

Subcortical Volumes in Long-Term Abstinent Alcoholics: Associations with Psychiatric Comorbidity

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Abstract

Background: Research in chronic alcoholics on memory, decision making, learning, stress and reward circuitry has increasingly highlighted the importance of subcortical brain structures. In addition, epidemiological studies have established the pervasiveness of co-occurring psychiatric diagnoses in alcoholism. Subcortical structures have been implicated in externalizing pathology, including alcohol dependence, and in dysregulated stress and reward circuitry in anxiety and mood disorders and alcohol dependence. Most studies have focused on active or recently detoxified alcoholics, while subcortical structures in long-term abstinent alcoholics (LTAA) have remained relatively uninvestigated.

Methods: Structural MRI was used to compare volumes of eight subcortical structures (lateral ventricles, thalamus, caudate, putamen, pallidum, hippocampus, amygdala and nucleus accumbens) in 24 female and 28 male long-term abstinent alcoholics (LTAA) (mean abstinence = 6.3 years, mean age = 46.6 years) and 23 female and 25 male non-alcoholic controls (NAC) (mean age = 45.6 years) to explore relations between sub-cortical brain volumes and alcohol use measures in LTAA, and relations between subcortical volumes and psychiatric diagnoses and symptom counts in LTAA and NAC.

Results: We found minimal differences between LTAA and NAC in subcortical volumes. However, in LTAA, but not NAC, volumes of targeted subcortical structures were smaller in individuals with vs. without comorbid lifetime or current psychiatric diagnoses, independent of lifetime alcohol consumption.

Conclusions: Our finding of minimal differences in subcortical volumes between LTAA and NAC is consistent with LTAA never having had volume deficits in these regions. However, given that imaging studies have frequently reported smaller subcortical volumes in active and

recently detoxified alcoholics compared to controls, our results are also consistent with recovery of subcortical volumes with sustained abstinence. The finding of persistent smaller subcortical volumes in LTAA, but not NAC, with comorbid psychiatric diagnoses, suggests that the smaller volumes are a result of the combined effects of chronic alcohol dependence and psychiatric morbidity, and suggests that a comorbid psychiatric disorder (even if not current) interferes with the recovery of subcortical volumes.

Keywords: long-term abstinent alcoholics; psychiatric comorbidity; subcortical brain structures; MRI.

Introduction

In recent years, several different lines of research have highlighted the importance of subcortical structures for understanding chronic alcohol dependence and its effects. First, animal experiments (Bonthius et al., 2001), human post mortem brain studies (Harding et al., 1997) and human imaging studies (Agartz et al., 1999; Sullivan et al., 1995; 1996) all show that chronic heavy alcohol consumption damages the hippocampus, and deficits in visuospatial learning and memory, believed to be hippocampus-related functions (Berthoz, 1997; Ghaem et al., 1997; Hartley et al., 2007; Iaria et al., 2003; Lavenex et al., 2006; O'Keefe, 1990; Santin et al., 2000), are among the most consistently found sequelae of chronic alcoholism in humans and in rodent models (Beatty et al., 1996; Bowden, 1988; Corral-Varela and Cadaveira, 2002; De Renzi et al., 1984; Matthews and Morrow, 2000; Nixon et al., 1987; Oscar-Berman and Ellis, 1987; Riege, 1987; Shelton et al., 1984) . The damaging effects are believed to be due to the combined effects of ethanol-induced glucocorticoid elevation, compromised nutrition, and oxidative stress. The hippocampus is rich in receptors for glucocorticoids, making hippocampal neurons vulnerable to reduced glucose uptake and energy supply (Eskay et al., 1995).

A second line of research has focused on the association between alcohol abuse and disruption of brain reward pathways (Koob and Kreek, 2007; Koob, 1999; Kreek and Koob, 1998; Makris et al., 2008; Wise, 1998) which, along with prefrontal cortex, include the subcortical striatopallidal and extended amygdala systems. Chronic consumption and withdrawal

of abusive substances, including alcohol, can lead to alterations in hedonic set point (e.g., raising thresholds), leading to increasing dependence. Magnetic resonance morphometric analysis (Makris et al., 2008) has revealed that, compared to non-alcoholic controls, chronic alcoholics have smaller volumes of reward-related structures (right nucleus accumbens and left amygdala, along with right dorsolateral prefrontal cortex and anterior insula). Smaller volumes were associated with impaired memory.

A related line of research has focused on the role of the amygdala in decision making and learning (Baxter et al., 2000; Bechara et al., 1999; Kahn et al., 2002; Rogers et al., 2004; Tabert et al., 2001; Winstanley et al., 2004). Amygdala activation has been associated with alcohol craving in recently abstinent alcoholics (Schneider et al., 2001), and morphometric analyses have revealed smaller amygdalar volumes in chronic alcoholics (Fein et al., 2006a; Makris et al., 2008), as well as in subjects at high risk for alcohol dependence (Benegal et al., 2007), when compared to controls. A hallmark of alcohol abuse is poor decision making with regard to use. That is, users persist in behaviors that have short-term benefits (i.e., intoxication) despite long-term major negative consequences. A PET activation study (Ernst et al., 2002) revealed increased amygdala activation during decision-making in the Simulated Gambling Task (SGT) (Bechara et al., 1994), which simulates real-life decisions regarding uncertainty, reward and punishment, and several investigations (Bechara et al., 2003; 1999; Verdejo-Garcia and Bechara, 2009) have reported impaired performance on the SGT in subjects with amygdala damage. Impaired performance has been interpreted in terms of an inability to attach appropriate negative emotional valence to negative consequences. Currently active or recently detoxified alcoholics have demonstrated impaired performance on the SGT (Bechara and Damasio, 2002; Bechara et al., 2001; Mazas et al., 2000), and a recent study by Fein and colleagues (2004b) has shown

persistent impaired performance on the SGT in LTAA (abstinence duration ranging from 6 months to 13 years, with a mean abstinence of 6.7 years). Indeed, Fein and colleagues (2006a) found that multi-year abstinent alcoholics who were impaired on the SGT had bilaterally smaller amygdalar gray matter volumes compared to controls.

Finally, large sample epidemiological studies conducted during the last 20 years (Grant et al., 2004a; 2004b; Kessler et al., 1997; Regier et al., 1990; Sher and Trull, 1994), along with studies of samples in treatment (Brady et al., 1998; Brady and Sinha, 2005; Hunter et al., 2000), establish the pervasiveness of co-occurring psychiatric diagnoses (including mood, anxiety, and externalizing disorders) in alcoholism and drug addiction. For example, the largest comorbidity study (N = 43,093), the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC) (Grant et al., 2004a; 2004b), found that about 28% of individuals with a past year alcohol dependence (55% with a drug dependence) had a co-occurring mood disorder, 23.5% (43% with a drug dependence) had a co-occurring anxiety disorder, and 39.5% (69.5% with a drug dependence) had a co-occurring personality disorder (PD) (not including borderline, schizotypal and narcissistic PDs), the most frequent of which was anti-social personality disorder (ASPD). The National Comorbidity Study revealed that 78% of alcoholic men and 86% of alcoholic women met criteria for a non-substance use related lifetime psychiatric diagnosis (Kessler et al., 1997).

Substance abuse frequently follows behavioral problems, including externalizing disorders and anxiety and mood disorders (Kessler et al., 1996; Rohde et al., 2001; Stewart, 1996). Gray matter volume abnormalities in the amygdala, thalamus, and parahippocampal gyri are associated with increased externalizing symptoms in subjects at high risk for alcohol dependence (Benegal et al., 2007). Moreover, a number of investigators (Arborelius et al., 1999;

Plotsky et al., 1995; Yoo et al., 2005) have proposed that anxiety and mood disorders are a function of dysregulated stress circuitry in regions including the amygdala, hippocampus, nucleus accumbens, and putamen, all of which have been shown to be compromised in chronic alcoholics (Agartz et al., 1999; Makris et al., 2008; Sullivan et al., 2005; 1995; 1996; Volkow et al., 2007). This is consistent with findings of structural abnormalities in the hippocampus and amygdala in posttraumatic stress disorder (PTSD) (Bremner, 2002; Gurvits et al., 1996; Yoo et al., 2005), in the amygdala, hippocampus, and putamen in panic disorder (Uchida et al., 2003; Yoo et al., 2005), and the amygdala (Sheline et al., 1998), hippocampus (Bremner, 2002; Sheline et al., 2002; Steffens et al., 2000), and putamen (Beyer, 2006; Husain et al., 1991; Parashos et al., 1998) in major depressive disorder.

Psychiatric comorbidity findings have been extended by two recent studies by Fein and colleagues, who explored psychiatric comorbid diagnosis (Di Sclafani et al., 2007) and sub-diagnostic comorbidity (Fein et al., 2007) in a sample of LTAA with a mean abstinence duration of 6.3 years. Over 85% of LTAA had a lifetime psychiatric diagnosis, compared to 50% of non-alcoholic controls (NAC), and LTAA had significantly more frequent current mood and anxiety diagnoses than NAC. Although no LTAA or NAC met diagnostic criteria for a current ASPD, LTAA showed more ASPD symptoms than NAC.

In the present study, we used structural MRI to compare volumes of cerebral subcortical structures in LTAA and NAC in the sample from the above-cited studies by Fein and colleagues. In addition, we explored relations of sub-cortical brain volumes with alcohol use measures in LTAA, as well as relations between subcortical volumes and both psychiatric diagnoses and symptom counts in LTAA and NAC. We have previously shown a higher incidence of white

matter signal hyperintensities (Fein et al., 2009) and localized smaller cortical volumes (Fein et al., 2009a), compared to controls, in this sample.

Methods and Materials

Participants

A total of 100 participants were recruited from the community by postings at AA meeting sites, mailings, newspaper advertisements, a local internet site, and subject referrals. The study consisted of two subject groups: LTAA and NAC. Table 1 lists demographic and alcohol use information by group. The LTAA group included 24 women and 28 men with lifetime alcohol dependence, ranging from 35 to 58 years of age (mean = 46.6 years), who were abstinent from alcohol and drugs (except nicotine and caffeine) for 6 months to 21 years (mean = 6.3 years). The inclusion criteria for the LTAA group were: 1) met lifetime DSM-IV-R (American Psychiatric Association, 2000) criteria for alcohol dependence, 2) had a lifetime drinking average of at least 100 standard drinks per month for men, and 80 standard drinks per month for women, and 3) were abstinent for at least 6 months. A standard drink was defined as 12 oz. beer, 5 oz. wine, or 1.5 oz. liquor. The NAC consisted of 23 women and 25 men, ranging from 34 to 60 years of age (mean = 45.6 years). The inclusion criterion for the NAC group was a lifetime drinking average of less than 30 standard drinks per month, with no periods of drinking more than 60 drinks per month.

Exclusion criteria for both groups were: 1) lifetime or current diagnosis of schizophrenia or schizophreniform disorder using the computerized Diagnostic Interview Schedule (c-DIS) (Bucholz et al., 1991; Erdman et al., 1992; Levitan et al., 1991; Robins et al., 1998), 2) history of lifetime or current drug abuse or dependence (other than nicotine or caffeine), 3) significant history of head trauma or cranial surgery, 4) history of significant neurological disease, 5) history of diabetes, stroke, or hypertension that required an emergent medical intervention, 6) laboratory evidence of hepatic disease, or 7) clinical evidence of Wernicke-Korsakoff syndrome.

All individuals participated in the following assessments: 1) psychiatric diagnoses and symptom counts were gathered using the c-DIS (Robins et al., 1998), 2) participants were interviewed on their lifetime drug and alcohol use using the timeline follow-back methodology (Skinner and Allen, 1982; Skinner and Sheu, 1982; Sobell and Sobell, 1990; Sobell et al., 1988), 3) medical histories were reviewed in an interview by a trained research associate, 4) blood was drawn to test liver functions, and 5) the Family Drinking Questionnaire was administered based on the methodology of Mann and colleagues (1985) and Stoltenberg and colleagues (1998). The Family Drinking Questionnaire asked participants to rate the members of their family as being alcohol abstainers, alcohol users with no problem, or problem drinkers. Family History Density (FHD) was defined as the proportion of 1st degree relatives that were problem drinkers.

Approval for the study was obtained from a free-standing independent human subjects research review committee [Independent Review Consulting, Corta Medera, CA] and written informed consent was obtained from all research participants.

Insert Table 1 about here.

Psychiatric and Cognitive Assessments

The c-DIS (Robins et al., 1998) was administered to all participants by a research associate. The c-DIS generates a list of endorsed lifetime symptoms, determining whether individuals meet criteria for a lifetime or current psychiatric diagnosis. Results for psychiatric diagnoses and sub-diagnostic pathology were reported in prior publications (Di Sclafani et al., 2007; Fein et al., 2007). Eighty-seven percent of LTAA were found to have at least one lifetime psychiatric diagnosis (including mood, anxiety, and externalizing disorders), compared to 50% of NAC. Thirty-five percent of LTAA with at least 18 months of abstinence (so that only periods after six months of abstinence were counted) had a current psychiatric diagnosis (i.e., met diagnostic criteria within the last 12 months), compared to 6% of NAC. Table 2 shows prevalence rates of current and lifetime psychiatric disorder diagnoses in LTAA and NAC. In addition, there was significantly greater sub-diagnostic psychiatric pathology in LTAA than in NAC.

Insert Table 2 about here.

Cognitive function was assessed using the MicroCog battery (Powell et al., 1993), supplemented by a number of individual tests with demonstrated sensitivity to damage in brain regions frequently compromised by chronic alcoholism. The measures yielded scores in 9 cognitive domains (Abstraction/Cognitive Flexibility, Attention, Auditory Working Memory, Immediate Memory, Delayed Memory, Psychomotor, Reaction Time, Spatial Processing, and Verbal Fluency) reported in a prior publication (Fein et al., 2006b). LTAA performed comparably to NAC in all domains except for a suggestion of persistent deficits in spatial processing abilities. We also reported persistent cortical atrophy in LTAA, compared to NAC,

with parietal gray matter volumes negatively associated with spatial processing performance in LTAA, but not NAC (Fein et al., 2009a).

Image 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 x 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 x 256) at 5 mm thickness. A neuroradiologist read all MRI scans. All scans were free from abnormalities other than white matter signal hyperintensities (WMSH).

Image Analysis

We studied 16 of the 17 subcortical brain structures which are extracted by FSL's FIRST (FMRIB Image Registration and Segmentation Tool), a method that has been used successfully in a number of recent investigations (de Jong et al., 2008; Franke et al., 2010; Lee et al., 2010; Seror et al., 2010; Yun and White, 2010). The brainstem was excluded because the shape model used in FIRST extended beyond the inferior boundary of the image. The following structures (shown in Figure 1) were extracted and their volumes were measured for all 100 T1-weighted MR images: left and right Lateral Ventricles, left and right Thalamus, left and right Caudate, left and right Putamen, left and right Pallidum, left and right Hippocampus, left and right Amygdala, and left and right Nucleus Accumbens.

Insert Figure 1 about here.

Image analysis was performed using FSL (FMRIB Software Library), version 4.1 (Oxford, UK). FIRST is the FSL tool for segmentation of subcortical structures. To ensure a more accurate segmentation of the lateral ventricles, MRIs were first registered nonlinearly to the standard MNI152 space using FSL's FNIRT (FMRIB's Nonlinear Image Registration Tool) (see Appendix for more information). The warped (registered) MRIs are then processed through FIRST to extract the surface mesh of each of the 16 subcortical structures. The surfaces in the warped space are then transformed back (un-warped) to the original MRI space. The un-warped surfaces of the segmented subcortical structures were then filled and boundary corrected (preventing voxel overlap between structures). Figure 2 shows the processing steps we used for subcortical segmentation. For each of the 16 extracted structures, the volume is measured in cubic millimeters. Cranium size estimation (an estimate of premorbid brain size) (Fein et al., 2004a) was also performed on the data.

Insert Figure 2 about here.

Statistical Analyses

The General Linear Model (GLM) (SPSS version 17.0) was used to assess effects of Group (LTAA vs. NAC), Sex, and Age on subcortical volumes. First, GLM was used to determine whether subcortical volumes were associated with cranium size index, calculated by FSL's SIENAX program. Since all 16 subcortical volumes varied significantly as a function of cranium size, we used linear regression to adjust each structure volume for the cranium size index. Then, for each subcortical structure, we added the left and right volumes and conducted a

GLM analysis of the 8 structure volumes with Group and Gender as fixed factors and Age as a covariate.

Spearman correlational analyses were conducted to explore relations between subcortical brain volumes and alcohol use history, cognitive performance, psychiatric symptoms, and diagnoses. (Spearman correlations are robust with regard to underlying distribution assumptions and resistant to the effects of outliers.) Finally, *t*-tests were used to compare volumes of target subcortical structures in LTAA and NAC with and without lifetime or current psychiatric diagnoses.

Results

Cranium Size

Men had craniums 12.1% larger than women, with gender accounting for 34.8% of the variance of the cranium size index ($F_{1,96} = 51.18, p < 0.001$). In contrast, we found no cranium size difference between groups (group accounting for only 0.5% of the variance of the cranium size index; $F_{1,96} = 0.49, p > 0.48$), nor a group by gender interaction (effect size 0.2% of the variance, $F_{1,96} = 0.22, p > 0.63$). Cranium size adjusted values for all subcortical structure volumes were used in all subsequent analyses.

Subcortical Structure Volumes

Consistent with findings from previous brain imaging studies (Krishnan et al., 1990; McDonald et al., 1991; Murphy et al., 1996; Samanez-Larkin et al., 2010; Van Der Werf et al., 2001), correlational analysis showed normal age-related declines, in our NAC, in thalamus ($r_s(46) = -.262, p < .037$), caudate ($r_s(46) = -.339, p < .01$), putamen ($r_s(46) = -.422, p < .002$), and nucleus accumbens ($r_s(46) = -.373, p < .005$) volumes. The GLM analyses reported below

controlled for age. There were no main effects of group for any of the 8 structures, with the exception of a trend for larger ventricles in LTAA ($F_{1,94} = 3.54$, $p = 0.063$, all remaining F 's < 1.08, all remaining p 's > 0.302). Two subcortical structures showed significant interactions, one a group by age interaction and the other a group by gender interaction (See Figures 3 and 4). The effect of age on lateral ventricle volumes (i.e., larger volumes in older subjects) was greater in LTAA than in NAC ($F_{1,94} = 4.356$, $p = 0.040$, effect size 4.4% of variance). Nucleus accumbens volume was smaller in LTAA than NAC in females only ($F_{1,94} = 7.187$, $p = 0.009$, effect size 7.2% of variance). Table 3 shows the average subcortical volumes for male and female subjects in NAC and LTAA groups, as well as effect size for group, group by age and group by gender interactions.

Insert Table 3 about here.

Insert Figure 3 about here.

Insert Figure 4 about here.

Correlations between Subcortical Volumes and Cognitive and Psychiatric Variables

Correlational analysis of subcortical volumes with performance in the nine cognitive domains yielded no significant associations. Correlational analysis of psychiatric symptom counts (sums of Externalizing Disorder Symptoms, Anxiety Disorder Symptoms, and Mood Disorder Symptoms) and subcortical volumes in our NAC and LTAA samples also yielded no significant associations, although there was a trend, in LTAA, toward a negative association between the sum of lifetime externalizing symptoms and hippocampus volumes ($r_s(50) = -.257$, $p = .066$). Correlational analysis did reveal significant negative associations, in LTAA, between

sums of lifetime psychiatric diagnoses and hippocampus ($r_s(50) = -.358, p = .005$) and amygdala volumes ($r_s(50) = -.297, p = .016$) and, in NAC, trends toward negative associations with hippocampus ($r_s(46) = -.206, p = .08$) and amygdala ($r_s(46) = -.190, p = .098$) volumes.

Subcortical Volumes in LTAA and NAC with and without Psychiatric Diagnoses

Figure 5 shows mean volumes of targeted subcortical structures in LTAA and NAC with and without psychiatric diagnoses. One-tailed t -tests were conducted in order to determine whether volumes of structures that were negatively correlated with sums of lifetime psychiatric diagnoses (hippocampus and amygdala) were smaller in LTAA and NAC with lifetime or current psychiatric diagnoses than in those without. One-tailed t -tests were also performed to determine whether volumes of accumbens and putamen, frequently implicated in psychiatric disorders (Beyer, 2006; Husain et al., 1991; Parashos et al., 1998; Sheline et al., 1998; Uchida et al., 2003), were smaller in LTAA and NAC with lifetime or current psychiatric diagnoses than in those without. Results revealed that, in LTAA, hippocampus and accumbens volumes were significantly smaller ($t_{50} = -1.948, p < .03, r^2 = .071$ and $t_{50} = -1.866, p < .034, r^2 = .066$, respectively) in subjects with a lifetime anxiety disorder diagnosis ($N = 19$) than in those with no lifetime anxiety disorder ($N = 33$). In NAC there were no significant differences (or trends toward differences) in targeted subcortical volumes between subjects with a lifetime anxiety diagnosis ($N = 7$) and those with no lifetime anxiety diagnosis ($N = 41$).

Given that the difference in prevalence of lifetime anxiety diagnoses between LTAA and NAC was due entirely to more PTSD diagnoses in LTAA, additional t -tests were conducted to determine whether smaller hippocampus and accumbens volumes in LTAA with lifetime anxiety diagnoses were specific to PTSD, or were associated with lifetime anxiety diagnoses in general. Results revealed that LTAA with a lifetime PTSD diagnosis ($N = 13$) had significantly smaller

($t_{50} = -2.29$, $p < .014$, $r^2 = 0.095$) nucleus accumbens than those without ($N = 39$), while there was no difference in accumbens volumes between LTAA with ($N = 6$) and without ($N = 46$) a non-PTSD lifetime anxiety diagnosis ($p = .840$). In contrast, hippocampus volumes were significantly ($t_{50} = -1.844$, $p < .036$, $r^2 = 0.064$) smaller in LTAA with a non-PTSD lifetime anxiety diagnosis ($N = 6$) than in those without ($N = 46$), while there was no difference in hippocampus volumes between LTAA with and without a lifetime PTSD diagnosis ($p = .441$).

T-tests also revealed that, in LTAA with a *current* anxiety disorder ($N = 10$), accumbens volumes were smaller ($t_{50} = -2.005$, $p < .026$, $r^2 = .074$) than in those without ($N = 42$). There was also a trend ($t_{50} = 1.522$, $p < .067$, $r^2 = .044$) toward smaller putamen volumes in LTAA with a current anxiety diagnosis than in those without. Although this trend is consistent with the pattern of reported significant findings, caution should be taken in interpreting trends, particularly when multiple comparisons are involved. None of the NAC met criteria for a current anxiety diagnosis. Finally, in LTAA, amygdala ($t_{50} = -2.168$, $p < .018$, $r^2 = .086$) and hippocampus ($t_{50} = -1.703$, $p < .048$, $r^2 = .055$) volumes were significantly smaller in subjects with a lifetime externalizing disorder diagnosis ($N = 14$) than in those without ($N = 38$). In NAC there were no differences (i.e., significant effects or trends) in targeted subcortical volumes between subjects with a lifetime externalizing disorder ($N = 4$) and those without ($N = 44$). No LTAA or NAC met criteria for a current externalizing disorder diagnosis.

Associations between Subcortical Volumes and Alcohol Consumption Measures

First, *t*-tests were performed to determine whether LTAA with a lifetime or current anxiety diagnosis, or lifetime externalizing disorder diagnosis, differed from LTAA without a diagnosis in terms of the alcohol consumption measures--Alcohol Peak Dosage, Alcohol Peak

Use (Peak Dosage x lifetime days of Peak Use), Alcohol Lifetime Dosage and Alcohol Lifetime Use. Results revealed no alcohol consumption differences (effects or trends) between LTAA with and without diagnoses. Correlational analysis yielded the following associations between subcortical volumes and the alcohol consumption measures: In LTAA, there was a trend toward a negative association between thalamus volumes and Alcohol Peak Dose ($r_s(50) = -0.254, p = 0.069$), negative associations were found between Alcohol Lifetime Dosage and hippocampus volumes ($r_s(50) = -.303, p = .029$) and between Alcohol Lifetime Use and hippocampus volumes ($r_s(50) = -.337, p = .015$), and a positive association was found between Lifetime Use and lateral ventricle volumes ($r_s(50) = .355, p = .01$). Finally, in LTAA, hippocampus volumes were negatively associated with Alcohol Peak Use ($r_s(50) = -0.312, p = 0.024$) and Alcohol Peak Use was positively associated with lateral ventricle volumes ($r_s(50) = 0.286, p = 0.039$). After partial correlations controlling for age, Alcohol Lifetime Use was still significantly associated with lateral ventricle volumes ($r_s(50) = .302, p = .031$), but not with hippocampus volumes ($r_s(50) = -.170, p = .233$). Similarly, with partial correlations controlling for age, the association between Alcohol Peak Use and lateral ventricle volumes still reached significance ($r_s(50) = .279, p = .047$), but the association between Alcohol Peak Use and hippocampus volumes was non-significant ($r_s(50) = -.085, p = .552$).

Insert Figure 5 about here.

Discussion

The central findings of the current study were 1) minimal differences between LTAA and NAC in volumes of subcortical brain structures, and 2) in LTAA, but not NAC, differences in

subcortical volumes between subjects with and subjects without lifetime or current psychiatric diagnoses. These results, discussed separately below, have implications regarding recovery of subcortical brain volumes with sustained abstinence, and combined effects of chronic alcohol dependence and psychiatric morbidity on subcortical volumes. Since our analyses used cranium size-adjusted values for all subcortical structure volumes, it should be noted that our LTAA and NAC did not differ with regard to mean cranium size, in contrast with a finding of Schottenbauer and colleagues (2007) of smaller intracranial volumes in alcoholics vs. non-alcoholic controls. However, the different findings may be due to sampling differences, since Schottenbauer and colleagues obtained their alcoholics from in-patient settings, while ours were recruited from the community. In addition, their intracranial volumes excluded the cerebellum and the cerebrospinal fluid space of the posterior fossa, while ours did not.

First, the effect of age on lateral ventricle volumes (i.e., larger volumes in older subjects) was greater in LTAA than in NAC, and, after controlling for age, lateral ventricle volumes of LTAA were positively associated with alcohol lifetime use and alcohol peak use (peak dosage x lifetime days of peak use). Brain imaging studies of active or recently detoxified alcoholics (Jernigan et al., 1991; Pfefferbaum et al., 1992) show that ventricular enlargement may be associated with volume reductions of cortical, subcortical or both cortical and subcortical cerebral structures. In addition, within our LTAA sample, hippocampus volumes were negatively associated with alcohol lifetime dose. However, we found no overall differences between LTAA and NAC in volumes of specific subcortical structures. Although it is possible that our LTAA never had smaller volumes than controls in those regions, it is unlikely, given that imaging studies focusing on active and recently detoxified alcoholics (Agartz et al., 1999; Charness, 1993; Jernigan et al., 1991; Makris et al., 2008; Pfefferbaum et al., 1996; 1992; Sullivan et al.,

2005; 1995; 1996) have consistently found smaller subcortical volumes in alcoholic subjects, compared to non-alcoholic controls. Notably, Sullivan and colleagues (2005) found greater nucleus accumbens volumes in “sober alcoholics” (1 month to > 1 year abstinence) compared to “recent drinkers” (1 to 20 days abstinence). It is also unlikely that the lack of group differences in our study is due to a lack of sensitivity of our measures, since our NAC demonstrated the age-related declines in subcortical volumes (reported above), consistent with findings from previous imaging studies (Krishnan et al., 1990; McDonald et al., 1991; Samanez-Larkin et al., 2010; Van Der Werf et al., 2001). Accordingly, our finding of no differences between LTAA and NAC in individual subcortical structure volumes suggests that LTAA recover subcortical brain volumes with sustained abstinence. This proposal is consistent with published evidence from our current sample for cortical region-specific recovery from alcohol-related smaller gray matter volumes with long-term abstinence (Fein et al., 2009a). For example, although Fein and colleagues (2009a) found persistently smaller gray matter volumes in LTAA vs. controls within the parietal lobe, they found no difference from controls in the prefrontal and temporal lobes (and intact executive and memory functions). This contrasts with findings of smaller gray matter volumes in these regions in active heavy drinking and recently detoxified alcoholics, compared to controls (Cardenas et al., 2005; Jernigan et al., 1991; Pfefferbaum et al., 1992; Sullivan et al., 1996). Moreover, the overall lack of differences between LTAA and NAC in individual subcortical volumes is consistent with published findings from our current sample (Fein et al., 2006b) of relatively spared cognitive function in LTAA, for whom we found only a suggestion of impaired performance in spatial processing, which was associated with parietal gray matter loss (Fein et al., 2009a). Taken together, the above findings indicate that, with long-term abstinence, alcohol-

related damage may be at least partially reversible in subcortical structures, along with prefrontal and temporal cortex.

Although we found no overall differences between our LTAA and NAC samples in individual subcortical volumes, a group by gender interaction revealed that in LTAA, but not NAC, women had smaller nucleus accumbens volumes than men. The nucleus accumbens is believed to mediate the rewarding effects of alcohol through dopamine release (Besheer et al., 2003; Weiss and Koob, 2001), and positron emission tomography has shown profound disruptions in dopamine release in the ventral striatum (which includes nucleus accumbens) in detoxified alcoholics (Volkow et al., 2007). Moreover, human post-mortem analysis of RNA expression in chronic alcoholics (Flatscher-Bader et al., 2005) has shown a neuroadaptive dysregulation of expression of genes involved in the organization of cellular architecture in the nucleus accumbens. Notably, *in vivo* analysis of acute ethanol-induced dopamine release in rats (Blanchard et al., 1993) provides evidence that accumbens sensitivity to ethanol is greater in female rats than in male rats. The difference between male and female nucleus accumbens volumes in our LTAA could reflect a sex difference in accumbens sensitivity (and consequent morphological vulnerability) to alcohol. However, caution should be taken in extrapolating from animal studies to human brain morphometry.

Second, our finding that in LTAA, but not NAC, there were differences in subcortical volumes between subjects with and subjects without lifetime psychiatric diagnoses is consistent with the proposal that chronic alcohol dependence and psychiatric comorbidity work synergistically to reduce subcortical brain volumes. This proposal is also consistent with our finding of smaller subcortical volumes in LTAA with a current anxiety diagnosis, compared to those without (although no NAC had a current anxiety disorder diagnosis). Moreover, the

associations in LTAA between subcortical brain volumes and lifetime and current psychiatric diagnoses persist even after extended periods of abstinence, suggesting that a comorbid psychiatric disorder (even if not current) interferes with recovery of subcortical volumes.

Critically, the differences in subcortical volumes between our LTAA with and without comorbid psychiatric diagnoses cannot be attributed to differences in alcohol use history, since LTAA with and without psychiatric diagnoses did not differ in terms of alcohol consumption measures. It should be noted that small Ns for our NAC with psychiatric diagnoses afford low statistical power for showing differences in subcortical volumes between subjects with and without diagnoses. However, as can be seen in Figure 5, volume of key subcortical structures associated with lifetime anxiety and externalizing diagnoses are consistently smaller for LTAA than for NAC.

Specifically, we found that, in LTAA, nucleus accumbens and hippocampus volumes were smaller in individuals with a lifetime anxiety disorder than in those without, and accumbens volumes were smaller, with a trend toward smaller putamen volumes, in subjects with a current anxiety disorder than in those without. Nucleus accumbens, hippocampus, and putamen are important components of a system of stress circuitry that is believed to be disrupted in anxiety and mood disorders (Arborelius et al., 1999; Plotsky et al., 1995; Yoo et al., 2005).

Cerebrospinal concentrations of corticotropin-releasing factor (CRF) are elevated in some anxiety disorders, including obsessive-compulsive disorder, Tourette's syndrome, and post-traumatic stress disorder (PTSD), as well as during alcohol withdrawal (see Arborelius (1999) for a review). Notably, in our current sample, LTAA had more than twice the rate of a lifetime anxiety disorder than NAC, an effect that was carried entirely by PTSD diagnosis (Di Sclafani et al., 2007). Stressful experiences promote synthesis and release of CRF from the hypothalamus,

ultimately triggering the synthesis and release of glucocorticoids (GCs), and alcohol consumption also elevates circulating GC levels. Glucocorticoids make neurons vulnerable to damage by inhibiting glucose uptake and reducing energy supply (Eskay et al., 1995). Brain areas that are high in GC receptors are most susceptible to GC-induced damage. GC receptors are found in the nucleus accumbens and putamen, and are especially abundant in the hippocampus. Abnormal morphometry of nucleus accumbens and putamen in co-occurring alcohol dependence and anxiety disorders is further consistent with accumulating evidence (Oswald et al., 2005) that vulnerability to abuse of drugs (including alcohol) is increased by frequent stress resulting in chronic exposure to high glucocorticoid levels which, in turn, increase sensitivity of dopaminergic neurons in mesolimbic structures (including nucleus accumbens and putamen) to drugs of abuse.

We also found smaller hippocampus and amygdala volumes in LTAA, but not NAC, with a lifetime externalizing disorder diagnosis, compared to those without, as well as a trend in LTAA toward a negative association between the sum of lifetime externalizing symptoms and hippocampus volumes. Gray matter volume abnormalities in the amygdala and parahippocampal gyri are associated with increased externalizing symptoms in subjects at high risk for alcohol dependence (Benegal et al., 2007). Numerous studies, reviewed by Zimmermann and colleagues, (2007), have demonstrated that substance abuse frequently follows behavioral problems, including externalizing disorders such as attention deficit hyperactivity disorder (ADHD), oppositional defiant disorder (ODD), and conduct disorder (CD), and is especially likely to co-occur with ASPD (Kessler et al., 1996; Rohde et al., 2001; Stewart, 1996). As reported in a previously published study (Di Sclafani et al., 2007), 25% of LTAA in our current sample had a lifetime externalizing disorder, compared to 8.3% of NAC, a difference carried entirely by

ASPD. Moreover, symptom counts indicate that the difference in presence and severity of psychiatric illness between LTAA and NAC was more than twice as large for ASPD as for mood or anxiety disorders (Fein et al., 2007). Notably, lifetime ASPD was associated with drinking at an earlier age, consistent with associations between disruptive behavior disorders in adolescence and early age at first drink (Kuperman et al., 2005; McGue et al., 2001) which in turn is a predictor of adult alcohol use disorder (AUD) (Kuperman et al., 2001). The symptomatology of ASPD (impulsivity, low harm avoidance, boredom susceptibility/thrill and adventure seeking, poor decision making, and poor learning from negative consequences) is very similar to the traits of addiction. Note that Fein and colleagues (2004b) found persistent impaired decision making in a sample of LTAA (largely overlapping with our current sample), interpreted in terms of poor learning from negative consequences. In fact, results from large sample studies (Krueger et al., 2002; 2005) support the contention that externalizing symptoms and alcohol/substance related problems may be traced to a single risk factor. Krueger and colleagues (2002) presented a model with a heritable ‘externalizing’ factor that fit best to their data for 1048 17-year-old participants from the Minnesota Family Twin Study. The externalizing ‘liability’ was highly associated with adolescent antisocial behavior, CD, and alcohol dependence, all good predictors of adult ASPD and AUD. More recently, Krueger and colleagues (2005) and Markon and Krueger (2005) presented evidence that, in adults, comorbidity among externalizing disorders (including substance use disorders and ASPD) is best modeled by a single heritable continuum of risk for multiple disorders within the externalizing spectrum. Findings of hippocampal and amygdalar abnormalities associated with increased externalizing disorders in subjects at high risk for alcohol dependence (Benegal et al., 2007) suggest that a common liability for externalizing disorders (including AUD and ASPD) may subsume neurobiological deficiencies in these

structures in individuals with comorbid alcohol dependence and ASPD, consistent with findings in the present study of persistent smaller volumes of these structures in LTAA with comorbid lifetime externalizing disorders compared to those without.

Both anxiety disorders and externalizing disorders indicate dysregulation of the hypothalamic-pituitary-adrenal (HPA) response to stress and HPA interactions with mesolimbic reward circuitry, albeit via different neurobiological mechanisms (see Sinha (2001) for a review). Mood and anxiety disorders are associated with oversensitization of the HPA axis, and concomitant hypercortisolism (Arborelius et al., 1999; Plotsky et al., 1995). In contrast, baseline and stress-induced cortisol is low in a substantial proportion of patients with oppositional defiant disorder and conduct disorder, and, in individuals with ASPD (including substance abusers with ASPD), there is an undersensitization of the HPA axis, marked by hypocortisolism (Deroche et al., 1997; King et al., 1990; Moss et al., 1995; Vanyukov et al., 1993). However, anxiety disorders, such as PTSD, and externalizing disorders, such as ASPD, both interact over time with genetic risk, environmental stress, and alcohol exposure to alter neural circuitry in stress and reward systems in a way that is maladaptive to future coping with stress (see reviews by Sinja (2001) and Zimmermann et al. (2007)). Finally, alcohol dependence and psychiatric disorders both accrue stressful negative consequences in daily life, which may result in a continuing downward spiral, with increasing maladaptive changes to stress and reward circuitry. In sum, the emerging pattern reflects synergistic roles of alcohol dependence and psychiatric disorders, altering stress and reward circuitry in subcortical structures (including accumbens, putamen, hippocampus, and amygdala), a pattern that is consistent with our finding of persistent smaller volumes of these structures in LTAA with lifetime or current psychiatric diagnoses compared to those without.

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Appendix

By visual inspection of the lateral ventricles defined by FIRST using the conventional pipeline (i.e. alignment of the models using linear registration) we found that 12% and 14% of the left and right lateral ventricles were grossly mis-estimated. Figure 6 shows an example of such segmentation error. This erroneous estimation is prevalent only in subjects with larger ventricles. The errors in FIRST can potentially come from two sources: 1) The variation in the training set used to construct the models may not cover large ventricles, and 2) the initial alignment to the ventricles, using FLIRT (FMRIB's Linear Image Registration Tool), places the superior boundary of the ventricles entirely within the CSF, distant from the CSF boundary. This results in a local maxima being found within the CSF region. Given that the ventricle sizes that we were dealing with in this study are within the range contained in FIRST's training set, it is more likely the latter pitfall is the culprit.

Insert Figure 6 about here.

By using non-linear registration to align the image to MNI space, the distance between the surface and the boundary is reduced, providing better initialization of the model. In fact, the use of non-linear registration may also potentially help to compensate for an inadequately represented shape model in that the non-linear registration absorbs the extra residual variance remaining after alignment. The method shows better agreement in the lateral ventricles, with no obvious negative impact on the other subcortical structures. Figure 7 shows the left lateral ventricle of the same Figure 6 MRI which is now segmented correctly. Note that the red lines which represent the boundaries of the segmented structure are matching the actual boundaries of

the ventricle. All remaining cases with erroneous segmentation of the ventricles were also corrected when their MRIs were processed by the method of Figure 2.

Insert Figure 7 about here.

Figure captions

Figure 1. Sixteen brain subcortical structures (left and right) examined in this study.

Figure 2. Flowchart of the steps for subcortical structure segmentation and volume measurement when a non-linear registration is utilized.

Figure 3. Total volumes of the left and right lateral ventricles plotted versus age for LTAA and NAC groups. Volumes were adjusted by the cranium size.

Figure 4. Nucleus accumbens volume average versus gender for LTAA and NAC groups.

Figure 5. Individual subcortical volumes and means (mm^3) for LTAA and NAC with and without current or lifetime psychiatric diagnosis. (Note that no NAC had a current anxiety diagnosis.) Lines connecting columns terminate at mean volumes.

Figure 6. An example of left lateral ventricle which was not segmented correctly when FSL's FIRST is applied on the original T1-weighted MR images. The red outline shows the boundary of the area which is incorrectly segmented as the left ventricle.

Figure 7. Corrected left lateral ventricle boundaries for the same MRI of Figure 6 after applying non-linear registration on the MRI before running FIRST. The red outline boundary of the area is segmented to be the left ventricle.

Table 1. Sample demographics.

Demographic Variables	Middle-Aged Normal Controls		Middle-Aged Abstinent Alcoholics		Effect Size (%)		
	Male (n=25)	Female (n=23)	Male (n=28)	Female (n=24)	Group	Sex	Group x Sex
Age (Years)	43.4 ± 6.3	48.0 ± 6.6	44.9 ± 6.9	48.4 ± 6.4	0.5	8.9**	0.2
Family Drinking Density ¹	0.14 ± 0.22	0.17 ± 0.21	0.40 ± 0.26	0.44 ± 0.30	23.9***	0.4	<0.1
Years Education	16.3 ± 2.2	16.0 ± 1.9	15.5 ± 2.0	15.5 ± 2.4	2.2	0.1	0.1
AMNART (Estimated Premorbid Verbal IQ)	1.20 ± 0.46	1.47 ± 0.42	1.21 ± 0.43	1.40 ± 0.35	0.2	7.4**	0.2
Alcohol Use Variables							
Duration of Active Drinking (months)	230 ± 130	290 ± 130	260 ± 90	280 ± 110	0.1	2.8	1.0
Average Lifetime Drinking Dose (std. drinks/month)	7 ± 8	7 ± 8	180 ± 150	130 ± 80	41.0 ²	1.1 ²	1.1 ²
Lifetime Alcohol Use (std. drinks)	1,800 ± 2,200	2,000 ± 2,300	54,000 ± 59,000	40,000 ± 38,000	28.3 ²	0.6 ²	0.7 ²
Duration of Peak Drinking (months)	70 ± 81	114 ± 113	61 ± 58	92 ± 82	0.8 ²	3.4 ²	0.1 ²
Average Peak Drinking Dose (std. drinks/month)	15 ± 14	17 ± 22	340 ± 250	270 ± 200	42.8 ²	0.6 ²	0.6 ²
Peak Alcohol Use (std. drinks)	900 ± 1,100	1,000 ± 1,500	26,000 ± 44,000	25,000 ± 29,000	17.3 ²	<0.1 ²	<0.1 ²
Abstinence Duration (Months)	N/A	N/A	75 ± 73	75 ± 68	N/A	<0.1	N/A

¹ Family 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.

² Statistical comparisons between groups are not valid since the group definitions are a function of the variable. Effect is significant: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Table 2. Prevalence rates of psychiatric disorder diagnoses in LTAA and NAC groups.

PSYCHIATRIC DIAGNOSIS	Middle-Aged Normal Controls (N=48)		Middle-Aged Abstinent Alcoholics (N=52)	
	Frequency	Percentage (%)	Frequency	Percentage (%)
Lifetime Mood Dx	19	39.6	35	67.3
Current Mood Dx	3	6.3	15	28.8
Lifetime Anxiety Dx	7	14.6	19	36.5
Current Anxiety Dx	0	0.0	10	19.2
Lifetime Externalizing Dx	4	8.3	14	26.9
Current Externalizing Dx	0	0.0	0	0.0

Table 3. Average subcortical structure volumes, adjusted for variations in head size with age as a covariate.

SUBCORTICAL STRUCTURES	Middle-Aged Normal Controls		Middle-Aged Abstinent Alcoholics		Effect Size (%)		
	Male (n=25)	Female (n=23)	Male (n=28)	Female (n=24)	Group	Group x Age	Group x Gender
Lateral Ventricles	17342	19427	19269	21700	3.5 ^{.063}	4.4*	0.2
Thalamus	16450	16042	15999	15754	0.2	0.5	0.3
Caudate	6680	6767	6780	6558	0.1	0.1	2.4
Putamen	9795	9570	9472	9454	0.5	0.8	0.6
Pallidum	3496	3369	3438	3292	0.5	0.7	0.0
Hippocampus	7428	7499	7614	7490	0.2	0.1	0.5
Amygdala	2866	2815	2855	2714	0.8	1.0	0.2
Nucleus Accumbens	999	1005	1019	899	1.1	0.7	7.2**

All volumes are reported in cubic millimeters.

Effect is significant: * $p \leq 0.05$, ** $p \leq 0.01$.

Trends are reported as superscripted p levels.

Figure 1.

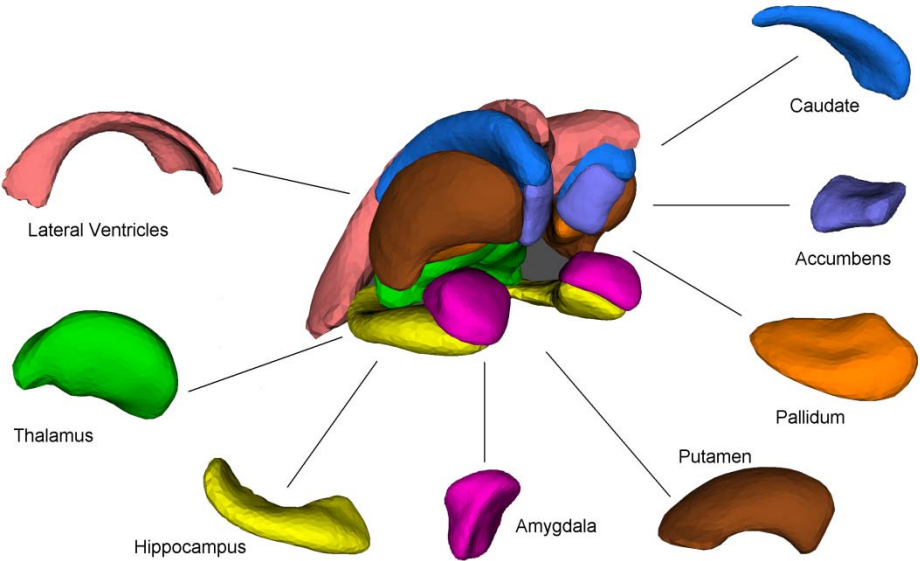


Figure 2.

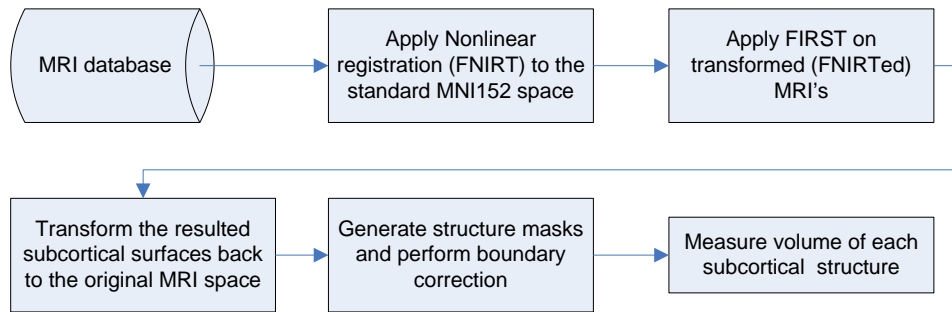


Figure 3.

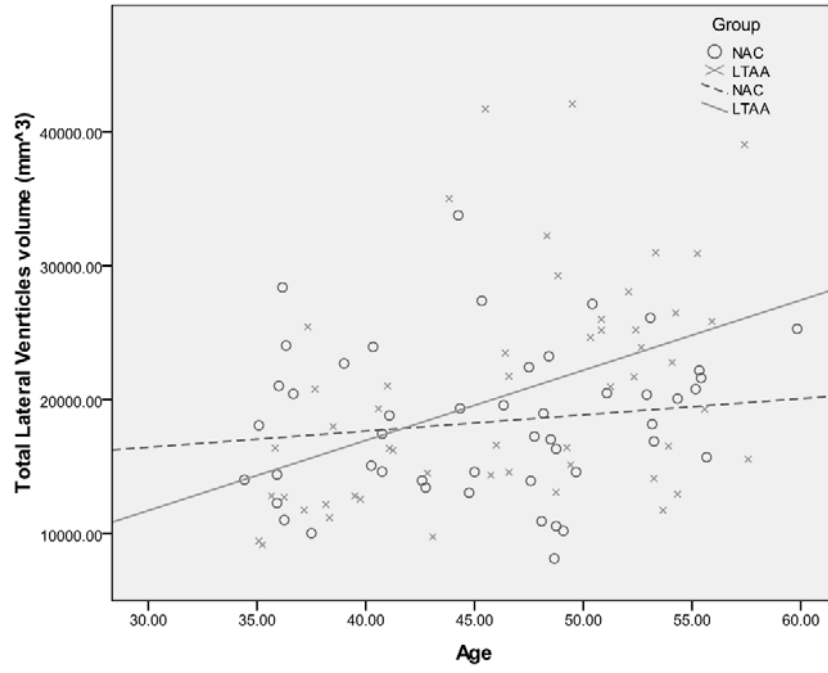


Figure 4.

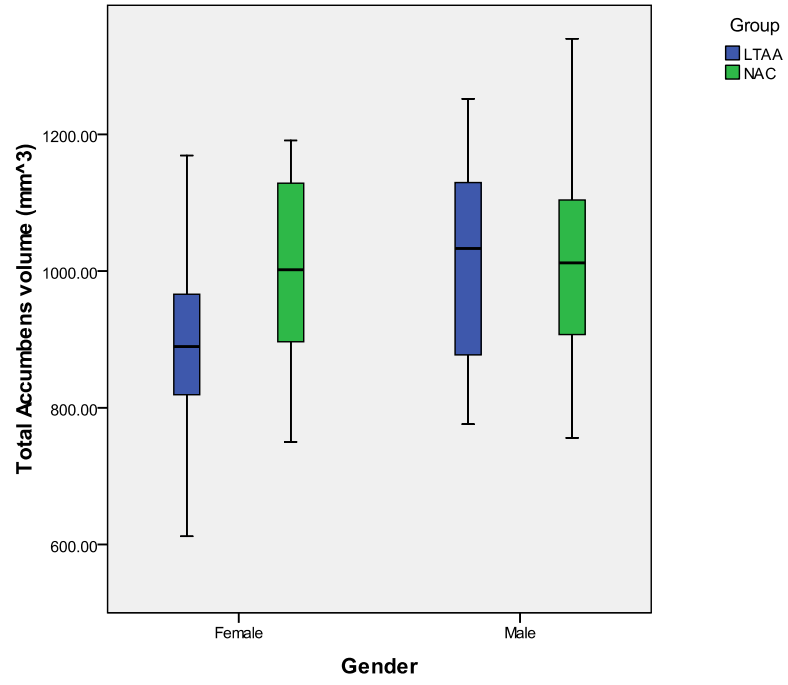
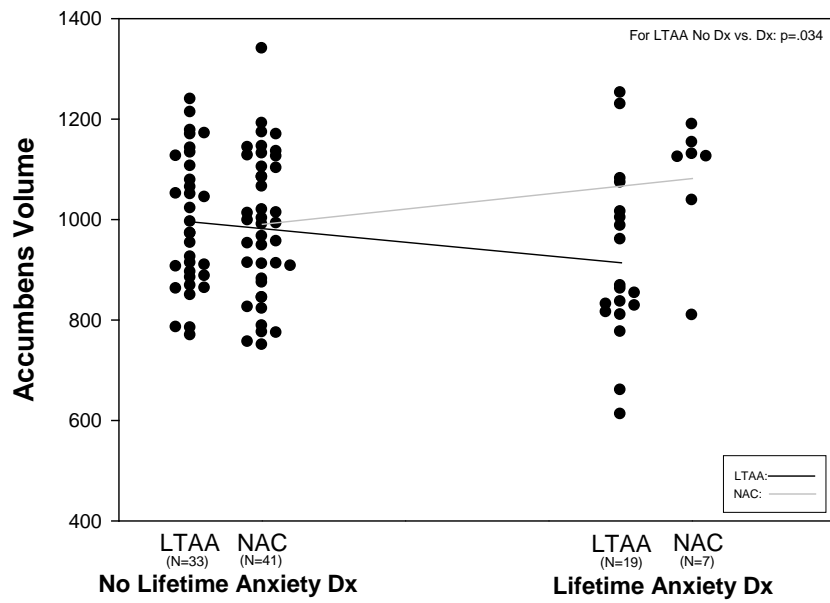
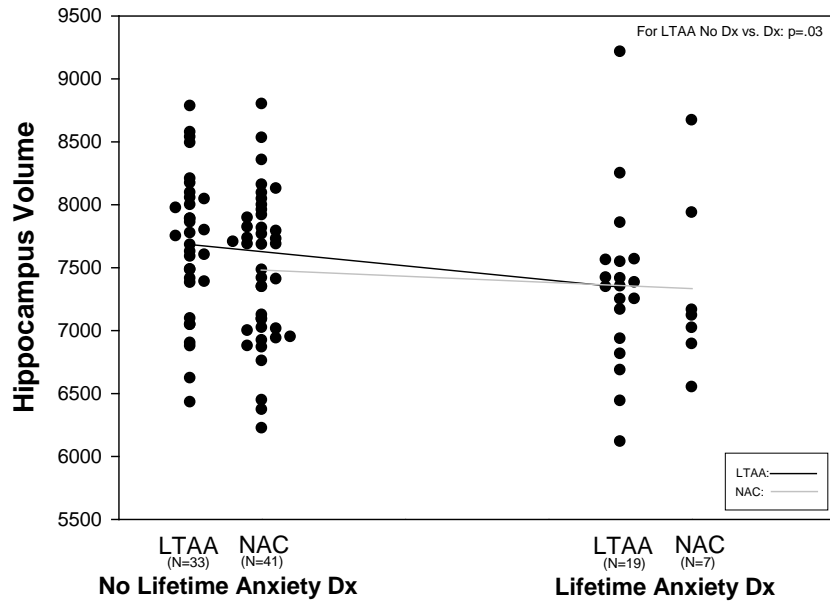
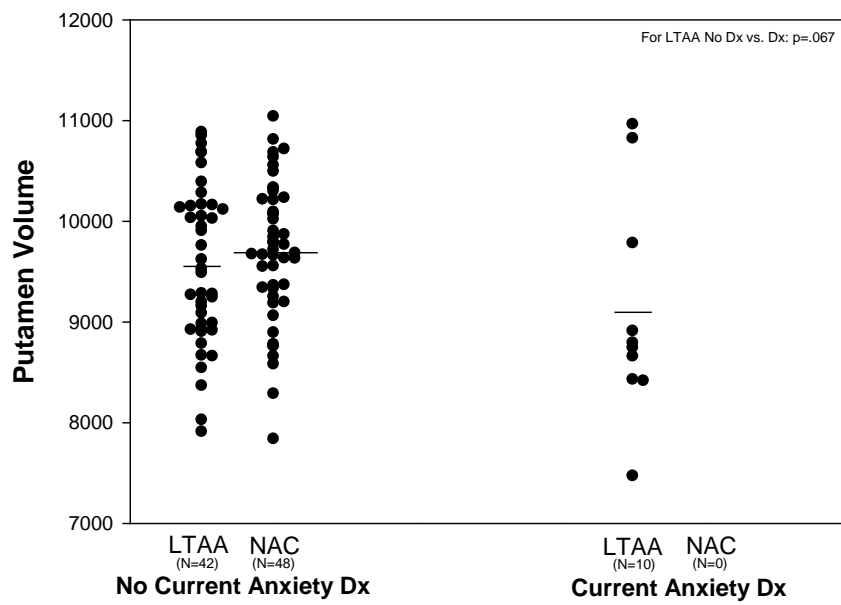
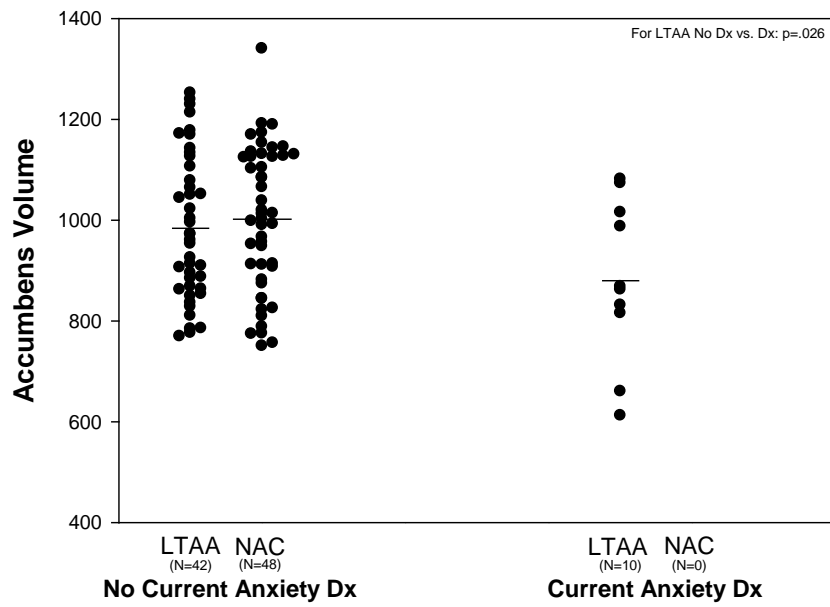


Figure 5.





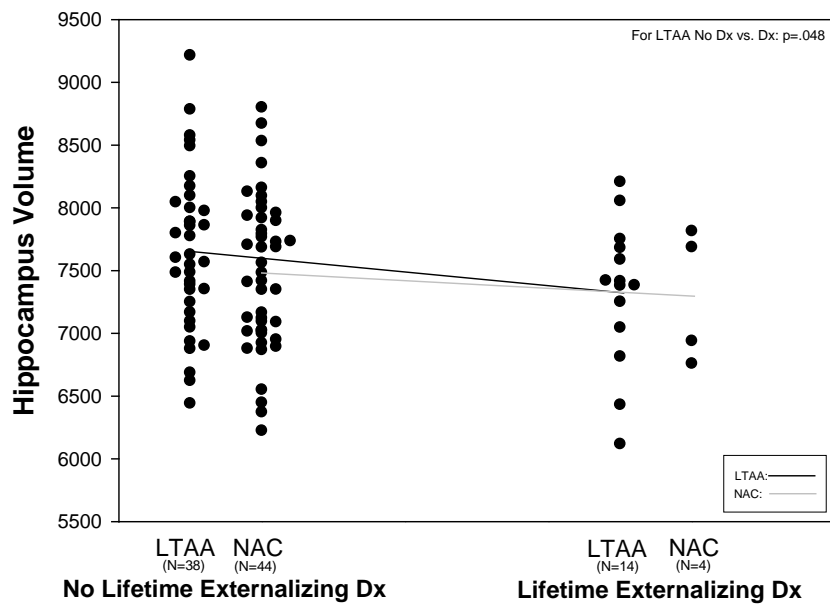
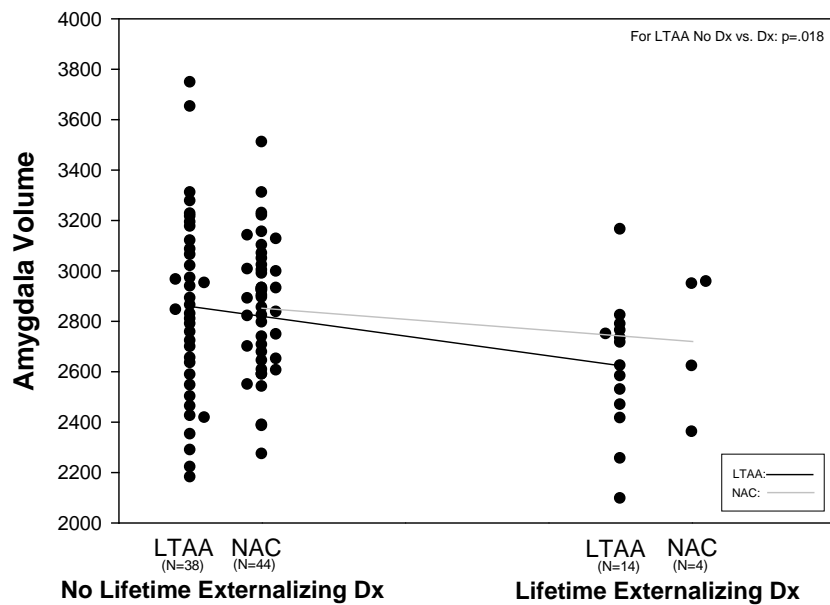


Figure 6.

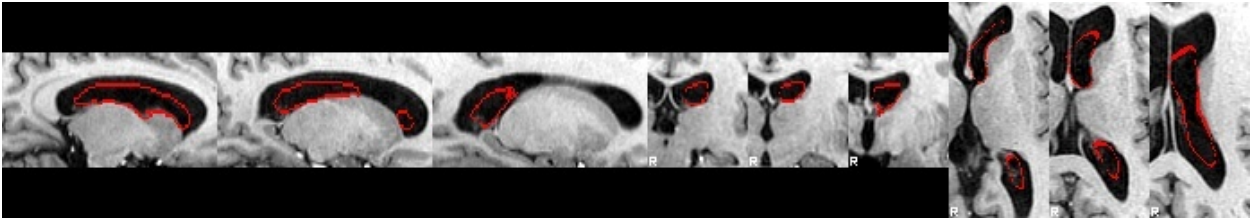


Figure 7.

