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Regional Brain Volume Changes in Alcohol-dependent Individuals during Short-term and Long-term Abstinence

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Abstract

Background—Widespread brain atrophy in alcohol-dependent individuals (ALC) has been consistently documented in pathological and magnetic resonance imaging (MRI) studies. Longitudinal MRI studies have shown that the regional brain volume losses in ALC are partially reversible during abstinence from alcohol. The goal of this study was to determine volume reductions in cortical and subcortical regions functionally important to substance use behavior and their changes during short-term (1 week to 1 month) and long-term abstinence (1 month to 7 months) from alcohol. The regions of interests (ROIs) were: anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (DLPFC), orbitofrontal cortex (OFC), insula, amygdala, and hippocampus.

Methods—A total of 85 unique ALC were assessed at 1 week (n = 65), 1 month (n = 82), and 7 months (n = 36) of abstinence. In addition, 17 light/non-drinking healthy controls (CON) were assessed at baseline and follow-up over a 10-month interval. Regional brain volumes were derived from FreeSurfer. Cross-sectional statistical analyses using one-way analysis of variance or Fisher's Exact Test were applied to identify group differences. Longitudinal statistical analyses using linear mixed models were applied to identify regional volume increases and non-linear volume recovery trajectories.

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Authors Contribution

DJM obtained funding for this study. TCD recruited the study participants, conducted their clinical and neuropsychological assessments, and performed cross-sectional image data processing. XZ, TCD and DJM conceptualized and designed the longitudinal analyses. XZ was responsible for longitudinal data processing, statistical analyses, data interpretation, and manuscript preparation. All authors critically reviewed the content and approved the final version of the manuscript for publication.

Results—We demonstrated significant regional volume reductions in ACC, DLPFC, and hippocampus. Older age was associated with smaller DLPFC and OFC, and higher average monthly drinking over 1 year prior to study was associated with smaller OFC. We also demonstrated significant volume increases of all ROIs except amygdala in ALC and significant non-linear volume recovery trajectories of DLPFC, OFC, and insula.

Conclusions—Results showed significant volume reductions in key regions of the executive control, salience, and emotion networks in ALC at entry into treatment and significant volume increases during short-term and long-term abstinence that were non-linear over the entire abstinence period for the DLPFC, OFC and insula. This gray matter plasticity during alcohol abstinence may have important neurobiological and neurocognitive implications in ALC, and it may contribute to an individual's ability to maintain abstinence from alcohol at different phases.

Keywords

Alcohol User Disorder; Abstinence; Recovery; Longitudinal MRI; Regional Brain Volume

Introduction

Widespread brain atrophy in alcohol-dependent individuals (ALC) has been consistently documented in pathological and magnetic resonance imaging (MRI) studies (Buhler and Mann, 2011, Chanraud et al., 2007, Fein et al., 2002, Kril and Halliday, 1999, Makris et al., 2008, Monnig et al., 2013, Moselhy et al., 2001, Sutherland et al., 2014, Topiwala and Ebmeier, 2017, Xiao et al., 2015, Zahr et al., 2011). Longitudinal MR studies have shown that the regional brain volume losses in ALC are partially reversible during abstinence from alcohol (Agartz et al., 2003, Demirakca et al., 2011, Gazdzinski et al., 2010, Gazdzinski et al., 2008, Hoefer et al., 2014, Pfefferbaum et al., 1995, van Eijk et al., 2013, Yeh et al., 2007). Brain volume increases can be demonstrated by quantitative structural MR imaging within as few as 14 days of abstinence (van Eijk et al., 2013). Longitudinal MRI studies using three time points (Agartz et al., 2003, Hoefer et al., 2014, Pfefferbaum et al., 1995, Yeh et al., 2007) also suggested that the trajectory of brain volume recovery in ALC during the entire abstinence period is not necessarily linear. In an early study of the temporal dynamics of brain volume recovery (Gazdzinski et al., 2005a), we observed that approximate 50% of whole brain tissue volume recovery during the entire 7 to 12 months of abstinence occurred during the first month of abstinence. We also showed that the data predicted from a non-linear mathematical formula were very similar to the experimentally measured data for all lobes and for both gray and white matter (Mon et al., 2011). Further analyses of structural imaging data from a similar cohort (Durazzo et al., 2017, Durazzo et al., 2015) revealed that ALC had significant increases in gray matter (GM) volumes in the frontal, parietal and occipital lobes between 1 week and 7.5 months of abstinence; the monthly GM volume change rates in the entire frontal and parietal lobes were significantly greater between 1 week and 1 month of abstinence than between 1 month and 7.5 months of abstinence, suggesting a non-linear trajectory of GM volume recovery in these lobes. Clinically relevant modulators of the degree of regional volume reduction in adult ALC and of the extent of volume recovery during abstinence have been identified, such as age, gender, genetic factors, family history of problem drinking, concurrent chronic cigarette smoking,

and comorbid medical, psychiatric, and substance use disorders (Cardenas et al., 2005, Demirakca et al., 2011, Durazzo et al., 2007a, Durazzo et al., 2007b, Durazzo and Meyerhoff, 2017, Gazdzinski et al., 2005b, Hoefer et al., 2014, Mon et al., 2013, Pennington et al., 2015). However, it is unclear if the volume reductions and longitudinal change trajectories also exist in functionally distinct sub-regions within the lobar GM regions that are critically important to addictive behavior.

Therefore, the goal of this study was to determine regional volume reductions and their changes during short-term (1 week to 1 month) and long-term abstinence (1 month to 7 months) from alcohol. Multiple GM regions contribute differentially to the development and maintenance of alcohol use disorders (AUD) as parts of interacting brain networks (Bota et al., 2005, Bressler, 1995, McIntosh, 2000, Mesulam, 1998). Particularly, three major brain networks (executive control, salience, and emotional regulation networks) contain cortical and subcortical regions of interests (ROIs) that are critical to AUD and the long-term maintenance of abstinence (Koob et al., 2014, Koob and Volkow, 2016, Volkow and Baler, 2013): anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (DLPFC), orbitofrontal cortex (OFC), insula, amygdala, and hippocampus. Our main hypotheses were:

1. Compared to light/non-drinking healthy controls (CON), ALC have significantly smaller volumes of the six ROIs at 1 week and 1 month of abstinence. At 7 months of abstinence, the volumes of the six ROIs do not differ significantly between ALC and CON.
2. Over both short-term and long-term abstinence, ALC have significant increases in volumes of the six ROIs. The volume recovery trajectories are significantly non-linear over the entire abstinence period.
3. Over both short-term and long-term abstinence, smoking ALC (sALC) have smaller rates of volume increases in these six ROIs than non-smoking ALC (nsALC).

Materials and Methods

Participants

The study was approved by the Committee on Human Research of the University of California San Francisco and the San Francisco Veterans Affairs Medical Center (SFVAMC). Prior to study participation, all participants provided their written informed consent per the Declaration of Helsinki. A total of 85 ALC were recruited from the SFVAMC Substance Abuse Day Hospital and the San Francisco Kaiser Permanente Chemical Dependence Recovery outpatient treatment clinics. The 65 out of 85 ALC (nsALC = 31, sALC = 34) were assessed at both 1 week (time point 1 = TP1) and 1 month (time point 2 = TP2) of abstinence, with 22 of them (nsALC = 11, sALC = 11) also re-assessed at 7 months of abstinence (TP3). Three sALC TP2 data had very poor image quality and were excluded from analysis. The remaining 20 ALC (nsALC = 8, sALC = 12) had no TP1 data and were first assessed at TP2, with 14 (nsALC = 6, sALC = 8) re-assessed at TP3. This resulted in a total of 65 abstinent ALC TP1 data (nsALC = 31, sALC = 34), 82 abstinent ALC TP2 data (nsALC = 39, sALC = 43), and 36 abstinent ALC TP3 data (nsALC = 17,

sALC = 19). The 49 ALC who were abstinent when assessed at TP2 but were not re-assessed at TP3 had either relapsed, changed their smoking status, or were lost to follow-up. In addition, 17 light/non-drinking CON (including 2 smokers) were recruited from the local community and assessed at baseline and follow-up over a 10-month interval.

Both ALC and CON were between the ages of 18 and 65. Primary inclusion criteria for ALC were: DSM-IV diagnosis of alcohol dependence at baseline, consumption of more than 150 standard alcoholic drinks (containing 13.6 gram of ethanol) per month for at least 8 years prior to enrollment for male participants, or consumption of more than 80 standard alcohol-containing drinks per month for at least 6 years prior to enrollment for female participants, and with no dependence on substances other than alcohol or nicotine within 5 years prior to enrollment. Other medical and psychiatric exclusion criteria for ALC were the same as detailed previously (Durazzo et al., 2015). Briefly, ALC with any medical and/or psychiatric condition known to influence the outcomes of this study were excluded except those with hepatitis C, type-2 diabetes, hypertension, unipolar mood disorders, and cigarette smoking. Primary inclusion criteria for CON were consumption of less than 30 standard alcohol-containing drinks per month, and no consumption of more than 100 alcohol-containing drinks in a single month over lifetime, and no history of medical and psychiatric conditions known or suspected to influence brain morphology and neurocognition, except cigarette smoking.

Clinical Assessment

The clinical assessment procedures were identical to those described in detail previously (Durazzo et al., 2015, Durazzo et al., 2014, Pennington et al., 2015): all participants at baseline completed the Structured Clinical Interview for DSM-IV Axis I Disorders (Patient Edition, Version 2.0), the Lifetime Drinking History interview yielding average number of standard alcoholic drinks/month over one year prior to enrollment and over life time, and an in-house interview questionnaire for other substance consumption (type, quantity, and frequency) based on the Addiction Severity Index and NIDA Addictive Drug Survey. At all TPs, all participants also completed standardized questionnaires that assessed depression (Beck Depression Inventory = BDI) and trait anxiety (State-Trait Anxiety Inventory Y-2 = STAI-Y2) symptoms, as well as the Fagerström Tolerance Test for Nicotine Dependence. Participants were classified as smoker if they reported smoking daily or nearly every day for at least 6 months prior to enrollment in the study (Durazzo et al., 2014) and at the time of assessment. The Timeline Follow-Back Interview was used to assess for any alcohol or other substance use between TPs to verify abstinence status in ALC and no significant substance use in CON.

To assess effects of common comorbid disorders on volume recovery in ALC, three comorbid disorder factors were defined for ALC participants, as described earlier (Durazzo et al., 2015): (1) the comorbid medical disorder factor was positive if the participant had a history of non-exclusionary medical conditions; (2) the comorbid substance use disorder factor was positive if the participant met the DSM-IV criteria for any substance abuse or dependence disorder other than alcohol or nicotine within 5 years prior to enrollment; and (3) the comorbid psychiatric disorder factor was positive if the participant met the DSM-IV

criteria for a unipolar mood disorder or anxiety disorder. The most common medical comorbidities were hypertension and hepatitis C. The most common substance use comorbidities were cocaine and methamphetamine abuse/dependence. The most common psychiatric comorbidities were major depressive disorder and substance/alcohol-induced mood disorder with depressive features.

MRI Acquisition and Processing

At each TP, T1-weighted structural MR images of each participant were acquired using a three-dimensional Magnetization-Prepared-Rapid-Gradient-Echo (MRPAGE) sequence with $1\times1\times1.5$ mm^3 resolution on a 1.5 Tesla Siemens scanner. The TR/TE/TI were 10/4/300 ms.

The intra-cranial volume (ICV) and regional brain volumes were obtained by applying the FreeSurfer v4.5 longitudinal processing pipeline (Reuter et al., 2012) on the T1-weighted MPRAGE images. First, all time points of all participants were processed cross-sectionally followed by rigorous quality control and manual adding of the control points in white matter. Then an unbiased template of each participant was created from the cross-sectional results. At the end, the longitudinal processing was performed on the cross-sectional results with the template for each participant. Volumes of brain regions in the left and right hemisphere were highly correlated, and, therefore, the left and right regional brain volumes were summed for volume analysis. Insula, hippocampus, and amygdala were directly labeled by FreeSurfer. In this study, ACC, DLPFC, and OFC were composite regions defined in terms of FreeSurfer labels as in a previous report (Pennington et al., 2015) and their volumes were calculated as: ACC volume = sum of the volumes of rostral and caudal anterior cingulate cortices; DLPFC volume = sum of the volumes of rostral and caudal middle frontal and superior frontal cortices; OFC volume = sum of the volumes of medial and lateral orbitofrontal cortices.

Statistical Analyses

All statistical analyses were performed in RStudio (Version 0.99.903, RStudio Inc., R version 3.3.1). Important covariates shown and thought to affect regional brain volumes in AUD or their changes during abstinence were identified through exploratory analyses (see “Supplement Material Section A: Determining Possible Covariates for Cross-sectional and Longitudinal Analyses”).

In cross-sectional analyses, group differences in demographic and clinical measures between CON at baseline and ALC at TP1 were examined using one-way analysis of variance (ANOVA) or Fisher’s Exact Test. A p-value < 0.05 was considered significant. General linear regression model (GLM) and ANOVA were used to examine group differences in the volumes of the six ROIs between the CON and ALC groups at TP1, TP2, and TP3. Because no significant volume changes were observed in CON over time, the regional brain volumes of CON at baseline were used to compare with the regional brain volumes of ALC at both TP1 and TP2; however, CON data obtained at follow-up were compared with ALC at TP3. The covariates were selected according to the exploratory analyses presented in “Supplement Material Section A: Determining Possible Covariates for Cross-sectional and Longitudinal Analyses”. In summary, the main predictor in these group comparisons was group and the covariates tested were ICV, age, and smoking status. Since CON did not have

substance use, psychiatric, or medical comorbidities, the comorbidity factors were not tested as covariates in cross-sectional group comparisons between CON and ALC. We corrected for multiple comparisons (six ROIs) by adjusting the significance level α using a modified Bonferroni procedure (Sankoh et al., 1997); with the average inter-correlation coefficient between the volumes of the six ROIs being 0.569, the adjusted significance level was $\alpha = 0.023$.

In longitudinal analyses, the volume changes in the six ROIs were evaluated with linear mixed models (LMM) in the "nlme" library. LMM is an extension of the generalized linear models that explicitly models individual change across time. LMM is more flexible than generalized linear modeling (e.g., repeated measures analysis of variance) for repeated measures/longitudinal assessments because it allows participants to have different numbers of observations across time points, and time can be modeled as a continuous variable, rather than a fixed set of points. LMM also permits flexible specification of the covariance structure among repeated measures to best fit the data structure.

LMM were applied for baseline and follow-up data from CON (2 TPs per participant, total number of TP data = 34) and separately for data from the short-term (TP1-TP2, 1 or 2 TPs per participant, total number of TP data = 147), long-term (TP2-TP3, 1 or 2 TPs per participant, total number of TP data = 118), and entire abstinence periods (TP1-TP2-TP3, 2 or 3 TPs per participant, total number of TP data = 183) of ALC. The outcome of each LMM was the volume of a ROI and the fitted intercept was the estimated ROI's average volume (mm^3). For ALC, random slopes were fit to account for participants having different numbers of observations across TPs as only 22 ALC had all three TPs, while the rest had subsets of the three TPs. In LMM, the main predictor was months of abstinence (i.e., days of abstinence/30, month) and thus the fitted coefficient of the main predictor was the estimated linear monthly volume change rate (mm^3/month). The covariates were selected according to the exploratory analyses presented in "Supplement Material Section A: Determining Possible Covariates for Cross-sectional and Longitudinal Analyses". In summary, the primary covariates were ICV, age, one-year average drinks/month, and smoking status (smoker or non-smoker). Other covariates per region were: comorbid medical factor for ACC; comorbid medical factor and BDI for DLPFC; comorbid medical factor, comorbid substance use factor, and BDI for insula; comorbid medical factor and STAI-Y2 for hippocampus; comorbid medical factor, comorbid psychiatric factor, BDI, and STAI-Y2 for amygdala. The effects of covariates on the linear monthly volume change rates were tested by statistically comparing the base LMM models with secondary LMM models containing the foregoing main predictor and covariates plus an interaction term (months of abstinence x one covariate).

To test for non-linearity of regional GM volume recovery trajectories, the base models were also statistically compared with secondary LMM models that contained the foregoing main predictor and covariates plus a quadratic term for months of abstinence (i.e. the second power of months of abstinence, $month^2$). The fitted coefficient of the quadratic term was the estimated quadratic monthly volume change rate ($mm^3/month^2$). A significant quadratic term for monthly volume change indicates that the ROI volume recovery trajectory was best fit with a parabolic curve rather than a linear fit. A significant positive quadratic term

indicates that the curve opens upward, i.e. the ROI volume increases faster during long-term abstinence than short-term abstinence. A significant negative quadratic term indicates that the curve opens downward, i.e. the ROI volume increases faster during short-term abstinence than long-term abstinence. As for the cross-sectional analyses, an adjusted p-value < 0.023 was considered statistically significant for all longitudinal analyses.

Results

Participant Demographics and Clinical Measures

Demographics and clinical measures of CON at baseline and ALC at TP1, TP2, and TP3 are presented in Table 1. Compared to the CON group at baseline, the ALC group at TP1 was older ($p = 0.024$) with fewer years of education and showed greater depression and trait anxiety symptoms (all $p < 0.001$). By design, the average abstinence duration of the ALC group was approximately 1 week for TP1, 1 month for TP2, and 7 months for TP3. Across the three TPs, the ALC group analyzed had largely similar demographic and clinical measures. Of note, compared to TP2, the ALC group analyzed at TP3 had a smaller percentage of individuals with comorbid psychiatric disorders ($p = 0.03$) as well as lower BDI and STAI-Y2 scores (both $p = 0.009$).

Graphic Visualization of ALC Regional Brain Volumes at All TPs

Volume distributions of the six ROIs in ALC across the TPs are displayed in Fig. 1 using box-and-whisker plots. The bottom edge, center band, and top edge of the boxes are the 25^{th}, 50^{th} (median), and 75^{th} percentiles, respectively. The bottom and top whiskers represent 1.5 times the distance between 25^{th} and 75^{th} percentiles from the bottom and top edges of the boxes, respectively. For better display, the brain volumes (mm^3) of each region were normalized with respect to the average volume of that region of all ALC over all TPs (mm^3, see “ALC TP1-TP2-TP3 average volume” in Table 2). The volume distributions across different ROIs were visibly different. ALC at TP2 had visibly higher median volumes of ACC, DLPFC, OFC, insula, and hippocampus than their respective median volumes of ALC at TP1. From TP2 to TP3, the median volumes of ACC, DLPFC, and OFC continued to increase visibly, whereas the changes in the median volumes for insula, hippocampus, and amygdala were barely visible.

Cross-sectional Analyses

The group differences between regional brain volumes of CON and ALC at all TPs are displayed in Fig. 2. For better display, the group volume differences (mm^3) of each region were normalized as the percentages (%) of the region’s average volume of all ALC over all TPs (mm^3, see “ALC TP1-TP2-TP3 average volume” in Table 2). Compared to CON, ALC had significantly smaller volumes of ACC and DLPFC at TP1 and TP2 (all $p < 0.023$) but ACC and DLPFC volumes in CON and ALC were equivalent at TP3 (both $p > 0.290$). ALC had smaller hippocampi than CON across all TPs (all $p < 0.004$). The volumes of the OFC, insula, and amygdala did not differ significantly between CON and ALC at any TP, but the insular volume tended to be smaller in ALC at TP1 and TP2 compared to CON (both $p < 0.07$). In all ROI group comparisons between CON and ALC at each TP using GLM and ANOVA, ICV was a significant predictor of regional brain volumes whereas smoking status

was not. Age was a significant predictor for the ACC volume at TP1 ($p = 0.015$) and for the DLPFC and OFC volumes at all TPs (all $p < 0.011$).

Longitudinal Analyses

The results of regional longitudinal volume changes using LMM are presented in Table 2 and the linear monthly volume change rates are displayed in Fig. 3. For better display, the linear monthly volume change rates (mm^3/month) of each region were normalized as the percentages (%/month) of the region's average volume of all ALC over all TPs (mm^3, see "ALC TP1-TP2-TP3 average volume" in Table 2). All fundamental model assumptions were met. The regional volumes in CON did not change significantly over the 10-months scan interval, attesting to the stability of our measurement paradigm over time. By contrast, in ALC, the volumes of DLPFC, OFC, insula, and hippocampus increased over the TP1-TP2 (all $p < 0.005$) and TP2-TP3 intervals (all $p < 0.012$). The ACC volume increased significantly only over TP2-TP3 ($p = 0.013$), but the volume did not increase significantly over the TP1-TP2 interval. The amygdala volume tended to increase only during the TP1-TP2 interval ($p = 0.058$) and remained practically unchanged after TP2. The linear monthly volume change rates in the DLPFC, OFC, and insula were about 4 to 6 times higher during short-term than long-term abstinence (2.5 times for the ACC), so that up to 50% of the observed volume increases in these ROIs over the entire 7-months abstinence period occurred during the first month of abstinence. Over the entire abstinence period, the DLPFC, OFC, and insula also showed significant non-linear volume recovery trajectories, i.e., the quadratic monthly volume change rates were significant (all $p < 0.009$) (see Table 2). The significant quadratic rates of these three ROIs were all negative (i.e., frowning parabolas), indicating that the volumes of these three ROIs increased faster during short-term abstinence than long-term abstinence.

The effects of smoking status, comorbid substance use, medical, and psychiatric factors on volume changes as well as their interaction terms with months of abstinence were not statistically significant in any region. Generally, no significant effects of age and one-year average drinks/month and their interaction terms with months of abstinence were found on regional volume changes. However, the LMM revealed that older age was associated with smaller DLPFC (0.31% smaller volume per one year older) and OFC (0.34% smaller volume per one year older), and higher average monthly drinking over 1 year prior to study was associated with smaller OFC (0.01% smaller volume per additional drink per month).

Discussion

The main purpose of this study was to determine the nature and extent of recovery of volume deficits in ACC, DLPFC, OFC, insula, amygdala, and hippocampus of treatment-seeking ALC and to characterize their changes during short-term (1 week to 1 month) and long-term abstinence (1 month to 7 months) from alcohol. We demonstrated significant regional volume reductions in ACC, DLPFC, and hippocampus during short-term abstinence. Older age was associated with smaller DLPFC and OFC, and higher average monthly drinking over 1 year prior to study was associated with smaller OFC. We also demonstrated significant volume increases of all ROIs except amygdala in abstinent ALC and significant

non-linear volume recovery trajectories of DLPFC, OFC, and insula. For all six ROIs, smoking status was not a significant predictor in any of the cross-sectional and longitudinal regional brain volume analyses, and smoking status did not interact with linear monthly volume change rates.

In cross-sectional analyses, ALC had significantly smaller volumes of ACC, DLPFC, and hippocampus at TP1 and TP2 (approximately 6–10%) compared with CON. At TP3, ACC and DLPFC volumes of ALC were not significantly different from CON, but hippocampal volume remained significantly smaller. This significant atrophy in ACC, DLPFC, and hippocampus at TP1 and TP2 is Consistent with neuropathologic findings (Kril and Halliday, 1999, Sutherland et al., 2014) and with many earlier MR neuroimaging reports. Studies of similar ALC populations with comparable abstinence duration to our TP1 and TP2 showed significant volume losses in the ACC and lateral prefrontal cortex (Beck et al., 2012, Demirakca et al., 2011, Rando et al., 2011), as well as hippocampus (Agartz et al., 1999, Hoefer et al., 2014, Laakso et al., 2000, van Eijk et al., 2013, Wrase et al., 2008). Unlike previous studies (Beck et al., 2012, Demirakca et al., 2011, van Eijk et al., 2013), the OFC and insula volumes did not differ significantly between our groups of CON and ALC across any time point. However, our longitudinal analyses found significant volume increases of OFC and insula over both short-term (TP1-TP2) and long-term abstinence (TP2-TP3; Fig. 3 and Table 2). Although we did not find a significant amygdala volume reduction nor volume increase during abstinence, two other 1.5T MR studies reported significantly smaller amygdala in ALC (Demirakca et al., 2011, Wrase et al., 2008), using manual tracing and editing methods to measure amygdala volume on MPRAGE images with $1\times1\times1$ mm^3 resolution. One possible explanation for these discrepant findings is that our MPRAGE images with $1\times1\times1.5$ mm^3 resolution at 1.5T may not have provided sufficient contrast to accurately delineate the amygdala from surrounding tissues using the FreeSurfer segmentation algorithm applied here.

Our longitudinal analyses showed significant regional volume increases in ALC over approximately 7 months of sustained abstinence. Specifically, we found significant volume increases during short-term abstinence (i.e., TP1-TP2 interval) in all ROIs except ACC and amygdala (which showed a trend) and significant long-term volume increases (i.e. TP2-TP3 interval) in all ROIs except amygdala; these increases over approximately 7 months of abstinence from alcohol lead to mean volumes in the DLPFC and ACC that were statistically equivalent to those in CON. The regional volume increases are consistent with earlier reports of significant volume increases in the frontal lobe GM (Durazzo et al., 2015, van Eijk et al., 2013), OFC (Demirakca et al., 2011), insula (Demirakca et al., 2011, van Eijk et al., 2013), and hippocampus (Demirakca et al., 2011, Gazdzinski et al., 2008) of abstinent ALC over similar durations of abstinence. Importantly, over the entire 7-month abstinence period, the DLPFC, OFC, and insula also showed significant non-linear volume recovery trajectories, consistent with our previous report of non-linear volume recovery of the entire frontal lobe in a similar population of ALC (Durazzo et al., 2015). Furthermore, our longitudinal data across three TPs showed that the monthly rates of volume increases in ACC, DLPFC, OFC, insula, and hippocampus were at least 2.5 times greater during short-term than long-term abstinence. This is consistent with previously reported rapid frontal and insular GM volume increases during the first 2 weeks of abstinence (van Eijk et al., 2013) and with our

preliminary report of approximately 6 times greater gains of the entire brain volume during the first month of abstinence than the following months (Gazdzinski et al., 2005a). The ACC volume increased significantly between TP2 and TP3 but not between TP1 and TP2; this difference may exist because most ALC studied at TP1 and TP2 (69%) relapsed later between TP2 and TP3. We showed previously that specifically smaller ACC volume in the first month of abstinence (but not that of other cortical regions) predicted future relapse (Durazzo and Meyerhoff, 2017, Durazzo et al., 2017). Therefore, ACC volume early in abstinence may be more variable across ALC participants than the volumes of other ROIs.

The volume reductions and recovery trajectories in these ROIs encompassing executive control, salience, and emotion networks have important neurobiological and neurocognitive implications for AUD. While volume reductions in AUD have been found in many brain regions (Agartz et al., 1999, Demirakca et al., 2011, Durazzo et al., 2015, Gazdzinski et al., 2005a, Pfefferbaum et al., 1995, Wrase et al., 2008), frank neuronal loss was observed specifically only in DLPFC but not in primary motor cortex and hippocampus (Harding et al., 1997, Harper and Kril, 1989, Kril and Halliday, 1999, Kril et al., 1997), suggesting selective neuronal vulnerability (Pulsinelli, 1985). Some plausible candidate mechanisms for GM plasticity during abstinence from alcohol are gliogenesis, synaptogenesis, and changes in neuronal morphology and vasculature (Zatorre et al., 2012). Changes in neuronal morphology (such as dendrite formation, axonal sprouting, increases in the size of neuronal cell bodies) and synaptogenesis could be accompanied by increases in cortical N-acetylaspartate, and such MR spectroscopic increases are commonly observed during abstinence from alcohol, including in the population of this report (Meyerhoff et al., 2013). Consistent with potential vascular changes, we observed in a subpopulation of the cohort of this analysis significant cortical perfusion increases in frontal and parietal lobes between 1 and 5 weeks of abstinence (Mon et al., 2009).

At 7 months of abstinence, while ACC, DLPFC, OFC, and insula volumes did not differ significantly from those in CON, hippocampal volume remained significantly smaller, despite its significant volume increases during both TP1-TP2 and TP2-TP3 intervals. This finding implies that neurobiological abnormalities in hippocampus are more persistent than those in cortex, which may be related to the persistent deficits in some domains of functioning after extended abstinence (Durazzo et al., 2014, Parsons, 1998) and to potential problems with learning new tasks important for managing long-term abstinence and practicing new adaptive behavior to prevent relapse. The significant non-linear volume recovery trajectories of DLPFC, OFC, and insula over 7 months of abstinence may underlie aspects of the previously reported significant non-linear improvements in common neurocognitive measures over 8 months of abstinence of a similar ALC cohort (Durazzo et al., 2014). Examination of the associations between changes in neurocognitive measures and the six ROIs used in this will be addressed in future analyses. Altogether, our regional structural findings may have implications for treatment outcome. In AUD, relapse frequently occurs within 6 months of conclusion of treatment (Boothby and Doering, 2005, Witkiewitz, 2011) and subsequent relapse has been related to regional and global frontal GM atrophy (Beck et al., 2012, Durazzo et al., 2017, Durazzo et al., 2011, Rando et al., 2011). We previously observed that individuals who relapsed within 12 months after treatment, compared to those who had sustained abstinence for at least 12 months, showed significantly

smaller bilateral total frontal GM volumes near entry into treatment as well as after approximately 5 weeks of abstinence (Durazzo et al., 2017). The macrostructural integrity of the six ROIs investigated in this study is associated with adaptive function of the executive control, salience, and emotional regulation networks (Durazzo and Meyerhoff, 2017, Volkow and Baler, 2013). Therefore, the differential changes in these regions during short-term abstinence may represent an endophenotype that distinguishes those who respond more favorably to the typical psychosocial and pharmacological interventions provided for AUD.

Smoking status was not a significant predictor in any of the cross-sectional and longitudinal regional brain volume analyses we performed here and smoking status did not interact with the linear monthly volume change rates. Our previous cross-sectional MR studies of cigarette smoking effects in similar cohorts of ALC showed that sALC only had significantly smaller GM volumes of the entire temporal and parietal lobe than nsALC, but that frontal GM was not as much affected by smoking status (Durazzo et al., 2007a, Gazdzinski et al., 2005b). As the six ROIs of this report were in the frontal cortex, amygdala, and hippocampus, it is not surprising that we did not find significant effects of smoking status on volume reductions and changes during abstinence. In addition, our other longitudinal analyses that used different MR image segmentation methods in similar ALC cohorts (Durazzo et al., 2015, Gazdzinski et al., 2008, Hoefer et al., 2014) also did not find significant main effects of smoking status or interaction effects of smoking status x months of abstinence on the volumes of frontal and temporal cortices.

This study has several limitations that may influence the generalizability of the findings. First, only ALC abstinent at each TP were studied, and some ALC entered the study at TP2. Therefore, not all ALC had data at all TPs. The ALC who were not re-assessed at TP3 (relapsed, changed smoking status, or lost to follow up) did not differ demographically or in histories of substance use from those re-assessed at TP3, but as a group they had significantly more psychiatric comorbidities and moderately higher trait anxiety (see “Supplement Material Section B: Demographics and Clinical Measures of the abstinent ALC at TP2 with and without TP3“), consistent with a previous report in a larger population (Durazzo and Meyerhoff, 2017). Second, we used all the data available for all participants in the longitudinal LMM analyses, recognizing that some ALC did not provide data to all the TPs involved in the longitudinal analyses. For example, in the short-term abstinence LMM analysis involving TP1 and TP2 (total number of TP data = 147, 65 TP1 and 82 TP2), three ALC had only TP1 data and twenty ALC had only TP2 data. The TP3 observations after 7 months of abstinence was rather modest, because at least 60% of treatment-seeking ALC typically relapse within six months of treatment (Boothby and Doering, 2005, Witkiewitz, 2011). The limited TP3 data increases the risk of over-fitting the longitudinal LMM models. However, we found no indications of over-fitting and violations of other critical assumptions (see “Supplement Material Section C: Linear Mixed Models (LMM) for Longitudinal Analyses for ALC”). To address the issue of selective survivorship, we also applied paired t-tests to the subset of ALC who had both TP1 and TP2 (n = 62), and the subset of ALC who had both TP2 and TP3 (n = 33). The results of these paired t-tests were very similar to the LMM results presented here (see “Supplement Material Section D: Paired t-tests for Longitudinal Analyses”). Third, the majority of our CON group were non-smokers, who were shown to have larger regional tissue volumes than their smoking counterparts (Durazzo

et al., 2010), while our ALC group consisted of equal proportions smokers and non-smokers. Therefore, our study may not have had enough power to detect effects of smoking and the cross-sectional group differences between CON and ALC may include effects of both chronic drinking and smoking on regional brain volumes. Fourth, this largely U.S. veteran cohort had only approximately 10% female and 20% non-Caucasian participants, which precluded any meaningful examination of potential gender and ethnicity effects. Fifth, the results may have also been influenced by genetic factors not assessed in this study. For example, we showed earlier that the ALC who were Val homozygous for the brain-derived neurotrophic factor genotype [Val66Met rs6265] tended to have greater hippocampal volume recovery over 7 months of abstinence than the ALC who were Met carriers of the Val66Met single nucleotide polymorphism (Hoefer et al., 2014). Finally, the 1.5T images may not have sufficient contrast to precisely detect subtle volume reductions and changes using the FreeSurfer segmentation algorithm we employed.

Taken together, the results of the current study showed significant volume reductions in key regions of the executive control, salience, and emotion networks in ALC at entry into treatment and significant volume increases during short-term (1 week to 1 month) and long-term abstinence (1 month to 7 months), that were non-linear over the entire abstinence period for the DLPFC, OFC and insula. The volume reductions and recovery trajectories of the regions investigated may have important neurobiological and neurocognitive implications in AUD, and in turn may contribute to an individual's ability to maintain abstinence from alcohol at different phases. This study, therefore, provides a basis for investigating the dynamic interactions between brain networks during abstinence and their clinical and behavioral correlates, which may inform individualized treatment modalities for AUD and prevention of relapse. Results from this study further substantiate previous neuroimaging research findings that significant neurobiological recovery is robustly associated with sustained abstinence from alcohol; they provide information of considerable clinical and psychoeducational relevance to how the human brain adaptively changes during the early and later stages of recovery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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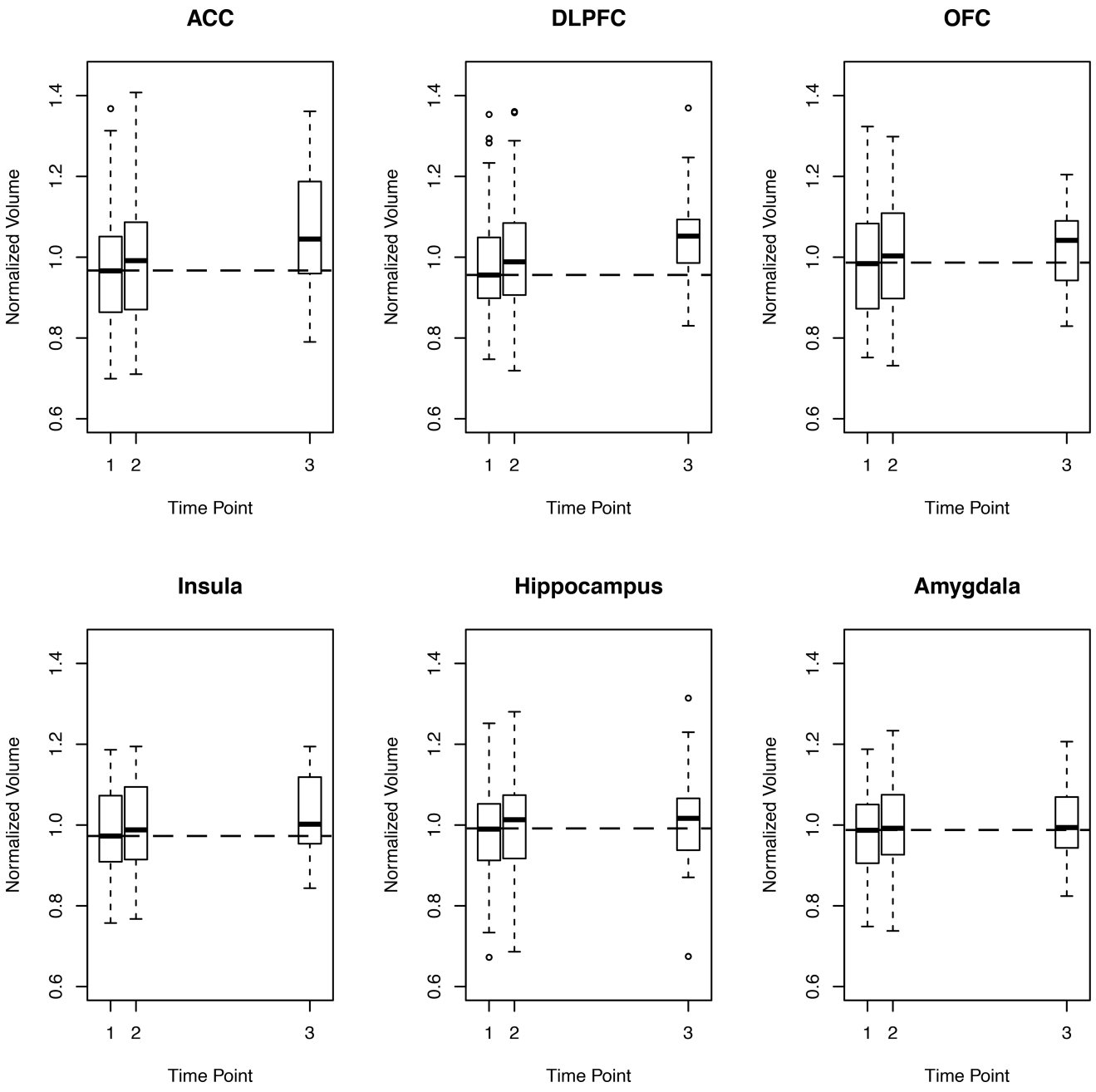

Figure 1.

Graphic visualization of ALC regional brain volumes at all TPs. ALC: alcohol-dependent individuals. TP1: time point 1, at 1 week of abstinence. TP2: time point 2, at 1 month of abstinence. TP3: time point 3, at 7 months of abstinence. ACC: anterior cingulate cortex. DLPFC: dorsolateral prefrontal cortex. OFC: orbitofrontal cortex. Brain volumes of each region were normalized with respect to the average volume of that region of all ALC and displayed using the box-and-whisker plots. The bottom edge, center band, and top edge of the boxes are the 25th, 50th (median), and 75th percentiles, respectively. The bottom and top whiskers represent 1.5 times the distance between 25th and 75th percentiles from the bottom and top edges of the boxes, respectively.

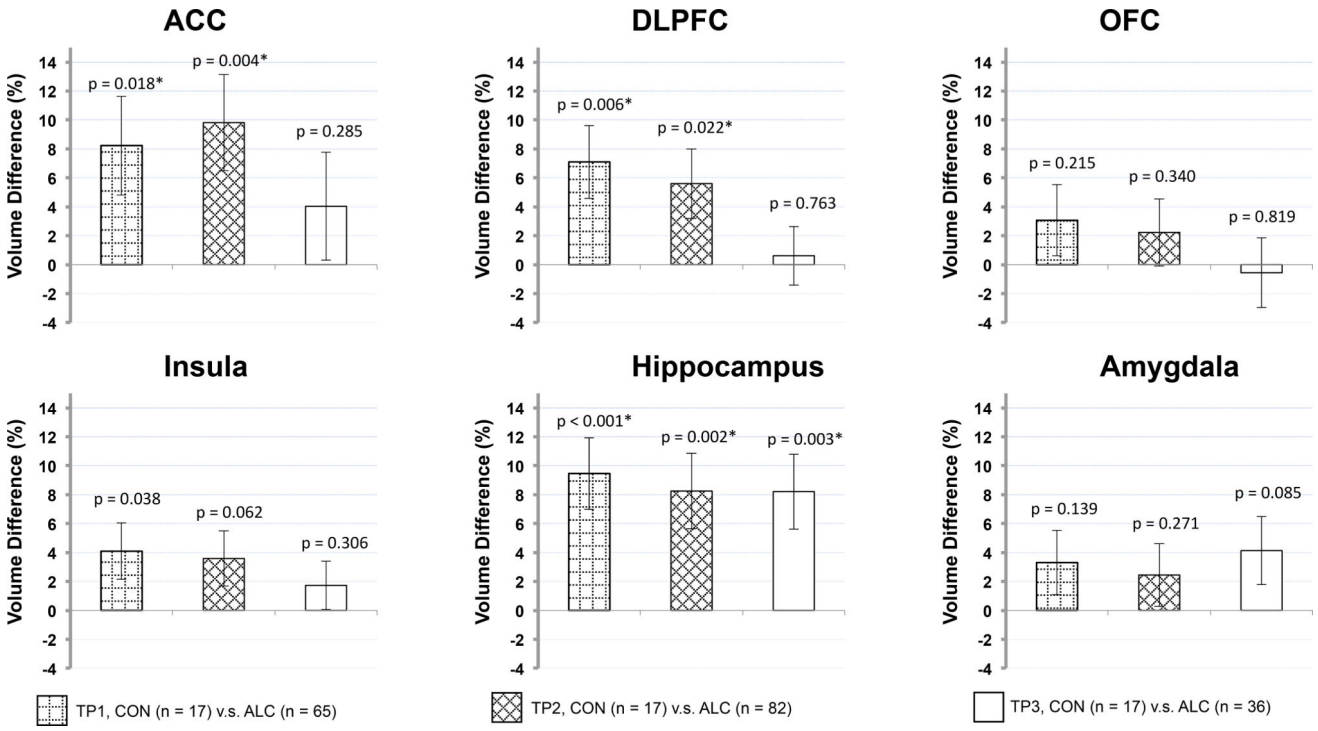

Figure 2.

The group differences between regional brain volumes of CON and ALC at all TPs using general linear regression model (GLM) and one-way analysis of variance (ANOVA). For better display, the group volume differences (mm^3) of each region were normalized as the percentages (%) of the region's average volume of all ALC over all TPs (mm^3, see " ALC TP1-TP2-TP3 average volume" in Table 2). *$p < 0.023$ was considered statistically significant (multiple comparison corrected). CON: light/non-drinking healthy controls. ALC: alcohol-dependent individuals. TP1: time point 1, at 1 week of abstinence. TP2: time point 2, at 1 month of abstinence. TP3: time point 3, at 7 months of abstinence. ACC: anterior cingulate cortex. DLPFC: dorsolateral prefrontal cortex. OFC: orbitofrontal cortex.

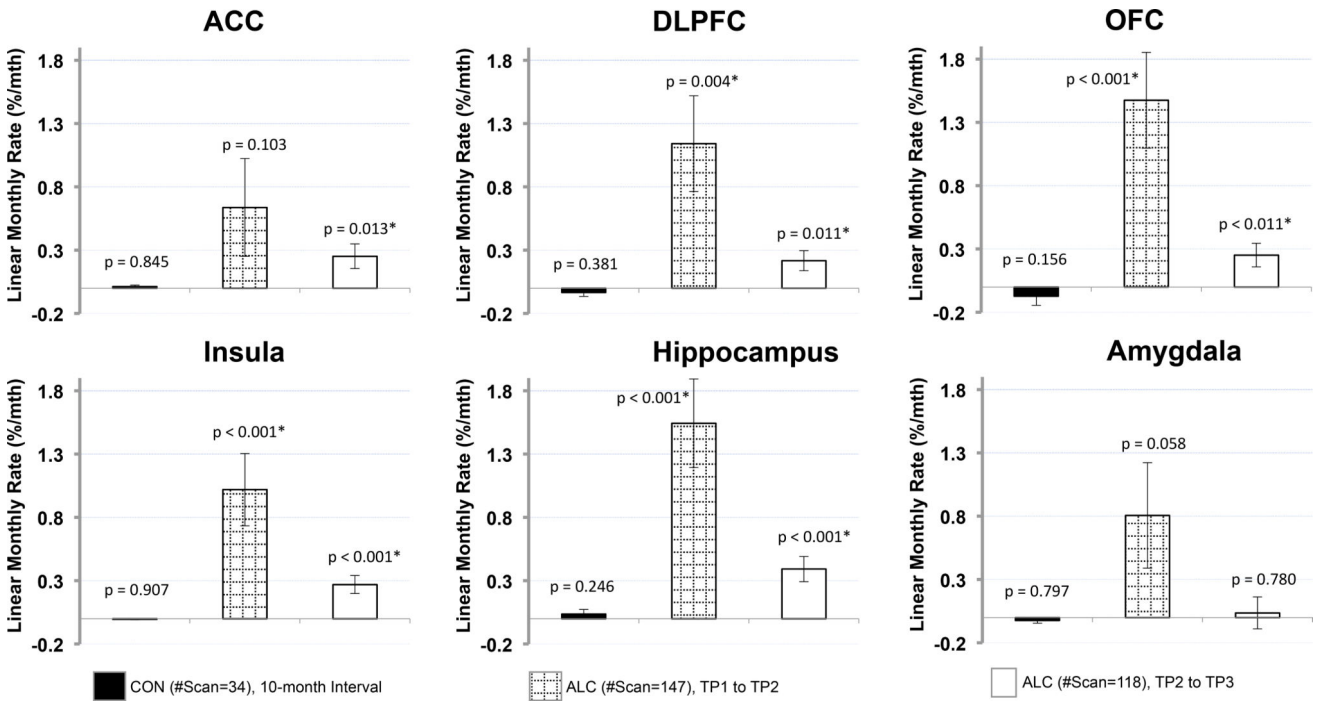

Figure 3.

Linear monthly volume change rates of CON and ALC using linear mixed model (LMM). For better display, the linear monthly volume change rates (mm^3/month) of each region were normalized as the percentages (%/month) of the region's average volume of all ALC over all TPs (mm^3, see " ALC TP1-TP2-TP3 average volume" in Table 2). *p < 0.023 was considered statistically significant (multiple comparison corrected). CON: light/non-drinking healthy controls. CON were assessed at baseline and follow-up over a 10-month interval (312.3 ± 109.2 days). ALC: alcohol-dependent individuals. TP1: time point 1, at 1 week of abstinence. TP2: time point 2, at 1 month of abstinence. TP3: time point 3, at 7 months of abstinence. ACC: anterior cingulate cortex. DLPFC: dorsolateral prefrontal cortex. OFC: orbitofrontal cortex.

Table 1

Participant Demographic and Clinical Measures.

	CON at baseline (n = 17)	**ALC at TP1 (n = 65)**	**ALC at TP2 (n = 82)**	**ALC at TP3 (n = 36)**
Days of abstinence	NA	6.5 ± 3.6	33.5 ± 8.8	218.3 ± 46.2
Age (years):	44.3 ± 9.9	50.5 ± 9.8	51.2 ± 9.5	52.6 ± 10.4
Caucasian (%):	71	77	79	81
Male (%)	88	89	89	89
Education (years):	16.7 ± 2.4	13.9 ± 2.2	14.1 ± 2.1	14.3 ± 2.2
One-year average drinks/month	17 ± 26	396 ± 221	402 ± 220	407 ± 235
Lifetime average drinks/month	20 ± 23	234 ± 153	222 ± 138	216 ± 119
Smoker (%)	12	52	52	53
History of comorbid substance use disorders (%)	0	18	18	14
History of comorbid psychiatric disorders (%):	0	40	38	17
History of comorbid medical disorders (%)	0	49	50	47
Beck Depression Inventory	4 ± 5	14 ± 9	9 ± 8	5 ± 4
State-Trait Anxiety Inventory Y-2	34 ± 8	48 ± 12	44 ± 11	37 ± 11

CON: light/non-drinking healthy controls. ALC: alcohol-dependent individuals. TP1: time point 1, at 1 week of abstinence. TP2: time point 2, at 1 month of abstinence. TP3: time point 3, at 7 months of abstinence.

Table 2

Average volumes (mm^3) and longitudinal volume change rates (mm^3/month) in different brain regions over different intervals by linear mixed model (LMM) analysis.

	CON				ALC									
	10-month Interval Average Volume (mm^3)		10-month Interval Linear Monthly Rate (mm^3/month)		TP1-TP2-TP3 Average Volume (mm^3)		TP1-TP2 Linear Monthly Rate (mm^3/month)		TP2-TP3 Linear Monthly Rate (mm^3/month)		TP1-TP2-TP3 Linear Monthly Rate (mm^3/month)		TP1-TP2-TP3 Quadratic Monthly Rate (mm^3/$month^2$)	
	β ± SE	p-value	β ± SE	p-value	β ± SE	p-value	β ± SE	p-value	β ± SE	p-value	β ± SE	p-value	β ± SE	p-value
ACC	8650.9 ± 245.0	< 0.001	1.1 ± 5.7	0.845	8017.9 ± 106.8	< 0.001	51.1 ± 30.9	0.103	20.2 ± 7.7	0.013	20.9 ± 6.0	< 0.001	−4.5 ± 2.9	0.126
DLPFC	87879.8 ± 1146.0	< 0.001	−29.1 ± 32.1	0.381	82512.7 ± 753.8	< 0.001	942.0 ± 312.8	0.004	179.5 ± 66.1	0.011	219.6 ± 59.3	< 0.001	−78.9 ± 28.0	0.006
OFC	24015.2 ± 401.3	< 0.001	−17.3 ± 87.7	0.156	23220.0 ± 197.0	< 0.001	342.5 ± 87.7	< 0.001	58.5 ± 21.5	0.011	75.0 ± 18.2	< 0.001	−23.2 ± 8.5	0.008
Insula	12630.1 ± 134.9	< 0.001	−0.5 ± 3.8	0.907	12323.6 ± 98.2	< 0.001	125.7 ± 35.3	< 0.001	33.2 ± 8.8	< 0.001	36.9 ± 7.3	< 0.001	−9.1 ± 3.5	0.001
Hippocampus	8966.8 ± 143.2	< 0.001	3.3 ± 2.8	0.246	8319.6 ± 89.0	< 0.001	128.4 ± 29.0	< 0.001	32.7 ± 8.4	< 0.001	39.0 ± 6.7	< 0.001	−0.4 ± 3.2	0.912
Amygdala	3083.2 ± 66.7	< 0.001	−0.7 ± 2.7	0.797	3026.2 ± 25.2	< 0.001	24.4 ± 12.6	0.058	1.1 ± 3.8	0.780	4.1 ± 2.1	0.127	0.5 ± 1.3	0.699

CON: light/non-drinking healthy controls. CON were assessed at baseline and follow-up over a 10-month interval (312.3 ± 109.2 days). ALC: alcohol-dependent individuals. TP1: time point 1, at 1 week of abstinence. TP2: time point 2, at 1 month of abstinence. TP3: time point 3, at 7 months of abstinence. ACC: anterior cingulate cortex. DLPFC: dorsolateral prefrontal cortex. OFC: orbitofrontal cortex. A p value < 0.023 was considered statistically significant (multiple comparison corrected).