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Cerebellar morphometry and cognition in the context of chronic alcohol consumption and cigarette smoking

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Abstract

Background: Cerebellar atrophy (especially involving the superior anterior cerebellar vermis) is among the most salient and clinically significant effects of chronic hazardous alcohol consumption on brain structure. Smaller cerebellar volumes are also associated with chronic cigarette smoking. The present study investigated effects of both chronic alcohol consumption and cigarette smoking on cerebellar structure and its relation to performance on select cognitive/behavioral tasks.

Methods: Using T1-weighted MRIs, the Cerebellar Analysis Tool Kit segmented the cerebellum into bilateral hemispheres and three vermis parcels from four participant groups: smoking (s) and non-smoking (ns) abstinent alcohol-dependent treatment seekers (ALC) and controls (CON) (i.e., sALC, nsALC, sCON, and nsCON). Cognitive and behavioral data were also obtained.

Results: We found detrimental effects of chronic drinking on all cerebellar structural measures in ALC participants, with largest reductions seen in vermis areas. Furthermore, both smoking groups had smaller volumes of cerebellar hemispheres but not vermis areas compared to their non-smoking counterparts. In exploratory analyses, smaller cerebellar volumes were related to lower measures of intelligence. In sCON, but not sALC, greater smoking severity was related to smaller cerebellar volume and smaller superior anterior vermis area. In sALC, greater abstinence duration was associated with larger cerebellar and superior anterior vermis areas, suggesting some recovery with abstinence.

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Conclusions: Our results show that both smoking and alcohol status are associated with smaller cerebellar structural measurements, with vermal areas more vulnerable to chronic alcohol consumption and less affected by chronic smoking. These morphometric cerebellar deficits were also associated with lower intelligence and related to duration of abstinence in sALC only.

Keywords

cerebellum; alcohol; smoking; ataxia; cognition; MRI

Introduction

Consistent evidence demonstrates a co-occurrence of alcohol use disorder (AUD) and cerebellar pathology (Andersen, 2004, Baker et al., 1999, Harper, 1993, Torvik and Torp, 1986, Victor et al., 1959, Victor et al., 1989), including alcohol-related cerebellar atrophy e.g., (Sullivan et al., 2000a, Sullivan et al., 2000b, Davila et al., 1994), which contributes to the hallmark impairments of gait and balance in chronic alcohol abusers (Baker et al., 1999, Victor et al., 1959, Sullivan, 2003, Sullivan et al., 2000a, Sullivan et al., 2010, Sullivan et al., 2006, Sullivan et al., 2009, Currie et al., 2013, Vassar and Rose, 2014). With the neocerebellum also involved in neurocognition, alcohol-related cerebellar pathology has also been associated with impairments in non-motor and higher-order cognitive functions, such as attention shifting (Allen et al., 1997), working memory (Hayter et al., 2007, Chanraud et al., 2010, Chen and Desmond, 2005a, Chen and Desmond, 2005b), executive skills (Nakamura-Palacios et al., 2014) and language/verbal tasks (Booth et al., 2007). Moreover, patients with cerebellar pathology unrelated to excessive alcohol use demonstrate deficits in executive functioning, language skills, and affective behaviors (Schmahmann and Sherman, 1998). Such findings support the theory that cerebellar dysfunction may mechanistically contribute to cognitive dysfunction, motor impairment and postural instability in AUD.

Recent work has also linked smaller cerebellar volumes to chronic cigarette smoking. An early analysis found smaller cerebellar gray matter density in smokers versus non-smokers, but no difference in gray matter volume (Gallinat et al., 2006). A later voxel-wise analysis localized a cluster of significant gray matter volume reduction in Crus I, a region in the posterior lobe of the cerebellar hemispheres, in smokers compared to never-smokers (Kuhn et al., 2012), although these findings were not replicated in a similar study (Fritz et al., 2014). More recently, smaller bilateral cerebellar gray matter volumes have been reported in smokers (Franklin et al., 2014, Vnukova et al., 2017). Lastly, 3-week-abstinent alcohol dependent treatment seekers (ALC) who smoke (sALC; many of whom contributed to this cerebellar structural analysis) showed poorer performance on postural stability tasks compared to their non-smoking counterparts (nsALC) (Schmidt et al., 2014), an effect that could be mediated by cerebellar structural abnormalities (Vassar and Rose, 2014).

In addition to studies showing group differences between substance users and controls, research has suggested that substance use contributes to accelerated brain aging. Excessive alcohol use and smoking have both been reported to amplify age-related volume loss in the brain (Pfefferbaum et al., 1992, Durazzo et al., 2017, Durazzo et al., 2014b). A recent voxel-based structural analysis measured gray matter volumes in 110 brain regions in those with

AUD and controls aged 25–65 years to examine for potential alcohol-related accelerated aging. Results indicated that, in the later decades of life, the brain age of the chronic drinkers was increased by an impressive 12 years, consistent with the accelerated brain aging theory in substance users (Guggenmos et al., 2017). Similarly, we recently compared cortical and subcortical volumes in non-smokers and smokers aged 22–70 years without any other substance use disorder and also found chronic smoking associated with accelerated age-related volume loss in subcortical white and gray matter regions, including the cerebellum (Durazzo et al., 2017).

In this study, we investigated effects of both chronic alcohol consumption and cigarette smoking on cerebellar structure and their relations to performance on select cognitive tasks, as their joint and separate effects on the cerebellum have not previously been reported. The Cerebellar Analysis Tool Kit (CATK; (Cardenas et al., 2014, Price et al., 2014) was used to segment the cerebellum as visible on T1-weighted MR images into left and right hemispheres and three vermis parcels. From two separate neuroimaging research studies conducted concurrently, we constructed four groups of participants: sALC, nsALC, smoking controls (sCON), and non-smoking CON (nsCON). We hypothesized that (i) all cerebellar measurements (area and volume) are smaller as a function of both chronic smoking and alcohol status, with the largest effect of alcohol consumption on the superior anterior vermis, (ii) smoking exacerbates the effects of alcohol on cerebellar morphometry, and (iii) smaller cerebellar measures are associated with worse performance on select cognitive/clinical tasks. We also predicted that greater smoking and alcohol consumption severity are associated with smaller cerebellar measures. In addition, we explored whether group differences in cerebellar measures related to smoking and alcohol were better explained by a fixed factor model or by a model of accelerated aging.

Materials and Methods

Participants

Participants were drawn from two different research projects on alcohol and tobacco use disorders conducted in the greater San Francisco area. Treatment-seeking ALC participants were recruited from substance abuse treatment programs at the San Francisco VA Medical Center and Kaiser Permanente. The treatment seekers participating in this research were clients of outpatient treatment programs at the local VA and Kaiser Permanente, with whom we have had longstanding research relationships. Importantly, clinical staff at these treatment centers distributed research study-related information to treatment clients and those who were interested to learn more about the study then contacted research study personnel for screening. As such, the study participants were fully self-referred into this research. CON, without histories of medical or psychiatric conditions known or suspected to influence brain structural outcome measures, were recruited from the local community. All participants were between the ages of 25 and 70 years (see Table 1 for demographics). Medical exclusion criteria were a current or past history of intrinsic cerebral tumors, human immunodeficiency virus or acquired immune deficiency syndrome, cerebrovascular accident, aneurysm, insulin dependent diabetes, chronic obstructive pulmonary disease, non-alcohol-related seizures, significant exposure to known neurotoxins, demyelinating and

neurodegenerative diseases, Wernicke-Korsakoff Syndrome, alcohol-induced persisting dementia, and traumatic brain injury resulting in loss of consciousness for more than 15 minutes. Psychiatric exclusion criteria included schizophrenia or other thought disorders, bipolar disorder, dissociative disorders, posttraumatic stress disorder, obsessive compulsive disorder, and panic disorder (with or without agoraphobia), all according to DSM-IV-TR criteria. Hepatitis C, type-2 diabetes, hypertension, and unipolar mood disorders were not exclusionary given their high prevalence in substance use disorder.

At baseline, all ALC treatment-seekers met DSM-IV-TR criteria for alcohol dependence and were abstinent from all substances except tobacco for an average of 20±11 days (nsALC: 18±10; sALC: 21±11; $p>0.05$). At the time of assessment, all smoking participants were actively smoking at least 10 cigarettes per day, had been doing so for the past 5 years or more, had no periods of smoking cessation greater than 1 month in the 5 years prior to study, and were not concurrently using other tobacco or non-tobacco nicotine products. No smoker was engaged in any pharmacological/behavioral smoking cessation program. Non-smoking participants never smoked or smoked less than 40 cigarettes during their lifetime and used no cigarette/tobacco products for 10 years prior to study. Participants provided written informed consent according to the Declaration of Helsinki, and all procedures were approved by the institutional research review boards of the University of California San Francisco and the San Francisco VA Medical Center.

All participants with T1-weighted MRIs deemed usable by experienced imagers (i.e., full cerebellum in the field of view, no movement, good signal-to-noise ratio assessed by visual review), and accurate cerebellar segmentations were included in the analyses. This resulted in a final sample of 17 nsCON (48±12 yrs, 14 males), 31 sCON (49±9 yrs, 27 males), 21 nsALC (51±12 yrs, 16 males), and 23 sALC (49±7 yrs, 21 males).

Clinical and Neurocognitive Measures

Each participant completed the Structured Clinical Interview for DSM-IV Axis I Disorder Patient Edition, Version 2.0, as well as questionnaires assessing depressive (Beck Depressive Inventory, second edition (Beck et al., 1996)) and anxiety symptoms (State-Trait Anxiety Inventory, form Y-1 (state) and Y-2 (trait), STAI, (Spielberger, 1983)). Lifetime alcohol consumption was assessed with the Lifetime Drinking History semi-structured interview (Skinner and Sheu, 1982, Sobell et al., 1988). The average number of standard alcoholic drinks (containing 13.6 g of ethanol) consumed per month was derived for one and three years before enrollment and over lifetime. The Fagerstrom Tolerance Test for Nicotine Dependence (Heatherton et al., 1991) was used to assess level of nicotine dependence, years smoking at current level, total years of cigarette smoking, and average number of daily cigarettes currently smoked.

Participants completed a comprehensive neurocognitive battery (Durazzo et al., 2013b). We focused on cognitive measures that have been shown to be associated with cerebellar morphological integrity: Wisconsin Card Sorting Test-64 (WCST; shifting, self-monitoring and use of verbal feedback to guide decision making (Heaton and Staff, 1993)), Trail Making Test (TMT) (Reitan and Wolfson, 1985): Trails A (processing speed) and B (set-shifting, visuomotor scanning, and graphomotor speed), Grooved Pegboard (fine motor

dexterity (Oldfield, 1971)), and the Sharpened-Romberg task with eyes-closed (static postural stability (Fregly et al., 1972)). Premorbid verbal intelligence was assessed with the American National Adult Reading Test (AMNART VIQ) (Grober and Sliwinski, 1991) and general intelligence with the Ward-7 Full Scale IQ (using a z-transformed composite score based on WAIS-III Arithmetic, Block Design, Digit Span, Digit Symbol, Information, Picture Completion, and Similarities subtests). All measures, with the exception of the Sharpened-Romberg, are well normed and commonly used in clinical and/or research settings (Strauss et al., 2006). In order to mitigate the potential for nicotine withdrawal effects on function, smokers were allowed to smoke *ad libitum* prior to all assessments and were allowed to take cigarette smoking breaks, if requested.

We obtained gross lab markers of recent drinking for most subjects (e.g., GGT, AST, ALT, mean corpuscular volume). These markers were approaching or were within normal limits at the time of assessment for all participants. The VA and Kaiser hospitals also routinely conducted breathalyzers and urine substance use assays on patients in their substance abuse treatment program, from which we drew our participants. Prior to assessment at our laboratory, participants' urine was tested for five common substances (THC, opiates, PCP, cocaine, and amphetamines) and participants were breathalyzed for recent ethanol consumption. No participant was positive for the above common substances or ethanol at the time of assessment.

MRI Acquisition and Image Processing

MRI data were acquired on a 4.0 T Bruker MedSpec system using an 8-channel transmit-receive head coil (Siemens, Erlangen, Germany). A Magnetization Prepared Rapid Gradient Echo sequence (TR/TE/TI = 2300/3/950 ms, 7° flip angle, 1.0 mm isotropic resolution) was used to acquire 3D sagittal T1-weighted images for cerebellar segmentation.

We used CATK (Cerebellar Analysis Tool Kit, (Cardenas et al., 2014, Price et al., 2014)) to segment T1-weighted MR images of the cerebellum into left and right hemispheres and three vermis parcels (superior anterior I-V, superior posterior VI-VII, and inferior posterior VIII-X); measures of cerebellar volume (total and hemispheric) or area of the three vermis parcels are the focus of this report. CATK functions as a fully-automated T1 MRI cerebellum delineation and parcellation tool. It uses an active profile-appearance modeling (AAM, (Patenaude et al., 2011, Cootes, 2000, Cootes, 2001)) framework, which combines surface-based registration with statistical models of shape and texture derived from high-resolution T1-weighted images acquired from 43 healthy participants (mean age 44 years, range 5–96 years, 49% male), providing delineation of the cerebellar hemispheres and three vermal lobes. The advantage of this is that strong prior knowledge about the cerebellum inherent in the data (such as the overall shape of the cerebellar vermal lobes) are taken into account during segmentation of new data, resulting in a segmentation method that enforces smoothness according to probable variations specific to the structures. Figure 1 shows an example segmentation output for CATK. All cerebellar segmentations were visually reviewed for accuracy by authors VAC and CMH, and poor segmentations were excluded.

Statistics

Comparisons of demographic and clinical data between all four groups were conducted with univariate analysis of variance. Multivariate analyses of covariance (MANCOVA) using a 2 × 2 design (alcohol-by-smoking status) examined the effects of alcohol and smoking on three cerebellar volume measures (total, left cerebellar hemisphere, right cerebellar hemisphere) and three vermis cross-sectional areas (superior anterior, superior posterior, and inferior posterior), while controlling for age, sex, and intracranial vault volume (ICV; estimated using FreeSurfer). The non-significant sex term and non-significant alcohol-by-smoking status interaction terms were removed from final models. Age was not a significant covariate in the model for vermis areas and was removed in the final model. A sensitivity analysis conducted using G*Power (Faul et al., 2007) using our sample of 92 subjects and assuming equal group sizes, conventional levels of $\alpha = 0.05$ and $\beta = 0.20$ (80% power), showed that an effect size of 0.08 could be detected. Using our smallest group size of 17 and assuming 4 equal groups (i.e., assuming a sample of only 68 subjects), an effect size 0.11 could be detected. These effect sizes are considered “small” according to Cohen (Cohen, 1988). Because of our *a priori* hypotheses and relatively small number of outcome measures, we did not correct for multiple comparisons.

To explore whether the accelerated aging hypothesis better explained our cerebellar data than the fixed factor statistical model, we employed an alternative model of the effects of smoking and alcohol on cerebellar volume using MANOVA with cerebellar measures as dependent variables and age, ICV, and alcohol-by-smoking-status-by-age interactions as independent variables; this allowed us to model potential age-related differences in cerebellar structures among the four groups.

General linear modeling was used to explore relationships of cerebellar measures (after correcting for the effects of ICV by regressing each cerebellar measure on ICV and using the residual predicted values as the independent variables) to measures of neurocognition. Measures of neurocognition included individual tests that focus on cerebellar function, as described above. Since the left, right, and total cerebellar measures were highly collinear (all $r>0.93$), a composite cerebellar measure was created (0.5(total+right+left)); the composite measure and all three vermis measures were included as independent variables in these models with education, age, smoking and alcohol status as covariates. In each group separately (sCON, nsALC, and sALC), Pearson correlations were computed using the predicted values derived from the general linear models to examine associations between cerebellar measures with measures of smoking severity in sCON, and with measures of drinking severity and abstinence in nsALC. In sALC, associations between cerebellar and smoking severity measures were adjusted for the monthly average of alcoholic drinks consumed over one year before study, and associations between cerebellar and drinking severity and abstinence measures were adjusted by the Fagerstrom total score. Given our *a priori* hypotheses and relatively small number of outcome measures, we did not correct the statistical significance of these relationships for multiple comparisons.

Results

Sample characterization

Sample characteristics are summarized in Table 1. Age and proportion of female participation did not differ between the four study groups (both p>0.55). sALC drank more than nsALC (3-yr monthly avg, p<0.01; 1-yr monthly avg, p=0.06; lifetime monthly avg, p<0.01). nsCON had more years of education than sCON (p=0.04) or sALC (p<0.01), who had fewer years of education than nsALC (p=0.03). sCON and sALC did not differ on measures of smoking severity (all p>0.10).

Main effects of smoking and alcohol on cerebellar measures

Cerebellar and intracranial volumes are summarized in Table 2. ICV was not significantly different among the four groups (p>0.47). Total, left, and right cerebellar volumes showed significant multivariate effects of alcohol status ($F_{3,81}$=3.47, p=0.02), age ($F_{3,81}$=5.22, p=0.002), and ICV ($F_{3,81}$=15.04, p<<0.01), but not smoking status (p=0.21). The ALC groups had significantly smaller (approximately 3.2%) left and right hemisphere volumes relative to CON groups. Multivariate effects of age indicate a decrease of approximately 2 ml in cerebellar volume with each additional decade of age. Although the multivariate effect of smoking status was not significant, univariate models showed significant smoking effects (all p<0.05 uncorrected), and plots revealed 2% smaller cerebellar volumes in sCON and sALC relative to their non-smoking counterparts, as shown in Figure 2. Vermal cross-sectional areas showed significant multivariate effects of alcohol status ($F_{3,82}$=2.67, p=0.05) and ICV ($F_{3,82}$=2.89, p=0.04), with ALC groups showing 4.4–5.9% smaller vermal areas relative to CON. Age and smoking status showed no significant effects on vermis measures (both p>0.32), and plots comparing smoking to non-smoking groups showed no consistent effect of smoking (all univariate p>0.67), as shown in Figure 2.

Accelerated cerebellar aging model

Because age was not significantly associated with any cerebellar vermis measure, only the total, left and right hemisphere cerebellar volumes were examined with an accelerated aging model. All three cerebellar volumes showed significant multivariate effects of age ($F_{3,80}$=5.17, p<0.02), ICV ($F_{3,80}$=14.61, p<<0.01), and age-by-alcohol-by-smoking status interactions ($F_{9,246}$=1.91, p=0.05). An examination of the parameter estimates from the univariate model showed that, compared to nsCON, significantly greater volume losses with increasing age were observed in both the left and right cerebellar hemispheres of sCON (p=0.04 and p=0.05, respectively) and sALC (both p=0.01), as illustrated in Supplement 1.

Neurocognitive Results

Performance on individual neurocognitive tests previously associated with cerebellar function is summarized in Table 3. There were no group differences due to alcohol or smoking status for the AMNART, Grooved Pegboard, time to complete the TMT Trails-A or TMT Trails-B tests, time to complete the WCST, and number of nonperseverative errors on the WCST (all p>0.11). However, smokers (sALC and sCON combined) made more perseverative errors and perseverative responses (both p<0.02) than all non-smokers, both

alcohol groups had markedly shorter standing times on the Sharpened-Romberg eyes-closed tasks ($p<<0.01$) compared to all CON, and there were group effects on the WCST total number correct (smoking $p=0.04$) and total errors (smoking $p=0.03$, alcohol $p=0.04$). The significant pairwise differences on these measures reported in Table 3 are consistent with these overall smoking and alcohol effects.

Associations between cerebellar and neurocognitive measures

Since the 2 × 2 MANOVAs reported above showed that ICV was related to all cerebellar measures, we regressed each cerebellar measure on ICV and used the residual predicted values as independent variables in our cognitive model. As summarized in Figure 3, smaller cerebellar composite volume was associated with lower AMNART (parameter estimate $\beta=0.001$, $p=0.02$) and Ward-7 Full Scale IQ (parameter estimate $\beta=2.3E-05$, $p=0.04$) scores, over and above the effects of education. The interpretation is that each 1000 mm^3 increase in residual cerebellar volume was associated with a 1 point increase in AMNART score and a 0.023 increase in the IQ z-score. None of the cerebellar measures significantly predicted the Grooved Pegboard, TMT-Trails A, time to complete the TMT Trails B, or any WCST measure (time to complete, total correct, total errors, or number of perseverative errors). Given the reports in the literature (Sullivan et al., 2010, Bernard et al., 2015, Bernard and Seidler, 2013, Medina et al., 2010), we were surprised to find that no cerebellar measures predicted Sharpened-Romberg performance over the entire group. However, when we examined groups separately, we found that the composite cerebellar volume measures was associated with performance on the Sharpened-Romberg task in nsALC ($\beta=0.006$, $p<0.01$), such that each 1000 mm^3 increase in cerebellar volume corresponded to a 1 second increase in standing time.

Associations between cerebellum and smoking/drinking severity and abstinence

Several measures of smoking and alcohol consumption showed correlations of moderate strengths with our cerebellar measures (see Figure 4). Among sCON, the number of years smoked over lifetime was associated with smaller total cerebellar volume ($r=-0.31$, $p=0.05$) and superior anterior vermis area ($r=-0.36$, $p=0.02$. Among sALC, smoking or drinking severity measures were not associated with cerebellar measures, but the number of days of alcohol abstinence were significantly correlated with total ($r=0.52$, $p=0.01$), left ($r=0.48$, $p=0.02$), and right ($r=0.46$, $p=0.02$) cerebellar volume, and superior anterior vermis area ($r=0.38$, $p=0.05$), with larger measures associated with longer abstinence. There were no significant associations between drinking severity and cerebellar measures in nsALC.

Discussion

Cerebral atrophy is commonly reported in AUD and has also been described with chronic cigarette smoking e.g., (Durazzo and Meyerhoff, 2007). Here, we investigated the effects of alcohol dependence and chronic cigarette smoking on cerebellar function and morphometry using a previously described and validated MRI segmentation tool kit. As hypothesized, detrimental effects of alcohol dependence in 3-week-abstinent treatment seekers were found on volume measures of bilateral cerebellar hemispheres and on sagittal area measures of all three vermis sections segmented, with the largest reductions observed in vermis areas.

Though our hypothesis that sALC would display the smallest cerebellar measurements was not confirmed, the results did support our hypothesis of smaller cerebellar volume as a function of smoking status. Both smoking groups (sCON and sALC) had smaller volumes of cerebellar hemispheres than their non-smoking counterparts. Vermis areas, however, did not differ significantly between smokers and nonsmokers. The morphometric deficits in these cross-sectional analyses were related to long-term drinking and smoking measures, suggesting that cerebellar structures may respond to chronic substance use rather than be premorbidly determined. Furthermore, the variance in cerebellar volumes is explained almost equally well by an accelerated aging model or one modeling group differences between nsCON and both smoking groups (sCON and sALC).

Our observation that alcohol status was related to 3% smaller cerebellar hemisphere volumes and up to 5.9% smaller vermis areas compared to controls agrees with prior studies that observed smaller vermis (Karhunen et al., 1994, Sullivan et al., 2000b, Sullivan et al., 2000a, Yokota et al., 2006) or volume loss in the cerebellar hemispheres (Sullivan et al., 2000b, Sullivan et al., 2000a, Sullivan, 2003, Chanraud et al., 2007, Anderson et al., 2010). The congruence of our findings with previous research provides compelling evidence that CATK, the automated cerebellar measurement software used here, is valid in clinical and non-clinical samples. The studies cited above counted Purkinje cells in autopsy patients, manually traced the cerebellum, or used voxel-based morphometry. Though cell counting and manual tracing are gold standards, their time-consuming nature limits their application to small samples, whereas voxel-based methods, although automatic, may not be sufficiently sensitive after necessary correction for multiple comparisons. CATK provides a straightforward, uncomplicated and convenient way of measuring cerebellar volumes and areas noninvasively, quickly, and automatically, being accurate and sensitive enough to confirm previously reported group differences related to chronic alcohol consumption in new samples.

In prior ataxia research involving largely the same ALC and CON participants of this study (89% of the ALC and 92% of the CON participants studied in this manuscript), we showed that chronic smoking was associated with reduced performance on the Sharpened-Romberg eyes-closed task in both groups (Schmidt et al., 2014). Since this postural stability task is a test of cerebellar integrity, we were not surprised to find cerebellar structural deficits related to chronic smoking. Other work had reported smoking-related volume deficits in ventrolateral and dorsolateral prefrontal cortices and cerebellum (Vnukova et al., 2017, Fritz et al., 2014, Franklin et al., 2014, Brody et al., 2004, Hanlon et al., 2016). Moreover, we have repeatedly demonstrated that smoking exacerbates neuropsychological and brain structural deficits in ALC and impedes their neurobiological and functional recovery during abstinence from alcohol (Durazzo et al., 2013a, Durazzo et al., 2014b, Durazzo et al., 2014c, Mon et al., 2009, Pennington et al., 2015, Pennington et al., 2013). Although the multivariate test for an overall effect of smoking in the present study was not significant at alpha=0.05, the results of our uncorrected univariate analyses were strongly suggestive of smaller cerebellar volumes in smokers, whereas cerebellar vermis areas were not related to smoking status (Figure 2). Although the vermis has the same cell types as the flocculonodular, anterior, and posterior lobes of the cerebellum, there is some evidence in mice that the morphology of Purkinje cells may be different in the vermis (Nedelescu et al., 2018),

potentially offering some explanation as to why our vermis measures were not smaller in smokers. Another possibility is that smoking affects the lateral boundaries of the vermis which are extraordinarily difficult to measure reliably and are not reflected in our mid-sagittal vermis cross-sectional area.

In sCON, greater severity of smoking was related to smaller cerebellar volumes and smaller area of the superior anterior vermis, even though we did not observe a significant effect of smoking status on any vermis measure. One prior study found putamen volume in male smokers positively correlated with severity of smoking but did not report a similar relationship with cerebellar volume (Franklin et al., 2014); however, it is possible that the stringent multiple comparison corrections used in their voxel-based analyses may have obscured any such a relationship. In sALC but not nsALC, we found that greater abstinence duration was related to greater measures of total, left, right, and superior anterior cerebellum. If sALC had had smaller cerebellar measures upon treatment entry (corresponding to an additive effect of alcohol and smoking) that had partially recovered before they were imaged, it might explain why a significant multivariate effect of smoking was not observed in our main analyses. Further research with imaging at treatment entry and longitudinal follow-up is warranted to investigate whether this is the case.

In exploratory analyses across all subjects, we found that worse performance on the AMNART, a measure of premorbid intelligence, and the Ward-7 Full Scale IQ, a measure of general intelligence, were associated with smaller cerebellar volumes. General intelligence has been linked to distributed neocortical gray matter (Menary et al., 2013, Colom et al., 2006), raising the possibility that large corticocerebellar networks also contribute to intelligence. In a recent study, independent components analysis was used to identify brain networks based on similar gray matter patterns across 92 healthy individuals aged 17–48 years. The cerebello-parietal network identified in this analysis was associated with an estimate of IQ, where greater loading of this network (i.e., greater inferior parietal lobe and cerebellar crus II gray matter co-occurrence) predicted higher IQ (Yoon et al., 2017). Earlier work using voxel-based morphometry showed that bilateral cerebellar gray matter was associated with general cognitive ability in older adults with a mean age of 69 years (Hogan et al., 2011). Our results are consistent with these previous studies, and show that the relationship between cerebellar volume and IQ extend to a greater age range and clinical samples. Across all subjects, the number of perseverative responses on the WCST (a classic measure of executive functioning) was weakly associated with the superior anterior vermis area (trend level $p=0.08$), although smoking explained more of the variance in WCST performance than the vermis area. Previous reports related cerebellar structure to WCST performance in those with an AUD (Chanraud et al., 2007, Sullivan, 2003), but those studies did not account for any effects of smoking on WCST performance and cerebellar structure. Thus, it is possible that these previously reported relationships of cerebellar structure to WCST performance were mediated by potential effects of smoking in the samples. We did not replicate previously reported associations of cerebellar measures with Sharpened-Romberg eyes-closed (Sullivan et al., 2010, Bernard et al., 2015, Bernard and Seidler, 2013, Medina et al., 2010), or associations between trail-making tasks and cerebellum as previously hypothesized (Zahr et al., 2010) when examined over the entire sample. However, when the groups were examined separately, in nsALC we observed that larger cerebellar

volumes were associated with longer times on the Sharpened-Romberg task. Pooling all subjects to increase our sample size limited our detection of this association, perhaps due to ceiling effects where more subjects obtained the maximum time on the Sharpened-Romberg tasks within the CON groups, and the standard deviation was also smaller. In sALC we did not find an association between cerebellar volumes and Sharpened-Romberg. Examination of scatterplots revealed that some sALC had maximal times despite small cerebellar volumes, suggesting that smoking may have compensated for the effects of alcohol in these participants.

We found a significant effect of age on cerebellar hemisphere volume measures consistent with previous work on the effects of normal aging on cerebellar volumes (Bernard et al., 2015, Bernard and Seidler, 2014). Using a model with the age-by-alcohol-by-smoking interaction, we found evidence for accelerated aging of the cerebellar hemispheres related to both alcohol and smoking status, where both sCON and sALC showed steeper negative slopes with aging than nsCON, suggesting accelerated aging for cerebellar volumes as a function of chronic smoking, with and without chronic drinking. The findings for greater age-related cerebellar hemisphere volume loss in sCON relative to nsCON is consistent with our findings in a larger sample, using FreeSurfer to quantitate cerebellar cortical volumes (Durazzo et al., 2017). As in the original model, the vermis area measures were not associated with age. A model comparison showed that this alternative model had almost exactly the same goodness of fit (adjusted R^2=0.349) as our original fixed factor model (adjusted R^2=0.354) and explained the same amount of variance in our data.

Amplified cerebral oxidative stress (OxS) has been proposed as a mechanism contributing to neurobiological abnormalities related to both heavy alcohol consumption and cigarette smoking in humans (Kim et al., 2004, Kim et al., 2003, Moriarty et al., 2003, Bloomer, 2007, Northrop-Clewes and Thurnham, 2007, Durazzo et al., 2014a) and animal models (Mendez-Alvarez et al., 1998, Panda et al., 2000, Kovacic, 2005, Anbarasi et al., 2006, Das et al., 2009, Valavanidis et al., 2009, Waly et al., 2011). It is well established that OxS is directly associated with damage to membrane lipids, proteins, carbohydrates, DNA and RNA of brain neuronal, glial, and vascular tissue [for review see (Durazzo et al., 2014a)]. Granular neurons of the cerebellar cortex are highly susceptible to OxS (Wang and Michaelis, 2010). While not universally accepted [see (Salmon et al., 2010)], increasing OxS burden with aging is also suggested to be a fundamental mechanism contributing to neurodegeneration in normal aging (Halliwell, 2006, Zimniak, 2011). Collectively, our findings of greater age-related cerebellar volume loss in sCON and sALC suggest the chronic OxS associated with alcohol dependence and/or chronic cigarette smoking may interact with the OxS associated with normal aging, thus amplifying degeneration of the cerebellar structures investigated here.

Prior research using measures of gray matter density in an atlas that segments the cerebellum into 28 parcels has shown sex differences in the human cerebellum (Fan et al., 2010). There have also been reports that smoking and alcohol have differential effects on women and men (Sung et al., 2015, Sawyer et al., 2017). In our study, we did not observe significant effects of sex on any cerebellar measure. However, our sample was overwhelmingly male (see Table 1), limiting our ability to detect sex effects. Moreover, we examined only a small number of

cerebellar parcels and did not differentiate between gray and white matter, further limiting our ability to detect cerebellar sex effects previously reported. Future work should examine more women and explore differences among cerebellar lobules, potentially revealing more effects of smoking, alcohol, and sex.

Summary and Conclusions

Overall, our results demonstrate that alcohol dependence and chronic cigarette smoking are associated with smaller cerebellar structural measurements, with vermis areas more vulnerable to alcohol dependence and less affected by smoking. We observed some evidence that these cerebellar deficits were associated with lower intelligence. Further evidence within subgroups indicated that the severity of smoking or alcohol abstinence duration was related to cerebellar structure, reflecting injury related to chronic substance use rather than premorbid abnormalities. Although the CATK provides a quick, reliable, and automated method for cerebellar segmentation, our results are limited due to the relatively small number of cerebellar parcels examined; methods that segment the cerebellum into further anatomically/functionally defined subdivisions might reveal more specific cerebellar-cognitive associations. Our results are also limited by the modest sample sizes, especially our correlations within subgroups, although a power analysis demonstrated sufficient sample sizes to detect group alcohol and smoking differences with small effect sizes. Despite these limitations, CATK's agreement with previous findings of alcohol effects in the cerebellum using gold standard manual tracing convince us that the method is valid and accurate and that our findings of smoking exacerbating cerebellar hemisphere deficits while relatively sparing the vermis are robust. Our findings showing associations between cerebellar structure and intelligence, smoking severity, and abstinence duration were more exploratory and hypothesis-generating, and suggest that further studies on the interaction of smoking, AUD, and age on brain structure, function, and recovery are warranted to help develop more effective AUD treatment interventions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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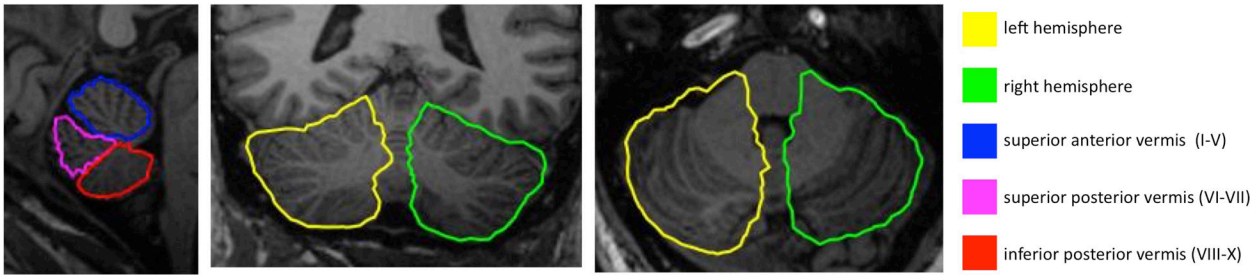


Figure 1:

3D parcellation of: vermis superior anterior lobe (lobules I–V) blue; vermis superior posterior lobe (lobules VI–VII) magenta; vermis inferior posterior lobe (lobules VIII–X) red; hemispheres, yellow and green, from a representative participant in the study.

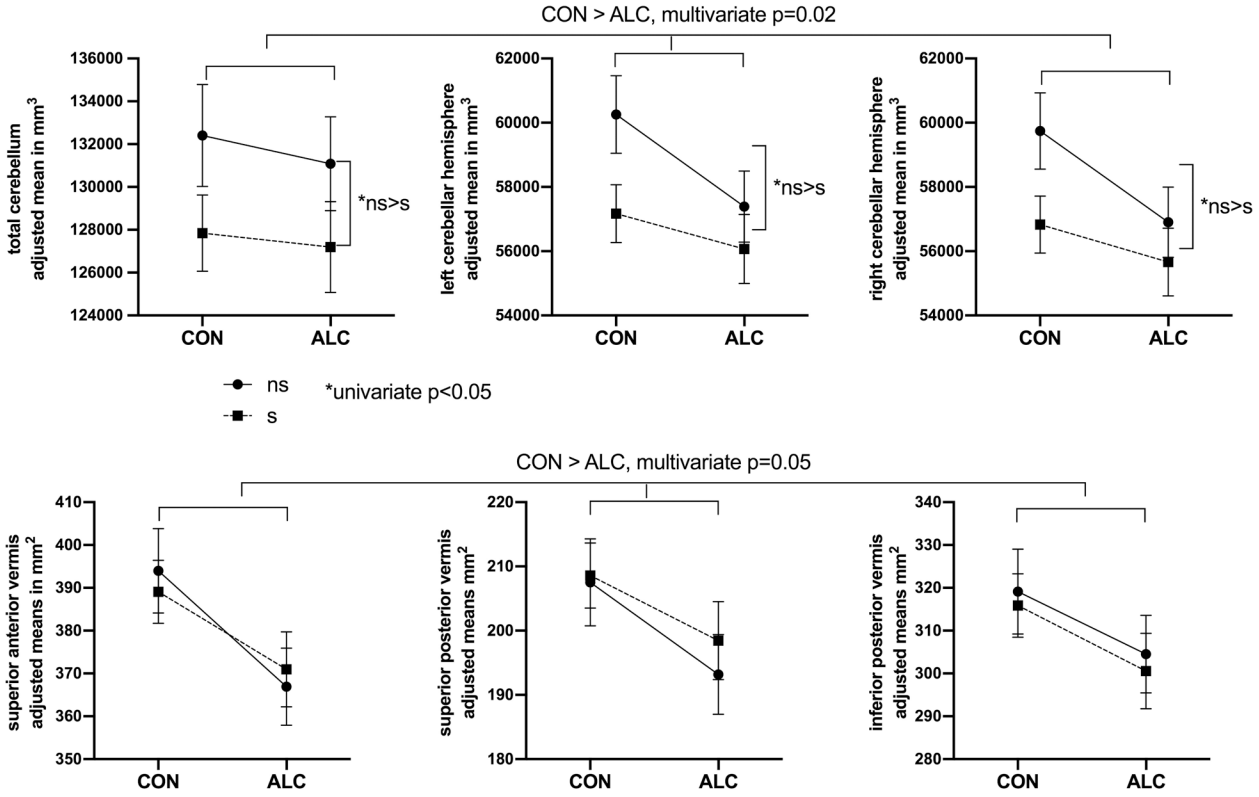
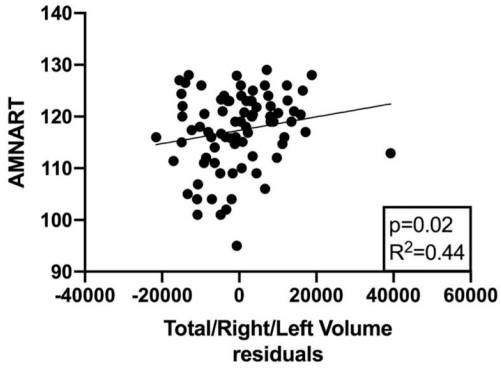


Figure 2:

Cerebellar measurements (mean ± SEM, adjusted for age and ICV) are shown for the four groups. All cerebellar parcels (total, left hemisphere, and right hemisphere volumes shown on top; superior anterior, superior posterior, and inferior posterior vermis areas on the bottom) were smaller in ALC; cerebellar volumes but not vermal areas were smaller in smokers.



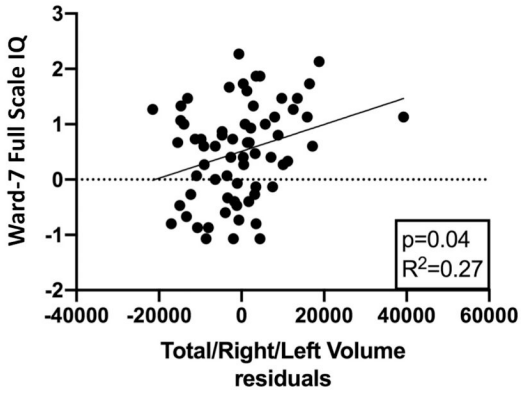


Figure 3:

Scatterplots of AMNART scores (American National Adult Reading Test) and the Ward-7 Full Scale IQ versus total cerebellar volumes (residuals after regression on intracranial volume); solid line shows the best linear fit.

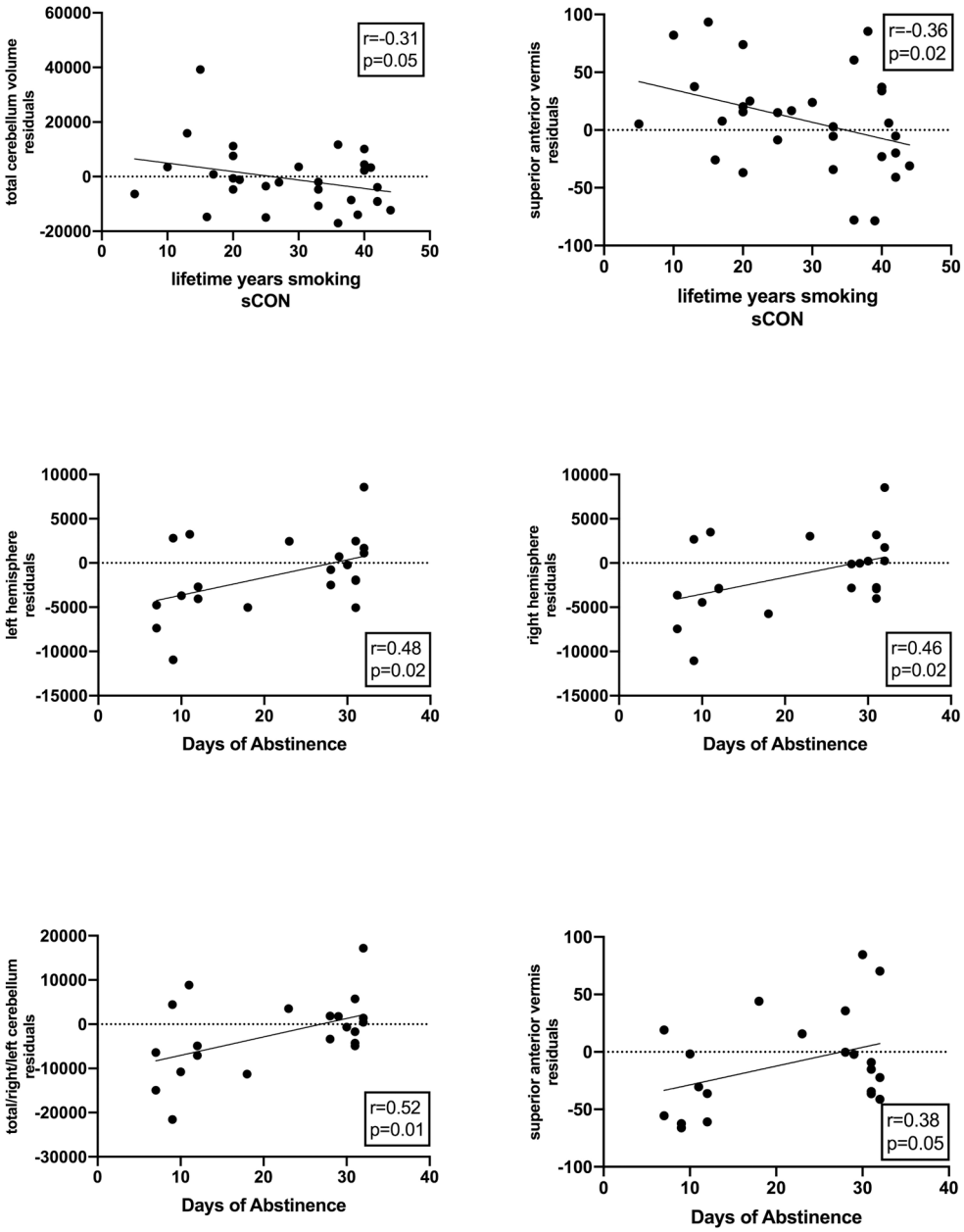


Figure 4:

Scatterplots of measures of smoking severity or days of alcohol abstinence versus cerebellar measurements (residuals after regression on intracranial volume); solid line shows the best linear fit.

Table 1:

Participant Demographics, drinking and smoking measures

	nsCON N=17	sCON N=31	nsALC N=21	sALC N=23
% Male	82	87	76	91
Age (yrs) mean ± SD (min-max)	48±12 (26–69)	49±9 (33–64)	51±12 (25–71)	49±7 (33–60)
Education (yrs)	16±2	15±2 †	15±2	13±2 ‡*
1-yr avg drinks per month &	12±13	22±19	291±152	387±194 *
3-yr avg drinks per month &	14±13	22±19	248±135	378±202 *
Lifetime avg drinks per month &	18±14	26±13	171±93	256±129 *
Total lifetime drinks ˇ	6759±5642	9540±6691	72403±53646	100697±60623
Fagerstrom total score	NA	4.8±1.6	NA	4.0±1.6
Cigarettes per day	NA	18.3±6.7	NA	15.1±7.1
Years smoking at current level	NA	16±12	NA	15±12
Total years smoking	NA	29±11	NA	26±9

Abbreviations: nsCON=non-smoking controls, sCON=smoking controls, nsALC=non-smoking abstinent alcohol-dependent treatment seekers, sALC= smoking abstinent alcohol-dependent treatment seekers.

& 1 standard alcoholic drink contains 13.6 g of ethanol

† sCON < nsCON, uncorrected p≤0.05

‡ sALC < nsCON, uncorrected p≤0.05

* sALC < nsALC (edu) and sALC > nsALC (drinking severity measures)

All other pairwise comparisons, uncorrected p>0.05

Table 2:

Cerebellar Measures by Group

	nsCON N=17	sCON N=31	nsALC N=21	sALC N=23
Total Cerebellum (mm^3)	131,106±12,559	128,595±11,969	131,162±13,036	126,285±11,043
Left Cerebellar Hemisphere (mm^3)	59,848±6,518	57,509±5,837†	57,443±5,813	55,885±4,788‡
Right Cerebellar Hemisphere (mm^3)	59,301±6,335	57,168±5,785†	56,922±5,564	55,459±4,871‡
Superior Anterior Vermis (mm^2)	391±33	392±46	372±39$^{\&}$	370±45
Superior Posterior Vermis (mm^2)	208±28	209±28	193±25	198±26
Inferior Posterior Vermis (mm^2)	316±33	317±50	307±34	297±39
ICV (mm^3)	1,376,572±220,548	1,469,520±190,085	1,458,896±196,621	1,430,624±205,340

Abbreviations: nsCON=non-smoking controls, sCON=smoking controls, nsALC=non-smoking abstinent alcohol-dependent treatment seekers, sALC= smoking abstinent alcohol-dependent treatment seekers.

$^{\&}$nsALC < nsCON, uncorrected p≤0.05

†sCON < nsCON, uncorrected p≤0.05

‡sALC < nsCON, uncorrected p≤0.05

All other pairwise comparisons, uncorrected p>0.05

Table 3:

Cognitive Measures by Group

	nsCON N=17	sCON N=31	nsALC N=21	sALC N=23
AMNART (Verbal IQ estimate)	120±8	117±6	119±6	113±9
Ward-7 Full Scale IQ (general intelligence)	1.25±0.63	0.31±0.85†	0.49±0.97$^{\&}$	0.34±0.81
Grooved Pegboard – Dominant (sec)	69±8	76±13	84±34	74±11
Grooved Pegboard – Nondominant (sec)	73±12	70±22	67±29	78±21
Sharpened-Romberg – eyes closed (sec) **	171±73	161±65§	101±99$^{\&}$	101±99‡*
TMT Trails – A (sec)	28±9	32±9	34±15	31±9
TMT Trails – B (sec)	56±21	71±20	76±28$^{\&}$	63±27
WCST – total time (sec)	286±27	374±76†	392±137$^{\&}$	386±129
WCST – total correct **	52±5	42±13†§	50±6	47±11*
WCST – total errors $^{**, *}$	11±5	23±13†§	14±6	17±11*
WCST – perseverative errors *	6±4	12±6†§	6±2	9±7*
WCST – perseverative responses *	6±5	13±8†§	6±3	10±9*
WCST – nonperseverative errors	5±2	10±8†	8±5	8±5*

Abbreviations: AMNART=American National Adult Reading Test, sec=seconds, TMT=Trail Making Test, WCST=Wisconsin Card Sorting Test, nsCON=non-smoking controls, sCON=smoking controls, nsALC=non-smoking abstinent alcohol-dependent treatment seekers, sALC= smoking abstinent alcohol-dependent treatment seekers.

* overall smoking effect, uncorrected p≤ 0.05

** overall alcohol effect, uncorrected p≤0.05

$^{\&}$ nsALC < nsCON (Sharpened-Romberg, IQ) or nsALC > nsCON (WCST–total time, TMT-Trails B), uncorrected p≤0.05

† sCON < nsCON (WCST – total correct, IQ) or sCON > nsCON (WCST – all other measures), uncorrected p≤0.05

‡ sALC < nsCON, uncorrected p≤0.05

§ sCON <nsALC (WCST – total correct), uncorrected p≤0.05

* sCON < sALC (WCST – total correct) or sCON > sALC (Sharpened Romberg, other WCST measures), uncorrected p≤0.05

All other pairwise comparisons, uncorrected p>0.05