

Age-Related Gray Matter Shrinkage in a Treatment Naïve Actively Drinking Alcohol-Dependent Sample

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Background: We previously demonstrated, in a small sample, steeper age-related gray matter shrinkage in treatment naïve alcohol-dependent (TxN) men compared to nonalcoholic controls, but could not separate out the contributions of age and lifetime duration of alcohol use (which were highly correlated) to this effect. In the current study, we have quadrupled the sample size and expanded it to include both men and women to try to replicate and extend the previous findings and to separate the contributions of age and alcohol use to the phenomenon.

Methods: In the current study, we examine cortical gray matter volumes in 18- to 50-year-old TxN ($n = 84$) versus age and gender comparable controls ($n = 67$). We used a new Region of Interest Analysis method which accounts for differences in sulcal and gyral enfolding between individuals (Fein et al., 2009a).

Results: We found greater age-related gray matter shrinkage in TxN than in controls. Partial correlation analysis showed that the effect was a function of age and not lifetime alcohol burden.

Conclusions: Implications of the findings are discussed in terms of their contribution toward our knowledge of differences between different subpopulations of alcoholics and in terms of their implications for the morbidity of alcohol dependence in an aging national population.

Key Words: Alcohol Dependence, Structural MRI, Brain Shrinkage, Gray Matter, Aging

HERE IS AN ever-growing body of evidence documenting the adverse effects of alcohol dependence on the brain. Structural magnetic resonance imaging has revealed an association between alcohol dependence and cortical gray matter shrinkage (Sachdev et al., 2008), and hazardous drinking alone has been shown to damage the brain (Garcia-Valdecasas-Campelo et al., 2007; Jernigan et al., 1991; Kril et al., 1997; Paul et al., 2008). Most studies on the effects of alcohol dependence on brain structure have used treated samples, leading to a bias known as Berkson's Fallacy (Berkson, 1946, 1955), which arises when the association between variables, such as alcoholism and brain morphology, differs between the sample studied (i.e., treated alcoholics) and the population to which the results are generalized (all alcoholics).

Many previous studies on brain structure in alcohol-dependent individuals agree on finding smaller gray matter volumes in areas of the cerebral cortex, although the localities of these reductions occasionally disagree. In an early MRI study, Jernigan and colleagues (1991) found cerebral gray matter reductions in the frontal, parietal, and mesial temporal

lobes of treated alcoholics. Other more recent studies on treated alcoholics have found reductions within the dorsolateral prefrontal cortex and insula (Makris et al., 2008), the precentral gyrus, middle central gyrus, and insula (Mechtcheriakov et al., 2007), and the lateral cortices (Shear et al., 1992). In a recent analysis of middle-aged long-term abstinent alcoholics, we found reduced gray matter volumes in the parietal and occipital lobes (Fein et al., 2009a).

The fact that the majority of the publications on alcohol research are based on a disproportionately large sampling of the treated subset of alcoholics must be considered when extending conclusions to the general alcoholic population. In contrast to treated alcoholics, untreated alcoholics comprise the majority of alcohol-dependent individuals in the United States. According to the 2001 to 2002 National Epidemiologic Survey of Alcoholism and Related Conditions (NESARC), of the 4,422 respondents who met criteria for prior-to-past-year alcohol dependence, only 25.5% reported ever having received treatment (Dawson et al., 2005). In addition to being a majority of the alcohol-dependent population, treatment naïve alcoholics have less severe alcoholism (Fein and Landman, 2005), less severe comorbid psychiatric illness (Di Sclafani et al., 2008), evidence less impaired performance on decision-making tasks, and do not suffer from the full extent of the tissue loss seen in treated alcoholics (Gazdzinski et al., 2008). In light of this evidence, generalizing results from studies on treated samples, which comprise the bulk of the current literature, to the general alcohol-dependent population may not be valid.

The relatively few studies done on untreated alcohol-dependent individuals have yielded mixed results. Our 2002

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study comparing treatment-naïve alcoholics ($N = 24$) and light drinkers ($N = 17$) found no significant group differences in cortical gray matter volumes (Fein et al., 2002), but did find stronger relationship between age and overall cortical gray matter shrinkage in the alcohol-dependent group. In a study by Cardenas and colleagues (2005), actively drinking untreated alcohol-dependent individuals ($N = 49$) presented with significantly lower gray matter volumes than light drinkers ($N = 49$) in all lobes except the frontal lobe. In a comparison of treated and untreated alcohol-dependent individuals versus controls, Gazdzinski and colleagues (2008) found numerically but not significantly lower gray matter volumes in the untreated group compared with controls, whereas those in the treated sample had more shrinkage in the frontal, occipital, parietal, and temporal lobes than the untreated sample.

A potential confounding factor in alcohol research is normal age-related brain shrinkage accompanied by declines in mental abilities. It is widely accepted that brain volume is lost in normal aging, and the total lifetime alcohol consumption often correlates with age due to longer periods of drinking. While the cumulative effects of long periods of drinking may be difficult to separate from the normal atrophic effects of age, the effects of extended periods of heavy drinking have been shown to independently contribute to brain volume declines. Early CT- and MRI-based studies by Pfefferbaum and colleagues (1992, 1993) reported that the rate of age-related brain tissue loss, measured by decreases in gray matter and white matter or increases in cerebrospinal fluid, is larger in older alcoholics than in younger alcoholics compared with controls. A later study by the same group found that alcoholics ranging in age from 45 to 63 years had significant gray and white matter deficits compared with age-matched controls, and significantly more volume loss in the prefrontal cortex compared to younger alcoholics between 26 and 44 years old (Pfefferbaum et al., 1997). The presence of the age-related differences despite the lack of group differences in disease duration and total estimated lifetime alcohol consumption in the cohort studied led Pfefferbaum and colleagues to the conclusion that the accelerated age-related brain volume declines in older alcoholics was due to the increased vulnerability of the aging brain rather than a cumulative effect of alcohol consumed.

The purpose of the current study was to assess volumetric differences in the cerebral cortex of treatment-naïve alcoholics (TxN) compared to light- or nondrinking nonalcoholic controls using structural MRI. Our previous study on a separate sample of TxN men found greater age-related volume reductions in TxN than in controls. There was a strong inverse association between brain size and lifetime duration of alcohol use, but because the duration of alcohol use was highly correlated with age, these measures were highly confounded in this sample ($n = 24$), and could not be disentangled. Moreover, we did not find any associations between regional cortical gray matter volumes with total or average lifetime alcohol consumption, supporting our previous assertion that treated and untreated alcoholics come from different populations. With the larger sample size of the current study

($N_{\text{TxN}} = 84$, $N_{\text{controls}} = 67$), we hoped to further explore the relationship between age and cortical gray matter volumes in both male and female alcoholics versus controls, and to have power to separate the contributions of age and alcohol use measures to this association.

MATERIALS AND METHODS

Participants

The sample for this study consisted of treatment-naïve actively drinking alcohol-dependent individuals (TxN, 49 men and 35 women, ranging in age from 19 to 51 years), and age and gender comparable light/nondrinking controls (39 men and 28 women, ranging in age from 18 to 51 years). Both groups had comparable years of education. All participants were recruited from the community through restaurant and bar postings, newspaper advertisements, and a local internet site. TxN participants met DSM-IV (American Psychiatric Association, 2000) criteria for current alcohol dependence and were actively drinking. Information on each subject's lifetime drinking history was obtained during an interview covering lifetime use of alcohol and drugs and broken into phases of when use changed significantly using a timeline follow back procedure (Skinner and Sheu, 1982). Average lifetime consumption and average consumption during periods of peak drinking were computed as monthly totals. The inclusion criteria for the control group was a lifetime drinking average of less than 30 drinks per month, with no periods of drinking more than 60 drinks per month. Exclusion criteria for both groups were (i) lifetime or current diagnosis of schizophrenia or schizophreniform disorder, (ii) history of drug (other than nicotine or caffeine) dependence or abuse, (iii) significant history of head trauma or cranial surgery, (iv) history of diabetes, stroke, or hypertension that required medical intervention, or of other significant neurological disease, or (v) clinical evidence of Wernicke-Korsakoff syndrome. Subjects underwent 4 sessions of clinical, neuropsychological, electrophysiological, and MRI assessments. All subjects were administered the computerized version of the Diagnostic Interview Schedule in which Mood, Anxiety, and Externalizing Disorder symptom counts and diagnoses were measured. This data has been published previously where we showed that TxN compared to controls had more psychiatric symptoms, but not more diagnoses (Di Sclafani et al., 2008). Moreover, TxN had fewer symptoms and diagnoses than Long-Term Abstinent Treated Alcoholics. A breathalyzer was used to confirm abstinence before each session.

MR Acquisition

All MRIs were collected on a 1.5T GE Signa Infinity with the LX platform (GE Medical Systems, Waukesha, WI) located at the Pacific Campus of the California Pacific Medical Center in San Francisco. For each subject, we acquired a transaxial T1-weighted Spoiled Gradient image (TR = 35 ms, TE = 5 ms, acquisition matrix = 256×192) at 1.3 mm thickness and a Fluid Attenuated Inversion Recovery (FLAIR) image (TR = 8800 ms, TE = 144.7 ms, inversion time = 2200 ms, acquisition matrix = 256×256) at 5 mm thickness. A neuroradiologist read all MRI scans. All scans were free from abnormalities other than white matter signal hyperintensities (WMSH). Both image types had an in-plane resolution of 0.86×0.86 mm.

Image Processing

Preprocessing of the data, including skull removal, registration, segmentation, and noise reduction was performed using FMRIB's Software Library (FSL), version 4.1 (Oxford, U.K.).

Region of Interest Definitions

In the Talairach coordinate system (Talairach and Tournoux, 1988), the brain is subdivided into a 12 (superior-inferior) \times 9 (anterior-posterior) \times 8 (lateral) grid, with a total of 864 compartments. The 5 cerebral regions of interest (frontal, limbic, occipital, parietal, and temporal) were defined in the Talairach atlas using the Talairach Daemon resource (Lancaster et al., 2000), as were many of the subcortical structures bounding these lobes. We transformed the Talairach ROIs (regions of interest) into standard Montreal Neurological Institute (MNI) space using a nonlinear transformation (Brett, 1999). We then used nearest-neighbor interpolation to assign every voxel in MNI space to its closest defined region. This nearest neighbor approach allowed the ROI masks to reliably capture the gray matter boundaries in subject space after registration. See Fig. 1 for an example of the lobar definitions used in the analysis.

White Matter Signal Hyperintensity Correction

White matter lesions appear darker than the surrounding white matter in T1-weighted images, often causing automated segmentation algorithms to mislabel these areas as gray matter or cerebrospinal fluid (CSF). Automated methods were used to delineate these white matter lesions on FLAIR images, where they appear as WMSH. To prepare the FLAIR images for WMSH delineation, each subject's T1-weighted image was registered to his or her own FLAIR image using a 6 degree-of-freedom registration (without deformation or scaling). Each subject's hand-edited brain mask was then transformed from T1 space into FLAIR space and used to remove the skull from the T1 and FLAIR images. FSL's multispectral segmentation tool (FAST) then used both images to create 3-class segmentation in FLAIR space. After these preprocessing steps, an in-house application was used to detect and delineate white matter lesions based on intensity and location (Fein et al., 2009). The resulting WMSH mask was transformed back into T1 space and used to reclassify affected areas as white matter. The corrected segmentation was then used for gray matter region measurements.

Cortical Gray Matter Volume Measurement

The gray matter volumes of the 5 lobes were automatically computed using computer-based methods. The image processing engineer was blind to subject demographics and group membership. The T1-weighted image was used for segmentation into tissue categories. The

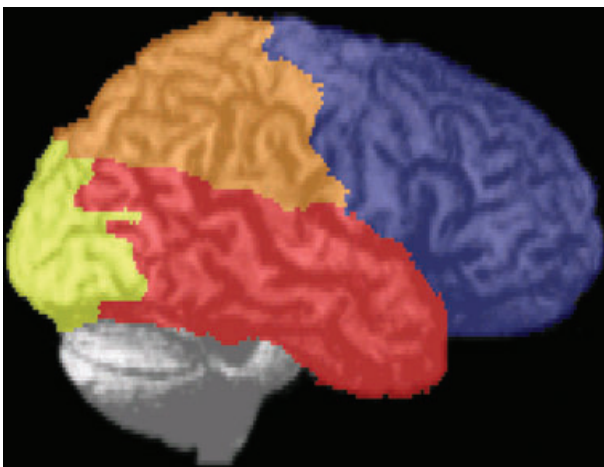


Fig. 1. The regions of interest defining the frontal lobe (blue), parietal lobe (orange), occipital lobe (yellow), and temporal lobe (red) overlaid on the gray matter of a 45-year-old male control subject. The limbic lobe was not included in the image because it is not on the surface of the brain.

segmentation process began with automatic stripping of the skull from the images using FSL Brain Extraction Tool (BET) (Smith, 2002), followed by manual corrections by trained technicians. After skull removal, the MNI 152 average brain was registered to each subject's T1-weighted image with the skull removed using an affine transformation with 12 degrees of freedom. This was done using FMRIB's Linear Image Registration Tool (FLIRT) (Jenkinson and Smith, 2001), and the transformation matrix was used to transform pre-defined ROIs of the cerebral lobes and sublobar regions from MNI space into subject space. A 3-class tissue segmentation (gray matter, white matter, and cerebrospinal fluid) was then performed on the skull-stripped T1-weighted image using FMRIB's Automated Segmentation Tool (FAST) (Zhang et al., 2001). In-house software was used to perform gyral and sulcal correction to accurately capture the gyri belonging to each lobe (a full description of this method is presented in Fein and colleagues (2009a)). The resulting ROIs were specific to each subject but reflect a common Talairach definition.

Statistics

Statistical analyses were performed using the General Linear Model implementation of analysis of covariance within the Statistical Analysis Software (SAS), version 9.1.3. Since we are interested in inferring shrinkage (i.e., loss of gray matter) from our gray matter volumes, we first used a linear regression analysis to adjust each subject's gray matter volumes for premorbid brain size using the cranium size index from FSL's SIENAX program. We have previously shown that the cranium size index has an almost perfect correlation with intracranial vault volume (Fein et al., 2004). We examined age by group and age by sex interaction effects to test for the appropriateness of using age as a covariate (i.e., no significant age by group or age by sex interactions). All effects were statistically significant at the 0.05 significance level.

RESULTS

Demographic and Alcohol Use Measures

The demographic and alcohol use measures for both groups are summarized in Table 1. The control and TxN groups did not differ in age, years of education, or estimated verbal IQ (effect sizes $< 1\%$, p 's > 0.23). There was a strong trend for a greater density of problem drinking in the family histories of TxN versus controls (effect size = 2%, $p = 0.072$). Within TxN, men had a significantly higher average lifetime alcohol dose (measured in standard drinks per month) than women ($F_{1, 82} = 15.52$, $p < 0.001$, effect size = 15.91). However, using weight as a covariate removed this effect ($F_{3, 80} = 0.85$, $p = 0.36$).

Cranium Size

Men had craniums 12.5% larger than that of women, with sex accounting for 36.7% of the variance in head size between subjects ($F_{3, 147} = 85.05$, $p < 0.001$). There was a trend for larger craniums in the TxN men and women ($F_{3, 147} = 3.02$, $p = 0.08$), with group accounting for 2% of the sample variance. There were no group by sex interactions ($F_{3, 147} = 0.37$, $p = 0.54$, effect size = 0.2%). We adjusted the volumetric data to account for between subjects differences in head size for use in all subsequent gray matter correlation analyses.

Table 1. Sample Demographics

| Demographic variables | Nonalcoholic controls | | Treatment-naïve alcoholics | | Effect size (%) ^a | | |
|---|-----------------------|--------------------|----------------------------|--------------------|------------------------------|-------------------|-------------------|
| | Male (n = 39) | Female (n = 28) | Male (n = 49) | Female (n = 35) | Group | Sex | Group × Sex |
| Age (years) | 32.2 ± 8.3 | 32.4 ± 8.8 | 31.4 ± 7.8 | 30.7 ± 7.8 | 0.6 | <0.1 | <0.1 |
| Family drinking density ^b | 0.12 ± 0.17 | 0.13 ± 0.24 | 0.14 ± 0.20 | 0.25 ± 0.29 | 2.0 | 0.9 | 1.6 |
| Years education | 16.0 ± 1.8 | 16.3 ± 1.4 | 16.1 ± 1.8 | 15.9 ± 1.6 | 0.1 | <0.1 | 0.5 |
| AMNART (estimated premorbid verbal IQ) ^c | 1.22 ± 0.41 | 1.12 ± 0.48 | 1.29 ± 0.33 | 1.19 ± 0.36 | 0.9 | 1.6 | <0.1 |
| Alcohol use variables | | | | | | | |
| Duration of active drinking (months) | 134 ± 93 | 132 ± 110 | 183 ± 96 | 177 ± 88 | 5.5 ^d | <0.1 ^d | <0.1 ^d |
| Average lifetime drinking dose (std. drinks/month) | 6.9 ± 6.9 | 5.2 ± 4.6 | 100 ± 46 | 66 ± 31 | 61.5 ^d | 8.3 ^d | 6.9 ^d |
| Lifetime alcohol use (std. drinks) | 989 ± 1,303 | 809 ± 1,139 | 17,600 ± 11,500 | 11,600 ± 7,300 | 45.1 ^d | 4.0 ^d | 3.5 ^d |
| Duration of peak drinking (months) | 75 ± 109 | 80 ± 116 | 53 ± 50 | 52 ± 55 | 2.2 ^d | <0.1 ^d | <0.1 ^d |
| Average peak drinking dose (std. drinks/month) | 15.3 ± 16.0 | 11.5 ± 10.8 | 182 ± 121 | 122 ± 65 | 44.6 ^d | 4.1 ^d | 3.2 ^d |
| Peak alcohol use (std. drinks) | 600 ± 905 | 505 ± 772 | 8,400 ± 9,100 | 5,700 ± 5,200 | 23.6 ^d | 4.4 ^d | 1.2 ^d |

^aEffect size is presented as percent of variance accounted for by the named effect:

$$\eta_p^2 = \text{SS}_{\text{effect}} / (\text{SS}_{\text{effect}} + \text{SS}_{\text{error}}) \times 100\%$$

^bFamily drinking density is the proportion of first-degree relatives who were problem drinkers; statistical comparisons and estimates of effect size for family drinking density were performed after normalizing the proportions via the arcsine transformation. We do not have family drinking history information for 1 treatment-naïve alcoholic male subject.

^cWe do not have AMNART data for 1 male control subject.

^dStatistical comparisons between groups are not valid since the group definitions are a function of the variable.

Effect is significant: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

White Matter Signal Hyperintensity Measurements

We performed a separate test to examine group differences in white matter signal hyperintensities (WMSH) by adjusting the data for differences in total cerebral white matter volume. Very low quantities of WMSH were found in both groups, with the controls having slightly (and nonsignificantly) higher mean volumes than the TxN for both deep and periventricular WMSH.

Gray Matter Volumes

We examined the raw total gray matter volumes using group and sex as fixed effects, and cranium size and age as covariates, with age by sex and age by group interactions assessed. There were no significant group differences in cortical gray matter volumes in any of the 5 major lobes (frontal, limbic, occipital, parietal, and temporal), but significant age by group interactions were present in the frontal ($F_{6, 144} = 14.09$, $p < 0.001$), limbic ($F_{6, 144} = 3.96$, $p = 0.049$), parietal ($F_{6, 144} = 6.31$, $p = 0.013$), and temporal lobes ($F_{6, 144} = 12.32$, $p < 0.001$), as well as for total cortical gray matter ($F_{6, 144} = 12.79$, $p < 0.001$), indicating that the relationships between age and gray matter volumes differed between groups. See Table 2 for a listing of adjusted volumes based on the covariates mentioned above and the effect sizes of the age by group interactions in the various regions.

Associations of Gray Matter Volumes With Age

To test the nature of the age by group interactions, we performed correlations between age and the cranium size index-adjusted cortical gray matter volumes in each group. There were age-related gray matter volume reductions in nearly all regions in both groups, with generally larger correlations in

the TxN (see Table 2). To investigate whether the age-related cortical gray matter reductions were due to localized shrinkage or an overall reduction, we performed partial correlation analyses between individual ROIs and age, controlling for overall cortical gray matter volume in both groups to see if any of the cortical regions experienced a greater degree of age-related atrophy than the cortex as a whole. Neither group had significant regional atrophy when adjusting for total cortical gray matter volumes, suggesting general age-related cortical gray matter loss in both groups. Regression plots illustrating the age-related declines in both groups are shown in Fig. 2.

Total cortical gray matter volumes were adjusted across all subjects for differences in age using the regression parameter estimates from the controls. This adjustment removed the age-related gray matter decline within the controls and isolated the additional (i.e., beyond normal) age-related shrinkage in TxN. A significant negative relationship between the age adjusted total cortical gray matter volumes and age remained in the TxN ($r = -0.456$, $p < 0.001$). To test the effect of recent drinking on this trend, we performed a partial correlation between the age adjusted cortical gray matter volume and age controlling for average alcohol consumption during the 6 months prior to the first interview. The correlation remained significant ($r = -0.455$, $p < 0.001$).

Separating Age and Lifetime Alcohol Burden Contributions to Brain Shrinkage in TxN

Significant correlations between age- and cranium size-adjusted cortical gray matter volumes and lifetime alcohol use were found ($r = -0.257$, $p = 0.018$). Within controls, all of the age-ROI correlations were essentially zero because of the age adjustment.

Table 2. Adjusted Mean Volumes and Pearson Correlations Between Age and Cranium Size Index-Adjusted Regional Gray Matter Volumes

| | Treatment-naïve alcoholics | | Nonalcoholic controls | | Age correlations | | Age × Group interaction effect sizes (%) |
|------------------------------|----------------------------|------------------------|-----------------------|------------------------|------------------|------------------|--|
| | Men <i>n</i> = 49 | Women <i>n</i> = 35 | Men <i>n</i> = 39 | Women <i>n</i> = 28 | TxN | Controls | |
| Cortical gray matter | 452 | 460 | 447 | 460 | -0.626*** | -0.389** | 8.2*** |
| Frontal lobe | 153 | 159 | 150 | 157 | -0.588*** | -0.315** | 8.9*** |
| Prefrontal | 102 | 106 | 102 | 106 | -0.595*** | -0.306* | 8.2*** |
| Posterior prefrontal | 16.0 | 17.7 | 16.3 | 17.3 | -0.572*** | -0.151 | 8.8*** |
| Lateral prefrontal | 17.7 | 17.9 | 17.5 | 17.4 | -0.588*** | -0.267* | 6.3** |
| Dorsolateral prefrontal | 25.4 | 26.8 | 25.4 | 26.6 | -0.598*** | -0.412*** | 6.2** |
| Orbital frontal | 42.1 | 43.9 | 42.4 | 44.3 | -0.512*** | -0.242* | 5.3** |
| Primary motor | 4.68 | 5.12 | 4.31 | 4.72 | -0.213 | -0.363** | 0.2 |
| Premotor/supplementary motor | 32.0 | 33.7 | 29.9 | 32.0 | -0.492*** | -0.109 | 8.8*** |
| Insula | 14.3 | 14.5 | 14.8 | 14.8 | -0.471*** | -0.344** | 2.2 |
| Limbic lobe | 58.0 | 60.7 | 59.2 | 59.8 | -0.484*** | -0.282* | 2.7 |
| Anterior cingulate | 17.3 | 18.2 | 17.9 | 18.2 | -0.452*** | -0.399*** | 0.8 |
| Occipital lobe | 54.0 | 54.9 | 51.9 | 54.7 | -0.402*** | -0.397*** | 0.6 |
| Anterior occipital | 2.59 | 2.60 | 2.59 | 2.61 | -0.079 | -0.190 | 0.2 |
| Visual association | 39.6 | 40.0 | 37.7 | 39.8 | -0.344*** | -0.369** | 0.4 |
| Primary visual | 4.01 | 4.05 | 3.85 | 4.00 | -0.206 | -0.167 | 0.3 |
| Parietal lobe | 71.2 | 71.5 | 70.8 | 71.9 | -0.556*** | -0.371** | 4.2* |
| Mesial parietal | 29.1 | 28.6 | 28.5 | 28.4 | -0.495*** | -0.277* | 3.6* |
| Lateral parietal | 25.1 | 25.8 | 25.6 | 26.5 | -0.608*** | -0.383** | 3.8* |
| Primary sensory | 9.69 | 9.89 | 9.67 | 9.63 | -0.409*** | -0.278* | 1.9 |
| Temporal lobe | 101 | 99.3 | 101 | 102 | -0.554*** | -0.150 | 8.1*** |
| Superior temporal | 19.0 | 19.4 | 19.2 | 19.9 | -0.570*** | -0.235 | 6.5** |
| Mesial temporal | 36.1 | 35.2 | 36.2 | 35.9 | -0.495*** | -0.277* | 5.0** |
| Inferior temporal | 16.5 | 15.9 | 16.5 | 16.6 | -0.136 | 0.144 | 2.2 |

Volumes are reported in cm³. Effect size is presented as percent of variance accounted for by the named effect: $\eta_p^2 = SS_{\text{effect}} / (SS_{\text{effect}} + SS_{\text{error}}) \times 100\%$. Adjusted volumes were obtained from a general linear model using group and sex as fixed effects, and cranium size and age as covariates, with age × group, age × sex, and group × sex interactions assessed. Correlations were computed between raw cranium size-adjusted gray matter volumes and age.

Effect is significant: **p* ≤ 0.05, ***p* ≤ 0.01, ****p* ≤ 0.001.

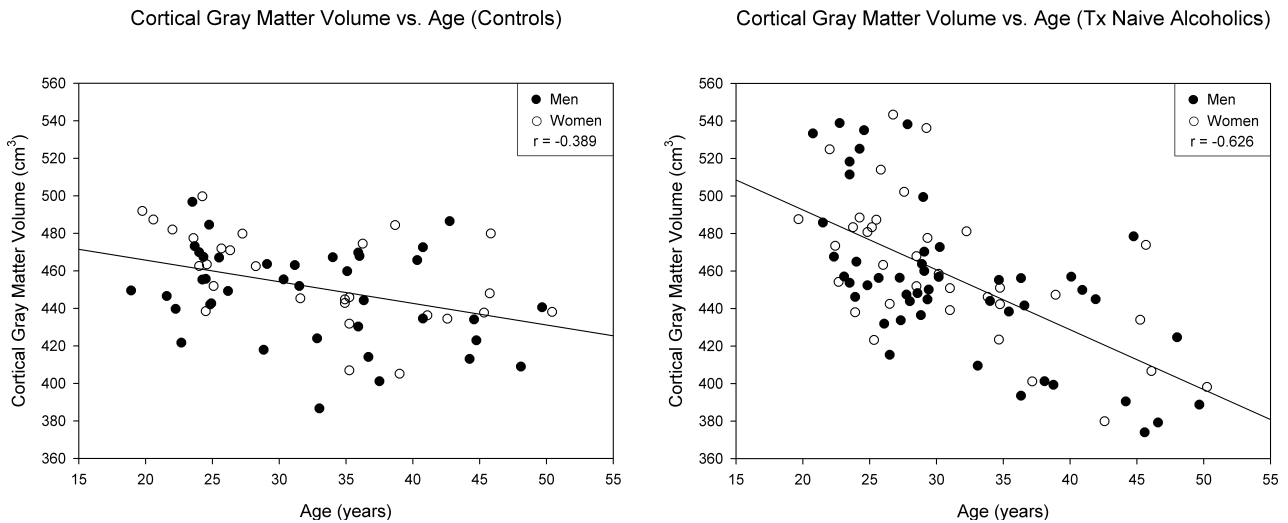


Fig. 2. The relationship between age and cranium size-adjusted cortical gray matter is much steeper in the TxN (right) than controls (left). Removing the large number of TxN under 30 years old somewhat reduced the magnitude of the correlation, but it remained significant (*r* = -0.485, *p* = 0.003).

Given the strong association of age and total lifetime alcohol consumption in TxN (*r* = 0.58, *p* < 0.001), partial correlations were used to disambiguate the contribution of these measures to the gray matter shrinkage. Controlling for age removed all associations of total lifetime alcohol consumption

with gray matter shrinkage. In contrast, controlling for lifetime alcohol consumption did not remove the age-related shrinkage. A significant negative partial correlation between age and cranium adjusted cortical gray matter volume remained (*r* = -0.390, *p* < 0.001).

Age was also associated with total alcohol consumption during periods of peak drinking ($r = 0.47, p < 0.001$), lifetime drinking duration ($r = 0.97, p < 0.001$), and duration of peak drinking ($r = 0.64, p < 0.001$). Age was not significantly correlated with average lifetime alcohol dose ($r = -0.12, p = 0.27$) or average alcohol dose during periods of peak drinking ($r = -0.09, p = 0.40$).

There were no significant associations between the amount of alcohol consumed in the 6 months prior to the interview and age or total cortical gray matter volume, indicating that recent alcohol use in the TxN was not a contributing factor to their accelerated age-related gray matter decline.

DISCUSSION

The major finding of this study is the presence of greater than normal age-related cortical gray matter volume reduction in TxN that is independent of lifetime alcohol consumption (average and total). In contrast, the effect of lifetime alcohol consumption on brain shrinkage was the result of its high correlation with age. These results are consistent with a synergistic effect of heavy alcohol use and aging on gray matter shrinkage in TxN. In our previous study on treated long-term abstinent alcoholics (LTAA, on average 10 to 15 years older than the current study) (Fein et al., 2009a), we noticed separate and additive effects of alcohol dependence and aging on localized gray matter shrinkage. It is impossible to say whether the difference in findings between the 2 studies is the result of the greater age of the LTAA cohort, their longer period of alcohol dependence, or their greater severity of alcohol dependence (Fein and Landman, 2005) and comorbid psychiatric disorders (Di Sclafani et al., 2008) than TxN in the current study. We are now gathering data on a TxN sample of comparable age to the LTAA sample.

The fact that we did not find any significant overall or regional gray matter reductions in the alcohol-dependent sample replicates the result from our previous study (Fein et al., 2002), supporting the hypothesis that the neurological effects on nontreatment seeking alcohol-dependent individuals are less severe than in those who seek treatment. Similarly, we did not find any significant associations within the TxN between average lifetime alcohol dose and cranium size-adjusted cortical gray matter volumes. However, unlike the previous study, we were able to determine that the accelerated decrease in cortical gray matter volume within the TxN was a function of age that was independent of any alcohol consumption measures.

Despite the lack of highly controlled studies on the mechanisms of aging-related brain shrinkage, a number of hypotheses for this phenomenon have been proposed. One is that cerebrovascular changes can contribute to the natural aging of the brain. Decreases in the growth of new capillaries and cerebrovascular blood flow have been known to occur in old age (Sonntag et al., 2007), and evidence suggests that prolonged exposure to glucocorticoids, a hormone released in response to stress, has a negative impact on brain function

and may contribute to the age-related decline in brain function (Goosens and Sapolsky, 2007). Calcium ions (Ca^{2+}) have also been associated with neurodegeneration and accelerated aging of the brain. Homeostatic intracellular Ca^{2+} systems, which are responsible for important transducing processes, have been shown to regulate neuronal death through the process of excitotoxicity (Olney and Ho, 1970), and tend to show dysfunction with increasing age (Toescu, 2007). Finally, oxidative stress (associated with free radical metabolism and inflammation) is thought to play a role in the overall decline of the central nervous system over time.

Alcohol abuse and withdrawal has been found to be associated with elevated glucocorticoid levels in mice and rats (Little et al., 2008) as well as increased oxidative stress in humans (Huang et al., 2009; Lecomte et al., 1994; Peng et al., 2005). Although our data do not provide the information necessary to pinpoint the mechanism responsible for the accelerated age-related decline of the brain in the TxN, it is likely that the heavy drinking in the TxN subjects influenced the concentrations of the neurodegenerative or neuroprotective substrates involved in the associated aging pathway or pathways. However, the fact that we did not see significant effects for cumulative alcohol exposure or average alcohol dose during periods of drinking indicates that the increasing vulnerability of the aging brain to these pathways is a significant reason for this pattern of gray matter volume loss.

Within the white matter, we found no group differences in WMSH severity, in contrast to our finding of increased WMSH in LTAA (Fein et al., 2009b). The absence of any significant group differences in either periventricular or deep WMSH volumes is not surprising, due to the relatively young age of our sample (on average over a decade younger than the sample in our study of long-term abstinent alcoholics). Although age is the most important predictor of WMSH load, appreciable amounts of WMSH are not usually found in individuals under 40 (Awad et al., 1986).

On average, TxN had slightly larger craniums than controls, indicating that TxN may have enjoyed a slightly higher brain reserve capacity than our sample of controls. This is opposite to the finding of reduced intracranial vault size (i.e., cranium size) in early-onset-treated alcoholics (Gilman et al., 2007). Our results are suggestive of a selection bias in TxN toward individuals with higher brain (and cognitive) reserve capacity. These results demonstrate the importance of examining selection bias indicators in complex and demanding studies. This selection bias, if real (note that it was only a trend in the analyses presented here), would attenuate any cognitive effects of alcohol dependence in the TxN sample.

A limitation of this study is that we did not measure tobacco smoking in our samples. There is evidence that smoking is highly associated with white matter damage, and that between 80 and 95% of alcoholics smoke cigarettes (Patten et al., 1996), 3 times higher than among the population as whole. Cigarette smoking is thought to be associated with increased WMSH burden, an indicator of white matter damage, independent of hypertension (Fukuda and Kitani, 1996).

Gazdzinski and colleagues (2005) found preliminary evidence that comorbid cigarette smoking in alcoholics accounts for some of the cortical gray matter loss. Moreover, Mon and colleagues (2009) found that cerebral perfusion improved in nonsmoking recovering alcoholics, while this improvement was hindered in alcohol abstainers who continued to smoke.

In conclusion, we found a synergistic effect of alcohol dependence and aging on gray matter shrinkage, implying that the shrinkage will get worse as the TxN sample ages. This has potential important public health consequences, portending increasing brain morbidity of alcoholics as individuals age. Our results in the current study and in our study of elderly LTAA also point out the presence of selection bias in studies of clinical samples of alcoholics, and suggest that such selection bias will make it very difficult to measure the phenomena of increased brain morbidity of alcohol dependence in elderly cohorts. These issues are exacerbated when other neurodegenerative diseases associated with aging (e.g., Alzheimer's disease, cerebrovascular disease, etc.) add their morbidity to the aging cohort. Nonetheless, the possibility of increased brain morbidity of alcohol dependence in elderly cohorts is a very important area of study, with increasing public health implications as the population of the country ages, and underlines the importance of developing creative and innovative sampling plans to study this phenomenon.

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