APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

2. DATE SUBMITTED	Applicant Identifier
3. DATE RECEIVED BY STATE	**State Application Identifier**

1. * TYPE OF SUBMISSION

☐ Pre-application ☑ Application ☐ Changed/Corrected Application

4. Federal Identifier

5. APPLICANT INFORMATION *** Organizational DUNS:** 624218814

* Legal Name: Palo Alto Institue of Research and Education, Inc.

Department: | Division:

* Street1: 3801 Miranda Ave | Street2: PO Box V-38

* City: Palo Alto | County: | * State: CA: Californ

Province: | * Country: JNITED ST | * ZIP / Postal Code: 94304-0038

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6. * EMPLOYER IDENTIFICATION *(EIN) or (TIN):*

1770207331A1

7. * TYPE OF APPLICANT:

M: Nonprofit with 501C3 IRS Status (Other than Institution of Higher Education)

Other (Specify):

8. * TYPE OF APPLICATION: ☑ New

☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

Small Business Organization Type

☐ Women Owned ☐ Socially and Economically Disadvantaged

If Revision, mark appropriate box(es).

☐ A. Increase Award ☐ B. Decrease Award ☐ C. Increase Duration

☐ D. Decrease Duration ☐ E. Other *(specify)*:

9. * NAME OF FEDERAL AGENCY:

Dept. of the Army -- USAMRAA

* Is this application being submitted to other agencies? Yes ☐ No ☑

What other Agencies?

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:

12.420

TITLE: Military Medical Research and Development

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:

MEMTRAX – A Flexible Computer Game System for Assessing Cognitive and Brain Pathology Associated with TBI and for Augmenting and Monitoring Ref

12. * AREAS AFFECTED BY PROJECT *(cities, counties, states, etc.)*

N/A

13. PROPOSED PROJECT:

* Start Date	* Ending Date
09/01/2008	08/31/2012

14. CONGRESSIONAL DISTRICTS OF:

a. * Applicant	b. * Project
CA-014	US-all

15. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix:	* First Name:	Middle Name:	* Last Name:	Suffix:
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Position/Title: Staff Psychiatrist | * Organization Name: VA Palo Alto Health Care System

Department: | Division: War Related Illness & Injury

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OMB Number: 4040-0001

Expiration Date: 04/30/2008

Technical Abstract

Traumatic brain injury (TBI) has been estimated to be present in over 20 percent of the injured soldiers returning from the conflicts in Iraq and Afghanistan. However, the extent of brain injury is problematic to assess because the resulting deficits are frequently difficult to evaluate. In many cases when a head injury has been reported, possibly even including an episode of loss of consciousness, there is no obvious residual problem and standard brain scans show no pathology, though the individuals may be noted to have changes in cognitive, behavioral, and neuropsychiatry function. Further, for many of these patients, these problems have impaired their ability to function near levels that they had prior to their injuries. To aid with clinical diagnosis, clinicians have utilized recent advances in brain imaging, particularly MRI-DTI (magnetic resonance imaging with diffusion tensor imaging). These scans have shown damage to fragile axons that are critical to many higher brain functions, providing a reasonable explanation for the first time why TBI causes so much dysfunction. However, currently treatment for victims of TBI focuses on teaching patients to compensate for their loss in brain function or motor skill. There is no medication or direct intervention aimed at brain substance that can restore lost connections. However, brain neuroplasticity is active throughout life and can be called into action for rehabilitation. Therefore, our hypothesis is that the key to rehabilitating a patient with TBI that has cognitive impairment is intense cognitive stimulation directed at the specific deficits being suffered.

The specific aims of this project are in two parts, those that address the measurement of the brain injury and those that address the cognitive rehabilitation. First, we hope to determine the relationship between local axonal shearing as measured on MRI-DTI scans and cognitive dysfunction. The second aim is to determine whether one year of intense cognitive-rehabilitation will enhance connectivity of brain regions subserving similar functions to those areas where axonal shearing occurred. Thirdly, we will strive to determine whether one year of intense cognitive-rehabilitation provides benefit to TBI patients on any other measures of brain or cognitive function. Finally, we hope determine whether any neuropsychiatric diagnoses, medication treatments, or other interventions interact with baseline conditions to moderate treatment outcomes.

The study design of this project has two parts, the first to evaluate a series of 100 mild TBI cases to determine the relationship between axonal disconnection and cognitive dysfunction, with comparison to 50 normal subjects, 50 moderate TBI patients (200 subjects total). Second, a computer-based cognitive evaluation and stimulation system, MEMTRAX, will be adapted for prescribing the intensive brain rehabilitation that is needed individually for these injured soldiers to recover mental function to return to productive lives. This program will be provided to 75 of the mild TBI cases, 25 of the normal subjects, and 25 of the moderate TBI cases, while a placebo condition (routine care) will be used for 25 of the mild TBI cases and 25 of the moderate TBI cases, with no specific instructions for 25 of the normal controls. The benefit of the rehabilitation program will be assessed with the MRI-DTI imaging to provide solid evidence of the effect of the MEMTRAX intervention. A full panel of traditional and innovative assessment techniques, including neuropsychological assessment, PET/CT, and qEEG/ERP/RT, will also be used to confirm and support the findings of this study.

Public Abstract:

With advances in body armor technology and acute trauma care, many military service members are now surviving injuries that would have been fatal in previous wars. However, the brain remains susceptible to non-penetrating injuries from high impact collisions and explosive blasts. In fact, it has been estimated that over 20 percent of the injured soldiers returning from the conflicts in Iraq and Afghanistan will have some form of traumatic brain injury (TBI).

This study will demonstrate how cognitive rehabilitation can make a significant difference in the brain connectivity, cognitive function, and daily lives of those suffering from TBI, as measured by both brain imaging studies, neuropsychological testing, and functional assessment tools.

By conducting neurological tests, we expect that the specific problems such as memory impairment, language difficulties, visuospatial disorganization, decision making problems, etc. can be directly related to brain damage. We will then relate these specific problems to identified portions of the brain through the use of imaging studies. The next step is to use this information to prescribe and assign assessment and training programs (e.g., computer exercises and games) to the veteran in hopes that we will produce valuable data as to what types of cognitive stimulation affects what areas of the brain and benefits brain injury. Our plan is to use this information to further enhance the types and variety of assessments and training tools available to veterans with TBI. Eventually, it is our hope to be able to match a specific brain injury to a specific rehabilitation plan that has proven to be the most effective treatment for that injury.

Because the study's design utilizes non-invasive, readily available, computer-based, cognition stimulation, the positive outcomes from this project can be directly and immediately translated into the clinic setting. It is our opinion that this novel approach to brain rehabilitation will provide an effective yet inexpensive and non-invasive method to improve the lives of not only the veterans of OEF/OIF but all individuals suffering from TBI and related problems.

BUDGET JUSTIFICATION

The Palo Alto Institute for Research & Education (PAIRE) is the VA-affiliated nonprofit corporation established to facilitate research and education activities at the Department of Veterans Affairs Palo Alto Health Care System (VAPAHCS). There is an executed agreement between PAIRE and Stanford University that defines the conditions and terms whereby grants for VA-based Stanford faculty are administered by PAIRE rather than by Stanford as is done for faculty based on campus.

Key Personnel

J. Wesson Ashford MD, PhD (Principal Investigator, 1.2 cm in-kind effort)—Dr. Ashford is the Acting Director for the VAPA-HCS WRIISC (War Related Illnesses and Injuries Study Center), which was activated in August, 2007 by VA Central Office to address the issue of combat injuries with a specific focus on mild TBI. Dr. Ashford was selected for this position because of his extensive experience in Neuropsychiatry and Neuroscience, including significant contributions to understanding brain function and Alzheimer's disease and pioneering work in brain imaging and assessment of cognitive function. Dr. Ashford will manage the Memtrax project, recruit subjects, supervise their evaluations, initiate their involvement in the prescribed cognitive rehabilitation program, monitor the activities of the subjects, assure their one-year follow-up assessments, direct the analysis of the data, and prepare reports about the progress of the project.

Shereif Gamie, MD (Co-investigator, effort-as-needed)—Dr. Gamie is a staff Nuclear Medicine physician. He will coordinate the PET/CT scanning procedures at the time of the initial evaluation and at the one-year follow-up. He will also collaborate in the coregistration of the PET/CT scans with the VAPA 3T-MRI scans and the analyses of cortical metabolism.

Arlene Kasprisin, PhD (Co-investigator, effort-as-needed)— Dr. Kasprisin is the Chief of the Audiology/Speech Pathology service. She will supervise the speech & language assessments of all subjects at the time of the initial evaluation and the one-year follow-up. She will also consult on the decisions regarding cognitive rehabilitation strategies, particularly with regard to dysfunctions involving speech or language.

Henry L. Lew, MD (Co-Investigator, effort-as-needed)— Dr. Lew is a staff Physical Medicine and Rehabilitation physician. He is in charge of the clinic that evaluates outpatients with traumatic injuries. He sees about 150 patients with traumatic brain injury each year. He will be involved in the recruitment and initial and follow-up evaluations of the patients. Dr. Lew also runs an electrophysiology laboratory where he studies EEG and Event-Related Potentials (ERPs) in his patients. Dr. Lew will obtain EEG & ERP studies of the patients at the initial evaluation and at the one-year follow-up.

John H. Poole, PhD (Co-Investigator, effort-as-needed)— Dr. Poole is a staff neuropsychologist, who conducts the neuropsychological evaluations of the traumatic brain injury patients. He will supervise the performance of the neuropsychological assessments at the initial evaluation and the one-year follow-up.

Allyson Rosen, PhD (Co-Investigator, effort-as-needed)— Dr. Rosen is a staff neuropsychologist with extensive experience in functional MRI analysis. Dr. Rosen will consult with the neuropsychologists conducting the evaluations. She will also continue her studies of cognitive stimuli and the activation of various regions of the cortex. She will accordingly consult on which particular types of cognitive rehabilitation stimuli are likely to activate specific injured brain regions.

Other Personnel

TBN, Psychologist (12 cm effort)— This position will be for a cognitive psychologist. This individual will review the neuropsychological and speech & language evaluations and link their results to findings from the brain imaging methods, particularly the DTI scans. If cognitive dysfunction can be related to the results of the

DTI scans, then the patient will be entered into the study and the psychologist will coordinate a cognitive rehabilitation plan for the following year designed to help the patient rehabilitate the specific cognitive deficits. This individual will be available for contact with the subjects throughout the one year of the study concerning their participation.

TBN, Research Assistant (12 cm effort years 1-3)— The research assistant will assist the patients that have been identified for possible participation in the study. This assistant will help the patients to get to all of the assessments. This individual will also work with Dr. Lew to help with the EEG/ERP recordings and analyses. The assistant will also visit the homes of the subjects (at least those that are geographically feasible to visit), to assure that they have adequate computer hardware and software in their homes to participate in the study, check to be sure that the Internet connections are stable. This assistant will also be available to the subjects throughout the one year of the study to help with any technical difficulties that the subjects might encounter.

TBN, Data Manager (12 cm effort) — The Data Manager will be responsible for collecting all data from the initial and follow-up evaluations. This individual will also be responsible for the collection and monitoring of all evaluation and performance data accumulated during the year of participation for each subject. This individual will also be responsible for preliminary data analyses to be sure that subjects' performances are being monitored to the level that subtle variations in performance or involvement can be identified for adjustment of the system.

Materials and Supplies

Computerized Cognitive Rehabilitation Tools, Software and Upgrades ($37,500 years 1-3) — The treatment component of this project is the involvement of the subjects in a range of interactive activities that are prescribed individually for each patient and designed to help the patient rehabilitate their cognitive deficits. In many cases, these cognitive tools might be referred to as video games, in other cases they will be exercises, but in all cases, they must be activities that the subject will perform. The central activity proposed at this time is a direct expansion of the current MEMTRAX memory assessment system, which involves showing a series of slides and requires various contingent responses. There will be a nominal cost for upgrading this software. Many of the cognitive rehabilitation activities will consist of software freely available on the internet. There are also several software companies that provide learning tools for children and adults and for developing many types of skills. Some of these tools may be free or available for nominal charge. Some of this software may have to be adapted for use on the project server. For example, POSIT-SCIENCE has been working on specific tools to improve neuroplasticity, and this company has expressed a willingness to provide their cognitive rehabilitation software to this project, but the software will have to be upgraded to be available on the server. The major video game makers, including X-Box (Microsoft - Halo), Play-Station (Sony), and Nintendo (Wii, Brain Age), provide software that could have a particular use for a patient with some specific brain injury. In such cases, particular equipment may need to be made available for a patient for some specified period of time.

Consultant Services

Michael Addicott, PhD (no salary requested) — Dr. Addicott is the Chief Executive Officer of CognitiveLabs, in internet-based company headquartered in Mountain View, California. He is committed to accumulating computer software that can assess and rehabilitate cognitive function. His website, www.cognitivelabs.com and the companion site, www.brain.com, are extensively visited. His company is working closely with SRI International to develop cognitive rehabilitation systems for elderly individuals with cognitive problems (see supporting letter from SRI). They are very interested in extending their developments to help veterans with TBI to recover from their injuries that affect their cognitive functions. Dr. Addicott will consult with Dr. Ashford and the cognitive psychologist to select cognitive rehabilitation tools for specific cognitive problems and for each subject, including which tools to use, how to obtain them, how to make them work most reliably, and how to make them most likely to continue to be used by the subjects.

James Clifford, PhD ($4,000 per year)— Dr. Clifford is the Chair of the Psychology Department at the College of San Mateo. He is extensively published in brain electrophysiology and has studied TBI. He will consult with Dr. Lew in the collection and analysis of EEG and ERP data.

Kerry Lee Coburn, PhD ($8,000 per year)—Dr. Coburn is a professor of Psychiatry at Mercer University. He is extensively published in brain electrophysiology and has studied TBI. He will consult with Dr. Lew in the collection and analysis of EEG and ERP data.

Ann Mehra ($18,000 per year)—Ms. Mehra has extensive experience with brain imaging and management of research studies. She is representing the resources of PriceWaterhouseCooper, one of the "big eight accounting firms" (see attached letter of support). This firm has had extensive experience in managing research projects for the government to bring highly useful products into existence to help soldiers and veterans. She will be consulting with the WRIISC to assure that all aspects of the project are moving forward in a timely fashion, including the brain imaging and the development of the cognitive rehabilitation system. She will assist in developing oversight to assure that the internet servers have maximum security, that the data is carefully stored, that all internet software is functioning optimally, and that the data analyses are completed correctly and timely.

Subawards

Subcontract with the San Francisco VA Medical Center with Dr. Michael Weiner. Total costs are $2,486,031 (directs and indirects). Dr. Weiner's lab will conduct 4T imaging studies of the subjects with DTI and tractography, to determine whether there is axonal damage in the brain and quantify that damage precisely. They will also perform SWI and perfusion scanning to look for evidence of bleeding (with long-term iron deposition) and perfusion (to see if particular region lack physiological activity). Subjects will have a repeat scan at this lab one year later to determine changes, which will be analyzed for relationship with the cognitive rehabilitation intervention. Their budget and justification is attached with the overall proposal.

Subcontract with Bowles-Langley Technology, Inc (BLT). Total costs are $324,424 (directs and indirects). BLT will coordinate the provision of rehabilitative cognitive activities to the subjects as well as placebo. They will visit the subjects' homes and assure that they have adequate personal computer equipment and access to the Internet server to participate in the computer activities (the research assistant will accompany Dr. Langley on these visits). They will work with other private agencies to develop the computer software, and host a website to provide tool access to the subjects and collect data about their interactions and performance. They will conduct analyses of performance to continually modify the rehabilitation activities to optimize the benefit for the subjects. At the end of each subjects' year of cognitive rehabilitation they will provide a summary of that subject's participation including number of hours over the year that the subject interacted with the software and the degree of change that occurred in their capabilities. Their budget and justification is attached with the overall proposal.

Other Direct Costs

Server Management ($5,000 per year)— It is planned for all subjects to interact with the cognitive rehabilitation system through one Internet server. This server will meet maximum levels of security and provide continual backup at sites in two other states. All data pertaining to subject logging onto the server and performance of all activities while logged on will be stored.

Indirect Costs

Facilities & Administrative costs are calculated at 40% of the modified total direct cost base each year. Our exclusions are the subawards with the exception of the first $25,000 of each subcontract. PAIRE's rate agreement with the Division of Cost Allocation, Department of Health and Human Services is dated 10/6/06.

MEMTRAX – A Flexible Computer Game System for Assessing Cognitive and Brain Pathology Associated with TBI and for Augmenting and Monitoring Rehabilitation

• Background:

The proposal is designed to test two fundamental concepts. The first is that the cognitive deficits associated with traumatic brain injury (TBI) are common to specific brain injuries - especially local axonal shearing as described below. The second concept is that the key to rehabilitating a patient with TBI associated cognitive impairment is with intense cognitive stimulation directed toward the specific deficits being suffered. The specific aims outlined below will provide scientific proof of these concepts.

While there is no medication or direct intervention that can restore lost brain connections, there is evidence that neuroplasticity remains active and can be called into action for rehabilitation. Therefore, the key to rehabilitating a patient with TBI is intense cognitive stimulation directed at the specific deficits being suffered. The most practical approach to providing this kind of intense stimulation is by interactive computer exercises.

This project will first measure axonal brain injury in combat veterans with mild TBI using the approach of MRI brain scans with diffusion tensor imaging (MRI-DTI). Then we will provide the patients with one year of intense, prescriptive cognitive stimulation using the MEMTRAX system. Then we will rescan the subjects to measure improvement of axonal connectivity. MRI-DTI is currently considered the best approach to assay axonal shearing injuries in the brain, which may represent the most significant damage of mild TBI. Assessing changes in axonal connections with MRI-DTI could provide specific anatomical evidence of the benefit of cognitive rehabilitation. The technologies of scanning and computerized cognitive stimulation are thus harnessed together in a comprehensive program of rehabilitation.

Background of TBI:
As battle-field mortality has diminished due to body-armor, the survival of the brain has come into focus. Advances have been made in reducing acute mortality from TBI, but more progress is needed in the diagnosis of the chronic pathology associated with TBI, and there is a tremendous need to develop effective rehabilitation strategies for cognitive and behavioral problems that become chronic problems for those who have had TBI (Kim et al., 2007). One particular problem has been to understand the neurological deficits that underlie the chronic problems of TBI. It has long been considered that coup-contra-coup forces and focal cortical contusion damage of basal forebrain, including orbito-medial frontal cortex and medial temporal lobe structures are the critical problems. However, to date, high-resolution scanning techniques have not been adequate to show the substantial lesions associated with such chronic problems.

Recently, it has been suggested that diffuse axonal injury may explain a high proportion of the chronic problems in TBI patients (see Levine et al., 2006 for review). More recently, damage to discrete axonal tracts of the cerebral white matter has been linked to specific cognitive deficits (Taber & Hurley, 2007). The critical problem of vulnerability appears to be the difference in density between gray matter (90% water) and white matter (70% lipids). As blast waves or other concussion waves pass through the brain, a differential displacement of gray and white matter regions may occur. While small arterioles may be as small as 20 um and consist of fibrous walls resistant to tension, axons are 0.5 um at the largest, and have walls made of a fragile lipid bilayer with no significant tensile strength. In the gray matter, axons pass through a matrix of similar density supported by glial cells. In the white matter, axons are surrounded by the axolemma, that includes a thick sheath of myelin. However, there is no support for the axon as the point that it traverses the gray-white matter boundary. Consequently, if a shock wave displaced the gray matter as little as 1 um relative to the white matter, axons at this location would be sheared completely. Such shearing may be

referred to as local axonal shearing, as opposed to diffuse axonal injury that has been considered in the literature.

With the development of the technique of MRI-DTI, it is now possible to visualize damage to specific axon tracts in patients with TBI (Nakayama et al., 2006) as well as mild cognitive impairment (Rose et al., 2006). Tractography can delineate specific axonal tracts that are potentially vulnerable to axonal shearing (Taber & Hurley, 2007), and the extent of local axonal shearing can be quantified and related to specific cognitive, behavioral, and neuropsychiatric problems. Further, shearing lesions at the gray-white matter interface may be focally localized and be related to the actual vector of force that caused the TBI.

To assess the loss of brain axonal connectivity associated with mild TBI, MRI-DTI appears to be the only approach that can provide information about the extent of the injury. Further, to determine whether intense cognitive rehabilitation directed at the impaired brain location, the most objective and reliable outcome measure would be to determine if improvement in axonal connectivity had occurred as measured using MRI-DTI.

Brain Neuroplasticity:
Critical periods are episodes during development when new connections are formed in the brain. For example, the primary visual area in the occipital cortex undergoes a critical period soon after birth when the primary afferent axonal pathways connect with appropriate neurons. Primary cortical areas undergo such connections early in life but still have some capability for forming new connections later (Wall & Kaas, 1985). Secondary cortical regions are also thought to undergo such critical periods during childhood, and then have limited capacity for altering connections later. However, tertiary cortical regions (higher association cerebral cortex) – including large areas of the frontal, temporal, and parietal lobes, have later periods of maturing and still maintain the capacity to form new connections throughout life. The frontal lobes do undergo a critical period in late adolescence and early adulthood (Feinberg, 1983), but the temporal lobes appear to maintain the capacity to change connections throughout life, to the extent that their neuroplasticity provides vulnerability to later life disease processes such as Alzheimer's disease (Teter & Ashford, 2002). Of relevance to TBI, axons of the central nervous system are able to reestablish connections after damage (Aguayo, 1985). The continued neuroplasticity of associative cortical regions after maturity indicates their capacity to form new axonal connections and recover from the impairments of cognitive function caused by axonal-shearing deficits caused by TBI. Just as physical impairments related to neuronal dysfunction can frequently improve with intense rehabilitative therapy, this project is based on the presumption that more plastic higher cortical brain structures can similarly recover function with intense rehabilitative stimulation. The most objective and reliable approach available at this time to measure a change of axonal connectivity is MRI-DTI. A concern with the above formulation is that temporal and parietal areas may actually have more remaining plastic capability than the frontal lobes, so cognitive rehabilitation may be more successful than rehabilitation of thinking and personality deficits.

MEMTRAX System for Cognitive Assessment and Rehabilitation:
MEMTRAX is an internet-server based system to be accessed by computers using a web-browser for testing cognitive function and rehabilitating cognitive deficits. The core MEMTRAX programs for testing cognitive functions consist of the presentation of a sequence of images and the measurement of the response of pressing the keyboard space-bar. Programs to run the test have been written using several computer-based and web-browsing languages (e.g., FLASH, HTML, Javascript, JAVA, PERL, C++). Images are generated by digital photography and/or computer-design. MEMTRAX was initially used for testing retentive memory and screening elderly individuals for dementia, and it compared favorably to numerous longer screening tests in a systematic study (see: Brodaty et al., 2006 - as the Bowles-Langley Technology / Ashford Memory Test). Testing is easy and fun, while being highly flexible, so it can potentially assess many cognitive functions. MEMTRAX can be adapted to assess highly specific cognitive functions by adjusting the pictures and the response contingencies. Accordingly, the test has the potential to examine and determine the specific

deficits found in any individual TBI patient. Test images and contingencies targeting specific deficits can serve as a fun video game. This system can be supplemented with a broad variety of available and developing computer-based mental exercises and video games so that even impaired patients will play eagerly for prolonged sessions each day, and rehabilitate and develop their cognitive impairments associated with TBI.

To supplement the core MEMTRAX program and provide optimal cognitive stimulation for brain rehabilitation, plans are being developed to incorporporate a broad assortment of additional assessment tools (including attention tests developed by Bowles-Langley Technology and others assembled by Cognitive Labs) as well as cognitively stimulating interactions targeting prescriptively the deficits of individual patients. Such cognitive stimulation will include the unlimited resources of puzzles and games available for personal computers and on the internet. The MEMTRAX system will be expanded to include all computerized stimulation - training, exercising, gaming - that is considered to be potentially beneficial for TBI patients. Also under development are collaborations with "Bright-Minds-Institute" (see letter of support), which develops computerized learning methods for children, and PositScience (see letter of support), both of San Francisco, which has developed some specific rehabilitation tasks to improve brain functioning (personal communication, Zelinsky et al., Gerontological Society of America, 2007).

To maximize the technical performance of MEMTRAX, SRI International has offered to provide additional development and implementation assistance to strengthen the functionality of this system. PriceWaterhouse Cooper will coordinate, supervise, and monitor the progress of MEMTRAX development in the private sector. Their coordination will provide a structure that will foster the optimal development and reliability of the MEMTRAX system. Together, these two organizations will assure the provision of optimal rehabilitation to the veterans.

Brain Assessment Approaches Including MRI-DTI:

Conventional radiological study of patients with the post-concussion syndrome may reveal few or no abnormalities, and metabolic and blood-flow imaging studies generally provide little or no indication of loss of cerebral functional activity. Similarly, the major structural changes of more severe injury provide little insight regarding accompanying impairment of brain function. Thus, the identification of markers of neurological function which may permit more precise diagnosis, and enhanced evaluation of the response to treatment and of recovery prediction, must be sought elsewhere.

a) Diffusion tensor imaging (MRI-DTI): MRI-DTI (Basser, 1994) represents an advance over older diffusion weighted imaging techniques, which provided a spatially averaged apparent diffusion coefficient (ADC), not specific for tissue structures. In contrast, MRI-DTI can, in addition, characterize the directionality of water diffusion in 3-dimensional space, providing more specific information. Within coherently organized white matter tracts with parallel fiber bundles, water diffuses more freely along the direction of the white matter fibers than across the fibers, since diffusion orthogonal to the fibers is impeded by structural elements such as the myelin sheath of axons and their plasma membranes, the axolemma. This phenomenon is known as diffusion anisotropy, and can be quantified within white matter tracts using MRI-DTI. The most commonly used metric is fractional anisotropy (FA) (Abe, 2006), which ranges in value from zero (i.e., perfectly isotropic diffusion) to one (i.e., perfectly linear diffusion). Recent MRI-DTI studies have shown that FA is reduced at sites of traumatic axonal shearing injury, corresponding to a loss of microstructural fiber integrity, resulting in the reduced directionality of microscopic water motion (Arfanakis, 2002; Huisman, 2004). While an increasing number of MRI-DTI studies in traumatic brain injury are emerging (Naganawa, 2004; Inglese, 2005; Ducreux, 2005; Ewing-Cobbs, 2006; Le, 2005; Nakayama, 2006; Niogi et al., 2007; Salmond, 2006; Tisserand, 2006; Xu, 2007; Benson, 2007), so far, very few studies on the possible relationships between MRI-DTI findings and neurocognition have been undertaken (Salmond, 2006; Niogi et al., 2007), and no data concerning the specific impact of military brain injury (blast or impact injury) on the integrity of white matter tracts are currently available.

b) Susceptibility-weighted MR imaging (SWI): Recently, SWI, originally designed for MR venography by using the paramagnetic property of intravascular deoxyhemoglobin, has been introduced (Reichenbach et al., 1997; Haacke, et al., 2004}. Based on a high-spatial-resolution three-dimensional gradient-echo technique, SWI is extremely sensitive to local susceptibility changes and can be performed with conventional MRI instrumentation. Very recently, the SWI technique has been applied on a clinical 1.5 T MRI scanner in several studies of pediatric traumatic brain injury (Babikian et al., 2005; Tong et al., 2003a; 2003b; 2004). These studies demonstrated that SWI allows detection of hemorrhagic lesions in brain-injured children with significantly higher sensitivity than conventional gradient-echo MR imaging (Tong et al., 2003a). The number and volume of hemorrhagic lesions was shown to correlate with the Glasgow Coma Scale score (Tong et al., 2003b), as well as with other clinical measures of TBI severity, and with outcome at 6 to 12 months post-injury (Tong et al., 2004). Significant differences were detected between children with normal outcome or mild disability and children with moderate or severe disability when comparing regional injury to clinical variables (Tong, et al., 2004). In addition, negative correlations between lesion number and volume with measures of neuropsychologic functioning at 1-4 years post-injury were demonstrated (Babikian et al., 2005. No parallel studies in adult brain injury have been reported, and there are currently no SWI data available from subjects who have suffered a traumatic brain injury by either blast or impact during combat.

c) Perfusion-weighted MR imaging (pMRI): While dynamic contrast enhanced pMRI of TBI patients has shown that regions of both normal appearing and contused brain may have an abnormal regional cerebral blood volume (rCBV) and that alterations in rCBV may play a role in determining the clinical outcome of patients (Garnett et al., 2001), no studies using arterial spin labeling (ASL) pMRI in TBI have been published so far. Taken together, these imaging studies imply that TBI is associated with a range of structural, functional, and metabolic alterations.

Preliminary Results:

a) Quantitative parcellation of hippocampal subfields: A high resolutionT2 weighted fast spin echo sequence was acquired for manual marking of hippocampal subfields (acquisition and processing methods described in our publication (Mueller et al., 2006}). The hippocampal subfields, ERC, subiculum, CA1, CA1&CA2 transition zone (CA1&CA2), CA& dentate gyrus (CA3&DG), were marked on 5 consecutive slices. (Mueller et al., 2006) (Fig. 1).

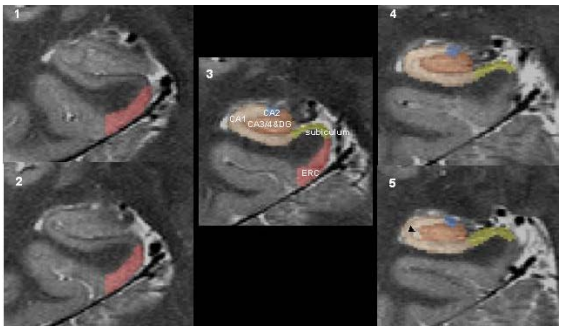


Figure 1: Subfield marking scheme

b) Segmentation/Voxel-based-morphometry: T1 weighted image were acquired using a magnetization prepared rapid gradient echo sequence (TR/TE 2300/3.37 ms, TI 950 ms, 176 slices, resolution 1x1x1 mm) for single channel segmentation using Expectation Maximization Segmentation (EMS) algorithm (Van Leemput, et al., 1999a;b). For VBM, the gray matter maps in subject space obtained from EMS were normalized to a customized but not project specific gray matter prior. An 8 mm FWHM Gaussian smoothing kernel was applied to all gray matter maps (Fig. 2).

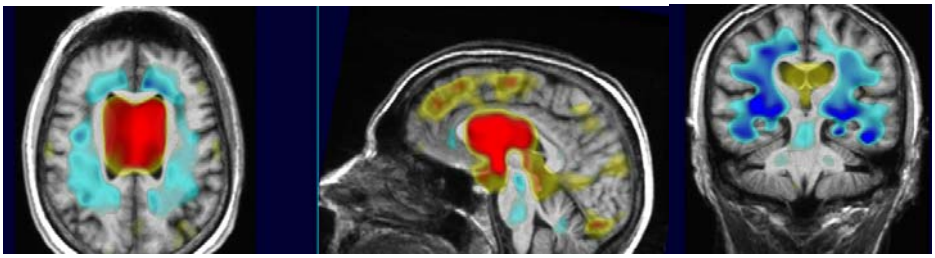


Figure 2: The two left panels show effects of age; the right panels shows regions where AD patients have tissue atrophy compared to controls (blue).

c) Arterial Spin Labeling perfusion-weighted MRI (ASL pMRI): For measurement of cerebral blood flow in resting state, a continuous arterial spin labeling sequence (TR/TE 5200/9 ms, delay in TR 1590 ms, 80 measurements, resolution: 5.0 x 3.8 x 5.0 mm, 16 slices) was acquired. Subjects were resting quietly with their eyes closed. Correction for partial volume effects between tissue/csf was performed by dividing the tagged and un-tagged image with a tissue probability map (gray and white matter probability maps derived from EMS) Finally, gray matter CBF was obtained by subtracting CBF measured in deep white matter regions (centrum semiovale) (Muller-Gartner et al., 1992). Statistical analysis was performed using statistical parametric mapping (SPM) (Fig. 3).

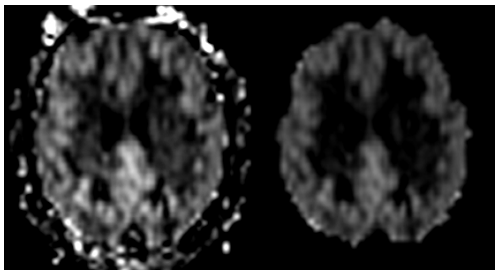


Figure 3: CBF map before (left) and after partial volume correction (right).

d) Diffusion Tensor Imaging (MRI-DTI): MRI-DTI was acquired using an echo planar imaging (EPI) sequence (TR/TE 6000/77 ms, GRAPPA acceleration factor 2, b = 800 s/mm^2, 2 x 2 x 3 mm, 40 slices, and 6 diffusion sensitization directions). After motion correction, fractional anisotropy (FA), mean diffusivity (MD), maps were calculated offline using DTIstudio and Volume-One (Jiang et al., 2006; Masutani et al., 2003). The FA map was used to identify the cingulum (FA threshold 0.18) and corpus callosum. In addition to this, voxel based analysis, was performed using tract based spatial statistics (TBSS) (Smith et al., 2006) or SPM. More preliminary studies with MRI-DTI are outlined below.

e) Cortical Thickness Measurements with Freesurfer: The T1 weighted images were bias corrected using the bias field provided by EMS and loaded into the FreeSurfer {http://surfer.nmr.mgh.harvard.edu/ #13} software package for calculation of cortical thickness and parcellation into different cortical and subcortical region. The hippocampal labels of the latter were extracted, manually edited and used to calculate total hippocampal volumes (Fig. 4).

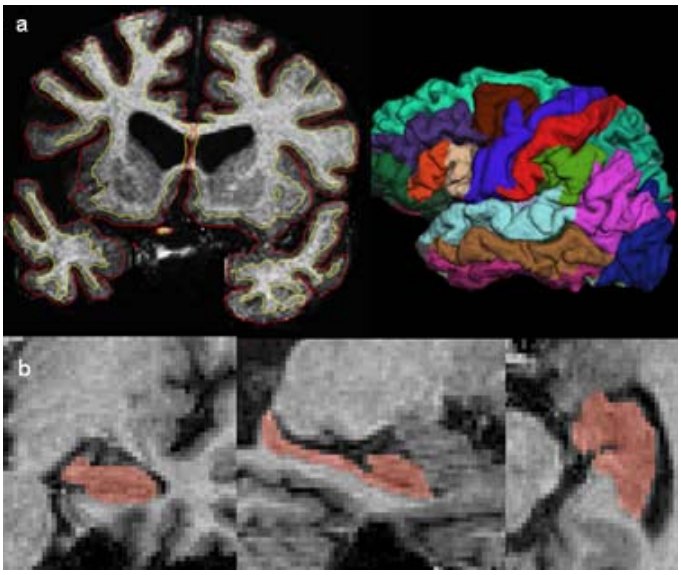


Figure 4: (a) Cortical thickness measurement and parcellation in a 66-year-old Alzheimer's patient. (b) Hippocampal labels derived from the parcellation were used to calculate total hippocampal volume.

f) Susceptibility-weighted MR imaging (SWI): SWI (Haacke et al., 2004) has been implemented on the 4 Tesla MRI magnet at the CIND. Extensive experience with acquisition, processing, and analysis of SWI data, as well as software for these tasks, is available in Dr. Weiner's lab. Preliminary data obtained with this method are shown in Fig. 5. This demonstrates the ability of the SWI technique to detect small hemorrhagic lesions, such as those occurring in cerebral amyloid angiopathy (see arrows in Fig. 5) and in traumatic brain injury.

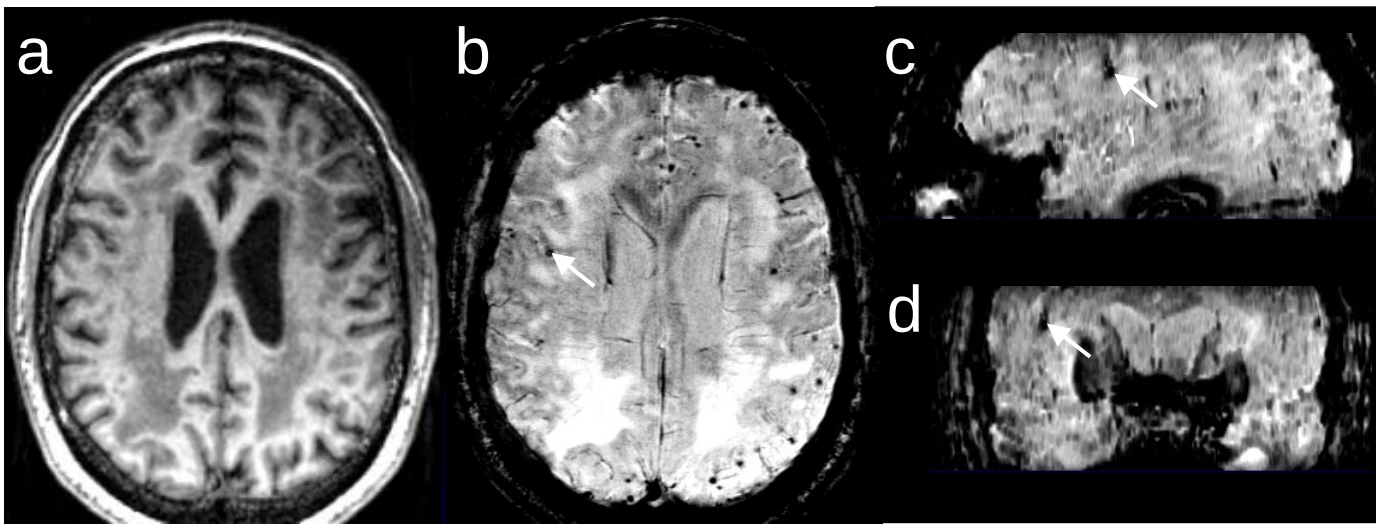


Figure 5: Structural MR image (a) and susceptibility-weighted MR images (b-d), representing three orthogonal sections through a hemorrhagic lesion (arrows), acquired at 4 Tesla from a patient suffering from cerebral amyloid angiopathy.

g) Effects of Mild Cognitive Impairment (MCI) and Alzheimer's Disease (AD): Subfield measurements and total hippocampal volumes were available for 14 AD, 14 MCI and 47 age-matched control subjects. Compared to controls, AD had significantly smaller volumes of ERC, subiculum, CA1, CA1-2 transition, and total hippocampal volumes, and MCI had smaller CA1 and CA1-2 transition volumes (Table 1).

	Control N = 47	MCI N = 14	AD N = 14
ERC	202.4 ± 54.0	168.4 ± 48.0	145.0 ± 53.4*
Subiculum	200.2 ± 36.1	184.7 ± 38.1	154.2 ± 44.9*
CA1	331.4 ± 47.0	285.1 ± 42.5*	264.4 ± 63.1*
CA1-2 transition	20.5 ± 5.5	15.1 ± 3.4 *	14.1 ± 3.8*
CA3&DG	224.4 ± 37.7	227.2 ± 24.3	230.3 ± 54.7
Total Hippocampus	5520.6 ± 770.4	5154.9 ± 817.7	4450.8± 1285.2*

Table 1: volumes in mm^3; *, significant $p<0.05$ than controls.

The patterns of subfield atrophy in AD and MCI are consistent with patterns of neuronal cell loss/reduced synaptic density described in histopathological studies. Discriminant analysis and power analysis showed that CA1-2 transition, i.e. the region in the dorsal medial aspect of the hippocampus, was superior to total hippocampal volume for distinction between controls and subjects diagnosed with MCI.

These preliminary findings suggest that hippocampal subfield volumetry is superior to total hippocampal volume as a measure for diagnosis of very early AD. Cortical thickness measurements using FreeSurfer (13 AD, 57 controls) showed significant reductions in the region of ERC, temporo-lateral, precuneus, parietal, and frontal (Fig. 6 a). Perfusion measurements showed CBF in the precuneus and parietal and temporal in AD (6 AD, 26 controls) (Fig. 6b). A similar but milder pattern of hypoperfusion was found in MCI (Fig. 6c).

Voxel-based MRI-DTI analysis using TBSS showed FA reductions in parietal and posterior cingulate white matter, similar to our published work at 1.5 Tesla (Zhang et al., 2007). We also found FA reductions in frontal WM consistent with frontal higher white matter lesion load in AD compared to controls.

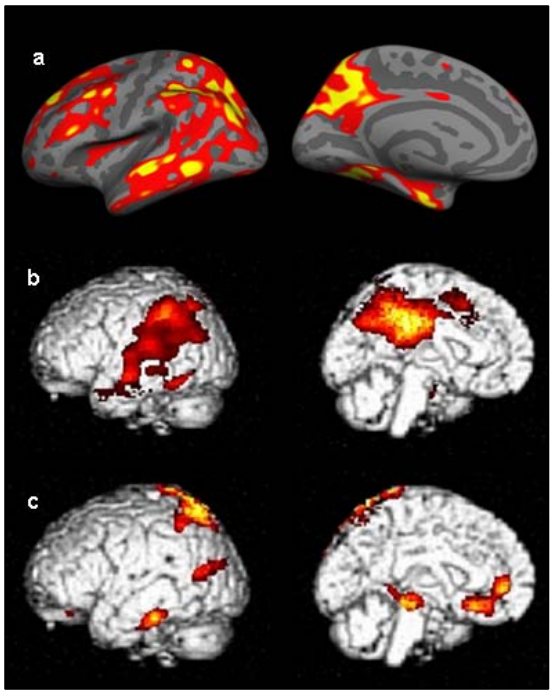


Figure 6: Gray matter loss and hypoper-fusion in AD and MCI.

h) MRI-DTI fractional anisotropy and tractography applied to specific clinical cases:

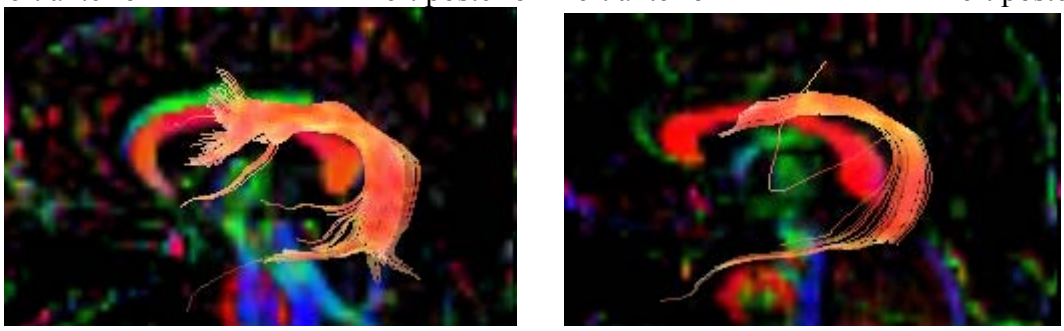
There have been several hundred patients with TBI who have been assessed recently at the VA Palo Alto Health Care System (VAPAHCS); (about 150 per year since onset of OIF/OEF). The VAPAHCS War Related Illness and Injury Study Center (WRIISC) program will conduct an inventory of these patients and determine their status. Several cases from the VAPAHCS have already been evaluated at the San Francisco Veterans Affairs Medical Center (SFVAMC).

To demonstrate the resources available at the VAPAHCS and SFVAMC, there is a case of a 24 year old patient who was still active-duty military. Though he survived the explosion of an improvised explosive device in Iraq and suffered 10 minutes of unconsciousness, he was scheduled to return to Iraq. The 1.5T MRI and the PET/CT was read as normal. He was thoroughly evaluated by the Speech Pathology Service and found to have conduction aphasia that was questioned as relevant or significant for his return to active duty. On referral to the SFVAMC, loss of volume in the arcuate fasciculus, which connects Wernicke's and Broca's areas on the Left side of the brain was discovered. Retrospective analysis of the MRI and PET scans showed abnormalities.

Firgure 7: 4T-MRI with MRI-DTI imaging

25 y/o control — 24 y/o patient

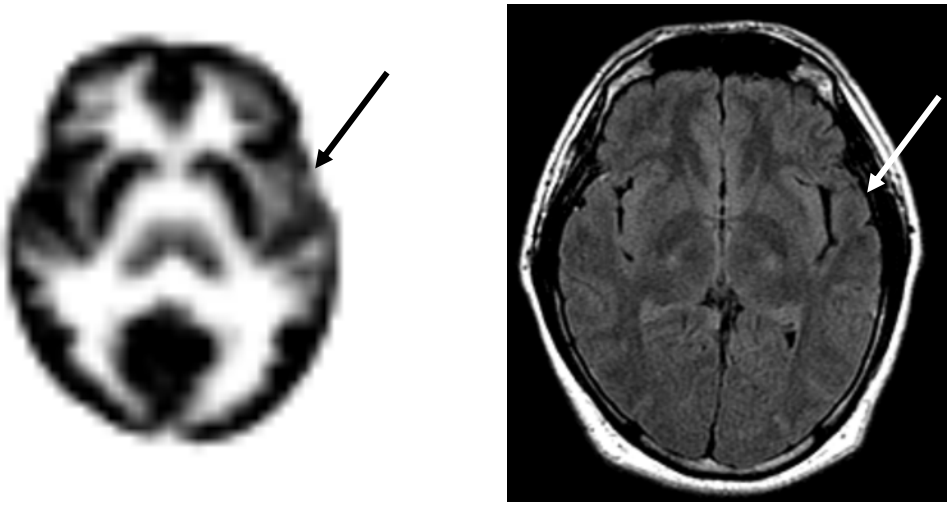
Left anterior — Left posterior — Left anterior — Left posterior



Arcuate fasciculus in orange. Note abrupt termination anteriorly (Right), may be related to fractional anisotropy, likely due to loss of fiber integrity. One possible causal explanation is local axon shearing.

PET — 1.5T-MRI

Right — Left — Right — Left



PET and MRI were both read as normal, but there is decreased activity seen on the PET scan in the Left frontal region near Broca's area and there is apparent atrophy in this region on the MRI.

Figure 8: Other Brain Circuits Measured with MRI-DTI: Fronto-temporal paralimbic circuit (uncinate fasciculus, pink) and anterior corpus callosum (yellow) in a patient with fronto-temporal dementia (71 y/o, left), which is frequently accompanied by disinhibition, apathy, and altered social regulation. (Control 70 y/o on Right). Green represents the visual radiations (from lateral geniculate to striate cortex).

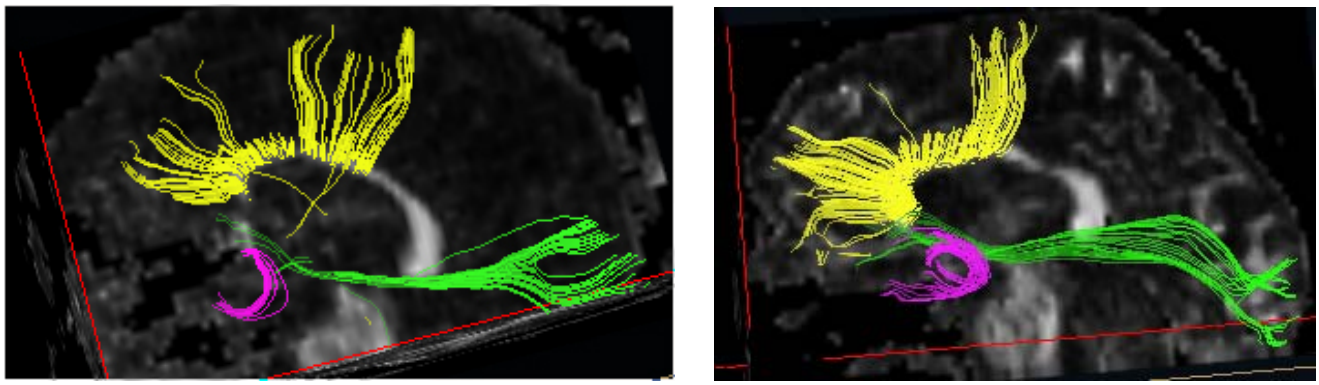


Figure 9: Shows various parts of the corpus callosum, with less fractional anisotropy in the older subject

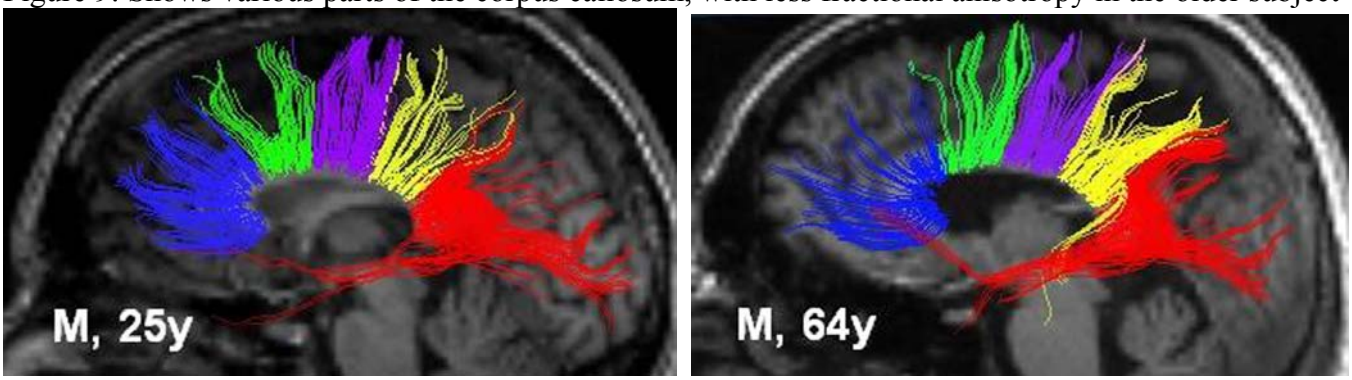
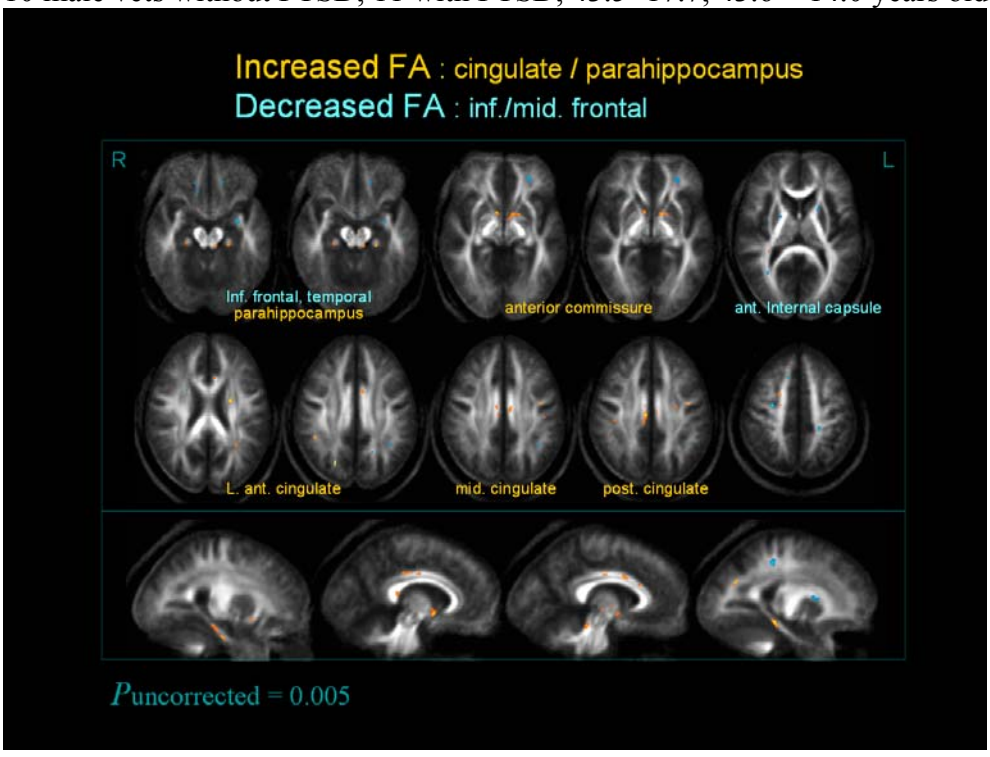
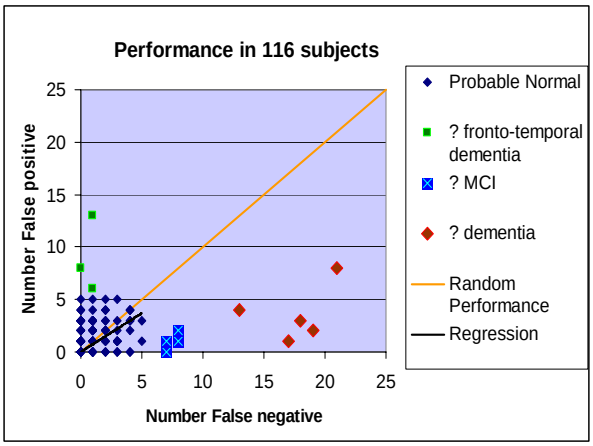


Figure 10: Disorder specific MRI-DTI changes: PTSD: DTI by SPM2 - Fractional Anisotropy ~ Group+age: 10 male vets without PTSD, 11 with PTSD, 43.5±17.7; 43.6 ± 14.0 years old.



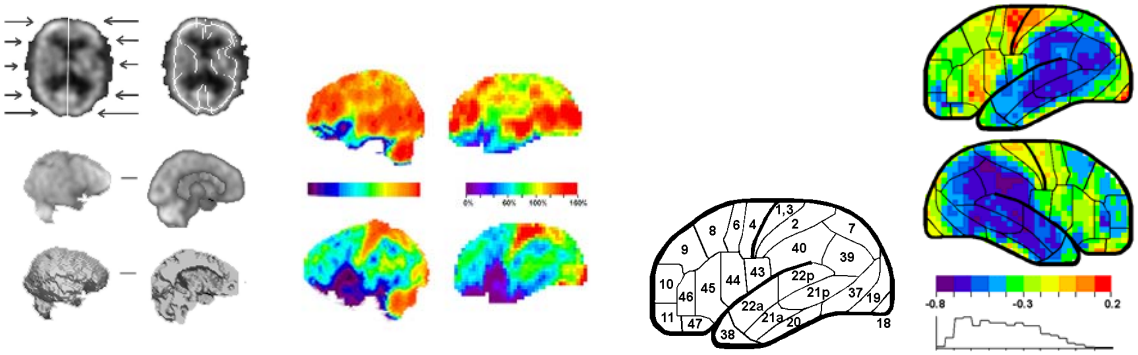
i) MEMTRAX clinical experience:

The MEMTRAX screen, as a series of 50 pictures, 25 repeated, 5 seconds per picture, and paper-form response, has been given to over seven hundred subjects. The performance of each item was studied in a preliminary sample of 116 elderly subjects who took the test anonymously as part of their participation inn a presentation on Alzheimer's disease. In this group, 5 subjects were significantly impaired in recognizing the pictures, 4 had borderline difficulty with correct recognition, and 3 subjects made an excess number of inappropriate responses (reporting more than 5 pictures as repeats falsely). Consistent variation was noted in the scores on individual pictures, related to similarity to other pictures and visual uniqueness (Ashford, 2007). Figure 10:



j) Metabolic brain image analysis:

SPECT images from Alzheimer patients were analyzed with a cortical surface mapping program. Surface values had high correlation with severity in regions following a pattern essentially identical to the known distribution of Alzheimer pathology (Ashford et al., 2000). Figure 11:



A similar, but more advanced dimensionally, cortical surface analysis approach will be used for comparing the PET data with the MRI-DTI indicators of axonal disconnection.

● Hypothesis and Objective:

The central thesis of this study is that intensive cognitive stimulation for one year will increase the measured connectivity in the injured cerebral circuits in patients with mild traumatic brain injury (TBI) associated with limited neuropsychogical deficits, and related to localized injuries in specific cerebral axonal pathways.

The main objectives of this proposal are:

- to determine the utility of MRI-DTI as a central tool for determining brain changes secondary to TBI that explain the common, diffuse neuropsychological and cognitive problems found in these patients,
- to coordinate an assortment of computerized cognitive assessments, training programs, cognitive rehabilitation tools, and brain-enhancing entertainment systems to optimally activate the damaged brain mechanisms, as uniquely determined for each patient, and
- determine if the participation in the computer interactions is associated with improvements in the injured cerebral pathways.

The specific hypotheses to be tested are as follows:

Hypothesis 1: In mild TBI patients, axonal damage seen on MRI-DTI will correspond to neuropsychological, speech & language deficits, the pattern of brain metabolism, and variations in neurophysiological activity.

Hypothesis 2: After one year of intense prescriptive cognitive rehabilitation directed at those deficits, beneficial changes will be seen in axonal connectivity indicated by MRI-DTI

Hypothesis 3: After 1 year, axonal connectivity changes seen on MRI-DTI will correspond with changes seen on follow-up neuropsychological testing, speech & language assessment, changes in brain metabolism, and neurophysiological activity.

Hypothesis 4: Improvements in all basic measures will correspond with improvements in ADL function and QOL indicators

Numerous secondary hypotheses will be tested, including the following:

- MRI-DTI indicators of axonal injury will occur in regions of the brain indicated as dysfunctional by other assessment modalities, including neuropsychological testing, speech & language assessment, PET brain metabolism using high resolution coregistration with CT and MRI, and electrophysiological measures, including quantitative EEG and event-related potential (ERP) parameters.
- After one year, improvements in MRI-DTI connectivity will be reflected by improvements in neuropsychological testing and speech & language assessment measures, PET metabolism, and electrophysiological indices.
- EEG/ERP measures at baseline will predict the degree of recovery after one year and improvements in the EEG/ERP parameters will correspond to the improvements seen in the other modalities.

● Specific Aims:

The specific aims of this project are in two parts, those that address the measurement of the brain injury and those that address the cognitive rehabilitation:

1) Determine the relationship between local axonal shearing as measured on MRI-DTI scans and cognitive dysfunction:
MRI-DTI scans will be obtained in 100 mild TBI cases (20 - 50 years of age), 50 normal age-range matched controls, and 50 moderate TBI cases (20 - 50 years of age), all of whom will have complete medical evaluations including neuropsychological testing, speech & language assessment, PET/CT scans, EEG/ERP measures. Further MRI studies will include SWI and perfusion assessments. This project will determine the relationship between axonal impairments seen in MRI-DTI scans and the other measures of brain function.

2) Determine whether one year of intense cognitive-rehabilitation will enhance connectivity of brain regions where axonal shearing occurred or in areas subserving similar functions to those areas where axonal shearing occurred:

Cognitive rehabilitation methodology will be coordinated and applied to 75 mild TBI cases, 25 moderate TBI cases, and 25 normal individuals, while placebo (no additional or specific therapy) will be provided to 25 mild TBI cases, 25 moderate TBI patients, and 25 normal individuals. The MEMTRAX test will be expanded to evaluate and meet the needs and deficits of each of the TBI patients, for neurocognitive assessment, tracking recovery, and rehabilitative training. The efficacy of the MEMTRAX system for TBI rehabilitation will be primarily assessed with assessment of change on the MRI-DTI scans obtained after one year of treatment. Secondary measures will include comparison of performance on specific aspects of this tool with brain imaging – PET/CT, MRI (anatomic measures, SWI, perfusion), fMRI, as well as neuropsychological testing and speech & language assessment, and quantitative EEG and ERPs.

3) Determine whether one year of intense cognitive-rehabilitation provides benefit to TBI patients on any other measures of brain or cognitive function:
The active treatment group will intensively use the MEMTRAX directed rehabilitation system for a year while the placebo group participates in standard rehabilitation and/or non-directed computer/internet activity. The progress of the subject groups will be monitored and assessed weekly to determine acceptability and benefit and appropriate prescriptive adjustments to the levels of stimulation. At the end of the year, the subjects will be reassessed with the same brain imaging techniques, including 4T-MRI-DTI (primary outcome measure), PET metabolism (co-registered to CT and MRI), neuropsychological testing and speech & language assessment, and EEG/ERP parameters (secondary outcome measures), to determine if there is any change, from the measurements made at the study start, attributable to the MEMTRAX intervention.

4) Determine whether any neuropsychiatric diagnoses, medication treatments, or other interventions interact with baseline conditions to moderate treatment outcomes.

● Research Strategy:

Clinical and Research Setting :
The clinical aspect of this project is to be conducted at the VA Palo Alto Health Care System (VAPAHCS), in the clinical space of the VAPAHCS Polytrauma Center (Dr. Lew), under the coordination of the VAPAHCS WRIISC (War-Related Illnesses and Injuries Study Center, Dr. Ashford, Acting Director, Dr. Yesavage supervising, funded directly by VA Central Office for developing brain imaging approaches to TBI). The focus of the WRIISC is mild TBI evaluation and rehabilitation. The WRIISC will coordinate the evaluation of local and national veterans and service personnel with TBI using an extensive assessment battery, including neuropsychological testing and speech & language assessment, physical, neurological, and psychiatric examination, brain imaging, including PET/CT and 3T-MRI imaging, and neurophysiological assessment. The WRIISC will also seek approaches to improve rehabilitation strategies for TBI patients and conduct follow-up examinations. The WRIISC is expected to comprehensively evaluate at least 2 new TBI cases per week. Specific developments in assessment are ongoing, including 3T-MRI, PET/CT scanning (Dr. Gamie, Nuclear Medicine), EEG/ERP (Dr. Lew; Drs. Clifford and Coburn consulting), and neuropsychological testing (Drs Poole, Groff, Tomander, Kinoshita, and Rosen). Currently, there is an ongoing collaboration between the VAPA and the 4T-MRI laboratory at the San Francisco VA Medical Center (Dr. Weiner, Director) to perform higher resolution MRI scans with DTI imaging.

Project Outline:

a) Adaptation of MEMTRAX for TBI assessment and rehabilitation

- Development of MEMTRAX protocols to assess all relevant cognitive functions in TBI cases (Bowles-Langley Technology, Mr. Bowles, Dr. Langley; CognitiveLabs, Dr. Addicott; PriceWaterhouse Cooper, Ms. Mehra).
- Testing of MEMTRAX stimuli with fMRI in 50 normals (age-range of mild TBI cases) to determine which regions of the cortex are activated by specific categories of stimuli (Dr. Rosen).
- Accumulation of a large array of MEMTRAX stimuli to provide rehabilitative stimulation and assessment of progress for one year (Bowles-Langley Technology).
- Development of computer gaming strategies to enhance user interest (CognitiveLabs, Dr. Addicott; PriceWaterhouse Cooper, Ms. Mehra).
- Implementation of MEMTRAX system on secure internet server for providing interactive tools for subjects, collecting data, and monitoring subject performance and change (Bowles-Langley Technology, Mr. Bowles, Dr. Langley; CognitiveLabs, Dr. Addicott; PriceWaterhouse Cooper, Ms. Mehra; SRI)

b) Clinical Assessment

- Complete evaluation of 100 mild TBI patients (Polytrauma Center, WRIISC).
- Additional evaluation of 50 moderate TBI patients.
- Assessment of 50 age-range matched normal vets.
- Coordination of 4Tp-MRI scans at Fort Miley VA (WRIISC).
- Testing of TBI cases with MEMTRAX (Bowles-Langley Technology, Dr. Addicott; PriceWaterhouse Cooper, Ms. Mehra).
- Implementing computer rehabilitation (Bowles-Langley Technology, CognitiveLabs, Dr. Addicott; PriceWaterhouse Cooper, Ms. Mehra).
- One-year follow-up evaluation – MRI-DTI; PET/CT, neuropsychological testing, speech & language assessment, neurophysiological recording (EEG/ERP/RT).

c) Experimental Design

- Mild TBI cases will randomly be assigned to treatment or comparison groups, 75 cases in the active treatment group, 25 cases in a placebo comparison group.
- All patients will be interviewed at their personal residence to determine what computer resources they possess. Since most OIF/OEF veterans are familiar with computers and many are closely acquainted with computer games and have high performance computers in their homes, the level of each subject will be evaluated to determine their capability to access the secure server which will host the MEMTRAX system. Subjects who do not have adequate computer availability and internet access will be provided with a minimal system in their home for one year. (The Stanford / VA Alzheimer Center is now in a 4 year study supplying elderly with a similar setup in their homes.)
- The treatment group will be provided a code to access the MEMTRAX program via the internet, and the use of this program will be monitored by the central server.
- The comparison group will be given general instructions to access the server on a regular basis and be asked standard questions about their level of function.
- The primary outcome measure will be cortical metabolic changes as assessed with PET, using PET/CT co-registered to 3TMRI structural scan.
- The secondary outcome measures will be changes in axonal structure measured on 4TMRI-DTI and in neuropsychological tests.
- Analysis of results will be conducted using intent to treat statistics, as in standard drug trials.

d) Assessment - Standardized WRIISC Assessment:

The WRIISC assessment is conducted over a 5 day period. After an initial Monday morning interview, the patient is seen by a psychiatrist and a neurologist. Neuropsychological testing is begun in the afternoon. The next morning, Tuesday, before breakfast, the PET/CT is conducted. Then, the neuropsychological testing is continued. Speech & Language assessment is started Wednesday morning. After noon, the 3T-MRI brain scan is performed. On Thursday, the patient has EEG, ERP, and driving assessment. Friday morning will be scheduled for a trip to San Francisco, to the San Francisco VA, for 4T-MRI scanning. A summary meeting will be conducted with the patient and any significant others on Friday afternoon.

Neuropsychological testing (Drs. Poole and Rosen; additional staff psychologists with certification in Neuropsychology that will be involved in assessments include Drs. April Groff, Darryl Tomander, Lisa Kinoshita).

Minimum Core Neuropsychological Assessments consist of the following:

Pre-morbid verbal intelligence	Wechsler Test of Adult Reading (WTAR)
Executive function	Trail Making Test (Reitan) Letter-Number Sequencing (WAIS-III) Wisconsin Card Sorting Test-64 (computer version) Verbal fluency (animals; fruits & vegetables; 3 letters) Design Fluency (Jones-Gotman)
Memory for new information	California Verbal Learning Test (CVLT-II) Brief Visuospatial Memory Test (BVRT-R)
Symptom Ratings	PTSD Check List, Military version (PCL-M) Neurobehavioral Symptom Inventory (Ciceroni)
Functional Ratings	Community Integration Questionnaire (CIQ)

Disability Rating Scale (DRS)

The RBANS (Repeatable Battery for the Assessment of Neuropsychological Status) is being considered for use at the VAPAHCS for routine use in this population (McKay et al., 2007).

Speech & Language assessment (Dr. Arlene Kasprisin, Chief of Service))

A full battery of standard speech and language assessments are routinely given to all TBI patients. This battery will serve as the standard battery at the initial evaluation and will be repeated at the one-year follow-up visit.

e) Description of Subjects (all male veterans, 18 to 50 years of age):

There will be a total of 200 subjects in three groups:

- Group A will consist of 100 patients ages 18 - 50 with mild TBI, who have documented OIF/OEF combat head injuries, mild cognitive impairment, and no penetrating head injuries.
- Group B will consist of 50 moderate TBI (ages 18 - 50 years) TBI comparison subjects.
- Group C will consist of 50 normal controls, OIF/OEF combat returnees with injuries but no documented evidence of head injuries, age range matched to the 100 mild TBI cases.

Inclusion Criteria for patients:

OIF/OEF combat injuries (including blast related head injuries)

- at least 3 months after the injury
- up to 5 years after the injury

Exclusion Criteria:

- Major medical problems or disabilities that would interfere with participation in the study.

Diagnostic criteria:

- Mild TBI patients will be selected for a history of non-penetrating head injuries.
- Histories will be examined for evidence of cognitive impairment or social dysfunction that has occurred since the injury.
- Complete neuropsychological testing (including personality assessment) and speech/language assessments will be reviewed for objective evidence of impairment. .Subjects will be assessed for Rancho Los Amigos - Revised Levels of Cognitive Functioning (Original Scale co-authored by Chris Hagen, Ph.D., Danese Malkmus, M.A., Patricia Durham, M.A. Communication Disorders Service, Rancho Los Amigos Hospital, 1972. Revised 11/15/74 by Danese Malkmus, M.A., and Kathryn Stenderup, O.T.R. Revised scale 1997 by Chris Hagen):
- Mild TBI - Levels IX (purposeful, appropriate, stand-by assistance on request), X (purposeful, appropriate, modified independent), and above.
- Moderate TBI - Levels VII (Automatic, appropriate: minimal assistance for daily living skills) or VII (Purposeful, appropriate: stand-by assistance).

Patients meeting these criteria will have complete medical evaluations, including PET/CT and MRI brain scans. Patients with brain lesions larger than 5 cc's total will be excluded. Patients with marked atrophy or hydrocephalus will also be excluded.

Patients who still meet the above criteria will be referred for MRI-DTI scanning.

For the 100 mild TBI cases, only patients with neuropsychological deficits and abnormalities of cerebral connectivity found on the MRI-DTI scans corresponding to the neuropsychological deficits will be included.

For the 50 moderate TBI cases who have brain injuries that exceed the above criteria, but are able to function at an adequate level for frequent computer interactions will be provided to a randomized sample of 25 of these patients --- included in the moderate TBI patient group.

The 50 controls may include any OIF/OEF veterans with a history of exposure to blasts, with or without a history of mild head-injury, but with no documented neuropsychological impairments.

Other treatments: Patients with TBI are susceptible to a variety of neuropsychiatric conditions (Kim et al., 2007) and may be treated with a variety of pharmacologic agents, including anti-psychotic and anti-depressant medications as well as stimulants. A log will be maintained of all neuropsychiatric diagnoses and medications used.

f) Randomization into treatment groups:

100 mild TBI will be randomized so that 75 patients get intensive rehabilitation for one year and 25 patients will be provided with non-specific but comparable stimulation. Randomization will attempt to balance for age and severity. Person guiding the randomization will be a psychologist (or neurpsychologist) who will try to balance the comparison 25 with the types of cognitive problems of the treatment 75, as well as age.

The 50 moderate patients will be divided into 2 groups, 25 patients will be provided with the intensive cognitive rehabilitation, to the extent that they can manage this type of activity. The remaining 25 will not be provided any further treatment beyond what is routinely available. Randomization will attempt to match types of cognitive problems in treatment and comparison groups, along with age.

50 controls will be apportioned so that 25 will get the intensive measures comparable to those prescribed individually for the mild TBI patients and 25 will be provided with no recommendations.

g) Outcome Measures:

The primary outcome measures in this study will be baseline evaluation using MRI-DTI and the change over one year in MRI-DTI measurements. The secondary outcome measures are a) the change over one year in neuropsychological and speech-language assessments, b) change over one year in brain imaging PET (coregistered to MRI), c) neuroscan analyses of EEG/ERP/RT, d) perfusion / SWI scan changes, e) MRI changes in atrophy, and f) PIB changes (tentative, including decrease in amyloid).

h) Intervention - Therapeutic (Implementation of the MEMTRAX System) vs Standard Care:

There are 3 functional components to the cognitive rehabilitation therapy (CRT): a) periodic assessments (requiring 1 hour per week of testing, b) regular and directed cognitive training (involving 1 hour per day, 5 days per week, and c) entertainment enticements which also have some cognitive therapeutic value (target 2 hours per day, maximum allowed on this server, 10 hours per day or 70 hours/week). The cognitive rehabilitation will consist of interacting with a personal computer that is linked through a dedicated internet service provided to a secure server (FIPS-140-B compliant). All patients randomized to CRT will receive treatment for one year.

At the beginning of each training session, there will be a review to assure than an assessment occurred within the prior 7 days. The assessments will include cognitive performance measurement and questions about the level of interest and preferences for continued enticement video games. If the weekly assessment has been completed, it will then be checked to determine whether the subject has completed an hour of cognitive training in the prior 48 hours (requiring 5 hours per week). Assessments, cognitive training sessions, and game levels will be continuously adapted to the functional level of the individual patient.

Assessments will be targeted most specifically for regions of the brain that have been shown on the MRI-DTI scans to have damage.

Areas of assessment will relate to brain regions known to be most affected by TBI that are associated with specific neuro-cognitive deficits (Taber & Hurley, 2007), and will specifically address (as examples):

Dysfunction	Associated brain region	Specific test
memory difficulties	fornix, cingulum	Slide repetition
visuo-spatial difficulties	Right parietal lobe	Location of stimulus on slide
speech/language impairment	Left Broca/Wernicke's areas	Use of words, word combinations
decision-making problems	Frontal lobes	Bowles-Langley and CogLabs
hand coordination	Motor cortex	Finger tapping
speed of processing	Occipital cortex	Simple/complex RT
facial recognition	Right temporal lobe	Face slide-shows

Cognitive training tasks (to be developed progressively as indicated for each subject) will address each of the above mentioned areas of assessment. Other cognitive training systems will be evaluated and prescribed on a case-by-case basis to provide the best possible rehabilitation therapy for each patient. The additional systems may include those from Posit Science (Letter of support attached), Bright Minds (Letter of support attached), Brain Age (Nintendo), Learn Rx, and Sun Burst.

Participants in the treatment group will also be provided access to computer games enticements, which will be monitored and time limited to 10 hours maximum per day. Such games might include World of Warcraft, Myst, multiple available video games, puzzle games such as tic-tac-toe, and Myst. (examples).

Participants in the control / placebo group (25 mild TBI patients, 25 moderate TBI patients, and 25 normal controls) will not be provided any special access. They will be provided the VA Standard of Care. No special attention will be provided to these subjects. They will be seen quarterly for evaluation with questionnaires to determine what rehabilitation activities they have received and how much time they spend working with a computer.

i) Providing Computers for Subjects:

After a subject is selected, the additional evaluation process will start out with a survey about each subjects technical sophistication, including inventory subject's computer availability and inventory of subject's internet availability. After participant enrollment, a researcher and a research assistant will visit the homes of all participants to assess each subject's technical sophistication, and to determine the availability of an adequate personal computer and internet access for those randomized to receive intensive treatment. For subjects without adequate personal computer access, a one year lease and a contract for an internet service provider for one year will be arranged. Subjects will be provided a code to access a virtual private network (VPN).

Overview of MRI-AT (MRI-advanced technology) studies:

The MRI-AT brain imaging studies will be carried out at the SFVAMC facility, equipped with a Siemens 4T-MRI system. The MRI sessions will include structural, susceptibility-weighted, diffusion, and perfusion MRI. Before start of the study, the MRI system will be calibrated. During the study, regular scans of phantoms will be performed for quality control of the scanner. The personnel at the SFVAMC facility will not be informed of the diagnoses of the subjects or their treatment assignment. Hence, all of the brain scan analyses done at this facility will be blinded with respect to diagnosis or treatment arm.

a) Structural MRI.:

The protocol, optimized for the 4T GE platform will be used for standardized structural MRI scans that will include a volumetric T1-weighted magnetization-prepared gradient-echo sequence (MPRAGE: TR/TE/TI = 2300/3/900 ms, flip angle = 9º, 1x1x1 mm^3 resolution) and a T2-weighted, turbo spin-echo sequence (TSE:

TR/TE = 4000/30 ms, same resolution as MPRAGE). MPRAGE provides high gray/white matter contrast, and will be used for tissue segmentation, spatial normalization, and voxel-wise analysis of brain atrophy. TSE will primarily be used for intracranial volume estimates, brain masking, and for a supplementary step in registering EPI data, which have T2-weighted features, to MPRAGE. The protocol will be augmented by high-resolution TSE imaging (TR/TE= 4000/21 ms; base matrix size of 512x512 yielding 0.5x0.4 mm^2 in-plane resolution, 24 slices, each 2 mm thick, aligned perpendicular to the main axis of the hippocampus) for volume measurements of hippocampal subfields.

b) Susceptibility-weighted MR imaging (SWI): SWI (Reichenbach et al., 1997; Haacke et al., 2004), originally designed for MR venography by using the paramagnetic property of intravascular deoxyhemoglobin, will be used to detect microhemorrhage with substantially higher sensitivity than would be possible with conventional gradient-echo MRI (Tong et al., 2003a). Based on a high-spatial resolution three-dimensional gradient-echo technique, SWI is extremely sensitive to the susceptibility changes related to small hemorrhagic lesions and can be performed with conventional MRI instrumentation. The method acquires both magnitude and phase image data and employs a post-acquisition processing step, including high-pass filtering of the phase data, to create enhanced contrast between tissues with different magnetic susceptibilities. SWI will be implemented at 3T with the following parameters: TR/TE 25/32ms; 0.6 x 0.5 x 1.5mm resolution, 0.5mm gap between slices, flip angle 12 degrees. Magnitude and phase images will be analyzed in a semi-automatic fashion to measure lesion volumes.

c) Diffusion Tensor imaging (MRI-DTI): We will use a multislice single-shot EPI sequence (TR/TE = 6000/90 ms, 3x3 mm^2 resolution, 40 contiguous slices, 3 mm each). Susceptibility distortions and signal loss due to T2* will be reduced by first using parallel imaging with two–fold acceleration, second by using a relatively high bandwidth for EPI of at least 2,200 Hz/pixel, and third by employing the reversed-gradient methods, in which two EPI images are collected under equivalent imaging settings except that one will traverse k-space top-to-bottom and the other bottom-to-top (Chang et al., 1992; Andersson et al., 2003). Although the reversed-gradient method will prolong scan time two-fold, this is not a disadvantage since signal averaging is needed. The MRI-DTI sequence is augmented by diffusion encoding gradients and incorporates two refocusing pulses to minimize eddy-currents. Diffusion-weighting gradients will be applied along 25 to 30 directions, depending on the final harmonization between GE and Siemens scanners. The directions for the 25/30 directions will be taken from a capped dodecahedron (also known as the molecular structure of a Buckyball) to minimize directional noise bias. The minimum of 25 directions is chosen based on methods developed by Dr. Singh of USC (who is collaborating with Dr. Weiner on other studies) to resolve multiple fibers without necessity for Q-ball diffusion imaging (Tuch et al., 2004) which requires substantially longer scan time than MRI-DTI. EPI-based diffusion sequences will be optimized to ensure comparable settings, such as image resolution, bandwidth per pixel, background noise level, phase-encoding direction, b-value, and diffusion-weighting gradient table. We will use MRI-DTI (Basser et al., 1994) to investigate white matter integrity by employing Tract-Based Spatial Statistics (TBSS) {Smith et a, 2006} #14911} for voxel-wise MRI-DTI analysis, and the fiber tractography tool DTI Studio (http://lbam.med.jhmi.edu/DTIuser/DTIuser.asp) to generate tract-based regions of interests (ROI). Other useful tools from FMRIB Software Library (FSL) (Smith et al., 2004) and Analysis of Functional Neuro-Images (AFNI) (http://afni.nimh.nih.gov) are integrated into our MRI-DTI processing package for image reconstruction, including raw image artifact correction (eddy current and susceptibility artifacts), optimized diffusion-tensor estimation, spatial normalization, and non-parametric multivariate analysis methods involving randomized permutation tests. Compared to other voxel-based tools, TBSS has advantages with regard to the nonlinear co-registration quality for MRI-DTI images and the unique skeleton projection technique, evidenced by a sharper mean FA map that reduces the need for spatial smoothing to account for misregistrations. DTI-based tractography serves to generate voxel-wise images of specified fiber tracts, on which FA and mean diffusivity (MD) can be analyzed in a ROI-based manner to test tract-relevant hypotheses with enhanced statistical significance. Group comparisons (by permutation tests) will be used to detect white matter differences, whereas the associations between DTI and non-DTI measurements, established by multiple linear regression and/or multivariate analysis, are expected to reveal the WM alteration contributions attributable to targeted PTSD/TBI biomarkers.

d) Arterial Spin Labeling (ASL) perfusion imaging: We plan to measure brain perfusion using the pulsed ASL-MRI protocol established by the functional brain imaging network (fBIRN) group for 3T Siemens and GE platforms. Drs. Weiner and Schuff are members of the calibration group of the fBIRN. ASL-MRI sequence of fBIRN consists of a single-shot EPI (TR/TE=4000/11 ms, 2890 Hz per pixel bandwidth, 3.4x3.4 mm^2 inplane resolution, 24 axial slices, each 4 mm thick. However, pulsed ASL sacrifices some sensitivity compared to continuous ASL (Wong et al., 1998), whereas the RF requirements for continuous spin labeling are generally prohibitively high and pulses too long for 3T MRI systems. Dr. Alsop of Harvard University, a consultant to this study, developed pseudo-continuous ASL (Garcia et al., 2005) with similar sensitivity than continuous labeling for GE scanners and Drs. Detre and Wang of the University of Pennsylvania, also consultants to this study, independently developed pseudo-continuous ASL for Siemens 3T (Wang et al., 2005). Pseudo-continuous labeling can acquire one slice in approximately 50 ms and approximately up to 10 slices can be acquired without excessive signal loss. Interleaved acquisition of more slices to cover the brain is supported. We will therefore attempt to implement pseudo-continuous ASL at the various study sites during the initial MRI calibration phase of this study, but will default back to pulsed ASL should calibration of pseudo-continuous ASL-MRI across sites fail.

e) Phantom scans: Scanner calibration will be done using specially designed phantoms. The structured ADNI phantom will be used to verify gradient calibration and geometry. A sophisticated diffusion phantom (currently being built by Dr. Le Bihan, Orsay, France) that allows fiber separation will be used to verify DTI. No adequate phantom currently exists for perfusion. However, we will adapt the quality assurance protocol from the fBIRN to verify signal-to-noise and stability of EPI, which is the backbone of the ASL sequence, on a silicone oil phantom. During the main study, the ADNI phantom and silicone oil phantom will regularly be scanned at each site for quality assurance. We will determine how often phantoms need to be scanned based on the data we collect at each site during the preparation phase.

Central QC and Data Processing:

The central laboratory for MRI quality control and image processing will be the CIND. Upon receipt, all images will be immediately inspected for quality. All subsequent steps in data checking and processing are automatically logged and recorded by our work flow management system. The sites will be notified of any problems concerning data quality of a specific subject or image sequence. If we see any abnormal patterns emerging we will immediately contact the site and take steps to correct the problem.

The CIND will perform a variety of automated image processing steps (most of which have been developed by other outside investigators, validated, published, and are available via the world wide web) on all of the MRI data. It is our experience, however, that even the most "completely automated techniques" require a great deal of visual checking to insure that the programs function correctly. The only completely manual image processing analysis to be done in this study is the manual measurement of hippocampal subfields. The automated processing steps for structural MRI involve spatial normalization using affine alignment for initial registration and progressing towards non-linear registration using in-house software based on large-deformation fluid diffeomorphisms (Christensen et al., 1997). Tissue segmentation is accomplished using a single channel Expectation Maximization Segmentation (EMS) algorithm (Van Leemput et al., 1999a;b). Cortical thickness measurements will be performed using Freesurfer Software using published methods (Fischl & Dale, 2000). Freesurfer is also used for parcellation of different brain structures to derive regions of interest (ROI) for structural perfusion, diffusion, and spectroscopy data.

Perfusion analysis, including atrophy and gray/white matter partial volume correction will be performed using established methods, which we previously published (Johnson et al., 2005; Du et al., 2006; Hayasaka et al., 2006).

Data Analysis:

For the primary outcome measure, the MRI-DTI, there are two aspects of the data, the fractional anisotropy and the tractography. The fractional anisotropy provides specific values that can be compared across subjects and within subjects to determine change over time. However, even for the fractional anisotropy, we expect that there will be substantial fluctuations in values between patients. Therefore, in the analysis of the

baseline measures, we expect that specifically quantified neuropsychological / speech & language deficits will show significant point correlations with the fractional anisotropy of the related brain region. Significant correlations are also expected with PET, EEG, EPR localization, and specific types of RT.

When the second MRI-DTI scan is done a year later, the two scans from each individual will be co-registered and precise assessments of change over the year will be calculated. For those patients participating in the computerized rehabilitation therapy, a correlation is expected between estimates of the severity of the brain damage and the change in behavior.

Specific analysis directions include:

1) Determine the relationship between local axonal shearing as measured on MRI-DTI scans and cognitive dysfunction:

Mild TBI patients frequently have abnormalities on MRI-DTI scans. At this time, it is unclear whether mild TBI patients have more such injuries than age-matched controls. This aspect of this study will provide critical foundational information about the presence of axonal injuries in mild TBI specifically with respect to normal individuals and to the cognitive deficits.

2) Determine whether one year of intense cognitive-rehabilitation will enhance connectivity of brain regions subserving similar functions to those areas where axonal shearing occurred:

Mild TBI patients are expected to improve their cognitive function over the course of a year of intense cognitive rehabilitation. However, the physical confirmation of this improvement would be to see actual regrowth of neuronal connections, which this specific aspect of this study proposes. This outcome measure, if it shows an effect, would be considerably more robust than any functional measures, which have substantial variability and less direct relationship with the underlying brain functions. The comparison with normal subjects is essential to determine if benefits could be non-specifically related to the rehabilitation measures or are related to targeted enhancement of individual patient deficits.

3) Determine whether one year of intense cognitive-rehabilitation provides benefit to TBI patients on any other measures of brain or cognitive function:

A comprehensive set of measures of brain, cognitive, and social function will be studied to determine whether individual patients have made improvements that are related to changes in axonal connectivity and improvements that will benefit their lives. These changes will be contrasted to those seen in normal subjects and more severely impaired and older subjects, to determine if there is specificity for the benefit for mild TBI cases or if the benefits are non-specific.

4) Determine whether any neuropsychiatric diagnoses, medication treatments, or other interventions interact with baseline conditions to moderate treatment outcomes.

Additional analyses will be made for possible interactions. For example, the tabulation of neuropsychiatric disorders and medications will be analyzed for relationships to treatment outcomes.

There is no estimation of effect-size or the number needed to demonstrate a particular power to detect significant effects, because the method to estimate the variety of axonal disconnections that will be seen are not yet established. While a close connection is expected between type of neuropsychological test and the region of local axonal shearing, we expect to find a very wide assortment of localizations of the shearing.

Other Brain Imaging Studies (Secondary outcome measures):

a) PET with CT and MRI Coregistration and Cortical Element Analysis:

PET/CT (FDG) and 3T-MRI images will be obtained as part of the WRIISC assessment, prior to the Cognitive-Training, and after one year, at the end of the training. The 3T-MRI scans will be segmented into

gray and white matter. The CT scans will be coregistered to the MRI images. Then the PET metabolism values will be apportioned to the regions identified as gray matter of the cerebral cortex. Changes between the preliminary and post scans will be calculated and analyzed for significant change. Abnormal axonal terminations will be studied for their relationship to the gray-white matter boundary (suspected location of shearing). Decrease cortical metabolism will also be studied for its relationship the points of axonal shearing.

An important part of the development of this project will be the development of co-registration techniques to integrate data from the 4T-MRI (including DTI), 3T-MRI, fMRI, PET/CT, and EEG/ERP to precisely determine brain pathology (Drs Ashford, Rosen). This co-registration will be essential to determine exactly which MEMTRAX stimuli are associated with specific regions of brain function and dysfunction, to validate the expanded MEMTRAX cognitive assessment system as a brief, accurate cognitive assessment technique that can be used in many environments, and to develop specific brain region targets for focal rehabilitation.

The basic evaluation tools used in this part of the project, 3T-MRI, CT, PET, to be used in this project are not new and are available for routine clinical use at many VA hospitals. However, it is only since about 2006 that the higher grade machines for performing these procedures have become available as standard clinical equipment. As of 2007, many centers have a 3 Tesla MRI (3T MRI) scanner and a PET/CT scanner. While 3T MRI scans offer very high resolution of anatomical structures (.5 x .5 x .5 mm), the magnetic inhomogeneity problems preclude rigorous quantification of specific volume elements (voxels) of brain tissue. Further, the measures of MRI, the T1 and T2 characteristics and the proton density characteristic, have not been clearly related to subtle pathological cortical function. CT scanning offers relatively stable numbers about tissue electron density (Hounsfield units), but provides considerably less resolution (1 x 1 x 1 mm) than MRI. PET scanning shows metabolism of brain tissue with relatively stable proportions across regions, but has even lower resolution (2 x 2 x 2 mm). The methods proposed in this sub-section use the CT scan, collected during the same head-fixation session as the PET scan, for coregistration with the MRI scan.

Anatomical analysis: the principle issue anatomically is assigning brain voxels as white matter, gray matter, CSF, or other. To achieve this delineation, CT and MRI will be coregistered and used to estimate the classification of all brain tissue. After coregistration, estimates will be made of the localization of all gray matter, which will then be subtracted from the image array (and added to a different array), leaving the white matter as the external surface. In the subsequent step, the external white matter surface will be applied to the inner side of the gray matter (gray matter array) and vectors will be traced directly outwards from the large array of external white matter surface points, testing the gray matter voxels, to determine the width of gray matter at each point surrounding the white matter. Additional analysis will be made of the gray-white matter distribution, which is abnormal in TBI patients, in proportion to the severity of the TBI (Thatcher et al., 1997). At this step, the gray matter will be defined for the brain, for use in analysis of brain metabolism measured by PET.

Density analysis: the density of the cortical gray matter will be estimated by determining proton density (from MRI) and electron density (Hounsfield units from CT) apportioned to the gray matter voxels, for all cortical regions.

Metabolic activity: With gray matter defined, PET activity will be apportioned to the gray matter. White matter activity is expected to be very low and CSF values essentially zero. However, the average value of these tissues will be estimated in areas with no apparent partial volume effects, so that each PET voxel can be appropriately apportioned to nearby gray-matter anatomical voxels. Averaging will then be done across cortical thickness with cortical surfaces of 1 mm x 1 mm. 3-D surface renditions will also be made for evaluating regional function. The surface of the resulting 3D image will be composed of an array of cortical elements, serving as the individual units for analysis, including metabolism, proton and electron density, and thickness. PET data will then be analyzed with 3-dimensional stereotactic surface projections using

Neurostat software (Minoshima et al., 1998). (Dr. Ashford has developed similar soft-ware which will be used for comparison.)

Statistical analysis for PET metabolic measures:

1) Each of the 100 mild TBI cases will be examined individually for pathological brain findings.

2) Then, each cortical element of each patient brain will be assessed with respect to the 50 control subjects, to determine if that individual has a significant number of cortical elements with density or metabolic values that are abnormal. Such abnormalities will be compared to the behavioral and psychiatric test values, and the patient treated accordingly.

3) Third, an analysis will be performed on all cases to determine the typical constellation of cortical element abnormalities associated with behavioral and psychiatric abnormalities. Then, each patient will be reanalyzed to determine if their cortical patterns have a significant match to any pathological constellation.

4) In cases without statistically significant abnormalities of cortical elements or any match to a pathological constellation, a reanalysis will be performed for specific neuropsychological, speech and language, behavioral, and psychiatric problems of that individual, to see if a clinically significant match is present, according to specifically identified behavioral or psychiatric problems, to determine if their distribution of cortical element density, metabolism or thickness fits a pattern consistent those problems.

5) Change over the course of the year will be analyzed to determine if the cortical elements in the mild TBI patients are stable, improving, or deteriorating over time and with respect to the cognitive rehabilitation. Change will be assessed with respect to the normal control subjects and to determine if moderate TBI patients or older TBI patients show different patterns of change over the year.

b) EEG / ERP / RT Analysis:

Another set of methods to be used to as a secondary outcome measure is quantitative EEG (qEEG) and event-related potential (ERP) recording. Quantitative EEG and ERPs will be measured at the time of the initial evaluation and at the one-year follow-up visit. Also, reaction times will be measured for simple and complex decisions.

ERPs can be employed to assess cognitive processes related to stimulus gating (Arciniegas & Topkoff, 2004), sensory processing (Lew et al., 2004), and attentional allocation (Potter et al., 2001; Yucel et al., 2005). We have demonstrated the reliability of the ERP component (N1) to quantify perception of auditory stimuli (Lew et al., 2007). ERP abnormalities have been associated with both severity of neuropsychological deficits (Viggiano et al., 1996) and poor functional outcomes in patients with severe TBI (Lew et al., 2003).

The ERP referred to as the P300 is the manifestation of a brain response indicating the recognition of an unexpected event and may be part of the initiation of memory encoding. Shorter P300 latencies and larger amplitudes are associated with better cognitive performance (Segalowitz et al., 1997; Lew et al, 2004). Changes in amplitude and latency of the P300 component of the ERP waveform have also been widely studied as indicators of cognitive function and probable outcomes in patients with TBI (Lew, 2006). Additionally, P300 amplitude is suggested to reflect the extent to which cortical attentional resources have been utilized in a stimulus recognition task (Polich et al., 1995; Potter et al., 2001). The P300 clinical literature has demonstrated numerous factors associated with this potential. One recent example of uncertainty associated with the P300 noted the marked variability of P300 in elderly patients with dementia during a single day (Uemura & Hoshiyama, 2007), indicating the importance of diurnal rhythms affecting ERPs, but ERPs are relatively stable in TBI patients (Lew et al., 2007). Given the long history of P300 studies, it will be important to see how P300 latencies and amplitudes (and distributions) vary according the other results.

Thus, ERP offers the methodology to objectively quantify measures of cognitive function that do not depend on observer ratings (Lew, 2004; Yucel, 2005) in order to verify perception and attentional state for cognitive testing.

Analyses of "odd-ball" stimuli, both auditory tones and simple visual patterns, for ERPs will be done, following the traditional method of generating the P300. An option under consideration is the link the various complex stimuli from the MEMTRAX system with the ERP recording system to examine the P300 and later waves of the MEMTRAX stimuli, which may generate response patterns that reflect damage to specific cortical regions (Taylor & Olichney, 2007).

The available electrophysiologic system in Dr. Lew's lab for the assessments is a Neuroscan Synamps2 system with up to 32+ channels. There is an electrode cap system for placement of up to electrodes using the 10-20 system. ERP data will be obtained using a Physiometrix, e.Net (Billerica, MA) with 21 electrode sites (International 10-20 System) utilizing disposable gel electrodes. A NeuroScan STIM system (Compumedics USA, El Paso, TX) will be used for auditory and visual stimulus presentation and collection of behavioral responses. The NeuroScan portable SynAmps2, 32 channel digital amplifier, and Acquire software (version 4.3) will be utilized on a computer (Dell) for EEG acquisition, storage, and digital production and processing of ERP waveforms. ERP waveforms will be measured using Matlab (The MathWorks, Natick, MA) scripts previously developed by our laboratory (Lew, 2004).

Continuous electroencephalogram (EEG) will be recorded by a trained research assistant (RA). Baseline EEG recording will consist of two minutes each of eyes open, fixed gaze and eyes closed resting state. Event related potentials (ERP) will be extracted following each test session using tools available in the NeuroScan Edit software. To assess cognitive state, the following ERP components from a standard oddball paradigm in both the auditory and visual modality will be examined: 1) N1 for basic perceptual processing, 2) P300 for attention and working memory, and 3) the motor potentials associated with behavioral reaction time (RT), including response preparation and accuracy. Resultant amplitude and latency data for these components will be statistically analyzed as described below. Time to prepare participant and place electrodes for ERP collection is approximately 20 minutes. Total time to perform all ERP procedures (including preparation and testing) will be about 45 minutes.

Quantitative EEG will be used to a) assess the degree of functional impairment prior to treatment, and b) assess the degree of functional recovery after treatment. Analyses will use the Thatcher system (R.W. Thatcher's "NeuroGuide" TBI qEEG software; Thatcher, 2000; Thatcher et al., 2001). Since The Thatcher system uses a brief segment of resting EEG as its input, there will be no added data collection burden to the subject or researchers. Since this system has received both FDA approval for clinical use and also approval by a court as admissible evidence of functional brain impairment, and since it has been well validated in the literature (and extensively within the VA system), this system will be included in the assessment battery. This method represents a simple, valid, and widely recognized assessment that can be used both to determine a relationship between MRI-DTI axonal changes at baseline and at follow-up and with the cognitive rehabilitation measures.

Additionally, the EEG recordings and analyses will include LORETA (Thatcher et al., 2005) to examine the cortical distributions of impairment and recovery as measured by EEG. LORETA (which uses the same resting EEG input data as NeuroGuide) gives a complimentary and supplementary topographic dataset that can be compared with the MRI and PET results.

Information processing speed is also known to be slowed in TBI (Mathias & Wheaton, 2007; Tombaugh et al., 2007). The MEMTRAX system will measure a variety of reaction times, and this data will be collected at the initial evaluation, frequently throughout the year of intense cognitive rehabilitation, and at the follow-up assessment at the end of the year.

All EEG, ERP, and RT data will be analyzed for baseline signals that are associated with the presence and severity of TBI, including possible localizing effect, that may predict outcome a year later, and that may change over the course of a year to indicate successful rehabilitation.

Importance of additional secondary measures:
While the MRI-DTI is expected to show the actual brain injury that causes the cognitive and psychological problems associated with TBI, it is important to determine how traditional measures relate to the localized axonal injuries. It is hypothesized that demonstration of the axonal injuries will give a clear explanation for complex neuropsychological impairments and speech and language deficits. Further, there could be direct relationships between axonal loss and subtle changes of brain metabolism and cerebral atrophy (as suggested in the preliminary case study). Also, changes on EEG and ERP measures could be explained by the loss of axonal connections.

The change between the initial MRI-DTI scan and the year follow-up scan will reveal whether there is any regrowth of axonal endings or establishment of new connections to subserve lost cognitive abilities. If such reconnections are seen, then it will be important to link these changes with changes in neuropsychological test patterns, speech and language assessments, as well as PET metabolism, EEG/ERP, and other brain function parameters.

Functional level, Quality of Life, Interest in surroundings:
Activities of Daily Living (ADLs) will be assessed at the initial evaluation and at the end of the year to see if there has been any improvement in functional level. ADL scales, including "Basic" and "Instrumental" measures (Ashford et al., 1992) will be used to optimally assess the overall level of function of each subject on a linear continuum. The continuum will be expanded to include occupation and occupational attainment.

A health assessment will be performed using a validated questionnaire (SF-36; Ware & Sherbourne, 1992) during the initial assessment and at the end of the treatment year. Generic measures of Quality of Life are broadly applicable and can therefore be used across patient populations. The most widely measure used is the SF-36, which accounts for over 10% of the total number of reports on this subject (Garratt et al., 2002). The SF-36 (also known as the RAND 36 Health Survey, Version 1.0), encompasses 8 concepts: physical functioning, bodily pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, emotional well-being, social functioning, energy/fatigue, and general health perceptions. The questionnaire also includes a single item that provides an indication of perceived change in health. This questionnaire has also been used to assess changes in coping strategies, social support, optimism and health-related quality of life (QOL) in patients following TBI. The SF-36 was used in a previous study that assessed the changes in coping strategies, social support, optimism and health-related quality of life following traumatic brain injury.

The Apathy Inventory (Robert et al., 2002) will be administered to subjects as part of the initial assessment and at the end of the year of study. This inventory is sensitive to changes in brain function related to mild cognitive impairment and frontal lobe dysfunction.

ADL performance change on the linear continuum, QOL change, and difference in level of apathy over the year will be assessed with respect to which treatment group the 100 mild TBI cases were in, to determine whether the intensive cognitive rehabilitation program had any impact (positive or negative) on the subjects' lives. The changes in these measures will also be analyzed for the other groups.

Ancillary studies:
The VAPAHCS-WRIISC will be coordinating the assessment of numerous veterans with TBI in addition to the subjects of this study. Further, the subjects evaluated in the present study will represent a rich source of data for further analysis of other clinical questions. For example:

a) PET scanning with Pittsburg Compound B (PIB) in TBI:

There has been an extensive literature relating head trauma and Alzheimer's disease. Further, there have been reports of increased cerebral beta-amyloid following brain trauma (Uryu et al., 2002). The PIB compound has been shown to provide a reliable in vivo, in human assay of cerebral beta-amyloid. This compound could be used to determine if TBI patients have an elevation of beta-amyloid in their brains, and its relationship to the severity of the residual deficits and recovery from those deficits.

The Stanford Department of Nuclear Medicine has developed the first automated system for producing PIB. Preliminary discussions have suggested that imaging of TBI patients at the Palo Alto VA is a priority. The study described in the proposal would be ideal for initial studies of PIB as an assay for cerebral beta-amyloid in TBI patients. If significant elevations of PIB were found, an ancillary project could be developed to determine if PIB levels change over time or as a consequence of cognitive rehabilitation.

b) Cognitive stimulation and fMRI assessment of cortical activation:

Another issue is the determination of exactly which areas of the brain respond to a particular environmental stimulus. Activation paradigms using fMRI have shown specific brain regions responding to unique stimuli. Dr. Rosen, at the VAPAHCS, has been studying these aspects of fMRI (Rosen et al., 2005). For the purposes of this project, it would be useful to have information available to indicate what brain regions are activated by the various cognitive rehabilitation programs and stimulations. This information would allow more precise prescription of specific cognitive rehabilitation tools for the TBI patients. Plans are being developed to examine fMRI activation both in the normal subjects and in TBI patients. Activation in normal subjects will indicate what area of the brain responds to a stimulus under normal circumstances. An fMRI study of the same stimulus in a patient who lacks function in that usually activated cortical region, will show what secondary, back-up areas are available for the brain to utilize.

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ACRONYM LIST

aCing.....Anterior Cingulate
AD.....Alzheimer's Disease
ADL .....Activities of Daily Living
ADNI.....Alzheimer's Disease Neuroimaging Initiative
ASL .....Arterial Spin Labeling

BVMT-R .....Brief Visuospatial Memory Test-Revised
BVRT.....Benton Visual Retention Test

CBF .....Cerebral Blood Flow
CIQ.....Community Integration Questionnaire
COWAT .....Controlled Oral Word Association Test
CPT .....Continuous Performance Test
CRT.....Cognitive Rehabilitation Therapy
CSF .....Cerebral Spinal Fluid
CT .....Computed axial Tomography
CVLT.....California Verbal Learning Test

DOD.....Department Of Defense
DPI .....Driver Performance Inventory
DRS.....Disability Rating Scale
DSM-IV .....Diagnostic and Statistical Manual of mental disorders-revision IV
DTI.....Diffusion Tensor Imaging
DVBIC .....Defense and Veterans Brain Injury Center

EEG .....ElectroEncephaloGram
EMS .....Expectation Maximization Segmentation
EPI.....Echo Planar Imaging
ERC.....Entorhinal Cortex
ERP .....Event-Related Potential

FA .....Fractional Anisotropy
fBIRN.....functional Brain Imaging Network
FDA.....Food and Drug Administration
fMRI.....functional Magnetic Resonance Imaging

GCS.....Glasgow Coma Scale

HIPAA .....Health Insurance Portability and Accountability Act

IRB.....Institutional Review Board

MCI.....Mild Cognitive Impairment
MD .....Mean Diffusivity
MIDAS.....Metabolite Imaging and Data Analysis System
MIRECC .....Mental Illness Research Education and Clinical Center
MPRAGE.....Magnetization Prepared RApid Gradient Echo
MR .....Magnetic Resonance
MRI.....Magnetic Resonance Imaging

MRI-DTI.....Magnetic Resonance Imaging – Diffusion Tensor Imaging
MRSI.....Magnetic Resonance Spectroscopic Imaging

NAB .....Neuropsychological Assessment Battery

OEF .....Operation Enduring Freedom
OIF .....Operation Iraqi Freedom

PAIRE .....Palo Alto Institute for Research and Education
PASAT .....Paced Auditory Serial Addition Test
PCA.....Principal Component Analysis
pCing.....posterior Cingulate
PCL-M .....PTSD Checklist–Military version
PCS .....Post-Concussive Symptoms
PDF .....Portable Document Format
PET .....Positron Emission Tomography
PHI .....Protected Health Information
PI.....Principal Investigator
PIB .....Pittsburg Compound B
PM&R.....Physical Medicine & Rehabilitation
pMRI.....Perfusion-weighted Magnetic Resonance Imaging
PNS .....Polytrauma Network Site
PRC.....Polytrauma Rehabilitation Center
PTRP.....Polytrauma Transitional Rehabilitation Program
PTSD.....Post-Traumatic Stress Disorder

qEEG.....quantitative Electroencephalography
QOL .....Quality of Life

RA.....Research Assistant
RBANS .....Repeatable Battery for the Assessment of Neuropsychological Status
rCBV.....regional Cerebral Blood Volume
RFA.....Request for Applications
ROI.....Region Of Interest
RPQ .....Rivermead Postconcussive symptom Questionnaire
RT .....Reaction Time

SAE .....Serious Adverse Event
SF-36 .....RAND medical outcomes study short form
SFVAMC.....San Francisco Veterans Affairs Medical Center
SOW.....Statement of Work
SPECT.....Single Photon Emission Computed Tomography
SPM.....Statistical Parametric Mapping
SV-MRS.....Single Voxel Magnetic Resonance Spectroscopy
SWI .....Susceptibility Weighted Imaging
SWLS .....Satisfaction With Life Scale

TBI .....Traumatic Brain Injury
TBSS.....Tract Based Spatial Statistics
THQ .....Trauma History Questionnaire
TMT .....Trail Making Test
TOMM .....Test Of Memory Malingering

TSE .....Turbo Spin-Echo sequence
TSQ.....TBI Screening Questions

UCSF.....University of California, San Francisco
USAMRAA.....US Army Medical Research Acquisition Activity
USAMRMC.....United States Army Medical Research and Materiel Command

VA.....Veterans Administration
VAMC.....Veterans Affairs Medical Center
VAPAHCS.....VA Palo Alto Health Care System
VBM .....Voxel Based Morphology

WAIS-III .....Wechsler Adult Intelligence Scale–Third Edition
WMS-R.....Wechsler Memory Scale
WRIISC .....War-Related Illness and Injury Study Center
WTAR .....Wechsler Test of Adult Reading

VA Palo Alto Health Care System

Description of Facilities and Resources

The VA Palo Alto Health Care System (VAPAHCS) is a major tertiary care referral center. Full radiological, rehabilitative, laboratory and pharmacologic services support the mission of this Advanced Technology Proposal.

Polytrauma Rehabilitation Center (PRC):

Regarding Traumatic Brain Injury (TBI) and polytrauma, the core of the Polytrauma Rehabilitation Center (PRC) at VAPAHCS is a 12-bed ward located in Building 7D on the campus of the Palo Alto Division. The PRC building also has four general rehabilitation beds that are available to polytrauma patients on a priority basis, plus two additional beds for residential rehabilitation and/or women veterans. Since its inception (i.e., from February 2005 through early September 2007), the PRC has accepted 143 patients (mostly severe TBI), and 160 new outpatients (mostly mild TBI) since July 2006. Fifty full time rehabilitation professionals currently serve this mission.

Polytrauma Network Sites (PNS):

The VAPAHCS is also home to one of the 21 Polytrauma Network Sites (PNS) across the country. The PNS clinic provides key components of specialty rehabilitation evaluation and treatment that address the cognitive, mental health, and physical needs of individuals with polytrauma. The program provides outpatient and transitional rehabilitation to individual patterns of impairment sustained due to trauma as well as management of associated conditions through consultation with other specialties as necessary. Marked functional improvements occur with intensive rehabilitation services. Following the acute phase of rehabilitation, cognitive and behavioral difficulties often persist which prevent effective community reentry and/or return to duty. Seven full time rehabilitation professionals support the activities of this program. In April 2007, the VA Central Office issued a directive, implementing a mandatory procedure for the screening of symptomatic TBI, or post-concussive syndrome, among all OIF/OEF veterans. Since then, more than 150 new patients have been referred to the PNS. Dr. Henry Lew is the Director for Clinical Research for the PRC and the PM&R Service at VAPAHCS, as well as Staff Physician for the PNS. He will assure access to the resources required to fulfill the mission of this proposal.

Polytrauma Transitional Rehabilitation Center (PTRP):

In addition to PRC and PNS, a community re-entry program has been established. It consists of the specialty programs designed to be utilized in each of the PTRC rehabilitation settings (apartment, dormitory, day treatment and outpatient treatment) to provide intensive, focused treatments to increase functional and vocational independence. These programs will be housed within the PRTC to allow for an integrated approach to maximizing community independence and return to duty.

War Related Illness and Injury Study Center (WRIISC):

The WRIISC-VAPAHCS is one of three centers in the United States whose purpose is to provide an in-depth examination and evaluation of the medical problems of combat veterans with debilitating symptoms that remain unexplained after medical examinations by the local VA medical centers. The WRIISC is staffed by a full-time physician director, Dr. Ashford, specializing in traumatic brain injury, and a registered nurse, data analyst, risk/education coordinator, administrative officer, and three research assistants.

Community resources

Dr. Ashford and other investigators have excellent working relations with local community agencies. Dr. Ashford is a member of the Scientific Advisory Board for the Alzheimer's Association of Northern California, based nearby in Mountain View, CA. He is also the Chair of the Memory Screening Advisory Board for the Alzheimer's Foundation of America, based in New York City.

Dr. Yesavage, Associate Chief of Staff for Mental Health, is Dr. Ashford's supervisor. He and his staff have offered a number of cognitive retraining courses to local community agencies over the past six years.

Equipment

Quantitative Electroencephalograph equipment is located in the Polytrauma Rehabilitation Center at the VA Palo Alto Health Care System. This equipment includes Compumedics/Neuroscan Synamps2, version 4.3 software, 32 channel EEG collection and processing system; Neuroscan Stim, Stimulus presentation system; Physiometrix EzeNet, EEG electrode caps; Neuroscan SynAmps, 32 channel EEG collection and processing system; and Nicolet/VikingQuest version 8 software, EMG/EP acquisition system. It is exclusively used for research under the supervision of Dr. Henry Lew. Dr. Lew has endorsed the use of the equipment for the CORBISS study provided the study is able to fund its own EEG technician time and EEG supplies.

A magnetic resonance imaging (MRI) scanner (GE Healthcare SIGNA 1.5Tesla Excite HD, 1.5Tesla) is available at VA Palo Alto Health Care System. Gradients are EchoPlanar capable / slew is not the highest nor the lowest. Two Types of head coil are available 1). Birdcage Transmit and Receive 2). 8 channel phased array receive only. The current software platform/version running on the scanner is Linux Rev 12.1. This machine is located at VA Palo Alto Health Care System and will be available to the CORBISS study provided the study is able to fund its own MRI technician time.

For the Stanford Sleep Research Center, major equipment consists of ample computers, together with multiple printers, scanners and administrative accessories. All computers are connected to the internet. In addition, all computers and printers are connected to the university network. Sleep assessments are planned to become part of the routine clinical assessment for TBI patients admitted to the WRIISC program.

The Nuclear Medicine Department at VA Palo Alto Health Care System houses a GE Discovery VCT 64 slice PET/CT scanner as well as the technical staff and equipment to support its operation.

Single SPECT Measures of Cerebral Cortical Perfusion Reflect Time-Index Estimation of Dementia Severity in Alzheimer's Disease

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To determine the relationship between cerebral cortical blood flow loss and the temporal development of the dementia in Alzheimer's disease (AD), SPECT was studied in a cross section of AD patients with a broad range of impairment. **Methods:** Thirty patients with a diagnosis of probable AD had their mini-mental state examination scores transformed into time-index values to give an estimation of dementia severity relative to the developmental time course. SPECT images were obtained using ^{99m}Tc-ethyl cysteinate dimer and a 3-head camera. Cortical surface perfusion was analyzed, including modified Talairach standardization, to obtain cortical elements from the convexity (each representing about 0.25 cm^2 at the surface, 6.6-mm cortical depth) referenced to the mean perfusion of the full greater cerebellar hemisphere. These element ratios were analyzed (individually and by averages of estimated Brodmann's areas and brain regions) using linear regression with the time-index value. **Results:** For individual posterotemporal and inferoparietal Brodmann's areas (21, 22 and 39, 40, respectively) the correlation coefficients between cortical perfusion ratios and dementia severity ranged between −0.67 and −0.78 ($P < 0.001$). Perfusion ratios from these regions declined 2.5%–4.2% for each estimated year of progression. Prefrontal area perfusion showed less association with severity. Perfusion in primary cortical regions had no significant association with dementia severity. **Conclusion:** Cerebral cortical perfusion loss is temporally related to development of dementia. The spatial pattern of high, significant correlations between cortical perfusion and dementia severity shows a regional distribution that corresponds closely to the distribution of AD pathology described in autopsy studies.

Key Words: Alzheimer's disease; SPECT; computer-assisted image processing; cognitive dysfunction; dementia severity

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Alzheimer's disease (AD) is a condition that progresses over time (*1*) and impacts brain regions selectively, especially the posterotemporal and inferoparietal lobes (*2,3*). The pattern of changes in brain function can be observed with techniques that image glucose metabolism (*4–6*) or blood flow (*7–11*). These metabolic and perfusion changes correspond closely to the composite pattern of tissue degeneration seen at autopsy (*12,13*). The selective disruption of temporoparietal function seen with metabolic (*4,14,15*) or blood flow (*7–9*) imaging function is useful for confirming the AD diagnosis. Imaging techniques to identify this pattern may be useful in improving the detection of subjects early in the course of AD (*16,17*).

The general pattern of loss of temporoparietal function is not unique to AD. The same temporoparietal pattern of function loss is also seen in dementia caused by Parkinson's disease (*6,14,18–20*). However, to our knowledge, no other dementias have been found to produce this distribution.

In analyzing the relationship between regionally specific brain changes and cognition in living AD patients, investigators have reported associations between measures of dementia severity and decreases in cerebral function that are maximal in the temporal and parietal lobes, with correlations ranging from 0.4 to 0.77 (*7,21–24*). Because of the variability of the measures and the disease heterogeneity (*10*) and the imprecision of the relationship between specific neuropsychologic characteristics and local brain function deficits (*11*), controversy exists about the clinical usefulness of SPECT for the assessment of AD severity. For extending our understanding of the progression of AD and improving clinical assessment, determination of the relationship between cognitive and cerebral function in this disease is important. The explanation of this relationship depends partly on the precision and validity of the cognitive assessments and the resolution of the techniques for measuring cerebral function. One approach to improving the knowledge about this relationship is to better assess the time course of the clinical progression of the dementia in AD. Another approach is to improve the measurement techniques, using high-resolution computer image analyses with more precise quantification of cerebral cortical function.

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Analysis of the association between decline of function in specific locations of the cerebral cortex and progression of dementia severity may help to improve understanding of the developmental course of AD pathology.

It has recently been suggested that dementia severity could be represented by an estimate of the mean population time course of the disease's progression (*1,25,26*). Accordingly, the nonlinear relationship between mini-mental state examination (MMSE) score and time course can be estimated by either nonparametric (*1*) or parametric (*25,26*) means. For this study, a nonparametrically fitted model was used to transform the MMSE score into time-index values as described (*1*). For example, using the MMSE score and this model of loss of cognitive function for a mean population, 0 time-index year units correspond to a MMSE score of 25, and 7 or more time-index year units of disease progression correspond to a MMSE score of 0 (*27*). This transformation has recently been used on the dataset of the Consortium to Establish a Registry for Alzheimer's Disease (*28*). In addition to modeling this nonlinear change in mental status, use of units related to time course allows an adjustment of the chronologic differences between the date of the dementia severity evaluation and the date of the SPECT scan. This study analyzes the relationship between cerebral cortical perfusion and dementia severity in patients with probable AD relative to time course and thus estimates the rate of progression of the loss of cerebral blood flow in this disease.

MATERIALS AND METHODS

Subjects

In a dementia outpatient clinic and a long-term care unit, 90 male veteran patients had a standard battery of tests, which included the MMSE (*27*) and CT and SPECT scans, over a 2-y period (May 14, 1994, to June 13, 1996). Thirty patients (mean age, 74.5 ± 5.5 y; range, 64–87 y) had a diagnosis of probable AD (National Institute of Neurological Disorders and Stroke–Alzheimer's Disease and Related Disorders Association criteria [*29*]). Patients with a history of cerebrovascular disease, or whose structural brain scans indicated stroke or other cerebrovascular or neurologic disease, did not have a diagnosis of probable AD. None of these patients had a significant history of ethanol abuse within a decade of the development of dementia. For this study of the 30 patients with probable AD, no patient was included or excluded from analysis on the basis of the SPECT pattern. For these 30 patients, there was a small, nonsignificant, positive correlation between age and MMSE score ($r = 0.19$).

MMSEs were obtained an average of +31 ± 93 d before the SPECT scans (range, −114 to +443 d; median, 13 d), and scores ranged from 0 to 25 (median, 10; 7 patients had a MMSE score = 0). These MMSE scores were transformed into time-index values using a simple nonlinear equation (*1*); an MMSE score of 25 corresponds to a time-index score of 0 (usual point of onset of clinical symptoms), and an MMSE score of 0 is defined at 7.0 y of disease progression. The time-index scores were then adjusted to the date of the SPECT scans. The mean level of severity at the time of the scans in time-index year units was 4.3 ± 2.1 (range, 0–7.3). Patients were taking various chronic medications, including psychoactive medications such as haloperidol and trazodone, which may have had some effect on cerebral blood flow. However, none of the patients was taking γ-aminobutyric acid agonists or other medications that are known to have substantial effects on cerebral function. All patients had normal levels of consciousness during MMSE and SPECT testing.

SPECT Procedures

We used a 3-head SPECT scanner (PRISM; Picker International, Inc., Cleveland Heights, OH) with high-resolution collimators. While patients lay in a quiet, dimly lit room with their eyes open, an intravenous dose (925–1110 MBq) of ^{99m}Tc-ethyl cysteinate dimer (ECD) (≥90% radiochemical purity, Neurolite; DuPont Merck Pharmaceuticals, Billerica, MA) was administered. ^{99m}Tc-ECD has uptake characteristics that make it a satisfactory marker for measuring regional cerebral blood flow in AD and is best for maximizing image contrast and quality (*30*). ECD provides a high signal and is rapidly cleared from extracerebral tissue, yielding images suitable for computer analysis (*31*). This agent produces tracer concentrations in AD brains that are proportional to blood flow (*32*). Furthermore, it is suitable for diagnostic assessment, providing information that is useful for determining the degree of functional brain impairment in AD patients (*33*).

Thirty minutes after ECD injection, SPECT images were obtained (2.2 × 2.2 mm resolution, 128 pixels wide, 120 images, 30 s/image, continuous camera motion during data collection) with no corrections for attenuation. Raw data images were backprojected onto 128 × 128 horizontal matrices (ramp prefilter followed by a low-pass filter with order of 4.0 and cutoff frequency of 0.3; voxel size, 2.2 × 2.2 × 2.2 mm; resolution, 6.7 mm at 15-cm full width at half maximum).

Between-patient SPECT comparisons usually use the cerebellum as a reference (*15,34*). In AD, loss of blood flow probably occurs in the cerebellum, leading to an underestimation of the magnitude of the disease effect in structures being referenced (*32*). In this study the values of cortical surface elements were divided by the average perfusion of the full volume of the cerebellar hemisphere with the greater average value to obtain cortical perfusion ratios (CPRs).

Cortical surface analysis used parallel rays directed at the horizontal matrices to find the cortical surface (Figs. 1A and 1B). The cortical surface was defined as the first set of 3 consecutive points on the ray whose average exceeded a threshold of 35% of the value of the greater cerebellar hemisphere (although the starting point of the ray and the threshold could be adjusted to improve image quality). This average value was taken to represent the local cortical perfusion value. This method is similar to that described by Tachibana et al. (*18*), except parallel rays were used in the current study to find the surface, whereas the study by Tachibana et al. (*18*) used elliptic rays (we are currently developing a method that uses ovoid rays). For image viewing, 8 sets of parallel rays were directed at the brain, 1 set from each side (Figs. 1A, 1C, and 1E), superior and posterior, anterior and posterior, and outward laterally from an estimated medial plane (Figs. 1A, 1B, 1D, and 1F). Curved edge limits were compared between adjacent dimensions to reduce tangent artifacts (Fig. 1B), and threshold adjustments were used to improve resolution. The resulting views were assembled as a perfusion picture (Figs. 1C and 1D for left lateral and right medial views, respectively) and a surface 3-dimensionally rendered picture (Figs. 1E and 1F for left lateral and right medial views, respectively). The lateral surface structural images allowed visualization of external anatomic landmarks of the brain, such as the

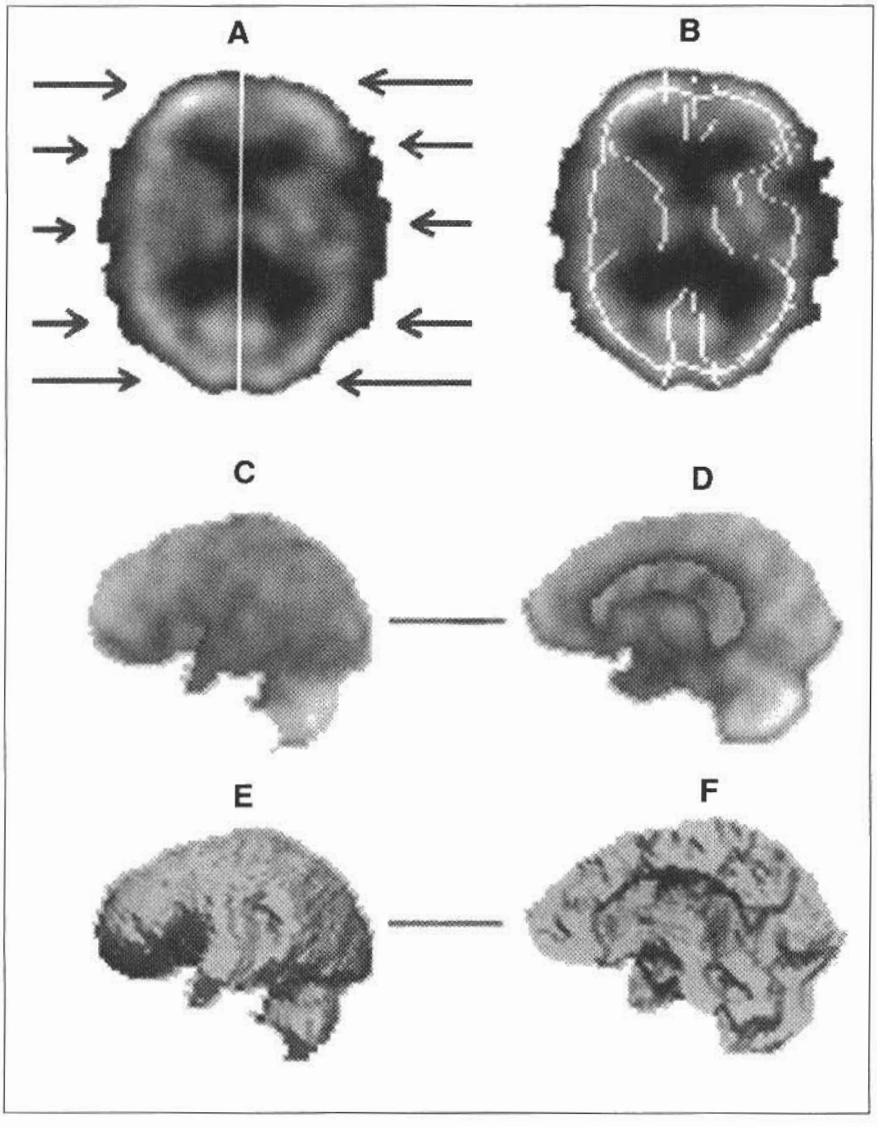

FIGURE 1. Cortical surface determination. (A and B) Horizontal matrices are analyzed by parallel rays (A, lateral rays are directed medially) to estimate cortical surface (B, lateral and medial surface estimates for both left and right hemispheres). (C and D) Raw, surface, cerebral perfusion values from across all horizontal matrices are used to construct cerebral perfusion pictures. (E and F) Information about location of surface values is used to construct 3-dimensional surface picture.

sylvian sulcus. The medial views portrayed the brain stem, the void of the corpus callosum just below the cingulate gyrus, the approximate location of the posterior commissure, and the suggested location of the anterior commissure (Figs. 1D and F).

Morphologic standardization was a modification of the Talairach and Tournoux procedure (*35*), applied to only the lateral views, left and right (Fig. 2). The line connecting the anterior and posterior commissure coordinates was estimated using the medial image processed by the 3-dimensional rendering algorithm. Because of the lack of clarity of the anterior commissure, reference was made to the external orientation of this line in Talairach and Tournoux's atlas (*35*) and the lateral images of the 3-dimensionally rendered external surface images to verify the position of this line. Because the anterior commissure frequently lies anterior to the tip of the atrophic temporal lobe of the AD brain (a violation of Talairach and Tournoux's morphometry), the tip of the temporal lobe was used as the midanterior limit instead of the anterior commissure, with separate adjustments for the left and right hemispheres. The cerebellum was then removed by estimating the boundary between the inferior aspect of the cerebrum on the lateral view (Fig. 2A). The full volume of each cerebellum between the lateral points and the estimated midline (Fig. 1A) was analyzed to define the mean voxel count values for each cerebellar hemisphere. The greater of the 2 values was used as the denominator for calculating the surface ratios of the cortical surface elements. Then the brain was stretched, using the established landmarks, to meet the standard outline (Fig. 3). Voxels from the raw image ($2.2 \times 2.2 \times 2.2$ mm) were grouped to form a standard size, resulting in the derived elements (each representing about 0.25 cm^2 at the surface, 6.6-mm cortical depth), presented in CPRs (Fig. 2B).

Statistical Analysis

The derived elements from the 2 hemispheres were transformed into a columnar matrix containing 1330 CPRs for each patient (representing a surface area of about 333 cm^2). On the basis of the description in Talairach and Tournoux's atlas (*35*), these CPRs were averaged for each Brodmann's area (Fig. 3) and for larger lobar regions (prefrontal, posterotemporal and inferoparietal, primary somatic) bilaterally (Table 1). We performed least-squares linear regressions of the average CPR for each CPR element, for 26 Brodmann's areas bilaterally (for each patient), and for these 3 global regions as a function of the time-index values (derived from

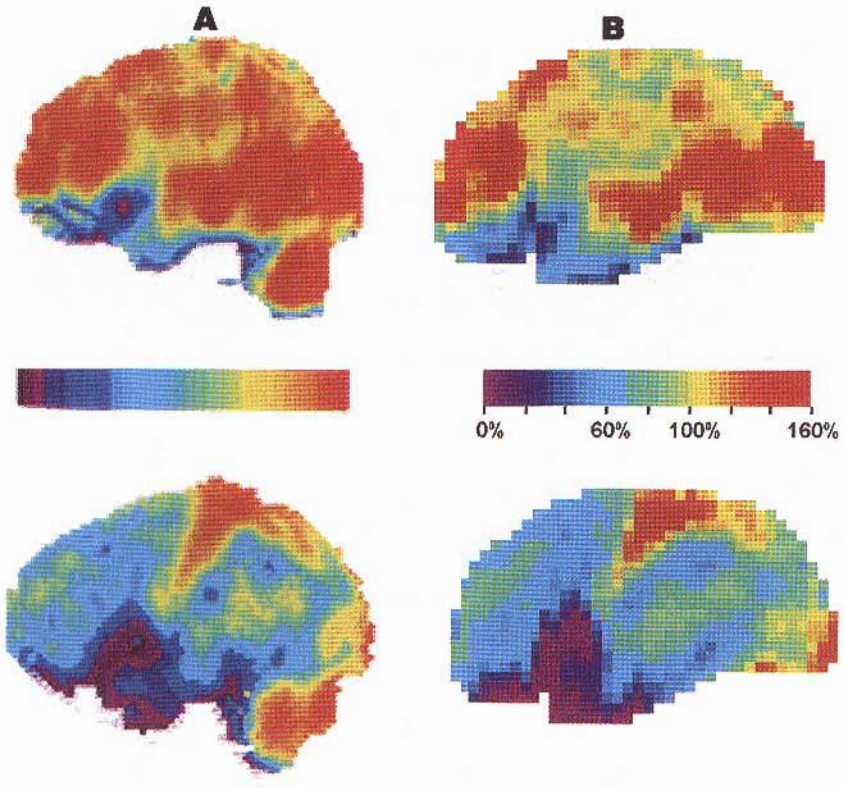

FIGURE 2. Cerebral perfusion image standardization. (A) Raw cerebral perfusion images are shown (highest value represents maximum value in dataset, far right on scale; pixels are 2.2 × 2.2 mm). (B) Values are transposed into lateral Talairach and Tournoux representation (*35*) (scale represents cerebral perfusion ratios relative to full greater cerebellar average raw-perfusion value).

the MMSE scores). The values from these calculations were used to assess the rate of decline in cerebral blood flow associated with increasing dementia severity. Correlations were examined to estimate the spatial distribution of CPR loss, to compare the degree of correlation between specific areas and regions, and to determine the significance of this loss.

RESULTS

A visual representation of the individual correlation coefficients for each surface element is shown superimposed on the Brodmann's areas from Talairach and Tournoux's atlas (*35*) (Fig. 4) to show the general distribution of the relationships between the CPRs and the time-index severity measures. The general pattern of correlations between cerebral blood flow and dementia severity (Fig. 4) suggested a negative relationship between CPRs and dementia severity that was most pronounced in the temporal and parietal regions, intermediate in the prefrontal regions, and minimal or positive in the primary regions. For most cortical elements in the temporal and parietal regions, regression values ranged from −0.40 to −0.80 (Fig. 4).

The posterotemporal or inferoparietal regions bilaterally showed a correlation between blood flow and dementia severity of −0.79 (Fig. 5A), with correlation coefficients for the individual Brodmann's areas in these regions ranging from −0.66 to −0.78 ($P < 0.01$) (Table 2). As expected, these results indicate that AD has a major impact on the posterotemporal and inferoparietal regions. Furthermore, the pattern of correlations (Fig. 4) indicates that the impact is distributed broadly across this relatively homogeneous associative cortex.

A correspondence was found between the rates of decline of the CPRs (calculated by least-squares regression) and the

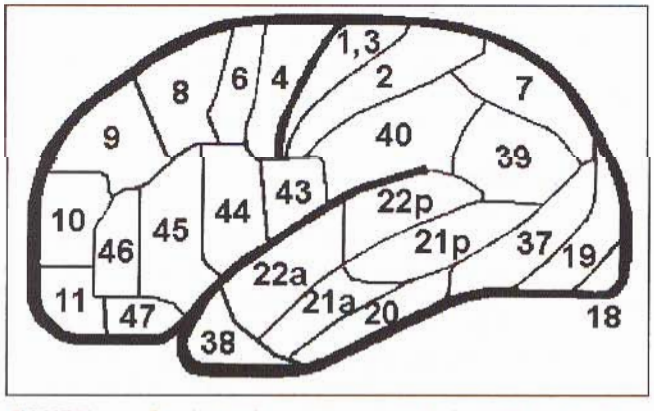

FIGURE 3. Brodmann's areas, adapted from Talairach and Tournoux (*35*). Areas 21 and 22 are divided into anterior (a) and posterior (p) sections.

TABLE 1
Brodmann's Areas of Designated Lobar Regions

Region	Brodmann's areas
Posterotemporal and inferoparietal	21p, 22p, 39, 40
Prefrontal	8–11, 46
Primary somatic (around central sulcus)	1, 3, 4

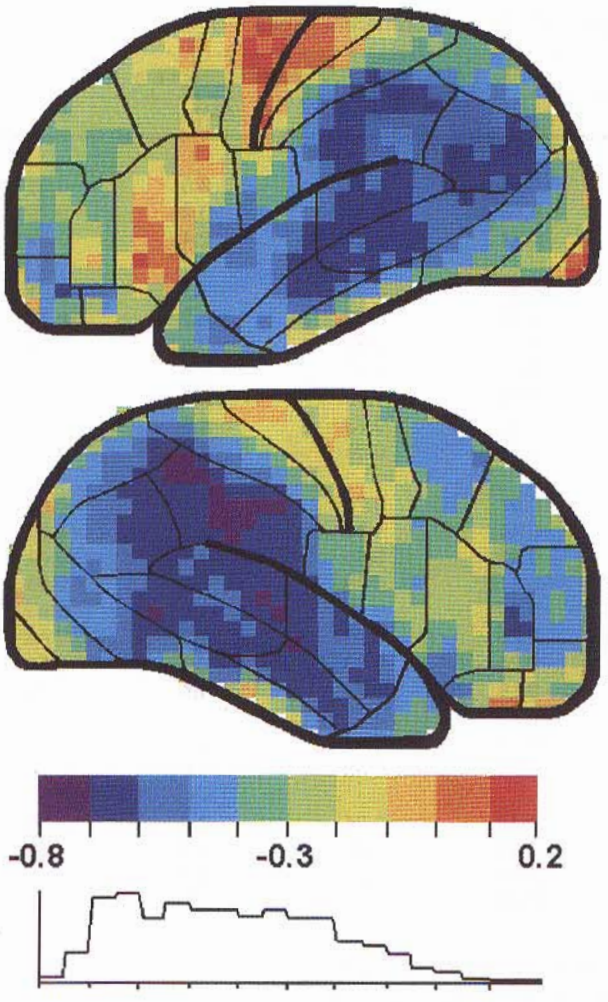

FIGURE 4. Lateral view of cortical surface (left side is on top). Scale indicates range for Pearson correlations with single decimal precision. Bottom graph shows relative frequency of each decimal range.

associated correlation coefficients between dementia severity and CPRs across all Brodmann's areas, with more rapid rates occurring in the areas with the higher correlations (Table 2). For example, the Brodmann's areas of the posterotemporal and inferoparietal regions showed rates of decline of 2.5%–4.3%/y (Table 2) and a rate of 3.3%/y for this region as a whole. Consequently, the CPR for this region decreased by 27% (from 1.08 to 0.80) for the time-indexed 7 y (MMSE decline from 25 to 0) between mild and severely impaired patients (Fig. 5A).

Generally, intermediate associations were found between severity and CPRs in the prefrontal areas (Fig. 5B; Table 2). The correlation with the average CPR for this region bilaterally was −0.53, and the least-squares linear regression showed a decline rate of 1.7%/y (Fig. 5B). Consistent with an observation reported earlier (*21*), there seemed to be more variability in the CPRs of the more severely impaired patients in these areas (note the increased dispersion of the values for the more impaired patients in Fig. 5B).

The CPRs in the primary somatic cortical regions (Fig. 5C) and the lateral visual areas of the occipital cortex (area 18) showed low correlations with severity, suggesting less change in CPR with the advance of AD. Another region relatively unaffected was Broca's speech area (Brodmann's areas 44 and 45 on the left side of the brain). The primary visual cortex, at the tip of the occipital lobe and medially

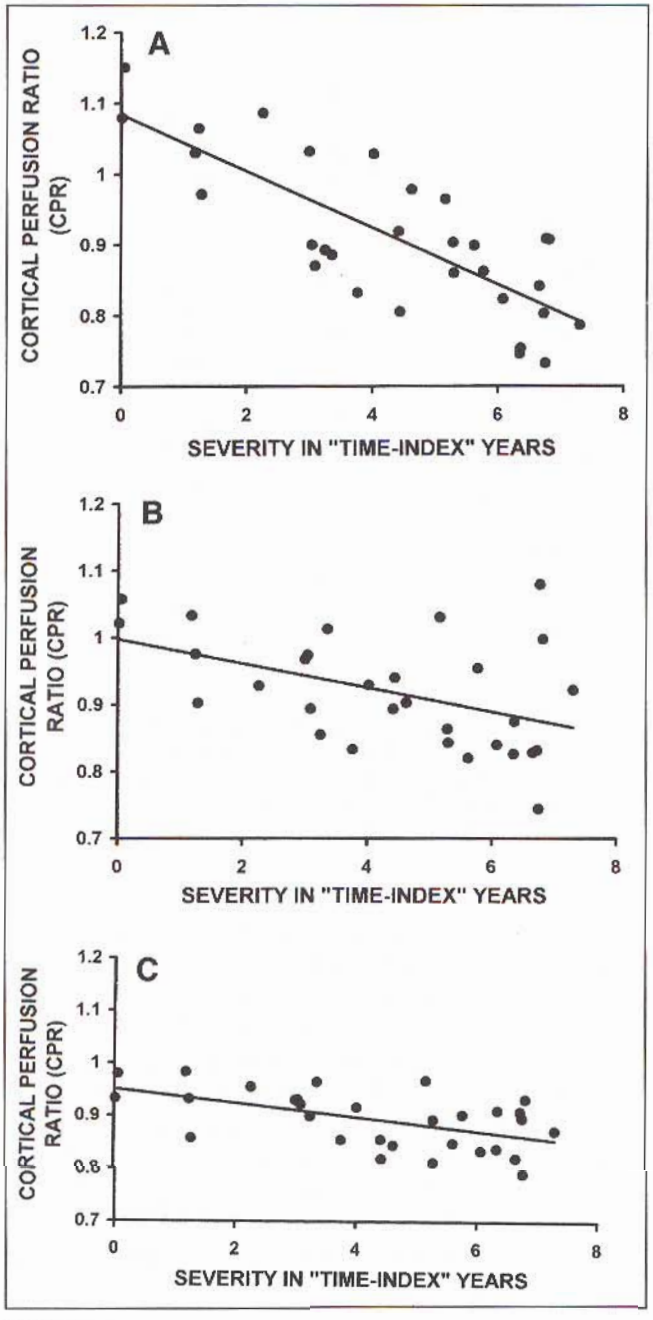

FIGURE 5. CPR values for regions (bilateral), plotted by time-index values for each patient; solid line is least-squares regression. (A) Posterotemporal (areas 21 and 22) and inferoparietal (areas 39 and 40); intercept = 1.08; slope = −3.3%/y; $r = -0.79$ ($P < 0.0001$). (B) Prefrontal (areas 8–11 and 46); intercept = 1.0; slope = −1.7%/y; $r = -0.53$ ($P < 0.01$). (C) Central gyri (areas around central sulcus) (areas 1, 3, and 4); intercept = 0.95; slope = −0.4%/y; $r = -0.14$ ($P > 0.05$).

TABLE 2
Correlation Between Dementia Severity and CPRs for Brodmann's Areas

Brodmann's area	n	Left hemisphere			Right hemisphere		
		Intercept	Slope	r^*	Intercept	Slope	r^*
Parietal							
39	36	0.91	−3.1	−0.69	0.95	3.6	−0.66
40	48	0.87	−2.9	−0.68	0.94	−3.9	0.78
7	28	0.86	1.9	−0.51	0.91	−2.4	−0.54
Temporal							
21p	30	0.91	−3.8	−0.71	0.95	−4.3	−0.71
22p	30	0.90	−3.3	−0.70	0.96	−4.3	0.72
21a	21	0.80	−2.8	−0.67	0.87	−3.8	0.72
22a	37	0.83	−2.5	−0.67	0.87	−3.5	−0.75
37	33	0.88	−2.2	0.56	0.92	−2.8	−0.59
20	20	0.68	−1.4	−0.55	0.73	−1.8	−0.56
38	21	0.64	−1.5	−0.50	0.66	−2.3	−0.56
Frontal							
11	30	0.77	−1.8	−0.57	0.78	−1.5	−0.40
10	26	0.93	−1.8	−0.44	1.00	−2.6	−0.50
47	13	0.59	−1.1	−0.43	0.59	−1.1	−0.41
46	19	0.81	1.3	−0.41	0.88	−2.2	−0.56
9	33	0.85	−1.4	−0.38	0.79	−1.6	−0.43
44	31	0.75	−1.0	−0.28	0.78	−1.6	−0.43
8	32	0.80	−0.9	−0.24	0.83	2.1	−0.54
45	38	0.68	0.5	−0.18	0.72	−1.4	0.44
Occipital							
19	25	0.88	−1.4	−0.31	0.91	−1.5	−0.35
18	5	0.85	0.4	0.08	0.93	−1.0	−0.20
Central							
43	18	0.84	1.7	−0.49	0.84	−2.0	−0.53
2	35	0.85	−1.4	−0.42	0.88	−2.1	0.49
6	19	0.83	−0.9	−0.25	0.83	−1.2	−0.33
1, 3, 4	47	0.84	0.0	−0.01	0.86	−0.7	−0.23

*For n = 30, 1-tailed tests indicate $r = -0.36$, $P = 0.05$; $r = -0.46$, P 0.01; $r = -0.57$, $P = 0.001$.
n = number of cortical SPECT elements in each Brodmann's area; r = Pearson correlation coefficient.
Linear regression results are given for CPRs of Brodmann's areas (arranged by cerebral region) versus time-index values. Intercept is calculated CPR (cortical blood flow ratio) at time index = 0 from least-squares linear regression line with slope calculated in units of %/y (loss in flow relative to that of cerebellum).

along the banks of the calcarine fissure, was similarly unaffected (Fig. 4, medial data not shown). Those patients with the most severe dementia still showed relatively normal blood flow ratios in all primary regions. The values of the correlation coefficients increased progressively from the primary regions through the corresponding secondary regions to the associative cortical areas (Fig. 4).

The calculated CPRs for all of these areas at the defined threshold of clinically observable dementia (time index = 0) showed small differences. None of the discrepancies between the intercepts was considered to significantly influence the correlation and rate analyses.

DISCUSSION

The aim of this study was to determine the relationship between cortical blood flow and the time-course of AD progression using cross-sectional data. Consistent with previous findings, impairment of CPRs was associated with dementia severity (*7,15,23,24*). Furthermore, these data showed highly significant correlations between blood flow losses in specific Brodmann's areas and the time course of dementia development. As expected, the most rapid decline of perfusion developed in the posterotemporal and inferoparietal areas of these patients. Flow changes in the frontal regions appear to develop at intermediate and variable rates, and no significant hypoperfusion develops in the primary cortical regions.

A notable geographic variation occurred between the pattern of Brodmann's areas affected in the right prefrontal regions (areas 8, 10, and 46) and the 1 prefrontal area on the left side (area 11). This pattern suggests that future studies of neuropathologic and functional brain changes in AD patients should report both left and right hemisphere results.

Several studies have used correlation analyses or linear regression to examine functional imaging measures and their association with clinical symptoms as measured by different dementia rating scales (*8,10,11,17,36*). In early cases of AD,

these scales have complex interactions with dementia severity (e.g., ceiling effects and education effects). Thus, a better method of dementia severity assessment is needed to grade progression of AD, especially for mildly demented patients. Brain scan analyses could help in the development of early assessment measures.

The mean time course of AD provides a framework to develop a transformation of any measure of dementia signs or symptoms, which are progressive in a defined population with AD, into a standard interval scale. Using values on a time-index scale, changes in mild ranges can be compared with changes in severe ranges using identical interval units (of time). In these analyses, rates of progression of blood flow loss over time could be estimated from cross-sectional data. The correlations between CPRs and dementia severity in posterotemporal and inferoparietal regions were as high as −0.79, meaning that the least-squares regression line explains 63% of the change in CPRs. These correlations are comparable with those found between different psychometric tests used to assess dementia severity (*36*).

A further issue is explanation of the variance in CPR data unexplained by dementia severity. Because of the concern that age could have contributed to this relationship, the correlation between age and MMSE score was examined. Age had a nonsignificant (slightly positive) correlation with MMSE score in this sample. Therefore, an age effect is unlikely to have affected the relationship between dementia severity and CPR. Also, because the relationship between age and MMSE score was (slightly) positive (older age, better score), the known relationship between age increase and CPR decline could not contribute to these findings. Similarly, cerebellar blood flow is believed to decline slightly over the course of AD, so use of the cerebellum as the reference would be expected to weaken, not contribute to, the findings (*32*). Because this study examined the cortical surface, correction for lobar atrophy was not required. AD is accompanied by minimal cortical thinning that could explain only a small part of the results. No other cerebral blood flow measurement factor, such as attenuation, was considered as a possible cause of the described correlation, but some such factor could have influenced the results.

In considering other possible explanations of the relationship between CPRs and illness time course, some unexplained variance could be associated with a wide range of patient factors. Prescribed centrally or peripherally active medications could have affected the SPECT values, including the variations in frontal lobe function, but few patients were taking medications, and those medications that were prescribed are not known to have such dramatic effects. Other factors include variability in performance on the MMSE; variations in brain anatomy and Brodmann's area location; disease-related issues, including genetics and environmental stresses; variation in the exact pattern of areas affected by the AD process; or concurrent additional neurodegenerative disease processes. Additional factors that could have affected the correlation include premorbid brain size or psychosocial variables, including education (*37*). More precise testing (*21,22,38*) and multidimensional testing (*8–11,36*) may also account for additional currently unexplained variations in the relationship between cerebral blood flow and dementia severity.

Prior studies suggest a pattern of sequential attack by AD of temporoparietal before frontocortical regions based on neuropathologic analysis (*2*) and metabolic brain scan results (*21,39*). These data indicate that the disease process does affect particular regions more severely according to the hierarchy of involvement suggested by the distribution of neurofibrillary pathology (*2*). However, more precise longitudinal studies are needed to define whether the attack is temporally sequential or related to a hierarchy of vulnerability (*1,38*). In the involved brain regions, AD pathology destroys neuronal processes (*40*). Presumably, the loss of metabolic demand associated with the loss of these processes causes the decrease in cerebral blood flow. Alternatively, other mechanisms, such as loss of projecting connections from activating regions, may account for such a decrease in intact structures, which could later contribute to the development of pathology in these structures.

This study shows that analysis of cerebral blood flow in patients with AD can help to determine the progression of AD and thereby may be useful for further understanding the evolution of the disease process, developing more reliable diagnosis, and testing new therapies. Multicenter SPECT studies of AD patients, particularly with respect to the time course of the disease, would help to clarify the usefulness of this tool, and the area in most need of study is the early phases preceding the dementia.

CONCLUSION

This study shows that SPECT data reflect the progressive loss of cerebral cortical blood flow in various brain regions relative to the time course of AD. Because the time-index method could be applied to any psychometric test used to assess dementia severity, it provides a framework to analyze disease progression across the full spectrum of AD and to make comparisons across different studies. Such analyses could be used as a standard to evaluate psychologic or radiologic tools that are needed to measure dementia severity and progression, especially for the investigation of treatment interventions and potential etiologic factors.

A similar correlation occurs between a clinical quantitation of 3-dimensional SPECT scans and interscan intervals to follow-up scans (*41*).

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Review

Neuroplasticity in Alzheimer's Disease

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Ramon y Cajal proclaimed in 1928 that "once development was ended, the founts of growth and regeneration of the axons and dendrites dried up irrevocably. In the adult centers the nerve paths are something fixed, ended and immutable. Everything must die, nothing may be regenerated. It is for the science of the future to change, if possible, this harsh decree." (Ramon y Cajal, 1928). In large part, despite the extensive knowledge gained since then, the latter directive has not yet been achieved by 'modern' science. Although we know now that Ramon y Cajal's observation on CNS plasticity is largely true (for lower brain and primary cortical structures), there are mechanisms for recovery from CNS injury. These mechanisms, however, may contribute to the vulnerability to neurodegenerative disease. They may also be exploited therapeutically to help alleviate the suffering from neurodegenerative conditions. **Published 2002 Wiley-Liss, Inc.**†

Key words: neuroplasticity; Alzheimer's; genetics; apolipoprotein E; therapeutics; pharmacogenetics

Abbreviations: AB, amyloid β protein; ADDLs, AB-derived diffusible ligands; AD, Alzheimer's disease; apoE, apolipoprotein E (gene or protein); APP, amyloid precursor protein; ChAT, choline acetyl transferase; CNS, central nervous system; CREB, cAMP response element binding protein; DRG, dorsal root ganglion; E4, E3, apoE isotypes epsilon 4, epsilon 3; EC, entorhinal cortex; ECL, entorhinal cortex lesion; ERT, estrogen replacement therapy; GFAP, glial fibrillary acidic protein; GT1-1, hypothalamic cell line; HC, hippocampus; HDL, high density lipoprotein; HNE, hydroxy-nonenol; IML, inner molecular layer of the hippocampus; ko, gene knockout mice; LDLR, low density lipoprotein receptor; LRP, LDLR-related protein; LTP, long term potentiation; MAP, microtubule associated protein; NCAM, neural cell adhesion molecule; NF-κB, nuclear factor kappa B; NFT, neurofibrillary tangles; NGF, nerve growth factor; NOS, nitric oxide synthetase; NO, nitric oxide; NSAIDs, nonsteroidal anti-inflammatory drugs; NSE, neuron-specific enolase; OML, outer molecular layer; OHSC, organotypic hippocampal slice culture; PNS, peripheral nervous system; PS, presenilin; PSD-95, post-synaptic density protein; TNFα, tumor necrosis factor α; VLDL, very low-density lipoprotein

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1. INTRODUCTION

Alzheimer's disease (AD) displays aspects of mechanisms related to all the major theories of aging: mitochondrial decline in energy production, deregulation of calcium homeostasis, ROS generation and accumulation of its damage products, immune/inflammation dysfunction, hormone deregulation, and loss of regenerative ability (Brewer, 2000). The information storage defect in AD is represented at all levels of systems functions: biological, psychological and sociological (Ashford et al., 1998a). All these factors and levels can be traced to basic mechanisms of memory storage and retrieval. The contribution of neuroplasticity to AD, as a compensatory response or a fundamental defect, is gaining recognition, from the original recognition of the implications of dystrophic neurites by Alzheimer and others to more recent evidence of plasticity at many levels (Fischer, 1907; Simchowicz, 1911; Scheibel and Tomiyasu, 1978; Scheibel, 1982). That AD is a fundamental defect in such mechanisms was first proposed in 1985 (Ashford and Jarvik, 1985) and has been recently reviewed (Neill, 1995; Mesulam, 2000; Arendt, 2001a,b). This common downstream target can explain how numerous genes and factors cause the same clinical and neuropathological phenotype.

AD is characterized by ongoing neurodegeneration, yet in AD and in normal aging neuronal loss is not a prerequisite for functional deficits (reviewed in Morrison and Hof, 1997; Mrak et al., 1997). Synaptic pathology is an early marker of both AD and aging (Greenough et al., 1978; Agnati et al., 1992; Martin et al., 1994). Is AD an inevitable consequence of aging-related processes, simply a faster deterioration of the capacity for plasticity? Even 'normal aging' can change its course at some point: enhanced dendritic growth in early aging (70s) is followed by regression of dendritic arbor in the oldest old (90s) (Flood et al., 1985). Plasticity in AD may be a process of compensatory, albeit futile sprouting in vulnerable neurons. In this scheme, mechanisms of plasticity and their physiological burden are overstimulated in AD, leading to secondary neurodegenerative effects, which then feed a vicious cycle of increasing plasticity burden (Mesulam, 2000; Joseph et al., 2001). The increasing burden of plasticity is initially an adaptive response that also includes upregulation of τ phosphorylation and APP turnover, with subsequent formation of neurofibrillary tangles (NFT) and amyloid plaques as consequences that eventually lead to neurodegenerative events including loss of synapses, axons, and dendrites, and eventually cell death (Mesulam, 2000). The two pathologic hallmarks of AD, neuritic plaques and NFT, could be both causative in memory deficits and result from more fundamental failures of memory, where positive feedback in vicious cycles could feed initially minor disturbances (Geddes et al., 1985). AD synaptic degeneration can also be viewed as an adaptive 'rescue program' in response to metabolic fuel deprivation, by pruning of the axonal tree to reduce energy-consuming neuronal activity, as suggested by the decrease in synaptic metabolic activity with age and in AD (Heininger, 2000). The vulnerability of neurons to the effects of such plasticity-elicited degeneration reflects their capacity for plasticity. A simplistic analogy is found in cancer, where cells with a predilection to divide are the most vulnerable to failure of mitogenic control. Failure of neuroplasticity ultimately unleashes the onset of clinical AD symptomology by disrupting the balance between degenerative and regenerative processes (reviewed in Mesulam, 2000; Arendt, 2001a).

It is remarkable that all genetic causes and risk factors of AD can impinge on neuroplasticity. Instead of causing AD, these genetic mutations can be viewed as interacting with ongoing, age-related impaired plasticity activity to accelerate the events that lead to its failure. Alternatively, they could initiate stress-related repair mechanisms that fail because of downstream defects in or blocks to plasticity. Are genetic factors in AD progeroid genes? For example, ApoE4 is associated with decreased longevity compared to E2 (Corder et al., 1994). What do genetic mutations tell about the distinction between AD and 'normal' aging? Do genetic mutations decrease the natural activity of the wild-type protein, or are they gain-of-function mutations that create altogether new activities? Only parallel analysis of wild-type and mutant forms address such questions. In addition, most of the genes and factors discussed here are pleiotropic and interact at various levels. Such interactions create many secondary, indirect effects that exponentially expand the complexity of AD etiology, and full coverage of these is beyond the scope of this review (extensively reviewed in Arendt, 2001a). Further, because most factors also show effects in neurodegeneration, the interactive relationship between neurodegeneration and the capacity for neuroplasticity adds yet more to this complexity (see Section 8).

2.1 NEUROPLASTICITY: AN OVERVIEW

Neuroplasticity is both a substrate of learning and memory and a mediator of responses to neuronal attrition and injury (compensatory plasticity). It is a continuous process in reaction to neuronal activity and neuron injury, death, and genesis, which involves modulation of structural and functional processes of axons, dendrites, and synapses. The varied structural elements that embody plasticity include LTP, synaptic efficacy, synaptic remodeling, synaptogenesis, neurite extension including axonal sprouting and dendritic remodeling, and neurogenesis and recruitment. In a broader sense, phenomenological processes that manifest plasticity are: synapses (electrical, biochemical, structural), neurite (axon, dendrite), neuron cell bodies, anterograde (toward distal neurites) and retrograde (from distal neurites) transport, cell interactions (neuron-glia), neural networks, and behavioral, psychological, and sociological activities.

The rules of synaptic strengthening postulated by Hebb (1949), which require a concerted activation of pre- and postsynaptic elements (see Sections 2.2, 8), subserve the phenomenon of LTP as a model of memory formation, and which is also associated with synapse dynamics including formation and removal of synapses and changes

in synapse morphology (Chang and Greenough, 1984; Toni et al., 1999; Martin et al., 2000) (see Section 2.2). Signals of plasticity include intraneuronal (anterograde and retrograde), interneuronal (transsynaptic and extra/parasynaptic) as well as intercellular signaling through glia (Cotman and Nieto-Sampedro, 1984; Neill, 1995). They include many molecules in the following families: extracellular matrix molecules, semaphorins/collapsins, immunoglobulins, myelin-associated inhibitors, tyrosine kinase receptors, netrins, neurotrophic factors, growth factors, inflammatory cytokines, and neurotransmitters; furthermore, many inhibitory molecules also come from the same classes (reviewed in Horner and Gage, 2000). Many mutant and transgenic mice have helped elucidate aspects of plasticity (reviewed in Chen and Tonegawa, 1997).

The adult central nervous system responds to injury with limited yet sometimes effective restoration of synaptic circuitry. Whether compensatory growth is widespread and whether it reverses cognitive deficits has been debated (Cotman et al., 1991; Poirier, 1994; Masliah et al., 1995b). Functional recovery requires that reactive synaptogenesis not exacerbate circuitry dysfunction, as has been proposed (Cotman et al., 1991; Masliah et al., 1991c). If reactive plasticity leads to aberrant misconnection by innervating the wrong target, there may be intrinsic, inhibitory or limiting mechanisms to attenuate such misguided synaptogenesis. Clearly, brain self-organization continuously balances synapse formation and removal as well as neurite sprouting and retraction, and in some conditions, inhibition of sprouting may actually be protective by sequestering dysfunctional neurons. Such inhibition of distal plasticity events could signal plasticity-related events in the perikaryon (Mesulam, 2000). Chronic stimulation, however, may become unsustainable resulting in a plasticity 'burden' that leads to degenerative events.

2.2 Synapses

The balance between dynamic stabilization and destabilization of synapses may provide the basis for failure of plasticity with age and disease. Aspects of LTP are mediated by rapid generation of new spines, presumably guided by actin-mediated shape changes (Engert and Bonhoeffer, 1999; Maletic-Savatic et al., 1999; reviewed in Luscher et al., 2000). The shape of dendrites, as well as cell survival, can be modified by neurotrophins (McAllister et al., 1999; reviewed in Huang and Reichardt, 2001). The cytoskeleton also mediates aspects of signal transduction, as shown by microtubule involvement with effector molecules in the hedgehog, Wnt, JNK, and ERK pathways (reviewed in Gundersen and Cook, 1999). Actin controls the generation and motility of growth cones, spines and dendrites. F-actin assembly at the leading edge of growth cones is regulated by many factors, especially those of the substrate (Suter and Forscher, 1998; Hynes, 1999) and by small receptor-activated GTPases including rac, rho and Cdc42 (Lanier and Gertler, 2000). Dendritic spines are enriched in actin (reviewed in Matus, 1999). Not only are they highly motile structures covered with presynaptic structures, they may coordinate with the postsynaptic complex, moving together, mechanically stabilizing the synapse (Barres and Smith, 2001). Synapse formation during development may be a collaborative process involving growth of a presynaptic element on a site where a postsynaptic spine is either present or ready to form (Horner, 1993) (see Section 9.1). Further, the cadherin/catenin systems play an important role in the recognition between presynaptic growth cones and its postsynaptic dendritic target (Brose, 1999). Subsequent actions of immediate early genes like Narp, Arc, and synaptotagmin recruit and localize synaptic protein components. Arc stimulates both activity and plasticity of synapses and is modulated by the insulin receptor signal cascade. The cellular sorting, directional transport, and specific accumulation of axonal and dendritic components (including certain mRNAs) (Schuman, 1999; Winckler et al., 1999; Wells et al., 2000) are affected by AD-related pathology like NFTs (see Section 6) and APP (see Section 5.2).

Interestingly, mRNAs for GAP-43 and Arc have been found in growth cones, and NR1 and Arc in dendrites, implicating the important need for their activities at these sites and their synapse-specific regulation (Crino and Eberwine, 1996; Gazzaley et al., 1997; reviewed in Huang, 1999; Martin et al., 2000; Campenot and Eng, 2000; Steward and Schuman, 2001). Translation-dependent synapse formation can occur even in the absence of cell bodies (Schacher and Wu, 2002).

Presynaptic markers include GAP-43, SNAP25, syntaxin, synaptotagmin, synaptoporin, synaptophysin, and the synapsins. GAP-43 is highly expressed in neural development, axon regeneration and neuritic sprouting (Neve et al., 1988; Masliah et al., 1991a ; de la Monte et al., 1995; Benowitz and Routtenberg, 1997). Postsynaptic markers include MAP-2, PSD-95, NR1, spinophillin, and dendritic actin (reviewed in McEwen, 2001).

2.3 Adhesion Molecules

Optimal cell adhesion is required for synaptic plasticity (Schubert, 1991). Presynaptic differentiation is triggered by molecules associated with the synaptic basal lamina (reviewed in McGowan and Marinkovich, 2000). Adhesion molecules also communicate directly with signaling cascades regulating cell proliferation and differentiation, like FAK and MAP cascade, which are also implicated in AD (Shirazi and Wood, 1993; Zhang et al., 1994; Gartner et al., 1999). L1 and PSA-NCAM are associated with regenerating hippocampal axons (Aubert et al., 1998; Seki and Rutishauser, 1998; Ronn et al., 1999; Weidner et al., 1999). NCAM-I, a marker of plasticity (Ronn et al., 1998), is increased in hippocampal regions, but in a disorganized way in more AD-affected hippocampal areas (Mikkonen et al., 1999). Proteolytic disassembly of the extracellular matrix is regulated by MMP-9 during dendritic remodeling in the adult hippocampus (Szklarczyk et al., 2002). Laminin stimulates neurite outgrowth (Baron-Van Evercooren et al., 1982), is reorganized with estradiol-induced neurite outgrowth (Rozovsky et al., 2002), is found around plaques in AD brain (McKee et al., 1991; Murtomaki et al., 1992), and its mRNA and protein

are elevated in AD brain. Laminin interacts with many factors and systems reviewed here (see Sections 3.3, 3.4, 5.3, 5.4, 6, 8, 9.3).

2.4 Glia: Astrocytes and Microglia

Astrocytes and microglia play critical roles in CNS response to and recovery from injury (Gage et al., 1988; Frederickson, 1992; Norenberg, 1994; Chao et al., 1996; Bechmann and Nitsch, 1997; Rabchevsky, 2002). Astrocytes have been shown to play important roles in nutrient supply, waste removal, and axonal guidance. More recent work reveals that astrocytes play a more active role in neuronal activity, including regulating ion flux currents, energy production, neurotransmitter release, and synaptogenesis. The latter includes the activity of glia cell apposition to synapses and the regulation of synapse elimination by ensheathment (known as glial swelling) (reviewed in Laming et al., 2000). Ultrastructurally, this is seen as close apposition of GFAP-positive processes (astrocyte end-feet) that undergo rearrangement associated with changes in GFAP expression and localization. This has been observed not only in the hypothalamus during estrus cycle-dependent synaptogenesis, but also in hippocampus and visual cortex, and may mediate the astrocyte control of synapse number in the developing cerebellum (Lino et al., 2001). Age-related increases in GFAP as an astrocyte activation marker, involved in astrocytic morphologic changes in responses to injury and stress (Nichols et al., 1993; David et al., 1997), may adversely affect their support of synaptogenesis (Vernadakis, 1996); indeed, repression of GFAP is associated with estradiol-induced neurite outgrowth (Rozovsky et al., 2002). Astrocytes can couple directly to neurons and directly regulate synaptic activity (Alvarez-Maubecin et al., 2000). Neurons signal to astrocyte through neuronally-derived glial growth factors (GGF) (Verdi et al., 1996). Glia (astrocytes, microglia and oligodendrocytes) secrete growth-promoting factors like neurotrophins (NT-3) and cytokines, and show age-dependent changes in this activity (Sievers et al., 1995; reviewed in Goldberg and Barres, 2000). Other possible mediators and modulators include S100b (Whitaker-Azmitia et al., 1997), taurine, PS-NCAM, tenascin, NT-3, and cytokines. Glia mediate many effects of estrogen on plasticity (Garcia-Segura et al., 2001) (see Section 9), and are major producers of apoE lipoprotein particles (see Section 2.4, 4).

Glia also play roles in failure of plasticity (reviewed in Lemke, 2001). When activated, microglia and astrocytes secrete potent inhibitors of neurite outgrowth (Snow et al., 1990; McKeon et al., 1991; Canning et al., 1996). White matter actively inhibits axon outgrowth through secretion of inhibitory proteins like myelin protein IN-1, proteoglycans, semaphorins and slit proteins; however, responsiveness of neurons to this kind of inhibition can depend on their ability to survive (Davies et al., 1997) (see Section 8). These may contribute to or mimic the astrocyte-induced physiological 'stop' signal to growth cone progression (Reier et al., 1983; Liuzzi and Lasek, 1987).

2.5 Age

Age diminishes many aspects of plasticity including LTP induction and maintenance, compensatory synaptogenesis after injury, and reactive synaptogenesis in response to complex experience (Scheff et al., 1980; Mori, 1993; Lanahan et al., 1997). The interplay of many factors contributes to decreased synaptoplastic potential in the aging brain, with resulting delay of axonal sprouting and less effective formation of new connections to replace those lost (McWilliams and Lynch, 1984; Anderson et al., 1986). The capacity of neurons to elaborate neurites is reduced with age but is not lost completely (Brewer, 2000; Brewer et al., 2001). The failure of granule cell axon sprouting is inherent in the age of the sprouting neuron, not the age of its targets (Li et al., 1995). Ca^{2+} homeostasis is disrupted with aging and can contribute to disrupted neuronal plasticity (Mattson et al., 1992; Teyler et al., 1994; Ghosh and Greenberg, 1995; Foster and Norris, 1997; O'Neill et al., 2001).

Is this age-related loss in plasticity capacity due to reduced intrinsic neuronal capacity, reduced stimulation, or increased inhibition, which can be the same as reduced stimulation in terms of neuronal permissiveness or responsiveness (Tuttle and O'Leary, 1998)? It seems all three are involved, and future experimental directions will focus on determining whether boosting the extrinsic signaling can ameliorate the reduction in intrinsic growth ability (Aubert et al., 1995; Neumann and Woolf, 1999; Cai et al., 1999; reviewed in Goldberg and Barres, 2000). The decreased capacity for plasticity with age might represent a continuous process of which AD is an inevitable endpoint, although there are many differences between normal aging and AD that support AD as a partly age-independent disease (see Section 1).

3. ALZHEIMER'S DISEASE

3.1 Temporal and Spatial Course

AD pathology progresses over a typical spatial and temporal course of events, with the sequential involvement of basal forebrain, entorhinal cortex, hippocampus, amygdala, and association cortices (Braak and Braak, 1991, 1997). This sequence of events can be understood from a perspective of the functional network through which these areas associate. AD-vulnerable regions like hippocampus and amygdala are related by ancient projections of the olfactory bulb. The entorhinal cortex sits at the evolutionary crossroads between the highly plastic olfactory system, with its distributed representation of information, and the archi-cortex (hippocampus), paleocortex (amygdala), and neocortex (see Section 3.6). These evolutionary relationships may underlie the neural network of initiating and propagating processes in AD (Ashford et al., 1998a).

AD pathology affects CNS regions involved in higher brain functions that are synaptically (structurally and functionally) plastic, and involved in acquisition of new epigenetic information. The limbic system has perhaps the highest potential for neuroplasticity compared to

other parts of the cerebral cortex (indicated by high level expression of GAP-43, particularly in the entorhinal-hippocampal pathway (see Section 3.6)) (Neve et al., 1988; Lin et al., 1992). Plasticity-related dendritic remodeling (length and branching) is most extensive in limbic and paralimbic regions (entorhinal-hippocampal), less in association cortices, and undetectable in primary sensory and motor areas (Arendt et al., 1998a). The lifelong increased neuroplasticity burden and chronic upregulation of plasticity-related cellular activities of the limbic system could increase its vulnerability to NFT formation. Degeneration in limbic structures could then spread to adjacent limbic and paralimbic neurons in reciprocally connected association cortices to increase their plasticity burden. This would induce reactive synaptogenesis to replace the synapses provided originally by the degenerating axons of NFT-bearing neurons and induce dendritic remodeling to receive synapses once associated with the dendritic trees of adjacent degenerating neurons. If these reactive neurons cannot respond to the challenge of this increased plasticity burden due to barriers to plasticity, they too might then be subjected to similar τ events and subsequent NFT formation with cytoskeletal disruption.

In AD there is extensive loss of cholinergic input into the hippocampus (reviewed in Francis et al., 1999). Cholinergic neurotransmission plays an essential role in reactive and experience-induced synaptic reorganization (Baskerville et al., 1997; Kilgard and Merzenich, 1998; Zhu and Waite, 1998), and induces production of neurotrophic secreted APP (Nitsch et al., 1992) (see Sections 5.3, 10.3). Cortical cholinergic depletion in AD (Geula and Mesulam, 1999) arises from loss of neurons that project from nucleus basalis of Meynert, a limbic structure that retains high plasticity in late adulthood (Arendt et al., 1995) and contains some of the first neurons to show NFT pathology (Mesulam, 1996).

3.2 Development, Differentiation Recapitulation

Mechanisms of plasticity in adults overlap those used in brain maturation in early development (Cotman et al., 1990; Eriksson et al., 1998; Wheal et al., 1998). Regions with the highest degree of structural plasticity are those that take the longest to mature during childhood (Braak and Braak, 1996) and are the same regions most vulnerable in AD (reviewed in Arendt, 2001b). Although many regions undergo critical periods of intense plasticity, many become relatively quiescent at maturity and the regions that retain high levels of plasticity correlate with AD vulnerability (Ashford et al., 1995, 2000; Alexander et al., 2002). This may allow for the evolutionary acquisition of higher brain functions; regions vulnerable in AD share a common evolutionary foundation in the massive enlargement of the association cortices and functionally linked regions (Rapoport, 1990; Neill, 1995) (see Section 3.1).

The differential susceptibility of AD-specific regions and neurons may be related to the degree of retained capacity for plastic remodeling (Arendt et al., 1998a). In vivo, synaptogenesis rates decline with developmental age (Gall et al., 1979) and there is recapitulation of developmental gene expression responses in adult lesion and aging, including AD (Kondo et al., 1996; Styren et al., 1999). The *Nun Study* indicates that the risk of AD can be determined as early as 20years of age, implicating genetic and developmental factors (Ashford and Mortimer, 2002). If such mechanisms controlling developmental plasticity are defective and are later reactivated (in aging, or AD, or pre-AD), they would contribute to ineffective plasticity responses and exacerbate the plasticity burden of aging and AD.

It has been hypothesized that a 'labile state of differentiation' of neurons allows for neuroplasticity after development but also renders these neurons vulnerable to degenerative effects (Arendt, 2000, 2001a,b). In AD, the differentiation control may be in some way disrupted, involving expression or re-expression of genes (dedifferentiation) that contribute to making new neuronal connections in regenerative plasticity, i.e., genes involved in both growth cones and synaptic connections (Pfenninger et al., 1991) (see Sections 3.1, 3.3), as a necessary component of the ability to maintain a high degree of plasticity throughout life. This retention of plasticity potential leads to or may require re-expression of developmentally regulated genes, alteration of posttranslational modifications and imbalance of gene products, and re-activation of cell cycle genes such as cyclin B and E, as observed in neurons in healthy, elderly individuals (Nagy et al., 1997; Smith et al., 1999) and in phospho-τ-expressing neurons (Nagy et al., 1997). This confounds irreversible block of entry into the cell cycle of the neuron, a situation that may trigger cell death (Heintz, 1993) (see Sections 8.2, 8.3). For example, developmentally regulated genes like MAP1B-P, involved in axon growth (Gordon-Weeks and Fischer, 2000; Mack et al., 2000), are downregulated postnatally but remains active in regions of plasticity (Nothias et al., 1996). Its distribution parallels PSA-NCAM, involved in neurite growth and synaptogenesis (Seki and Arai, 1993; Muller et al., 1996; Cremer et al., 1997). The capacities for plasticity may depend on specific kinases, high levels of neurofilaments, and τ isoforms (Myoken et al., 1990; Hof and Morrison, 1994; Bahr and Vicente, 1998; Delacourte et al., 1998; Esclaire et al., 1998; Morrison et al., 1998), some of which also mark neurons destined for degeneration in AD (see Section 8).

3.3 Synaptic Loss

Synaptic loss is an early event in AD and is a structural correlate of cognitive dysfunction (Gonatas et al., 1967; Gibson, 1983; Davies et al., 1987; Bertoni-Freddari et al., 1989; Hamos et al., 1989; Scheff et al., 1990; Weiler et al., 1990; Brunelli et al., 1991; Terry et al., 1991; Honer et al., 1992; Lassmann et al., 1992, 1993; Zhan et al., 1993; Martin et al., 1994; Masliah et al., 1994, 1995a; Dickson et al., 1995; Heinonen et al., 1995; DeKosky et al., 1996; Sze et al., 1997; Cotman and Anderson, 2000; Mattson et al., 2001; reviewed in Arendt, 2001a). Memory loss in AD may result from synaptic dysfunction that precedes large-scale neurodegeneration, where the synapse-to-neuron ratio is decreased by about 50% (Cullen et al., 1997; Lambert

et al., 1998; Chapman et al., 1999; Hsia et al., 1999; Chen G et al., 2000; Tezuka et al., 2001; reviewed in Arendt, 2001a). Synapse and dendrite loss in AD exceeds that seen with normal aging (reviewed in Terry et al., 1994; Anderton et al., 1998). Synaptic degeneration, like early AD, progresses slowly at first, perhaps reflecting attempts for compensatory plasticity, and as such could be initially reversible, but eventually becomes irreversible due to marked synapse loss (Rapoport, 1999).

In early AD, a number of growth-associated proteins are upregulated, which may reflect attempts to stimulate plasticity, including GAP-43, MARCKS, spectrin, heparansulfate, laminin (see Sections 2.3, 6), NCAM, various cytokines and neurotrophic factors including NGF (see Section 10.4), bFGF, EGF, IL-1, Il-2, IL-6, IGF-1, IGF-2, PDGF, HGF/SF, and several growth factor receptors (reviewed in Arendt, 2001b: see Section 3.2). Deregulation of proteins involved in structural plasticity of axons and dendrites (Jorgensen et al., 1990; Hatanpaa et al., 1999; Lubec et al., 1999; Mikkonen et al., 1999) and computational studies (Horn et al., 1996; Hasselmo, 1997) indicate a failure of plasticity mechanisms, and support a disruption of synapse turnover as a primary mechanism in AD (Arendt, 2001b) (see Section 3.2). For example, synapsin IIa mRNA is downregulated in early AD, as detected by gene chip microarray analysis (Ho et al., 2001; Pasinetti, 2001). Synaptic remodeling in AD brain is detected also by elevation in the NCAM/SNAP-25 ratio (Jorgensen et al., 1990, 1997; Jorgensen, 1993, 1995). Although these structural and biochemical changes in AD provide understanding of aspects of plasticity, their relation to the properties of LTP in relation to AD are poorly understood (Dawson et al., 1992; Farooqui and Horrocks, 1994; Nalbantoglu et al., 1997).

3.4 Axonal and Dendritic Remodeling

Extracts of AD brain increase axonal branching of neurons grown on laminin (Kittur et al., 1992; Jorgensen, 1993), and AD brain and CSF extracts sustain neuron growth and survival (Uchida et al., 1988; Uchida and Tomonaga, 1989; Pauwels et al., 1993; Erickson et al., 1994). Axonal and dendritic remodeling in AD show restricted regional and temporal localization (Arendt et al., 1995, 1997, 1998a). Neocortex and hippocampus exhibit increased sprouting and synaptogenesis in AD (Grady et al., 1989; Masliah et al., 1991a,c; Jobst et al., 1994). Sprouting of commissural and associational fiber axons in AD is indicated by expansion of kainic acid receptor distribution that matches that seen in entorhinal cortex lesions (see Section 3.6); hippocampal sprouting of septal afferents is indicated by the pattern of AchE innervation in the perforant path terminal zone (Geddes et al., 1985; Gertz et al., 1987; Hyman et al., 1987; Masliah et al., 1991c) (see Section 3.6). In AD, axon length correlates with dementia severity suggesting regressive axonal events may be more relevant than dendritic attrition or neuron loss (Anderson, 1996). This is consistent with degeneration of presynaptic termini that then leads to secondary transneuronal degeneration of postsynaptic dendrites (Su et al., 1997).

Dendritic extent in the hippocampus can increase with age itself, possibly a compensatory response to loss of synaptic connections (Flood and Coleman, 1990). This may not be sustainable, however, because enhanced dendritic growth in the early aging (70s) is followed by regression of dendritic arbor in the oldest old (90s) (Flood et al., 1985). Neocortex and hippocampus in AD also show massive somatodendritic sprouting (Ihara, 1988; Jorgensen et al., 1997), which may reflect unsuccessful remodeling in response to presynaptic or axonal damage (Scott, 1993). Such somatodendritic sprouts, which have filopodium-like structures resembling growth cones, contain τ and MAP2, which recapitulates their codistribution in neurite sprouting during development (reviewed in Arendt, 2001a). These dendritic changes therefore may be secondary to deafferentation, signal transduction failures, or cytoskeletal abnormalities (Anderton et al., 1998). As in aging, dendritic sprouting in AD may not be sustainable, as dendritic extent can decline (Flood and Coleman, 1990), particularly dendrites of hippocampal granule cells (Einstein et al., 1994). Some neuropil threads (curly fibers) show preferential development at dendritic branch points, suggesting that blocking dendritic transport could lead to dendrite pruning, and the loss of associated synapses (Ashford et al., 1998b).

3.5 Aberrant Sprouting and Dystrophic Neurites as Dendritic Sprouting

Neuronal sprouting in AD can be aberrant based on its localization, morphology, cytoskeletal composition (Arendt et al., 1986, 1998b; Arendt and Zvegintseva, 1987; McKee et al., 1989; Ferrer et al., 1990; Phinney et al., 1999), and synaptic protein expression (Geddes et al., 1985; Ihara, 1988). Aberrant sprouting can be an early feature of AD (Ihara, 1988), preceding detectable tangle formation and extensive neuron loss (Su et al., 1993; Arendt et al., 1998b), and therefore might represent a fundamental defect in AD rather than an overt response to ongoing degeneration (Geddes et al., 1991; Cotman et al., 1993; Masliah et al., 1993a,b). Abnormal neurite growth might be associated with the elevation of NGF receptors (Ernfors et al., 1990; Mufson and Kordower, 1992) that precedes neurofibrillary degeneration (Arendt, 1993). APP transgenic mice also show behavioral and synaptic changes before plaque formation (Holcomb et al., 1998; Hsia et al., 1999; Moechars et al., 1999; Chen G et al., 2000) (see Section 5). Transgenic mice expressing APP show increased hippocampal synaptophysin that correlates with impaired learning and memory (King and Arendash, 2002).

Dystrophic neurites (mainly dendritic) within or near plaques (Gonatas et al., 1967; Probst et al., 1983; Benzing et al., 1993; Su et al., 1993), as a consistent component of AD pathology, were originally regarded as aberrant sprouts by Alzheimer and others (Fischer, 1907; Simchowicz, 1911; Scheibel and Tomiyasu, 1978). This is supported by Golgi (Scheibel and Tomiyasu, 1978; Ferrer et al., 1983, 1990; Arendt et al., 1986; Ihara, 1988),

ultrastructure (Paula-Barbosa et al., 1980), and association of GAP-43, MARCKS, spectrin, and synaptic, axonal, and cytoskeletal proteins (Geddes et al., 1985, 1986, 1990; Masliah et al., 1989, 1990, 1991a,Masliah et al.,b,d, 1992, 1993a; Cotman et al., 1990; Kosik, 1991; Saitoh et al., 1993; Phinney et al., 1999) (see Section 5.4). Abnormally dilated synaptic terminals, indicative of a compensatory response, are found in both aged and demented brains (Braak and Braak, 1988; DeKosky and Scheff, 1990; Ferrer et al., 1990) (see Section 5.4).

Mega neurites, which represent a specific subpopulation of dystrophic neurites (>10 μM diameter), are often associated with plaques, contain synaptophysin, hyperphosphorylated PHF-τ, GAP-43, and are modified by sialic acid addition and glycosylation. These characteristics suggest that they are abnormal neuritic sprouts of atrophic dendritic structures (Espinosa et al., 2001). Such modifications may represent early events in neurofibrillary degeneration mediated by microtubule depolymerization at the growth cone and adhesion interactions (Araujo et al., 1997).

3.6 Entorhinal Cortex, Hippocampal Pathway, and Lesion Models

In AD, the entorhinal cortex (EC) shows extensive loss of neurons (Hyman et al., 1984; Geddes et al., 1985) and neuronal cytoskeletal disruption (McKee et al., 1991) whereas the hippocampal region that receives EC innervation (the molecular layer) shows plaque-independent granule cell dendritic pathology (Einstein et al., 1994) and loss of synaptophysin immunoreactivity (Heinonen et al., 1995). In response to these degenerative effects, some patients show regenerative changes in the dentate gyrus (Geddes et al., 1985; Arendt et al., 1998a) and apoE4 patients are impaired in this compared to apoE3 (Arendt et al., 1997; reviewed in Arendt, 2001b). Aged human brain shows increased granule cell axon sprouting, suggesting that the molecular layer might be partially deafferented with age (Cassell and Brown, 1984). The dendritic spine density of granule cells is reduced in AD only in distal segments, possibly indicating sprouting of undamaged proximal segments.

In mice and rats, the entorhinal cortex lesion (ECL) is a well-established model of synaptic plasticity (Poirier et al., 1991a,b; Masliah et al., 1995d, 1996; Danik and Poirier, 1998) and behavioral correlates (Miwa and Ueki, 1996; Good and Honey, 1997; Hardman et al., 1997). ECL-induced deafferentation of the EC input models aspects of AD, albeit in an acute model, and has been used to show age-dependent reduction in sprouting in response to ECL (Scheff et al., 1980). The major neuron type that undergoes sprouting is the granule cell of the dentate gyrus, whose dendritic field is the target of EC innervation. Granule cell axons, so-called mossy fibers, sprout and are detected by Timm's stain (for vesicular zinc) (Danscher, 1981; Gaarskjaer, 1986), or other markers of neurite sprouting GAP-43, synaptophysin; these latter markers also detect sprouting of commissural/associational fibers (Cotman et al., 1991). The ECL paradigm recapitulates developmental gene expression responses seen in adult lesion models, aging, and AD (Kondo et al., 1996; Styren et al., 1999) (see Section 3.2). Such similarities to developmental events include expression of synapsin I, eNCAM, and fetal ALZ-50 reactive clone 1 (FAC1) (reviewed in Bulinski et al., 1998; Styren et al., 1999) and partial similarity to changes in dendritic structure, microtubule (MAP2) metabolism, intermediate filament expression (nestin, vimentin), trkB expression, and glutamate and GABA receptor expression.

Organotypic hippocampal slice culture (OHSC) is a semi-simplified yet physiologically and neuro-organo-relevant in vitro system of postnatal hippocampal tissue, widely regarded as a bridge between in vivo and in vitro models, powerful in elucidating mechanisms of complex and necessarily emergent CNS phenomena (Zimmer et al., 1999). OHSCs are typically derived from early postnatal rodents although adult OHSC methods are now available (Temple and Malouf, 2000; Xiang et al., 2000). OHSCs continue to develop and retain organotypic features of the intact hippocampus (Bruce et al., 1995), including development of the mossy fiber pathway that arises from dentate granule cells and projects to the CA3 pyramidal cells (Zimmer and Gahwiler, 1987; Sutula et al., 1989), as well as other synaptic development phenomenon that parallel those observed in vivo (Buchs et al., 1993; Muller et al., 1993; Stoppini et al., 1993).

OHSC is an in vitro model of deafferentation-induced hippocampal neuron sprouting that replicates aspects of ECL. In addition to C/A connections (Frotscher, 1992), the preparation of OHSC transects the perforant path and thereby removes the major extrinsic innervation by the entorhinal cortex (EC) to the granule cell dendritic field in the OML, as well as the commissural projection to the IML. Like ECL in vivo, this deafferentation stimulates sprouting of granule cell mossy axon collaterals into the dentate molecular layer, where they are not normally found in abundance (Gaarskjaer, 1986; Sekiguchi et al., 1996). There they make aberrant synapses (Rudling and Angelin, 1993) with dendrites of the deafferented granule cells that are electrophysiologically functional (Wong and Moss, 1992). Granule cell axon sprouting is altered by intrinsic neural excitability in the absence of cell death (Stringer et al., 1997) (see Sections 3.1, 3.4, 4.2, 8.1).

3.7 Cholesterol in the CNS and AD

Metabolism of cholesterol in the brain and cross-talk with peripheral lipid metabolism (reviewed in Dietschy and Turley, 2001; Rapoport, 2001) is an emerging consideration for AD etiology and possible therapeutic targets (Roses and Saunders, 1997; Vance et al., 2000). Levels of cholesterol in the brain are critical for synapse formation and maintenance and recent studies identify cholesterol as a limiting factor in synaptogenesis (reviewed in Koudinov and Koudinova, 2001). Reduced cholesterol may place a limit on plastic processes thus reducing the tendency to develop AD. An issue for very long axons is the ability to supply sufficient cholesterol for rapid axonal growth, especially in regeneration. What proportion of axonal mem-

brane phospholipid is synthesized in situ in axons compared to that made in cell bodies and transported to axons? (reviewed in Vance et al., 2000).

AD brain contains less cholesterol, perhaps because of enhanced efflux of derivatized cholesterol from the brain (Koudinov and Koudinova, 2001). This contributes to AD-related alterations in membrane composition (Bertoni-Freddari, 1988; Majocha et al., 1989; Svennerholm and Gottfries, 1994; Gottfries et al., 1996), membrane fluidity (Scott et al., 1994; Fernandes et al., 1999; Zubenko et al., 1999), and lipid bilayer structure and dynamics (Mason et al., 1992; Mulder et al., 1998) (see Section 4.4). Cholesterol also influences the phosphorylation status of τ (Distl and Meske, 2000; Fan et al., 2001; Koudinov and Koudinova, 2001; Ohm et al., 2001), MAP2 phosphorylation in the context of dendrite outgrowth (Fan et al., 2002), and amyloid metabolism (including AB production) and its related membrane fluidity effects (Hartmann, 2001; Buxbaum et al., 2002; Ji et al., 2002; Runz et al., 2002; Wahrle et al., 2002) (see Section 5).

Statins, as inhibitors of cholesterol synthesis, may reduce the prevalence of AD (Jick et al., 2000; Wolozin et al., 2000; Buxbaum et al., 2002; Rockwood et al., 2002; Yaffe et al., 2002), possibly by reducing cholesterol turnover in the brain (Locatelli et al., 2002). Axonal growth ceases when cholesterol synthesis is inhibited by pravastatin and could be reactivated by addition of cholesterol to either cell bodies or distal axons (Posse de Chaves et al., 1997). LTP is inhibited by cholesterol biosynthesis inhibitors (Matthies et al., 1997) and LTP induction is associated with pathway-specific increases in lipid production (Koudinov and Koudinova, 2001). An important contribution of glia is their production of apoE-bound lipoprotein particles to deliver rate-limiting cholesterol to neurons, stimulating both synaptogenesis and stable maintenance of synapses, as measured by synapsin and synaptophysin (Kosik, 1992; Pfrieger and Barres, 1997; Barres and Smith, 2001; Mauch et al., 2001; Ullian et al., 2001) (see Section 4.4). Possible mechanisms include cholesterol as a limiting factor in the structural demands of synaptogenesis including membrane formation, synaptic vesicle formation, and clustering of postsynaptic receptors (Gimpl et al., 1997; Martens et al., 2000; Thiele et al., 2000; Bruses et al., 2001; Lang et al., 2001), activation of synaptogenesis by cholesterol signaling through the apoE receptor LRP (see Section 4.1), or other pathways such as hedgehog, Wnt, and reelin (Herz, 2001a; Rice et al., 2001). Future goals should include determining whether these cellular effects can be generalized to synaptogenesis during learning and memory, whether astrocyte-derived cholesterol is a limiting factor in vivo (see Section 2.4), and evaluating the differential effects apoE isotypes in these phenomena.

4.1 APOLIPOPROTEIN E

Apolipoprotein E (apoE) is a component of several classes of lipoproteins regulating lipid metabolism and redistribution (Mahley and Huang, 1999; LaDu et al., 2000; Mahley and Rall, 2000). ApoE isotype E4 is a risk factor for familial and late-onset AD, showing increased risk particularly in the 60–80 year age group (Breitner et al., 1999), and earlier age of onset (Roses et al., 1995; Blacker et al., 1997; Meyer, 1998) ApoE4 influences the risk of AD through pleiotropic effects on both the pathology of AD and the environmental and developmental factors influencing its etiologies (reviewed in Teter, 2000; Teter et al., 2002). This pleiotropy obscures the mechanism for apoE4, and may involve a balance or interaction between neurodegenerative (Poirier, 1994; Buttini et al., 1999; reviewed in Teter et al., 2002) and neuroregenerative effects (see Section 8). The major epidemiological effect of E4 in AD is to promote an earlier age of onset than E3, typically by ~5 years but as much as 15 years (reviewed in Hyman et al., 1996; Blacker et al., 1997; Meyer, 1998; Mesulam, 1999; Arendt, 2001b; Ashford and Mortimer, 2002). Because AD is characterized by ongoing neurodegeneration, accelerated clinical onset could be caused by defects in apoE-related compensatory mechanisms that repair circuitry (reviewed in Mesulam, 1999; Teter, 2000; Arendt, 2001a) This is only one of several mechanisms that could delay the onset of AD.

A great deal of evidence implicates a role for apoE in AD-associated plasticity (Poirier, 1994; Masliah et al., 1995d, 1996), possibly through its isoform-specific functions in cholesterol and phospholipid metabolism and membrane lipid recycling and trafficking, which facilitate neuronal sprouting (Mahley, 1988). ApoE plays a role in both PNS and CNS synaptic remodeling (Poirier et al., 1993a; Poirier, 1994; Laskowitz et al., 1998) although apoE deficiency does not compromise PNS regeneration, perhaps by compensatory overproduction of another apolipoprotein (Popko et al., 1993), it seems to be essential in the CNS (Poirier et al., 1993a; Masliah et al., 1995b). Evolutionary perspectives of apoE allele frequencies are consistent with roles in diet and lipid metabolism (Corbo and Scacchi, 1999).

Differential intracellular trafficking may underlie apoE isotype effects on plasticity. ApoE isotypes localize differentially and accumulate in neurons and astrocytes (Xu et al., 1998). ApoE isotypes may be sorted into late endosomes, escaping lysosomal hydrolysis, where they can then differentially mediate intracellular process like stimulating neurite outgrowth (Mahley and Rall, 2000). E4 may not be able to escape the endocytic pathway to interact with τ or contribute other functions (Hardy et al., 1998; Tesseur et al., 2000).

Many of the activities of apoE are dependent on receptor-mediated events, involving any of a number of low-and high-affinity receptors, including the LDL receptor family of lipoprotein receptors (reviewed in Herz, 2001a), like LRP and HSPG. Several of the neurite outgrowth-promoting properties of apoE isotypes have been shown to be dependent on LRP, both in vitro (Table I) and in vivo (Veinbergs et al., 2001). LRP decreases with age (Kang et al., 2000; Herz, 2001a) and is implicated in AD (Rebeck et al., 1993), with LRP and VLDL polymor-

TABLE I. ApoE4 is Defective in Supporting Neurite Sprouting In Vitro

apoE source	Neurite source	apoE4 effect (apoE3 stimulates)	Depends on	References
Pure	DRG and 1° cortical neuron	Inhibit	Lipoprotein, apoE levels	Handelmann et al., 1992; Nathan et al., 1994; Nathan et al., 2002
Pure	N2A	Inhibit	β-VLDL, LDLR/LRP	Nathan et al., 1994; Nathan et al., 1995
Transfected N2A low expressing	N2A	Inhibit	β-VLDL, HSPG/LRP	Bellosta et al., 1995
Transfected N2A high expressing	N2A	Neutral		De Mattos et al., 1998
Pure	GT1-1 (a HT line)	Neutral	β-VLDL, LRP	Holtzman et al., 1995b
Human plasma HDL, CSF lipoproteins	GT1-1 (a HT line)	Neutral	LRP	Fagan et al., 1996
GFAP transgenic astrocyte	1° HC neuron	Neutral	LRP	Sun et al., 1998
Pure (no lipid) + laminin	1° HC neuron	Stimulates (=E3)		Huang et al., 1995
Transfected HEK cells	1° HC neuron	Stimulates (=E3)		Puttfarcken et al., 1997
Human APOE transgenic OHSC	Granule neurons	Stimulates (=58% E3)		Teter et al., 1999b
		"Inhibits" by dose	apoE levels	Teter et al., 2002

phisms increasing AD risk (Kang et al., 1997; Helbecque et al., 1998). LRP is implicated in LTP in OHSC (Zhou et al., 2000). LRP signaling roles may modulate synaptic plasticity because it interacts with NMDA receptors via the multivalent scaffold protein PSD-95 in postsynaptic membranes, among many possible mechanisms (Gotthardt et al., 2000; reviewed in Herz, 2001a,b; Herz and Strickland, 2001). LRP may mediate the effect of E4 but not E3 stimulating the ERK cascade and CREB (Ohkubo et al., 2001). ApoE isotypes show other signaling-dependent effects (reviewed in Ohm et al., 2001).

ApoE expression is increased in early postnatal development (Muller et al., 1997), which correlates with the onset of synaptic development. ApoE is upregulated by estrogen and in association with estrogen-stimulated, apoE-dependent plasticity (Tam et al., 1986; Stone et al., 1997; Srivastava et al., 1997), and in glia (primarily astrocytes) in regions that undergo estrus cycle-dependent synaptic remodeling (Stone et al., 1997) (see Section 4.11). ApoE mRNA is upregulated in AD (Poirier, 1994) and in the entorhinal cortex lesion model (Poirier et al., 1991a; McRae et al., 1997). Besides effects of apoE levels on plaque development (Bales et al., 1997), levels of expression of the apoE protein have a profound effect on the isotype-specific activity in supporting compensatory sprouting in vitro and in lesion responses and behavior effects in vivo (see Section 4.10). The dose-responsiveness of isotype-specific activities also bears on the therapeutic implications of altering apoE expression levels (see Section 4.12).

4.2 ApoE-Dependent Sprouting (reviewed in Teter, 2000; Teter et al., 2002)

4.3 Alzheimer's Disease. There is epidemiologic evidence for failure of plasticity in E4 patients with Alzheimer's disease. For example, in later stages of AD, E4 brains show reduced dendritic remodeling of pyramidal and subcortical neurons in addition to more severe degeneration. ApoE E4 copy number also affects the relationship between (and possible coupling between) neuronal loss and dendritic growth (see Section 8), with E4/E4 showing no relationship, and shows a shift toward proximal branching (Arendt et al., 1997; 1998a; reviewed in Arendt, 2001b). Interestingly, basal dendrites do not consolidate LTP unlike apical dendrites (Arai et al., 1994a) (see Sections 3.4, 3.6, 4.9 for proximal branching effects).

4.4 Sprouting mechanisms and the lipid metabolism model. The role of apoE in stimulating neuronal regeneration has received much support. E4 consistently shows defects (reviewed in Poirier, 1994, 1995; Danik and Poirier, 1998; Holtzman and Fagan, 1998; Laskowitz et al., 1998; Kerr and Kraus, 1998); unfortunately, no studies have examined the relative capacity of E2 to support neurite sprouting. A well-established mechanism involves the role of apoE in lipid metabolism. Among the many activities that apoE has demonstrated that could account for its CNS effects (see Sections 4.1, 4.4), its definitive role in cholesterol and phospholipid scavenging, metabolism, and transport has defined its role in CNS and PNS plasticity after injury (Masliah et al., 1995d, 1996). The central model of this latter role has been described (Boyles et al., 1989; Poirier et al., 1993a, 1994; Laskowitz et al., 1998) where glia phagocytosing degenerating terminals esterify cholesterol from scavenged membrane lipid, repackage it with apoE as a lipoprotein particle and deliver it to neurons to supply cholesterol for neurite growth via their apoE receptors, LDLR or LRP. Aspects of this mechanism were demonstrated originally in the PNS (Boyles et al., 1989; Saada et al., 1995). Recently, apoE and the cholesterol it carries was identified as the glial factor that stimulates new synapse formation in cultured neurons (Mauch et al., 2001; Ullian et al., 2001).

Besides lipid metabolism, isotype-specific effects could be mediated by specific association with lipoprotein particles, inter- and intracellular apoE trafficking, and oxidative effects of apoE. The defective ability of E4 to support neurite sprouting could involve the isotype- and cell type-specific differential localization and accumulation of apoE (see Section 4.1). Stimulation of neurite outgrowth by E3 in vitro is associated with greater neuronal apoE accumulation (Nathan et al., 1994) and E3 extends along neurites more than E4 (Nathan et al., 1995). Studies have shown that apoE isotypes experimentally directed to cytoplasmic compartmentalization exhibited the E4 defect in sprouting of N2A cells; that the carboxy terminus determined intracellular distribution whereas the amino terminus mediated neurite sprouting suggests that the E4 defect may be due to differential cytoplasmic compartmentalization (Huang et al., 1999).

ApoE could play a role in lipid metabolism through its oxidative effects (see Section 10.2). ApoE-dependent effects on oxidative stress could modulate its ability to support neurite sprouting and could account for synaptic disruption observed in apoE-ko mice. Lipid peroxidation toxicity could inhibit sprouting by the inability to efflux such toxins. In humans, E4 genotype shows higher plasma lipid peroxide that correlates with apoE levels (Smith et al., 1998) and higher lipid peroxidation in brain (Ramassamy et al., 2000). The lack of apoE in the apoE-ko mice results in oxidative stress in the periphery and CNS, e.g., increased CNS F2-isoprostanes (Montine et al., 1999; Pratico et al., 1999), which are suppressed in the plasma by vitamin E (Pratico et al., 1998). Vitamin E ameliorates cognitive deficits in apoE-ko mice (Veinbergs et al., 2000); apoE-ko animals demonstrate increased susceptibility to oxidative stress conditions including global ischemia where neuronal damage correlates with 4-HNE (Horsburgh et al., 1999). Lack of apoE, however, (in apoE-ko mice) was found to increase CNS lipid peroxidation without neurodegenerative or synaptic changes, perhaps because of an oxidative magnitude issue (Montine et al., 1999) (see Section 10.2).

4.5 Model systems

4.6 ApoE-knockout mice. Studies of the apoE-ko mouse (Piedrahita et al., 1992) reveal insight into functions of apoE, peripherally and centrally. Although neuropathologically normal, apoE-ko mice show numerous CNS defects including impaired memory and learning deficits, some of which are age-dependent (Gordon et al., 1995; Masliah et al., 1995b,c, 1996; Krzywkowski et al., 1997, 1999; Veinbergs et al., 1997; Veinbergs and Masliah, 1999; Keller et al., 2000; Bi et al., 2001), changes in cholinergic responses (Gordon et al., 1995), age-related disruption in the dendritic cytoskeleton, and reduced synaptophysin and MAP2 in the hippocampus (Masliah et al., 1995a; Veinbergs and Masliah, 1999; reviewed in Masliah et al., 1996;). Some effects, however, may be strain-dependent (Gandy et al., 1995; Masliah et al., 1996). ApoE-ko mice also demonstrate deficits in response to injury, including cerebral ischemia (Connolly et al., 1996; Laskowitz et al., 1997), and impaired synaptic regeneration (recovery of synaptophysin to entorhinal cortex deafferentation) (Masliah et al., 1995b; Chen Y et al., 1997; Laskowitz et al., 1997; Fagan et al., 1998) (see Section 3.6). Neurotrophic compounds like cerebrolysin that ameliorate behavioral and neurodegenerative changes in apoE-ko mice are associated with upregulation of GAP-43 (Masliah et al., 1999). Some evidence suggests, however, that synapse formation in development is normal in apoE-ko mice, and humans who lack apoE are apparently cognitively normal (Feussner et al., 1996), suggesting the existence of redundant pathways replacing some apoE functions. It may be that in the aging brain these redundant pathways are ineffective, increasing the reliance on apoE activity for plasticity. Loss of synaptic and dendritic density seen separately with age or with absence of apoE expression are synergistic in aged apoE-ko mice (Masliah et al., 1995d).

4.7 Human apoE isotype transgenics. Several lines of transgenic mice have been developed that express the human apoE isotypes under the transcriptional control of various promoters: the natural human apoE promoter (*human apoE*); the astrocyte-specific GFAP promoter (*GFAP*); the neuron-specific NSE promoter (*NSE*); and recently, the natural mouse apoE promoter (*mouse apoE*), so-called knock-in mice. Clearly, each has strengths and limitations experimentally and in their relevance to AD. For example, the *GFAP* transgenic mice have provided what is considered a natural source of lipoprotein particles as synthesized by astrocytes. E3 produced by primary astrocyte cultures from transgenic mice (*GFAP*) is better than E4 at promoting neurite outgrowth in primary cultured neurons (Sun et al., 1998, Table I). This is also an advantage of the *human apoE* transgenic mice (see Section 3.6); transgenic line also expresses apoE in vivo with cellular specificity like that seen in humans (Xu et al., 1996, 1998, 1999) (see Section 4.1). Sprouting responses and synaptic disruptions in hippocampal pyramidal neurons of aged apoE-ko mice (GAP-43, MAP-2) and behavioral deficits are better ameliorated by E3 than E4 transgene (*human apoE*) expression (Veinbergs et al., 1999). Similar results were obtained by infusion of apoE isotypes directly into the brain of apoE-ko mice (Masliah et al., 1997). Behavioral and structural alterations are seen in female E4 but not E3 transgenic mice (*NSE*) (see Section 4.11). E4 transgenic mice (*human ApoE*) are unable to compensate for age-related neuronal loss by synaptic remodeling of the residual neurons (Hoffman and Chernak, 1994; Cambon et al., 2000). E4 transgenic mice (*NSE*) show less synaptophysin (a presynaptic marker) and MAP-2 (a dendritic marker) and behavioral deficits (Buttini et al., 1999; Raber et al., 1998, 2000, 2002). The effects of neuronal expression of human ApoE on sprouting have not been addressed adequately.

4.8 In vitro sprouting systems. The mechanisms by which apoE facilitates neuronal sprouting have been studied extensively in vitro. In most studies, E4 was defective in supporting neurite sprouting (Table I). In

these studies, the apoE source varied between pure (recombinant) protein, lipoprotein particles produced by transfected neurons or liver cells (HEK-293), particles produced by transgenic astrocytes (GFAP promoter-driven), or by a balanced production by all CNS cells (human ApoE transgenics). Neurite sprouting was measured in neuron cell lines N2A or GT1-1 (a hypothalamic line), primary hippocampal neurons, or hippocampal granule neuron mossy fibers in OHSC (see Section 3.6). In all these studies, E3 stimulated sprouting, whereas E4 showed an inhibitory effect, no effect, or weakly stimulatory effects on sprouting, always less than (or equal to) E3 (reviewed in Teter, 2000).

Important findings include consideration of the lipidation state of the apoE isoforms to reveal isoform-specific activities. For example, pure E4 inhibits N2A sprouting only when reconstituted with b-VLDL or with other lipid sources (also required for the defect in N2A-expressed apoE4) (Nathan et al., 1994, 1995; Bellosta et al., 1995; Holtzman et al., 1995b), and apoE isoforms expressed in lipoprotein particles by transfected HEK cells do not reveal the E4 defect (Puttfarcken et al., 1997) whereas those produced by transgenic astrocytes do (Sun et al., 1998). The lipidation state of apoE is a critical issue yet to be resolved fully because not all apoE isotype-specific effects, including sprouting, depend on lipidation (reviewed in Nathan et al., 1994, 2002; Jordan et al., 1998; Teter, 2000).

Possible mechanisms of isotype-specific sprouting include isotype-specific effects on lipid efflux (see Sections 3.7, 4.9); apoE cellular accumulation (Ji et al., 1998) (see Section 4.1), microtubule depolymerization and destabilization (Nathan et al., 1995; Pitas, 1996; Roses et al., 1996; Pitas et al., 1998), and neurotoxicity (Marques et al., 1996; reviewed in Teter, 2000; Teter et al., 2002). Several studies show a dependence of E3-stimulated sprouting on the LRP receptor or the HSPG/LRP receptor system (Table I) (Bellosta et al., 1995; Holtzman et al., 1995b; Nathan et al., 1995; Fagan et al., 1996; Sun et al., 1998). Interestingly, exogenous E3 does not rescue the E4 defect in stimulating sprouting (Nathan et al., 1994; Holtzman et al., 1995b).

4.9 Isotype-dependent granule cell mossy fiber sprouting. In early development and in OHSC in vitro (see Section 3.6), the early postnatal development of the granule cell mossy fiber system (Gaarskjaer, 1986; Slomianka and Geneser, 1997) parallels the large increase in apoE expression at this time (Muller et al., 1997). Mossy fiber sprouting in OHSC is found to be regionally dependent on apoE expression, where only dorsal dentate granule cells fail to sprout in apoE-ko OHSC (Teter et al., 1999a). These studies indicate that apoE-dependent spouting is region-specific, perhaps reflecting a developmental age-dependent difference in the capability of the granule cells to react to deafferentation. Aspects of this region-specific, apoE-dependent sprouting have been demonstrated independently in adult animals, where apoE-ko mice show deficient sprouting in response to ECL (Masliah et al., 1995b; Stone et al., 1998) (see Section 4.11).

Granule cell sprouting in OHSC derived from E3 and E4 transgenics (*human ApoE*) showed that E4 induced sprouting to a level only 50% of that induced by E3 (Teter et al., 1999b). This E4 defect in sprouting was demonstrated recently in vivo using the same transgenic mice and the ECL paradigm, where compensatory sprouting measured by GAP-43 and synaptophysin immunoreactivity did not recover as effectively in E4 as in E3, nor did morphometric measures of sprouting extent (White et al., 2001). ApoE4 transgenic mice (*NSE*) also show poorer recovery from other lesion paradigms, such as excitotoxic injury (Buttini et al., 2000). The reduced distal mossy fiber sprouting measured in E4 OHSC (outer molecular layer sprouting) may be explained by effects on neurite branching. The effect of apoE4 on proximal neurite branching in AD (see Section 4.3) is also seen in sprouting responses in vitro (Nathan et al., 1995).

4.10 ApoE gain-of-function defect in sprouting. Although E4 reduced sprouting activity in most studies, several studies indicate that the E4 activity in the inhibition of neurite sprouting actually represents a gain-of-negative function. First, Nathan et al. (1994) found that dorsal root ganglion (DRG) neurons, Neuro2A cells (Bellosta et al., 1995), and primary cortical neurons (Nathan et al., 2002) extend neurites in the presence of E3, but decreased neurite extension with E4 is dose-dependent. Importantly, E4 inhibition dominates over E3 stimulation, an effect seen in other in vitro systems (Holtzman et al., 1995b) and in bigenic mice (Nathan et al., 1995; Buttini et al., 2000) (see below). Second, in the OHSC model of denervation-induced fiber sprouting (see Section 4.9), transgenic (*human apoE*) expression of E4 is not only defective in supporting neurite sprouting compared to E3, but increased expression of E4 (by doubling the transgene copy number) inhibited sprouting whereas increasing E3 expression stimulated sprouting (Teter et al., 2002). The apparent gain-of-negative activity of apoE4 could be a form of toxicity (reviewed in Teter, 2000; Teter et al., 2002) that, at higher expression levels, dominates its weak sprouting activity. This could be relevant at the apoE levels measured in OHSC media because similar levels are found in human CSF and brain (2–6 μg/ml) (Hesse et al., 2000). Two studies show in vivo evidence consistent with E4 dominant negative inhibition of neurite sprouting. First, in the loss of synaptic markers (synaptophysin, MAP2 and neurofilament) in response to kainate lesioning, whereas E4 transgenics (*NSE*) show reduced synaptophysin that is equal to apoE-ko mice, doubling the gene dose causes even greater reductions; notably, the E4 effect dominates over E3 (Buttini et al., 2000). Second, E4-specifc cognitive impairments in these same mice (*NSE*) are not present in nontransgenic apoE-ko littermates (this "gain of function" is in comparison to apoE-ko, not a dose response of E4) (Raber et al., 1998, 2000; reviewed in Teter et al., 2002).

4.11 ApoE, Gender, Estrogen (see Section 9). Gender has an impact on ApoE4 effects, further increasing AD risk and diminishing ERT treatment response in post-

menopausal women (Corder et al., 1993; Poirier et al., 1993b; Farrer et al., 1995, 1997; Schneider and Farlow, 1997; Yaffe et al., 1997, 2000; Bretsky et al., 1999). These results from human studies are paralleled to some extent by results from studies of transgenic animals.

In OHSC, granule cell sprouting is regionally dependent on apoE expression (see Section 4.6). Although sprouting in wild-type, apoE-expressing OHSC is stimulated by physiological levels of estrogen, an effect that is blocked by both progesterone and tamoxifen, as seen in purified neuron cultures (Chawen et al., 1992; Woolley and McEwen, 1993), estrogen does not stimulate sprouting in apoE-ko OHSC, showing that neuronal sprouting is increased by estrogen in the same hippocampal region where sprouting is dependent on apoE. Likewise, whereas apoE-ko animals show compromised compensatory sprouting in response to ECL lesion in vivo (Masliah et al., 1995a; Masliah et al., 1996; Anderson et al., 1998; Stone et al., 1998), estrogen replacement in ovariectomized mice stimulates sprouting only in wild-type but not apoE-ko mice (Stone et al., 1998). Like the region-specific apoE dependency of estrogen-stimulated granule cell sprouting in OHSC, granule cells in the dorsal region are sensitive specifically to estrogen-stimulated increases in spine density (Miranda et al., 1999). Sprouting may be stimulated by estrogen through upregulation of apoE expression (see Section 4.1). Upregulation of apoE synthesis in glia (primarily astrocytes) occurs in CNS regions that undergo estrus cycle-dependent synaptic remodeling (Stone et al., 1997). Estrogen and apoE may therefore interact in their modulation of both AD risk and CNS plasticity. This is consistent with a postmenopausal decline in peripheral apoE levels (Kushwaha et al., 1991; Muesing et al., 1992). Other possible mechanisms of apoE and estrogen interaction include estrogen receptor polymorphism (Mattila et al., 2000) and oxidation (Inestrosa et al., 1998) (see Section 10.2).

Only female E4 transgenic mice (*NSE*) develop age-related progressive impairments in spatial learning and memory in the water maze and nonspatial novel object recognition memory (Raber et al., 1998; 2000, reviewed in Teter et al., 2002). These cognitive impairments are independent of the cellular source of apoE as they are observed in mice expressing E4 in neurons (*NSE* transgenics) and in astrocytes (*GFAP* transgenics) (see Section 4.7). The findings that the detrimental effects of E4 are greater in female than in male transgenic mice is consistent with the epidemiological interaction of apoE4 and female gender on increased risk to develop AD.

4.12 ApoE Therapeutic Implications: Drug Interactions and Pharmacogenetics

ApoE4 plays a major role in the risk and onset of AD for ~50% of AD cases in the United States (Ashford and Mortimer, 2002); therefore, therapies that target the mechanism of increased risk for apoE4 and reduced risk for apoE3 and E2 would greatly impact AD prevalence. Possible targets include apoE expression levels and regulation, apoE protein structure or gene replacement, and primary targets or secondary effects of apoE activity. The protein structural determinants of apoE4 are known (Weisgraber, 2001); with further understanding of how structure modulates various apoE activities, this avenue holds promise for drugs that convert the E4 protein to a structure that resembles E3 or E2. Gene replacement may capitalize on emerging stem cell technology, or using cell precursors in the bone marrow that can cross the blood brain barrier and differentiate into a variety of CNS cell types (see Section 10.5).

Reducing the expression of the human apoE4 gene could reduce apoE4-related risk, however, the relevance of apoE gain- and loss-of-function effects are not well understood. Further, human apoE gene regulation is very poorly understood, particularly with respect to effects of current and candidate therapeutic drugs. Estrogen is known to regulate apoE expression, and this may mediate, at least in part, the effect of estrogen replacement therapy (ERT) in improving the cognitive deficits in postmenopausal women with AD and the poorer response of apoE4 women (see Section 4.11). These effects of the efficacy of ERT in AD will be better understood with results from several clinical trials currently in progress (WHIMS and others) as apoE genotype is monitored routinely.

Other therapeutic drugs show apoE isotype-dependent effects that may interact with primary targets or secondary effects of apoE activity (reviewed in Poirier, 1999). Tacrine (an anti-cholinesterase) therapy has lower efficacy in E4 (Poirier et al., 1995) and in women with E4, no effect by genotype in men (Farlow et al., 1998), and lower efficacy in E4 women on combination tacrine plus ERT (Schneider and Farlow, 1997). There are indications, however, that apoE genotype may affect only longer-term tacrine therapy (MacGowan et al., 1998). The efficacy of a noradrenergic and vasopressinergic activity facilitator is also higher in E4 (Richard et al., 1997). Citicoline, an intermediate of lipid and acetylcholine biosynthesis that increases cerebral blood flow, shows greater efficacy with E4 (Alvarez et al., 1999). Growth hormone therapy is poor in E4 (Johannsson et al., 1995), possibly involving the mechanisms of GH regulation of plasma ApoE levels (Sjoberg et al., 1994). Other drugs that could modulate apoE expression include therapeutic agents that target oxidative mechanisms, such as vitamin E, selegiline (Sano et al., 1997b), and *Ginkgo biloba* extract (EGb 761) (Le Bars et al., 1997) (see Sections 4.4, 10.2), anti-inflammatory drugs like NSAIDs that could impact apoE expression through glial responses to inflammation (see Section 10.3), and statins that modulate cholesterol levels and may thereby regulate apoE expression (see Section 3.7).

The gain-of-negative function of E4 could have important clinical implications for the pharmacogenomic efficacy of therapeutic drugs that impact or target apoE expression (Poirier, 1999; Saunders et al., 2000) to the extent that E4-defective sprouting contributes to neuroregenerative events in neurodegenerative conditions (or, for that matter, any toxic activity of E4 that prevents neuroregeneration or promotes neurodegeneration). A drug that increases apoE expression might show efficacy in

E3 but not in E4, and may even exacerbate the E4 condition, whereas the opposite is predicted for E4 defects that are simple loss-of-functions. As in the ERT trials, it will be important to evaluate apoE genotype effects in trials of other drugs that can modulate apoE expression. With the implementation of pharmacogenetic approaches to therapeutic drug design and with testing and efficacious matching of treatment protocol to the genetic polymorphism fingerprint of the patient, understanding of genetic influences on neurodegenerative disease will see rational therapeutic application.

Therapeutic strategies must also consider both when and how the drug target contributes to disease etiology. For example, the apoE4 phenotype of accelerating the age of onset requires prevention strategies and may not respond to drugs designed to slow disease progression. The pleiotropic effects of apoE and its isotypes raise the strong possibility that the isotypes differ in the mechanism by which they contribute to AD etiology. Although apoE has emerged as the strongest genetic risk factor for sporadic AD and is implicated in other neurodegenerative diseases, many other genes are also implicated. Identification of these genes (Tanzi, 1999) has been slow methodologically, but the availability of human genomic sequence to reveal other polymorphisms linked to AD will help pharmacogenetic drug design.

5.1 APP AND AB

Other than production of AB, the functions of APP relevant to AD etiology include neuronal development, synaptogenesis, synaptic plasticity, and cell signaling (Luo et al., 1992; Moya et al., 1994; Mucke et al., 1994; Muller et al., 1994; Roch et al., 1994; Qiu et al., 1995; reviewed in Neve et al., 2000, 2001; Neve, 2001; Arendt, 2001a). APP and PS are expressed at higher levels in neurons in regions most affected in AD: hippocampal CA fields, amygdala subregions, and neocortex (Lee et al., 1996). APP gene expression and processing is regulated by NF-κB as an injury-responsive cytokine/neurotrophic factor (Mattson and Camandola, 2001; Weggen et al., 2001) (see Section 10.3). Injury and denervation that induces plasticity also upregulate APP (Banati et al., 1993; Wallace et al., 1993; Beeson et al., 1994; Chauvet et al., 1997), as does cholinergic innervation (Nitsch et al., 1995) (see Section 3.1). APP interacts with substrate adhesion in many ways, including association with adhesion patch components, integrins, transglutaminase, glycosaminoglycans, and collagen (reviewed in Arendt, 2001a).

5.2 APP Trophic Effects and Axonal Transport

The superfamily of amyloid precursor proteins (APP, APLP1,2) is associated with axonal outgrowth in several neural systems (Ohta et al., 1993; Arai et al., 1994b; Moya et al., 1994; Thinakaran et al., 1995; Lyckman et al., 1998) and neurite outgrowth that is APP isoform-dependent (Milward et al., 1992; Qiu et al., 1995). APP is secreted from neurons in response to electrical activity and induces neurite outgrowth, synaptogenesis, and LTP (Roch et al., 1994; Huber et al., 1997; Ishida et al., 1997; Mattson, 1997). Transgenic mice expressing various forms of APP exhibit both degenerative and regenerative changes that depend on age and APP genotype. Transgenic mice expressing APP show increased synaptophysin (King and Arendash, 2002), even at low levels of APP (Mucke et al., 1994), and secreted APP promotes dendrite outgrowth at pM concentrations (Mattson, 1994). APP transgenic mice undergo synaptic, electrophysiologic, and behavioral changes before plaque formation and in the absence of overt neurodegeneration (Hsia et al., 1999; Mucke et al., 2000), although some models do show plaque-associated neuron loss (Calhoun et al., 1998). APP transgenic mice have increased numbers of synapses (Mucke et al., 1994) and increased cortical neuron number before plaque formation (Bondolfi et al., 2002) (see Section 3.5). APP may enhance proliferation of neural stem cells (Ohsawa et al., 1999) (see Section 10.5). In contrast, degenerative changes are expressed in older animals, perhaps reflecting accumulated AB/amyloid or soluble AB toxicity (see Section 5.4).

Members of the APP superfamily of proteins are transported by and play a role in the fast anterograde transport system (Koo et al., 1990; Sisodia et al., 1993); they also accumulate in presynaptic membranes. Axonal pathology is reflected by diminished axonal transport (Geinisman et al., 1977). This may involve the role of APP in chaperoning NCAM and sialic acid to the presynapse. Aging is associated with decreased axonal transport, which may be caused by AB (Kasa et al., 2000). The insulin receptor signaling cascade also affects APP trafficking. Reduction in axonal transport (anterograde) would deplete APP at the presynapse and cause accumulation in other cell compartments where AB production may be favored (Golde et al., 1992). Conversely, reducing APP proteolysis to AB is predicted to lead to trophic APP accumulation at the presynapse (see Sections 3.4, 5.3).

5.3 APP Processing Balance

The processing of APP by α secretase produces soluble/secreted APP that promotes new synapse formation. Decreasing the amount of functional APP or shifting toward β secretase products could contribute to failure of plasticity and elimination of synapses. This situation could be induced by mutations that inhibit any of these trophic activities of APP, or by mutations or other factors that shift the APP processing balance to produce nonfunctional fragments. Mutations in APP and other situations that shift the processing balance away from secreted APP toward AB42 would interfere with APP-induced plasticity. Transgenic mice expressing such mutated APP show decreased synaptic and dendritic density in the hippocampus, impaired LTP, decreased compensatory synaptogenesis in response to injury, and impaired spatial memory (Games et al., 1995; Masliah et al., 1995b; Chapman et al., 1999). Conceivably, any event that promotes AB deposition could do so by shifting the processing toward more AB production (albeit, ignoring clearance effects). With age, the processing of APP also shifts away from producing the neurotrophic secreted APP form (Palmert et al., 1990; van Gool et al., 1994). APP metabolism is also influenced by

laminin (Monning et al., 1995; Narindrasorasak et al., 1995; Bronfman et al., 1996), cholesterol (Jick et al., 2000; Refolo et al., 2000; Wolozin, 2001), and NSAIDs (see Section 10.3). APP-knockout mice show loss of presynaptic markers, reduced CA1 dendritic length, impaired LTP and cognitive performance, and reduced axon and dendrite growth in vitro (reviewed in Arendt, 2001a).

5.4 Amyloid-β and Amyloid Plaques

A major issue facing the amyloid hypothesis of AD is the relative contributions of the various forms of Aβ, the peptide released from β secretase-processed APP. Aβ forms a continuum of aggregation species: monomeric Aβ, soluble Aβ, ADDLs, insoluble Aβ, diffuse amyloid, compact amyloid, and neuritic amyloid, the latter two being the pathologic and diagnostic hallmarks of AD. Independent of fibril or plaque formation, however, Aβ can alter membrane potential and firing, synaptic transmission, synaptic plasticity, and learning (Cullen et al., 1997; Lambert et al., 1998; Hartley et al., 1999; Chen G et al., 2000; Chen QS et al., 2000; Chapman et al., 2001). Aβ, especially Aβ1–42, shows neurotoxic, neurite-inhibiting, and LTP-inhibiting properties (Freir et al., 2001; Dewachter et al., 2002). Soluble Aβ and Aβ oligomers inhibit LTP but not LDP, resulting in a 'neuroplasticity imbalance' in the competition for synaptic stabilization (Cullen et al., 1997; Wang et al., 2002), as occurs in development (Constantine-Paton, 1990). Oligomeric, but not monomeric Aβ inhibits LTP in vivo (Lambert et al., 1998; Walsh et al., 2002).

Negative effects of Aβ on plasticity are also revealed in studies of vaccination/immunization in mouse models. Active immunization reduces both brain Aβ/amyloid burden and cognitive impairments in APP transgenic mice (Janus et al., 2000; Morgan et al., 2000). Recent studies using the passive immunization approach in APP transgenic mice also indicate such a correlation, but importantly, very rapid behavioral effects are achieved (within days), even without changing global Aβ levels in the brain (Dodart et al., 2002). Perhaps relatively minor compartments of Aβ that inhibit learning are reduced rapidly by peripheral immunization, which is consistent with reports that soluble Aβ is a better correlate of memory impairment (Lue et al., 1999; McLean et al., 1999; Koistinaho et al., 2001). Clinical immunization for Aβ, despite an early setback, remains a potential therapeutic strategy.

These neurotoxic effects of Aβ may act to destroy synapses that are no longer required or are underutilized. In contrast to these neurotoxic effects, however, low concentrations of Aβ can show neurotrophic effects (Calabrese, 2001), and laminin and Aβ act synergistically in stimulating neurite outgrowth (Koo et al., 1993). Low levels of Aβ can modulate the activity of the transcription factor CREB (Sato et al., 1997), a factor necessary for neuronal plasticity (Segal and Murphy, 1998; Silva et al., 1998). Aβ may play a functional role in membrane lipid dynamics (Muller et al., 2001; Chochina et al., 2001). Aβ enhances the transport, uptake, and oxidative metabolism of lipids, acting much like an apolipoprotein (Wood et al., 1999), a process that takes place in the ER/trans Golgi and endolysosomal pathways, which are also utilized by apoE (Jensen et al., 1994). The possible equilibrium between plaque-bound Aβ and soluble Aβ is not understood. Plaques could be a localized source of soluble Aβ which, when released, could impact plasticity responses, and contribute to the plaque-association of dystrophic neurites, growth-promoting factors, and synaptic proteins (SNAP-25, synaptophysin, synaptotagmin, chromogranins, NT75, spectrin), as reviewed by Arendt (2001a) (see Section 3.5). Numerous growth-promoting factors associated with plaques could contribute to stimulated sprouting, such as S100b, bFGF, HGF, PDGF, Trk receptors, proteoglycans, EGF-R, ICAM, integrins, collagen, laminin (reviewed in Arendt, 2001a). Other plaque constituents like perlecan, agrin, and laminin could also contribute to localized sprouting responses (Phinney et al., 1999).

6. TAU

Tau is a member of the MAP family (reviewed in Maccioni and Cambiazo, 1995). Aggregated, hyperphosphorylated τ forms neurofibrillary tangles (NFT), the intracellular pathological hallmark of AD (reviewed in Lovestone and Reynolds 1997; Lovestone et al., 2001). Its expression and phosphorylation is associated with increased neuroplasticity in vivo and in vitro (Busciglio et al., 1987; Viereck et al., 1989; Trojanowski et al., 1993; Brion et al., 1994; Black et al., 1996; Lovestone and Reynolds, 1997). Hyperphosphorylation may also cause deleterious effects on plasticity, however, and may underlie its role in the etiology of AD (Maccioni and Cambiazo, 1995; Mandelkow et al., 1995). Tau modulates cytoskeletal and microtubule dynamics that contribute to growth cone migration and collateral branching (Gallo and Letourneau, 1999). Tau plays a major role in the outgrowth of neurites and axonal development (Maccioni and Cambiazo, 1995). NFT-bearing hippocampal neurons show more extensive dendritic trees, suggesting a concurrent or previous induction of reactive plasticity (Gertz et al., 1990) (see Section 3.4). NFT-bearing neurons contain numerous growth-associated proteins (reviewed in Arendt, 2001a). Antisense to tau mRNA suppresses neurite formation in B103 cells (Lambert et al., 1995). Tau overexpression by PC12 cells induces neurite extension, and NGF-induced extension is associated with large upregulation of τ (Drubin et al., 1985; Esmaeli-Azad et al., 1994).

The equilibrium between τ phosphorylation and dephosphorylation modulates the stability of the cytoskeleton and thereby the axonal morphology. Tau is phosphorylated by several kinases including GSK3b and Cdk5, and broken down by several phosphatases including A and B. Breakdown of this equilibrium causes structural and conformational changes in τ, thus affecting binding with tubulin and the capacity to promote microtubule assembly (Mandelkow et al., 1995; von Bergen et al., 2000). This may promote NFT, particularly in limbic structures (see Section 3.1), leading to cytoskeletal dysfunction. Dentate granule cell mossy fiber axons that undergo deafferentation-induced sprouting (see Sections 3.6, 4.6) display

excessive τ phosphorylation (Koudinov and Koudinova, 2001). It is not clear whether neurofibrillary-induced neurodegeneration is a later event in AD or whether its pathology simply cannot be detected in early AD (see Sections 3.4, 3.5).

Tau can link trophic signaling with cytoskeletal rearrangements involved with dendritic sprouting (see Sections 8.2, 8.3). The protein kinase Cdk5 and its neuron-specific activator p35 are essential molecules for neuronal migration and regulate axonal extension through phosphorylation of MAPs including τ (Pigino et al., 1997; Paglini et al., 1998). The formation of a stable Cdk5/p35 complex in hippocampal neurons (Alvarez et al., 1999) may lead to constitutive activation of the protein kinase with a consequent increase in τ phosphorylation. The complex concentrates at the leading edge of the growth cone (Pigino et al., 1997). Laminin stimulates p35 expression, increasing its redistribution to the growth cone (Ramirez et al., 1999), contributing to the axon-outgrowth activity of laminin (Paglini et al., 1998). Cdk5 may link τ hyperphosphorylation and AB (Alvarez et al., 1999; Maccioni et al., 2001). Therefore, the Cdk5 system may provide an important regulatory link between extracellular signals like laminin and the intracellular organization of MAPs and other cytoskeletal proteins involved in axon elongation (Maccioni et al., 2001). Cdk5 interacting proteins include τ, synapsin, CK1, b- and g-catenins, N-cadherins, Rac GTPase and Pak1 (which impacts actin cytoskeleton dynamics) (reviewed in Maccioni et al., 2001) (see Sections 3.4, 8.2).

7. PRESENILINS

PS-1 is necessary for normal neurogenesis and survival (Shen et al., 1997; Wong et al., 1997) and localizes to synaptic membranes and neurite growth cones. Presenilins are involved in intracellular trafficking, developmental signaling pathways, and Ca^{2+} homeostasis (Shen et al., 1997; Wong et al., 1997; Naruse et al., 1998; Nishimura et al., 1999). Ca^{2+} dysregulation could underlie effects of PS-1 on LTP: wild-type PS-1 underexpression impairs LTP in mice (Morton et al., 2002) and rats (Dewachter et al., 2002), mutant PS-1 alters LTP (Parent et al., 1999; Zaman et al., 2000); however, mutant but not wild-type PS-1 and mutant PS-2 facilitate weak-stimulation LTP in brain slices (Schneider et al., 2001). Mutant PS-1 may interfere with metabolism of β- and γ-catenin, which are involved in synapse formation and stabilization (Zhang et al., 1998; Kang et al., 1999) and cell adhesion (Noll et al., 2000) (see Section 6). Wild-type PS-1 stimulates whereas mutant PS1 inhibits NGF-induced neurite outgrowth (Furukawa et al., 1998; Dowjat et al., 1999; reviewed in Arendt, 2001a). PS-1 cleaves Notch, which inhibits neurite outgrowth (Berezovska et al., 1999; Sestan et al., 1999; Figueroa et al., 2002). Presenilins are also considered as a therapeutic target for AD (Golde and Younkin, 2001), however, the negative secondary effects of inhibiting presenilin need further investigation (Dewachter et al., 2002).

8. NEURODEGENERATION AND NEUROREGENERATION INTERACTIONS

8.1 Degeneration-Regeneration Cross Talk and Combinatorial Signaling

The same trophic signals that control survival can also promote neurite outgrowth (Campenot, 1994; Meyer-Franke et al., 1995). This could allow for a mechanism whereby simply promoting survival stimulates plasticity (Goldberg and Barres, 2000). Trophic responsiveness can be dependent on continuous trophic stimulation, where competition for limited target-derived trophic factors can ultimately decide cell fate, and thus it is possible that continuous availability of trophic stimulation could be limiting for plasticity mechanisms as well (Goldberg and Barres, 2000).

Neurons do not extend axons by default but must be signaled specifically to do so (Goldberg and Barres, 2000). Promotion of plasticity requires both presentation of an extrinsic stimulus and the intrinsic responsiveness of the neuron, which includes states that can be induced transcriptionally (Smith and Skene, 1997). Responsiveness of neurons to intrinsic and extrinsic signals that promote plasticity may come from combinatorial signaling, e.g., simultaneous presentation of electrical activity and growth factors, like BDNF. (McAllister et al., 1996; (reviewed in Goldberg and Barres, 2000). Electrically and biochemically active neurites would therefore survive and grow in response to trophic stimulation; for example, granule cell axon sprouting can result from alteration of the intrinsic neural excitability in the absence of cell death (Stringer et al., 1997). Thus, reduced neuronal activity would reduce its responsiveness to stimulation of plasticity.

Many factors influence both neuronal death and neurite sprouting, for example, c-Jun and GAP-43 (Herdegen et al., 1997; Gagliardini et al., 2000; Wehrle et al., 2001), and neurotrophins that promote neurite growth (Levi-Montalcini, 1987; Campenot, 1994; Meyer-Franke et al., 1995; Henderson, 1996). Other factors include substrate molecules like laminins, although this may be insufficient (Goldberg and Barres, 2000). Retrogradely transported signals like CREB (Silva et al., 1998) and signaling by the ras/raf/MAP pathway may play important roles in intra-neuronal signaling of plasticity (Perron and Bixby, 1999) (see Sections 3.2, 6).

The relationship between plasticity and the classical plaque pathology of AD is unclear. Granule cell dendritic regression is not modified by plaque association (Einstein et al., 1994). Transgenic mice expressing anti-NGF antibody develop amyloid plaques, NFT-like inclusions, neuron losses, and behavioral deficiencies with age (Capsoni et al., 2000) including impaired spatial learning (Van der Zee et al., 1995a,b; Chen KS et al., 1997). Ex-boxers with an increased plasticity burden (injury-induced) have AD-like neuropathological changes (Tokuda et al., 1991; Geddes et al., 1996). The increased risk of AD with head injury and stroke (Salib and Hillier, 1997; Snowdon et al., 1997) may require widespread or chronic injury combined

with factors or events that inhibit neuroplasticity responses (see Sections 3.5, 5.4).

8.2 Cell Cycle

If a neuron that is committed to permanent cessation of cell division is forced, through ectopic expression of cell cycle proteins, to reenter the cell cycle, it may die. In AD frontal cortex during the early Braak I/II stage, when there in no τ or amyloid pathology or related dementia, mitotic events are activated including increased MAP2 and ERK1/2, which may lead to cell death (Arendt, 2001b). Tau kinases like MAP kinases, Cdk5, and others, are all associated with the cell cycle (reviewed in Arendt, 2001a,b). MAP kinases are activated by cell surface receptors through p21ras (Stokoe et al., 1994), which also plays a role in dendritic proliferation and synaptogenesis (Phillips and Belardo, 1994) (see Section 8.3).

8.3 Nitric Oxide (NO)

NO participates in axonal remodeling at the growth cone and synaptogenesis during development and regeneration (Hess et al., 1993; Wu et al., 1994; Yu, 1994; Luth et al., 1995; Rossiter et al., 1996; Yan et al., 1996; Downen et al., 1999). NO may be the retrograde messenger in LTP and may serve to help maintain normal LTP; however, NO also mediates some excitotoxicity (glutaminergic) mechanisms and has anti-proliferative effects (Arendt, 2001b). The NO synthesizing enzyme nNOS is dynamically regulated in neuronal development, plasticity, and responses to injury (Dawson et al., 1994, 1998; Dawson and Snyder, 1994; Forstermann et al., 1995). Activation of NF-κB in astrocytes increases iNOS expression and NO production. Changes of NOS (nNOS, neuronal) and iNOS (glial) (Srivastava et al., 1997) in AD are inconsistent (Law et al., 2001). Endothelial eNOS mediates neuroprotective actions of NO in ischemia and multi-infarct dementia (Law et al., 2001).

NO and other oxidative stress intermediates activate p21ras, a potential endogenous NO-redox-sensitive effector molecule (Yun et al., 1998). p21ras is highly colocalized with nNOS expression in AD and in NFT-bearing neurons (Luth et al., 2000). P21ras is overexpressed in advanced AD (Gartner et al., 1995), and is upregulated in early AD in affected regions before neurofibrillary degeneration (Gartner et al., 1999) (see Sections 3.4, 10.4), paralleling nNOS (Luth and Arendt, 1998; Luth et al., 2000). This may set up an autocrine loop (Lander et al., 1997) and may exacerbate neurofibrillary degeneration and limit the ability to terminate the vicious cycle. This relationship may switch two potentially neuroprotective mechanisms of NO and p21ras into a chronic neurodegenerative process (Arendt, 2001b).

9.1 GENDER AND ESTROGEN

Estrogen plays a powerful, pleiotropic role in many neurodegenerative conditions including AD (reviewed in Brinton, 2001; Garcia-Segura et al., 2001; McEwen, 2001; Wise et al., 2001a,b). Women have been shown to have increased risk, earlier onset, and more rapid progression of AD than men, although gender-specific morbidity is an issue (Sanderson et al., 2002). Postmenopausal loss of estrogens leads to generally reversible decreases in memory that respond to ERT (Sherwin and Tulandi, 1996). Besides mechanisms of blocking neurotoxicity directly, estrogen acts at various levels of plasticity: axon sprouting, synaptogenesis, and promoting synaptic transmission (electrophysiologically and biochemically). These effects can be ascribed to either receptor-dependent mechanisms, primarily transcriptional, including direct effects of ER in transcription and indirect effects through other transcription factors like CREB and Akt, as well as their retrograde transport (McEwen, 2001) or receptor-independent (rapid) mechanisms involving activational effects of second messenger systems, coexisting neurotransmission, or coordinated activation of both (Kelly et al., 1977; Nabekura et al., 1986; Wong and Moss, 1992; Garcia-Segura et al., 2001) (see Section 8.1), as well as oxidative effects of the estrogen molecule (see Section 9.3). Other secondary effects could be mediated through effects on AB, τ, microtubules, apoE, GAP-43, BDNF, ERK, IGF-1, NF-κB, CREB, gliosis, neurogenesis (Blanco et al., 1990; Gould et al., 1999; Tanapat et al., 1999), differentiation, or many other modulators of plasticity.

9.2 Estrogen Replacement Therapy (ERT)

Estrogen replacement decreases the risk of AD in postmenopausal women (Paganini-Hill, 1996; Kawas et al., 1997), delays the age of onset (Tang et al., 1996), and perhaps slows the decline; however, it remains controversial whether ERT can treat the disease once it has reached the clinical stage (Henderson et al., 2000; Marder and Sano, 2000; Mulnard et al., 2000; Wang et al., 2000). This latter effect is consistent with experimental results indicating that neuroprotective effects of estrogen occur only when administered before or during the neurodegenerative stimulus, but not after (Garcia-Segura et al., 2001). The therapeutic efficacy of ERT depends on its administration protocol: time course, treatment window, endogenous vs. exogenous hormone, and neuron population-specific effects on promoting survival vs. death. The mechanism of ERT efficacy is unlikely to include antioxidant effects as they require very high hormone concentrations to reduce lipid peroxidation (Vedder et al., 1999) (see Sections 4.4, 10.2); however, whether hormone concentrations are modulated by local aromatase expression are not known (Garcia-Segura et al., 2001). This is critical for considerations of testosterone therapy for men, in terms of its estrogenic actions (Cyr et al., 2000; Goodenough et al., 2000; Twist et al., 2000; Bowen, 2001). Testosterone also has estrogen-independent, potentially beneficial actions on amyloid toxicity (Pike, 2001).

9.3 Estrogen/Plasticity

(Reviewed in Toran-Allerand et al., 1999; Brinton, 2001; McEwen, 2001; Kelly and Levin, 2001).

Estrogen stimulates axon and dendrite plasticity in the limbic neurons of both male and female brain (Ferreira and Caceres, 1991; Lorenzo et al., 1992; Woolley and

McEwen, 1992; Woolley et al., 1996; McEwen et al., 1997; Teter et al., 1999a). Estrogens may support neuronal functions and confer resistance to neural damage by their ability to maintain synaptic connections (McEwen, 2001). Many studies demonstrate neuroprotective effects of estrogen in a variety of systems, and these are often associated with changes in gene expression, including genes that effect axonal elongation and synaptogenesis (GAP-43, τ, microtubules) (Ferreira and Caceres, 1991; Shughrue and Dorsa, 1993). Estrogen modulates plasticity during development and in adult CNS (Matsumoto, 1991; Garcia-Segura et al., 1994; McEwen, 1996; Woolley, 1998). Estrogen enhances neurite outgrowth by repressing GFAP and reorganizing laminin (Rozovsky et al., 2002). Estrogen can activate neurite mRNA translation (Pierce et al., 2000; Tiedge et al., 2001; Steward and Schuman, 2001).

Granule cell sprouting is stimulated by physiological levels of estrogen (which is blocked by progesterone and tamoxifen) in both wild-type OHSC (Teter et al., 1999a) (see Sections 4.6, 4.11) and in purified neuron cultures (Chawen et al., 1992; Woolley and McEwen, 1993). This effect is hippocampal region-specific, occurring only in the dorsal dentate region, both in OHSC (in the same region that is also dependent on apoE expression) and in vivo in adult, female rats, where short-term estrogen replacement in long-term estrogen-deprived females increases dentate granule cell spine density primarily by the dorsal region (Miranda et al., 1999). Sprouting of hippocampal neurons in response to ECL (see Section 3.6) was reduced by ovariectomy in rats and mice (Stone et al., 1998, 2000), and estrogen replacement rescues sprouting (Morse et al., 1986, 1992; Stone et al., 1998) (see Section 4.11).

Estrogen stimulates cyclic induction of synapses and dendritic spines in the hypothalamus and hippocampus of female rats (reviewed in Woolley and McEwen, 1992; McEwen, 2001). Synapse formation induced by estrogen may differ from that occurring during development, however (see Section 2.2): estrogen increases the number of synapses on multiple synaptic boutons between neurons not connected previously (Yankova et al., 2001). Estrogen induces both pre- and postsynaptic markers where new spines are formed (Brake et al., 2001).

10.1 TREATMENTS FOR PLASTICITY (see Sections 3.7, 4.12, 5, 9.2)

The public health impact of AD is predicted to rise at least three-fold in the next 50 years (Sloane et al., 2002). Clearly, all rational therapeutic avenues should be tested, but therapeutic stimulation, stabilization, or recovery of plasticity mechanisms could impact all neurodegenerative diseases. Current AD therapy targets cholinergic dysfunction, which may be linked to effects on plasticity through modulation of APP metabolism (and τ phosphorylation) by affecting the coupling of M1 muscarinic ACh receptors to G proteins (Fisher et al., 2000) (see Section 5.3). Other therapies under development focus specifically on Aβ, τ, inflammation, and oxidation (Galasko, 2001); if these pathologic phenotypes contribute to AD indirectly through interaction with an underlying process of plasticity, the effectiveness of interventions targeting these hallmarks may be enhanced if age-related plasticity failure is also treated. New drug strategies that target mediators of either neurodegenerative processes or neuroplastic processes must be considered for their pleiotropic and potentially confounding roles in both, as exemplified by apoE, NF-κB, etc. Of critical importance for the efficacy of plasticity-stimulating therapies is whether they create neural networks that are competent to replace lost function (see Section 10.5).

There are several avenues for stimulating plasticity in the damaged CNS, with targets at all levels of plasticity failure (Horner and Gage, 2000). Enhancing regrowth has been targeted as a therapeutic strategy. Putative neurotrophic agents such as Cerebrolysin have been reported to have positive effects in clinical trials, with sustained improvement after short treatment of AD (Bae et al., 2000; Ruther et al., 2000; Xiao et al., 2000); Cerebrolysin also ameliorates behavioral deficits and neurodegeneration in apoE-ko mice (Masliah et al., 1999). Propentofylline shows neurotrophic effects on glia function (Wilkinson, 2001), and cholesterol inhibitors like statins may reduce the incidence of AD (see Section 3.7). Memory rehabilitation, which targets mechanisms of cognitive reserve and compensatory reorganization to activate alternative, intact brain structures (facilitation of residual explicit memory, or, the 'use it or lose it' phenomenon), can be clinically effective (Grady, 1996), and alternative and innovative techniques are still under refinement (De Vreese et al., 2001).

10.2 Oxidation

As a lipid rich organ, the CNS is particularly susceptible to effects of lipid peroxidation in modulating cellular signaling pathways, cell dysfunction, and cell death in the nervous system (Keller and Mattson, 1998). In AD, emerging evidence provides strong support for a role for oxidative stress in neurodegeneration, as multiple indices of oxidative stress have been observed, including protein oxidation, decreased polyunsaturated fatty acids, mitochondrial and nuclear DNA damage, as well lipid peroxidation markers 4HNE (Sayre et al., 1997; Markesbery and Carney, 1999), and F2 and F4 isoprostanes (Nourooz-Zadeh et al., 1999), variously detected in brain and CSF. Vitamin E slows cognitive decline in AD (Sano et al., 1997a) and in rat models (Yamada et al., 1999) (see Section 4.4). Although it is not clear what causal relation oxidation has to AD etiology, e.g., whether it is a secondary effect of the stress caused by synaptic or neuronal loss, antioxidant therapies have shown limited but promising efficacy in treating AD (Pitchumoni and Doraiswamy, 1999).

Lipid peroxidation toxicity could inhibit sprouting by the inability to efflux such toxins; an efflux defect is shown by oxidized HDL (Therond et al., 1999). This oxidation-induced lipoprotein aggregation is neurotoxic to primary neurons and is accompanied by cytoskeletal microtubule disruption and inhibition of neuritogenesis

(Kivatinitz et al., 1997). Importantly, 4HNE disruption of microtubule organization inhibits neuronal sprouting (Neely et al., 1999). Effects of lipid peroxidation toxicity on inhibiting neuritogenesis could also involve apoE (see Sections 4.4, 8, 8.3).

10.3 Inflammation/NF-κB

NF-κB is directly required for synaptic plasticity, as shown in in vitro hippocampal slices (Albensi and Mattson, 2000). NF-κB is activated in association with LTP (Worley et al., 1993; Meberg et al., 1996). NF-κB is located in synapses at considerable distance from its canonical nuclear site of action, suggesting that it modulates synaptic function locally (O'Neill and Kaltschmidt, 1997).

NF-κB activation (reviewed in Mattson and Camandola, 2001) is implicated in AD (Perez-Otano et al., 1996; Lukiw and Bazan, 1998). NF-κB activity is increased in AD brain, including cholinergic neurons in the basal forebrain (Boissiere et al., 1997; O'Neill and Kaltschmidt, 1997). This could represent a neuroprotective and a cytoprotective response to plaques, because AB and secreted APP can activate NF-κB (Barger et al., 1995), and is associated with neuroprotective response to metabolic/excitotoxic events (Barger and Mattson, 1996) and mutant PS-1 (Guo et al., 1998). NSAIDs that target NF-κB have been shown to reduce the incidence of AD (Akiyama et al., 2000), even at low NSAID doses (Broe et al., 2000). Some NSAIDs reduce AB production, however, by modulating γ-secretase (Weggen et al., 2001) and could thereby influence the mechanism of plasticity involving the processing balance of APP (see Sections 4.1, 5.3, 8.3, 9, 10).

10.4 Growth Factors

NGF can induce sprouting and outgrowth, particularly after injury (Ramer et al., 2000; reviewed in Sofroniew et al., 2001), consistent with retrograde transport of trkA signaling complexes that alters gene expression in NGF-responsive neurons, including cholinergic neurons (Knipper et al., 1994; Holtzman et al., 1995a), which account for most of the NGF-responsive neurons in the adult CNS. The extent to which NGF is necessary for cholinergic survival of adult cholinergic neurons is controversial (reviewed in Rattray, 2001) (see Section 8). Indeed, AD brain shows increased NGF in the cortex and hippocampus (Jette et al., 1994; Scott et al., 1995; Fahnestock et al., 1996; Hock et al., 1998), which may reflect an increased demand for cholinergic input with a decreased ability of cholinergic neurons for retrograde transport of NGF. Although NGF may not have a classical neurotrophic role in cholinergic survival, i.e., through actions that are independent of retrograde signaling and gene expression, it is an important regulator of neuron morphology and function that would be predicted to maintain or improve cholinergic function in AD by promoting survival of degenerating neurons, promoting sprouting and enhancing neurotransmitter synthesis, and enhancing neuronal firing (reviewed in Rattray, 2001). Abnormal neurite growth might be associated with elevated NGF receptors (Ernfors et al., 1990; Mufson and Kordower, 1992) that precedes neurofibrillary degeneration (Arendt, 1993) (see Sections 3.4, 8.3).

NGF has been considered as a therapeutic target; however, problems with CNS delivery and side effects (pain) limit its clinical application (Eriksdotter Jonhagen et al., 1998). NGF application to the uninjured CNS causes cholinergic neurons to grow, sprout, increase ChAT, and increase choline uptake (Mobley et al., 1985; Higgins et al., 1989; Lapchak et al., 1992; Heisenberg et al., 1994). Experimental methods of delivery include gene transfer, cell grafts, or direct administration of NGF by intracerebroventricular infusion (reviewed in Rattray, 2001). These approaches have been successful in enhancing cholinergic neuron function and restoring some behavioral function in response to deafferenting lesions or impaired cognitive function. Drugs that increase NGF synthesis in astrocytes include propentofylline, a phosphodiesterase inhibitor (Rother et al., 1998), and various quinone derivatives (Takeuchi et al., 1990; Yamaguchi et al., 1993; Yamada et al., 1999). Nicotinic treatment that targets NGF production is also a possibility (Rattray, 2001). NGF mimetic drugs, like Neotrofin and AIT-082, are being tested in clinical trials (Emilien et al., 2000).

Neurotrophins are pivotal regulators of neurite outgrowth (Crutcher, 1986; Kang and Schuman, 1995). For example, BDNF acts at the synaptic level and is altered in AD (Murer et al., 2001). Further, GDNF clinical trials are underway (Maimone et al., 2001) and other neurotrophic strategies are being considered (Siegel and Chauhan, 2000).

10.5 Neurogenesis

Adult hippocampal neurons retain their proliferative capacity (Brewer, 1999, 2000; Seaberg and van der Kooy, 2002), where they provide a continuous replacement of neurons in the dentate gyrus (Seaberg and van der Kooy, 2002), particularly in conditions of enhanced learning (Kempermann et al., 1997, 1998a,b; Huang et al., 1998; Gould et al., 1999). Neurogenesis in the hippocampus declines with age (Cameron and McKay, 1999). An unanswered question is whether neurogenic capacity declines more in AD. Regardless, replacing lost neurons and reversing the age-related decrease in neurogenesis could be a therapeutic target using neural stem cell technology or other neuronal sources. This approach is attractive because neurogenesis occurs naturally and replacement does not suffer from caveats invoked for stimulation of aberrant sprouting, although it is not clear whether neural networks created by these new neurons are competent to replace lost function (see Section 2). Of critical importance is the orchestration of topographically accurate migration, targeted differentiation, and synaptic functionality of transplanted cells (Gage et al., 1995; Flax et al., 1998; Zhou et al., 1998; reviewed in Horner and Gage, 2000). Multipotent cells from the blood lineage, injected peripherally, migrate through the blood brain barrier; these may also provide a source and therapeutic avenue for CNS neuron

replacement (Bartlett, 1982; Bjornson et al., 1999; Brustle et al., 1999; Ono et al., 1999) (see Sections 5.2, 9.1).

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Perspectives

Should older adults be screened for dementia? It is important to screen for evidence of dementia!

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Abstract Multiple arguments for considering routine dementia screening have been presented. Furthermore, dementia diagnoses are widely unrecognized. As a result, persons with dementia are missing important clinical care and treatment interventions. By distinction, the problems of defining, diagnosing, and treating mild cognitive impairment (MCI) are not yet resolved, and MCI is not ready for a screening recommendation. Dementia screening approaches, including cognitive testing and functional assessment, must be evaluated on their scientific merits, including sensitivity and specificity for recognizing affected individuals in at-risk populations. Screening tests must be "cost-worthy", with the benefits of true-positive test results justifying the costs of testing and resolving false-positive cases, with due consideration for proper diagnostic evaluation and potential harms. With the tremendous number of new cases projected in the near future and the expected emergence of beneficial therapies, considerably more research is needed to develop more efficient screening systems.
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Editor's Note: This paper was written in response to a comment submitted to this Journal on the consensus statement by a group of scientists concerned about screening for dementia, which was published in this Journal in April 2006 [1]. The submitted manuscript was withdrawn after this response was submitted. However, this response is being published because it addresses concerns about screening recommendations and provides clarification and additional information on key points concerning dementia screening.

Keywords: Dementia; Alzheimer's disease; Screening; Diagnosis; Case-finding; Mild cognitive impairment

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1. The clinical evidence justifies screening for dementia

In April 2006, a group of clinicians and scientists concerned about dementia screening [1] presented their consensus that screening at-risk populations for evidence of dementia was an important matter to consider (A&D Consensus Group). The A&D Consensus Group addressed the well-documented and widely recognized problem of inadequate recognition of dementia in clinical practice [2–6]. Freund [7] estimated that the missed diagnoses are greater than 25% of the dementia cases and might be as high as 90%. Dementia exerts a substantial burden on patients' lives and the lives of those close to them [8]. The A&D Consensus Group reviewed the responses of numerous national and international organizations to this worsening crisis and noted that none recommended screening for dementia, although essentially all of the reviewed organizations did recommend a diagnostic evaluation when memory problems or dementia were suspected. It is commonly accepted that most dementia patients are cared for in the primary care setting, and clinicians working in this setting do not have adequate time for in-depth consideration of unrecognized cognitive difficulties that their patients might have. Furthermore, there are a variety of reasons that the clinicians, the patients, and those close to the patients do not express concerns about the presence of dementia when symptoms are first noticed. Multiyear delays from first symptom occurrence to clinical assessment have been documented and attributed mostly to uncertainty about the severity of the cognitive deficit (47%) and attributing observed changes to normal aging (37%) [9].

To respond to the acknowledged need to improve recognition of dementia in primary care settings, the A&D Consensus Group recommended a systematic approach to enhancing suspicion of dementia that would otherwise go unnoticed. Accordingly, the A&D Consensus Group recommended that the process for suspecting and recognizing possible early dementia be carried out. The process required to detect unrecognized or unacknowledged disease is commonly referred to as screening. Given the abundance of adequate tests for recognizing mild dementia, the numerous benefits in doing so, the slight costs associated with such testing, and the minimal nature of the potential harms from such investigation (Table 1), this group recommended the consideration of implementation of procedures to screen for dementia. The perspective that it is reasonable to recommend screening for dementia has only recently developed and has been championed independently by other groups [10–12].

Table 1
Benefits and harms of dementia screening

- Psychological and social benefits from early dementia recognition
  - Early education of caregivers on how to handle the patient
  - Advance planning while patient is competent, establishing a will, proxy, power of attorney, advance directives
  - Reduced patient and family anxiety, uncertainty, and stress and improved family understanding of demented patient, reduced caregiver burden, blame, denial
  - Promote safety in driving, medication compliance, cooking, etc
  - Patient's and family's right to know especially about genetic risks
  - Promote advocacy for research and treatment development
- Medical benefits from early dementia recognition [11,22]
  - Early diagnosis and treatment and appropriate intervention might improve overall course substantially, including lessening disease burden on caregivers and society
  - Specific treatments are now available for Alzheimer's disease (anticholinesterases, memantine). These medications have been shown to:
    - Temporarily improve cognitive dysfunction
    - Temporarily improve function (ADLs)
    - Delay conversion from MCI to Alzheimer's disease
    - Decrease development of behavior problems
    - Delay nursing home placement
- Harms from failure to recognize early dementia
  - Dangerous behaviors: cooking, operating machinery
  - Driving problems [26]
- Listing and accounting for the harms of not screening for dementia [12]
  - Missed opportunities for:
    - the application of available treatments
    - participation in research
    - advance care planning
    - support of caregivers
- Listing and accounting for the harms of dementia screening [24]
- Harms that might occur to those with positive screening test result
  - Clinical error of equating positive screen with diagnosis (education about screening and proper dementia diagnostic implementation can address this issue).
  - Complications arising from further testing (only additional clinical questions necessary to inquire about the patient's history should be considered at this point, as is recommended for evaluation of suspicion of dementia by the AAN).
  - Adverse effects of treatment must be considered with respect to the benefits, on their own merits, based on the opinion of the clinician that makes the decision for treatment.
  - Anxiety generated by investigation and treatment; such anxiety must be balanced against the already considerable and appropriate anxiety about Alzheimer's disease in our society. Screening in the context of proper diagnosis and medical management can help manage that anxiety.
  - Costs and inconvenience incurred during evaluation; the cost of dementia evaluation needs to be entered in the calculation of whether screening is cost-worthy
- Harms that might occur to those with negative screening test result
  - False reassurance: a false negative might wrongly diminish concern and motivation to participate in future evaluation. The consequences of incorrect results are factors that can be accounted for in the decision to screen.

Abbreviations: ADL, activities of daily living; AAN, American Academy of Neurology.

2. Defining the dementia-related conditions for screening consideration

There is a long-term problem in the field of dementia of defining the basic diagnostic issues and symptom constellations. The American Psychiatric Association (APA), during the prolonged period of development of the Diagnostic Manual, versions III and IV (DSM-III, DSM-IV), has es-

tablished a diagnostic spectrum of dementia, including "Dementia of the Alzheimer Type," "Vascular Dementia," and "Dementia Due to Other General Medical Conditions" [13]. The core description of dementia includes development of multiple cognitive deficits, including memory and other disturbances, causing impairment in social or occupational functioning. Generally, dementia does not have a defined onset, and the rate of progression varies extensively. The dementia course might be "characterized by gradual onset" that progresses insidiously, as is typical with Alzheimer's disease, or it might begin suddenly and progress in discrete increments, as might be seen with vascular dementia. The uncertainty of the point of dementia onset is one of the basic reasons that a screening system is needed; with a variable course, early dementia is difficult for clinicians to notice. There are now many widely acceptable management interventions that are not properly applied because the presence of the disease is missed. Because of the difficulty in recognizing this problem, along with the perceived value of recognition, many scientists and clinicians have sought to develop screening tests for this difficult problem.

Recently, there have been increasing discussions and extensive considerations of what follows normal function but precedes dementia, a concept now widely referred to as mild cognitive impairment (MCI) [14]. Although MCI has received a considerable amount of research attention, it has not been formally defined as a diagnostic entity for routine clinical purposes. MCI is being characterized as an early clinical stage of diseases that lead to dementia. Although MCI is of considerable academic and research interest, the core issue in primary care is early detection of Alzheimer's disease and related disorders, because the benefits afforded are considered to be substantial. From a purely pragmatic perspective, primary care physicians are not likely to have the time to know when a patient crosses the line from having MCI to having mild dementia, so that the physician's focus should simply be on detecting early dementia. Simple screening tests have not yet been developed to recognize or detect MCI [15], although there are a few tests that accurately distinguish normal aging from MCI [16] at levels comparable to tests for other conditions for which screening is widely accepted, such as breast cancer and Down syndrome.

3. Reviewing the screening principles

Because dementia has been such a difficult syndrome to recognize in the primary care setting when symptoms are mild, it is important to use the best available screening principles to decide how to evaluate a subject for this problem. It is the contention of the A&D Consensus Group that all of the criteria for conducting a screen for dementia (as opposed to MCI) are met. This is a brief review of those principles for dementia:

3.1. It must be common

Dementia is admittedly very common, but it must also be noted that the prevalence increases steeply with age, more so than mortality [18]. As yearly incidence increases with age, the imperative to screen increases proportionally. Depending on ancillary considerations, the threshold for recommending routine screening to the population might be reached by age 75 years.

3.2. It must have sensitive and specific tests available for its detection

There are an abundance of tests available for dementia screening whose sensitivities and specificities that are acceptable for dementia screening purposes [10,15].

3.3. It must have efficacious treatment

There are five Food and Drug Administration–approved medications for Alzheimer's disease as well as recommended treatments for several other types of dementia. There are a few groups who have questioned the efficacy of the cholinesterase inhibitors for Alzheimer's treatment (originally Ashford et al [19]), and the most prominent has been the statement of the National Institute for Health and Clinical Excellence (NICE) in Great Britain in response to data from Courtney et al [20]. The NICE appraisal was revised in November 2006 to include a recommendation for subsidizing use of cholinesterase inhibitors for moderately demented patients with Alzheimer's disease [21]. However, the preponderance of the studies have shown that the approved dementia medications have useful benefit for many patients [11,22,23]. Beyond specific treatments for Alzheimer's disease, there are efficacious biologic, psychological, and social interventions that should be at least considered as soon as possible in the Alzheimer's disease course and the types of dementia associated with other etiologies.

3.4. If treatment exists, treated patients must have better outcomes than untreated patients

As noted earlier, there has been some debate about this point, but many studies have shown the benefits of the treatments on biologic (brain scans), psychological (cognitive testing and behavioral testing), and social (ADLs, activities of daily living) measurements [11,22]. Furthermore, there are many types of dementia beyond Alzheimer's disease in which a specific early intervention is clinically superior to no intervention. Beyond specific clinical outcomes, the value of general supportive care for dementia patients and their families are outcomes that are being studied, and these outcomes must be included in the evaluation of the criteria for judging screening tests. Several studies have shown better outcomes for caregivers of treated patients, and these outcomes add further value to dementia screening.

3.5. The benefits from screening must outweigh the harms

There are multiple benefits and comparatively few and minor harms associated with the administration of specific screening tests for dementia (Table 1). The issue of harmful side effects from treatment is not directly related to screening. Instead, the cost-benefit of implementing treatments is a decision that is made on the basis of the diagnostic evaluation, independent of the rationale for initiating the diagnostic work-up. There is a concern that some clinicians may equate a positive screen with diagnosis, rather than making the proper secondary enquiries and initiating the diagnostic evaluation only when indicated; education for appropriate implementation and patient communication for screening is essential to address this quality of care issue.

For Alzheimer's disease, the side effects from the available treatments are usually manageable clinically. The concern that there might be increased mortality with the use of cholinesterase inhibitor treatment in patients with MCI is not directly germane to the issue of treating patients with diagnosed dementia of the Alzheimer type. However, the question of harms of treatment does introduce the issue that screening tests need to be followed by appropriate diagnostic evaluation before treatments are initiated. In addition, some other effects of cholinesterase inhibitors might have clinical benefit (eg, reduced constipation, slower heart rate, and improved behavior).

4. The need to distinguish the concepts of dementia and MCI

An important distinction must be drawn between dementia and MCI. MCI is a prodromal condition to dementia in many cases, but it is not a diagnosis of dementia. Screening for dementia is addressing a problem that has already been recognized by numerous official entities as requiring a diagnosis. In spite of popular interest in screening for MCI [17] and widespread concern about MCI in primary care practice, diagnostic criteria for MCI still need clarification, as was noted in the original A&D Consensus Group article. Further research is needed on screening methodology and treatment benefits before population screening for MCI will be ready for consideration. This concern applies to MCI screening, not to dementia screening as discussed in the A&D Consensus Group article.

5. Delineation of screening-related risks from adverse consequences of diagnosis

It is important to distinguish the risks involved in a screening test from the results that might occur as the direct outcome of clinical care. These factors include the potential adverse consequences of diagnostic tests and treatments. In the "Guidelines and Recommendations about Screening" [24], there are diagrams describing systems to implement screening tests; this article does not apply to dementia screening as discussed by the A&D Consensus Group because it presents a direct advance from screening tests to treatment. What is discussed in the A&D Consensus Group article is screening tests to determine when diagnostic tests should be considered. It is an adverse development if the results of any of the dementia screening tests are interpreted wrongly as a diagnosis of "Alzheimer's disease" without proceeding through the Standard of Care diagnostic procedures for dementia diagnosis. Furthermore, the routine dementia diagnostic tests, including progressively: history and physical examination, blood tests, focused neuropsychological assessment, and a brain scan, are generally safe. However, it is reductionistic to indiscriminately apply the logic of attributing the numerous potential adverse consequences of complex clinical interventions to the use of a brief check for early dementia signs for triggering a complete evaluation.

After clarifying the separation of screening and diagnostic procedures, the correct point about screening tests needs to be reiterated, as expressed in the original Consensus Group statement, "The only negative impact of a false positive could be at most a brief secondary assessment!" In this circumstance, a screening test only leads to a recommendation of a second step in assessment. Such a test should be considered to be of no greater consequence than the commonly used screening question, "Do you have a cough?" for which the positive response should lead to auscultation of the lungs, not diagnosis and treatment of lung cancer. Thus, a dementia screening test by itself should carry limited social, psychological, or ethical concerns.

There is a related commonly expressed issue suggesting that a screening test can result in "labeling." Labeling should not occur without a diagnosis. Because screening does not provide a diagnosis, there is no reason for labeling to occur. There have been appropriate concerns raised about psychological and social consequences of making a diagnosis of Alzheimer's disease. This concern is appropriate and needs to be addressed as part of the refinement of dementia diagnosis. However, this is not a problem that should be related to appropriate screening. Accordingly, education should accompany screening implementation to delineate limitations of screening clearly and to outline the appropriate uses of derived information.

Another concern is that individuals participating in a screening test would make inappropriate decisions about the recommendations resulting from the testing. Failure of a subject with a positive screen to get further diagnostic assessment is a concern, but compliance with medical recommendations is a widespread problem, not just related to screening tests. Compliance problems represent one of the points in which modern medicine needs broad-based improvement that appears to be difficult to address within an overburdened health care system. The opposite problem, that an individual with a negative screen might see this

result as permanent freedom from worry about dementia, is also a concern and a misunderstanding. The negative screening result only suggests that the concern about dementia can be reduced for a limited period of time, for example, 1 year. Such inappropriate patient responses to screening test results should not be considered harms of screening but areas for attention and patient education in which the quality of the whole screening system could be progressively improved, and population education can lead to an overall improvement in the health of society.

6. Financial costs associated with screening

There are monetary costs incurred by screening and the resulting increase of care burden. Analyses of this issue should formally address the "cost-worthiness" of the medical test [25]). Such analyses have been reported and published [11], and looking at limited health economic data, the results tend to support screening. However, the A&D Consensus Group further argued that the benefits from having information about mental dysfunction can help the patient's support system function more efficiently and with less stress and plan for more effective management of the issues that will develop as the patient deteriorates.

7. Conclusions

(1) In the development of cognitive and memory screening tests, a clear distinction must be drawn between screening for dementia and screening for MCI. Tests are currently available that should be considered cost-worthy for dementia screening. The basis for screening for MCI is not yet established.
(2) The decision to screen for dementia should be based on sound scientific consideration of all relevant issues and public health benefits, not political issues. Further attention to dementia screening is clearly warranted, although implementation will require careful development of practice guidelines and public education that might vary considerably across different settings.
(3) Dementia screening in clinical settings is clearly appropriate for those whose risk is above a certain threshold, for example, persons older than the age of 75 years. Widespread screening of the whole elderly population also has merit, although systematic recommendations need to be developed. Full public health screening will become justifiable when more substantive therapeutic and/or preventive interventions are developed, and such therapies are currently under intense testing. Consequently, now is the time to prepare for the future by developing dementia screening systems and memory testing programs that will be able to detect patients with early phases of dementia.

8. Addendum

8.1. Motivations of professionals in the field of dementia screening

A concern is always present about whether there are irreconcilable conflicts of interest when recommendations are made with broad social implications, in this case, either to screen or not to screen for dementia. There are financial and professional incentives that operate in all human beings. Financial motivations are clear for the pharmaceutical industry. However, as is apparent from problems that certain pharmaceutical companies have had recently, attempts to obtain results in violation of accepted ethical principles for scientific conduct lead to direct financial risk and harm to company reputation with attendant financial consequences. More often and more profitably, pharmaceutical companies do their best to follow all of the rules very carefully, although at least in part because of the careful scrutiny. Furthermore, all scientists, even the peer reviewers at the National Institutes of Health, have their own issues and biases, and they are working in a political arena that also is fraught with personal ambitions. The co-authors of the original article are admittedly those interested in screening test development. That interest is a direct result of their interest in providing optimum care for patients. The motivations of all parties involved in making comments that can influence social policy need to be similarly scrutinized.

8.2. Notes about potential conflicts of interest for funded or unfunded researchers

Opinions of funding organizations cannot be taken as accepted opinions of all of those funded by those organizations. Simply because a group has received funding for their research from various organizations, some of which may support or are opposed to screening, does not mean that opinions expressed by them are biased by those organizations. Similarly, opinions or implicit agendas of funding organizations associated with the A&D Consensus Group do not necessarily bias the expressed opinions.

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The Effects of Increasing Stimulus Complexity in Event-Related Potentials and Reaction Time Testing: Clinical Applications in Evaluating Patients with Traumatic Brain Injury

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Summary: This study compared the effectiveness of P300 event related potentials (ERPs) and reaction time (RT) in discriminating patients with traumatic brain injury (TBI) from healthy control subjects. In particular, we examined how the use of more complex, ecologically relevant stimuli may affect the clinical utility of these tasks. We also evaluated how length of posttraumatic amnesia (PTA) and loss of consciousness (LOC) related to P300 and RT measures in our patient sample. There were 22 subjects (11 patients with TBI and 11 age-matched healthy control subjects). Four stimulus detection procedures were used: two using simple, conventional stimuli (auditory tone discrimination, AT; visual color discrimination, VC), and two using complex, ecologically relevant stimuli in the auditory and visual modalities (auditory word category discrimination, AWC; visual facial affect discrimination, VFA). Our results showed that RT measures were more effective in identifying TBI patients when complex stimuli were used (AWC and VFA). On the other hand, ERP measures were more effective in identifying TBI patients when simple stimuli were used (AT and VC). We also found a remarkably high correlation between duration of PTA and P300 amplitude.

Key Words: Event related potential, P300, Reaction time, Traumatic brain injury, Posttraumatic amnesia.

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The event-related potential (ERP) is a reflection of cortical brain activity during which attention is directed toward a novel stimulus event (Polich and Kok, 1995). Changes in amplitude and latency of the P300 component of the ERP waveform have been widely studied as indicators of severity of traumatic brain injury (TBI) (Lew et al., 2006). Reaction time (RT) is a measure of the speed with which cognitive tasks are completed by a measured motor response, usually a button press. Slowing of RT is one of the cardinal sequelae of TBI (Cicerone, 2006; Levinson and Reeves, 1997; van Zomeren and Deelman, 1978; Warden et al., 2001). Simultaneous recording of ERP and RT allows investigators to capture both the attentional and processing speed deficits that commonly follow TBI (Eger et al., 2003; Lew et al., 2004; Lew et al., 2005a; Starbuck et al., 1995).

In the field of TBI, most ERP studies have utilized simple tasks that require minimal cognitive activity, e.g., recognizing a target tone or color (Lew et al., 2004). Fewer ERP studies have utilized complex stimuli that require more cognitive processing. Lew et al. (2002) demonstrated the feasibility of using words rather than tones to generate ERPs in TBI patients. Eger et al. (2003) obtained measurable ERPs with affective facial stimuli in normals, and Lew et al. (2005b) were successful in using ERPs to differentiate TBI patients from control subjects with facial expressions as stimuli.

The present study used simultaneously collected ERP and RT measures to compare how well four cognitive tasks that varied in stimulus complexity differentiated TBI patients from control subjects. We hypothesized that complex stimuli would be superior to simpler stimuli for this purpose because they may tap higher levels of cognitive processing. They may also better simulate the cognitive activities of every-day life. We also hypothesized that ERP and RT measures from complex tasks would be more strongly related to indices of injury severity than those based on simpler tasks, again because performance on complex tasks may be affected by the higher level cognitive processing deficits that are characteristic of TBI.

METHODS

Participants

TBI patients were recruited from the brain injury rehabilitation unit of a university affiliated medical center to undergo simultaneous ERP/RT assessment. Eleven of them

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met the selection criteria for the present analysis: severe TBI with >24-hour loss of consciousness, neuroimaging positive for intracranial injury and a good recovery (Glasgow Outcome Scale = 5, Rancho level VIII). Participants were excluded if they had any of the following (1) a history of permanent hearing loss or color blindness; (2) took sedatives, anticholinergic agents, dopamine agonists or antagonists (medications with the potential to distort the morphology of the waveforms (Fowler and Mitchell, 1997; Nishimura et al., 1995) within 72 hours before testing (3) possession of a rare talent for absolute pitch. Individuals who posses this talent have an intrinsic advantage in processing pure tones (Wayman et al., 1996).

Control subjects were age-matched volunteers having no history of traumatic brain injury, psychiatric or neurologic disorder. They were recruited from the patients' friends and family and from hospital staff and volunteers. They were ten males and one female with an average age of 25.0 years (age range: 18 to 49). There was no statistical difference in age (Wilcoxon $Z = -0.42$, $P = 0.67$) or gender (Wilcoxon $Z = -0.58$, $P = 0.56$) between the TBI and control groups. All subjects were fully oriented, able to follow instructions, had visual acuity within normal limits, and bilateral upper-limb strength of 5/5 on neurologic examination. Informed consent forms, approved by the local institutional review board, were signed by all twenty-two subjects.

Information utilized regarding the patients' injuries and recovery (e.g., cause of injury, neuroimaging results, loss of consciousness (LOC), posttraumatic amnesia (PTA), current medication, Glasgow Outcome Scale score, Rancho level, and neuropsychological test results) was extracted from medical records, clinical tracking forms, and from patient interviews. Eight patients were injured in motor vehicle accidents, two had sustained combat related blast injuries and one was injured in a physical assault. They averaged 13-day LOC and 36 days of PTA. The duration of PTA was calculated beginning with the onset of injury including the period of coma. All patients had evidence of intracranial injury on neuroimaging. Their average level of education was 12.6 years.

Neuropsychological testing was performed an average of 14.3 months after injury and the ERP and RT data were collected an average of 15.5 months after injury. The patients' mean IQ score was about ten points lower than expected given their level of education (WAIS-III Full Scale IQ for the TBI patients was 90, range: 79 to 95, Verbal IQ 93, range: 82 to 105 and Performance IQ 86, range: 73 to 106). Their below average mean WAIS-III Digit Symbol score ($Z = -1.44$, SD = 0.90) suggested slowed processing speed. Impaired memory for new learning was evident from their well below average mean score on the California Verbal Learning Test Trials 1 to 5 total ($Z = -2.03$, SD = 1.88). Some of their mean cognitive scores were in the average range: WAIS-III Digit span ($Z = -0.21$, SD 0.74), Trail Making A ($Z = 0.78$, SD 0.62) Trail Making B, ($Z = -0.13$, SD – 0.76) Wisconsin Card Sorting Test total errors ($Z = 0.56$, SD 1.19).

Stimulus Tasks

Four stimulus detection procedures were employed to obtain ERP and RT data, two using simple conventional stimuli: auditory tone (AT) discrimination and visual color (VC) discrimination. The other two used more complex, ecologically relevant stimuli in the auditory and visual modalities: auditory word category (AWC) discrimination and visual facial affect (VFA) discrimination. All tasks utilized an oddball paradigm with a total of 150 stimuli, 120 frequent/nontargets (80%) and 30 randomly presented rare/target stimuli (20%). Each participant was seated 65 cm. from the display monitor and was asked to respond as quickly as possible when a rare/target stimulus was presented by pressing a button located on a response pad. The tasks were presented in the same sequence to all subjects, with AWC as the first task, VFA second, AT third, and the VC task last. Testing was completed in a single session for all participants. The entire session lasted approximately 45 minutes per subject, including the application of electrodes, task instructions, and the presentation of all four tasks.

(I) *Auditory Tone (AT) Discrimination.* The tone task used 1000 Hz tones as frequent stimuli with the rare stimuli being 500 Hz tones. The tones were presented to the subject using binaural earphones at a sound pressure level of 80 dB SPL with a duration of 500 ms. and stimulus onset asynchrony (SOA) (time between onset of the tones) of 2.11 seconds. A cross appeared continuously on the display monitor during the presentation of the tones. The subjects were asked to fix their eyes on the cross to minimize eye movement.

(II) *Visual Color (VC) Discrimination*. Green circles were used as frequent stimuli and red circles as rare stimuli in the visual color task. All circles measured $1^{13}/_{16}$ inches in diameter, thus maintaining a constant visual angle. The images of the circles were displayed on the computer monitor for 500 ms, with an SOA of 2.11 seconds.

(III) *Auditory Word Category (AWC) Discrimination*. Monosyllabic nouns were used in the auditory word task. The frequent stimuli were the names of various inanimate objects (e.g., book, desk) and the rare stimuli were the names of animals (e.g., dog, cat). Fifty different words were used three times each to make up the 150 stimuli. The subjects' task was to press the button as soon as they heard the name of an animal. The words were presented using binaural earphones at a sound pressure level of 80 dB SPL, with a duration of between 300 and 600 ms, and an SOA of 2.11 seconds. As with the tone task, a fixation cross appeared continuously on the display monitor during the presentation of the words to minimize eye movement.

(IV) *Visual Facial Affect (VFA) Discrimination*. The face task utilized Ekman's published series of facial expressions (Ekman and Friesen, 1976). Pictures of four faces were employed as stimuli. The frequent stimuli were a male and a female face showing neutral affect and the rare stimuli were a male and a female face showing angry expressions. Of the 150 images, half were male faces and half were female faces. All facial images measured 7.75 inches tall by 5.55 inches

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wide to maintain a constant visual angle. Each face was displayed for 1.0 seconds, with an SOA of 2.11 seconds.

Instrumentation

The electroencephalogram (EEG) was obtained using a Physiometrix (Billerica, MA) electrode cap with disposable gel electrodes. The cap utilized the international 10 to 20 system with data collected at electrode sites Fz, C3, Cz, C4, Pz, M1, and M2 with the grounding electrode at FP2 and reference electrode on the nose tip. Vertical electro-oculograms and horizontal electro-oculograms were collected from bipolar electrode arrays placed above and below the right eye and at the outer cantus of each eye, respectively. We followed the usual convention of reporting P300 at site Pz where it is nearly always maximal. The other sites allowed for verification of cognitive stimulus processing. The Neuroscan STIM system (El Paso, TX) was used for the presentation of stimuli. A portable Neuroscan SynAmps2 digital amplifier (El Paso, TX) and Acquire version 4.3 software on a Dell laptop were employed to collect EEG and RT data, and for data processing and storage. Stimulus-locked ERPs (–100 to 1000 ms) were corrected for eye movements, HHz baselined to the prestimulus interval and rejected for movement artifacts. ERP waveforms were produced after frequent and rare trials were sorted and averaged. Epochs were digitally filtered (15 Hz low pass) before peak P300 amplitude and latency at electrode Pz were measured relative to baseline in the 270 to 600 ms interval for the rare stimuli using Matlab (MathWorks, Natick, MA) scripts. ERP latency was measured in msec. from stimulus onset to the P300 peak.

Behavioral data (RT duration and response accuracy) were recorded simultaneously during the acquisition of the electroencephalography waveforms. Reaction time was measured in msec. from stimulus onset to button press. Response accuracy was measured as the percentage of correct response to the rare (target) stimuli.

Statistical Analysis

Statistical analyses were carried out with the Statistical Package for Social Sciences (version 14.0) to compare ERP amplitudes, latencies, and RT measures between the TBI and control groups. Because previous literature suggested that the TBI patients tend to have lower ERP amplitudes, longer ERP latencies, longer RTs and lower response accuracy, one-tailed tests of significance were employed. Due to the limited sample size, nonparametric statistics (Wilcoxon signed ranks test) was used to test for group differences. Significance was defined as $P < 0.05$ and effect size was measured with the value of Wilcoxon Z.

To examine general trends in relationships between the major measures (P300 amplitude, latency, RT, hit rate) with a limited N, composite scores were created. This was accomplished by first computing the measures' internal consistency (Chronbach's α) using various sets of tasks for each measure. Since the alphas were highest when all four tasks were included in this computation, all four tasks were used to obtain a composite score [(AT+AWC+VC+VFA)/4] for each subject on each major measure: P300 amplitude composite score, latency composite score and percent correct composite score. These composite scores were then used to generate Spearman Rho correlations.

RESULTS

The grand average ERP waveforms and RTs for each group on the four tasks are shown in Fig. 1. Group means and tests of significance for the size of group differences for ERP amplitudes and latencies and for RT and accuracy are presented in Table 1. Both Fig. 1 and Table 1 illustrate the differences between the TBI and control groups on these measures. Compared with the control group, the TBI group had lower P300 amplitudes and more prolonged P300 latencies on three of the four tasks (AT, VC and VFA), as well as slower RTs on all four tasks.

Table 1 shows that with regard to the traditional stimuli (AT and VC), the TBI group had a significantly lower mean P300 amplitude, longer mean P300 latency and slower mean RT than the control group. Regarding the VFA complex stimulus, the TBI group had a significantly lower mean P300 amplitude and longer mean RT. The other complex stimulus (AWC), however, did not produce significant differences between the two groups on ERP measures (P300 amplitude and latency), but the TBI group had a significantly longer mean RT on this task than the control subjects. It should be noted that the AWC task was the only one that successfully differentiated the two groups with regard to response accuracy. The TBI group averaged 6% more errors than control subjects on the complex tasks, but the variability was too high on VFA for this difference to reach significance.

By inspecting Fig. 1 and by comparing analogous boxes in Table 1, it can be seen that P300 peak latencies occurred before the button push on all tasks for each group. When the tasks were ranked by mean RT (Fig. 2), the same hierarchy was present for both the TBI patients and the control subjects; the AT task was done most quickly, followed by VC, then VFA, with the AWC task having the longest reaction time. This parallel ranking between groups suggests that the tasks were of equivalent relative difficulty for the TBI patients and the control subjects.

Table 2 shows the correlations among the four composite scores and between each of the composite scores and group (bottom row). As was found with tests of group differences on the individual tasks (Table 1), the significance and direction of the correlations between composite scores (bottom row of Table 2) indicate that the TBI group tended to have a lower P300 amplitude, longer P300 latency, longer RT and lower accuracy. Of the four composite scores, P300 amplitude had the highest correlation with group, i.e., it was the best single measure for differentiating the TBI patients from the control subjects.

When the TBI patients and control subjects were combined into one group, the composite P300 latency score was positively correlated with the composite RT score (Rho = 0.69, $P = 0.01$). Supplementary correlational analyses with the separate tasks making up the composite P300 latency and composite RT scores, showed that this relationship was strongest ($P \leq 0.05$) for the visual tasks (VC and VFA). The composite RT score was negatively related to the composite

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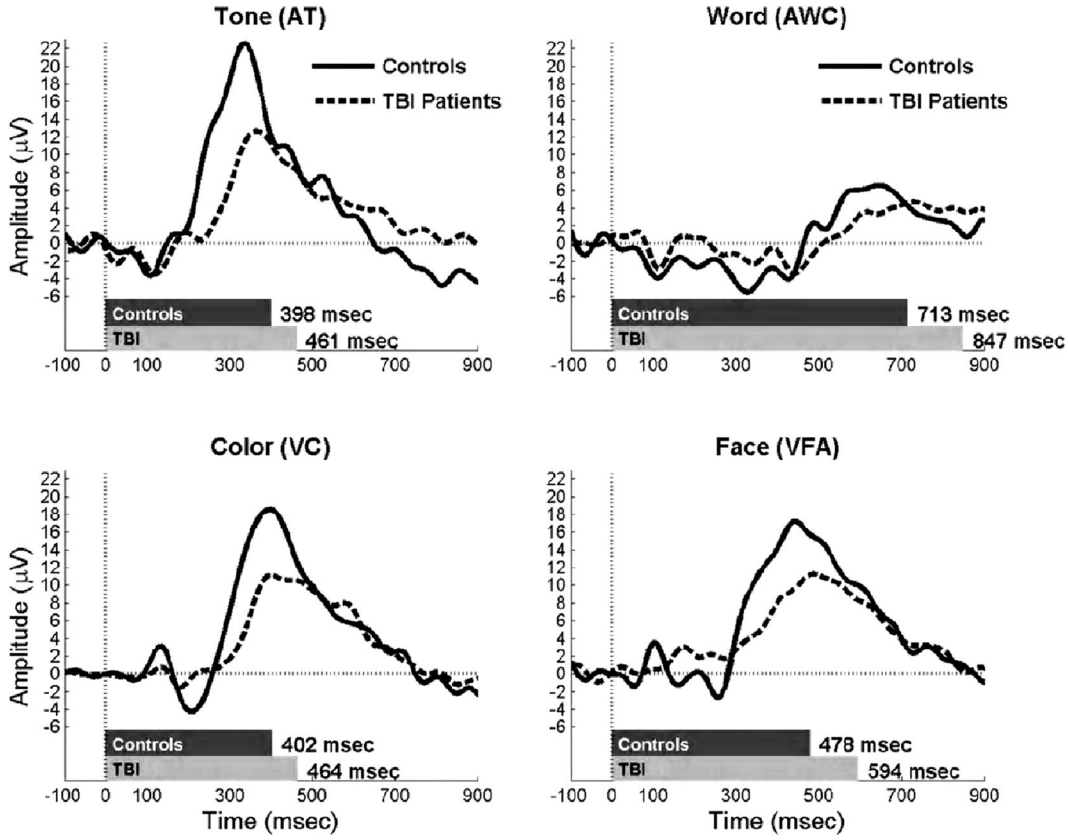

FIGURE 1. Stimulus locked ERP (Pz) waveforms for TBI and control groups on four tasks. RTs are shown at the base of each image.

percent correct (Rho = −0.56, $P = 0.01$) indicating that faster responders were also more accurate.

To investigate how TBI severity may relate to the P300 and RT measures in our patient sample, Spearman correlations were calculated between duration of loss of consciousness, length of posttraumatic amnesia and the composite RT and ERP scores. A strong negative relationship was found between the length of PTA and the composite P300 amplitude scores (Rho = −0.75, $P < 0.01$, two-tailed) (Fig. 3). No significant correlations were present between PTA and LOC or between PTA, LOC, and the composite scores or with any of the cognitive test scores. When we looked at correlations between PTA and RT, RT accuracy, P300 amplitude and P300 latency separately for each of the four tasks, PTA was significantly correlated with P300 amplitude only on the simple tasks (AT, Rho = −0.80, $P < 0.01$, and VC, Rho = −0.66, $P < 0.05$), with P300 latency only for AT (Rho = −0.63, $P < 0.05$) and with accuracy only for AWC (Rho = −0.72, $P < 0.05$). No significant relationships were found between P300 amplitude and duration of LOC or the number of months since injury that these measures were taken.

DISCUSSION

Simple Versus Complex Tasks

We compared traditional, simple ERP tasks (AT and VC) to more complex, ecologically relevant tasks (AWC and VFA) for their efficacy in identifying impaired cognitive functioning in patients with history of severe TBI. It was hypothesized that complex stimuli (such as AWC and VFA) would be better than simpler stimuli at identifying the TBI group with ERP. The results we obtained regarding this hypothesis were mixed. When P300 amplitude was used, both simple tasks (AT and VC) and one of the two complex tasks (VFA) clearly separated the TBI patients from the control subjects. When P300 latencies were used, only the simple AT and VC tasks differentiated the TBI patients. This advantage for the simple tasks was probably because they generated a steeper, sharper P300 peak than did the complex stimuli and had less variance in P300 latency. This lower level of variability is presumably because the simple tasks exerted lower attentional or mental processing demands on the subjects than did the complex tasks. Considering both P300 amplitude and latency to identify severe TBI in our sample, traditional, simple tasks (tone and color discrimination) performed best.

When RT was used to differentiate TBI patients from control subjects, both the complex (AWC and VFA) and simple (AT and VC) tasks were effective, but the complex tasks had larger effect sizes (average $Z = 2.4$ versus 1.7). This is because the differences between the RT means for the TBI and control groups were larger for the complex tasks (avg. diff. = 125 ms) than for the simple tasks (avg. diff. = 63 ms). This comparison illustrates the more pronounced

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TABLE 1. TBI vs. Control Group Comparisons on ERP and RT Measures

	TBI		Control		Wilcoxon Test 1	
Measure	**Mean**	**SD**	**Mean**	**SD**	**Z**	***P***
P300 amplitude						
AT	13.43	5.08	22.79	8.95	−2.22	**.013†**
VC	12.52	7.65	19.91	7.20	−2.05	**.020***
AWC	6.35	2.04	7.82	6.07	−0.89	.187
VFA	13.15	2.94	18.32	6.06	−2.40	**.008†**
P300 latency (ms)						
AT	365	26	332	27	−2.05	**.020***
VC	451	63	392	30	−2.14	**.016***
AWC	696	82	645	74	−0.98	.164
VFA	463	84	448	57	−0.36	.359
Reaction time (ms)						
AT	461	109	398	130	−1.69	**.046***
VC	464	89	402	66	−1.69	**.045***
AWC	847	103	713	94	−2.49	**.006†**
VFA	594	115	478	90	−2.31	**.010†**
Percent correct responses						
AT	98.8	2.25	98.2	3.45	−0.43	.67
VC	97.9	4.30	98.5	3.11	0.00	1.00
AWC	90.9	9.08	97.9	4.02	−2.09	**.04***
VFA	91.5	14.6	97.0	4.78	−0.94	.35

N = 11 per group; [1]Wilcoxon signed ranks test, one-tailed; *$P \leq 0.05$, †$P \leq 0.01$.

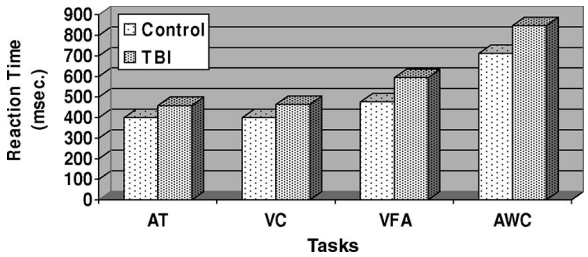

FIGURE 2. Reaction times on the four tasks for TBI patients and controls.

TABLE 2. Spearman Correlations Between Composite Measures (N = 22)

Composite Measure	Amplitude	Latency	RT	% Correct
P300 amplitude	1.0	.34	−.39	.18
P300 latency	.34	1.0	**.69†**	−.22
RT	−.39	**.69†**	1.0	**−.56†**
% correct	.18	−.22	**−.56†**	1.0
Group (TBI or Control)	**−.51***	**.48***	**.47***	**−.43***

*$P = 0.05$, †$P = 0.01$ (two-tailed).

slowing effect of severe TBI on complex tasks compared with simple tasks.

It has been observed that more complex tasks tend to generate P300s of lower amplitude (Polich, 1987; Segalowitz et al., 2001; Segalowitz et al., 1997).

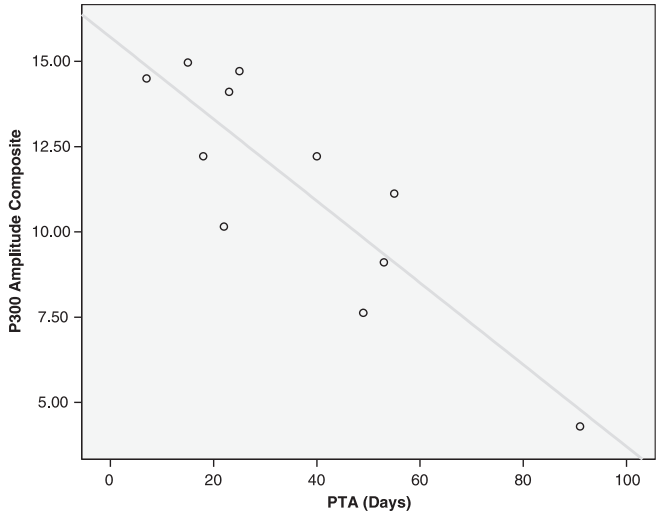

FIGURE 3. Scatter plot of correlations between length of posttraumatic amnesia and the P300 amplitude composite measure (Rho = −.75, p < .01, two-tailed).

Considering the two complex tasks we used, AWC did not generate a distinct P300 response, but VFA did. Examination of the waveforms show that the trend for P300 latency prolongation in VFA was similar to that of VC and AT, while that of AWC was different. Like the AT and VC tasks, the VFA task required classification at a sensory level based on just a few perceptual characteristics. The AWC task, on the other hand, was qualitatively different in that it required semantic processing of many different words, i.e., classification of the words according to their meaning. Since VFA was effective in differentiating the groups using both RT and P300 amplitude, we would pick it from among the four psychophysiologic tasks used in this study as the best single task choice for differentiating a group of TBI patients from control subjects.

P300 latency is a measure of the speed with which a subject recognizes that a given stimulus is a member of the rare category. It is generally shorter than RT because it does not include the time required to execute a motor response. In the present study, using composite scores, we found a significant relationship between RT and P300 latency. Previous investigators, using measures derived from single tasks, have reported inconsistent findings in this regard (Lew et al., 2002; McCarthy and Donchin, 1981; Novitski et al., 2004; Ohnuma et al., 1994; Segalowitz et al., 1997). The correlation between the P300 latency and RT in TBI deserves further study, especially in combination with motor related potentials.

ERP and RT Characteristics of TBI

P300 amplitude is thought to be an indicator of the extent to which cortical attentional resources have been utilized in the stimulus recognition task. Shorter P300 latencies and larger amplitudes are associated with better cognitive performance (Lew et al., 2005a). ERP abnormalities have also been associated with both severity of neuropsychological deficits (Viggiano, 1996) and poor functional outcomes

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(Lew et al., 2003) after brain injury. Solbakk et al. (2000) have hypothesized that impaired information processing speed is the cause of most of the attentional problems that follow TBI. The relationship found in the present study between composite RT and P300 latency scores seems to support this hypothesis.

When composite scores were created by obtaining an average across all four tasks, we found that, compared with control subjects, the TBI patients tended to have four characteristics: lower P300 amplitudes, longer ERP latencies, longer RTs and lower accuracy (Table 2). These psychophysiological findings are consistent with clinical observations that TBI patients tend to have trouble allocating attentional resources to cognitive tasks, are slower in generating motor responses and are more prone to error.

Severity of Injury

Both lower P300 amplitudes and longer P300 latencies have been associated with TBI severity (Lavoie et al., 2004; Lew et al., 2006; Olbrich et al., 1986). Prolonged RT has been associated with impaired attention and slower processing speed (Potter et al., 2001; Segalowitz et al., 1997). It may be the diffuse axonal loss common to severe TBI that underlies reported changes in both ERP (Cardenas et al., 1996; Clark et al., 1992; Hogan et al., 2006; Mathias et al., 2004a; Mathias et al., 2004b; van Zomeren and Deelman, 1978) and RT (Mathias et al., 2004a; Mathias et al., 2004b) after TBI. When we used composite scores, we found a remarkably high correlation between duration of PTA and P300 amplitude in these severely injured patients, but no significant relationship between PTA and speed of processing (RT or P300 latency). Our findings, although in contrast with that of Clark et al. (1992), provide further validation of P300 amplitude as an index of TBI severity. The size of the correlation between duration of PTA and P300 amplitude found in this small group of patients is surprisingly high (Rho = −0.75) and it should be replicated before being accepted as a generalizable finding.

We expected that the strength of the relationship between PTA and the composite P300 amplitude score would be contributed mostly by the complex tasks (AWC and VFA) rather than the simple tasks (AT and VC), however, the opposite was the case. Length of PTA was significantly related to AT and VC P300 amplitudes but not the amplitudes for the AWC and VFA tasks. Thus, if P300 amplitude is to be used as an index of injury severity, simple tasks like AT and VC may be best for generating the ERP waveforms.

Since length of PTA is one of the best predictors of future independent functioning (Brown et al., 2005) the relationship between PTA and P300 amplitude, latency and RT deserves further investigation. We also intend to investigate the predictive validity of ERP and RT measures, with regard to various aspects of functional outcome.

CONCLUSIONS

ERP measures were more effective in identifying TBI when simpler stimuli were used (AT and VC). This reflected the production of clearly defined ERP waveforms in response to simple stimuli, but more dispersed waveforms for complex stimuli, even in normal control subjects. On the other hand, RT measures were more effective in identifying TBI when stimuli of greater complexity and ecological relevance were used (AWC and VFA). RTs on the complex tasks may have been longer in the TBI group because interpreting the meaning of AWC and VFA required higher-order cognitive processes that are more impaired in TBI subjects than is simple perceptual discrimination. We also found a remarkably high correlation between duration of PTA and average P300 amplitude, adding to the validation of both as indices of TBI severity. These data highlight how results may be influenced by the nature of the task and help guide researchers and clinicians in choosing test stimuli when using ERP and RT paradigms to detect brain injury.

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Diffusion tensor imaging of cingulum fibers in mild cognitive impairment and Alzheimer disease

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Abstract—*Background:* Neuroimaging in mild cognitive impairment (MCI) and Alzheimer disease (AD) generally shows medial temporal lobe atrophy and diminished glucose metabolism and cerebral blood flow in the posterior cingulate gyrus. However, it is unclear whether these abnormalities also impact the cingulum fibers, which connect the medial temporal lobe and the posterior cingulate regions. *Objective:* To use diffusion tensor imaging (DTI), by measuring fractional anisotropy (FA), to test 1) if MCI and AD are associated with DTI abnormalities in the parahippocampal and posterior cingulate regions of the cingulum fibers; 2) if white matter abnormalities extend to the neocortical fiber connections in the corpus callosum (CC); 3) if DTI improves accuracy to separate AD and MCI from healthy aging vs structural MRI. *Methods:* DTI and structural MRI were preformed on 17 patients with AD, 17 with MCI, and 18 cognitively normal (CN) subjects. *Results:* FA of the cingulum fibers was significantly reduced in MCI, and even more in AD. FA was also significantly reduced in the splenium of the CC in AD, but not in MCI. Adding DTI to hippocampal volume significantly improved the accuracy to separate MCI and AD from CN. *Conclusion:* Assessment of the cingulum fibers using diffusion tensor imaging may aid early diagnosis of Alzheimer disease.

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Structural MRI studies found prominent volume losses in the entorhinal cortex and hippocampus in mild cognitive impairment (MCI)[1-3] and Alzheimer disease (AD)[4-6] while PET and SPECT studies found functional reductions primarily in the posterior cingulate.[7-12] The regional dissociation between structural and functional abnormalities may be due to reduced neuronal traffic between medial temporal lobe and posterior cingulate. Thus the cingulum fibers which connect the medial temporal lobe and the posterior cingulate[13,14] may be involved in early AD. In late AD, as the disease spreads to the cortex,[15] neocortical connections, such as corpus callosum (CC), may also be afflicted.

Diffusion tensor imaging (DTI) is used to detect degradation of white matter fiber bundles,[16-18] by measuring fractional anisotropy (FA) and mean diffusivity (D).[19] Several DTI studies of AD found abnormal FA and D in posterior cingulate,[20-22] temporal, parietal lobes,[23,24] and CC.[22,25-27] However, the regional patterns of abnormal DTI values were not consistent. Moreover, previous DTI assessments were limited to the posterior cingulate, while fiber extensions to medial temporal lobe were not evaluated. Furthermore, the diagnostic utility of DTI for MCI and AD as compared to conventional hippocampal volumetry has not been determined.

The main goals of this study were 1) to test if abnormal DTI values along the cingulum fibers are associated with MCI and AD pathology; and 2) to test if DTI measures improve the accuracy to separate MCI and AD from healthy aging over hippocampal volume loss.

Methods. *Population.* Seventeen subjects diagnosed with MCI (age 73.1 ± 7.4 years; 9 men, 8 women), 17 patients

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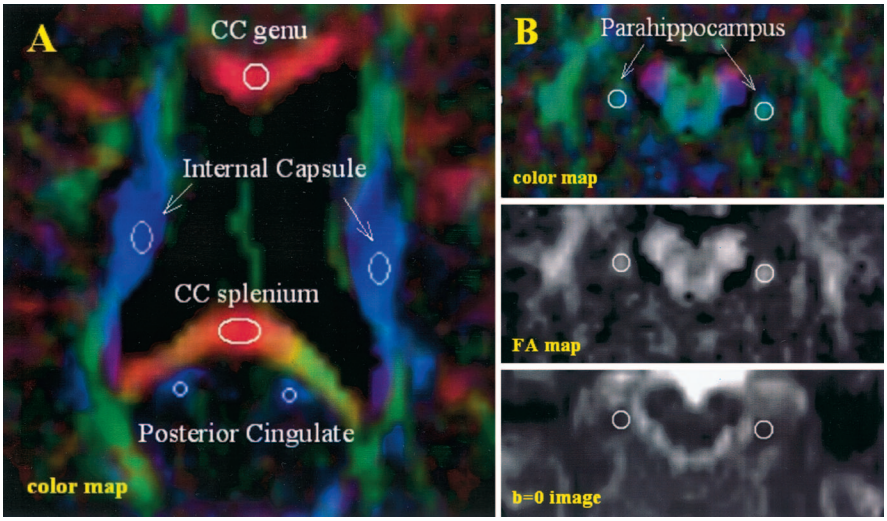

Figure 1. Illustration of the region of interest (ROI) selection: (A) ROIs at bilateral posterior cingulate, internal capsule, and corpus callosum (genu and splenium); (B) the ROIs at the parahippocampal region, and same regions overplayed on the FA and b = 0 images.

diagnosed with AD (age 77.1 ± 8.8 years; 8 men, 9 women), and 18 cognitive normal (CN) subjects (71.6 ± 9.2 years; 10 men, 8 women) were included in this observational study. Patients with AD and MCI were referred from the University of California at San Francisco (UCSF) and Davis (UCD) AD treatment centers, and CN subjects were recruited from populations, between July 2002 and April 2005. All participants underwent a series of neurologic tests and a battery of neuropsychological assessments, which included the Mini-Mental State Examination (MMSE)[28] and the Clinical Dementia Rating Scale (CDR)[29] at UCSF. MCI was determined according to either the Petersen criteria[30] or the criteria established by the AD Cooperative Study (ADCS).[31] Three of the MCI subjects were diagnosed with amnesic MCI, according to Petersen criteria, while the remaining MCI subjects presented a broader range of cognitive impairments. All patients with AD fulfilled the National Institute of Neurologic and Communicative Disorders and Stoke and AD and Related Disorders Association (NINCDS-ADRDA) criteria[32] for probable AD. The CN subjects had no history of a psychiatric or neuropsychological disease, major heart disease, diabetes, cardiovascular disease, epilepsy, or head trauma, and furthermore, scored within the normal range on all cognitive tests. A neuroradiologist reviewed the MR images from each subject to confirm absence of major neuropathologies, such as tumors and infarctions. Furthermore, subjects with extensive white matter lesions (WML) involving fibers of interest were not included in this study. In general, however, subjects with WML not extending into the regions of interest were included in this study. An experienced radiologist reviewed all T1, T2-weighted, and proton density images to determine severity of WML. In addition, the severity of WML was classified as mild (score 1), moderate (score 2), or severe (score 3), according to Scheltens' rating scale.[33] All subjects or their legal guardians gave written informed consent before participation in the study, which was approved by the committees of Human Research at UCSF and the VA Medical Center.

MRI acquisition. All examinations were preformed on a 1.5 Tesla MR system (Siemens Vision System, Germany), using a standard head coil. Structural MRI included volumetric T1-weighted magnetization-prepared rapid acquisition gradient-echo (MPRAGE) images (repetition time [TR]/echo time [TE]/inversion time [TI] = 10/7/300 msec, flip angle = 15°, 1 × 1 × 1.4 mm^3 resolution) for hippocampal tracing and tissue segmentation and multi-slice proton density and T2-weighted images based on a dual-echo sequence (TR/TE1/TE2 = 5000/20/80 msec, 1.25 × 1 mm^2 in-plane resolution, 3 mm slice thickness, without gap between slices) for measuring total intracranial volume and the extent of white matter hyperintensity. DTI was performed using an inversion-prepared double refocused single-shot echoplanar imaging (EPI) sequence[34] (TR/TE/TI = 6000/100/2000 msec; 2.34 × 2.34 in-plane resolution, 19 contiguous slices, each 5 mm thick), with bipolar diffusion sensitizing gradients of b = 1000 s/mm^2 applied along six directions. Additional spin-echo EPI scans without diffusion gradients (b = 0 image) were also acquired for normalizing diffusion measurements. Inversion recovery reduced contributions from CSF to the diffusion signal and double-refocusing RF pulses with bipolar gradients reduced geometric distortions in DTI due to eddy currents.

Hippocampal volumetry. Hippocampal boundaries were traced semi-automatically on MPRAGE images using a high dimensional brain-warping algorithm (Medtronic Surgical Navigation Technologies, Louisville, CO). We previously validated this method in comparison to manual tracing hippocampus in patients with AD and healthy subjects.[35] Manual and semi-automated volume measurements of the hippocampus generally correlated better than 90%. Furthermore, hippocampal boundaries from semi-automated tracing were visually reviewed scan by scan and manually corrected if misregistrations occurred, thus further diminishing differences between manual and automated measurements. Hippocampal volumes were furthermore normalized to total intracranial volumes (TIV) to account for variations of head size.

DTI data postprocessing. The DTI images were processed offline. DTIstudioV2 software[36] (Johns Hopkins University, Baltimore, MD) was used to create FA, D, and color-coded directionality maps of diffusion, that were overlaid on each other. The color-coded directional maps (red: left-to-right direction, green: anterior-to-posterior direction, blue: superior-to-interior direction) provided easy visualization of the white matter fiber tracts. Elliptical regions of interest (ROI) were drawn based on the identification of white matter tracts on the color-coded maps.[21,25] A total of four pairs of ROIs were placed on axial slices to select the following white matter regions (figure 1): 1) bilateral parahippocampal regions on the medial temporal portion of the cingulum fibers at a slice level where the full view of the hippocampal formation could be identified; 2) bilateral posterior cingulate regions, at the middle level of the dorsal curve of the cingulum fibers; 3) genu and splenium ROIs at the center of anterior and posterior CC; 4) for reference, bilateral regions at the posterior limb of internal capsule, where sensorimotor fiber converge and no degradations related to MCI or AD are expected. FA and D values within each ROI were averaged. ROIs were placed to maximally encompass each white matter fiber tract, and had generally an in-plane size of 4 × 4 mm^2 to 6 × 9 mm^2, with 5 mm slice thickness. The ROI sizes were kept fixed for all subjects. To account for potential variation of fiber thickness with head size, TIV was included as a covariate in the group analysis of DTI data. An experienced radiologist (Y.Z.), blinded to subject information including diagnosis, performed the ROI drawings. To determine reliability of the ROI measurements, the same rater repeated ROI drawings on 12 randomly selected subjects, blinded to the previous readings. Reliability, expressed as an intraclass correlation coefficient, was 0.96 for the DTI measurements.

Statistics. Variations of FA and D of each ROI were analyzed separately as a linear function of diagnosis with adjustments for age, sex, and TIV. A similar linear model was used to analyze

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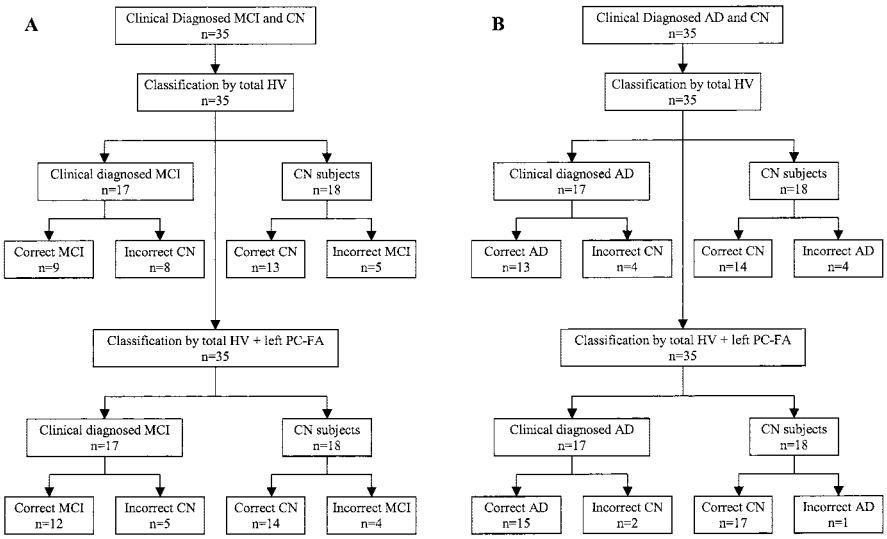

Figure 2. Classification of mild cognitive impairment and cognitively normal (CN) (A), Alzheimer disease and CN (B) by using total hippocampal volume (total HV) alone vs using total HV + left posterior cingulate fractional anisotropy (FA) (left PC-FA).

variations of hippocampal volumes. To determine if diagnosis and age added explanatory power and were therefore needed in the models, three nested models were fitted by maximum likelihood: the first (base) model included only the covariates, while diagnosis and a diagnosis × age interaction were sequentially added in the second and third model, respectively. The resulting fits were compared sequentially via *F*-tests to determine if diagnosis or diagnosis × age added explanatory power ($p < 0.05$) to the base model. To estimate the magnitude of group effects, effect sizes were calculated according to the following:

$$Effect\ \ Size = \frac{Mean_1 - Mean_2}{\sqrt{\frac{(n_1 - 1)Std_1^2 + (n_2 - 1)Std_2^2}{(n_1 - 1) + (n_2 - 1)}}}$$

where $Mean_{1/2}$ and $Std_{1/2}$ represent mean and SD, respectively, of measures in Groups 1 and 2, and $n_{1/2}$ are the number of subjects in Group 1 and 2. The powers of DTI and hippocampal volume measures to correctly classify CN, MCI, and AD were estimated based on logistic regressions and sensitivity and specificity of the classifications were expressed in terms of a receiver operator characteristics analysis as area under the curve (AUC). The logistic regressions were further adapted to a random leave-one-out procedure for cross-validation of the classifications. Finally, AUCs from cross-validations were compared using Wilcoxon signed rank tests. The statistical computations were performed using Splus 6.3 (Insightful Inc., Seattle, WA). The significance level was $\alpha < 0.05$ in all tests.

Results. All the subjects had satisfactory MRI quality and their data were included in the statistical analyses (figure 2). Demographics and clinical information of the subjects are summarized in table 1. There was no difference between the groups in age ($p = 0.14$, ANOVA), sex ($\chi^2 = 0.08$, $p > 0.7$), or years of education ($p = 0.25$, ANOVA). Furthermore, WML severity, which is also listed in table 1, and broken down into mild:moderate:severe, was similar between the groups ($p = 0.82$). As expected, patients with AD had markedly lower MMSE scores than

Table 1 *Demographics*

	CN	MCI	AD
Number	18	17	17
Age, y, mean ± SD	71.6 ± 9.2	73.1 ± 7.4	77.1 ± 8.8
M:F	10:8	9:8	8:9
Education, y, mean ± SD	15.5 ± 2.7	15.8 ± 2.6	14.2 ± 3.6
WML, mild:moderate:severe	13:0:5	9:5:3	11:4:2
MMSE, mean ± SD	29.5 ± 0.8	27.9 ± 2.0	22.1 ± 4.0
CDR, mean ± SD	0.0 ± 0.0	0.5 ± 0.0	1.0 ± 0.4

CN = cognitive normal; MCI = mild cognitive impairment; AD = Alzheimer disease; WML = white matter lesions; MMSE = Mini-Mental State Examination; CDR = Clinical Dementia Rating Scale.

Table 2 *Group effects of diagnosis on fractional anisotropy*

Dependent variable	Coefficients value	SE	*p* Value	Effect size
AD vs CN				
Left PH	−0.27	0.08	0.002	−1.14
Right PH	−0.18	0.09	0.04	−0.72
Left PC	−0.42	0.08	<0.0001	−1.76
Right PC	−0.28	0.07	0.003	−1.36
sCC	−0.37	0.11	0.003	−1.22
gCC	−0.11	0.12	0.39	−0.59
MCI vs CN				
Left PH	−0.24	0.09	0.01	−0.96
Right PH	−0.18	0.07	0.02	−0.76
Left PC	−0.23	0.07	0.003	−1.07
Right PC	−0.04	0.07	0.59	−0.14
sCC	−0.12	0.07	0.11	−0.5
gCC	−0.07	0.13	0.6	−0.26
AD vs MCI				
Left PH	−0.03	0.09	0.69	−0.1
Right PH	0.03	0.09	0.79	−0.06
Left PC	−0.15	0.09	0.12	−0.6
Right PC	−0.24	0.08	0.004	−1.09
sCC	−0.29	0.11	0.01	−0.89
gCC	−0.03	0.14	0.81	−0.29

AD = Alzheimer disease; CN = cognitive normal; PH = parahippocampus; PC = posterior cingulate; sCC = splenium and gCC = genu of the corpus callosum; MCI = mild cognitive impairment.

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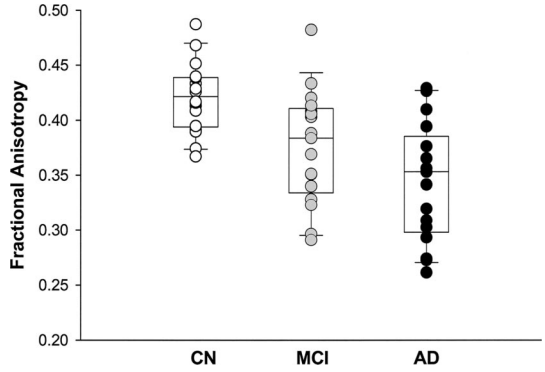

Figure 3. Scatter and box plots of fractional anisotropy in the left posterior cingulate in cognitive normal (○, mean ± SD = 0.42 ± 0.03), mild cognitive impairment (◍ , 0.38 ± 0.05), and Alzheimer disease (●, 0.34 ± 0.05).

CN ($p < 0.0001$) or MCI ($p < 0.0001$), and further, MCI subjects had lower MMSE scores than CN ($p = 0.02$). CDR scores for each group are listed as well.

Fractional anisotropy. Results from modeling FA as a function of diagnosis are summarized in table 2, separately for each ROI region. Compared to CN, patients with AD had lower FA values bilaterally in both parahippocampal (left $p = 0.002$; right $p = 0.04$) and posterior cingulate regions (left $p < 0.0001$; right $p = 0.003$). Moreover, patients with AD had markedly lower FA values in the splenium ($p = 0.003$) than CN subjects, while the genu was spared ($p = 0.39$) in AD. Similar to AD, patients with MCI—when compared to CN—had lower FA values bilaterally in parahippocampal regions (left $p = 0.01$; right $p = 0.02$), and in the left posterior cingulate ($p = 0.003$). However, MCI showed no change in the splenium ($p = 0.11$) and the genu ($p = 0.6$). Comparing AD to MCI showed that the two groups had similar FA reductions in parahippocampal regions (left $p = 0.69$; right $p = 0.79$) and in the left posterior cingulate ($p = 0.12$), while patients with MCI had less FA reductions than AD in the right posterior cingulate ($p = 0.004$) and the splenium ($p = 0.01$). Finally, CN, MCI, and AD had similar FA values in the internal capsule ($p > 0.05$), as expected. FA reductions in the left posterior cingulate yielded the largest effect size between CN subjects and MCI (effect size = –1.07) or AD (effect size = –1.76). The scatter plot in figure 3 depicts the distribution of FA values of the left posterior cingulate, separately for CN, MCI, and AD subjects. Notably, FA differences between the groups remained significant after accounting for age. In addition, adding years of education into the model did not alter the significance of FA differences between the groups.

Diffusivity. Results for D as a function of diagnosis are summarized in table 3. Compared to CN subjects, patients with AD had increased D values bilaterally in parahippocampal (left $p = 0.03$; right $p = 0.002$) and posterior cingulate regions (left $p = 0.01$; right $p = 0.01$), while patients with MCI had slightly increased D values only in left posterior cingulate regions ($p = 0.04$). Compared to MCI, patients with AD had increased D values in the right posterior cingulate ($p = 0.01$). Similar to FA, differences in D between groups remained significant after accounting for age and years of education. Furthermore, no differences

Table 3 *Group effects of diagnosis on diffusivity*

Dependent variable	Coefficients value	SE	*p* Value	Effect size
AD vs CN				
Left PH	0.22	0.09	0.03	0.78
Right PH	0.39	0.12	0.002	1.25
Left PC	0.24	0.09	0.01	0.95
Right PC	0.24	0.09	0.01	1.04
sCC	0.18	0.17	0.31	0.44
gCC	0.11	0.16	0.5	0.45
MCI vs CN				
Left PH	0.1	0.09	0.28	0.4
Right PH	0.17	0.09	0.06	0.73
Left PC	0.16	0.08	0.04	0.82
Right PC	−0.04	0.07	0.59	−0.08
sCC	−0.07	0.12	0.53	−0.15
gCC	−0.21	0.13	0.13	−0.35
AD vs MCI				
Left PH	0.1	0.1	0.34	0.41
Right PH	0.21	0.12	0.1	0.58
Left PC	0.06	0.1	0.55	0.21
Right PC	0.23	0.09	0.01	1.06
sCC	0.21	0.17	0.23	0.55
gCC	0.27	0.16	0.1	0.77

AD = Alzheimer disease; CN = cognitive normal; PH = parahippocampus; PC = posterior cingulate; sCC = splenium and gCC = genu of the corpus callosum; MCI = mild cognitive impairment.

in D ($p > 0.05$) were found across groups in bilateral internal capsule and the genu.

Hippocampal volume. The scatter plot in figure 4 depicts the distributions of left hippocampal volumes, separately in CN, MCI, and AD. Comparing MCI to CN, hippocampal volumes were smaller only on the right side ($p = 0.03$) but not on the left side ($p = 0.16$), while age made contributions to the volume differences between MCI and CN on both sides (left $p = 0.001$, right $p = 0.002$). For

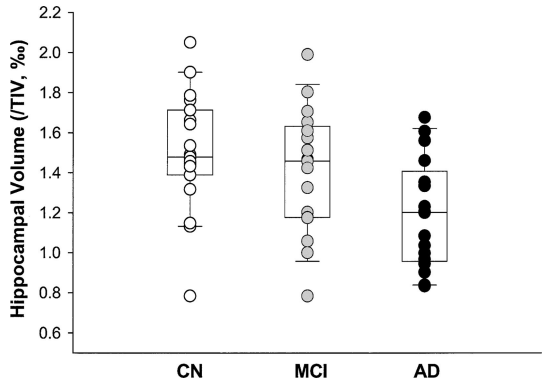

Figure 4. Scatter and box plots of the left hippocampal volume (in thousandth of total intracranial volume) in cognitive normal (○, mean ± SD = 1.5 ± 0.2), mild cognitive impairment (◍ , 1.4 ± 0.3), and Alzheimer disease (●, 1.2 ± 0.3).

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Table 4 *Group effects on hippocampal volumes, accounting for age*

Dependent variable	Independent variables	Coefficient	SE	*p* Value	Effect size
MCI vs CN					
Left HV	Age	−257	70	0.001	N/A
	Dx	−774	541	0.16	0.5
Right HV	Age	−241	70	0.002	N/A
	Dx	−1,225	536	0.03	0.76
AD vs CN					
Left HV	Age	−110	65	0.1	N/A
	Dx	−2,226	588	<0.001	−1.38
Right HV	Age	−87	72	0.23	N/A
	Dx	−2,094	651	0.003	−1.35
AD vs MCI					
Left HV	Age	−101	93	0.29	N/A
	Dx	−1,506	695	0.04	−0.74
Right HV	Age	−125	112	0.27	N/A
	Dx	−748	837	0.38	−0.45

MCI = mild cognitive impairment; CN = cognitive normal; AD = Alzheimer disease; HV = hippocampal volume; Dx = diagnosis; N/A = not available.

patients with AD, however, smaller hippocampal volumes compared to CN were entirely explained by diagnosis (left $p < 0.001$; right $p = 0.003$) while age made no contribution ($p \geq 0.1$) (see also table 4). Finally, compared to MCI, patients with AD had smaller hippocampal volumes on the left side ($p = 0.04$), but not on the right side ($p = 0.38$), while age made no significant contributions. Similar to DTI measures, years of education did not make any significant contribution to hippocampal volume differences across groups.

Classifications. Since the FA reduction in the left posterior cingulate yielded the largest effect size between CN and MCI or AD, this measure was used to compare the powers of DTI and hippocampal volumes to correctly classify MCI, AD, and CN subjects. Results from a logistic regression analysis and a receiver operator characteristics analysis are summarized in table 5.

Hippocampal volume alone could not reliably separate MCI from CN subjects ($p = 0.1$), yielding no more than 63 ± 3% (mean ± SD) accuracy, 55 ± 8% sensitivity, and 70 ± 5% specificity with an area under the receiver operator characteristics curve (AUC) of 0.67 ± 0.02. The addition of FA improved classification ($p = 0.02$), increasing accuracy to 74 ± 2%, sensitivity to 69 ± 3%, and specificity to 78 ± 2% with an increased ($p < 0.001$) AUC of 0.78 ± 0.02. Hippocampal volume alone, however, separated AD from CN subjects reliably ($p = 0.007$), yielding 78 ± 1% accuracy, 75 ± 3% sensitivity, and 81 ± 3% specificity with an AUC of 0.85 ± 0.01. The addition of FA improved the classification further ($p < 0.001$), resulting in 91 ± 1% accuracy and 88 ± 1% for sensitivity and 94 ± 2% for specificity. The AUC also increased ($p < 0.001$), reaching a value of 0.98 ± 0.002, which indicates almost complete separation of the groups.

Discussion. We found that 1) MCI is associated with FA reductions particularly in the cingulum fibers, predominantly in the left posterior cingulate. In AD, FA is further reduced in the cingulum fibers and FA reductions extend to the splenium, consistent with previous reports. 2) FA reductions in the posterior cingulate improved the classification of MCI and AD from cognitively normal elderly compared to the classifications using hippocampal volume loss alone.

To explain the dissociation between PET/SPECT and MRI findings in MCI and AD, we hypothesized that the integrity of the cingulum fibers, which connect the posterior cingulate gyrus and the hippocampus,[13,14] may be compromised in the early stage of AD. Although the exact nature of the fiber disintegration is unclear, FA reduction, coupled with increased diffusivity, is thought to reflect axonal loss or demyelination.[37,38] Our finding of prominent FA reduction in posterior cingulate regions in MCI is consistent with previous reports,[21,22] and the substantial FA reductions in parahippocampal regions in MCI further establishes the vulnerability of the cingulum fibers in the early stage of AD. Taken together, these findings support our hypothesis that the entire connections between hippocampus and posterior cingulate are affected in the early stage of AD. Furthermore, we found that the FA values of the cingulum fibers in MCI are significantly lower

Table 5 *Group classifications by hippocampal volume and fractional anisotropy of posterior cingulate*

Classification	Factors	Sensitivity (%)	Specificity (%)	Accuracy (%)	*p* Value*	AUC	*p* Value†
MCI vs CN	Total HV	55 ± 8	70 ± 5	63 ± 3	0.1	0.67 ± 0.02	
	TotalHV+LeftPC-FA	69 ± 3	78 ± 2	74 ± 2	0.02	0.78 ± 0.02	<0.001
AD vs CN	Total HV	75 ± 3	81 ± 3	78 ± 1	0.007	0.85 ± 0.01	
	TotalHV+LeftPC-FA	88 ± 1	94 ± 2	91 ± 1	<0.001	0.98 ± 0.002	<0.001

* From logistic regression.
† From a Wilcoxon signed rank test, comparing two areas under the curves (AUC).

MCI = mild cognitive impairment; CN = cognitive normal; HV = hippocampal volume; left PC-FA = FA of the left posterior cingulate; AD = Alzheimer disease.

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than in controls but very close to the FA values in AD group, whereas the hippocampal volumes in MCI are intermediate between AD and control and in fact not significantly different from control. If we conceptualize MCI as representing early AD, our findings suggest that DTI measures of FA in the cingulum fibers may be a more sensitive marker of early AD pathology than hippocampal volume. Therefore, if these findings are replicated, DTI measures of FA in the cingulum fibers may be used as an early predictor of future cognitive decline and development of AD.

Consistent with previous DTI studies in AD, we also found FA reductions in posterior aspects but not anterior aspects of the CC.[22,25] Anatomically, the posterior CC connects the temporal and parietal cortices, while the anterior CC connects frontal cortices.[39] Thus, our finding supports the suggestion that the temporoparietal connections are more impacted than frontal connections by AD.

We also found that FA reductions of cingulum fibers, especially in the left posterior cingulate region, improved the classification between MCI and elderly controls over using hippocampal volume alone. Hippocampal volume could not accomplish reliable classification between MCI and CN, independent of whether adjustments for age effects were made. Moreover, FA of the cingulum fibers significantly improved the separation of AD from control subjects, achieving almost complete separation between the groups, compared to an already high classification power using hippocampal volume alone. Taken together, the results suggest that measuring the FA value in the cingulum fibers could be a supplementary marker to the hippocampal volume in diagnoses of MCI and AD. Prospective studies are underway to determine if DTI predicts cognitive decline and conversion to AD.

Our study has several limitations. Since we did not follow patients with MCI longitudinally to determine conversion to AD, it remains to be seen whether the DTI findings in MCI were related to AD or indicates perhaps other types of dementia. Another limitation is that DTI observations in AD and MCI could be related to other factors than the AD pathology, such as WML due to cerebral vascular disease, although the groups were matched by WML severity and the subjects with WML involving the ROI were not included in this study. The relation between fiber integrity and cerebral vascular disease needs to be further explored. Lastly, it cannot be excluded that inclusion of CSF, as a consequence of brain atrophy, may have biased DTI results in AD and MCI toward lower FA and higher D values, despite our attempts to suppress the CSF signal by using an inversion-recovery DTI sequence. Therefore, our DTI results may not entirely be decoupled from underlying brain atrophy and more work needs to be done to develop techniques that further reduce or eliminate this confound from DTI measurements.

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Neuro*Images*

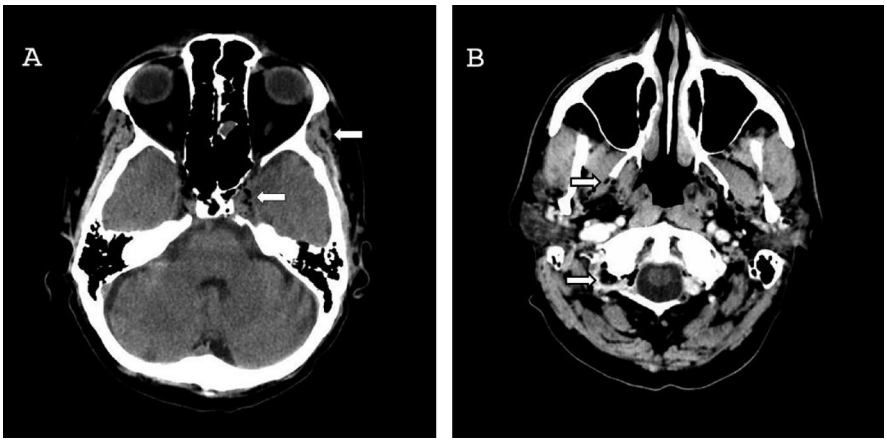

Figure. Extracranial venous air embolism: first arrow from the top in A and white arrows in B. Cavernous sinus gas embolism: second arrow from the top in A.

Headache and cerebral venous air embolism

Stephan A. Botez, MD, Lausanne, Switzerland

A 47-year-old man with migraine without aura presented with bilateral throbbing sudden onset headache resistant to usual triptan medication. He had dental implants revised 3 days earlier. General and neurologic examinations were normal. Brain CT showed cerebral venous gas embolism (figure). The headache resolved with 100% oxygen therapy in flat supine position. Brain CT was normal 48 hours later. Cerebral venous air embolism was probably secondary to an aberrant communication between air and venous system during dental surgery resulting in retrograde cerebral venous air embolism,[1] an underestimated diagnosis because it is often clinically silent.[2]

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Disclosure: The author reports no conflicts of interest.

Address correspondence and reprint requests to Dr. Botez, Neurology Department, Centre Hospitalier Universitaire Vaudois (CHUV), Rue du Bugnon 46, 1011-Lausanne, Switzerland; e-mail: Stephan.Botez@chuv.ch

1. Schlimp CJ, Loimer T, Rieger M, Lederer W, Schmidts MB. The potential of venous air embolism ascending retrograde to the brain. J Forensic Sci 2005;50:906–909.
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Diffusion tensor imaging of cingulum fibers in mild cognitive impairment and Alzheimer disease

Y. Zhang, N. Schuff, G. -H. Jahng, W. Bayne, S. Mori, L. Schad, S. Mueller, A. -T. Du, J. H. Kramer, K. Yaffe, H. Chui, W. J. Jagust, B. L. Miller and M. W. Weiner

Neurology 2007;68;13-19
DOI: 10.1212/01.wnl.0000250326.77323.01

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Palo Alto Institute for Research & Education

P.O. Box V-38 • Palo Alto, CA 94304-0038

October 31, 2007

Department of Defense
Congressionally Directed Medical Research Programs
Post-Traumatic Stress Disorder and Traumatic Brain Injury Research Program 2007
US Army Medical Research and Material Command (USAMRMC)

Re: **Award Mechanism:** TBI Advanced Technology – Therapeutic Development Award
Proposal Title: MEMTRAX – A Flexible Computer Game System for Assessing Cognitive and Brain Pathology Associated with TBI and for Augmenting and Monitoring Rehabilitation
Principal Investigator: J. Wesson Ashford, M.D., Ph.D.

Dear Review Committee:

On behalf of the VA Palo Alto Health Care System this is to certify that Dr. Ashford has adequate resources available to conduct the work outlined in the enclosed proposal. As described in detail in the application, adequate space, equipment and staffing are available and dedicated for his use.

It is our policy within this VA center to ensure that adequate research time is available to Principal Investigators conducting the studies proposed and approved for activation. The conduct of research is strongly emphasized and encouraged, and is backed up with active support in the form of appropriate resources.

VA Palo Alto Health Care System is uniquely situated to provide rich support to the investigators responding to the multiple Program Announcements for research that will ultimately assist the growing numbers of veterans affected by both TBI and PTSD syndromes. The National Center for PTSD, Clinical Laboratory and Education Division was established here in 1989. We have one of four full-service Polytrauma Centers in the VA system, have for more than a decade been a key site in DoD's Defense and Veterans Brain Injury Center, have a richly supported Rehabilitation Research and Development Center and a robust Mental Illness Research, Education and Clinical Center (MIRECC). Additionally funds to support a War Related Illness and Injury Study Center were recently provided to our facility. With such an abundance of interrelated centers and activities whose focus is research on neurologic and mental health issues, we are confident that grants awarded to our investigators will yield important and timely results that, hopefully, will translate quickly into effective care models.

Sincerely,

Fredric B. Kraemer, M.D.
Associate Chief of Staff, Research and Development
VA Palo Alto Health Care System

Intellectual and Material Property Plan:

As all VA approved research is subject to the rules and regulations outlined in VHA Handbook 1200.18 entitled "Intellectual Property", VA Palo Alto Health Care System (VAPAHCS) will adhere to the requirements of the Handbook when addressing intellectual property. The Palo Alto Institute for Research and Education (PAIRE), because of its status as a VA-affiliated nonprofit, must also follow the intellectual property rules and regulations outlined in VHA Handbook 1200.18. Additionally, VAPAHCS, PAIRE, and their collaborators will utilize a Cooperative Research and Development Agreement (CRADA) to address any background intellectual property and future developments as a result of this research project.

cognitivelabs.com

October 30, 2007

US Army Medical Research and Materiel Command (USAMRMC)
Office of the Congressionally Directed Medical Research Programs (CDMRP)
1077 Patchel Street
Fort Detrick, Maryland 21702-5024

Subject: Letter of Collaboration and Endorsement for Project Titled "MEMTRAX – A Flexible Computer Game System for Assessing Cognitive and Brain Pathology Associated with TBI and for Augmenting and Monitoring Rehabilitation" in response to DoD PTSD/TBI Research Program W81XWH-07-TBI-AT-TDA Solicitation, TBI Advanced Technology-Therapeutic Development Award, Principal Investigator - J.Wesson Ashford, MD, PhD

Dear Sir and/or Madam:

I am writing to confirm that I have agreed to serve as a collaborator for the above named project. I understand that this project is being submitted for scientific review to the Department of Defense Post-Traumatic Stress Disorder and Traumatic Brain Injury (PTSD/TBI) Research Program. I understand further that my role as Collaborator for this study will include the development, implementation and evaluation of computer-based cognitive assessment tools.

My background is primarily entrepreneurial; I have been involved in founding several software companies, developing large scale information systems in areas as diverse as supply chain management, digital media, and healthcare. Since 2004 I have been focused on developing tests and games to monitor and enhance cognitive performance that are effective and also highly scalable. Ever since the outbreak of the current conflict, I have been focused on supporting our soldiers in any way possible. This is natural for me since my father (retired) is a Viet Nam veteran (serving as C.O. of an LST on the Mekong) and was C.O. of a cruiser operating in the Persian Gulf at the time of the Iran-Iraq War, and later helped manage the logistics for Operation Desert Storm, including the USNS Mercy, which deployed to the Persian Gulf, as C.O., Military Sealift Command-Pacific. Numerous family members have served in the Army, Navy, and Air Force.

I believe that Dr. Ashford has identified an important treatment application that could result in an innovative intervention for returning OIF/OEF soldiers who are suffering from TBI, in terms of understanding the condition and potentially treating it through cognitive exercise. I look forward to dedicating the skills and expertise developed over the years to working with him on this important project.

Respectfully,

Michael Addicott

Michael Addicott, PhD
President
Cognitive Labs, Inc.

BOWLES-LANGLEY TECHNOLOGY, INC.

851 West Midway Avenue.
Alameda, CA 94501

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October 30, 2007

US Army Medical Research and Materiel Command (USAMRMC)
Office of the Congressionally Directed Medical Research Programs (CDMRP)
1077 Patchel Street
Fort Detrick, Maryland 21702-5024

Subject: Letter of Collaboration and Endorsement for Project Titled "MEMTRAX – A Flexible Computer Game System for Assessing Cognitive and Brain Pathology Associated with TBI and for Augmenting and Monitoring Rehabilitation" in response to DoD PTSD/TBI Research Program W81XWH-07-TBI-AT-TDA Solicitation, TBI Advanced Technology-Therapeutic Development Award, Principal Investigator - J.Wesson Ashford, MD, PhD

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Dr. Ashford has identified an important treatment application that could result in an innovative intervention for returning OIF/OEF soldiers who are suffering from TBI. I look forward to working with him on this project.

Respectfully,

Henry M. Bowles
President
Bowles-Langley Technology, Inc.

COLLEGE of SAN MATEO

1700 West Hillsdale Boulevard · San Mateo, California 94402
(650) 574-6161

October 30, 2007

US Army Medical Research and Materiel Command (USAMRMC)
Office of the Congressionally Directed Medical Research Programs (CDMRP)
1077 Patchel Street
Fort Detrick, Maryland 21702-5024

Subject: Letter of Collaboration and Endorsement for Project Titled "MEMTRAX – A Flexible Computer Game System for Assessing Cognitive and Brain Pathology Associated with TBI and for Augmenting and Monitoring Rehabilitation" in response to DoD PTSD/TBI Research Program W81XWH-07-TBI-AT-TDA Solicitation, TBI Advanced Technology-Therapeutic Development Award, Principal Investigator - J.Wesson Ashford, MD, PhD

Dear Sir and/or Madam:

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Dr. Ashford has identified an important treatment application that could result in an innovative intervention for returning OIF/OEF soldiers who are suffering from TBI. I look forward to working with him on this project.

Respectfully,

James Clifford, PhD
Professor and Chair
College of San Mateo
Department of Psychology

DEPARTMENT OF PSYCHIATRY AND BEHAVIORAL SCIENCES
655 First Street,
Macon, GA 31201
TEL: (478) 301-4033 FAX: (478) 301-5337

October 30, 2007

US Army Medical Research and Materiel Command (USAMRMC)
Office of the Congressionally Directed Medical Research Programs (CDMRP)
1077 Patchel Street
Fort Detrick, Maryland 21702-5024

Subject: Letter of Collaboration and Endorsement for Project Titled "MEMTRAX – A Flexible Computer Game System for Assessing Cognitive and Brain Pathology Associated with TBI and for Augmenting and Monitoring Rehabilitation" in response to DoD PTSD/TBI Research Program W81XWH-07-TBI-AT-TDA Solicitation, TBI Advanced Technology-Therapeutic Development Award, Principal Investigator - J. Wesson Ashford, MD, PhD

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Dr. Ashford has identified an important treatment application that could result in an innovative intervention for returning OIF/OEF soldiers who are suffering from TBI. I look forward to working with him on this project.

Respectfully,

Kenny Lee Coburn, MA, PhD
Director of Psychiatric Research,
Director of Brain Mapping Laboratory
Mercer University School of Medicine

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October 30, 2007

US Army Medical Research and Materiel Command (USAMRMC)
Office of the Congressionally Directed Medical Research Programs (CDMRP)
1077 Patchel Street
Fort Detrick, Maryland 21702-5024

Subject: Letter of Collaboration and Endorsement for Project Titled "MEMTRAX – A Flexible Computer Game System for Assessing Cognitive and Brain Pathology Associated with TBI and for Augmenting and Monitoring Rehabilitation" in response to DoD PTSD/TBI Research Program W81XWH-07-TBI-AT-TDA Solicitation, TBI Advanced Technology-Therapeutic Development Award, Principal Investigator - J.Wesson Ashford, MD, PhD

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Dr. Ashford has identified an important treatment application that could result in an innovative intervention for returning OIF/OEF soldiers who are suffering from TBI. I look forward to working with him on this project.

Respectfully,

Theodore D. Langley, Ph.D.

Dr Theodore D. Langley, Ph.D.
Senior Vice President
Bowles-Langley Technology, Inc.

NCIRE
NORTHERN CALIFORNIA INSTITUTE
FOR RESEARCH AND EDUCATION

November 1, 2007

J. Wesson Ashford, MD, PhD
Director, War Related Illness & Injury Study Center
VA Palo Alto Health Care System
3801 Miranda Avenue
Palo Alto, CA 94304-1290

Dear Dr. Ashford:

This institutional support letter certifies that the Principal Investigator, Michael W. Weiner, MD, Professor of Medicine, Radiology, Neurology and Psychiatry, and Co-Investigators Norbert Schuff, PhD, Susanne Mueller, MD, Andreas Ebel, PhD, and Wang Zhan, PhD, of the Northern California Institute for Research and Education (NCIRE), has been assigned the respective laboratory space, equipment, and common resources for the proposed research, as described in the subaward application. The PI and Co-I's will be relieved of academic or administrative responsibilities, to the extent of their effort commitments, as stated in the application, to pursue their research goals. NCIRE is a non-profit research institute affiliated via an MOU with the VA Medical Center, San Francisco.

Sincerely,

Robert Obana
Executive Director

4150 Clement Street, 151NC, San Francisco, CA 94121-1545
t 415 750 6954 f 415 750 9358 www.ncire.org

PositScience™
YOUR MIND, VITAL

November 7, 2007

J. Wesson Ashford, M.D., Ph.D.
Director of WRIIC
Veterans Administration of Palo Alto Health Care System
3801 Miranda Avenue
Palo Alto, CA 94304-1290

Dear Wes,

I am writing to express Posit Science Corporation's strong support for your grant "MEMTRAX – A Flexible Computer Game System for Assessing Cognitive and Brain Pathology Associated with TBI for Augmenting and Monitoring Rehabilitation." The need for validated interventions in TBI is tremendous, and the research in your proposal will clearly advance the field's basic and applied knowledge in this critical area.

Posit Science Corporation will supply the experimental training programs required by your grant free of charge and will provide ongoing technical support for the use and deployment of these training programs in your studies. In addition, we look forward to working with you closely on using the information from this study to design improved training programs for TBI patients going forward.

Best regards,

Henry W. Mahncke, Ph.D.

Vice President, Research & Outcomes

www.PositScience.com 225 Bush Street 7th Floor San Francisco California 94104 tel 415 394 3100 fax 415 986 2829

PRICEWATERHOUSECOOPERS

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November 8, 2007

US Army Medical Research and Materiel Command (USAMRMC)
Office of the Congressionally Directed Medical Research Programs (CDMRP)
1077 Patchel Street
Fort Detrick, Maryland 21702-5024

Subject: Letter of Agreement to serve as a Consultant and Endorsement for Project Titled "MEMTRAX – A Flexible Computer Game System for Assessing Cognitive and Brain Pathology Associated with TBI and for Augmenting and Monitoring Rehabilitation" in response to DoD PTSD/TBI Research Program W81XWH-07-TBI-AT-TDA Solicitation, TBI Advanced Technology-Therapeutic Development Award, Principal Investigator - J. Wesson Ashford, MD, PhD

Dear Sir and/or Madam:

I am writing to confirm that PricewaterhouseCoopers LLP (PwC) has agreed to serve as a consultant for the above named project. PwC understands that this project is being submitted for scientific review to the Department of Defense Post-Traumatic Stress Disorder and Traumatic Brain Injury (PTSD/TBI) Research Program. PwC understands further that our role for this study will include consultation regarding the collection, processing and analysis of cognitive data for subjects at the Palo Alto VA.

Dr. Ashford has identified an important treatment application that could result in an innovative intervention for returning OIF/OEF soldiers who are suffering from TBI. We look forward to working with him on this project.

Respectfully,

Sincerely,

Melissa S. Glynn

Melissa S. Glynn, Ph.D.
Principal
PricewaterhouseCoopers LLP

NCIRE
NORTHERN CALIFORNIA INSTITUTE FOR RESEARCH AND EDUCATION

November 1, 2007

J. Wesson Ashford, MD, PhD
Director, War Related Illness & Injury Study Center
VA Palo Alto Health Care System
3801 Miranda Avenue
Palo Alto, CA 94304

Dear Dr. Ashford,

I am happy to collaborate with you on your Department of Defense CDMRP grant submission entitled "MEMTRAX – A Flexible Computer Game System for Assessing Cognitive and Brain Pathology Associated with TBI and for Augmenting and Monitoring Rehabilitation" and to provide collaboration, consultation, and imaging services.

In particular, I will be working with you to determine how to maximize the information from MRI imaging to contribute to the success of your project by helping develop specific hypotheses, specifying imaging sequences, helping to schedule the subjects for imaging, performing the imaging, performing QC/QA of imaging data, analyzing the images, providing the results of image analysis to you and your group, discussing the results and interpretation, and preparing abstracts and papers of our findings.

Sincerely Yours,

Michael W. Weiner, M.D.
Director, Center for Imaging of Neurodegenerative Diseases
San Francisco Veterans Affairs Medical Center
Professor of Medicine, Radiology, Neurology and Psychiatry
University of California, San Francisco

MW/gs

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Patents and Permissions:

To address any existing patents owned by collaborators or permissions required to conduct this project, VAPAHCS will utilize a Cooperative Research and Development Agreement (CRADA).