Published in final edited form as:
Adv Exp Med Biol. 2006 ; 576: 241–363.

N-ACETYLASPARTATE AS A MARKER OF NEURONAL INJURY IN NEURODEGENERATIVE DISEASE

Norbert Schuff, **Dieter J. Meyerhoff**, **Susanne Mueller**, **Linda Chao**, **Diana Truran Sacrey**, **Kenneth Laxer**, and **Michael W. Weiner***
* *Magnetic Resonance Unit VA Medical Center, Department of Radiology, University of California, San Francisco, CA 94121 USA. Email: mweiner@itsa.ucsf.edu.*

1. NAA IN NORMAL AGING

Considerable evidence suggests that normal aging is associated with gradual impairment of memory functioning [1]. The medial temporal lobe, especially the hippocampus, plays a central role in declarative memory processing [2]. However, magnetic resonance imaging (MRI) studies have produced controversial results concerning the age-related hippocampal volume loss, which could be due in part to the non-specificity of volume shrinkage as an indicator for neuron loss. In contrast to volume, NAA is generally considered a marker for viable neurons, because NAA reaches detectable concentrations only in neuronal tissue but not in other brain tissues, including glial cells. Using proton magnetic resonance spectroscopic imaging (^{1}H MRSI) and MRI together, we studied hippocampal metabolites and volumes in 24 healthy adults from 36 to 85 years of age. Our goals were to test whether NAA levels vary in the hippocampus as a function of normal aging and 2) to determine the relationship between hippocampal NAA and volume changes. We found NAA/Cho ratios decreased by 24% ($r = -0.53$, $p = 0.01$) and NAA/Cr ratios decreased by 26% ($r = -0.61$, $p < 0.005$) over the age range studied, while Cho/Cr remained stable, implying diminished NAA levels. In the same population, hippocampal volume shrank by 20% ($r = -0.64$, $p < 0.05$). The relationships of these measures with aging are depicted in Figure 1. Since NAA is considered a marker of neurons, these results provide stronger support for neuron loss in the aging hippocampus than volume measurements by MRI alone.

Since contributions to the NAA signal may arise from both gray and white matter tissue, it is critical to differentiate between metabolite changes of gray and white matter, and other tissue types. One approach for differentiation is the use of linear regressions to predict the relationship between metabolite intensity changes and gray/white matter variations in MRSI voxels (4,8–12). However, most previous MRSI studies that used linear regression averaged metabolite concentrations over different lobes of the brain, ignoring regional variations. In addition, tissues other than gray and white matter were ignored or not determined, such as white matter lesions, which occur frequently in the aged brain. We developed an approach for obtaining metabolite concentrations of gray, white matter, as well as of white matter lesions in different lobes of the brain using linear regression. We applied the new technique to measure NAA concentrations in the frontal and the parietal lobe in 40 normal elderly subjects (56 to 89 years, mean age 74 ± 8, 22 female, 18 male). NAA was about 15% lower in cortical gray matter and 23% lower in white matter lesions when compared to normal white matter. Cr was 11% higher in cortical gray than in white matter, and also about 15% higher in the parietal cortex compared to the frontal cortex. Cho was 28% lower in cortical gray matter than in white matter. Furthermore, NAA and Cr changes correlated with age. The results suggest that in addition to the hippocampus, age-related neuronal changes can also occur in cortical regions, while white matter regions seem to be spared.

2. NAA IN DEMENTIA

2.1 Alzheimer's Disease

The first ^{1}H MRSI study on AD from this laboratory [8] showed abnormalities of metabolite ratios of NAA, and choline (Cho) and creatine (Cr) containing compounds in white and gray matter of the centrum semiovale. Decreased levels of NAA in white matter suggest diffuse axonal loss or damage. Decreased NAA in gray matter suggests loss of neurons, while increased Cho may result from membrane breakdown products. The second study from this laboratory [9] using similar ^{1}H MRSI methods extended the observation of metabolic abnormalities in the centrum semiovale to a larger population of AD patients and controls, and in addition, included a group of patients with subcortical ischemic vascular dementia (SIVD). While the findings from the AD and control groups were similar to the first study, different metabolite changes were noted in SIVD patients, supporting the possibility that ^{1}H MRSI may provide information to differentiate AD from SIVD. Finally, the third report from this laboratory [10] investigated the extent to which these metabolic differences between patients and controls were independent of variations in the tissue composition of the MRSI voxels (e.g. enclosed amounts of gray matter, white matter, and WM signal hyperintensities). This analysis was made possible by using information from MRI tissue segmentation coregistered with the ^{1}H MRSI data. Although the analysis revealed significant variations of the tissue composition in the regions of interest, these changes did not contribute significantly to the metabolite differences, indicating that reduced NAA/Cho and increased Cho/Cr in posterior mesial gray matter of AD were not simply an artifact of these structural variations. However, limitations of these previous studies were a relatively small number of subjects and an early MRI technology with relatively (compared to today's standards) thick slices (5 mm) and interslice gaps (0.5 mm), which compromised accuracy of tissue segmentation. Furthermore, acquisition of the ^{1}H MRSI data was performed at a relatively long spin-echo time (TE) of 272ms and further metabolite ratios rather than concentrations were reported. Subsequent MRSI studies from this lab tried to overcome these limitations.

We subsequently studied 28 AD patients and 22 healthy elderly using ^{1}H MRSI and MRI for image segmentation. ^{1}H MRSI data were aligned with MRI segmentation data to obtain volume-corrected metabolite concentrations. The results were: NAA levels were significantly reduced in frontal and posterior mesial cortex of AD, consistent with previous results from NAA ratios. Furthermore, the NAA reductions were independent from structural variations as measured by MRI, and in parietal mesial cortex correlated mildly with dementia severity. But NAA combined with MRI measures did not improve discrimination power for AD over that of MRI alone. While these results from fronto-parietal brain showed reduced NAA in AD is not an artifact of underlying structural variations, and thus may provide useful information in addition to MRI, these NAA reductions were of limited use for diagnosis of AD.

Since the hippocampus is thought to be earlier involved in AD pathology than cortical regions, we tested in another MRSI study the hypothesis that hippocampal NAA and volume used together provide greater discrimination between AD and normal elderly than does either measure alone. We used proton magnetic resonance spectroscopic imaging (^{1}H MRSI) and tissue segmented and volumetric MR images to measure atrophy corrected hippocampal NAA and volumes in 12 AD patients (mild to moderate severity) and 17 control subjects of comparable age. In AD, atrophy corrected NAA from the hippocampal region was reduced by 15.5% on the right and 16.2% on the left (both $p<0.003$), and hippocampal volumes were smaller by 20.1% ($p < 0.003$) on the right and 21.8% ($p < 0.001$) on the left when compared to control subjects. The NAA reductions and volume losses made independent contributions to the discrimination of AD from control subjects. When used separately, neither hippocampal NAA nor volume achieved to correctly classify AD patients better than 80%. When used together, however, the two measures correctly classified 90% of AD and 94% of control

subjects. The separation between AD and controls using NAA and volume of the hippocampus together is depicted in Figure 2. In conclusion, hippocampal NAA measured by ^{1}H MRSI combined with quantitative measurements of hippocampal atrophy by MRI may improve diagnosis of AD.

2.2. Subcortial Ischemic Vascular Dementia

The contribution of subcortical ischemic vascular disease (SIVD) to cognitive impairment and dementia is poorly understood. Disruption of subcortical-cortical connections by strategically located infarctions are considered one important mechanism for cognitive impairment in SIVD.[1] MRI studies from our group found that cognitive deficits in patients with SIVD were strongly correlated with brain atrophy, while subcortical infarctions made little contributions.[2, 3] Aside from greater numbers of ischemic lesions and more extensive white matter hyperintensities (WMH), other MRI studies comparing SIVD and AD have shown atrophy in the hippocampus and entorhinal cortex in both dementias, though less prominent in SIVD than in AD.[4, 5] Furthermore, some SIVD patients without AD pathology confirmed by autopsy had reduced hippocampal and cortical gray matter volumes.[2] Therefore, MRI has limited ability to differentiate between SIVD and AD. Although we found a stereotypical regional pattern of NAA losses in AD that involved the hippocampus and parietal gray matter, but not frontal gray matter and white matter, regional differences in NAA reductions between SIVD and AD have not thoroughly been investigated before. Therefore, in a new MRSI study that included 13 SIVD patients (71 ± 8 years old), 43 AD patients of comparable age and dementia severity to SIVD, and 52 cognitively normal subjects with and without lacunes, we sought to determine the regional pattern of NAA in gray matter, white matter, and WMH in SIVD. We found that compared to controls, SIVD patients had lower NAA by 18% ($p < 0.001$) in frontal cortex and by 27% ($p < 0.003$) in parietal cortex, but no significant NAA reduction in white matter and medial temporal lobe. Compared to AD, SIVD patients had lower NAA by 13% ($p < 0.02$) in frontal cortex and by 20% ($p < 0.002$) in left parietal cortex. Cortical NAA decreased in SIVD with increasing white matter lesions ($r = 0.54$, $p < 0.02$) and number of lacunes ($r=0.59$, $p < 0.02$). In particular, thalamic lacunes were associated with greater NAA reduction in frontal cortex than lacunes outside the thalamus ($p < 0.02$) across groups, after adjusting for cognitive impairments, as shown in Figure 3.

The finding in SIVD that [NAA] losses in cortical regions correlated with subcortical infarction load and WMH, implies that subcortical vascular disease is responsible for cortical changes in SIVD. There are several possible explanations for this finding. First, and most likely, is that subcortical infarctions cause functional deafferentiation of the cerebral cortex, sometimes termed subcortical-cortical diaschisis. This is consistent with PET studies showing in SIVD hypometabolism and hypoperfusion in cortical regions,[6] especially in the frontal lobe.[7] In support of this view, MRS studies on animals have shown trans-synaptic decrease of NAA levels following acute deafferentation without neuronal loss.[8] It is therefore conceivable that the cortical [NAA] losses in SIVD could indicate deafferented neurons in a state of functional inactivity with a possibility for recovery rather than frank neuron loss. A second explanation is that neurons are damaged or lost via transneuronal degeneration, secondary to subcortical infarctions.[9] With the assumption that [NAA] reflects neuron density, the results imply further that a secondary degeneration causes disproportionately greater loss of neuronal than to non-neuronal cells.

A third possibility is that cortical [NAA] reductions are due to cortical ischemia, with or without micro-infarctions in the cortex, undetectable with MRI. There are two arguments against this view: First, quite remarkably, we found no [NAA] reduction in white matter in SIVD (or in AD). It would seem reasonable that a widespread ischemic process, which affected cortex and caused subcortical infarctions and WMH would also produce [NAA] reduction in white matter.

Second, [NAA] reductions can occure preferentially in the frontal lobe, as the comparison between subjects with and without thalamic lacunes showed. In presence of a generalized ischemic process, however, one would expect that the entire cortex is affected and not the frontal region singled out. Eventually, it will be necessary to obtain autopsy information to exclude micro-infarctions and concurrent AD as potential cause of cortical [NAA] reduction in SIVD.

2.3 Reduced Medial Temporal Lobe NAA in CIND

We have previously showed that AD patients have significantly less NAA concentration in the medial temporal lobe (MTL) and parietal lobe gray matter (GM) than cognitively normal subjects.[10] This study sought to determine whether cognitively impaired but non-demented (CIND) elderly individuals who are at risk for developing dementia exhibit a similar pattern of reduced NAA in the MTL and parietal lobe GM. In addition, we also compare regional NAA patterns and hippocampal volumes in CIND patients who remained cognitively stable with those who later became demented during follow-up (mean follow-up duration: 3.6 + 1.7 years; range: 1–7 years). Seventeen CIND patients (mean age: 75.4 + 6.8 years), 24 AD patients (mean age: 74.8 + 6.9 years), and 24 cognitively normal subjects (mean age: 76.0 + 6.3 years) were studied using MRSI and MRI. There were no significant hippocampal volume differences between CIND patients and cognitively normal subjects. However, CIND subjects had 21% less MTL NAA ($p = 0.005$) than controls. Moreover, dichotomizing CIND patients revealed greater MTL NAA reductions in patients who later became demented than patients who remained cognitively stable during follow-up. Together, these results suggest that NAA reduction in the MTL can be detected in the absence of significant hippocampal atrophy and before the development of dementia. Thus, MTL NAA could potentially serve as an early marker for AD.

3. NAA IN POST TRAUMATIC STRESS DISORDER (PTSD)

Posttraumatic stress disorder (PTSD) is characterized by exposure to markedly distressing traumatic event(s), re-experiencing symptoms, emotional numbing, and increased arousal. Biological alterations include adrenergic hyperresponsiveness [11], increased thyroid activity [12], low cortisol levels, and increased negative feedback sensitivity of the hypothalamic-pituitary-adrenal (HPA) axis following low-dose dexamethasone administration [13]. In addition, magnetic resonance imaging (MRI) studies reported decreased volumes of the hippocampus in both, Vietnam combat veterans [14, 15] and noncombat trauma victims [16, 17] with PTSD. However, laterality was inconsistent across these MRI studies, with volume decreases being reported in the right, the left, and both hippocampi. In a preliminary ^{1}H MRSI study [18], we found decreased hippocampal NAA in a small number of veterans with PTSD, many of whom had been recently abusing alcohol, compared to healthy controls without a history of alcohol abuse. Another ^{1}H MRS study reported NAA reductions in medial temporal lobe structures of veteran PTSD subjects [19]. Therefore, in a new MRSI study on a new group of PTSD subjects we sought to determine if ^{1}H MRSI measurements could detect NAA changes in the hippocampus of PTSD, separate from volume changes.

Eighteen male patients with combat-related PTSD (mean age 51.2 ± 2.5 years) and 19 male control subjects (mean age 51.8 ± 3.2) were studied using MRI and Proton MR spectroscopic imaging. Both groups had no alcohol and drug abuse during the past 5 years. PTSD and control subjects had similar volumes of hippocampus and entorhinal cortex. We found NAA was significantly reduced by about 23% and creatine containing compounds were reduced between 11% and 26% bilaterally in the hippocampus of PTSD when compared to control subjects (Table 1). However, there were no significant differences in hippocampal or ERC volumes between PTSD patients without recent history of alcohol abuse and control subjects. This

contrasted previous reports of hippocampal atrophy in PTSD, suggesting that alcohol abuse may have been at least in part responsible for these previous findings.

The finding of 23–24 % reduction of hippocampal NAA in PTSD subjects of this study, in the absence of hippocampal volume loss, were surprising for two reasons. First, the magnitude of the NAA reduction is very similar to that, which we previously reported for patients with Alzheimer's dementia [20]. Notwithstanding this similarity of NAA reductions, our PTSD patients showed no gross cognitive memory impairments. Second, hippocampal NAA reductions in Alzheimer's disease were accompanied by substantial hippocampal volume losses in the range from 20% to 40% [20]. Another explanation for NAA decrease in PTSD is impaired metabolism of neuronal processes, resulting in secondary NAA loss. Reversible NAA losses have been found in amyotrophic lateral sclerosis [21], epilepsy after surgery [22], in multiple sclerosis [23], and more recently in schizophrenic patients after treatment with antipsychotics [24, 25]. These changes have been attributed to reversible impairment of oxidative metabolism of which NAA is a product. Therefore, we cautiously interpret the finding of decreased hippocampal NAA in PTSD to reflect either neuron loss in the presence of gliosis and/or neuronal metabolic impairments. In this regard, NAA changes would be expected to be more sensitive to neuronal damage in PTSD than volume loss. We also examined the relationship of hypothalamic-pituitary-adrenal (HPA) measures and hippocampal N-Acetyl Aspartate in 11 PTSD and 11 control subjects of this study, using morning salivary cortisol samples before and after low dose dexamethasone (0.5mg) as measure of cortisol levels. We found left hippocampal NAA was strongly associated with both pre-dexamethasone cortisol levels (N= 22, r= 0.53, p= 0.013) and post dexamethasone cortisol (N=22, r= 0.63, p=0.002). After accounting for clinical symptom severity and hippocampal volume, cortisol levels accounted for 21.9% of the variance (F = 5.6, p = .004) in left hippcampal NAA and 12.6% of the variance (F = 3.2, p = .035) in right hippocampal NAA. These results show a positive relationship between cortisol levels and hippocampal NAA in subjects without hypercortisolemia. Within the range of values seen in our subjects, cortisol may have a trophic effect on the hippocampus.

4. CORRELATIONS BETWEEN NAA AND FDG-PET

The *in vivo* neuronal contribution to human cerebral metabolic rate of glucose (CMRglc), measured by ^{18}FDG-PET, is unknown. Since NAA is thought to reflect neuron density, evaluating how CMRglc varies as a function of NAA concentration ([NAA]) should reflect the way in which brain glucose metabolism is affected by neuron density and/or by NAA content per neuron. The CMRglc-to-[NAA] relationship could be derived for an individual subject by plotting local CMRglc against local [NAA] across that subject's brain. The CMGglc-to-[NAA] relationship might be expected to vary from subject-to-subject depending on factors such as subject cognitive status. However, there are limitations to this approach. First, while cortical gray matter is the tissue of primary interest in evaluating brain metabolic activity, PET and ^{1}H MRSI data are often expressed in terms of whole, unsegmented brain tissue, rather than as values for cortical gray matter alone. Second, even if regional data are compared, the spatial resolution of MRSI is lower than that of PET, a source of possible signal infidelity. In a study that included 19 demented, cognitively impaired, and control subjects, who had whole-brain PET data, MRI, and MRSI we aimed to establish a method for the assessment of CMRglc and [NAA] in cortical gray matter, accounting for differences between PET and ^{1}H MRSI image resolution. Furthermore, we looked for the quantitative relationship between gray matter CMRglc and [NAA] in individual cognitively normal, cognitively impaired, and demented subjects and explored whether this CMRglc-to-[NAA] relation varies with cognitive status across subjects. In 18 of 19 subjects, a significant linear regression ($P < 0.05$) resulted when gray matter PET was plotted against gray matter NAA, whereby gray matter PET was higher

for higher gray matter NAA. A representative example of this PET-NAA correlation is shown in figure 4.

To the extent that [NAA] can be taken as a marker of neurons (5–6), the correlation with FDG-PET suggests that the metabolic activity measured by ^{18}FDG-PET in a sample of gray matter increases with the density of neurons present in that gray matter sample and/or with the quantity of NAA within those neurons. Furthermore, the slope of the GMCMRglc-against-GMNAA regression decreased with increasing CDR across subjects, suggesting that CMRglc per neuron is lower in cognitively impaired and demented subjects than in cognitively normal subjects. This explanation is consistent with the idea that diminished cortical metabolism is a physiologic substrate of dementia, regardless of etiology (7, for a review see 18). It also suggests that such hypometabolism may be due not simply to losses in neuron numbers in gray matter, but to an alternative or concomitant decrease in the metabolic activity per neuron of those neurons remaining. This method may be used to investigate the relationship of CMRglc to neurons in various conditions.

5. DEEP GRAY MATTER STRUCTURES IN HIV: A ^{1}H MRSI STUDY (26)

The goal was to determine the concentrations of the neuronal marker N-acetylaspartate (NAA) and of choline-containing metabolites (Cho) in the subcortical brain of HIV-seropositive patients as a function of their cognitive impairment and clinical symptoms. Pathological studies suggest that subcortical gray matter carries a heavy HIV load, and neuropsychological test results are consistent with involvement of subcortical and fronto-striatal brain systems in HIV disease. Single-volume ^{1}H MRS studies suggested neuronal preservation (i.e., unchanged NAA) and macrophage infiltration (i.e., high Cho) in subcortical brain of cognitively impaired and clinically symptomatic HIV+ individuals. Improved ^{1}H MRS methods may allow the early detection of metabolite alterations in subcortical brain of asymptomatic HIV+ individuals.

Two-dimensional ^{1}H MR spectroscopic imaging with volume preselection was performed in 30 HIV- controls and 70 HIV+ participants with varying severities of systemic disease and neuropsychological impairments.

Subcortical NAA was about 20% lower than control only in HIV+ patients with severe cognitive impairments; asymptomatic patients or those with mild cognitive impairments had normal subcortical NAA. Subcortical Cho was about 11% higher compared to controls in HIV + patients regardless of the presence or absence of cognitive impairment or clinical symptoms. Subcortical NAA correlated with performance on a variety of neuropsychological tests but not with Center for Disease Control clinical stage. High thalamic Cho was associated with low CD4 lymphocyte counts. The NAA findings suggest functionally significant neuronal subcortical injury only in severely cognitively impaired HIV+ patients. High subcortical Cho throughout all stages of HIV disease is consistent with early and persistent macrophage infiltration. The findings are consistent with the lack of significant subcortical neuron loss in neuropathological studies. Quantitative ^{1}H MRSI may play a role in the objective assessment of the presence, magnitude and progression of brain involvement in HIV infection.

6. BRAIN DAMAGE IN TREATED HIV-INFECTED INDIVIDUALS (27)

This was the first clinical study of the effects of HIV infection and its progression on brain metabolites using short-TE multi-slice 1H MRSI. Figure 5 shows a representative slice of a multislice ^{1}H MRSI experiment at TE=25ms from a control. Metabolite images of mI, Cho, Cr, and NAA were generated using the automatic fitting program developed in this lab. The raw (solid line) and fitted (dashed) ^{1}H MR spectrum was selected from a region in white matter. We co-registered MRSI to segmented MRI data, determined atrophy-corrected absolute metabolite concentrations in major brain regions, and analyzed by region and tissue type, using

linear regression in a mixed effects model. All statistical analyses used a 2x2 ANOVA (with age as a covariate when appropriate), yielding main effects of HIV infection and heavy drinking, HIV-by-alcohol interactions and group contrasts. Statistical analyses were also done for HIV+ individuals on or off highly active antiretroviral treatment (HAART) and for CDC stages, to evaluate the contrast in HIV symptomatology. We detected effects of heavy drinking [see below or Meyerhoff et al. ACER 2004] and HIV infection, the latter reported here.

Main HIV effects in patients on HAART were observed for 7% higher mI in thalami, 6% higher Cr in temporal WM and trends to lower parietal GM NAA (−5%) and Chow. Additional data analyses including the 30% of subjects who were not on HAART, suggested that stable HAART appears to ameliorate metabolite abnormalities in HIV samples.

When 21 neurologically asymptomatic HIV+ participants in CDC A were compared to 35 symptomatic HIV individuals in CDC B and C, we found main effects of CDC status: symptomatic patients had higher mI in parietal and temporal WM, frontal WM, higher Cho in parietal WM and trends to higher frontal GM Cho. NAA was largely normal in these patients. Thus, the primary impact of HIV symptomatology was on WM, suggesting that inflammatory changes and perhaps myelin damage are the primary pathological events in mostly treated HIV + samples, whereas neuronal/axonal deterioration is largely absent.

Nevertheless, lower NAA in left parietal WM and in frontal WM NAA were associated with higher viral load. CD4 did not correlate with NAA measures in this heavily treated group. Thus, these correlations support our hypotheses that lower NAA levels are associated with greater viral load.

In conclusion, the magnitude and anatomical distribution of the cross-sectional HIV effects differ from previous reports (see our previous studies above), which showed dramatic metabolic abnormalities in WM, GM and subcortical brain in pre-HAART era patients with cognitive impairments of greater severity than today. In this heavily treated population, some NAA loss was only observed in parietal GM, while WM Cho and mI increases are still significant but are not as ubiquitous a finding as in previous HIV-infected samples.

7. ^{1}H MRSI REVEALS BRAIN METABOLITE INJURY IN TREATED HIV+ PATIENTS AND IN CHRONIC HEAVY DRINKERS [28]

The above study showed that cross-sectional HIV effects were relatively subtle and it is unclear if they are premorbid or a function of HIV infection. Therefore, we studied HIV+ individuals about 2 years apart by repeated 1H MRSI to test the hypothesis of ongoing brain damage in HIV patients, who are not treated or have high viral loads despite antiretroviral therapy (HAART). Twenty-four HIV+ patients on HAART showed lower rates of frontal GM and parietal WM NAA loss than 6 HIV+ off HAART. However, even the 13 virally suppressed HIV+ (i.e., those on “successful” HAART) had NAA decreases in temporal WM at a rate of 4±6% compared to 9 viremic patients on HAART at 0±3% (p=0.09). Temporal WM Cho and lenticular Cho further decreased over time in virally suppressed patients on HAART.

Using 2x2 ANOVA in HIV+ participants on HAART (viral status x alcohol status), we found greater rates of NAA loss in suppressed patients than viremic patients in frontal GM and temporal WM, and greater increases of Cho in viremic than suppressed HIV+ patients in frontal and parietal GM, temporal WM, and thalami. This suggests that in virally suppressed subjects on HAART, neuronal damage continues while inflammatory processes are arrested.

In summary, untreated HIV infection presents with ongoing neuronal injury, in particular in parietal WM. HIV+ patients on HAART show less severe longitudinal brain metabolite damage

than those off HAART, but even suppressed HIV+ patients tend to have ongoing regional NAA loss. These results demonstrate the ability of 1H MRSI to detect longitudinal metabolite changes due to HIV infection.

8. ^{1}H MRSI SEPARATES NEURONAL FROM GLIAL CHANGES IN ALCOHOL-RELATED BRAIN ATROPHY [29]

Eleven elderly alcoholics (61 ± 7 years), abstinent for approx. 9 months at the time of MRSI study, were compared to 9 age-matched light-drinking controls. 1H MRSI with PRESS volume preselection was used in a single slice above the ventricles. Cortical spectra were extracted from frontal and parietal gray matter regions, corrected for possible atrophy using tissue-segmented MR images and cortical metabolite ratios were compared. We found a location-by-group effect, which indicated significantly lower NAA/Cr in frontal than parietal cortex of recovering elderly alcoholics. Alcoholics with the most ventricular CSF showed the lowest NAA/Cr in frontal cortex. Together with findings of no significant tissue atrophy in these elderly alcoholics, the MRS results suggest neuronal damage or loss in frontal cortex associated with glial hyperplasia (or gliosis). This report demonstrates that: 1) that absolute metabolite measures are needed, 2) that by studying recovering alcoholics several months after cessation of drinking, tissue recovery may mask the full impact of chronic alcohol consumption on neuronal injury, and 3) that longitudinal studies in recovering alcoholics are needed to better understand the reasons for deficits in NAA and its potential recovery: Neuronal loss would not be associated with NAA recovery, whereas loss of dendritic arborization and neuronal cell body shrinkage would be reversible. Thus, this study pointed the way to further studies being performed in this lab.

9. SEPARATE AND INTERACTIVE EFFECTS OF COCAINE AND ALCOHOL [30]

In a ^{1}H MRSI study of the effects of concurrent cocaine and alcohol dependence on the brain, we compared non-dependent controls to subjects dependent on crack cocaine alone and to subjects dependent on both cocaine and alcohol. Our findings included lower NAA concentrations in cocaine-dependent and in cocaine and alcohol-dependent subjects, especially in dorsolateral prefrontal gray matter and in posterior parietal white matter, suggesting damage to neurons and axons in these brain regions of substance abusers. In that study, it was not possible to determine whether effects observed in subjects dependent on both cocaine and alcohol were due to cocaine abuse, to alcohol abuse, or to both. To answer that question, we examined in a follow-up study a fourth group of individuals dependent on alcohol alone and augmented the original sample by additional subjects, allowing to address the question of separate and interactive effects of chronic cocaine dependence and alcohol dependence on regional brain metabolites. We found main effects of alcohol dependence on brain atrophy and NAA concentrations. Alcohol-dependent individuals abstinent for 1–2 years had brain atrophy and about 8% lower NAA concentrations in both cortical and subcortical gray matter, but no NAA loss in white matter. NAA loss was most significant in frontal gray matter regions. There was no significant main effect of cocaine dependence on NAA concentrations, despite some regional atrophy in these cocaine-dependent individuals abstinent from cocaine for about 4 months. Cocaine-dependent individuals showed higher posterior parietal white-matter creatine concentrations than controls. In addition, no significant cocaine dependence-by-alcohol dependence interactions were found for any metabolite in any tissue or brain region. Thus, alcohol dependence, but not cocaine dependence, was associated with long-lasting NAA loss in cortical and subcortical gray matter throughout the brain, suggesting widespread neuronal injury. However, past chronic alcohol use did not aggravate any chronic cocaine-induced metabolite deficits.

10. EFFECTS OF HEAVY DRINKING

10.1 Effects of Abstinence on the Brain: Quantitative MRI and MR Spectroscopic Imaging in Chronic Alcohol Abuse [31]

Structural brain damage, especially to white matter, is well documented in chronic alcohol abuse, and there is also evidence for brain metabolic abnormalities in this condition. It is unknown, however, to what extent these structural and metabolic changes are still detectable in long-term abstinent alcoholics compared to active chronic drinkers. Therefore we compared 12 recovering alcoholics, who had been abstinent from alcohol for an average of 2 years, to 8 active heavily drinking subjects with similar alcohol use variables.

Metabolite concentrations in whole-brain and in gray matter and white matter of brain lobes did not differ significantly between the recovering alcoholics and active drinkers. However, active heavily drinking subjects had less frontal white matter than abstinent alcoholics and less gray matter in the orbital frontal pole and postcentral gyrus. However, abstinent alcoholics had smaller gray matter volumes in the anterior cingulated than active heavy drinkers.

Our cross-sectional ^{1}H MRSI measures were largely ineffective in revealing metabolic effects of abstinence on the alcohol-damaged brain. The study, however, suggests region-specific structural recovery from chronic alcohol-induced brain injury, but also region-specific long-term structural damage in abstinent alcoholics.

10.2 Brain Recovery During Abstinence from Alcohol [32]

In our ongoing studies of recovering alcoholics, we investigate the nature of brain injury in alcoholics and the potential improvements of brain metabolite concentrations and cognition during prolonged sobriety.

At one week of sobriety, NAA in alcoholics was lower by 6–19% in frontal, parietal and temporal gray matter and white matter, in the basal ganglia, the brain stem, and cerebellum, while Cho concentrations were lower by 7–13% in lobar grey and white matter regions and in the thalami. NAA deficits correlated with cognitive impairments.

Over the first month of sobriety, NAA, myo-inositol, and Cho concentrations in frontal white matter increased significantly and to a greater extent than in gray matter. Similarly, neurocognitive performance increased significantly and showed some correlation with neurochemical improvements. Seven months after cessation of drinking, abstinent alcoholics had normal concentrations of myo-inositol and Cho. Regional NAA concentrations in white and gray matter increased over 7 months of sobriety, however, they did not normalize. These long-term changes were accompanied by continued cognitive improvements in most domains except visuospatial learning and memory. Increases of Cho and myo-inositol concentrations over time are consistent with remyelination and astrocytosis. Slower increases of NAA suggest slower recovery from axonal and neuronal injury, and that NAA loss in alcoholics is not completely due to neuronal loss.

10.3 Effects of Heavy Drinking, Binge Drinking, and Family History of Alcoholism on Regional Brain Metabolites [33]

Abstinent alcoholics show regional NAA loss, primarily in frontal lobes and cerebellum. The main goals of this project were to investigate the effects of chronic active heavy drinking on NAA and other metabolites throughout the brain, and to determine if they are affected by family history (FH) of alcoholism and long-term drinking pattern.

We used quantitative MRI and multi-slice ^{1}H MRSI at a short echo-time to compare 46 chronic heavy drinkers (HD) and 52 light drinkers (LD) on regional, tissue-specific and atrophy-corrected concentrations of NAA, myo-inositol (mI), creatine- and choline-containing metabolites.

NAA in frontal white matter was 6% lower in HD than LD. NAA loss was greater in female than male heavy drinkers despite similar drinking severity and greater in FH-negative HD than FH-positive HD. FH-negative compared to FH-positive HD also had higher mI in the brainstem and tended to have lower NAA and higher mI in frontal GM. In addition, greater frontal NAA loss in HD was found as a function of age. Lower frontal white matter NAA in HD correlated with lower executive and working memory functions and with greater P3 latency.

Thus, heavy drinkers in their forties who are not in alcoholism treatment have frontal axonal injury, which is associated with lower brain function and is likely of behavioral significance. Family history of alcoholism modulates brain metabolite abnormalities. Brain injury in active heavy drinkers is less pronounced than in abstinent alcoholics and presents with a different spatial and metabolite pattern.

10.4 Magnetic Resonance Detects Brainstem Changes in Chronic, Active Heavy Drinkers [34]

Neuropathological and neuroimaging studies show cortical and subcortical volume loss in alcohol dependent individuals. The brainstem is considered critical in the development and maintenance of drug and alcohol dependence, but it has not been the focus of neuroimaging studies. Using quantitative MRI and ^{1}H MRSI, we compared the size and metabolite measures of potential cellular injury of the brainstem in 12 chronic, active heavy drinkers and 10 light drinkers. Chronic heavy drinking was associated with a significantly smaller overall brainstem volume and with significantly smaller midsagittal areas of the brainstem, midbrain, and pons. Heavy drinking was also associated with significantly lower ratios of N-acetyl-aspartate (NAA) and choline-containing metabolites (Cho) compared with creatine-containing compounds (Cr) in a region including midbrain and pons, independent of brainstem atrophy. These structural and metabolite findings are consistent with neuronal injury of the midbrain/pons of untreated chronic heavy drinkers.

EFFECTS OF EPILEPSY ON NAA

11.1 Identification of the Epileptogenic Focus

From the first studies in the early 1990's on, the most consistent finding in the epileptogenic focus has been a reduction of NAA. This has been first demonstrated in temporal lobe epilepsy (TLE) with evidence for hippocampal atrophy or mesial temporal sclerosis (MTS) where hippocampal NAA reductions correctly identify the epileptogenic hippocampus in up to 100%. Because early studies found a strong correlation between the degree of neuronal loss and the degree of NAA reduction, the NAA reduction in the epileptogenic hippocampus was thought to represent mainly neuronal loss [35]. However, newer evidence suggests that a substantial component of the NAA reduction is due to a not further specified, potentially reversible neuronal dysfunction in the epileptogenic tissue. This is also supported by studies in TLE without MRI evidence for MTS where histopathological studies show only mild neuronal loss despite clear hippocampal NAA reductions. However, NAA reductions in TLE without MTS are different from those found in TLE with MTS as has been demonstrated by a recent study from our laboratory. This study compared patterns of hippocampal NAA loss in 10 TLE without MTS with the patterns found in 15 TLE with MTS. The number of voxels with reduced NAA/(Cr+Cho) in the ipsilateral hippocampus was higher in TLE with MTS than in TLE without MTS (1.9 ± 1.3 vs 0.6 ± 1.3, $p = 0.02$). Furthermore, the NAA reductions in TLE without MTS

were more often diffuse (p = 0.007) and less often concordant (p = 0.015) to the epileptogenic hippocampus than in TLE with MTS.

Consequently, hippocampal NAA reductions in TLE without MTS are less accurate for the identification of the epileptogenic focus but nonetheless helpful for predicting the chance of becoming seizure free after epilepsy surgery. A study in 15 TLE without MTS from our laboratory [(36)] found that patients who became not seizure free had lower ipsilateral hippocampal NAA/(Cr+Cho) z scores than contralateral (p = 0.04). Furthermore, in comparison with patients who became seizure free, patients who did not had lower ipsilateral (p= 0.005) and contralateral (p=0.02) hippocampal NAA/(Cr+Cho) z sores. Taken together, TLE patients without MTS who became seizure free had milder and less well lateralized hippocampal NAA reductions than patients who did not.

Preliminary results show, that NAA reductions may also be helpful for focus identification in patients suffering from neocortical epilepsy (NE), i.e., a form of epilepsy where identification of the seizure focus is often challenging, particularly in patients with no structural abnormality on the MRI. We studied 21 patients with NE (10 with evidence for cortical malformations on the MRI, 11 with normal MRI) and 19 age-matched controls. In controls, NAA/Cr and NAA/Cho of all voxels of a given lobe was expressed as a function of white matter content and thresholds for pathological values determined by calculating the 95% prediction intervals for NAA/Cr and NAA/Cho. Voxels with NAA/Cr or NAA/Cho below the 95% prediction interval were defined as “pathological”. Z-scores were used to identify regions with a high percentage of pathological voxels. MRSI correctly identified the lobe containing the epileptogenic focus as defined by EEG in 62% of the NE patients. MRSI localization of the focus was correct in 70% of the patients with a lesion on the MRI and in 55% of the patients with normal MRI.[(37)]

NAA reductions are also a common finding in different types of cortical malformations. These lesions result from a disruption of the developmental processes during neuroblast proliferation and differentiation, neuroblast migration, or postmigrational cortical organization. Using a similar method as for focus localization in NE, 30% (range 0 – 78%) of all voxels in cortical malformations (8 patients with 10 malformations) were found to be metabolically abnormal. The most common abnormalities were areas with reduced NAA or increased Cho which were interspersed within metabollically normal areas. Cortical malformations are not only characterized by a disturbed tissue architecture but also by an intrinsic epileptogenicity and those NAA and Cho abnormalities probably reflect both disturbances.[(38)]

11.2 NAA in Brain Regions Secondarily Involved Seizure Spread

Recent studies found NAA reduction not to be restricted to the focus but also in brain areas which are involved in seizure spread. This is well known in the TLE where in up to 50% of the patients NAA reductions are not only found in the ipsilateral hippocampus but also contralaterally.[(39)] These contralateral NAA reductions increase to normal values after successful epilepsy surgery, but stay decreased, if surgery did not lead to seizure freedom [(40)]. This finding further supports the hypothesis that NAA reductions in epileptic tissue are not necessarily due to neuron loss but rather present the disturbance of the neuronal metabolism caused by epileptogenic activity. Those extrafocal NAA reductions are not restricted to the limbic system but may involve even more remote brain areas as a study done in our laboratory recently demonstrated.

In this study, we used MR spectroscopic imaging in combination with tissue segmentation in 14 TLE patients with MTS, 7 TLE patients without MTS and 12 age-matched controls. To identify voxels with abnormally low NAA, NAA/(Cr+Cho) of all voxels of a given lobe was expressed as a function of white matter content to determine the 95% prediction interval for

any additional voxel of a given tissue composition. Voxels with NAA/(Cr+Cho) below the lower limit of the 95% prediction interval were defined as “pathological”. Z-scores were used to identify regions with a higher percentage of pathological voxels than in controls. Additional regions with reduced NAA/(Cr+Cho) were found in the ipsilateral temporal and parietal lobes and bilaterally in insula and frontal lobes. Temporal abnormalities identified the epileptogenic focus in 70% in TLE with MTS and 83% of TLE without MTS. Extratemporal abnormalities identified the hemisphere containing the epileptogenic focus in 78% of TLE with MTS but in only 17% of TLE without MTS. Therefore, temporal and extratemporal NAA/(Cr+Cho) reductions might be helpful for focus lateralization in TLE [41]. Because volumetric studies found no evidence for tissue atrophy beyond the ipsilateral temporal lobe [42], it is reasonable that these NAA reductions probably also reflect neuronal dysfunction rather than actual neuronal loss. Furthermore, smaller, extrafocal areas with metabolic abnormalities were also found in 24% of the NE patients.[37]

12. REDUCED NAA IN ALS

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder that results in the loss of motor neurons both in the brain and spinal cord leading to paralysis and ultimately death. There is no definitive test for ALS and diagnoses are currently made based on clinical data. The development of a surrogate marker for disease progression would be useful for identifying individuals suffering from the early stages of ALS and for monitoring treatment effects. Previous MRSI research from our group found that the ratios NAA/Cre, NAA/Cho and NAA/Cre+Cho are reduced in probable/definite subjects as compared to controls and that NAA, Cre, and Cho decrease over time in ALS.[36] Because most previous MRS studies of ALS examined regions of interest that were sufficiently distant from the skull to avoid interference from lipids, sampling of motor cortex was often limited.[43–55] Using a Multiplanar ^{1}H MRSI technique to sample a larger region that includes brain surface, reductions of the ratio NAA/Cre+Cho, have been observed in the region of the motor cortex and corticospinal tract.[56] A quantitative analysis of these data showed that decreased NAA was responsible for the ratio changes in the motor cortex and increased Cho for ratio changes in the corticospinal tract.[57, 58].The specific aims of this project were to: 1) determine if previously reported ^{1}H MRSI differences between ALS patients and control subjects are limited to the motor cortex; and 2) determine the longitudinal metabolic changes corresponding to varying levels of diagnostic certainty. Toward this end, 21 patients with possible/suspected ALS, 24 patients with probable/definite ALS and 17 control subjects underwent multislice ^{1}H MRSI co-registered with tissue-segmented MRI to obtain concentrations of the brain metabolites NAA, Cre and Cho in the left and right motor cortex and in gray matter and white matter of non-motor regions in the brain. Of these subjects, 13 possible/suspected and 15 probable/definite patients received repeated scans (every 3 months for up to 12 months) and were studied longitudinally.

In the more affected hemisphere, Reductions in the ratios, NAA/Cho and NAA/Cre+Cho were observed both within (12.6% and 9.5% respectively) and outside (9.2% and 7.3% respectively) motor cortex in probable/definite ALS (Table 2). However, these reductions were significantly greater in motor cortex ($p<0.05$ for NAA/Cho and $p<0.005$ for NAA/(Cre+Cho). Longitudinal changes in NAA were observed at three months within the motor cortex of both possible/suspected ALS patients ($p<0.005$) and at 9 months outside the motor cortex of probable/definite patients ($p<0.005$). However there was no clear pattern of progressive change over time. NAA ratios are reduced in motor cortex and outside of motor cortex in ALS, suggesting widespread neuronal injury. Longitudinal changes of NAA are not reliable, suggesting that NAA may not be a useful surrogate marker for treatment trials.

13. QUESTION AND ANSWER SESSION

DR. BARKER: We have time for one or two questions. Yes?

DR. MATALON: I find this very fascinating. You had a slide where you showed six people with lower NAA or whatever. Two of them, you showed that they, when you saw that, later on had -- One had stroke, and one had vascular abnormality.

You mean to say that you can predict that before the event that destroys cells or there was a problem with profusion that you were not aware of?

DR. WEINER: We don’t know. We don't know the predictive value of NAA here, but what is interesting is that, as I showed you earlier, subjects have subcortical ischemic vascular dementia NAA reductions in their hippocampus. Therefore, NAA reductions in hippocampus are not at all specific for Alzheimer's disease. Whether the NAA reductions reflect a generalized ischemic process or whether it represents microscopic hippocampal sclerosis, it is hard to infer any specific mechanism when you see a reduction of NAA in a patient.

DR. WEINBERGER: Michael, these are really lovely studies. Let me ask a question which is inspired by your question of me, but fundamentally out of curiosity.

If you analyzed all your data by NAA-creatine ratios, how different would the data look?

DR. WEINER: We haven't done that very much because early on, we had some reviewers saying the ratio is going down, how do you know it’s not because the creatine is going up? Lots of people were doing spectroscopy measurements; Brian Ross in fact started doing single voxel spectroscopy in Alzheimer’s disease. It is difficult to interpret changes in ratios. A change in NAA/Creatine could be due to a change in NAA, a change of creatine, or both. One of the issues that must be dealt with concerns the effects of loss of gray matter and white matter and expansion of CSF. That is, it is important to understand the tissue composition of each MRS voxel to determine the extent to which the NAA measurements were changing independent of changes of tissue composition. To accomplish this, we developed segmentation programs and programs that co-register the spectroscopy voxels with the segmentation information, also correcting for the pulse profile, the slice offset and other instrumental parameters. This allows calculation of the absolute amount of NAA and other metabolies. We have published some work, especially in ALS where we calculated both rations and absolute values, because in some circumstances you obtain---more of this classification value (diagnostic value) by calculating ratios.

I mean, the classic example is what Brian Ross showed, is that if you just look at the NAA over creatine in Alzheimer's or look at the myo-inositol over creatine in Alzheimer's, you get one kind of classification. But if you look at the myo-inositol/NAA ratio, then you get much greater classification values.

If you are asking the question, is NAA changing? then our view has always been the best thing to do is to calculate it absolutely.

There is data that creatine does change in many circumstances, and I think we had some work we did in ALS some years ago that showed that the creatine vlaues were significantly changing in ALS patients. Those changes in creatine were giving us spurious data, making it more difficult to interpret the changes of NAA to creatine ratios.

DR. MATALON: My question is -- you didn't answer it, only partially. When you have decrease in NAA without any known reason, no dementia, no Alzheimer's, like those two you

show, should we follow that with another test? profusion studies? blood supply? because some of these things may be preventable. This is really the crux of my question.

DR. WEINER: Well, first of all, what we are doing is purely research at this stage. I think it would be very difficult to start using this information to try to manage patients. The whole goal of my research is to try to identify risk factors that predict cognitive decline.

I think that NAA is a potential useful risk factor in this area. However, there is nothing you can do which prevents or slows cognitive decline in patients. There are no drugs that have been shown to slow the progression of Alzheimer's.

What we are waiting for is a drug that blocks the production of amyloid or a neuroprotective agent. Some of these are currently under clinical trials. Once one of those drugs becomes available, the importance of being able to predict future development of Alzheimer's disease becomes huge in this country, and we need to have good, robust measures that can be used widely in many MRI centers, and maybe spectroscopy will be shown to have a unique role. So we'll see.

DR. BARKER: Okay, thank you very much, Mike.

References

1. Chui HC. Dementia due to subcortical ischemic vascular disease. Clin Cornerstone 2001;3(4):40–51. [PubMed: 11432121]
2. Fein G, Di SV, Tanabe J, et al. Hippocampal and cortical atrophy predict dementia in subcortical ischemic vascular disease [In Process Citation]. Neurology 2000;55 (11):1626–35. [PubMed: 11113215]
3. Mungas D, Jagust WJ, Reed BR, et al. MRI Predictors of Cognition in Subcortical Ischemic Vascular Disease and Alzheimer's Disease. Neurology 2001;57(12):2229–35. [PubMed: 11756602]
4. Du AT, Schuff N, Laakso MP, et al. Effects of subcortical ischemic vascular dementia and AD on entorhinal cortex and hippocampus. Neurology 2002;58(11):1635–41. [PubMed: 12058091]
5. Laakso MP, Partanen K, Riekkinen P, et al. Hippocampal volumes in Alzheimer's disease, Parkinson's disease with and without dementia, and in vascular dementia: An MRI study. Neurology 1996;46:678–81. [PubMed: 8618666]
6. Baron JC, D'Antona R, Pantano P, Serdaru M, Samson Y, Bousser MG. Effects of thalamic stroke on energy metabolism of the cerebral cortex. A positron tomography study in man. Brain 1986;109 (Pt 6):1243–59. [PubMed: 3491655]
7. Kwan LT, Reed BR, Eberling JL, et al. Effects of subcortical cerebral infarction on cortical glucose metabolism and cognitive function. ArchNeurology 1999;56:809–14.
8. Rango, M.; Spagnoli, D.; Tomei, G.; Bamonti, F.; Scarlato, G.; Zetta, L. MRM ed. Williams & Wilkins; 1995. Central Nervous System Trans-Synaptic Effects of Acute Axonal Injury: A 1H Magnetic Resonance Spectroscopy Study.
9. Escobar A. Cerebral changes associated with senility. I. The role of transneuronal degeneration in the neocortex. Bol Estud Med Biol 1973;28(1):1–8. [PubMed: 4804954]
10. Schuff N, Capizzano AA, Du AT, et al. Selective reduction of N-acetylaspartate in medial temporal and parietal lobes in AD. Neurology 2002;58(6):928–35. [PubMed: 11914410]
11. Southwick SM, Paige S, Morgan CA, Bremner JD, Krystal JH, Charney DS. Neurotransmitter alterations in PTSD: catecholamines and serotonin. Semin Clin Neuropsychiatry 1999;4(4):242–8. [PubMed: 10553029]
12. Wang S, Mason J, Southwick S, Johnson D, Lubin H, Charney D. Relationships between thyroid hormones and symptoms in combat-related posttraumatic stress disorder. Psychosom Med 1995;57 (4):398–402. [PubMed: 7480570]

13. Yehuda R, Southwick SM, Krystal JH, Bremner D, Charney DS, Mason JW. Enhanced suppression of cortisol following dexamethasone administration in posttraumatic stress disorder. AmJPsychiatry 1993;150(1):83–6.

14. Bremner JD, Randall P, Scott TM, et al. MRI-based measurement of hippocampal volume in patients with combat-related posttraumatic stress disorder. Am J Psychiatry 1995;152:973–81. [PubMed: 7793467]

15. Gurvits TV, Shenton ME, Hokama H, et al. Magnetic resonance imaging study of hippocampal volume in chronic, combat-related post-traumatic stress disorder. Biological Psychiatry 1996;40:1091–9. [PubMed: 8931911]

16. Bremner JD, Randall P, Vermetten E, et al. MRI-based measurement of hippocampal volume in posttraumatic stress disorder related to childhood physical and sexual abuse-A preliminary report. Biological Psychiatry 1997;41:23–32. [PubMed: 8988792]

17. Stein MB, Koverola C, Hanna C, Torchia MG, McClarty B. Hippocampal volume in women victimized by childhood sexual abuse. Psychol Med 1997;27(4):951–9. [PubMed: 9234472]

18. Schuff N, Marmar CR, Weiss DS, et al. Reduced hippocampal volume and n-acetylaspartate in post traumatic stress disorder. The Annals of the New York Academy of Sciences 1997;(821):516–20.Supplement on Psychobiology of Posttraumatic Stress Disorder

19. Freeman TW, Cardwell D, Karson CN, Komoroski RA. In vivo proton magnetic resonance spectroscopy of the medial temporal lobes of subjects with combat-related posttraumatic stress disorder. MagnResonMed 1998;40(1):66–71.

20. Schuff N, Amend D, Ezekiel F, et al. Changes of hippocampal n-acetylaspartate and volume in Alzheimer's disease: A proton MR spectroscopic imaging and MRI study. Neurology 1997;49:1513–21. [PubMed: 9409338]

21. Kalra S, Cashman NR, Genge A, Arnold DL. Recovery of N-acetylaspartate in corticomotor neurons of patients with ALS after riluzole therapy. Neuroreport 1998;9(8):1757–61. [PubMed: 9665596]

22. Hugg JW, Kuzniecky RI, Gilliam FG, Morawetz RB, Faught RE, Hetherington HP. Normalization of contralateral metabolic function following temporal lobectomy demonstrated by h-1 magnetic resonance spectroscopic imaging. Ann Neurol 1996;V40:236–9. [PubMed: 8773605]

23. De Stefano N, Matthews PM, Arnold DL. Reversible decreases in N-acetylaspartate after acute brain injury. Magn Reson Med 1995;34:721–7. [PubMed: 8544693]

24. Bertolino A, Callicott JH, Mattay VS, et al. The effect of treatment with antipsychiatric drugs on brain N-acetylaspartate measures in patients with schizophrenia. Biological Psychiatry 2001;49:39–46. [PubMed: 11163778]

25. Heimberg C, Komoroski RA, Lawson WB, Cardwell D, Karson CN. Regional proton magnetic resonance spectroscopy in schizophrenia and exploration of drug effect. Psychiatry Res 1998;83(2):105–15. [PubMed: 9818736]

26. Meyerhoff DJ, Weiner MW, Fein G. Deep gray matter structures in HIV infection: a proton MR spectroscopic study. AJNR Am J Neuroradiol 1996;17(5):973–8. [PubMed: 8733976]

27. Meyerhoff DJ, Cardenas V, Studholme C, et al. Evidence for Brain Damage in Treated HIV-Infected Individuals. Neurology 2003;60(5):A186.

28. Meyerhoff DJ, Truran D, Flenniken D, Song E, Studholme C, Weiner MW. Longitudinal Multi-Slice Short-Te 1H MRSI Reveals On Going Brain Metabolite Injury In Treated HIV+ Patients And In Chronic Heavy Drinkers. Proc Intl Soc Mag Reson Med 2004;11:290.

29. Fein G, Meyerhoff DJ, Di Sclafani V, et al. 1H magnetic resonance spectroscopic imaging separates neuronal from Glial changes in alcohol-related brain atrophy. Chapter in NIAAA Research Monograph No 27/Alcohol and Glial. Cells 1994:227–41.

30. O'Neill J, Cardenas VA, Meyerhoff DJ. Separate and interactive effects of cocaine and alcohol dependence on brain structures and metabolites: quantitative MRI and proton MR spectroscopic imaging. Addiction Biology 2001;6:347–61. [PubMed: 11900613]

31. O'Neill J, Cardenas VA, Meyerhoff DJ. Effects of abstinence on the brain: quantitative magnetic resonance imaging and magnetic resonance spectroscopic imaging in chronic alcohol abuse. Alcohol Clin Exp Res 2001;25(11):1673–82. [PubMed: 11707642]

32. Gazdzinski, S.; Durazzo, TC.; Meyerhoff, D. Brain Recovery During Abstinence from Alcohol: MRI, MR Spectroscopic Imaging, and Neurocognitive Studies; American Academy of Neurology 56th

Annual Meeting; 2004; Moscone Convention Center. San Francisco, CA USA: American Academy of Neurology; 2004. p. A542

33. Meyerhoff D, Blumenfeld R, Truran D, et al. Effects of heavy drinking, binge drinking, and family history of alcoholism on regional brain metabolites. Alcohol Clin Exp Res 2004;28(4):650–61. [PubMed: 15100618]
34. Bloomer CW, Langleben DD, Meyerhoff DJ. Magnetic resonance detects brainstem changes in chronic, active heavy drinkers. Psychiatry Res 2004;132(3):209–18. [PubMed: 15664792]
35. Duc O, Trabesinger AH, Weber OM, et al. Quanititative 1HMRS in the evaluation of mesial temporal lobe epilepsy in vivo. Magn Reson Imaging 1998;16:969–79. [PubMed: 9814780]
36. Suhy J, Laxer KD, Capizzano AA, et al. 1H MRSI predicts surgical outcome in MRI-negative temporal lobe epilepsy. Neurology 2002;58(5):821–3. [PubMed: 11889252]
37. Mueller, S.; Laxer, K.; Barakos, J., et al. Focus identification in neocortical epilepsy with MR-spectroscopic imaging. Submitted
38. Mueller, S.; Laxer, K.; Barakos, J., et al. Metabolic characteristics causing epilepsy. Submitted
39. Ende GR, Laxer KD, Knowlton RC, et al. Temporal lobe epilepsy: bilateral hippocampal metabolite changes revealed at proton MR spectroscopic imaging. Radiology 1997;202(3):809–17. [PubMed: 9051038]
40. Cendes F, Andermann F, Dubeau F, Matthews PM, Arnold DL. Normalization of neuronal metabolic dysfunction after surgery for temporal lobe epilepsy - Evidence from proton MR spectroscopic imaging. Neurology 1997;49:1525–33. [PubMed: 9409340]
41. Mueller S, Laxer K, Cashdollar N, Flenniken D, Matson G, Weiner M. Identification of Abnormal Neuronal Metabolism Outside the Seizure Focus in Temporal Lobe Epilepsy. Epilepsia 2004;45(4): 355–66. [PubMed: 15030498]
42. Mueller S, Suhy J, Laxer K, et al. Reduced extrahippocampal NAA in mesial temporal lobe epilepsy. Epilepsia 2002;43:1210–6. [PubMed: 12366737]
43. Pioro EP, Antel JP, Cashman NR, Arnold DL. Detection of cortical neuron loss in motor neuron disease by proton magnetic resonance spectroscopic imaging in vivo. Neurology 1994;44(10):1933–8. [PubMed: 7936250]
44. Jones AP, Gunawardena WJ, Coutinho CM, Gatt JA, Shaw IC, Mitchell JD. Preliminary results of proton magnetic resonance spectroscopy in motor neurone disease (amytrophic lateral sclerosis). J Neurol Sci 1995;129 (Suppl):85–9. [PubMed: 7595630]
45. Gredal O, Rosenbaum S, Topp S, Karlsborg M, Strange P, Werdelin L. Quantification of brain metabolites in amyotrophic lateral sclerosis by localized proton magnetic resonance spectroscopy [see comments]. Brain 1997;48(4):878–81.
46. Giroud M, Walker P, Bernard D, et al. Reduced brain N-acetyl-aspartate in frontal lobes suggests neuronal loss in patients with amyotrophic lateral sclerosis [published erratum appears in *Neurol Res* 1997 Aug;19(4):456]. NeurolRes 1996;18(3):241–3.
47. Cwik VA, Hanstock CC, Allen PS, Martin WR. Estimation of brainstem neuronal loss in amyotrophic lateral sclerosis with in vivo proton magnetic resonance spectroscopy. Neurology 1998;50(1):72–7. [PubMed: 9443460]
48. Ellis CM, Simmons A, Andrews C, Dawson JM, Williams SC, Leigh PN. A proton magnetic resonance spectroscopic study in ALS: correlation with clinical findings. Neurology 1998;51(4): 1104–9. [PubMed: 9781537]
49. Block W, Karitzky J, Treaber F, et al. Proton magnetic resonance spectroscopy of the primary motor cortex in patients with motor neuron disease: subgroup analysis and follow-up measurements [see comments]. Arch Neurol 1998;55(7):931–6. [PubMed: 9678310]
50. Pioro EP, Majors AW, Mitsumoto H, Nelson DR, Ng TC. 1H-MRS evidence of neurodegeneration and excess glutamate + glutamine in ALS medulla. Neurology 1999;53(1):71–9. [PubMed: 10408539]
51. Bradley WG, Bowen BC, Pattany PM, Rotta F. 1H-magnetic resonance spectroscopy in amyotrophic lateral sclerosis. J Neurol Sci 1999;169(1–2):84–6. [PubMed: 10540013]
52. Bowen BC, Pattany PM, Bradley WG, et al. MR imaging and localized proton spectroscopy of the precentral gyrus in amyotrophic lateral sclerosis. Am J Neuroradiol 2000;21(4):647–58. [PubMed: 10782773]

53. Chan S, Shungu DC, Douglas-Akinwande A, Lange DJ, Rowland LP. Motor neuron diseases: comparison of single-voxel proton MR spectroscopy of the motor cortex with MR imaging of the brain. 1999;212(3):763–9.

54. Tarducci R, Sarchielli P, Presciutti O, et al. Study of the primary motor cortex in amyotrophic lateral sclerosis by quantitative 1HMRS. ISMRM 2000;1:632.

55. Petropoulos H, Mandler RN, Qualls C, et al. 1H-MRS reveals diffuse neuronal injury in Amyotrophic Lateral Sclerosis. ISMRM 2000;1:633.

56. Kalra S, Arnold DL, Cashman NR. Biological markers in the diagnosis and treatment of ALS. J Neurol Sci 1999;165 (Suppl 1):S27–S32. [PubMed: 10448978]

57. Rooney WD, Miller RG, Gelinas D, Schuff N, Maudsley AA, Weiner MW. Decreased N-acetylaspartate in motor cortex and corticospinal tract in ALS. Neurology 1998;50(6):1800–5. [PubMed: 9633731]

58. Schuff N, Rooney WD, Miller RG, et al. Reanalysis of Multislice 1H MRSI in Amyotrophic Lateral Sclerosis. Magnetic Resonanc in Medicine 2001;45:513–6.

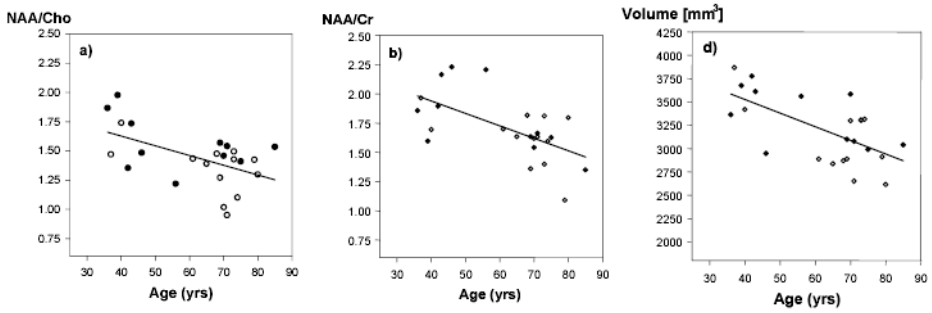

Figure 1.
Age-related changes in metabolite concentrations and volume in hippocampus.

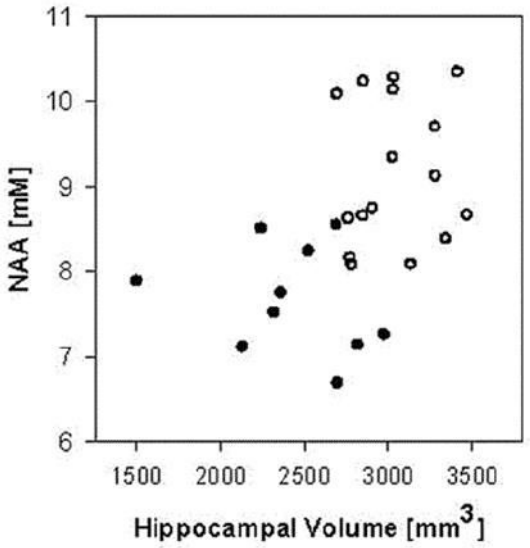

Figure 2.
Comparison of hippocampal volume and NAA levels in Alzheimer's patients (filled circles) and control subjects (open circles).

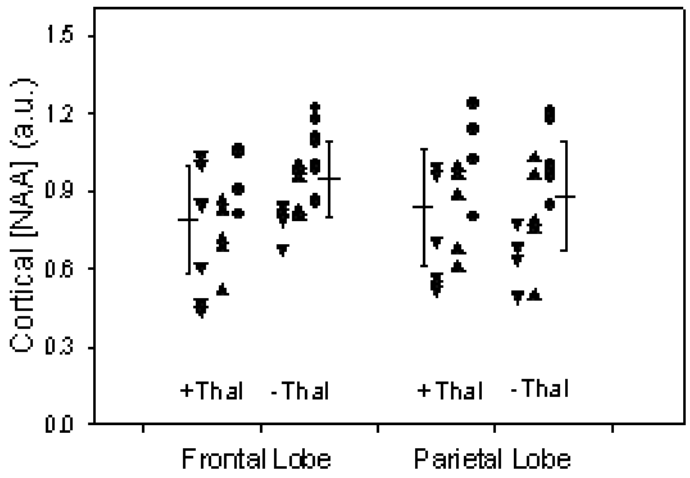

Figure 3.
Thalamic lacunes associated with decreases in NAA

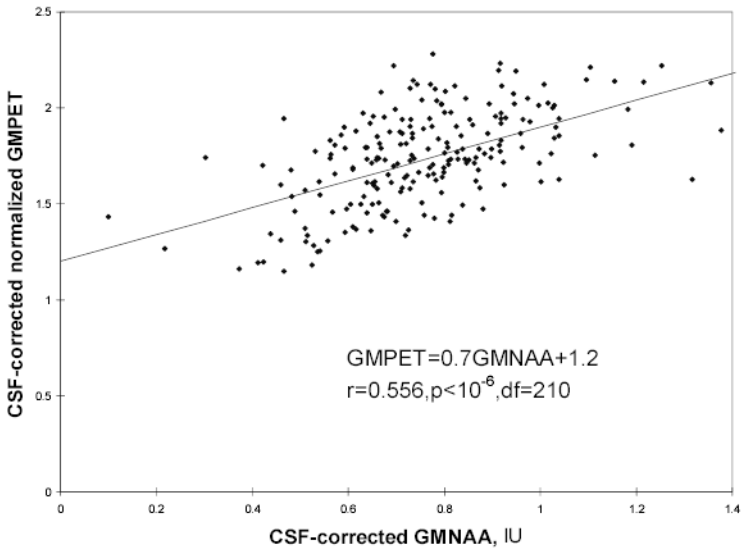

Figure 4.
Correlation between FDG-PET (glucose metabolism) and NAA levels.

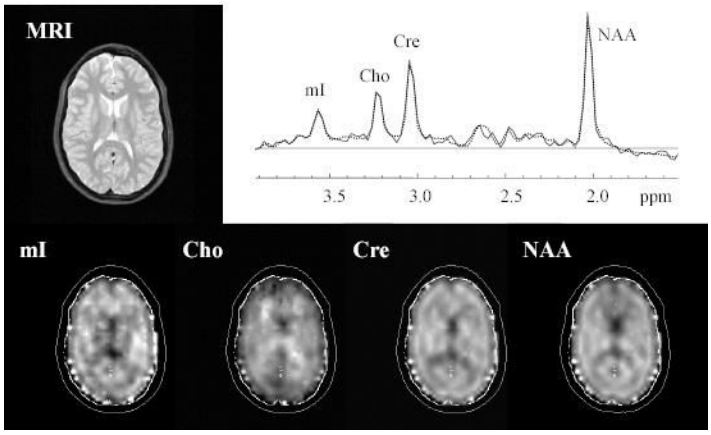

Figure 5.
Metabolic imaging of myo-inositol, Choline, Creatine and N-acetylaspartate in control.

Table 1

NAA reductions in PTSD patients.

Metabolites	PTSD	Control	Difference[b]	Effect Size
Left-NAA	2.8 ± 0.8	3.7 ± 0.8	−24*	1.12
Right-NAA	2.9 ± 0.9	3.8 ± 0.7	−23*	1.12
Hippocampal tissue[c]	0.4 ±0.7	0.4 ± 0.9	0	

[a] Concentrations in arbitrary units

[b] Difference in percent compared to controls

[c] Fraction of hippocampal to total gray and white matter tissue within a MRSI voxel

* $p < 0.01$

Table 2

More Affected Hemisphere	Control		Probable/Definite	
	Motor	Other	Motor	Other
NAA/Cre	2.16 ± 0.16	2.08 ± 0.14	2.02 ± 0.25 (−7.3%)	1.96 ± 0.17 (−5.8%)
NAA/Cho	3.60 ± 0.39	3.08 ± 0.36	3.14 ± 1.46 (−12.6%)†	2.79 ± 0.32 (−9.2%)*
NAA/(Cre+Cho)	1.34 ± 0.10	1.22 ± 0.09	1.21 ± 0.14 (−9.5%)*	1.13 ± 0.10 (−7.3%)*

Less Affected Hemisphere	Control		Probable/Definite	
	Motor	Other	Motor	Other
NAA/Cre	2.16 ± 0.16	2.08 ± 0.14	1.92 ± 0.20 (−11.6%)‡	1.94 ± 0.17 (−6.4%)*
NAA/Cho	3.60 ± 0.39	3.08 ± 0.36	3.14 ± 0.43 (−10.8%)*	2.79 ± 0.32 (−10.3%)*
NAA/(Cre+Cho)	1.34 ± 0.10	1.22 ± 0.09	1.21 ± 0.14 (−11.5%)‡	1.13 ± 0.10 (−8.1%)*

* $p<0.05$,

† $p<0.005$,

‡ $p<0.0005$

Numbers in parentheses are percent change from control subject mean