Hippocampal Subfield Volumes in Abstinent Men and Women with a History of Alcohol Use Disorder

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Abstract

Alcohol use disorder (AUD) has been associated with abnormalities in hippocampal volumes, but these relationships have not been fully explored with respect to sub-regional volumes, nor in association with individual characteristics such as gender differences, age, and memory. The present study examined the impact of those variables in relation to hippocampal subfield volumes in abstinent men and women with a history of AUD. Using Magnetic Resonance Imaging at 3 Tesla, we obtained brain images from 67 participants (31 women) with AUD and 63 healthy control (NC) participants (30 women) without AUD. We used Freesurfer 6.0 to segment the hippocampus into 12 regions. These were imputed into mixed models to examine the relationships of brain volume with AUD group, gender, age, drinking history, and memory. The AUD group had approximately 5% smaller CA1, hippocampal tail, and molecular layer regions than the NC group. Age was negatively associated with volumes for the AUD group in the hippocampal tail, subiculum, and presubiculum. The relationships for delayed and immediate memory with hippocampal tail volume differed for AUD and NC groups: Higher scores were associated with smaller volumes in the AUD group, but larger volumes in the NC group. Length of sobriety was associated with decreasing CA1 volume in women (0.02% per year) and increasing volume size in men (0.03% per year). These findings confirm and extend evidence that AUD, gender, age, and abstinence differentially impact volumes of component parts of the hippocampus. The course of abstinence on CA1 volume differed for men and women, and the differential relationships of subregional volumes to age and memory could indicate a distinction in the impact of AUD on functions of the hippocampal tail.

Introduction

Magnetic resonance imaging (MRI) has been used extensively to study morphological changes in the brain associated with alcohol use disorder (AUD), a widespread and harmful condition [1,2]. Because memory impairments are associated with long-term chronic AUD, one neuroanatomical focus of investigation has been the hippocampus [3]. Moreover, findings from previous research have shown that the largest subcortical volume loss observed in the brains of people with chronic AUD was in the hippocampus [4], and a meta-analysis [5] found a negative association between total hippocampal volume and degree of alcohol use (clinical vs. subclinical).

Although results of MRI studies have suggested gender-specific susceptibility of the brain to alcohol abuse [6–15], few studies have examined gender differences in the relationship between hippocampal volume and AUD. Instead, researchers have assessed AUD-related hippocampal abnormalities in men [16–20], or in AUD groups comprised of a combination of men and women [21–23]. In studies that did analyze men with AUD (AUDm) and women with AUD (AUDw) separately, investigators observed lower hippocampal volumes in the AUDm and in the AUDw, but they did not find significant interactions between gender and diagnostic group [21,23], while we previously observed smaller hippocampal volumes for AUDm than AUDw [9].

Similarly, the relationships observed between hippocampal volume and cognitive ability also have been inconsistent. It has been widely reported that lesions to the hippocampus result in memory deficits [24,25]. Positive correlations between hippocampal volume and memory

performance also have been reported in clinical populations such as patients with Alzheimer's disease or amnesic disorders [26–29], but this positive correlation has not been systematically observed in healthy populations [30]. In the context of AUD, some research has indicated the presence of functional deficits with lower hippocampal volumes. For example, decreases in hippocampal gray matter volume in alcohol dependent men have been associated with executive functioning deficits [18] as measured by the Trail Making Test Part B, an assessment of visual-conceptual and visual-motor tracking skills [31]. However, among participants with AUD in these earlier studies, smaller volumes of the anterior portion of the hippocampus were not significantly associated with memory impairment [32].

The inconsistent findings relating volume and function may be impacted by the distinct functions of hippocampal subfields and the processing streams that exist within the hippocampus (Figure 1, which overlays this processing stream on an MRI atlas; [33]). Traditionally, based upon research involving anatomical dissection, histology, and physiology, hippocampal subfields have been defined as the subiculum, dentate gyrus, and *cornu ammonis* regions (CA1 through CA3) [34]. Researchers have focused on two major neural pathways, as follows: First, major input to the hippocampus originates in entorhinal cortex and proceeds along a trisynaptic circuit (1) to the dentate gyrus, then (2) to CA2 or CA3, and finally, (3) to CA1 (and then, subiculum), which serve as outputs of the hippocampus. Second, the entorhinal cortex (not part of hippocampus) additionally has a direct connection to CA1, among other regions. All the input connections from CA1, CA2, CA3, and subiculum are made within an area called the molecular layer (also known as *stratum lacunosum moleculare* or SLM).

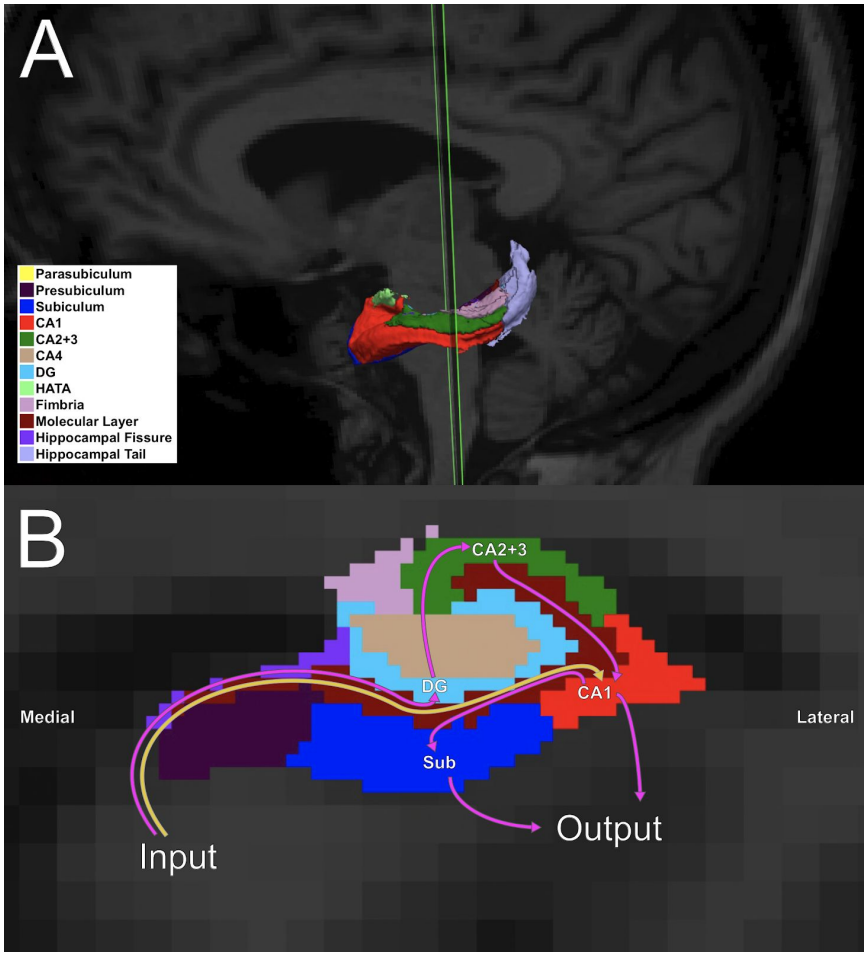

Figure 1. Hippocampal subfield segmentation using the procedure by Iglesias and colleagues [33]**.**

A. This three-dimensional model of the left hippocampus depicts its segmentation into 12 subfields (although not all subfields are visible from the angle presented). B. This coronal section through the hippocampus illustrates subfields that are visible in the slice, and it shows the two major hippocampal neural pathways originating in the entorhinal cortex and as described in the text: The pink arrows indicate the trisynaptic circuit; the yellow arrow shows the direct circuit. Although the connection from CA1 to the subiculum is not explicitly part of the trisynaptic circuit, it is important as an output region, in addition to CA1. Abbreviations: CA1 = cornu ammonis 1; CA2+3 = cornu ammonis 2 and 3; CA4 = cornu ammonis 4; DG = dentate gyrus; HATA = hippocampal-amygdaloid transition area; Sub = subiculum.

The molecular layer is the point of entry for information, including the direct pathway from entorhinal cortex that is theorized to contain information regarding presently experienced stimuli [35,36]. The dentate gyrus has been shown to play a role in distinguishing different contexts [37]. For the structures involved in the trisynaptic pathway, the CA2 and CA3 regions have been ascribed to learning, encoding [38], early retrieval of verbal information [39], and disambiguation and encoding of overlapping representations [40]. The output fields, CA1 and subiculum, are thought to compare current context with remembered contexts [41]. Their projections include regions implicated in addictive disorders: the prefrontal cortex, amygdala, nucleus accumbens, and ventral tegmental area. This processing stream is repeated along the axis of the hippocampus, described in the human as anterior to posterior, and in rodent models as ventral to dorsal. The anterior (or ventral) portion has been considered to involve a wide variety of contexts including stress, emotion, and affect [42], while the posterior (or dorsal) portion has been demonstrated to have involvement in spatial processing. White matter fibers that carry the projections of the hippocampus include the fimbria [43].

In a sample of men with ongoing AUD, Lee et al. [17], reported smaller volumes of the left presubiculum, bilateral subiculum regions, and fimbria, but in studies that have included women, significant differences in interactions of AUD and gender have not been detected for hippocampal subfield volumes [22,23], perhaps due to insufficient sample sizes. Research also has supported reports of smaller AUD-related volumes in subiculum, CA1, CA4, or other parts of the hippocampus (dentate gyrus, hippocampal-amygdaloid transition area [HATA], and the fimbria) [17,23], along with CA2 and CA3, which showed significant normalization two weeks following withdrawal [22]. Additionally, effects of age were found for the fimbria and the

hippocampal fissure, while a significant interaction between AUD diagnosis and age was seen in CA2 and CA3 regions. However, in neither the Lee et al.[17] nor the Kühn et al. [22] studies, was memory assessed, and Zahr et al. [23] found no significant memory impairment associated with reduced hippocampal subfield volumes.

In summary, the present study examined the impact of gender on hippocampal subfield volumes, and the relationships between age, memory, and volumes of these regions in abstinent individuals with a history of AUD. Examining the impact of AUD on individual subfield volumes and their interactions with age, gender, and memory can further elucidate which components of this brain network are associated with the problems contributing to, and resulting from, AUD.

Methods

As described in detail below, 67 AUD (31 women) and 63 NC participants (30 women) were included in analyses (Table 1). Memory and drinking history were assessed. T1-weighted 3T MRI scans were obtained with 1x1x1.5 mm voxels. The 12 hippocampal subfield volumes were obtained with FreeSurfer 6.0. In the mixed models, volume was entered as the dependent variable, and 'group,' 'gender,' 'region,' and 'age' were the independent factors. We examined the interaction of group-by-gender-by-region to assess how the impact of AUD differed for men and women. Also, we examined the interaction of group-by-gender-by-age to assess abnormal aging for men as compared to women. Following significant effects, additional models were constructed to examine relationships with memory measures, and with drinking history.

Participants

The study originally included 146 participants: 73 abstinent adults with a history of chronic AUD (33 AUDw; 40 AUDm) and 73 adult controls without AUD (34 NCw; 39 NCm), who were recruited locally through online and print advertisements. Participants provided written informed consent for participation in the study, which was approved by the Institutional Review Boards at the Boston VA Healthcare System, Massachusetts General Hospital, and Boston University School of Medicine. Exclusion criteria for participants included left-handedness, Korsakoff's syndrome, HIV, hepatic disease, head injury with loss of consciousness greater than 15 minutes, stroke, seizures unrelated to AUD, schizophrenia, Hamilton Rating Scale for Depression (HRSD) [44] score over 14, and illicit drug use (except marijuana) greater than once a week within the past five years. Sixteen individuals were excluded from the study for the following reasons: Three AUD participants (1 woman) were excluded for illicit drug use, and another three (1 woman) for brain lesions or head trauma. Six NCm were excluded for binge drinking, head trauma, or unusable scan data. Two NCw were excluded for claustrophobia or brain lesions, and another two NCw were identified as outliers, with brain hippocampal region volumes +/- 4 standard deviations, and removed from further analyses. The final sample of participants was comprised of a total of 67 AUD (31 women) and 63 NC participants (30 women) included in data analyses (Table 1).

Diagnostic criteria for study exclusion were based on a medical history interview, the HRSD, and a computer-assisted, shortened version of the Computerized Diagnostic Interview Schedule (DIS) [45]. The DIS provides diagnoses of lifetime psychiatric illnesses as defined by

criteria established by the American Psychiatric Association. Full-Scale IQ and cognitive performance were measured through the Wechsler Adult Intelligence Scale-IV (WAIS-IV) and the Wechsler Memory Scale-IV (WMS-IV) [46]. The memory assessments were conducted by trained researchers and consisted of tasks designed to measure memory for short stories, lists of words, shapes, and spatial locations. Incomplete WMS-IV scores were obtained from one NCm and two AUDm and were excluded from the memory analyses.

A number of participants were taking medications for a variety of conditions, had used drugs earlier than five years before enrollment, or had a potentially confounding medical history. We included these participants with confounding factors, so that our sample would be more representative of the conditions present in the United States, thereby allowing for greater generalizability of the results. However, the presence of confounding factors in the sample may limit the interpretability of our findings. Therefore, in the analysis of the results, a subsample of 77 participants (26 AUD; 51 NC) was created consisting of "unconfounded" participants who were not currently taking psychotropic medications, and reported never using illicit drugs more than once a week. Additionally, that subsample was restricted to individuals for whom no source indicated hepatitis or cirrhosis, nor any of the following disorders: major depressive, bipolar I or II, schizoaffective, schizophreniform, or generalized anxiety. All statistical effects of group reported in this manuscript (including group interactions) remained significant for this unconfounded subsample.

Drinking history was assessed using Duration of Heavy Drinking (DHD), i.e., years of consumption of 21 drinks or more per week, and Length of Sobriety (LOS), which measures abstinence duration in years. The amount, type, and frequency (ounces of ethanol per day,

roughly corresponding to daily drinks; DD) of alcohol use was measured for the last six months during which the participant drank alcohol [47]. Criteria for AUD participants included at least five years of previous alcohol abuse or dependence, and a minimum of four weeks of abstinence prior to testing. Two NCw and one NCm had no prior history of drinking, whereas the remaining NC participants drank occasionally. Compared to the AUDw, the AUDm had greater periods of heavy drinking and shorter periods of abstinence, which are consistent with national trends [48] and allow for generalizability of the results. However, to improve interpretability, we created a subsample in which the AUDw and AUDm were not significantly different by removing four AUDw with the longest LOS and shortest DHD values. All statistical effects of gender reported in this manuscript (including gender interactions) remained significant for this subsample.

MRI Acquisition and Analysis

MRI scans were obtained at the Martinos Center for Biomedical Imaging at Massachusetts General Hospital on a 3 Tesla Siemens (Erlangen, Germany) MAGNETOM Trio Tim scanner with a 32-channel head coil. Image acquisitions included two T1-weighted multiecho MPRAGE scans collected for volumetric analysis that were averaged to aid in motion correction (TR = 2530 ms, TE = 1.79 ms, 3.71 ms, 5.63 ms, 7.55 ms [root mean square average used], flip angle = 7 degrees, field of view = 256 mm, matrix = 256 x 256, slice thickness = 1 mm with 50% distance factor, 176 interleaved sagittal slices, GRAPPA acceleration factor = 2; voxel size = 1.0 mm x 1.0 mm x 1.5 mm).

Scans were analyzed using an automated hippocampal segmentation method [33] in FreeSurfer (https://surfer.nmr.mgh.harvard.edu), which more recently has shown high reliability

and agreement with manual segmentations [49]. Brain reconstructions were manually inspected and errors were corrected. Volumes of the hippocampal subfields (12 per hemisphere) were calculated using the hippocampal subfields subroutines for Freesurfer 6.0 (which omits the alveus due the poor reliability of the segmentation). These 12 volumes were used to define the extent of the hippocampus. Estimated total intracranial volume was taken from the segmentation volume estimate [50]. The volumes were divided by estimated total intracranial volume (eTIV) to adjust for head size.

Statistical Analyses

All statistical analyses were performed using R version 3.4.0 [51]. We used hierarchical linear models [52] to investigate the impact of several variables on regional hippocampal volumes. Data and code are available at https://gitlab.com/kslays/moblab-hippocampus. Because brain volumes vary with head size, we used normalized volume values (i.e., volume of each participant divided by total intracranial volume). Left and right hemisphere values of corresponding regions were averaged. Participants with outlier volumes (greater than or less than four standard deviations) within any region were removed from the analysis. In the regression models, we visually confirmed that other regression assumptions were satisfied (normality, equality of variance, homogeneity of regression), and set thresholds for multicollinearity (Pearson correlations among predictors were < 0.5) and influence (Cook's $D < 1.0$).

We first conducted an analysis of total hippocampal volume using multiple regression. We constructed a model predicting whole hippocampal volume from the interaction of 'group', 'gender', and 'age', along with the lower-order interactions and main effects. Next, for our

analysis of subfield volumes, we used mixed models. Volume was entered as the dependent variable, and 'group,' 'gender,' 'region,' and 'age' were the independent factors (all fixed effects). In order to account for multiple observations per subject (i.e., volumes for each 'region'), individual subject effects were specified as random intercepts.

Interactions were included to assess the primary aims of this project. We examined the interaction of group-by-gender-by-region to assess how the impact of AUD differed for men and women, and how gender differences impacted certain regions in comparison to others. Also, we examined the interaction of group-by-gender-by-age to assess abnormal aging for men as compared to women. The four way interaction of group-by-gender-by-region-by-age was examined to confirm homogeneity of regression slopes. All the non-significant (i.e., $p > 0.05$) interactions (except lower-order interactions included in higher-order interactions) that had been added to confirm homogeneity of regression slopes were removed, and the subsequent model was used to report results.

Following significant interactions, we evaluated mean differences within each region using Bonferroni multiple comparisons correction of a family-wise *p*-value threshold of 0.05. There were 12 hippocampal subfields, so this correction resulted in an adjusted *p*-value threshold of 0.0042. Results were reported as percent differences (i.e., mean difference/mean). Significant regions were then investigated for our secondary aims, i.e., analyses involving memory scores and drinking history. For interactions of group-by-gender, the comparisons of AUDm vs. NCm and AUDw vs. NCw were planned, while for region-by-group, only AUD vs. NC comparisons were planned. For age interactions, the effect of age for each of the subgroups was assessed, and the slope differences were compared in the same manner as mean differences.

To address our secondary aims, we started with the model that resulted from our primary aims. We then investigated interactions of group-by-gender with separate models for two memory measures from the WMS-IV: the immediate memory index (IMI) and the delayed memory index (DMI). These interactions were assessed in conjunction with each score's interaction with each region. We applied the same procedure used in the primary analyses to examine heterogeneity of regression slopes and to remove extraneous interactions. Following significant interactions, we examined the significance of each score's slope in the same 12 regions as for the primary analyses, and the same slope differences as for the primary analyses. The WMS-IV Index Scores and WAIS-IV Composite Scores were analyzed in separate ANOVA models, each of which contained between-subjects factors of group and gender, and a group-by-gender interaction term.

To investigate how alcohol-drinking history (DD, DHD, and LOS) impacted volume, we conducted an analysis with only the AUD participants included. The NC participants were not included because the participants did not have meaningful variation within the scores. We examined interactions of each of these three measures with gender-by-region in a single model. As with the primary and secondary aims, we tested for heterogeneity of regression slopes, and removed interactions as dictated by significance levels. Following significant interactions, we examined the significance of each score's slope in the same 12 regions as for the primary analyses, and we compared the slopes for men to the slopes for women.

Results

Participant Characteristics

Table 1 provides information about the participants. The groups consisted of 67 AUD (31 women) and 63 NC (30 women), with a mean age of 52 years for the 130 participants. Between-group (NC vs. AUD) differences in age were not significant (95% CI of mean difference = [5.4, -3.4] years). The AUD participants had 0.9 fewer years of education ($t(114.74) = -2.49$, $p < 0.05$) and 8.1 points lower FSIQ scores ($t(127.99) = -3.07$, $p < 0.01$). The AUDw had significantly longer LOS and DHD than the AUDm; other measures did not differ significantly. However, as described in the Methods, all gender effects reported remained significant in a subsample for which the AUDw and AUDm did not differ significantly by LOS or DHD.

	AUDw				AUDm			
Measure	Mean	SD	Min	Max	Mean	SD	Min	Max
Age (years)	54.5	12.0	31.1	76.0	51.2	10.8	28.1	76.8
Education (years)	14.7	1.8	12.0	19.0	14.1	1.9	12.0	18.0
FS	103.1	14.5	69.0	132.0	104.1	16.6	75.0	142.0
IMI	107.3	17.3	56.0	135.0	102.6	14.9	73.0	132.0
DMI	110.2	16.9	62.0	135.0	104.8	14.1	81.0	133.0
DHD (years)	13.7	5.6	5.0	25.0	17.5	9.1	5.0	37.0
DD (oz etOH/day)	8.8	7.2	2.5	34.8	11.8	6.8	2.3	26.6
LOS (years)	10.9	11.9	0.1	36.1	3.9	6.2	0.1	27.4

	NCw				NCm			
Measure	Mean	SD	Min	Max	Mean	SD	Min	Max
Age (years)	53.0	15.8	27.8	78.4	50.5	12.3	26.6	81.7
Education (years)	15.3	2.6	12.0	20.0	15.4	2.5	10.0	20.0
FS	110.8	15.1	82.0	141.0	112.7	14.7	89.0	141.0
IMI	113.0	16.1	89.0	143.0	110.5	15.4	84.0	140.0
DMI	115.0	12.5	93.0	146.0	114.2	17.4	78.0	147.0
DHD (years)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DD (oz etOH/day)	0.3	0.3	0.0	1.1	0.2	0.2	0.0	0.7
LOS (years)	2.2	7.4	0.0	31.9	2.1	7.9	0.0	40.2

Table 1. Participant Characteristics.

Minimum (Min), Maximum (Max), Means, and standard deviations (SD) are provided for the participant characteristics of AUDw (N=31) and AUDm (N=36) (women and men with a history of Alcohol Use Disorder), along with NCw (N=30) and NCm (N=33) (women and men without a history of AUD). DMI was not obtained from one AUDm and one NCm. IMI was not obtained from one AUDm and one NCm. Abbreviations: FS = Wechsler Adult Intelligence Full Scale IQ; DMI = Delayed Memory; IMI = Immediate Memory; DHD = Duration of Heavy Drinking; DD = Daily Drinks; LOS = Length of Sobriety.

Hippocampus Volumes, Group, Gender, and Age

All volumes were converted to proportions of head size (eTIV) before analysis. Linear regression analyses of whole hippocampus volume revealed a significant interaction of

group-by-age ($F(1, 122) = 7.15$, $p < 0.01$). The AUD group had 5.14% smaller volumes than the NC group ($t(122) = -3.855$, $p < 0.001$). Age was associated with a 0.44%/year decline for the AUD group, which was significantly steeper than the 0.13%/year decline observed for the NC group.

To analyze the subfield volumes, we used mixed-model regression. These analyses revealed significant three-way interactions (Table S2) for group-by-region-by-age ($F(11, 5366.83) = 3.02$, $p < 0.01$) and gender-by-region-by-age ($F(11, 5366.83) = 2.50$, $p < 0.01$). No significant group-by-gender interactions were observed. Group differences in means, and in slopes (for age), are reported below.

Mean differences between groups revealed significantly (Bonferroni adjustment of family-wise $p < 0.05$ for 12 regions: $p < 0.0042$) smaller CA1, hippocampal tail, and molecular layer volumes in the AUD group compared to the NC group, with marginal significance ($p = 0.0046$) for subiculum (Figure 2 and Table S2). The AUD group had 5.18%, 5.08%, 4.80%, and 3.70% smaller volumes in the CA1, molecular layer, hippocampal tail regions, and subiculum, respectively.

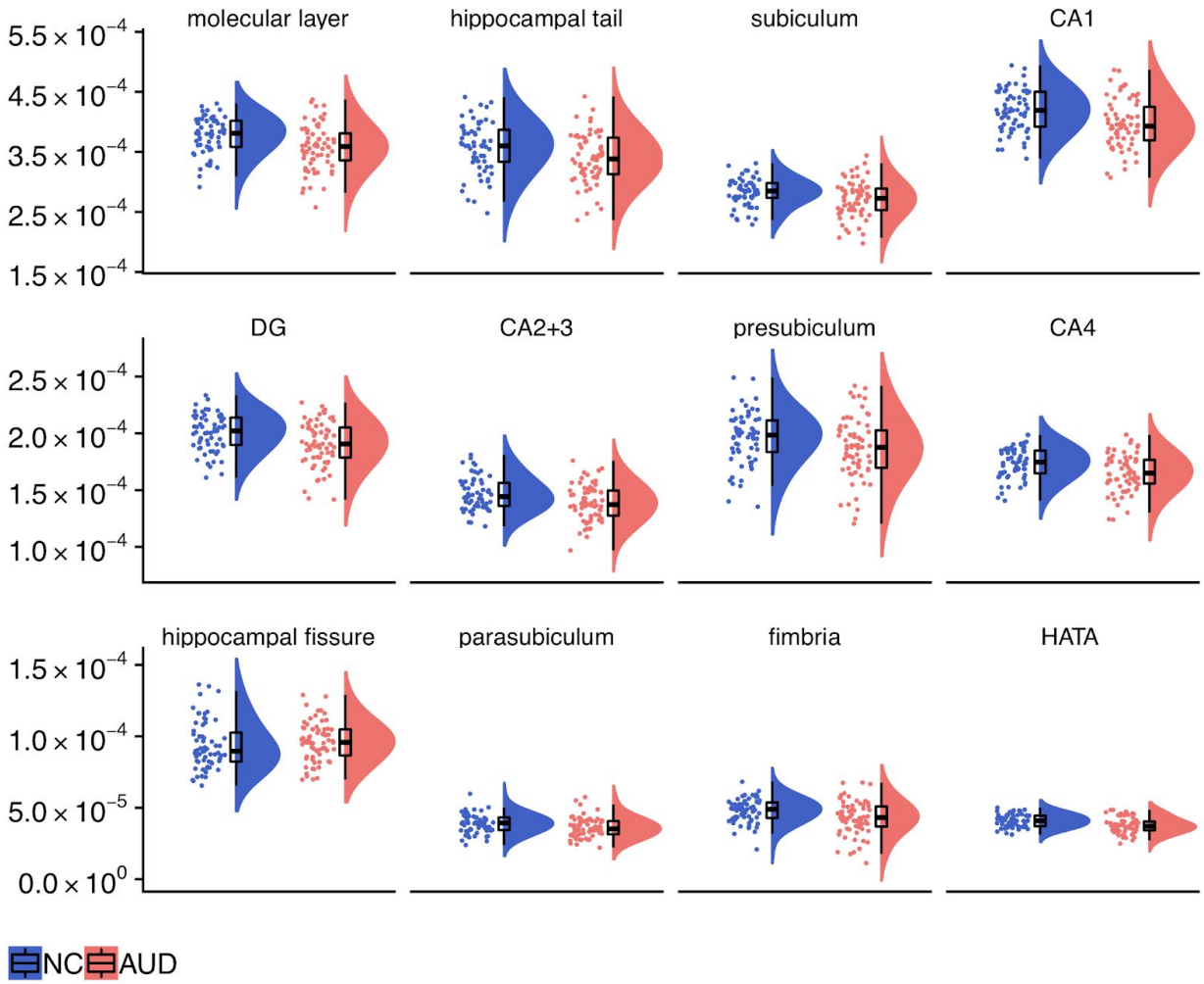

Figure 2. Regional volumes (proportion of estimated total intracranial volume).

Half violin raincloud plots [53] show hippocampal subfield volumes for the NC and AUD groups. See Table S1 for mean and standard deviation values. Abbreviations: CA1 = cornu ammonis 1; CA2+3 = cornu ammonis 2 and 3; CA4 = cornu ammonis 4; DG = dentate gyrus; HATA = hippocampal-amygdaloid transition area; Sub = subiculum. *Indicates regions where AUD < NC, $p < 0.0042$.

In the entire sample of the 130 participants, significant gender differences (with analyses including AUD and NC participants together) were found for the subiculum, presubiculum, CA1, and molecular layer regions. Women had 8.43%, 4.99%, 4.47%, and 3.30% larger volumes in the presubiculum, subiculum, molecular layer, and CA1, respectively. As with all analyses, volumes had been first converted to a proportion of cranial volume.

The impact of age on subfield volumes interacted significantly with group. For the AUD group, age was associated with significantly smaller hippocampal tail, subiculum, presubiculum, and molecular layer volumes (-0.52, -0.45, -0.58, and -0.47, respectively; all percent/year); these relationships were significantly more negative than those observed for the NC group (-0.17, -0.03, -0.04, and -0.17; all percent/year). The impact of age on subfield volumes also significantly interacted with gender. For women, age was associated with 0.4 percent/year lower CA1 volumes, a rate significantly less negative than that observed for men (1.0 percent/year).

Subfield Volumes and Memory

Following our main analyses, we assessed the relationship of memory scores and volume. We found significant interactions (Figure 3, Figure S1, Figure S2, Table S3, and Table S4) for group-by-region-by-IMI and for group-by-region-by-DMI. For better interpretability, slopes were divided by the grand mean for each region and are thus presented as percent volume/IMI or DMI unit.

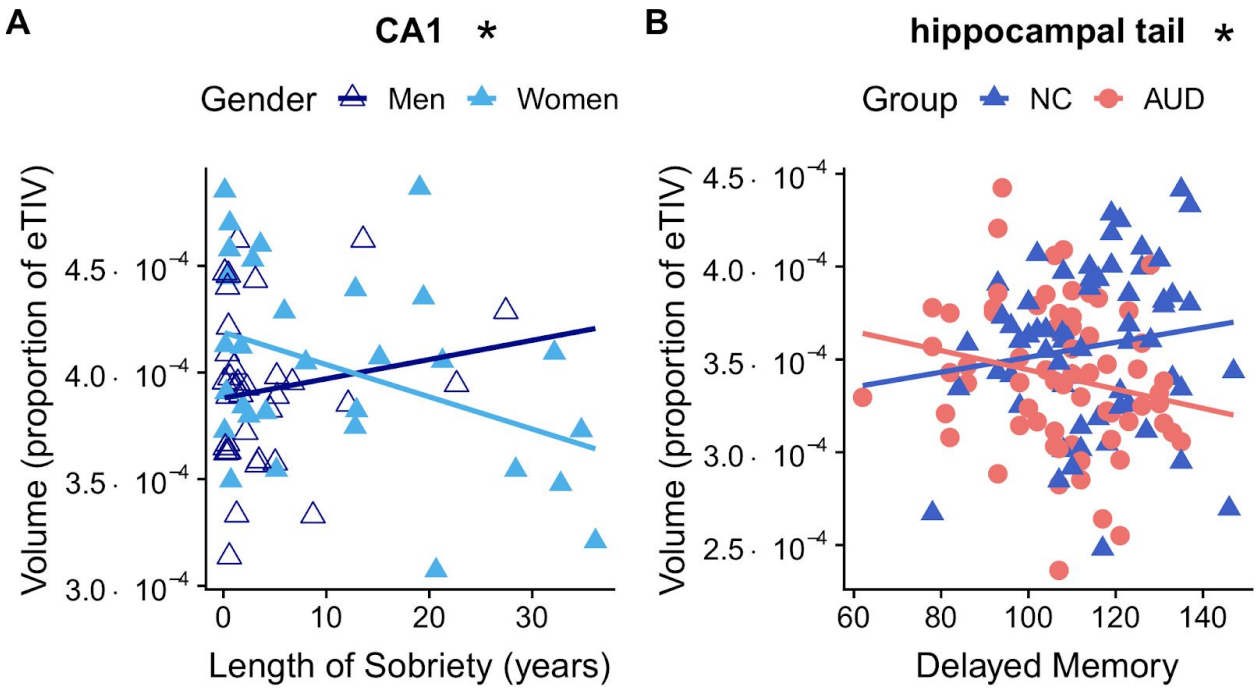

Figure 3. Distinct relationships of subfield volume for AUD and NC groups.

A. For AUD men, CA1 volumes (proportion of eTIV) were positively associated with Length of Sobriety (years), while for AUD women, a negative relationship was observed.

B. For the AUD group, Delayed Memory was associated with lower hippocampal tail volumes, while for the NC group, a positive relationship was observed.

Abbreviations: CA1 = cornu ammonis 1; eTIV = estimated total intracranial volume

* Indicates regions with interactions significant at $p < 0.0042$.

Immediate Memory Index. Following the identification of significant group effects for CA1, hippocampal tail, and molecular layer, and of marginal significant group effects in the subiculum ($p = 0.0046$), we found that associations of IMI with those regions differed for AUD and NC groups in the hippocampal tail ($t(594.84) = -3.51$, $p < 0.001$). In AUD participants, the volumes decreased by 0.12% per unit of IMI, while for NC they increased by 0.13% with each unit of IMI.

Delayed Memory Index. Also in the hippocampal tail, a significant interaction of AUD and NC participants was observed between DMI and subfield regions (Table S4). In AUD participants, the volumes were 0.14% lower with each unit of DMI, while for NC participants, volumes were 0.20% higher with each unit of DMI, relationships that differed significantly from each other ($t(599.16) = -4.55$, $p < 0.0001$).

Subfield Volumes and Drinking History

For the AUD group, we examined the relationships of DHD, DD, and LOS to volumes, and the interactions of those drinking history measures. All models included age as a covariate, and correlations between age, DHD, DD, and LOS were low (all $r < 0.5$; age with DHD = 0.36, DD = -0.37; LOS = 0.45; DHD with DD = 0.13; DHD with LOS = -0.26; DD with LOS = -0.28). Table S5 shows that the LOS-by-gender-by-region interaction was significant ($F(11, 2474.63) = 1.94$, $p = 0.03$). LOS was associated with smaller volumes in women and larger volumes in men in the CA1 ($t(257.95) = -2.493$, $p = 0.01$). CA1 volumes increased by 0.03% per year and decreased by 0.02% per year of sobriety in AUDm and AUDw, respectively.

Discussion

Results of this study confirmed findings of smaller volumes of hippocampal subfields in association with AUD [17,22,23]. Compared to the NC group, the AUD participants exhibited significantly smaller volumes in the CA1, subiculum (with marginal significance), molecular layer, and hippocampal tail. Smaller volumes in these regions could result in abnormal neural processing, coincident with impairments of distinct mental functions. We also found significant

associations between the volumes of individual subfields with age, memory, gender, and measures of drinking history.

Alterations in hippocampal subfield volumes have implications both for downstream targets and for hippocampal processing of upstream inputs. Accordingly, fewer neurons in the CA1 and subiculum may result in weaker output from the hippocampus, and smaller dendritic arborizations in the molecular layer may cause the hippocampus (particularly anterior regions) to be less effective in distinguishing between distinct contextual inputs, thereby resulting in forms of overgeneralization [54]. Both types of abnormality (input and output) could have severe consequences for emotion and motivation or sensitivity to reward. For example, the hippocampus projects to medial prefrontal areas, which are involved in conflict detection [55]. Weak or insufficiently precise contextual signals from hippocampus may bias these cortices towards greater sensitivity to emotional signals, such as those received from the amygdala. Elevated or overgeneralized processing of conflict may, in turn, bias the motivational and reward systems, particularly the nucleus accumbens, towards the use of poor coping strategies, including excessive drinking and other forms of self-medication. Abnormal inputs also can bias processing. For example, since the hippocampus receives connections from the amygdala [56], those signals may modulate the hippocampus more strongly if it has fewer neurons or smaller dendritic arborizations. This could act synergistically with weak contextual representations, thereby reinforcing their emotional valence. In other words, overgeneralized contextual representations might be more susceptible to "somatic markers" [57], as well as other forms of associative learning [54], which would increase the vulnerability to contextual signals that motivate drinking.

CA1 and Subiculum

Both output regions of the hippocampus (CA1 and subiculum) were reduced in volume, which could be related to two cognitive corollaries: (1) an abnormal ability to distinguish a currently experienced context from other similar previously learned contexts [58,59] and (2) higher susceptibility to emotion-driven actions [60]. That is, following a history of AUD, a given experienced context might be more likely to match a previous context in which drinking occurred.

The role of context in triggering alcohol craving is well established, and context has behavioral and treatment implications [61,62]. Evidence suggests that social support may be helpful for circumventing specific contexts entirely [63], which would avoid the aforementioned overgeneralizing activity of an impaired CA1 and subiculum. The subiculum in particular has been implicated in the prediction of future rewards [64], perhaps due to its projections (along with projections of CA1) to the nucleus accumbens and ventral tegmental area. Likewise, the CA1 has been shown to play a role in mental processes involving envisioning the self in the future and past [41]. Therefore, the abnormalities we observed in these structures in association with AUD could negatively impact motivation through the impairment of both future processing and reward prediction.

Molecular Layer

The molecular layer also was smaller in the AUD group. This region includes the apical dendrites of hippocampal pyramidal cells, and is traditionally viewed as an input region to the subfields of the hippocampus [65]. Smaller volumes of the molecular layer could suggest a

relationship of AUD with hippocampal inputs or with local computational processes of the hippocampus (as opposed to connectivity exiting the structure) and could lead to outcomes that are similar to those resulting from smaller CA1 and subiculum volumes. In the molecular layer, entorhinal cortex inputs are thought to convey information from the current sensory context, while intra-hippocampal projections to the molecular layer are thought to represent a memory representation of the current context [66,67]. With reduced volume of this circuitry in the molecular layer, the quality of the processing at the meeting of these two streams of input could be impacted. Therefore, it could result in a reduced ability to compare previously experienced contexts with the current experience, and might lead the hippocampus to overgeneralize the present context to previously experienced high-valence contexts. In turn, this could bias the system to addictive behaviors, i.e., similar outcomes to those discussed regarding reductions in CA1 and subiculum volumes.

Hippocampal Tail

While the anterior hippocampus has been associated with stress, emotion, and affect [42], reductions in cells and cell processes in the posterior tail could involve many elements of spatial processing. This possibility is congruent with previous findings of alcohol-mediated alterations of spatial processing [68–70], which in rats, has been hypothesized to occur through alterations of hippocampal place cells [71]. In our AUD group, hippocampal tail volume was negatively associated with both immediate and delayed memory scores, in contrast to the positive association observed in the NC group. One possible interpretation for this finding involves regional compensation within the brain [70,72,73]. That is, for AUD participants with smaller

hippocampal tail volumes, the extent of structural impairment might be sufficient to necessitate a shift to using other structures for the same function. Once this shift occurs, the new structures may provide good compensation for learning — but this shift may occur more often for the more extreme cases of hippocampal perturbation. In contrast, for AUD participants with mild hippocampal impairments, the brain may continue to rely on the impaired structures instead of shifting to an alternative compensatory region; relying on the impaired structures could result in impaired memory performance. (A metaphor that may assist in understanding is as follows: One may continue using a somewhat functioning toaster and make low quality toast; with a broken toaster one may switch to the oven broiler and make better toast.) Taken together, this explanation would fit with the observed tendency for larger hippocampal volumes to be associated with worse performance in the AUD group, and is consistent with findings that report how medial prefrontal cortex may compensate by increasing its efficiency for learning and memory after substantial hippocampal dysfunction [74].

Influence of Participant Characteristics

While our results of smaller volumes of the CA1, subiculum, and molecular layer in AUD subjects are in agreement with past findings [17,22,23], our results did not indicate significant reductions in other regions that were reported in those studies, including CA2+3, CA4, HATA, and fimbria. However, abstinence durations for the AUD participants examined in those papers were generally shorter than in the present study (an average of 7.1 years). Therefore, results reported in the earlier studies could reflect both acute effects of alcohol consumption and long-term sequelae of chronic alcohol use. For the regions that are consistent

across those studies and ours (CA1, molecular layer, and subiculum), the combined results support the view that they are vulnerable to chronic long-term AUD.

Gender differences also contribute to divergent findings across studies. As a proportion of head size, total hippocampal volumes are larger in women than in men [21,75], a finding we support in more detail with evidence from subfield volumetric analyses (subiculum, presubiculum, CA1, and molecular layer). Although the present study and the results reported by Agartz et al. [21] and Zahr et al. [23] did not show significant interactions between gender and diagnostic group, gender differences in volumes may be elucidated further by considering quantity and duration of alcohol consumption, and LOS. In the present study, CA1 volume was related to LOS differently for men and for women. In men, CA1 volume increased with LOS, suggesting recovery over time. However, in women, the volumes continued to decline with sobriety, even after statistically accounting for age. This might indicate an AUD-related abnormality in another biological system, as AUDw may be more susceptible to liver injury and heart disease, and have been reported to display lower drinking thresholds for systemic damage [76]. Thus, a brain abnormality could continue to result in reduced health over a long period, even following abstinence, explaining the divergent gender effects of LOS. Additionally, in mice, chronic ethanol administration induces astrocyte activation in a number of hippocampal regions, including the CA1, but exclusively in female mice [77]. Despite recovery from chronic ethanol intoxication, female mice exhibited altered astrocyte neurochemistry and function. Astrocytes are involved in neural health and vascular coupling, so abnormalities could have substantial neural consequences. While further studies are needed to translate these results to human populations, this study suggests that women could be more vulnerable to life-long

astrocyte inflammation-mediated brain damage, including in the hippocampus and associated regions [77].

Age, another factor that individuates the impact of AUD, was more negatively associated with hippocampal volumes in our AUD group than in our NC group for the subiculum, presubiculum, hippocampal tail, and molecular layer regions. These findings extend earlier reports based on pathology and MRI investigations, that brain regions, including the hippocampus, show greater volumetric reductions or abnormal blood flow in older than in younger individuals with AUD [19,32]. Moreover, cognitive ramifications of an interaction of age and AUD were reported to exert compounded abnormalities in memory and visuospatial abilities [78]. In the present study, age was negatively associated with volumes of the subiculum, presubiculum, hippocampal tail, and molecular layer regions, whereas Zahr and colleagues [23] found smaller volumes in the CA2+3 subfield. While not resolved, it should be noted that there is a long-running hypothesis discussing how age may be associated with greater volume losses and functional decline in AUD groups than NC groups [79,80].

Limitations

In the present study, the automated subfield labeling procedure that we employed relied upon a probabilistic atlas, rather than borders defined by image contrast at high resolution. Additionally, we used a cross-sectional design, whereas a longitudinal cohort may be better able to show how the observed abnormalities were related to pre-existing risk factors for AUD or consequences of AUD. We did not consider several other factors that could influence our findings (see Oscar-Berman et al. [70] for review). For example, we did not consider additional

participant characteristics (Table S6) such as family history of AUD [81] or smoking [82,83]. As described in the Methods, although we included participants with confounding factors to increase the generalizability to the United States population, we recognize that these confounding factors could limit interpretability. For that reason, we analyzed the data from a subsample of participants without the confounding factors. All statistical group effects, including group interactions, remained significant for this unconfounded subsample. As a separate issue, the AUDw group had longer LOS and shorter DHD than the AUDm group. As described in the Methods, we analyzed subsamples of AUDm and AUDw who did not differ significantly on LOS or DHD, and significant gender effects remained significant. For simplicity, measures of education and IQ were not included in the statistical models presented. However, when the models were re-run controlling for age and education, all statistical group effects, including group interactions, remained significant.

Conclusions

The results indicated smaller input (molecular layer) and output (CA1) volumes for the AUD group, abnormalities that could be related to distorted context processing in the AUD group. Smaller volumes also were evident for the hippocampal tail, implicating deficits in spatial processing. Memory scores were negatively associated with hippocampal tail volume in the AUD group, while a positive association was observed for the NC group, suggesting that the larger volumes were associated with better performance. This finding might further signal an AUD-related functional deficit in the hippocampal tail, and the spatial processing and memory functions performed by that region. Our observation of more extreme age-related hippocampal

volume reductions in the AUD group than in the NC group, not only are congruent with the notion of synergistic negative impacts of alcohol exposure and aging; they also refine the subregional implications of the abnormality to the hippocampal tail, subiculum, presubiculum, and molecular layer. Regarding gender differences, longer LOS was associated with larger CA1 volumes in AUDm, possibly indicative of recovery of contextual processing over time; however, the smaller volumes observed in AUDw in conjunction with longer sobriety periods, might suggest impaired recovery for women. We believe that our findings not only build upon other work that highlights brain structural and functional abnormalities in the impact of AUD, they also suggest that clinicians, educators, and public health officials could benefit by approaching prevention and treatment strategies with respect to individual differences.

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Supporting Tables and Figures

	AUDw (N=31)		AUDm (N=36)		NCw (N=30)		NCm (N=33)	
Hippocampus Region	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)
Hippocampal tail*	0.000338	(0.000039)	0.000340	(0.000044)	0.000357	(0.000034)	0.000357	(0.000050)
Subiculum	0.000278	(0.000034)	0.000264	(0.000026)	0.000288	(0.000020)	0.000278	(0.000024)
CA1*	0.000402	(0.000046)	0.000392	(0.000036)	0.000424	(0.000035)	0.000414	(0.000035)
Hippocampal fissure	0.000098	(0.000013)	0.000095	(0.000014)	0.000101	(0.000017)	0.000087	(0.000014)
Presubiculum	0.000194	(0.000028)	0.000180	(0.000025)	0.000203	(0.000020)	0.000189	(0.000024)
Parasubiculum	0.000037	(0.000007)	0.000036	(0.000007)	0.000040	(0.000005)	0.000038	(0.000007)
Molecular layer*	0.000365	(0.000040)	0.000350	(0.000034)	0.000383	(0.000028)	0.000372	(0.000032)
DG	0.000194	(0.000021)	0.000188	(0.000018)	0.000201	(0.000015)	0.000200	(0.000018)
CA2+3	0.000140	(0.000015)	0.000137	(0.000017)	0.000146	(0.000013)	0.000147	(0.000015)
CA4	0.000167	(0.000017)	0.000163	(0.000015)	0.000174	(0.000012)	0.000173	(0.000015)
Fimbria	0.000045	(0.000010)	0.000041	(0.000013)	0.000049	(0.000007)	0.000047	(0.000009)
HATA	0.000039	(0.000005)	0.000037	(0.000006)	0.000041	(0.000005)	0.000040	(0.000004)
Whole hippocampus	0.002199	(0.000219)	0.002129	(0.000198)	0.002307	(0.000144)	0.002256	(0.000184)

Table S1. Regional volumes (proportion of estimated total intracranial volume).

Means and standard deviations (SD) are provided for the hippocampal regional volumes of AUDw (N=31) and AUDm (N=36) (women and men with a history of Alcohol Use Disorder), along with NCw (N=30) and NCm (N=33) (women and men without a history of AUD). Abbreviations: CA1 through 4 = cornu ammonis 1 through 4; DG = dentate gyrus; HATA = hippocampal-amygdaloid transition area. *Indicates regions where AUD < NC, $p < 0.0042$.

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
group	1.3E-09	1.3E-09	1	78.97	4.11	0.05
region	1.8E-06	1.6E-07	11	5366.83	519.09	<0.01
age	7.4E-09	7.4E-09	1	78.97	23.77	<0.01
gender	1.3E-09	1.3E-09	1	78.97	4.18	0.04
group:region	6.2E-09	5.7E-10	11	5366.83	1.82	0.05
group:age	2.7E-09	2.7E-09	1	78.97	8.81	<0.01
region:age	5.4E-08	4.9E-09	11	5366.83	15.59	<0.01
region:gender	1.2E-08	1.1E-09	11	5366.83	3.41	<0.01
age:gender	6.3E-10	6.3E-10	1	78.97	2.01	0.16
group:gender	2.8E-10	2.8E-10	1	78.97	0.88	0.35
group:region:age	1.0E-08	9.4E-10	11	5366.83	3.02	<0.01
region:age:gender	8.6E-09	7.8E-10	11	5366.83	2.50	<0.01
group:region:gender	2.3E-09	2.0E-10	11	5366.83	0.66	0.78
group:age:gender	3.9E-10	3.9E-10	1	78.97	1.25	0.27

Table S2. Analysis of variance for model including group, gender, and age.

The analysis of variance obtained from the mixed model significant indicated significant group-by-region-by-age and gender-by-region-by-age interactions for volumes. Colons indicate interaction effects. Abbreviations: Sum Sq = sums of squares; Mean Sq = mean square; NumDF = numerator degrees of freedom; DenDF = denominator degrees of freedom; Pr(>F) = probability > F (i.e., *p* value).

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
group	3.5E-10	3.5E-10	1	75.01	1.12	0.29
region	4.9E-07	4.5E-08	11	5274.22	144.84	<0.01
IMI	3.3E-11	3.3E-11	1	75.01	0.11	0.75
gender	1.3E-09	1.3E-09	1	75.01	4.05	0.05
age	6.7E-09	6.7E-09	1	75.01	21.82	<0.01
group:region	6.1E-09	5.5E-10	11	5274.22	1.80	0.05
group:IMI	8.0E-11	8.0E-11	1	75.01	0.26	0.61
region:IMI	5.9E-10	5.4E-11	11	5274.22	0.17	1.00
region:gender	9.3E-09	8.4E-10	11	5274.22	2.73	<0.01
gender:age	5.7E-10	5.7E-10	1	75.01	1.85	0.18
region:age	4.3E-08	3.9E-09	11	5274.22	12.68	<0.01
group:gender	1.0E-11	1.0E-11	1	75.01	0.03	0.85
group:age	1.2E-09	1.2E-09	1	75.01	3.74	0.06
group:region:IMI	7.0E-09	6.3E-10	11	5274.22	2.06	0.02
region:gender:age	6.8E-09	6.2E-10	11	5274.22	2.01	0.02
group:gender:age	1.7E-11	1.7E-11	1	75.01	0.06	0.81
group:region:gender	1.2E-09	1.1E-10	11	5274.22	0.37	0.97

Table S3. Analysis of variance for model including Immediate Memory Index.

The analysis of variance obtained from the mixed model indicated a significant group-by-region-by-IMI interaction for volumes. Colons indicate interaction effects. Abbreviations: Sum Sq = sums of squares; Mean Sq = mean square; NumDF = numerator degrees of freedom; DenDF = denominator degrees of freedom; Pr(>F) = probability > F (i.e., *p* value).

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
group	5.5E-10	5.5E-10	1	73.62	1.80	0.18
region	4.6E-07	4.2E-08	11	5276.69	136.07	<0.01
DMI	7.5E-14	7.5E-14	1	73.62	0.00	0.99
gender	1.4E-09	1.4E-09	1	73.62	4.64	0.03
age	6.8E-09	6.8E-09	1	73.62	22.22	<0.01
group:region	9.0E-09	8.2E-10	11	5276.69	2.67	<0.01
group:DMI	2.2E-10	2.2E-10	1	73.62	0.71	0.40
region:DMI	7.4E-10	6.8E-11	11	5276.69	0.22	1.00
region:gender	1.1E-08	9.9E-10	11	5276.69	3.23	<0.01
gender:age	6.9E-10	6.9E-10	1	73.62	2.27	0.14
region:age	4.4E-08	4.0E-09	11	5276.69	13.15	<0.01
group:gender	1.1E-11	1.1E-11	1	73.62	0.04	0.85
group:age	1.1E-09	1.1E-09	1	73.62	3.63	0.06
group:region:DMI	1.0E-08	9.4E-10	11	5276.69	3.07	<0.01
region:gender:age	8.4E-09	7.6E-10	11	5276.69	2.49	<0.01
group:gender:age	1.9E-11	1.9E-11	1	73.62	0.06	0.80
group:region:gender	1.3E-09	1.2E-10	11	5276.69	0.38	0.97

Table S4. Analysis of variance for model including Delayed Memory Index.

The analysis of variance obtained from the mixed model indicated a significant group-by-region-by-DMI interaction for volumes. Colons indicate interaction effects. Abbreviations: DMI = Delayed Memory Index; Sum Sq = sums of squares; Mean Sq = mean square; NumDF = numerator degrees of freedom; DenDF = denominator degrees of freedom; Pr(>F) = probability > F (i.e., *p* value).

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
gender	8.2E-13	8.2E-13	1	36.00	0.00	0.96
region	4.4E-07	4.0E-08	11	2474.63	129.65	<0.01
DHD	4.1E-11	4.1E-11	1	36.00	0.13	0.72
DD	3.2E-10	3.2E-10	1	36.00	1.04	0.31
LOS	2.4E-10	2.4E-10	1	36.00	0.77	0.38
age	2.0E-09	2.0E-09	1	36.00	6.35	0.02
gender:region	3.6E-09	3.3E-10	11	2474.63	1.06	0.39
gender:DHD	1.3E-12	1.3E-12	1	36.00	0.00	0.95
region:DHD	2.2E-09	2.0E-10	11	2474.63	0.65	0.79
gender:DD	1.9E-11	1.9E-11	1	36.00	0.06	0.81
region:DD	3.7E-09	3.4E-10	11	2474.63	1.09	0.37
gender:LOS	7.0E-10	7.0E-10	1	36.00	2.28	0.14
region:LOS	7.3E-09	6.7E-10	11	2474.63	2.15	0.01
gender:age	1.5E-10	1.5E-10	1	36.00	0.49	0.49
region:age	1.4E-08	1.2E-09	11	2474.63	3.97	<0.01
gender:region:DHD	1.8E-09	1.6E-10	11	2474.63	0.52	0.89
gender:region:DD	3.8E-09	3.5E-10	11	2474.63	1.12	0.34
gender:region:LOS	6.6E-09	6.0E-10	11	2474.63	1.94	0.03
gender:region:age	4.5E-09	4.1E-10	11	2474.63	1.32	0.21

Table S5. Analysis of variance for model including drinking history (DHD, DD, and LOS) of the AUD group.

The analysis of variance obtained from the mixed model indicated a significant gender-by-region-by-LOS interaction for volumes, for the AUD group. Colons indicate interaction effects. Abbreviations: DHD = duration of heavy drinking; DD = daily drinks; LOS = length of sobriety; Sum Sq = sums of squares; Mean Sq = mean square; NumDF = numerator degrees of freedom; DenDF = denominator degrees of freedom; Pr(>F) = probability > F (i.e., *p* value).

Measure	Level	AUDm	AUDw	NCm	NCw
Confounding	Confounded	22	19	6	6
Confounding	Unconfounded	14	12	27	24
Current Smoking	No	22	19	29	28
Current Smoking	Yes	14	12	4	2
Five Year Drug History	No	31	27	31	27
Five Year Drug History	Less than once per week	4	2	1	2
Five Year Drug History	Once per week or more	1	2	1	1
Lifetime Drug History	No	9	11	26	23
Lifetime Drug History	Less than once per week	11	7	3	3
Lifetime Drug History	Once per week or more	16	13	4	4
Mother History	Alcoholic	8	9	2	6
Mother History	Alcoholic and drug abuse	2	3	0	2
Mother History	Drug abuse	0	1	0	0
Mother History	Never	6	5	7	7
Mother History	Social drinker	20	13	24	15
Father History	Alcoholic	16	19	7	6
Father History	Alcoholic and drug abuse	3	3	0	2
Father History	Don't know	3	1	2	3
Father History	Never	1	2	3	4
Father History	Social drinker	13	6	21	15
First Degree History	No	11	2	25	16
First Degree History	Yes	25	29	8	14
Second Degree History	No	14	5	22	18
Second Degree History	Yes	22	26	11	12

Table S6. Additional participant characteristics.

Counts of participants are given for each level of the measures listed for AUDw (N=31) and AUDm (N=36) (women and men with a history of Alcohol Use Disorder), along with NCw (N=30) and NCm (N=33) (women and men without a history of AUD). Confounded and unconfounded subsample assignment is described in the Methods. First Degree History was indicated by participant endorsement of ‘Alcoholic’ for mother, father, sibling, or offspring. Second Degree History was indicated by participant endorsement of ‘Alcoholic’ for grandparents, aunts, uncles, or grandchildren.

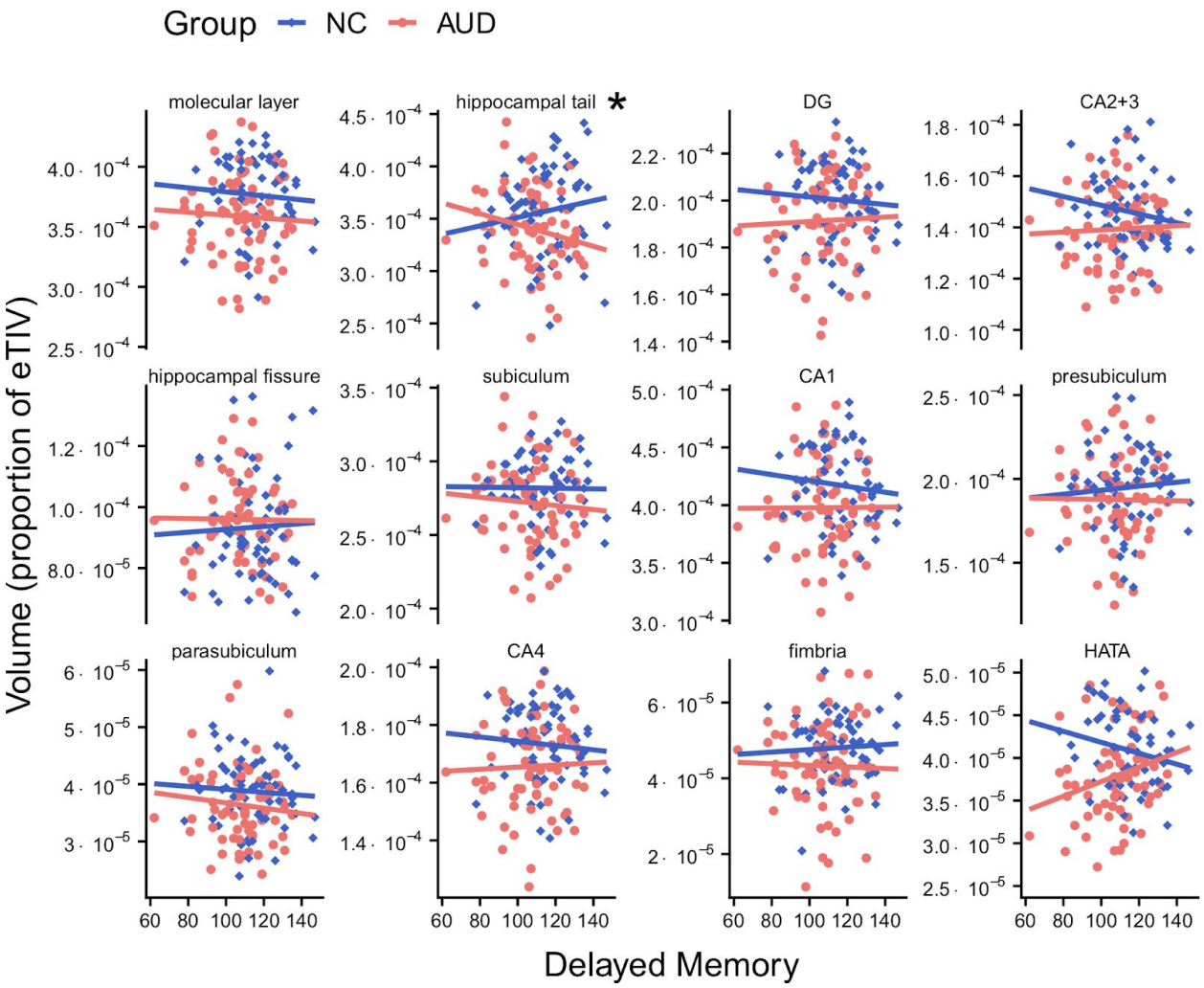

Figure S1. Relationships of volume with delayed memory for AUD and NC groups.

As described in Figure 3, for the AUD group, Delayed Memory Index was associated with smaller hippocampal tail volumes (proportion of eTIV), while for the NC group, a positive relationship was observed. This figure shows the relationships for all 12 regions. Abbreviations: CA1 = cornu ammonis 1; CA2+3 = cornu ammonis 2 and 3; CA4 = cornu ammonis 4; DG = dentate gyrus; HATA = hippocampal-amygdaloid transition area; Sub = subiculum; eTIV = estimated total intracranial volume. *Indicates regions where $p < 0.0042$ for the interaction of group-by-Delayed Memory Index.

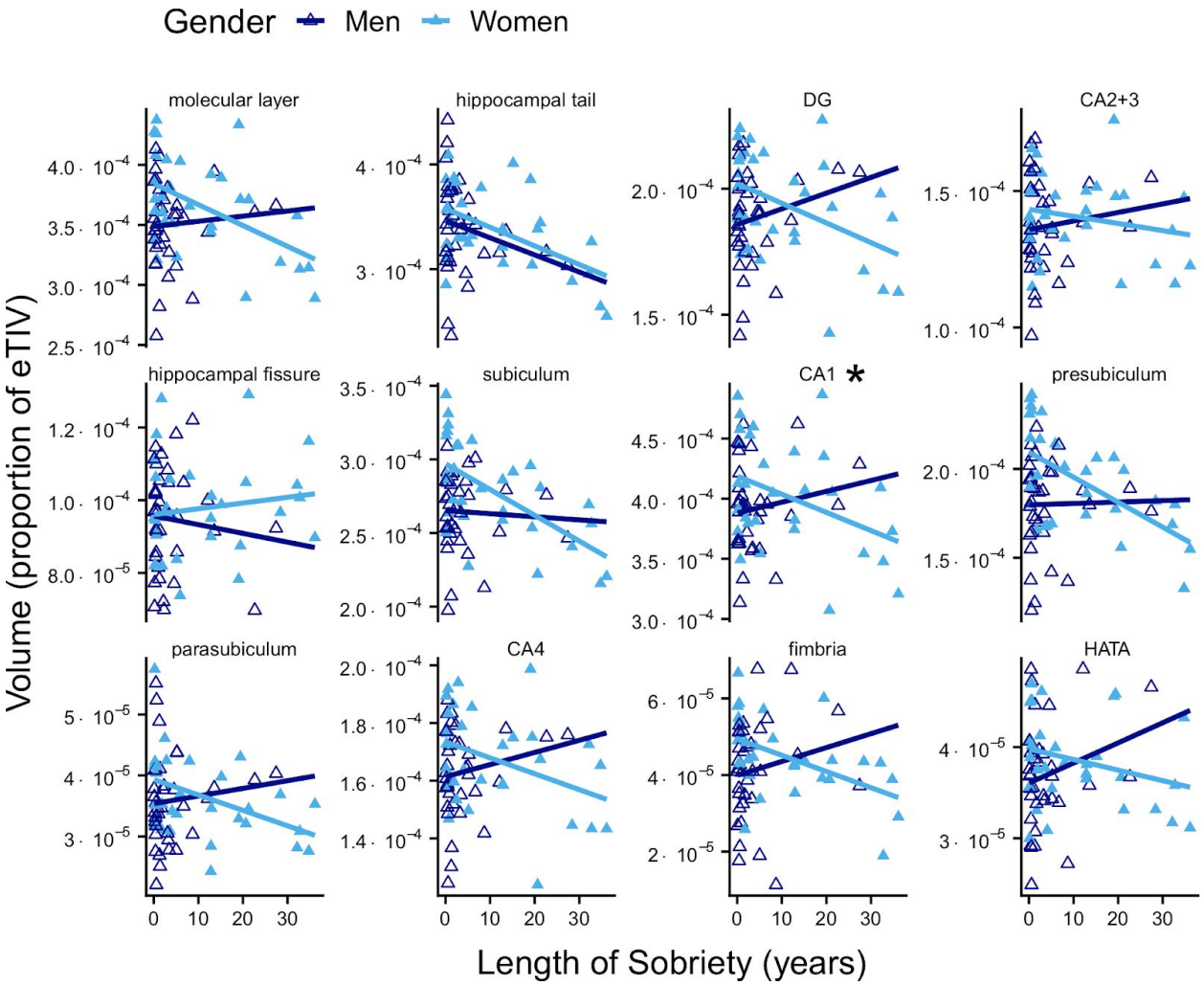

Figure S2. Relationships of volume with length of sobriety for AUD men and women.

As described in Figure 3, for AUD men, CA1 volumes (proportion of eTIV) were positively associated with Length of Sobriety, while for AUD women, a negative relationship was observed. This figure shows the relationships for all 12 regions. Abbreviations: CA1 = cornu ammonis 1; CA2+3 = cornu ammonis 2 and 3; CA4 = cornu ammonis 4; DG = dentate gyrus; HATA = hippocampal-amygdaloid transition area; Sub = subiculum; eTIV = estimated total intracranial volume. *Indicates regions where $p < 0.0042$ for the interaction of group-by-Length of Sobriety.

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