

# The Pathophysiology of 'Brain Shrinkage' in Alcoholics – Structural and Molecular Changes and Clinical Implications

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This article represents a symposium of the 2004 ISBRA Congress held in Mannheim. The presentations were: Review of the neuropathological and neurochemical changes seen in alcohol-related 'brain shrinkage' by Clive Harper; In Vivo Detection of Macrostructural and Microstructural Markers of Brain Integrity in Human Alcoholism and a Rodent Model of Alcoholism by Adolf Pfefferbaum, Elfar Adalsteinsson and Edith Sullivan; Gene and Protein Changes in the Brains of Alcoholics with 'Brain Shrinkage' by Joanne Lewohl and Peter Dodd; Cross sectional and longitudinal MR spectroscopy studies of chronic adult alcoholics by Michael Taylor; Brain Atrophy Associated with Impairment on a Simulated Gambling Task in Long-Term Abstinent Alcoholics by George Fein and Bennett Landman.

**Key Words:** Alcoholism, Brain Shrinkage, Neuropathology, Neuroimaging, Cognitive Deficits, Animal Model.

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**S**TUDIES DURING LIFE and after death have consistently revealed a reduction of the brain volume in alcoholic subjects (brain shrinkage). It is called 'shrinkage' because partial reversibility of the change has been reported in humans following significant periods of abstinence with concomitant improvement in cognitive function. The purpose of this symposium was to expand our understanding of the mechanisms underlying this change that could promote development of strategies for public education and new approaches to therapy. It is important for people who abuse alcohol to realize that some of the damage can be reversed. Moreover, there are specific factors in addition to alcohol that contribute to this shrinkage – for example asso-

ciated thiamin deficiency and the Wernicke/Korsakoff syndrome (WKS). Most of the data identifying the structural changes associated with brain shrinkage in alcoholics was derived from quantitative neuropathological studies using postmortem material. Technologies that can be employed to demonstrate and study this change *in vivo* include conventional structural magnetic resonance imaging (MRI), which yields high resolution images of regional brain morphology; magnetic resonance spectroscopy (MRS), which can measure major brain metabolites as well as track alcohol uptake and elimination kinetics in acute alcohol infusion experiments; MR diffusion tensor imaging, which is especially suited to interrogate the microstructure of white matter and functional MRI, which measures regional changes in blood oxygenation associated with changes in stimulus or response conditions. MRI is not limited to *in vivo* studies as high-resolution MRI scans of formalin-fixed brains can be obtained and these are invaluable for comparative ante and postmortem studies (Pfefferbaum et al., 2004c). Some of these neuroimaging techniques can also be applied to animal models of alcohol toxicity as discussed below. Other research tools that are useful include molecular methods using human postmortem tissues, particularly directed at changes in gene regulation and their 'downstream' proteins. The main focus of these studies has been the myelin-related genes and proteins as discussed below. Correlating imaging and cognitive studies of affected and recovering patients could also be useful in understanding this potentially reversible alcohol-related brain damage.

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REVIEW OF THE NEUROPATHOLOGICAL AND  
NEUROCHEMICAL CHANGES SEEN IN ALCOHOL-  
RELATED 'BRAIN SHRINKAGE'

*Clive Harper*

There are a number of ways in which alcohol is thought to affect the central nervous system. Direct neurotoxicity, the toxicity of metabolic by-products (e.g., acetaldehyde), the effects of secondary nutritional deficiency states and chronic liver disease have all been proposed to cause damage. These toxic, metabolic and nutritional factors interact in a complex fashion. Neuroradiological and neuropathological studies consistently reveal a reduction of the brain white matter volume in the cerebral hemispheres and cerebellum in uncomplicated alcoholic subjects (Harper and Kril, 1985; Pfefferbaum et al., 1995; Phillips et al., 1987). This has also been demonstrated in some animal models (Hansen et al., 1991). Technologies employed to demonstrate brain shrinkage include CT and MRI *in vivo* and quantitative neuropathology in autopsy studies. Partial reversibility of this change has also been reported in humans following significant periods of abstinence with concomitant improvement in cognitive function (Pfefferbaum et al., 1995). From a structural point of view, the decrease in the volume of white matter could be due to a change in extracellular space, a change of the nerve fibers within the white matter or a combination of these mechanisms (for *in vivo* evidence see (Pfefferbaum and Sullivan, 2004). There is a correlation between white matter loss and lifetime alcohol consumption, particularly in the cases that also have the WKS (caused by thiamin deficiency) (Kril et al., 1997). The shrinkage is reflected in thinning of specialized white matter structures such as the corpus callosum (Harper and Kril, 1988; Pfefferbaum et al., 1996). The reduced white matter volume is not related to changes in hydration or identifiable changes in the chemical structure of the myelin (Harper et al., 1988b; Harper et al., 1991). High-performance liquid chromatography was employed to study the effects of chronic alcohol consumption on the membrane lipid class composition of human brains (Olsson et al., 1996). Cholesterol, cerebroside, sulfatides, phospholipids and sphingolipids were measured in the gray and white matter of human brains. No significant differences were identified between the control and alcohol groups. Changes in the microstructure of the myelin sheaths could explain the reversible shrinkage. This has been noted in a mouse model of alcohol toxicity - when treated with alcohol for eight months the mean thickness of myelin sheaths of axons in the corpus callosum was 94nm compared with 115nm for the controls ( $p < 0.001$ ) (Unpublished data). Using a newly developed stereological method to measure myelinated fibers in the subcortical white matter of male alcoholics, Tang and colleagues found no significant differences between control and alcoholic subjects with respect to the total volume, total length, and mean diameter of myelinated fibers (Tang et al., 2004). However, this method might not be suitable for identifying subtle changes in myelin sheath thickness and the

authors noted that their findings do not exclude smaller or localized fiber changes in subregions of subcortical white matter.

Moreover, Lewohl and colleagues, using the same cases that were used in the human white matter volume studies, have shown a selective reprogramming of the expression of myelin related genes in the white matter (Lewohl et al., 2001). The neuropathological and related molecular studies discussed herein have been facilitated by the development of a brain bank that targets cases of alcohol-related disorders in Sydney, Australia (Harper et al., 2003). This bank or Tissue Resource Centre is supported by the National Institute on Alcohol Abuse and Alcoholism and provides tissues to research groups throughout the world for relevant alcohol-related projects.

Select populations of neurones appear to be susceptible to alcohol-related brain damage. There is a 20% reduction in numbers of neurones in the superior frontal cortex (Harper et al., 1987). This contributes to white matter loss due to Wallerian degeneration of myelinated axons but does not explain the partial reversibility of 'brain shrinkage' after significant periods of abstinence. Cortical neuronal dendritic arborization is also reduced in the alcoholic cases (Harper and Corbett, 1990) – another potentially reversible change. However, this would only impact on the volume of the cortical gray matter – not the white matter. A similar pattern of reversible shrinkage of dendrites has also been shown with abstinence in a rat model of chronic toxicity (McMullen et al., 1984).

Although alcohol alone appears to play a role in brain shrinkage, neuropathological studies suggest that vitamin B1 deficiency is a very important pathogenetic factor in this disorder (Harper, 1998). Similar reversible changes are described in anorexia nervosa where alcohol is not a causative factor. MRI studies reveal enlarged ventricles and high signal on T2-weighted images in subcortical regions (Drevelengas et al., 2001). There does appear to be a chemical change in the cerebral white matter as proton MRS in a patient with anorexia nervosa revealed a significant decrease of both myo-inositol and lipid compounds (Roser et al., 1999). There are precedents for changes in myelin sheaths in other diseases. In a study of progressive subcortical vascular encephalopathy of Binswanger type, nerve fibers had a tendency to have thinner myelin sheaths than in the control group but the difference was not significant (Yamanouchi et al., 1989).

IN VIVO DETECTION OF MACROSTRUCTURAL AND  
MICROSTRUCTURAL MARKERS OF BRAIN INTEGRITY  
IN HUMAN ALCOHOLISM AND A RODENT  
MODEL OF ALCOHOLISM

*Adolf Pfefferbaum, Elfar Adalsteinsson, and  
Edith V. Sullivan*

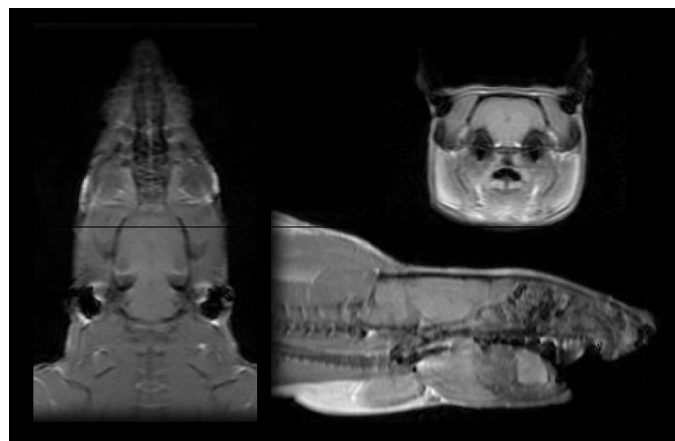
*In vivo* (Fein et al., 2002; Jernigan et al., 1991; Pfefferbaum et al., 1992; Pfefferbaum et al., 1997) and postmortem (Kril et al., 1997) studies report widespread gray mat-

ter pathology in prefrontal cortex and widespread white matter abnormalities as typical features in uncomplicated alcoholism. Controlled longitudinal MR structural imaging studies have revealed increase or decrease in gross gray or white matter brain volume depending on length of alcohol abstinence or amount of alcohol consumption by alcoholics (Pfefferbaum et al., 1995; Pfefferbaum et al., 1998; Shear et al., 1994). Because tissue volume recovery appears incomplete with abstinence, alcoholic brain pathology may have two components, one reflecting permanent change and one transient change (Carlen et al., 1978).

When complicated by nutritional deficiencies or electrolytic changes, the neurotoxic effects of alcohol on brain structure can be dramatic. Severe focal brain white matter pathology is a hallmark of several alcoholism-related clinical syndromes, such as Marchiafava-Bignami disease and central pontine myelinolysis (Victor et al., 1989). Thiamin deficiency (Martin and Fedio, 1983) has also been shown with animal models and by inference from patients with Wernicke-Korsakoff syndrome, to account for myelin loss (Harper, 1998; Langlais et al., 1996) and regional neuronal damage or loss in thalamus, mamillary bodies, and inferior colliculus (Victor et al., 1989). B12 vitamin deficiency is associated with peripheral neuropathy (Harper and Butterworth, 2002), although its role in central axonal loss has not been well established. Because malnutrition, liver disease, and head injury are frequent concomitants of chronic alcoholic use, these and other factors should be considered in addition to alcohol toxicity itself as direct factors contributing to brain abnormalities (Martin et al., 2003). Both Harper (1998) and Lancaster (1993) have argued, however, for an important role for alcohol consumption, per se, in the various white matter abnormalities for alcohol dependence. Supporting this position, a pathologic study (Krill et al., 1997) reported a relationship between reported maximum daily alcohol consumption and amount of white matter volume loss.

Translational neuroimaging studies focused on animal models of alcoholism can complement human research and permit control of factors not possible in naturalistic human study, including baseline measurement, nutrition, and amount, frequency, and type of alcohol consumed. Our initial animal MRI investigations have yielded novel data on both chronic and acute alcohol exposure of alcohol-preferring (P) rats (Li et al., 1979) and indicate substantial brain structural and metabolic variability that may underlie individual differences in alcoholism's untoward effects on brain structure and function.

We presented data from our longitudinal experiment of thiamin deficiency performed in 18 P-rats, which had prior exposure to either alcohol or water. Before and after the thiamin challenge, all animals were scanned with structural MRI protocols on a clinical 3T GE system, equipped with custom-made birdcage coils for rat brain imaging (Pfefferbaum et al., 2004a). All animals were fed thiamin-deficient rat chow for 15 days. In addition, 5 alcohol-exposed and 5



**Fig. 1.** Reformatted MR images of a 600 g Wistar rat collected on a human 3T clinical scanner equipped with a custom rat imaging RF coil. Slices were collected contiguously and were 0.5mm thick, permitting reformatting and reslicing in three orthogonal planes

water-exposed animals were given daily intraperitoneal (IP) doses of the thiamin-antagonist, pyriethiamin, whereas 4 alcohol-exposed and 4 water-exposed animals were given daily intraperitoneal doses of thiamin supplementation. Differences in MRI signal intensities between pre- and post-thiamin deficiency treatment revealed striking intensity increases, indicative of highly edematous tissue, in restricted brain regions, including the thalamus, inferior colliculus, mamillary nuclei, as well as enlarged lateral ventricles in animals in the thiamin-deficiency condition. The marked expansion of the ventricles replicates prior *in vivo* findings reported by Pentney and colleagues (Pentney et al., 1993).

The combined use of *in vivo* brain imaging with animal models of alcoholism provides unprecedented opportunities for systematic study of factors that have been suspected to cause alcohol-related syndromes or to interact with a history of excessive alcohol consumption to cause such syndromes. In the present experiment, longitudinal imaging enabled visualization and quantification of regionally specific pathologic changes resulting from thiamin deficiency. MR imaging modalities are safe and versatile. Principal modalities with broad clinical and research utility include conventional structural MRI, which yields high resolution images of regional brain morphology (Fig. 1); MR spectroscopy, which can measure major brain metabolites (Adalsteinsson et al., 2004) as well as track alcohol uptake and elimination kinetics in acute alcohol infusion experiments (Pfefferbaum et al., 2004b); MR diffusion tensor imaging, which is especially suited to interrogate the microstructure of white matter (Moseley et al., 1990); and functional MRI, which measures regional changes in blood oxygenation associated with changes in stimulus or response conditions (Febo et al., 2004). While each imaging modality has its own set of acquisition and analysis challenges, *in vivo* MR imaging can be applied in animals and

humans, making them useful vehicles for cross-sectional and longitudinal translational investigation.

#### GENE AND PROTEIN CHANGES IN THE BRAINS OF ALCOHOLICS WITH 'BRAIN SHRINKAGE'

*Joanne M Lewohl and Peter R Dodd*

Long-term alcohol abuse results in neurological and cognitive deficits in many alcoholic patients that are associated with localized neuropathological damage, including cerebral and cerebellar shrinkage. Studies of postmortem human brain confirm that there is a significant loss of brain tissue that can be correlated with lifetime alcohol consumption (Harding et al., 1996). Alcoholics have lower brain weights than controls (Harper and Blumbergs, 1982), and an increased peri-cerebral space which is predominantly due to a decrease in the volume of white matter in the cerebral cortex (de la Monte, 1988; Harper et al., 1985). There is also an increase in the size of the ventricles (de la Monte, 1988), widening of the cortical sulci, narrowing of the gyri and a regionally selective loss of certain populations of cortical and cerebellar neurons (Harper and Kril, 1990).

In an effort to understand the regional selectivity of the neuropathological effects of chronic alcohol abuse, genes that are differentially expressed in uncomplicated alcoholics were identified using DNA microarrays. This technique was used to generate expression profiles for the superior frontal cortex, a region of the brain that is particularly susceptible to alcoholic brain damage, and primary motor cortex, which is relatively spared. Comparison of the profiles revealed a selective reprogramming of the expression of myelin-related genes (Lewohl et al., 2000; Mayfield et al., 2002). A key question, however, is whether the changes in RNA expression so identified are reflected in altered protein expression. The expression of four myelin proteins – myelin basic protein (MBP), myelin proteolipid protein (PLP), myelin associated glycoprotein (MAG), cyclicnucleotide phosphodiesterase (CNP) – and an astrocytic marker, glial fibrillary acidic protein (GFAP), were measured in the superior frontal cortex and cerebellum of control and alcoholic cases. The subjects used were part of the original cohort used in pathologic studies, which included assessment of white matter volume changes (Harper, 1998). Overall, the expression of MBP and PLP showed similar patterns in different groups and may be concomitantly affected by chronic alcohol abuse. The difference in expression was most pronounced for PLP, which was differentially expressed between brain regions and case groups. CNP was expressed at greater levels in the superior frontal cortex than in cerebellum.

The extent of expression of myelin proteins, particularly MBP, was correlated with the age of the cases, suggesting that this protein declines naturally with advancing age. However, since the case groups used in the study were closely matched for age, this finding had no overall effect

on the analysis. The expression levels of each protein were also regressed on post mortem interval for combined subjects and for controls and alcoholics separately. None of the proteins studied declined significantly with postmortem delay.

The expression of myelin proteins was correlated with white matter volume, with a marked difference between controls and alcoholic cases. In general, the expression of myelin proteins increased proportionately to white matter volume in the controls, whereas the reverse was true for the alcoholic cases. This suggests that the structure of white matter may be altered in alcoholic cases, which may have a profound effect on the function of myelin sheaths in these brain areas. The expression of GFAP showed a somewhat different pattern. In alcoholic cases the level of GFAP expression was positively correlated with increasing white matter volume and advancing age in the cerebellum, suggestive of astrocytic proliferation in this brain region.

The effect of ethanol on myelin protein expression and myelination has primarily been studied in animal models of fetal alcohol syndrome. Maternal ethanol administration results in decreased myelination in the infant CNS (Lancaster, 1994). The offspring show decreased activity of CNP (Sedmak et al., 1978), and decreased expression of MBP (Ozer et al., 2000). The expression of MBP and MAG is also affected by exposure to alcohol during postnatal development, especially during periods of rapid myelination (Zoeller et al., 1994). Direct exposure of cultures of differentiating CG-4 oligodendrocytes to varying concentrations of ethanol alters the developmentally regulated expression of MBP but not CNP; these effects are dependent on the timing of ethanol exposure (Bichenkov and Ellingson, 2001). Cell culture studies have also shown that myelin protein expression is modulated by protein kinase C activity (Asotra and Macklin, 1993) and the effect of alcohol administration on MBP expression is counteracted by protein kinase C inhibitors (Bichenkov and Ellingson, 2002), suggesting that ethanol down-regulates MBP expression by activating PKC.

Neuroimaging studies have shown that a portion of the white matter loss that occurs with chronic alcohol abuse is reversible with abstinence (Pfefferbaum et al., 1995; Shear et al., 1994). Hence, the white matter loss seen in chronic alcoholic abuse may be the result of a combination of irreversible axonal degeneration and neuronal death, and reversible changes in myelination. Alcohol-induced changes in myelin gene expression may play a role in this reversibility. In this study the expression of MBP and PLP decreased with increasing white matter volume in alcoholic cases, while the reverse was true in controls. This finding suggests that an alteration in the protein composition of myelin occurs independently of any loss of white matter volume. Alternatively, the white matter volumes of some of the alcoholics may indicate that these cases have experienced some reversal of their white matter loss but that the

protein composition of their myelin sheaths is somewhat different from that seen in controls.

PLP and MBP are the major myelin proteins in the CNS and comprise approximately 50% and 30% of total CNS myelin proteins respectively (Eng et al., 1968). These proteins are required for the highly ordered and compact structure of myelin and are specifically involved in stabilization and compaction of the myelin sheath (Boison and Stoffel, 1994; Omlin et al., 1982; Weimbs and Stoffel, 1992). PLP and MBP are present in comparable amounts in myelin. Hence, alteration of the amount of PLP, in the absence of alterations in the expression of other myelin proteins, is likely to alter the structure and function of the myelin sheath and ultimately the conduction of action potentials. The alteration in expression is suggestive of a selective effect of chronic alcohol abuse or alcohol-related problem on PLP expression and should be investigated further.

#### CROSS-SECTIONAL AND LONGITUDINAL MR SPECTROSCOPY STUDIES OF CHRONIC ADULT ALCOHOLICS

*Michael J. Taylor*

Studies using various methodologies including structural MRI, neuropathology, and neuropsychology have demonstrated the deleterious effects of chronic alcoholism on the brain. Brain imaging and neuropsychological studies have also suggested improvement in the structure and function of the brain with continued abstinence. Several studies have been conducted in our laboratory at the VA San Diego Healthcare System using proton magnetic resonance spectroscopy (MRS) to evaluate the effects of chronic alcoholism on the brain and subsequent improvement with long-term abstinence. We have also used MRS to investigate the apparent susceptibility of the frontal lobes to alcohol-associated brain damage and the potential for greater brain impairment in alcoholics with a history of alcohol-withdrawal seizures.

MRS permits estimation of metabolite concentrations within anatomically localized regions of interest in the brain. In vivo studies of neurologic disease utilizing proton MRS have typically focused on the metabolites N-acetylaspartate (NAA), choline (a group of N-trimethyl compounds), creatine (free creatine plus phosphocreatine), and myo-Inositol. Typically, data are presented as ratios (NAA/Creatine), though recent technology permits estimations of absolute concentrations of metabolites. NAA is of interest because it is localized exclusively in neurons and neural processes. Therefore, reduction of NAA may reflect neuronal damage (retraction on neural processes or dendritic simplification) or cell loss. Choline compounds are widely distributed in the brain. Changes in choline signal could reflect alteration in the lipid constituents of cell membranes, whereas myo-Inositol increase might reflect glial activation, molecular shifts related to osmolar changes, or other processes reflective of cellular stress.

In our largest cross-sectional study, we compared age-matched groups of 100 recently detoxified alcoholics (mean age 43.9 years) with 20 nonalcoholic controls (mean age 41.0 years). Both groups were predominantly Caucasian males. The alcoholics had been sober from four to six weeks, had been drinking at least six drinks per day for the most recent five year period, and had been alcoholic for an average of 15 years. The alcoholic group had significantly lower NAA (ranging from 4.9% and 9.6%) in frontal white matter, frontal gray matter, and parietal white matter compared with controls. Concentrations of choline were also 5.5% lower in alcoholics compared with controls. Lower NAA in frontal white matter was significantly correlated with poorer performance on the Halstead Category Test (a measure of frontal/executive functioning). In addition, those alcoholics who were impaired on this test had 6.3% lower NAA compared to those who were not impaired.

The frontal lobes may be particularly vulnerable to the effects of alcoholism (Pfefferbaum et al., 1997). One study from our laboratory (Schweinsburg et al., 2001) addressed this issue using MRS to measure concentrations of NAA in frontal white matter and parietal white matter of 37 recently detoxified alcoholics (mean age 40.4 years; mean length of abstinence, 27.9 days) and 15 controls (mean age 38.0 years) to determine if a dissociation was evident. A statistically significant 14.7% reduction in frontal white matter NAA of alcoholics was observed compared with controls, whereas NAA levels in parietal white matter were similar in alcoholics and controls. Reductions in frontal white matter NAA were associated with a longer drinking history in the recently detoxified alcoholics group, but this result was not found when both age and drinking history were used as predictors. It is suggested that while alcohol-induced oxidative stress may cause global brain impairments in the metabolism and subsequent reduction of NAA, the frontal lobes are particularly rich in excitatory amino acid pathways, and axonal damage or destruction secondary to glutamate-mediated excitotoxicity during alcohol withdrawal might contribute to frontal lobe-specific reductions in NAA.

Examination of alcoholics who have had alcohol withdrawal seizures may provide indirect evidence of excitotoxicity-mediated brain injury. Supporting this, Sullivan and colleagues reported that alcoholics with a history of at least one withdrawal seizure had significantly lower temporal lobe white matter volume compared with alcoholic individuals without a history of seizure and nonalcoholic controls (Sullivan et al., 1996). We hypothesized that alcoholics with a past history of alcohol-related seizure would have reduced concentrations of NAA compared to alcoholics who report never having an alcohol withdrawal seizure and nonalcoholic control participants. In this study (Schweinsburg et al., 2002), MRS scans were performed on 10 recently detoxified alcoholics with a history of seizures, 16 recently detoxified alcoholics without a history of seizures, and 10 nonalcoholic controls.

The alcohol-withdrawal seizure group had 13.1% lower

NAA in frontal white matter compared to controls ( $p = 0.016$ ). In addition, there was a trend toward lower frontal white matter NAA in the alcohol-withdrawal seizure group compared to the alcoholics without a history of seizures ( $p = 0.081$ ). A similar pattern of results was evident in parietal white matter metabolites, although the results did not reach statistical significance.

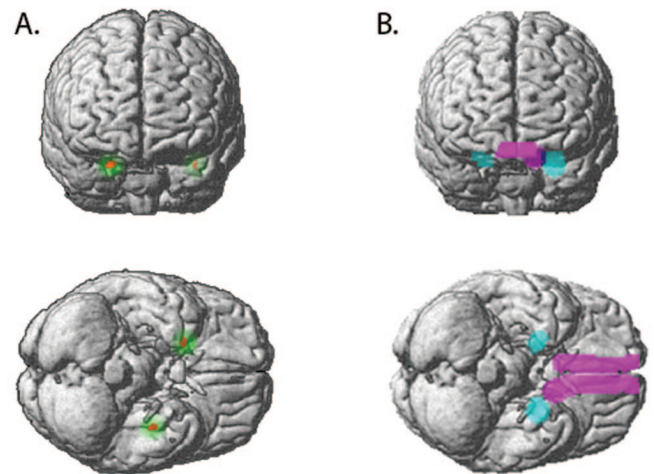
In a longitudinal study of alcoholics re-scanned after approximately two years we have found evidence for statistically significant 5.4% improvement in NAA/creatinine and 5.8% improvement in choline/creatinine in alcoholics who maintain abstinence. By contrast, alcoholics who relapsed during the follow-up period did not evidence such an improvement (1.4% improvement in NAA/creatinine and 1.0% improvement in choline/creatinine). These data suggest at least partial recovery from alcohol induced brain damage, and are consistent with neuropsychological studies indicating recovery of brain function with continued abstinence.

#### BRAIN ATROPHY ASSOCIATED WITH IMPAIRMENT ON A SIMULATED GAMBLING TASK IN LONG-TERM ABSTINENT ALCOHOLICS

*George Fein and Bennett Landman*

The simulated gambling task (SGT) was initially developed to study patients with acquired sociopathy due to damage to the ventromedial prefrontal cortex (VMPF) (Bechara et al., 1994; Bechara et al., 1997). VMPF patients often persist in behaviors that are immediately gratifying while ignoring negative future outcomes. It is hypothesized that they cannot see beyond short-term rewards to possible long-term negative consequences. It is almost axiomatic that substance abusers also make decisions in this mode. It is not surprising that currently active or recently detoxified substance abusers also make disadvantageous decisions on the SGT (Grant et al., 2000; Petry et al., 1998). Fein et al. (2004) recently extended that work to show that multi-year abstinent alcoholics, without antisocial personality disorder or conduct disorder, also have SGT impairments. Bechara and colleagues have extended their work to show that patients with amygdala damage also show SGT impairments (Bechara et al., 1999).

Fein and Landman presented imaging data on the sample of multi-year abstinent alcoholics and controls in whom they had previously demonstrated SGT impairments. That sample consisted of 44 abstinent alcoholics with a mean of 6.6 years of abstinence and 58 age and gender comparable controls. Their a priori hypothesis was that this sample would show atrophy in the regions of the VMPF and amygdala. T1-weighted MRIs were examined using a slight variant of the Voxel-Based Morphometry procedure developed by Baron and colleagues, (Baron et al., 2001): 1) the skull and scalp were removed using the FSL brain extraction tool, augmented by manual editing 2) the FSL Automated Segmentation Tool was used to segment the imaged brains into gray matter, white matter, and cerebro-spinal fluid 3)



**Fig. 2.** The two A images show two regions of reduced gray matter in abstinent alcoholics compared to their controls. These regions are displayed on an illustrative single subject template available in Statistical Parametric Mapping - Version 2. The red colored voxels denote the regions of reduced gray matter with a corrected  $p$ -value of less than 0.05. The green regions surrounding the red regions denotes the spatial uncertainty of the red regions. The B images show the VMPF (magenta) and amygdala (blue), as defined in the Talairach Daemon resource, superimposed on this single subject template.

the gray matter images were normalized to the Montreal Neurological Institute gray matter template (derived from the MNI-152 - the average of T1-weighted images from 152 normal individuals) 4) SPM2 (Statistical Parametric Mapping - Version 2) was used to smooth the gray matter images with a 12 mm FWHM Gaussian filter, and 5) SPM2 was then used to analyze the resulting images using their implementation of the General Linear Model.

Fig. 2A shows two regions of reduced gray matter in Fein and Landman's abstinent alcoholics compared with their controls. These regions are displayed on an illustrative single subject template available in SPM2. The Fig. 2B images show the VMPF and amygdala (as defined in the Talairach Daemon resource) superimposed on this single subject template. The asymmetries of these structures in the single subject template are illustrative of the spatial variability of anatomically defined regions across subjects. With caution related to that variability, comparing figures A and B, Fein suggests that the abstinent alcoholics demonstrate atrophy in the region of the amygdala.

Fein and colleagues have shown that alcoholics can achieve long-term abstinence despite persistent deficits in decision-making (Fein et al., 2004). In the current investigation they show that these decision-making deficits are associated with reduced gray matter in brain regions previously implicated in similar decision-making impairments in neurological samples. Thus, the decision-making deficits in these long-term abstinent alcoholics reflect an abnormality in brain structure.

This structurally based abnormality is likely to be the result of long-term alcohol abuse or dependence. However, it is also possible that the structural abnormality reflects a

pre-existing factor that predisposes to severe alcoholism. It is suggested that the latter possibility can be evaluated by structural brain imaging / SGT studies of samples at high genetic risk for alcoholism and of samples examined early in their alcohol abuse/dependence history.

#### SUMMARY

*Conclusions and future directions.* Brain shrinkage, quantified *in vivo*, is present in a high proportion of alcoholics and has even been demonstrated postmortem in those drinking between five and eight standard drinks each day (Harper et al., 1988a). In this latter group the volume of the cerebrospinal fluid (CSF) covering the brain was measured by subtracting the brain volume from the total intracranial volume and expressing the result as a percentage of intracranial volume (Harper et al., 1984). The percentage was increased from 7.3% in the control group to 9.8% in those drinking five to eight standard drinks per day. Those drinking more than eight standard drinks per day measured 11.0% and alcoholics with associated Wernicke/Korsakoff syndrome measured 15.9% (Harper et al., 1988a). Thus, the reduction of brain volume in this latter group is 8.6% or approximately 130 cc of brain tissue.

MRI studies have confirmed that some of this reduction in brain volume can be reversed by abstinence. Shear and colleagues followed 15 alcoholic subjects who abstained for three to four months and 13 of the 15 showed a reduction in CSF volume – that is, an increase in brain volume. In 11 of these cases the increase in volume was shown to be in the white matter compartment (Shear et al., 1994). Fein and Landman showed a volume reduction in the amygdala region in alcoholics with very long term abstinence. This might represent a partial recovery of volume, but nonetheless, significant atrophy remains, and this is associated with impaired abilities to weigh rewards and punishments in an atmosphere of uncertain outcomes.

MRS is another potentially useful tool for studying white matter changes. Recently detoxified alcoholics had an 11.8% increase in brain white matter myo-inositol relative to controls which may reflect astrocyte proliferation as well as an osmotic response to cell shrinkage (Schweinsburg et al., 2001). In another study concentrations of choline were 5.5% lower in alcoholics compared with controls. Changes in the choline signal could reflect alteration in the lipid constituents of cell membranes, whereas myo-Inositol increase might reflect glial activation, molecular shifts related to osmolar changes, or other processes reflective of cellular stress.

As discussed above, brain shrinkage has been confirmed by several neuropathological studies (de la Monte, 1988; Harper, 1998) and in animal experimental models (Hansen et al., 1991). The prefrontal white matter is the most severely affected region and there is a negative correlation between the white matter loss and life-time alcohol intake (Harding et al., 1996; Kril et al., 1997). There is a greater

proportion of white matter compared with cortical gray matter in frontal regions which might explain this finding – the ratio of gray matter with white matter is 1.22 in the frontal region and 1.40 in occipital lobes (Harper et al., 1998a). Microscopically, there are no obvious lesions in the white matter of the cerebral hemispheres of uncomplicated alcoholic subjects. The subtle nature of the white matter changes in the cerebral hemispheres is borne out by physical and chemical studies of the white matter that show very little change (Harper et al., 1988a). However, it should be noted that there are almost certainly two different abnormalities of the white matter. One is a permanent loss of white matter related to axonal degeneration subsequent to neuronal loss in cortical and/or subcortical regions. The second component appears to be a subtle structural change, probably in the microstructure of the myelin sheaths, which could account for the reversible white matter damage. This has been noted in a mouse model of alcohol toxicity – when treated with alcohol for eight months the mean thickness of myelin sheaths of axons in the corpus callosum was 94nm compared with 115nm for the controls ( $p < 0.001$ ).

Using a molecular approach Drs. Lewohl, Dodd and associates have used the same brain bank cases used in pathologic studies to look at changes in gene regulation and related proteins. The difference in gene expression was most pronounced for PLP, one of the major myelin proteins in the CNS. PLP and MBP are required for the highly ordered and compact structure of myelin and are specifically involved in stabilization and compaction of the myelin sheath (Boison and Stoffel, 1994; Omlin et al., 1982; Weimbs and Stoffel, 1992). Any alterations are likely to alter the structure and function of the myelin sheath and ultimately, the conduction of action potentials. The alteration in expression is suggestive of a selective effect of chronic alcohol abuse or alcohol-related problem on PLP expression and should be investigated further. REFS

There are precedents for changes in myelin sheaths in other diseases. In a study of progressive subcortical vascular encephalopathy of Binswanger type, nerve fibers had a tendency to have thinner myelin sheaths than in the control group but the difference was not significant (Yamanouchi et al., 1989). The study of cases of anorexia nervosa might also be fruitful as they appear to develop similar reversible white matter shrinkage (Drevelengas et al., 2001).

*Cognitive aspects.* The importance of understanding the mechanisms associated with this brain shrinkage is evident as it also correlates with cognitive deficits that are, at least partially, reversible. Functional sequelae of alcoholism that improve or reverse with abstinence include impairment in working memory, postural stability, and visuospatial ability (Brandt et al., 1983; Rosenbloom et al., 2004; Sullivan et al., 2000b). One study showed that the extent of shrinkage, that is, normalization, of the third ventricle was correlated with the degree of improvement in visual working memory, visuospatial ability, and visual long-term memory (Sullivan et al., 2000b).

The volume of white matter of the cerebellum is also reduced in alcoholics (Phillips et al., 1987; Sullivan et al., 2000a). Given the recent interest and potential importance of the cerebellum in executive function, abnormalities of the white matter (Schmahmann, 1997) and the critical role that the cerebellum and its input and output tracts serve in cerebellar-cerebral loops targeted by alcoholism (Sullivan, 2003). Connecting white matter tracts should be examined for disruption with drinking and recovery with abstinence. Studies based on MR diffusion tensor imaging, which is an *in vivo* method that provides an index of microstructural integrity of white matter, should provide the best quantification of such white matter systems (Pfefferbaum and Sullivan, 2004; Sullivan and Pfefferbaum, 2003).

Functional MRI, which measures regional changes in blood oxygenation associated with changes in stimulus or response conditions (Febo et al., 2004) tends to highlight regions of neuronal excitation rather than white matter pathways that form the interconnections between neuronal groups. Therefore, it is less likely to be useful in the context of studying white matter changes. Another brain region that warrants further investigation regarding specific cognitive deficits is the amygdala. Fein and Landman noted atrophy in the amygdala in their cohort of long-term abstinent alcoholics and have suggested that it could be the anatomic correlate for impaired decision-making.

*Clinical implications.* It is important that we develop a better understanding of the mechanisms underlying this white matter change. It could be important in developing strategies for public education and new approaches to therapy. People who abuse alcohol should be informed that some of the brain damage could be reversed. Moreover, there are specific factors, other than alcohol, that contribute to this shrinkage (associated thiamin deficiency) that can be treated or, better still, prevented with well-planned nutritional regimes or food enrichment programs (Harper et al., 1998b).

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