (SF-36): II. Psychometric and clinical tests of validity in measuring physical and mental health constructs. Med Care 31, 247-63 (1993).

38. Ware, J.E. Jr and Sherbourne, C.D. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 30, 473-83 (1992).

39. Vasterling, J.J., Brailey, K., Constans, J.I., and Sutker, P.B. Attention and memory dysfunction in posttraumatic stress disorder. Neuropsychology 12, 125-33 (1998).

40. Horner, M.D. and Hamner, M.B. Neurocognitive functioning in posttraumatic stress disorder. Neuropsychol Rev 12, 15-30 (2002).

41. Isaac, C.L., Cushway, D., and Jones, G.V. Is posttraumatic stress disorder associated with specific deficits in episodic memory? Clin Psychol Rev (2006).

42. Stuss, D.T., Eskes, G.A., and Foster, J.K. Experimental Neuropsychological Studies of Frontal Lobe Functions. In Boller, F. and Grafman, J. (eds.) Handbook of Neuropsychology. Elsevier, Amsterdam (1994).

43. Kitayama, N., Vaccarino, V., Kutner, M., Weiss, P., and Bremner, J.D. Magnetic resonance imaging (MRI) measurement of hippocampal volume in posttraumatic stress disorder: a meta-analysis. J Affect Disord 88, 79-86 (2005).

44. Geuze, E., Vermetten, E., and Bremner, J.D. MR-based in vivo hippocampal volumetrics: 2. Findings in neuropsychiatric disorders. Mol Psychiatry (2004).

45. Sweatt, J.D. Hippocampal function in cognition. Psychopharmacology (Berl) 174, 99-110 (2004).

46. Martin, S.J. and Morris, R.G. New life in an old idea: the synaptic plasticity and memory hypothesis revisited. Hippocampus 12, 609-36 (2002).

47. Gurvits, T.V. et al. Magnetic resonance imaging study of hippocampal volume in chronic, combat-related posttraumatic stress disorder. Biol Psychiatry 40, 1091-9 (1996).

48. Stein, M.B., Koverola, C., Hanna, C., Torchia, M.G., and McClarty, B. Hippocampal volume in women victimized by childhood sexual abuse. Psychol Med 27, 951-9 (1997).

49. Shin, L.M. et al. Hippocampal function in posttraumatic stress disorder. Hippocampus 14, 292-300 (2004).

50. Bremner, J.D. et al. MRI and PET study of deficits in hippocampal structure and function in women with childhood sexual abuse and posttraumatic stress disorder. Am J Psychiatry 160, 924-32 (2003).

51. Sass, K.J. et al. Verbal memory impairment correlates with hippocampal pyramidal cell density. Neurology 40, 1694-7 (1990).

52. Bremner, J.D. et al. Neural correlates of memories of childhood sexual abuse in women with and without posttraumatic stress disorder. Am J Psychiatry 156, 1787-95 (1999).

53. Bremner, J.D. et al. Neural correlates of exposure to traumatic pictures and sound in Vietnam combat veterans with and without posttraumatic stress disorder: a positron emission tomography study. Biol Psychiatry 45, 806-16 (1999).

54. Lanius, R.A. et al. Neural correlates of traumatic memories in posttraumatic stress disorder: a functional MRI investigation. Am J Psychiatry 158, 1920-2 (2001).

55. Liberzon, I. et al. Brain activation in PTSD in response to trauma-related stimuli. Biol Psychiatry 45, 817-26 (1999).

56. Rauch, S.L. et al. A symptom provocation study of posttraumatic stress disorder using positron emission tomography and script-driven imagery. Arch Gen Psychiatry 53, 380-7 (1996).

57. Clark, C.R. et al. Cerebral function in posttraumatic stress disorder during verbal working memory updating: a positron emission tomography study. Biol Psychiatry 53, 474-81 (2003).

58. Weber, D.L. et al. Abnormal frontal and parietal activity during working memory updating in post-traumatic stress disorder. Psychiatry Res 140, 27-44 (2005).

59. Geuze, E., Vermetten, E., Ruff, M., de Kloet, C. S., and Westenberg, H.G.M. Neural Correlates of Associative Learning and Memory in Veterans with PTSD. unpublished work.

60. Emdad, R. and Sondergaard, H.P. Impaired memory and general intelligence related to severity and duration of patients' disease in Type A posttraumatic stress disorder. Behav Med 31, 73-84 (2005).

61. Marder, S.R., Fenton, W., and Youens, K. Schizophrenia, IX: Cognition in schizophrenia--the MATRICS initiative. Am J Psychiatry 161, 25 (2004).

62. Green, M.F., Kern, R.S., and Heaton, R.K. Longitudinal studies of cognition and functional outcome in schizophrenia: implications for MATRICS. Schizophr Res 72, 41-51 (2004).

63. Danckwerts, A. and Leathem, J. Questioning the link between PTSD and cognitive dysfunction. Neuropsychol Rev 13, 221-35 (2003).

64. Lindem, K. et al. Neuropsychological Performance in Gulf War Era Veterans: Traumatic Stress Symptomatology and Exposure to Chemical-Biological Warfare Agents. Journal of Psychopathology and Behavioral Assessment 25, 105-119 (2003).

65. Stein, R.A. and Strickland, T.L. A review of the neuropsychological effects of commonly used prescription medications. Arch Clin Neuropsychol 13, 259-84 (1998).

66. Shalev, A.Y. What is posttraumatic stress disorder? J Clin Psychiatry 62 Suppl 17, 4-10 (2001).

67. Aleman, A., Hijman, R., de Haan, E.H., and Kahn, R.S. Memory impairment in schizophrenia: a meta-analysis. Am J Psychiatry 156, 1358-66 (1999).

68. Zakzanis, K.K., Leach, L., and Kaplan, E. On the nature and pattern of neurocognitive function in major depressive disorder. Neuropsychiatry Neuropsychol Behav Neurol 11, 111-9 (1998).

69. Vermetten, E., Vythilingam, M., Southwick, S.M., Charney, D.S., and Bremner, J.D. Long-term treatment with paroxetine increases verbal declarative memory and hippocampal volume in posttraumatic stress disorder. Biol Psychiatry 54, 693-702 (2003).

DISCUSSION

9

Summary and Concluding Remarks

Summary

The research described in this dissertation examined PTSD using different neuroimaging techniques and neuropsychological assessment. Part one discussed structural neuroimaging techniques such as hippocampal volumetry and cortical thickness. The results of an application of the cortical thickness technique to a sample of Dutch veterans with deployment-related PTSD were also reported. In part two, three functional neuroimaging studies, one [^{11}C]-flumazenil PET imaging study, and two functional MRI studies performed in Dutch veterans with deployment-related PTSD were described. Part three contains the results of a neuropsychological study on memory performance in PTSD and its relation to social and occupational functioning.

Part I. Structural Neuroimaging

The advance of neuroimaging techniques has resulted in a burgeoning of studies reporting abnormalities in brain structure and function in a number of neuropsychiatric disorders. Measurement of hippocampal volume has developed as a useful tool in the study of neuropsychiatric disorders. In PTSD, nearly all structural MRI studies have focused on measurement of hippocampal volume. **Chapters 2 and 3** report a review of the literature, in all human subject, data-driven papers on hippocampal volumetry were selected, yielding a database of 423 records. From this database the methodology of all original manual tracing protocols of the hippocampus were studied. In **chapter 2** it was reported that these protocols differ in a number of important factors for accurate hippocampal volume determination including magnetic field strength, the number of slices assessed and the thickness of slices, hippocampal orientation correction, volumetric correction, software used, inter-rater reliability, and anatomical boundaries of the hippocampus. These findings were discussed in relation to optimizing determination of hippocampal volume.

In **chapter 3** hippocampal volumetric findings in neuropsychiatric disorders were reported, using all 423 records in the database. Smaller hippocampal volumes have been found in epilepsy, Alzheimer's Disease, dementia, mild cognitive impairment, the aged, traumatic brain injury, cardiac arrest, Parkinson's disease, Huntington's disease, Cushing's disease, herpes simplex encephalitis, Turner's syndrome, Down's syndrome, survivors of low birth weight, schizophrenia, Major Depression, post-traumatic stress disorder, chronic alcoholism, borderline personality disorder, obsessive-compulsive disorder, and anti-social personality disorder. Significantly larger hippocampal volumes have been correlated with autism and children with fragile X syndrome. Preservation of hippocampal volume has been reported in congenital hyperplasia, children with fetal alcohol syndrome, anorexia nervosa, attention-deficit and hyperactivity disorder, bipolar disorder, and panic disorder. The specificity of hippocampal deficits for any psychiatric disorder is thus very low. Possible mechanisms of hippocampal volume loss in neuropsychiatric disorders were discussed.

Structural neuroimaging studies in PTSD have focused primarily on structural alterations in the medial temporal lobe, and only a few have examined gray matter reductions in the cortex. Advances

in computational image analysis provide new opportunities to use semi-automatic techniques to determine cortical thickness, but these techniques have not yet been applied in PTSD. In **chapter 4** twenty-five male Dutch veterans with deployment-related PTSD and twenty-five male veterans without PTSD matched for age, year and region of deployment were examined with structural MRI. Individual cortical thickness maps were calculated from the MR images, and all the subjects' brains were aligned using cortex-based alignment in a region of interest based approach. Cortex-based alignment substantially improves statistical analysis by reducing anatomical variability. Regions of interest examined included the bilateral superior frontal gyri, bilateral middle frontal gyri, bilateral inferior frontal gyri, bilateral superior temporal gyri, and bilateral middle temporal gyri. Veterans with PTSD revealed reduced cortical thickness in the bilateral superior and middle frontal gyri, the left inferior frontal gyrus, and the left superior temporal gyrus. Cortical thinning in these regions may correspond to functional abnormalities observed in these areas in patients with PTSD.

Part II. Functional Neuroimaging

$GABA_A$ receptors are thought to play an important role in orchestrating neural activity throughout the cerebrum. Preclinical and psychopharmacologic studies have provided support for a role of the $GABA_A$ system in PTSD. The objective of **chapter 5** was to assess differences in the benzodiazepine-$GABA_A$ receptor complex in Dutch veterans with and without deployment-related PTSD using [^{11}C]-flumazenil and PET. Nine drug-naïve male veterans with PTSD, and seven male veterans without PTSD, were recruited, and matched for age, region and year of deployment. Each subject received a [^{11}C]-flumazenil PET scan and a structural MRI scan. Dynamic 3D PET scans with a total duration of 60 minutes were acquired after intravenous injection of 371 MBq [^{11}C]-flumazenil. Both template-based and manual region-of-interest (ROI) analysis were performed using the simplified reference tissue model. In addition, parametric binding potential images were generated using Ichise plot analysis. These parametric images were compared on a voxel-by-voxel basis using Statistical Parametric Mapping (SPM2). ROI analysis using both template-based and manual ROIs revealed significantly reduced [^{11}C]-flumazenil binding in PTSD subjects throughout the cortex, hippocampus, and thalamus. SPM analysis of Ichise plots confirmed the ROI analysis. This is the first fully quantitative PET study demonstrating global reduction of [^{11}C]-flumazenil binding in patients with PTSD. This provides support for the role of the benzodiazepine-$GABA_A$ receptor in the pathophysiology of PTSD and is consistent with previous animal research and clinical psychopharmacological studies.

Clinical studies have described altered pain experience in patients with PTSD; however, few empirical studies have examined painprocessing in PTSD in detail. **Chapter 6** describes a functional MRI (fMRI) study of the neural correlates of pain processing in Dutch veterans with deployment-related PTSD. The experimental procedure consisted of a psychophysical assessment and neuroimaging with fMRI. Two conditions were assessed during fMRI in both experimental groups: one with administration of a fixed temperature of 43 °C (fixed temperature condition), and one condition with an individual temperature for each subject but with a similar affective label, equal to 40% of the subjective pain intensity (individual temperature condition). Twelve male veterans with PTSD, and twelve male veterans without PTSD, were recruited, and matched for age, region and year of deployment. Veterans with PTSD rated temperatures in the fixed temperature assessment as less painful compared to control veterans. In the fixed temperature condition, veterans with PTSD revealed increased activation in the left hippocampus, and decreased activation in the bilateral ventrolateral prefrontal cortex, and the right amygdala. In the individual temperature condition veterans with PTSD showed increased activation in the right putamen, and bilateral insula, as well as decreased activity in right precentral gyrus, and the right amygdala. These data provide evidence for reduced pain sensitivity in PTSD. The witnessed neural activation pattern is proposed to be related to altered pain processing in patients with PTSD.

Over the last decades, several studies have reported deficits in learning and memory in patients with PTSD. However, the conclusions from the majority of these studies were not warranted because of confounding factors such as medication use, substance abuse, age, and intelligence. In addition, the neural correlates of memory processing have not been adequately investigated. In chapter 7 and chapter 8 memory performance and processing was examined in Dutch veterans with and without deployment-related PTSD. The fMRI study described in **chapter 7** examined the neural correlates of associative learning and memory in veterans with PTSD and control veterans. Twelve male veterans with PTSD, and twelve male veterans without PTSD, were recruited, and matched for age, region and year of deployment. Changes in the fMRI BOLD response to encoding and retrieval of non-emotional word pairs reflecting deactivation and activation of brain areas involved in associative memory processing were assessed. Veterans with PTSD revealed underactivation of the frontal cortex, and overactivation of the temporal cortex during the encoding phase compared to control veterans without PTSD. Retrieval of the paired associates resulted in underactivation of right frontal cortex, bilateral middle temporal gyri, and the left hippocampus/parahippocampal gyrus in veterans with PTSD. These data support the long-held notion that altered activity in fronto-temporal circuits is related to deficits in memory performance in veterans with PTSD.

Part III. Neuropsychology

The neuropsychological data in **chapter 8** confirmed the finding of reduced memory performance in veterans with PTSD. Fifty Dutch veterans (25 with deployment-related PTSD and 25 without PTSD matched for age, and year and country of deployment) were assessed with a comprehensive neuropsychological test battery consisting of four subtests of the WAIS III (Picture Arrangement, Block Patterns, Similarities, and Vocabulary), WMS-R Figural Memory, WMS-R Logical Memory, California Verbal Learning Test (CVLT) and the Rey Auditory Verbal Learning Test (AVLT). Veterans with PTSD were free of medication and substance abuse. Multivariate analysis of variance was used to assess group differences of memory performance. Veterans with PTSD had similar total IQ scores com-

pared to veterans without PTSD, but displayed deficits of figural and logical memory. Veterans with PTSD also performed significantly lower on measures of learning and immediate and delayed verbal memory. Memory performance accurately predicted current social and occupational functioning.

Concluding Remarks

Very little is known about the biological basis of individual differences in stress response and vulnerability for stress-related mental disorders. The neuroimaging and neuropsychological studies described in this dissertation have provided support for neurobiological alterations in Dutch veterans with deployment-related PTSD. One of the major strengths of all the empirical studies in this dissertation is the use of matched trauma controls. The Veterans Institute in Doorn gave access to a unique population of Dutch veterans. This enabled matching of veterans with deployment-related PTSD to control veterans without PTSD. The groups were carefully matched with respect to year of deployment, region or country of deployment, and age. This means that control veterans had very similar experiences during deployment compared to the patients, and were also approximately the same age at the time of deployment. Studies have shown that the age at which traumatization occurs is an important factor that should be considered. Careful matching of the subjects ensured that this factor was controlled. This means that the results of the previously described studies are disorder related, and not solely due to trauma.

All studies performed, however, are only as good as the methods used. The neuroimaging methods used in this dissertation are state-of-the-art methods. They are generally proven and tried, some admittedly more than others, but there are a lot of methodological issues in neuroimaging that need resolution. Specific limitations of each of the studies presented in this dissertation have been discussed separately in each chapter and will not be repeated here, but it is important to be aware of them when drawing conclusions. Despite its limitations, neuroimaging is a valuable tool that enables a look at the active brain in a relatively noninvasive manner. This provides us not only with new insights into the brain and neurobiological mechanisms, but also sheds light on the manifestation of psychiatric disorders in the brain.

Research is often presented in an exceedingly pretentious way as if science has finally revealed 'truth'. Each new method is often looked upon as a new tool which will provide us with definitive answers. Structural MRI, functional MRI, PET imaging, PET receptor imaging, EEG, magneto-encephalography, diffusion tensor imaging, voxel-based morphometry, or cortical thickness measurements, each offers a new promise and researchers are always lured. However, in the case of complex psychiatric disorders or in the field of cognitive neuroscience, there is no reason to be pretentious. Ultimately, research culminates more research. New questions are raised during the course of every study performed, and unanswered questions always remain. Are the alterations witnessed in these patients with PTSD predisposing factors? Or are they a result of a maladaptive response to traumatic events? One way to approach this problem is to do prospective research. An attempt by the Dutch Department of Defense to provide some answers to this question is in the data acquisition stage. Other more direct questions raised by the studies described in this dissertation should be answered in future studies. It is true that each method will elevate research to new levels, expand the knowledgebase, and advance science, but there is probably no advanced cure that will be discovered by any of these methods. The exact nature of morphological and functional changes witnessed in PTSD and their ultimate effect on the course and nature of the disorder are still not clear. Nevertheless, each study is a small building block which provides another link in a multifaceted model of the etiology and nature of these complex disorders. As Thomas Kuhn stated, true progression in science is only achieved by a 'scientific revolution'.

The studies reported on in this dissertation, while providing a valuable contribution to our knowledge of the lasting consequences of traumatic stress and the pathology of PTSD, also illustrate the complexity of the human psyche and the human brain. It raises the question of whether our thirst for knowledge and our quest for truth will ever be satisfied.

***"War is an ugly thing"* J.S. Mill**

The ugliness of war is epitomized by the lasting deleterious effects of traumatic stress on neurocognition and brain function. For a large number of veterans, war does not end after they are removed from a combat zone, or after an armed conflict has ended. Traumatic stress affects nearly all veterans, but while the majority of veterans learn to live with their experiences, for some veterans traumatic stress seethes inside. These veterans (as many as 5-15% of all veterans) experience a 'war within'.

The war within experienced by a proportion of returning veterans after deployment is threefold in nature. For these veterans, (1) the war is not over, (2) they are at war with themselves, and (3) they experience a 'neurobiological war within'. For veterans with PTSD the end of a stressful six month tour is merely the beginning of a new period in their life which evokes profoundly more stress than that experienced during deployment and may still cost them their lives (both figuratively and literally). It may cost them their occupation, their relationship or marriage, their friends, their lifestyle. It costs them their health (physical and mental) and in several cases even their lives. For these veterans the 'war is still not over'. They are still 'on guard' or alert when they should be relaxed. Their sleep is troubled by frequent nightmares. During the day flashbacks or other reexperiencing phenomena haunt them. People and places, stimuli and events, are avoided because they are constant reminders that may trigger unwanted memories.

These veterans are also 'at war with themselves'. Their minds are troubled by anger, shame and disgust. Some find solace in a quest for recognition of their services for their country. Others crave an 'adrenaline kick,' engage in reckless behavior, or in the abuse of diverse substances, such as alcohol and drugs. Internal conflict within these veterans may provoke violent outbursts and cause anguish among relatives and friends, who do not always understand this 'war within'. Society, fuelled by the media, views these men, who have an unhealthy channeling of emotions, as pariahs and social

outcasts. For some veterans a terminal solution, such as suicide, is the only satisfying permanence left in their turbulent lives.
Finally, there is also another ‘war within’. On a neurobiological level the consequences of the ‘continuing war’ and the ‘war with themselves’ is also visible. Structural neuroimaging has identified a number of morphological changes including smaller hippocampal volume and thinner prefrontal cortex. PET receptor imaging revealed a global decrease in $GABA_A$-benzodiazepine receptor binding. Functional MRI showed that veterans with PTSD have decreased pain sensitivity and altered painprocessing. In addition, veterans with PTSD show altered prefrontal and temporal cortex activation during associative memory processing. Neuropsychological memory assessment confirmed a structural verbal and visual memory deficit which was related to current social and occupational functioning. These neurobiological alterations witnessed in veterans with PTSD provide some acknowledgement that the problems experienced by them are not just ‘figments of the imagination’ but very real neurobiological consequences of traumatic stress.

It is this neurobiological ‘war within’ that we should learn to wage and win.

10

Nederlandse samenvatting en slotbeschouwingen

Samenvatting

In dit proefschrift wordt onderzoek naar de neurobiologie van uitzendingsgerelateerde PTSS bij Nederlandse veteranen beschreven. Er werd hiervoor gebruik gemaakt van verschillende beeldvormende technieken en neuropsychologisch onderzoek.

Deel 1 beschrijft verschillende structurele neuroimaging-technieken waaronder het meten van hippocampaal volume en het bepalen van de dikte van de cortex. Daarnaast worden hierin de resultaten gerapporteerd van een toepassing van de semi-automatische corticale dikte techniek bij Nederlandse veteranen met uitzendingsgerelateerde PTSS. In deel 2 worden drie functionele neuroimaging studies beschreven; een [11C]-flumazenil positron emissie tomografie (PET) studie en twee functionele magnetische resonantie imaging (fMRI) studies. In deel 3 worden de resultaten gerapporteerd van onderzoek naar de relatie tussen de geheugenfunctie en het huidig sociaal en beroepsmatig functioneren bij patiënten met PTSS.

Deel 1. Structurele Neuroimaging

Door de introductie van neuroimaging-technieken en de veelvuldige toepassing ervan, is aangetoond dat er bij diverse neuropsychiatrische stoornissen sprake is van een abnormale hersenstructuur en hersenfunctie. Het meten van hippocampaal volume aan de hand van structurele MRI's heeft zich ontwikkeld tot een bruikbare onderzoeksmethode. Ook bij PTSS hebben nagenoeg alle structurele MRI studies zich gericht op het meten van hippocampaal volume. In de **hoofdstukken 2 en 3** wordt een review van de literatuur gerapporteerd van alle humane en empirische studies over hippocampaal volume. Dit heeft geleid tot een database met 423 studies. Van al deze studies is de gebruikte methodologie voor het meten van hippocampaal volume onderzocht. In **hoofdstuk 2** wordt gerapporteerd dat deze protocollen verschillen met betrekking tot belangrijke factoren voor het accuraat meten van hippocampaal volume. Hierbij moet gedacht worden aan de magnetische veldsterkte van de scanner, het aantal plakken én de dikte van de plakken die bestudeerd wordt, het wel of niet uitvoeren van een hippocampale oriëntatie correctie, de software die gebruikt wordt, interrater reliability en anatomische grenzen van de hippocampus. Deze bevindingen worden besproken in relatie tot het accuraat en optimaal meten van hippocampaal volume.

In **hoofdstuk 3** worden de bevindingen in neuropsychiatrische stoornissen weergegeven, waarbij alle 423 hippocampaal volume studies uit de database zijn gebruikt. Kleinere hippocampi worden voornamelijk gevonden bij personen met epilepsie, de ziekte van Alzheimer, dementie, mild cognitieve beperkingen, oudere mensen, mensen met traumatische hersenbeschadigingen, hart en vaat ziekten, ziekte van Parkinson, ziekte van Huntington, ziekte van Cushing, herpes simplex encephalitis, syndroom van Turner, syndroom van Down, premature geboorte, schizophrenie, depressie, PTSS, alcoholisme, borderline persoonlijkheidsstoornis, obsessief-compulsieve stoornis en anti-sociale persoonlijkheidsstoornis. Significant vergrote hippocampale volumes worden gevonden bij personen met autisme en kinderen met fragile X syndroom. Er wordt geen verandering in hip-

pocampaal volume gerapporteerd bij congenital hyperplasia, kinderen met fetal alcohol syndroom, anorexia nervosa, ADHD, bipolaire stoornis en paniek stoornis. De specificiteit van veranderingen in hippocampaal volume bij elke neuro-psychiatrische stoornis is dus zeer laag. Vervolgens worden mogelijke mechanismen die ten grondslag kunnen liggen aan de veranderingen in hippocampaal volume veranderingen in neuropsychiatrische stoornissen besproken.

Structurele neuroimaging studies bij PTSS focussen zich tot nu toe primair op structurele veranderingen in de mediale temporaal kwab. Er zijn maar enkele studies die af- of toename van de grijze stof in het brein onderzocht hebben. Recente ontwikkelingen in softwarematige beeldanalyse voorzien in nieuwe mogelijkheden om met semi-automatische technieken de dikte van de cortex te bepalen, maar deze technieken zijn nog niet eerder toegepast bij PTSS.

In **hoofdstuk 4** worden vijfentwintig mannelijke veteranen met uitzendingsgerelateerde PTSS en vijfentwintig mannelijke veteranen zonder PTSS, gematched op leeftijd, jaar van uitzending en uitzendingsgebied, vergeleken met behulp van structurele MRI. Er werden individuele corticale diktekaarten berekend van de MRI beelden. Daarnaast werden alle breinen van de individuen 'aligned' met behulp van 'cortex-based alignment' in een 'region of interest' (ROI) analyse. 'Cortex-based alignment' verbetert de statistische analyses aanzienlijk doordat de anatomische variabiliteit verminderd wordt. Geselecteerde ROI waren de bilaterale superieure frontal gyri, bilaterale midden frontale gyri, bilaterale inferieure frontale gyri, bilaterale superieure temporale gyri en bilaterale midden frontale gyri. Bij veteranen met PTSS was de cortex dunner in de bilaterale superieure en midden frontale gyri, de linker inferieure frontale gyrus en de linker superieure temporale gyrus. Een dunnere cortex in deze gebieden hangt samen met functionele veranderingen in deze gebieden bij patiënten met PTSS.

Deel 2. Functionele Neuroimaging

$GABA_A$ receptoren spelen een belangrijke rol in het arrangeren van neurale activiteit door het gehele brein. Preklinische en psychofarmacologische studies hebben tevens aangetoond dat het $GABA_A$ systeem betrokken is bij PTSS. Het doel van **hoofdstuk 5** was om verschillen in het benzodiazepine-$GABA_A$ receptor complex aan te tonen bij Nederlandse veteranen met uitzendingsgerelateerde PTSS door middel van [11C]-flumazenil en PET. Negen medicatie-naïeve veteranen met PTSS en zeven veteranen zonder PTSS werden gerekruteerd en gematched op leeftijd, uitzendingsgebied en jaar van uitzending. Elk individu onderging een [11C]-flumazenil PET scan en een structurele MRI scan. Er werden dynamische 3D PET scans gemaakt met een totale lengte van 60 minuten na een intraveneuze injectie van 371 MBq [11C]-flumazenil. De imaging data werd geanalyseerd met behulp van het 'simplified reference-tissue' model. Verder werden met behulp van Ichise plot analyse parametrische binding potential beelden gegenereerd. Deze parametrische beelden werden vergeleken op een voxel-voxel basis door middel van Statistical Parametric Mapping (SPM2). Dit onderzoek liet zien dat veteranen met PTSS een significant verminderde [11C]-flumazenil binding hadden in de hippocampus, thalamus en in corticale gebieden. Dit is de eerste volledig kwantitatieve PET studie bij veteranen met PTSS. Deze studie bevestigt dat de benzodiazepine-$GABA_A$ receptor een rol speelt in de pathofysiologie van PTSS. Dit is in lijn met de resultaten uit preklinisch en klinisch-farmacologisch onderzoek.

Verschillende klinische studies beschrijven een veranderde pijnbeleving bij patiënten met PTSS. Echter, tot op heden zijn er weinig empirische studies die pijnbeleving en pijnverwerking bij PTSS gedetailleerd onderzocht hebben. In **hoofdstuk 6** wordt een fMRI-studie beschreven, waarbij onderzoek is gedaan naar de neurale correlaten van pijnbeleving bij Nederlandse veteranen met uitzendingsgerelateerde PTSS. De experimentele procedure bestond uit een psychofysisch onderzoek en neuroimaging met fMRI. In beide experimentele groepen werden twee condities onderzocht met behulp van fMRI:

1. een conditie met een vaste temperatuur van 43 °C .
2. een conditie met een individuele temperatuur voor elke deelnemer, waarbij voor alle deelnemers de stimulus temperatuur gelijk was aan 40% van de subjectief bepaalde pijnintensiteit.

Twaalf veteranen met PTSS en twaalf veteranen zonder PTSS werden gerekruteerd en gematched op leeftijd, uitzendingsgebied en jaar van uitzending. Veteranen zonder PTSS ervaarden de temperaturen in de vaste temperatuur conditie als minder pijnlijk dan de controles. In de conditie met een vaste temperatuur van 43 °C hadden veteranen met PTSS meer activatie van de linker hippocampus en minder activatie van de bilaterale ventrolaterale prefrontale cortex en rechter amygdala. In de conditie met een individueel vastgestelde temperatuur toonden veteranen met PTSS meer activatie in de rechter putamen en bilaterale insula, en minder activatie van de rechter precentrale gyrus en rechter amygdala. Het veranderde neurale activatiepatroon wordt verondersteld ten grondslag te liggen aan de veranderde pijnbeleving en een verminderd pijngevoel bij veteranen met PTSS.

In de laatste jaren zijn er verschillende studies geweest die leer- en geheugenstoornissen aangetoond hebben bij patiënten met PTSS. Desondanks zijn de conclusies van het grootste gedeelte van deze studies niet zondermeer houdbaar als gevolg van verstorende factoren zoals medicatie gebruik, misbruik van alcohol en drugs, leeftijd en intelligentie. Bovendien zijn de neurale correlaten van deze geheugenstoornissen niet goed onderzocht. In **hoofdstuk 7 en hoofdstuk 8** zijn de geheugenprestatie en geheugenverwerkingsprocessen bij Nederlandse veteranen met én zonder uitzendingsgerelateerde PTSS onderzocht. De fMRI studie die in **hoofdstuk 7** is weergegeven laat een onderzoek zien van de neurale correlaten van associatief leren en geheugen bij veteranen met PTSS en 'controleveteranen'. Twaalf veteranen met PTSS en twaalf veteranen zonder PTSS werden gerekruteerd en gematched op leeftijd, uitzendingsgebied en jaar van uitzending. Er werden veranderingen in de 'fMRI BOLD respons' bepaald tijdens het encoderen en ophalen van non-emotionele woordparen. Veteranen met PTSS hadden minder activatie van de frontale cortex en meer activatie van de temporale cortex tijdens het encoderen van de woordparen. Tijdens het ophalen van de onthouden woordparen hadden veteranen met PTSS minder activiteit in de rechter frontale cortex,

bilaterale midden temporale gyrus en de linker hippocampus/parahippocampale gyrus. Deze resultaten bevestigen de hypothese dat de fronto-temporale cortex een rol speelt bij PTSS. Veranderingen in de fronto-temporale hersenactiviteit bleek meer samen te hangen met de ernst van PTSS dan met de prestatie op de geheugentaak.

Deel 3. Neuropsychologie

De neuropsychologische data in **hoofdstuk 8** bevestigde reeds eerder gerapporteerde bevindingen van geheugenstoornissen bij veteranen met PTSS. Vijftig Nederlandse veteranen (vijfentwintig met uitzendingsgerelateerde PTSS en vijfentwintig veteranen zonder PTSS gematched op leeftijd, uitzendingsgebied en jaar van uitzending) werden onderzocht door middel van een uitgebreide neuropsychologische testbatterij bestaand uit vier deelonderzoeken van de Wechsler Adult Intelligence Scale III (plaatjes ordenen, blokpatronen, overeenkomsten en woordenschat), Wechsler Memory Scale – Revised (WMS-R) figuren, WMS-R logisch verbaal geheugen, verbale leer en geheugen test en de vijftien woorden test. De veteranen met PTSS waren medicatievrij. Om groepsverschillen in taakprestatie aan te tonen werden multivariate analyses gebruikt. Veteranen met PTSS hadden vergelijkbare IQ scores als 'controleveteranen', maar presteerden significant slechter op visuele en logische-verbale geheugen taken. Veteranen met PTSS presteerden ook significant slechter op korte en lange termijn verbale geheugentaken. De prestatie op geheugentaken bleek een significante voorspeller voor het huidig sociaal en beroepsmatig functioneren te zijn.

Slotbeschouwingen

Er is weinig bekend over de biologische basis van individuele verschillen in de stress responsiviteit en vatbaarheid voor stress-gerelateerde psychische stoornissen. De beeldvormende en neuropsychologische studies die in dit proefschrift beschreven worden, bevestigen de aanwezigheid van neurobiologische veranderingen in Nederlandse veteranen met uitzendingsgerelateerde posttraumatische stress stoornis. De belangrijkste kracht van alle empirische studies in dit proefschrift, is het gebruik van gematchte traumacontroles. Het Veteranen Instituut in Doorn gaf toegang tot een unieke populatie van Nederlandse veteranen hetgeen de gelegenheid schiep, om veteranen met uitzendingsgerelateerde PTSS te matchen aan veteranen zonder PTSS. De groepen werden zorgvuldig gematched met betrekking tot jaar van uitzending, het gebied of het land van uitzending en leeftijd. Dit betekent dat 'controleveteranen' gelijkwaardige ervaringen tijdens uitzending hadden als de patiënten; ook waren zij tijdens uitzending ongeveer even oud. Verschillende studies hebben aangetoond dat de leeftijd waarop een traumatische ervaring wordt opgedaan een belangrijke factor is. Zorgvuldige matching zorgde ervoor dat deze factor constant gehouden kon worden tussen de twee groepen. Dit betekent dat de resultaten van de beschreven studies grotendeels stoornisspecifiek zijn en niet zondermeer zijn toe te schrijven aan het trauma.

Desondanks valt of staat de kwaliteit van de verrichte studies met de methode die gebruikt werd. De neuroimaging-methoden die zijn toegepast in dit proefschrift zijn ultramoderne technieken. Zij zijn algemeen onderzocht en beproefd, sommige meer dan anderen, maar er zijn veel methodologische kwesties in neuroimaging die nog geen aandacht gehad hebben. Specifieke beperkingen van elk van de studies in dit proefschrift zijn afzonderlijk besproken in elk hoofdstuk en zullen hier niet herhaald worden. Het is belangrijk dat men zich bewust is van de beperkingen van elke studie tijdens het vormen van conclusies of besluitvorming hierover. Ondanks de beperkingen is neuroimaging een waardevol instrument dat ons in de gelegenheid stelt om naar het actieve brein te kijken op een betrekkelijk niet-invasieve manier. Dit geeft ons niet alleen nieuwe inzichten in het brein en neurobiologische mechanismen, maar werpt ook het licht op hoe psychiatrische stoornissen zich manifesteren in het brein.

Onderzoek wordt dikwijls op een pretentieuze manier voorgesteld alsof de wetenschap de uiteindelijke "waarheid" heeft geopenbaard. Elke nieuwe onderzoeksmethodiek wordt gezien als het instrument dat ons van definitieve antwoorden zal voorzien. Structurele MRI, functionele MRI, PET imaging, PET receptor imaging, EEG, Magneto-encefalografie, diffusion tensor imaging, voxel-based morphometry of corticale dikte analyses, iedere techniek biedt weer hooggespannen verwachtingen en nieuwe beloftes en onderzoekers worden altijd geprikkeld. Evenwel, met betrekking tot complexe psychiatrische stoornissen of op het gebied van de cognitieve neurowetenschappen is er geen reden om pretentieus te zijn. Uiteindelijk culmineert onderzoek in meer onderzoek. Nieuwe vragen worden verwekt door elke onderzoek dat verricht wordt en er blijven altijd onbeantwoorde vragen. Waren de veranderingen in deze patiënten met PTSS predisponerende factoren? Of zijn zij het resultaat van een maladaptieve respons op traumatische gebeurtenissen? Een manier van benaderen van dit type vraag is om prospectief onderzoek te doen. Een poging door het Ministerie van Defensie om antwoorden op deze vragen te verkrijgen, is nu in het stadium van gegevensverzameling. Andere meer directe vragen die opgeroepen worden door het onderzoek dat beschreven wordt in dit proefschrift, moeten beantwoord worden in toekomstige studies. Het is waar dat elke methodiek het wetenschappelijk onderzoek naar een nieuw niveau tilt, leidt tot voortschrijdend inzicht en de wetenschap bevordert, maar er is vermoedelijk geen geavanceerde behandeling voor PTSS die door enig van deze onderzoeksmethodieken ontdekt zal worden. Het ontstaan van morfologische en functionele veranderingen bij PTSS en hun ultieme uitwerking op het beloop en het ontstaan van de stoornis is nog steeds niet helder. Niettemin is elke studie een kleine bouwsteen die een schakel vormt in een veelzijdig model van de etiologie van deze complexe stoornissen. Zoals Thomas Kuhn poneerde, wordt echte vooruitgang in de wetenschap enkel geboekt door een 'wetenschappelijke revolutie'. Het onderzoek dat is beschreven in dit proefschrift, verzorgt ongetwijfeld een waardevolle bijdrage naar onze kennis van de blijvende gevolgen van traumatische stress en de pathologie van PTSS, maar illustreert tevens de complexiteit van de menselijke psyche en het menselijke brein. Het roept de vraag op of onze honger naar kennis gestild zal worden en of we ooit onze zoektocht naar absolute zekerheid zullen volbrengen.

***"War is an ugly thing"* J.S. Mill**

De lelijkheid van oorlog wordt vervat door de blijvende schadelijke effecten van traumatische stress op neurocognitie en het functioneren van het brein. Voor een groot aantal veteranen eindigt de oorlog niet nadat zij uit de gevechtszone zijn verwijderd of nadat het gewapend conflict beëindigd is. Traumatische stress wordt door veel veteranen ervaren, maar terwijl de meerderheid van veteranen leert leven met hun ervaringen, is de traumatische ervaring voor anderen als een brandende maagzweer. Deze veteranen (ongeveer 5-15% van alle veteranen) ervaren een 'oorlog van binnen'. Deze 'oorlog van binnen' is drieledig. Voor deze veteranen (1) is de oorlog niet over, deze veteranen (2) zijn in oorlog met zichzelf en zij (3) ervaren een 'neurobiologische oorlog van binnen'. Voor veteranen met PTSS is het einde van een stresserende uitzending van zes of negen maanden slechts het begin van een nieuwe periode in hun leven welke soms meer stress en narigheid oproept dan tijdens de uitzending het geval was en hen nog steeds hun leven kan kosten (zowel figuurlijk als letterlijk). Hun beroep, relatie, vrienden of levensstijl worden er door op het spel gezet. Het kost hen hun gezondheid (lichamelijk en geestelijk). Voor deze veteranen is 'de oorlog nog steeds niet voorbij'. Zij zijn nog steeds 'op hun hoede' of alert wanneer zij ontspannen zouden moeten zijn. Hun slaap is gestoord door frequente nachtmerries. Tijdens de dag spoken flashbacks of andere herbelevingsfenomenen in hen rond. Mensen, plaatsen, gebeurtenissen en andere stimuli worden vermeden omdat het voortdurend herinneringen zijn die ongewenste herinneringen zouden kunnen oproepen.

Deze veteranen zijn ook 'in oorlog met zichzelf'. Hun geest wordt gekweld door boosheid, schaamte en gevoelens van machteloosheid. Sommige vinden rust in het zoeken naar erkenning van hun diensten voor hun land. Anderen hunkeren naar een 'adrenaline kick' en vertonen onbezonnen gedrag. Weer anderen misbruiken alcohol of drugs. Het interne conflict van deze veteranen kan leiden tot gewelddadige uitbarstingen en kan veel leed en onbegrip veroorzaken bij familie en vrienden die deze 'oorlog van binnen' niet altijd begrijpen. De maatschappij, gevoed door de media, beziet deze mensen die aan hun emoties op een ongezonde wijze uiting geven, als sociale buitenbeentjes. Voor sommige veteranen is een terminale oplossing, zoals zelfmoord, de enige tevredenstellende permanentie die nog overblijft in hun turbulente levens.

Maar er is ook nog een andere 'oorlog van binnen'. Op neurobiologisch niveau is het gevolg van 'de voortdurende oorlog' en de 'oorlog met zichzelf' ook zichtbaar. Structurele neuroimaging heeft een aantal morfologische veranderingen geïdentificeerd, zoals kleiner hippocampaal volume en een dunnere prefrontale schors. PET receptor imaging liet zien dat deze veteranen een globale afname van GABAA-benzodiazepine receptor binding vertonen. Functionele MRI toonde aan dat bij veteranen met PTSS de pijngevoeligheid is afgenomen en de pijnverwerking is veranderd. Tevens vertonen veteranen met PTSS een veranderd neuraal activatiepatroon tijdens geheugenverwerking. Neuropsychologisch onderzoek bevestigde een verstoring in verbaal en visueel geheugen die gerelateerd is aan het huidig sociaal en beroepsmatig functioneren. Deze neurobiologische veranderingen bij veteranen met PTSS getuigen ervan dat de klachten die door hen ervaren worden niet slechts 'hersenspinsels' zijn, maar echte neurobiologische gevolgen van traumatische stress.

Het is deze neurobiologische 'oorlog van binnen' die gevoerd en overwonnen moet worden.

PUBLIC
ATIONS

PUBLICATIONS

Papers

De Kloet, C. S., Vermetten, E., Geuze, E., Kavelaars, A. Heijen, C.J., and H.G.M. Westenberg. Assessment of HPA-axis function in posttraumatic stress disorder: Pharmacological and non-pharmacological challenge tests, a review. J Psychiatr Res. 2005 Oct 6

De Kloet Vermetten, E., Geuze, E., Lentjes, E.G.W.M., Heijnen, C.J., Stalla, G.K., and H.G.M. Westenberg. Elevated plasma corticotrophin releasing hormone levels in veterans with posttraumatic stress disorder. Submitted

De Kloet Vermetten, E., Geuze, E., Lentjes, E.G.W.M., Heijnen, C.J., and H.G.M. Westenberg. Enhanced cortisol suppression in response to dexamethasone administration in traumatized veterans with and without posttraumatic stress disorder. Submitted

De Kloet, C.S., Vermetten, E., Bikker, A., Meulman, E., Geuze, E., Kavelaars, A., Westenberg, H.G.M., and C.J. Heijnen. Leukocyte glucocorticoid receptor expression and immunoregulation in veterans with and without PTSD. Submitted.

De Kloet, C.S., Vermetten, E., Geuze, E., Heijnen, C.J., Wiegant, V.M., and H.G.M. Westenberg. Elevated plasma arginine vasopressin levels in veterans with posttraumatic stress disorder. Submitted

De Kloet, C.S., Vermetten, E., Geuze, E., Heijnen, C.J., and H.G.M. Westenberg. Dex-CRH

Geuze, E.; Vermetten, E., and Bremner, J. D. MR-based in vivo hippocampal volumetrics: 1. Review of methodologies currently employed. Mol Psychiatry. 2005 10 147-159.

Geuze, E.; Vermetten, E., and Bremner, J. D. MR-based in vivo hippocampal volumetrics: 2. Findings in neuropsychiatric disorders. Mol Psychiatry. 2005 10 160-184

Geuze, E., Westenberg, H.G.M., Jochims, A., de Kloet, C.S., Bohus, M., Vermetten, E., and C. Schmahl. Altered painprocessing in veterans with posttraumatic stress disorder. Arch. Gen. Psychiatry. 2006 in press.

Geuze, E., Vermetten, E., Jochims, A., Bohus, M., Schmahl, C., and H.G.M. Westenberg. Neuroimaging of pain perception in Dutch veterans with and without posttraumatic stress disorder: preliminary results. Ann. N.Y. Acad. Sci 2006 1071: 401-404.

Geuze, E., Vermetten, E., Ruf, M., de Kloet, C.S., and H.G.M. Westenberg. Neural correlates of associative learning and memory in veterans with posttraumatic stress disorder. Submitted.

Geuze, E., Vermetten, E., de Kloet, C.S., Hijman, R., and H.G.M. Westenberg. Neuropsychological performance in relation to current social and occupational functioning in veterans with posttraumatic stress disorder. Submitted.

Geuze, E., van Berckel, B.N.M., Lammertsma, A.A., Boellaard, R., de Kloet, C.S., Vermetten, E., and H.G.M. Westenberg. Reduced $GABA_A$ benzodiazepine binding in veterans with posttraumatic stress disorder. Submitted.

Geuze, E., Vermetten, E., Heinecke, A., de Kloet, C.S., Goebel, R., and H.G.M. Westenberg. Cortical thinning in prefrontal cortex of veterans with posttraumatic stress disorder. Submitted.

Van Liempt, S., Vermetten, E., Geuze, E., and H.G.M. Westenberg. Pharmacotherapy for disordered sleep in posttraumatic stress disorder: a systematic review. Int. Clin. Psychopharmacology 2006 21 193-202.

Van Liempt, S. Vermetten, E., Geuze, E., and H.G.M. Westenberg. Pharmacotherapeutic treatment of nightmares and insomnia in posttraumatic stress disorder: an overview of the literature. Ann. N.Y. Acad. Sci 2006 1071: 502-507.

Book Chapters

Geuze E., Vermetten E., and Westenberg H.: Neuroimaging in Anxiety Disorders. In: Anxiety Disorders Research. Velotis C Ed.:, Nova Publishers, 2005, pp 21-55.

Geuze, E. and Vermetten, E.: Disordered Sleep in Posttraumatic Stress Disorder: Clinical Presentation, Research Findings, and Implications for Treatment. Sleep Psychiatry: Ed.: A. Goldbin, Taylor and Francis, 2004

Vermetten, E., E. Geuze, and Joost Mertens: General Physiological Concepts of Sleep in Relation to Psychiatry. Sleep Psychiatry: Ed.: A. Goldbin, Taylor and Francis, 2004

PUBLICATIONS

Conference Abstracts

E. Geuze. Neural Correlates of Associative Learning and Memory in Veterans with Posttraumatic Stress Disorder. Koninklijke Nederlandse Akademie van Wetenschappen. Amsterdam, the Netherlands. August 30, 2006

E. Geuze, E. Vermetten, C.S. de Kloet, R. Hijman and H.G.M. Westenberg. Neuropsychological function in relation to current social and occupational functioning in posttraumatic stress disorder. International Society of Behavioural Neuroscience. Whistler, Canada. May 24-26, 2006

E. Geuze, E. Vermetten, C.S. de Kloet, R. Hijman and H.G.M. Westenberg. Neuropsychological function in relation to current social and occupational functioning in posttraumatic stress disorder. Society of Biological Psychiatry. Toronto, Canada. May 18-20, 2006

E. Geuze, B.N.M. van Berckel, R. Boellaard, A.A. Lammertsma, E. Vermetten, and H.G.M. Westenberg. Changes in benzodiazepine receptor binding in Dutch veterans with posttraumatic stress disorder assessed with [C-11]-Flumazenil and PET. European College of Neuropsychopharmacology. Nice, France. March 9-12, 2006

E. Geuze, A. Jochims, E. Vermetten, C. Schmahl, and H.G.M. Westenberg. Altered Pain Processing in Posttraumatic Stress Disorder. American College of Neuropsychopharmacology. Waikoloa Village, Hawaii, USA. December 13, 2005

E. Geuze, B.N.M. van Berckel, R. Boellaard, A.A. Lammertsma, E. Vermetten, and H.G.M. Westenberg. Changes in benzodiazepine receptor binding in Dutch veterans with posttraumatic stress disorder assessed with [C-11]-Flumazenil and PET. Neuroscience 2005. Washington D.C., USA. November 12, 2005-11-07

E. Geuze. Altered Pain Processing in Dutch Veterans with PTSD. ISTSS 2005. Toronto, Canada. November 3, 2005

E. Geuze, B.N.M. van Berckel, R. Boellaard, A.A. Lammertsma, E. Vermetten, and H.G.M. Westenberg. Changes in benzodiazepine receptor binding in Dutch veterans with posttraumatic stress disorder assessed with [C-11]-Flumazenil and PET. Society of Cerebral Blood Flow and Metabolism Brain ’05. Amsterdam, The Netherlands. June 10-11, 2005

E. Geuze, A. Jochims, H.G.M. Westenberg, C. Schmahl, and E. Vermetten. Pain Processing in Posttraumatic Stress Disorder. Society of Biological Psychiatry. Atlanta, GA, USA. May 20, 2005

E. Geuze. Benzodiazepine Receptors in PTSD. Annual Meeting Dutch Society of Psychiatry (Nederlandse Vereniging voor Psychiatrie), The Hague, The Netherlands. April 6, 2005

E. Geuze, E. Vermetten, D. Bremner. Hippocampal Volume in Neuropsychiatric Disorders. American College of Neuropsychopharmacology, San Juan, PR, USA. December 12-16, 2004

E. Geuze, E. Vermetten, D. Bremner. Hippocampal Volume in Neuropsychiatric Disorders. Rudolf Magnus Insitute of Neuroscience Summer School, Utrecht, The Netherlands. September 16, 2004

E. Geuze, E. Vermetten, D. Bremner. Hippocampal Volume in Neuropsychiatric Disorders. Annual Meeting Society for Neuroscience, San Diego, USA. October 21, 2004

BANK
WOORD

DANKWOORD

Toen ik in 2001 het symposium over Posttraumatische Stress Stoornis bijwoonde in het Universitair Medisch Centrum Utrecht en daar verschillende (inmiddels welbekende) sprekers hoorde over deze stoornis, had ik niet kunnen denken dat ik mij een jaar later ook met dit onderwerp bezig zou houden.

Aan alle patiënten en controleveteranen die met dit onderzoek mee hebben gedaan, wil ik mijn erkenning en dank uitspreken voor hun deelname aan verschillende onderzoeken. Een aantal van jullie hebben zelfs aan alle onderzoeken deelgenomen. Ik kan met recht zeggen dat er een sterke band is gegroeid met jullie, speciaal met degenen die mee zijn gegaan naar Mannheim. Veel van deze onderzoeken hadden voor de patiënten onder jullie een therapeutische waarde zoals jullie hebben laten weten. Dit boekje is een verantwoording naar jullie toe voor alles wat we met jullie uitgehaald hebben. Ik ben trots op het feit dat we hebben mogen samenwerken en hoop dat jullie je kunnen vinden in de slotbeschouwingen.

Geachte Professor Westenberg, beste Herman, u was de eerste die mij attendeerde op de mogelijkheid om een felbegeerde promotieplaats te verkrijgen bij het Centraal Militair Hospitaal. Na afloop van de sollicitatieprocedure zat ik nog erg in spanning en had de volgende dag om elf uur nog niets gehoord. Toen heb ik er toch maar een telefoontje aan gewaagd en kreeg u vervolgens aan de lijn. Het kleine beetje hoop wat ik nog had, nam u direct weg door te vragen “Heeft Eric nog niet gebeld dan?” Gelukkig bleek mijn gevoel niet goed te zijn en toen Eric om half één met ‘het nieuws’ belde was het niet moeilijk om nog steeds enthousiast te reageren. De eerste keer dat ik samen met Eric uw kamer binnenkwam, was ik nog erg timide en was verbaasd dat u naar mijn mening vroeg en die ook serieus nam. Het wederzijdse respect heb ik erg gewaardeerd. In de afgelopen jaren heb ik veel van u geleerd met betrekking tot het uitvoeren van wetenschappelijk onderzoek. Tijdens verschillende van onze bijeenkomsten heeft u een verbluffende inhoudelijke kennis over psychofarmacologie en neurobiologie laten zien. De drempel naar uw kamer was erg laag, het was nooit een probleem om even bij u binnen te vallen. Mijn oprechte dank voor uw integriteit, vertrouwen en inspanning.

Tevens gaat mijn oprechte dank uit naar **mijn co-promotor, Dr. E. Vermetten. Beste Eric,** in maart 2003 heb ik samen met een hoogzwangere Carien nog geholpen om jouw boekjes weg te brengen… het lijkt nog maar even geleden. Voor jou is dit nu weer een mijlpaal, de eerste promovendus die je hebt begeleid is nu ook zo ver. Jouw enthousiasme, respect en vertrouwen hebben mij zeer goed gedaan tijdens de afgelopen jaren en vormen de basis van onze hechte samenwerking. Slechts een dag was ik aan het werk en je vroeg me al mee naar een congres in Chicago - voor mij een hele onderneming - voor jou een terugkerend evenement ☺. Het werd toch nog een heel jaar later voordat ik de eerste keer met jou mee ging naar Neuroscience 2004, maar inmiddels zijn er al heel wat congressen gevolgd. Een groot aantal hebben wij samen bezocht en je hebt me altijd op sleeptouw genomen en me bij vele mensen geïntroduceerd. Dankzij jou zijn belangrijke internationale samenwerkingsverbanden met Doug Bremner, Christian Schmahl en Ruth Lanius gerealiseerd. Bedankt voor al je goede zorgen en de ruimte die je me gelaten hebt in het werk. Je hebt mij vanaf het begin serieus genomen, mijn opinie gevraagd en altijd gerespecteerd; ik waardeer dat enorm. Je blijft me verbazen door je ongetemperde enthousiasme, energie en het ongekende netwerken, wat misschien wel de belangrijkste les is die je mij hebt geleerd. Je bent een rasechte verkoper en zonder jouw inspanning was het nieuwe onderzoekscentrum van de militaire GGZ waarschijnlijk niet gerealiseerd.

Het voormalige Hoofd van de Dienst Militaire Psychiatrie, de kolonel-arts Unck, was de grote drijfveer van het wetenschappelijk onderzoek. **Beste Ferdi,** je hebt je altijd ingezet voor het belang van veteranen en dankzij jou is er een behandeling voor PTSS in het CMH gekomen. Deze multi-modulaire behandeling heeft door de jaren heen al vele vruchten afgeworpen. Met wat politiek manoeuvreren, wat jou wel is toevertrouwd, heb je de essentiële gelden voor het wetenschappelijk onderzoek binnen gehaald. Dit proefschrift is in dat opzicht ook een ‘kindje’ van jou. Je hebt oprechte interesse getoond voor de resultaten van dit onderzoek en ik hoop dat je er met een zeker gevoel van trots naar kunt kijken.

Zeer gewaardeerde WOPTSD collega’s, ik heb me altijd bevoorrecht gevoeld om met dit hechte team samen te werken. **Carien,** je bent zonder meer mijn meest gewaardeerde collega. Je vroeg al of er iets over beton in het dankwoord kwam, dus om je niet teleur te stellen nog even dit verhaal. Tijdens de sollicitatieprocedure vroeg je aan mij of ik veel dingen tegelijkertijd kon regelen en het overzicht kon bewaren; als moeder van (destijds) drie kinderen wist je maar al te goed hoe belangrijk dat was. Tja, moeder was ik niet… maar ik kwam met een verhaal over het afwerken van beton wat snel hard wordt en daarom ook wel enige flexibiliteit vereist. Of het verhaal je heeft overtuigd weet ik nog steeds niet, maar dat maakt gelukkig niet zo veel meer uit. Ik heb me zeer verbaasd over de ongelofelijke hoeveelheid werk die je verzet. En je blijft me verbazen: moeder van vier kinderen, promoveren en toch nog een hond, zodat je ook in het weekend om zeven uur op mag staan! Vooral in het patiëntcontact heb ik erg veel van je geleerd; het is ook het werk wat je volgens mij het liefste doet. In het begin zonderde ik me soms liever af in een kamertje, maar jij hebt daar wel verandering in weten te brengen. Ik heb erg veel waardering en respect voor hoe jij je werk doet. We hebben samen heel veel werk verricht en vullen elkaar goed aan; de dingen die mij wat minder liggen nam jij voor je rekening en andersom gebeurde dat ook. We hebben een hele goede band en hebben veel met elkaar gedeeld. Volgens mij kun je wel zeggen dat we meer een broer–zus relatie hebben. Het

DANKWOORD

schrijven van de Nederlandse samenvatting en dit dankwoord hebben mij al veel tijd en frustratie gekost. Ik heb echt moeite om mijn gevoelens in het Nederlands te uiten en kan me levendig invoelen hoe jij je soms voelt. Dank voor je opgewektheid, vertrouwen, geduld en respect.

Arthur, zeer gewaardeerde collega psycholoog. Jij bent een echte! Toen ik voor het eerst op de afdeling binnenkwam, maakte jij al onderdeel uit van de polikliniek, maar nog niet van het onderzoek. We hebben altijd met elkaar meegeleefd in goede en minder goede tijden en ik was ook zeer blij toen je eindelijk bij ons in dienst kwam. Ik weet nog dat ik je belde om je te feliciteren; je was toen net op weg naar je moeder voor de kerst en je zat in de bus. Als onze 'resident' psycholoog ben je een frequente vraagbaak voor ons team voor vragen over persoonlijkheid en diagnostiek. Je inhoudelijke kennis van zaken betreffende uitzendingsgerelateerde problematiek en de psychologie van PTSS is een belangrijke poot van ons wetenschappelijk onderzoek. Ik heb het altijd als zeer prettig ervaren om met je van gedachten te wisselen over een reeks uiteenlopende onderwerpen, je mening altijd erg gewaardeerd en je adviezen ook vaak opgevolgd (NB het woord 'klankbord' niet gebruikt☺). Ook het zeer bruikbare commentaar op de Nederlandse samenvatting was erg welkom en ik ben jaloers op het feit hoe jij je in onze taal weet uit te drukken.

Kim, je bent het zonnetje in huis. "Ik leef op zonne-energie" zeg je vaak en dat is je wel aan te zien. Vaak zijn we 's ochtends de enige die al (zo vroeg) aan het werk zijn en dan kom je altijd een bakje koffie doen. Je kritische noot tijdens de donderdag ochtend vergadering mag ik graag horen!

Harry, je bent een belangrijke collega voor mij geworden. Niet alleen als praatpaal, maar ook de praktische zaken van het onderzoek heb jij onder je hoede genomen. Zonder jouw hulp met al de financiële en administratieve kanten, kennis van de defensiecultuur en het schematisch denken was het niet goed gekomen. **Corine,** onze recente aanwinst, ook jij hartelijk bedankt. Samen met Kim geven jullie leiding aan het "keep Elbert fit team". De lunch wandeling kan niet altijd doorgaan, maar het is heel gezellig om even een 'luchtje' te scheppen. **Saskia,** ik weet nog goed dat je de eerste keer langs kwam om over het slaap-review te babbelen. Inmiddels heb je in Kempenhaege al weer veel kennis en ervaring opgedaan over slaap. Ik ben blij dat je straks weer bij ons komt werken en het slaaponderzoek vorm gaat geven. Marit, je hebt een jaar het 'aquarium' onder je hoede gehad. Bedankt voor de gesprekken, het vertrouwen en de gezelligheid. Ik hoop dat het hondenpension nog gerealiseerd gaat worden! Ook **alle andere WOPTSD medewerkers** met wie ik de afgelopen jaren samengewerkt heb, **zoals Joost Mertens, Dorith Harari, Daniel, Anjali, Mayaris en Walter,** zeer bedankt voor jullie collegialiteit en stimulerende gesprekken over wetenschappelijk onderzoek.

Ook mijn stagiaires wil ik bedanken. Dat zijn er eigenlijk maar twee geweest, namelijk **Valerio en Chris**. Valerio, jij was de eerste en samen met Ellen heb je een groot aandeel geleverd in het werven van de controleveteranen populatie. Ook ben je mee geweest naar Mannheim en heb je geholpen met de eerste data acquisitie van het pijn protocol. Later bij Doug Bremner in Atlanta heb ik je twee keer kunnen opzoeken. Ik ben blij te horen dat het zo goed met je gaat. Chris, via Valerio ben je ook op onze afdeling komen werken. Je echte hart ligt in de IT en in zeer korte tijd heb je de hele WOPTSD afdeling geprofessionaliseerd. Je bent nog meer een 'gadget freak' dan Eric. Ik heb veel van je geleerd (vooral met Nederlands 'het -hen en hun- en -als en dan- gebeuren') en heb je gezelligheid altijd gewaardeerd. **Ellen** je was wel 'van' Carien, maar je hebt ook een belangrijk aandeel geleverd in het werven van de controleveteranen en hebt altijd veel interesse getoond voor mijn onderzoek. Bedankt voor dit alles. **Ook de andere stagiaires** die een belangrijk onderdeel van onze afdeling gevormd hebben, de eerste twee **Inge en Sanneke**, toen alles nog op poten gezet moest worden - bedankt voor jullie geduld. **Anne, Marieke, Angela, Amanda, Krista, die andere Anne, Claudia, Martijn en Inez,** ik wens jullie allemaal het beste toe in jullie verdere carrières.

Toen ik die bewuste eerste ochtend binnenkwam op de afdeling Militaire Psychiatrie werd ik direct geïntroduceerd bij de ochtendoverdracht van de kliniek. Al die verwachtingsvolle blikken en het enige wat ik kon vertellen was dat ik iets met "neuroimaging en PTSD" ging doen. Inmiddels is het voor mezelf ook wel duidelijker wat ik doe en ik hoop dat ik met dit boekje een verantwoording naar jullie heb kunnen maken. **Alle mensen van de polikliniek,** ik heb even zitten dubben of ik jullie allemaal op moet noemen, maar ik ben eerlijk gezegd toch bang dat ik iemand vergeet. Hartelijk bedankt voor jullie interesse en hulp, vooral in het verwerven van patiënten. Het is belangrijk dat jullie vertrouwen hebben in het wetenschappelijk onderzoek wat wij verrichten en dat ook uitstralen naar de patiënt. Alleen op die wijze kan het wetenschappelijk onderzoek effectief uitvoering krijgen. Ik hoop dat we nog lang samen kunnen werken om zo het goede voor de patiënt en het belang voor hen te waarborgen. Een aantal van jullie hebben hun interesse voor het onderzoek specifiek getoond door af en toe even aan te waaien en een boompje op te zetten over de stand van zaken. Ik heb dat altijd zeer gewaardeerd en jullie zijn nog steeds van harte welkom. **Het secretariaat, Pauline, Elly, Ria en Tanja** allemaal bedankt voor jullie interesse, meeleven en jullie bijdrage in alles.

Ook de verpleging wil ik bedanken. Niet alleen voor de bakjes koffie die ik bij jullie gedronken heb, maar ook voor jullie meeleven in alles. **Ben, Corrie en al de anderen** bedankt, maar ook in het bijzonder **Robbert en Stephan** die beiden twee keer mee geweest zijn naar Mannheim, als begeleiding en als controle proefpersoon.

De leiding van het CMH, de voormalige Commandanten **de kapitein-ter-zee-arts Gerretse en de kolonel-arts Zijp**, het voormalige Hoofd Patiëntenzorg **de kolonel-arts Gerretsen**, maar ook met name het huidige Hoofd Patiëntenzorg **de luitenant-kolonel-arts Alwon**, bedankt voor het vertrouwen in dit onderzoek en het faciliteren er van. Ook **het MGFB en CDC** bedankt voor de gelden die jullie beschikbaar hebben gesteld voor dit onderzoeksproject. Tevens wil ik Jos Weerts van het Veteranen Instituut in Doorn bedanken voor zijn hulp met het rekruteren en selecteren van een trauma controle groep. **De kolonel-arts IJzerman**, het hoofd van de militaire GGZ, wil ik bedanken voor zijn vertrouwen en de effectieve wijze waarop hij de reorganisatie van de militaire GGZ vorm geeft. Ik vertrouw op een goede en vruchtbare samenwerking. Van het lab van het CMH wil ik

DANKWOORD

Joost Kiewik bijzonder bedanken voor zijn interesse en zijn deelname als controleproefpersoon. Als er weer een Mannheim project opgestart wordt, zul je wel weer benaderd worden…

Uiteraard gaat mijn dank ook uit naar de voorzitter van de leescommisie **Prof. Dr. R.S. Kahn** en de overige leden, **Prof. Dr. R. Kleber, Prof. Dr. A. Aleman en Prof. R. Lanius** voor het lezen van dit manuscript.

Van het **UMC Utrecht** bedank ik **al die medewerkers van de Divisie Hersenen** die mijn verblijf hier aangenamer hebben gemaakt. Een speciaal bedankje richting **Jeanette Sopacua** die altijd wel een uurtje van Hermans' tijd kon vrijplannen en altijd een heerlijke opgewekte verschijning is. Ook **Damiaan, Femke, Sara, André en alle anderen** die er ook nog zijn maar wiens namen ik al weer vergeten ben, bedankt voor jullie interesse en steun in de afgelopen jaren. **Sjoerd en Nienke** bedankt voor de inspirerende tijd tijdens de statistiekcursussen. Ik heb de 'bootstrapping' techniek nog niet toegepast, maar als de patiëntenstroom echter minder wordt… **Ron Hijman** wil ik nog bedanken voor de vruchtvolle samenwerking en zijn inhoudelijk commentaar op het neuropsychologie gedeelte van dit proefschrift. Ook **de neuropsychologie stagiaires,** die het niet erg vonden om al die neuropsychologische onderzoeken af te nemen en altijd interesse getoond hebben in onze veteranen, heel hartelijk bedankt. Een bedankje is ook op zijn plaats richting de medewerkers van de Divisie Beeld en het MRI personeel, met name **Anneke Hamersma** voor het inplannen en **Niels** voor het opzetten van het protocol en andere praktische zaken.

Van de **Vrije Universiteit Amsterdam** zijn er ook een heel rijtje die ik niet wil vergeten. **Dr. B.N.M. van Berckel en Prof. Dr. A.A. Lammertsma** wel in het bijzonder. Zonder jullie ondersteuning en inhoudelijk commentaar was het project nooit voltooid. **Bart,** ondanks alle tegenslagen, heb je me altijd weten te motiveren en me met raad en daad bijgestaan. Ik ben blij dat we dit project tot een goed einde hebben kunnen brengen. **Reina Kloet en Ronald Boellaard,** jullie hebben een hele warme zomer achter de rug in het huidige onderkomen. Bedankt voor al de hulp en raad tijdens de data analyse. **Alle andere medewerkers van Nucleaire Geneeskunde, PET Research en het Radionuclidencentrum,** mijn welgemeende erkenning voor het vele werk wat jullie gedaan hebben. Jullie verrichten een bijzonder staaltje van techniek.

The monthly trips to Mannheim have formed a regular part of my life. It started with a visit, but our collaboration was so good that we ended up doing two projects together. ***Dr. Schmahl, dear Christian****, I am indebted to you for your help and guidance with the "PTSD pain project". You must be thrilled to have two publications in the Archives with this protocol.* ***Anja,*** *thanks for all the work that you have done with and for me in these past years. We have spent many hours together in the scanning room and it amazes me that not one ill word has ever fallen between us; a big thank-you to you. Also to* ***Petra Ludäscher*** *where Valerio and I stayed on our first Mannheim trip.* ***Mattias Ruf*** *thanks for all the support and companionship that you have given.*

In addition, I would also like to extend my thanks to ***Prof. Doug Bremner,*** *who supervised the two reviews on hippocampal volumetry, and kindly invited me to the ACNP last year. I would also like to thank* **Prof. R. Lanius** *for agreeing to be part of the dissertation committee.* ***Dear Ruth,*** *I am very proud to have your acquaintance and am very happy that Eric introduced us. Thank you very much for allowing me to stay at your lab for several weeks.*
From ***Maastricht University,*** *I would like to thank* ***Prof. Rainier Goebel and Armin Heinecke*** *for their support during the cortical thickness analysis. It was two-months of hard work, but with your support I was able to complete it.*

Growing up in Canada has left an indelible stamp on me, and I am still thankful for that period of my life. It has left me with a number of friends who I still see from time to time. I would like to thank ***Anthony and Sherilyn*** *for their friendship and support,* ***Reina en Erica*** *for the late-night conversations, and* ***Les and Tanny*** *for their hospitality and interest in my work.* ***The Rotary Club of Chilliwack and Mount Cheam Christian School*** *for awarding me a scholarship and bursary to cover tuition costs in those first years. I would also like to thank my* ***high-school principal and English teacher, Mr. Stoutjesdijk*** *for teaching me how to write. Your English lessons on paragraph and essay writing still pay off today.* ***Leanne,*** *I am very happy that you agreed to proofread a substantial part of this manuscript.*

Dit dankwoord heb ik wel vijf keer gewijzigd en elke keer kwamen er namen bij… Mocht ik onverhoopt nog iemand zijn vergeten, bij deze mijn hartelijke dank.

Dan wil ik ook **mijn familie** niet vergeten. Op het werk zeggen ze wel eens "Jij hebt ook overal een oom zitten". Inderdaad, ik heb er ook 'maar' zevenentwintig. Een aantal verdienen wel een speciaal bedankje. Met name **Edo** bij wie ik tijdens mijn universitaire opleiding al gewerkt heb, maar waar ik nog steeds op zaterdagochtend kom. Dankzij dat werk heb ik mijn hoofd nog een beetje boven water kunnen houden. **Edo en Jannie,** jullie zijn altijd erg goed voor ons geweest en ook het afgelopen jaar hebben we weer van jullie gastvrijheid mogen genieten. **Geurt,** ook jij hebt je zwak voor familie weer getoond in de laatste maanden. Je hebt een bloeiend bedrijf met een hele goede sfeer die je direct proeft als je binnen komt. Jouw advies en het gratis verzorgen van de vormgeving waardeer ik zeer. **Kees Langerak** (onderdeel van de Muntz-familie), ik ben erg trots op de uiteindelijke vormgeving van dit proefschrift. Je hebt heel goed weergegeven wat ik graag wilde; het concept "oorlog, defensie, veteraan" moest er opliggen, maar toch net niet. Daar ben je naar mijn mening zeer in geslaagd.

DANKWOORD

Al mijn verdere familie bedankt. Met name zij die oprechte interesse hadden in het werk wat ik gedaan heb. **Coby,** bedankt voor alles. Je hebt een ontzettend moeilijke periode achter de rug en ik hoop dat je de kracht krijgt om alles te dragen. Ik vind het erg jammer dat mijn schoonvader dit niet meer mee kan maken. **Alle Nederlandse vrienden en vriendinnen, Bas en Josine, Bart en Klaaske, Gert-Jan en Gerlinde, Gerrit en Hanna, Andries, Henrike, Michelle en Henny,** bedankt voor het meeleven.

Opa en oma Kok, bedankt voor het aanhoren van de verhalen en de zakken snoep die ik (nog steeds) mee krijg als ik een buitenlands reisje moet maken. **Oma Geuze** ook bedankt voor het meeleven met het bereiken van deze nieuwe mijlpaal. Ik vind het heel erg jammer dat opa Geuze dit niet meer heeft mogen beleven. Hij was een van de weinige aan wie je je verhaal maar een keer hoefde te vertellen en het begreep. Ik weet hoe trots hij geweest zou zijn als hij dit mee had mogen maken.

Mijn broers, Diederik, Joost en Dinant. We zijn samen opgegroeid en hebben een zeer sterke band met elkaar, ik ben blij dat het nog steeds tot uiting komt. Jullie hebben altijd mijn verhalen aangehoord en oprechte interesse getoond. Ook hebben jullie alle drie inhoudelijk en bruikbaar commentaar gegeven op de inleiding van dit proefschrift, wat ik zeer gewaardeerd heb. Diederik, je bent een echte oudere broer die veel weg heeft van opa Geuze en zeker net zo glunderend kijkt naar dit boekje als hij gedaan zou hebben; ik ben blij dat je mijn paranimf wilde zijn. **Mijn zus Jennifer, broer Neil en zus Gwendolyn** ook bedankt voor het aanhoren van de verhalen van jullie broer. Wie weet gaat een van jullie dit ook nog eens doen…

Tenslotte wil ik nog vier belangrijke mensen in mijn leven bedanken. Dat zijn allereerst **mijn ouders**, die ons een buitengewone opvoeding gegeven hebben. Ik heb erg veel respect voor datgene wat jullie meegegeven hebben. Heel hartelijk bedankt voor alles wat jullie voor mij hebben betekend. Jullie hebben altijd veel interesse getoond in het werk wat ik uitgevoerd heb en me altijd gestimuleerd om verder te kijken. Dankzij pa, die vond dat ik toch wat nuttigs moest doen met mijn leven, ben ik toch geen geschiedenis gaan studeren en ik denk dat het een verstandige beslissing is geweest. De komende tijd gaat er voor jullie nog veel veranderen en ik wens jullie beiden en pa in het bijzonder daarbij Gods zegen toe.

Mijn lieve vrouw Evelinda wil ik in het bijzonder bedanken voor al haar begrip en toewijding. Evelinda, je bent altijd een opgewekte meid die mij in alle dingen hebt gesteund. Steun en oprechte interesse van het thuisfront is hard nodig bij een project als deze. Je hebt nooit geklaagd, geen ding was je te veel. Tijdens de vele Mannheim bezoeken of als ik op congres was stond je alleen voor alles, maar gelukkig regel jij alle belangrijke zaken (zoals aan- en verkoop van huizen ☺) prima zonder mij. Ik ben zeer bevoorrecht met jou; je opgeruimde en extroverte karakter heeft me bewaard voor sociale isolatie. Tijdens de promotie heb je vaak meegeholpen met allerlei klusjes, van het nakijken van mijn Nederlands, het regelen van de meer praktische zaken tot het aansturen van neuroimaging software toe. Ik ben nogal nuchter van aard, dat weet jij maar al te goed en zal niet vaak met een bloemetje thuis komen. Daarin schiet ik te kort - maar aan de andere kant heeft onze relatie dat gelukkig ook niet nodig. Je hebt ontzettend veel meegemaakt in de afgelopen jaren. Eerst het verlies van je moeder, de ziekte van Coby en van Aletta en toen het overlijden van je vader. Ondanks alles ga je niet bij de pakken neer zitten. Ik bewonder je daarvoor. Ik hoop dat we straks in ons nieuwe huis een iets rustiger vaarwater krijgen en dat we binnenkort ook weer samen, met Frédérique, naar Canada op vakantie kunnen.

Mijn lieve Frédérique, ik ben erg trots op je en laat graag je foto's aan iedereen zien. In tegenstelling tot mij ben jij tenminste nog fotogeniek. Jij betekent heel veel voor mij en mama en wat dat betreft zou elke promovendus een kind thuis moeten hebben om verantwoordelijk voor te zijn. Het bevordert de voortgang van een promotietraject op meer dan een wijze. Straks hoeft papa niet meer 's avonds achter de computer te zitten en ga ik samen met jou en mama lekker fietsen om de overtollige vergaarde kilo's kwijt te raken.

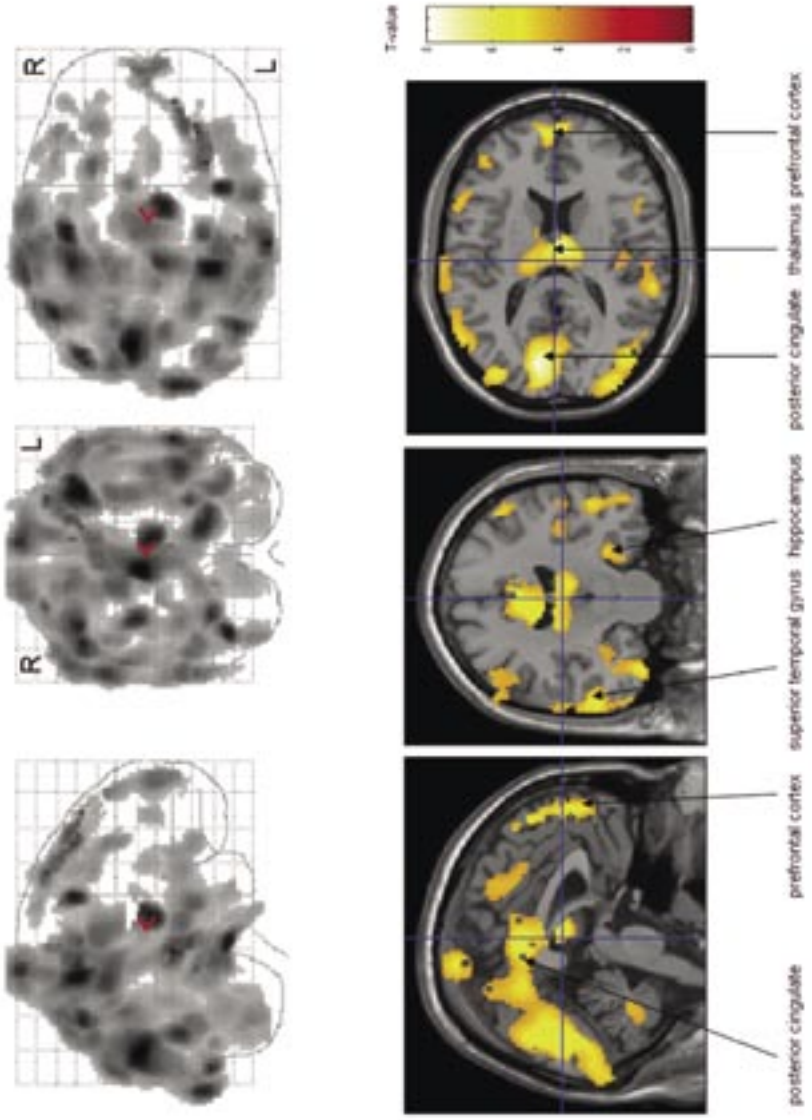
Tenslotte, het is mijn vaste overtuiging dat geen van dit alles tot stand was gekomen zonder de hulp van **God**. Zonder Zijn bewarende hand was ik nooit tot dit in staat geweest en ik heb ook tijdens dit werk gevoeld in alles diep afhankelijk van Hem te zijn. Als ik terugkijk op alles wat er de afgelopen jaren gerealiseerd is en hoe voorspoedig alles is gegaan, moet ik toch wijzen dat de eer daarvoor alleen God toekomt.

Elbert Geuze, zomer 2006

COLOR FIGURES

Organized

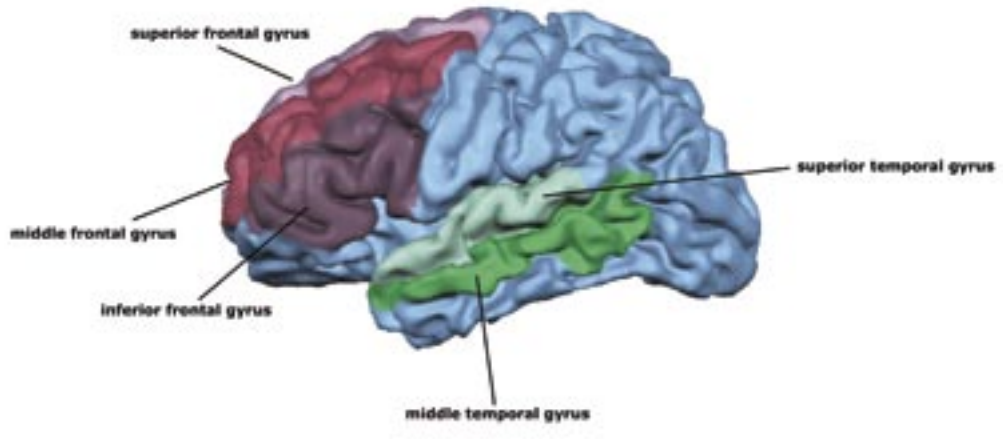
by chapter



SECTION 2 - chapter 5 page 129

Figure 3: SPM analysis of Ichise binding potential images.

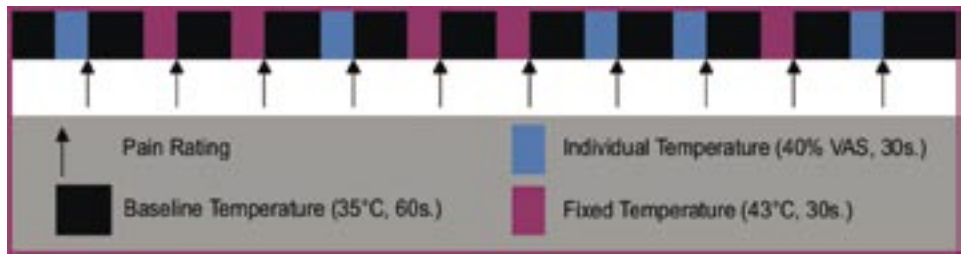
Veterans with PTSD showed decreased [^{11}C]-flumazenil binding in PTSD subjects compared to controls throughout the occipital cortex, temporal cortex, parietal cortex, prefrontal cortex, insular cortex, thalamus, and hippocampus.



SECTION 1 - chapter 4 page 113

Figure 1: Regions of interest.

This figure shows the selected regions of interest (ROI) for the left hemisphere (ROI for the right hemisphere were the same). These included the superior frontal gyrus, middle frontal gyrus, inferior frontal gyrus, superior temporal gyrus, and middle temporal gyrus.

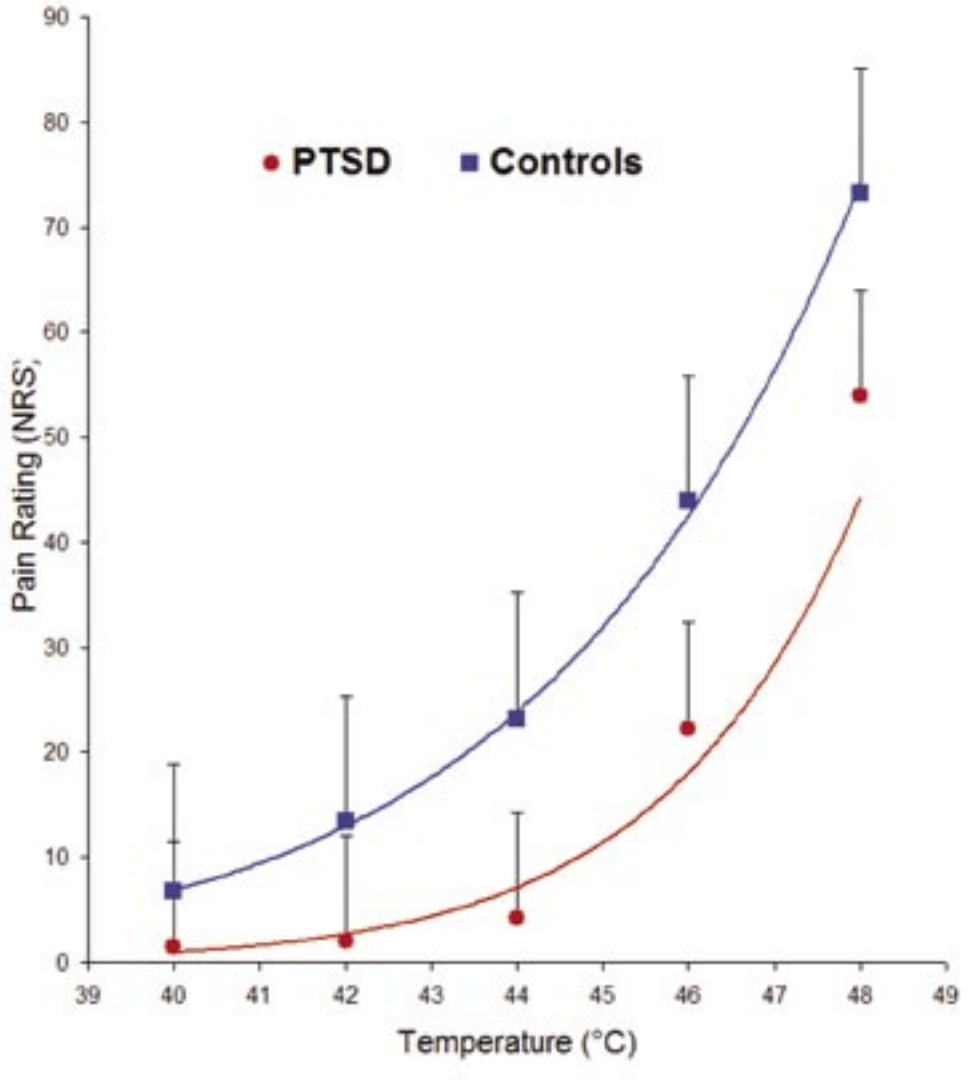


SECTION 2 - chapter 6 page 141

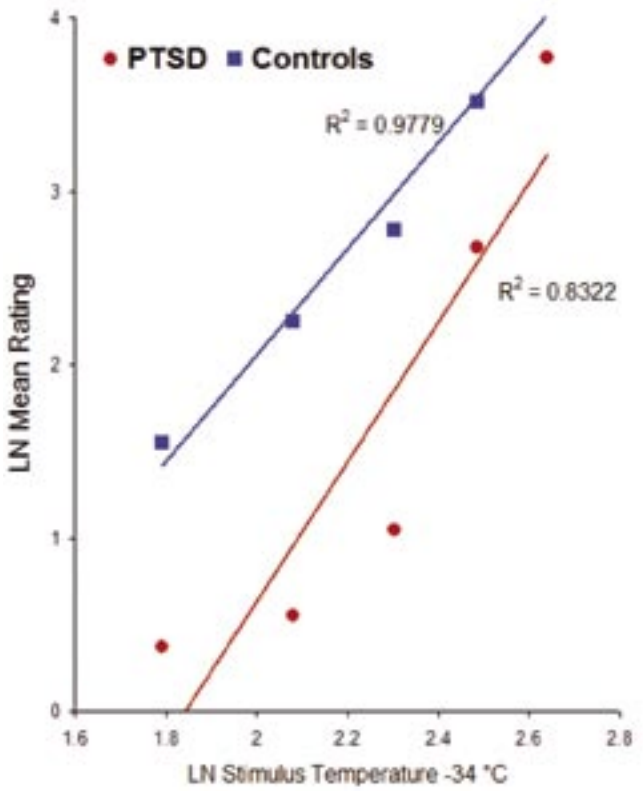
Figure 1: The fMRI scanning protocol.

Five 30s blocks of the individual temperature (equal to 40 on the NRS) were alternated with five 30s blocks of the fixed temperature (43 °C), and 60s baseline temperature blocks (35 °C).

(a)



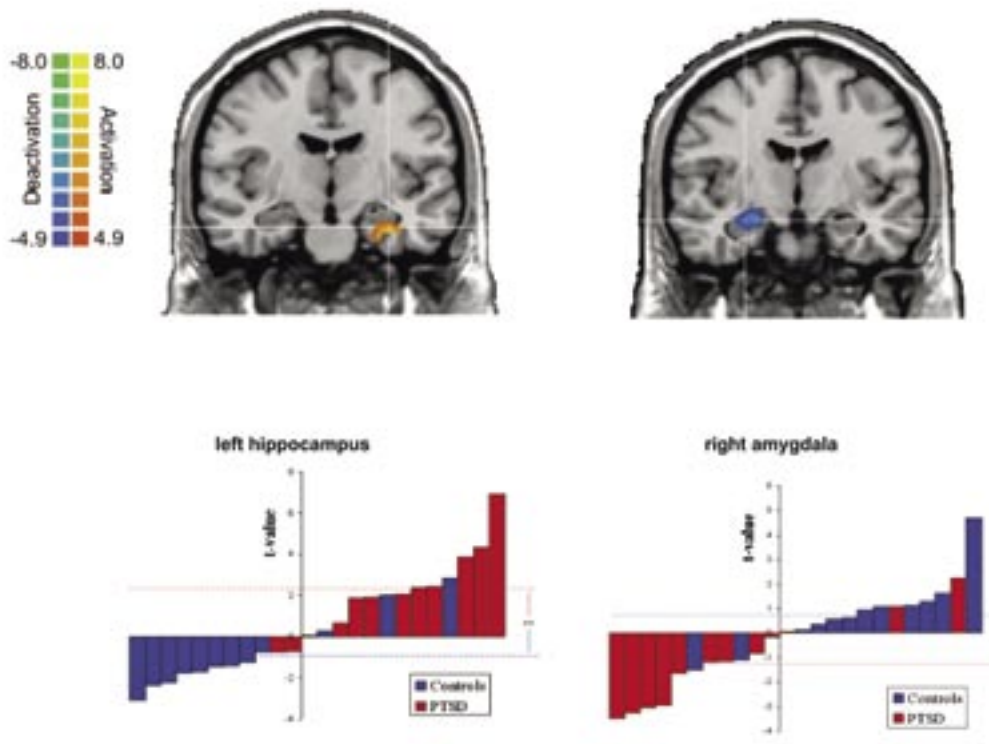
(b)



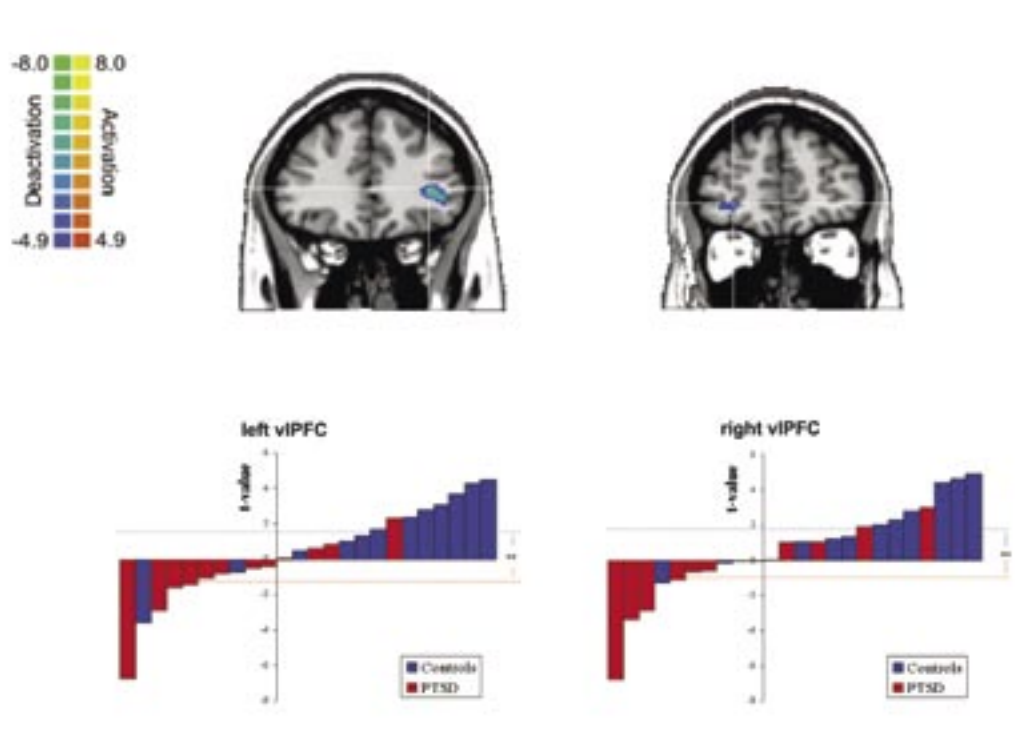
SECTION 2 - chapter 6 page 143

Figure 2a/b: Temperature and perceived pain intensity in patients and controls. Stimulus temperature-numerical rating functions plotted in linear coordinates (a). Red circles and blue squares represent means of perceived pain intensity (0-100) in patients and controls. Standard errors are indicated by vertical bars. Stimulus temperature-numerical rating functions plotted in log-log coordinates (b). Regression lines are displayed as solid red and blue lines for patients and controls, respectively. The slopes of these lines (and hence the power function exponents) are 4.03 for patients with PTSD, and 3.07 for controls.

(a)

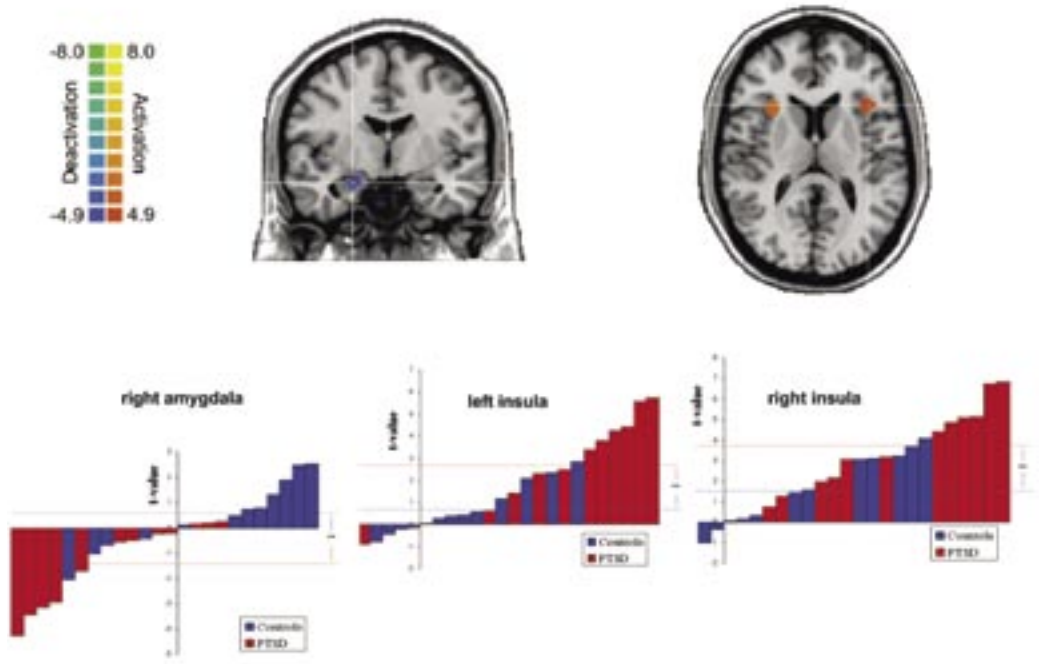


(b)



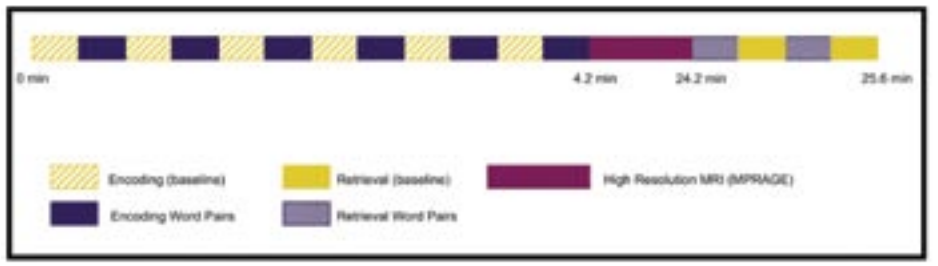
SECTION 2 - chapter 6 page 146

Figure 3a/b: Group comparison of responses to fixed temperature conditions.
Brain activity for the fixed temperature condition is shown in the left hippocampus and the right amygdala (a) and the left ventrolateral prefrontal cortex (vlPFC) and right vlPFC (b). The upper row displays the group differences between patients and controls in coronal slices ($p < 0.001$ cluster corrected with a minimum cluster size of 200 voxels, whole brain corrected). The lower part of the figure displays the ROI-based random effects analysis with individual t-values sorted by height in PTSD patients (red bars) and controls (blue bars). Positive values indicate that the condition-specific predictor explained a net signal increase from the mean signal during baseline, whereas negative values explained a net signal decrease. Group means are marked by red and blue dashed lines for patients and controls, respectively (** $p < 0.01$, two tailed t-test).



SECTION 2 - chapter 6 page 146

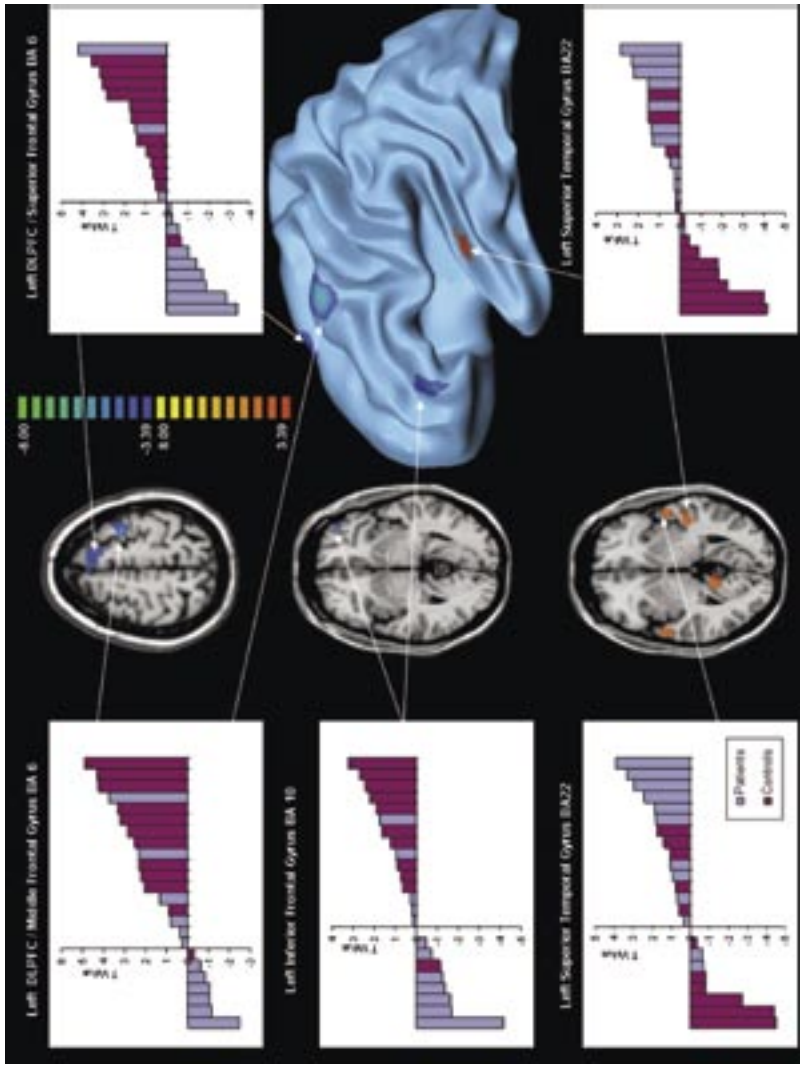
Figure 4: Group comparison of responses to individual temperature conditions.
Brain activity for the fixed temperature condition is shown in the right amygdala and bilateral insula. The upper row displays the group differences between patients and controls in coronal and transverse slices ($p < 0.001$ cluster corrected with a minimum cluster size of 200 voxels, whole brain corrected). The lower part of the figure displays the ROI-based random effects analysis with individual t-values sorted by height in PTSD patients (red bars) and controls (blue bars). Positive values indicate that the condition-specific predictor explained a net signal increase from the mean signal during baseline, whereas negative values explained a net signal decrease. Group means are marked by red and blue dashed lines for patients and controls, respectively (** $p < 0.01$, two tailed t-test).



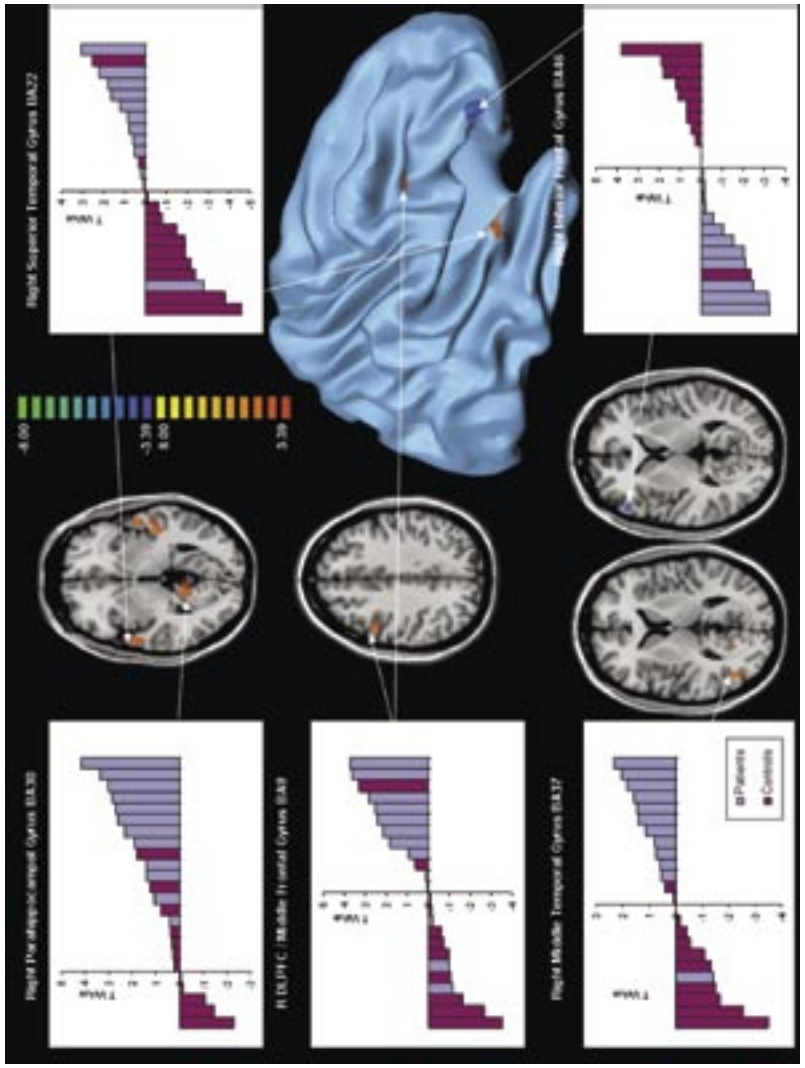
SECTION 2 - chapter 7 page 159

Figure 1: The fMRI scanning protocol.
The first task consisted of twelve 21 s blocks (6 stimuli per block, 3.5 s ISI) in which encoding blocks (presentations of word-pairs, e.g. rose-flower) were alternated with control blocks (presentations of successive numbers of two figures, e.g. 31–32), starting with the latter. This was followed by the MPRAGE, and the retrieval run. The retrieval run consisted of four 21 s blocks (6 stimuli per block, 3.5 s ISI) in which retrieval blocks (presentations of the first word of a pair, e.g. rose) were alternated with control blocks (presentations of one number of two figures, e.g. 28), starting with the latter.

(a)



(b)



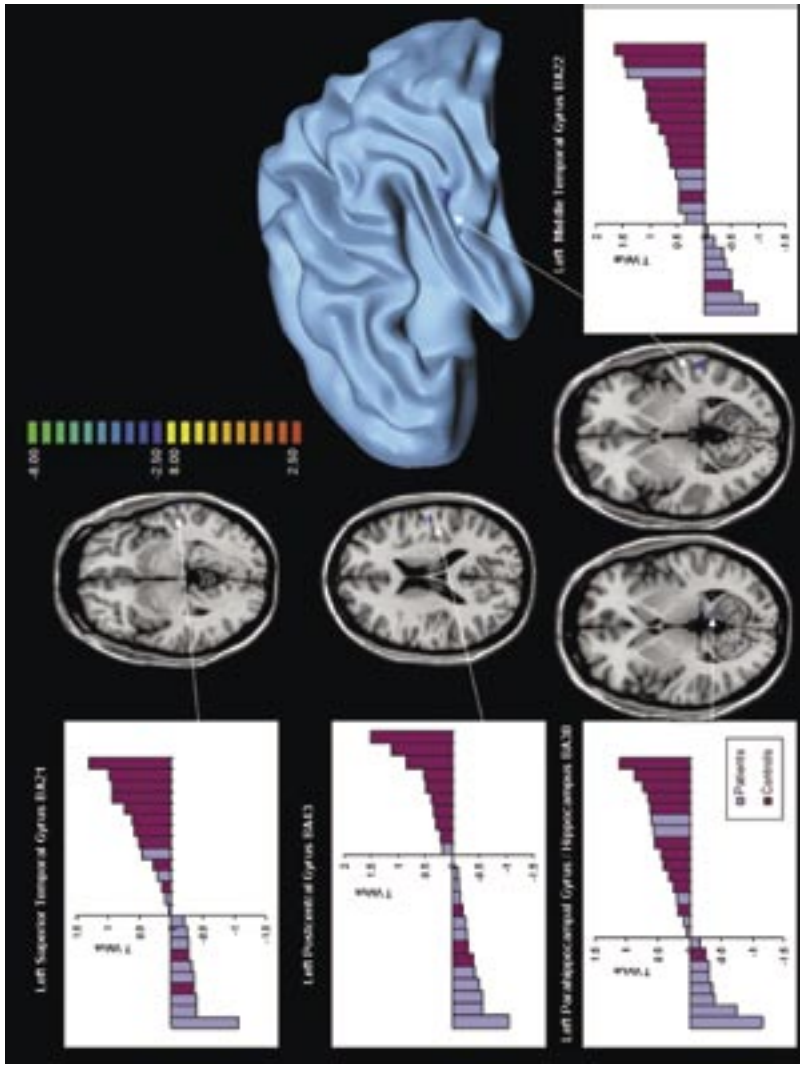
SECTION 2 - chapter 7 page 161

Figure 2a/b: Group comparison of responses to the encoding condition.

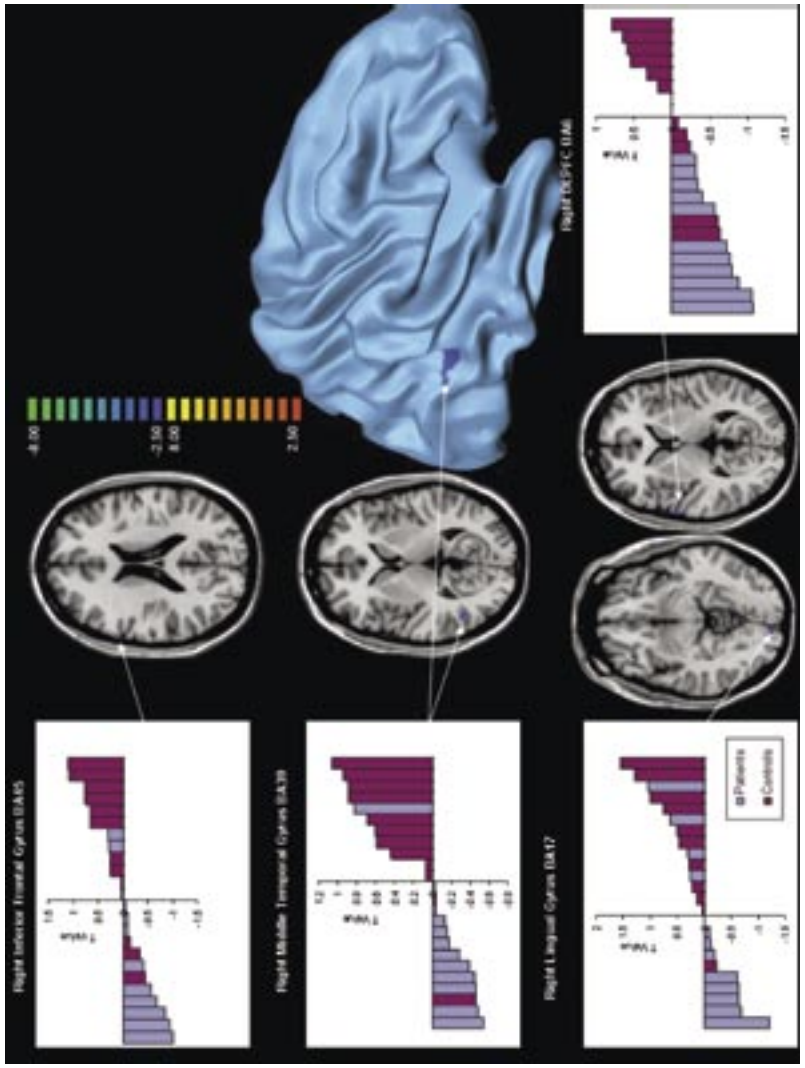
Brain activity for the encoding condition is shown for the left hemisphere (a) and the right hemisphere (b). Areas in which patients revealed more activity are displayed in red; areas in which patients revealed less activity are shown in blue. ($p < 0.01$ cluster corrected with a minimum cluster size of 50 voxels, random effects analysis). Transverse slices of the brain are in radiological orientation.

The left and right sides of the figure displays the ROI-based individual t-values sorted by height in PTSD patients (red bars) and controls (blue bars). Positive values indicate that the condition-specific predictor explained a net signal increase from the mean signal during baseline, whereas negative values explained a net signal decrease.

(a)



(b)



SECTION 2 - chapter 7 page 163

Figure 3a/b: Group comparison of responses to the retrieval condition.

Brain activity for the encoding condition is shown for the left hemisphere (a) and the right hemisphere (b). Areas in which patients revealed more activity are displayed in red; areas in which patients revealed less activity are shown in blue. ($p < 0.01$ cluster corrected with a minimum cluster size of 50 voxels, random effects analysis). Transverse slices of the brain are in radiological orientation. The left and right sides of the figure displays the ROI-based individual t-values sorted by height in PTSD patients (red bars) and controls (blue bars). Positive values indicate that the condition-specific predictor explained a net signal increase from the mean signal during baseline, whereas negative values explained a net signal decrease.