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Alcohol consumption during adolescence is associated with reduced grey matter volumes

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Alcohol consumption during adolescence is associated with reduced grey matter volumes in bilateral frontal and temporal lobes and insula

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Running head: Adolescent alcohol use and grey matter

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Abstract

Aims: Cognitive impairment has been associated with excessive alcohol use but its neural basis is poorly understood. Chronic excessive alcohol use in adolescence may lead to neuronal loss and volumetric changes in brain. Our objective was to evaluate possible abnormalities in grey matter volume connected to substantial alcohol consumption within sub-diagnostic limits.

Design: Longitudinal AUDIT-C survey, magnetic resonance imaging.

Setting: Heavy-drinking and light-drinking young adults were recruited from a follow-up cohort.

Participants: The 62 participants were aged 22–28; 35 alcohol users and 27 controls.

Measurements: Alcohol use was measured by AUDIT-C at three time points over 10 years. Grey matter volume was determined and compared between groups using voxel-based morphometry on 3D T1-weighted MR images.

Findings: Grey matter volumes were significantly smaller among heavy-drinking participants in the bilateral anterior cingulate cortex, bilateral prefrontal cortex, bilateral superior and middle frontal gyrus, right pars triangularis, bilateral superior temporal gyrus, right middle temporal gyrus and bilateral insular cortex as compared to the control group. In addition, the grey matter volume in these areas was found to correlate negatively with the AUDIT-C score.

Conclusions: The results support the hypothesis that excessive alcohol use especially in adolescence is associated with an abnormal development of the brain grey matter. Moreover, the structural changes detected in insula on the alcohol users may reflect a reduced sensitivity to alcohol’s negative subjective effects.
Introduction

A little over half of 15-16 year-olds have used alcohol in the past month in Europe [1]. In Finland, 50 % of 16-year-olds use alcohol more than monthly and as many as 20 % drink heavily at least once a month [2]. The onset age of alcohol use plays an important role in the development of alcohol dependence: 14.8 % of adults who started using alcohol at the age of 14 or under and 8.9 % of those who started between the ages of 15 to 17 have been later classified with alcohol dependence or abuse [3]. The updating of the new psychiatric Diagnostic and Statistical manual of Mental Disorders (DSM) V diagnostic criteria has been criticized with respect to its sensitivity in detecting adolescents with alcohol use disorder (AUD) [4], especially since substantial alcohol consumption that does not meet the current criteria for AUD limits has been found to be linked with cognitive impairments and a poor level of scholastic achievement [5].

Thus far, previous research on the structural and functional changes in the brain connected to drinking has focused mainly on chronic adult drinkers with a diagnosed AUD and often with several other clinical conditions after many years of heavy drinking [6]. Several areas in the brain have been discovered with less or thinner grey matter (GM) volumes in subjects with AUD as compared to light-drinking control subjects; these include anterior cingulate cortex (ACC) [7], superior [7-8], medial [9], middle [7,10-11] and inferior frontal gyrus [8-9], orbitofrontal gyrus [7], middle temporal gyrus [8], medial occipital gyrus [8], hippocampus [7,10,12], insular cortex [7,9-11], thalamus [10-11] and cerebellum [10-11].

Some studies have been conducted in adolescents or young adults with an AUD diagnosis; these have detected reductions in the GM volumes in hippocampus [13-15], prefrontal cortex [16-17] and lateral areas of the left temporal lobe [18]. In contrast, some research has been undertaken in examining structural brain changes connected to alcohol use that does not reach the threshold for an AUD diagnosis during adolescence or young adulthood. Mashoon and her research team studied young binge drinking adults and discovered their grey matter volume to be reduced in ACC and posterior cingulate gyrus (PCC) [19]. Squeglia et al studied a group of adolescents from the baseline age of approximately 15 years, who initiated heavy drinking during a three-year follow-up period. Those who transitioned later into heavy drinking showed thinner cortices in left cingulate and pars triangularis already at baseline, and the cortical thicknesses of left central diencephalon, inferior and middle temporal gyrus as well as left caudate and brain stem decreased during follow-up [20]. Another study from the team with a slightly longer follow-up of 3.5 years on average showed the grey matter to decrease in frontal and temporal cortices [21]. Luciana et al [22] studied adolescents, who were on average 17 years old at baseline and then transitioned to binge drinking during a follow-up period of two years. In this study binge drinkers exhibited decreased grey matter volume in middle frontal gyrus during the follow-up period, as compared to light-drinking adolescents. In addition, one study has discovered decreased GM volume in cerebellum [23] and another conversely increased GM volume in frontal areas [24]. One study discovered the left frontal and cingulate cortex to be thicker in female drinkers and thinner in male drinkers [25]. However, all studies apart from Doallo et al had participants with a relatively short history of heavy drinking. In addition, several studies had substance use comorbidities and the documentation of the participants’ alcohol use varies greatly between the
studies. Therefore, a study with participants with a longer history of heavy alcohol use as well as no substance use comorbidities is needed.

In the present study, we aimed to evaluate 1) whether adolescent alcohol use that does not reach the threshold for an AUD diagnosis would result in quantitatively smaller GM volumes and 2) if the degree of possible smaller GM volume would correlate with the pattern and cumulative quantity of alcohol used. We recruited a carefully selected group of young heavy-drinking participants who had a relatively long drinking history of at least ten years, but did not meet the criteria for AUD at the final time point, and their matched light-drinking controls. Based on the previous literature, we hypothesized that GM volume loss would be most pronounced in frontal lobes including the cingulate cortex and in temporal lobes.

Methods

Participants

This study is part of the Youth Wellbeing Study, which is monitoring Finnish adolescents’ health and alcohol use. Prior to participating in the study, written informed consent was requested from all participants. Permission for the study was provided by the Ethical committee of Kuopio University Hospital and University of Eastern Finland, the Finnish National Supervisory Authority for Welfare and Health, and the Finnish Ministry of Social Affair and Health. The baseline and follow-up study settings have been described in detail elsewhere [26-27]. Participants of the original cohort (year of birth 1986–1991, n = 4127) were gathered in 2004–2005 (time point 1). A similar follow-up questionnaire was sent in 2010-2011 by mail to those who had provided consent to be contacted for a follow-up study and whose postal address could be ascertained (time point 2, n = 1585). The participants for the present study (time point 3) concerning structural changes of the brain were selected from those who completed the follow-up questionnaire (n = 797).

The questionnaire at time points 1 and 2 included the Alcohol Use Disorders Identification Test (AUDIT), a structured questionnaire originally designed by the World Health Organization. In this study, we used only the first three questions of the AUDIT known as AUDIT-C. The questions were: “How often do you have a drink containing alcohol?”, “How many drinks containing alcohol do you have on a typical day when you are drinking?” and “How often do you have six or more drinks on one occasion?” The maximum score for each question was 4, amounting to a maximum total score of 12. Lifetime drug use was recorded using a questionnaire in which the participants were asked whether they had tried different drugs (as named in the questionnaire according to category) never, 1–5 times, or six or more times. The current smoking status and pack years were also recorded.
A participant was defined as a heavy alcohol user if the AUDIT-C score was 4 or more in males and 3 or more in females [28]. The light-drinking controls had low AUDIT-C scores (maximum 2) at both time points, and reported no binge drinking episodes. Alcohol users were listed in descending order based on AUDIT-C scores firstly at time point 2 and secondly at time point 1, and contacted in this descending order. Age-, gender- and education-matched light-drinking controls were recruited in parallel to match those alcohol users who had agreed to participate in this trial. The exclusion criteria were a history of a head injury needing medical treatment, neurological illness, severe mental disorder, metal or implanted devices in the body contraindicating MRI, regular use of other intoxicating substances, and pregnancy.

We sought to recruit altogether 40 alcohol users and 40 controls. Of the 80 participants, one heavy-drinking participant did not complete the MRI scanning due to technical problems and the images of another heavy-drinking participant as well as two controls were excluded due to congenital structural abnormalities (focal cortical dysplasia, a large cyst, enlarged ventricles). Five light-drinking control participants were excluded due to binge drinking episodes but no regular alcohol use, six light-drinking controls were excluded due to an AUDIT-C score of more than two, and three heavy-drinkers were excluded due to an AUDIT-C score of less than the cut-off point at one of the time points. Altogether thirty-five heavy-drinking and twenty-seven light-drinking participants were finally included in the analysis. Detailed subject demographics are presented in Table 1.

Psychiatric assessment

At time point 3, all participants were interviewed using a Structured clinical interview for DSM-IV axis I and II psychiatric disorders (SCID-I and SCID-II) [29-30]. Interview included clinical evaluation of current and previous psychiatric disorders, personality disorders, substance and alcohol use disorders. The interviews were conducted by specialists in adolescent psychiatry trained to undertake SCID interviews. In the interview, the participants filled in the AUDIT questionnaire as well as a questionnaire concerning their lifetime drug use and cigarette smoking. Their answers were then discussed and confirmed in the interview.

MR Image Acquisition

Participants underwent 3-T MRI of the brain (Philips Achieva 3.0T TX, Philips, Netherlands). A T1-weighted 3D TFE sequence (TR 8.24 ms, TE 3.82 ms, flip angle 8°, FOV 240, 190 contiguous sagittal slices with 0.94 x0.94 x1.0 mm voxels) as well as T2-weighted - and Flair-sequences were acquired. An experienced neuroradiologist evaluated all structural images for any abnormalities.

VBM Analysis
Voxel-based morphometry analysis was performed using the VBM8-toolbox (http://www.dbm.neuro.uni-jena.de/vbm/vbm8/) in SPM8 (Wellcome Department of Imaging Neuroscience, London; http://www.fil.ion.ucl.ac.uk/spm) running under Matlab R2007b (Mathworks Inc., Natick, MA). The T1 weighted images were normalized into the same stereotactic space and segmented into GM, white matter (WM) and cerebrospinal fluid (CSF). The GM images were then modulated to retain their original volume, and then smoothed with an 8 mm FWHM Gaussian kernel. In addition to a whole brain analysis, nine regions of interest (ROI) were selected based on the previous literature concerning heavy-drinking adolescents: ACC, PCC, middle and superior frontal gyrus, pars triangularis, superior, middle and inferior temporal gyrus and insula. The ROIs were defined using the WFU PickAtlas (version 2.5.2) [31].

Statistics

Analyses on the demographic variables were conducted with the SPSS statistical package (version 22.0; SPSS Inc., Chicago, IL). The differences in the gender, education and the prevalence of psychiatric diagnoses, marijuana and other drug use were examined with the chi-square test. The difference in age was tested with a 2-tailed independent samples t-test and differences in AUDIT-C scores as well as smoking in pack years were evaluated with Mann-Whitney test due to the non-normal distribution of the values. In order to observe possible linearity of the observed volumetric changes and the amount of alcohol consumption, we created a new variable “cumulative AUDIT-C” by adding together the AUDIT-C scores from all three time points for each participant.

The group difference of the GM volume between the heavy-drinking and light-drinking participants was analyzed using two-sample t-test with smoking in pack years as a covariate of no interest. Correlation analysis between the AUDIT-C scores and the GM volume was conducted using a single regression with pack years, gender and age as covariates of no interest. A Family Wise Error corrected threshold of $p<0.05$ was used in the whole brain analysis and an uncorrected threshold for multiple comparisons of $p<0.001$ was used in the ROI analyses. All results are shown with 50 voxels as the minimum cluster size.

Results

The basic characteristics of the study participants are shown in Table 1. The groups differed significantly in their AUDIT-C scores at every time point. The individual AUDIT-C scores of participants are shown in Fig. 1. The AUDIT-C scores of male and female participants did not differ significantly at any time point.

Age and educational background did not differ between the heavy- and light-drinking groups, nor did the number of psychiatric diagnoses. None of the participants had a current major depressive disorder or other severe mental disorder, but both groups included participants who had received a diagnosis for an anxiety disorder (heavy drinking and light drinking participants 14.3% and 14.8%
respectively) or a personality disorder (8.6% and 7.4%) at some point in their lives. Smoking was significantly more common among alcohol users (p<0.001) but there was no statistically significant difference between the groups in marijuana use. None of the participants had used other drugs more than 6 times and none of the participants had used any recreational drug during the preceding month.

Table 1. Demographic characteristics of the participants

![Figure 1. AUDIT-C scores of the participants at three time points, each line representing one participant. The lower set of lines depicts the light drinking participants, and the upper set of lines depicts the heavy drinking participants.](image)

VBM analysis

Whole brain VBM analysis showed no group differences between the light and heavy drinking participants. ROI analyses of the nine different predefined regions revealed smaller GM volumes in multiple areas among heavy alcohol users as compared to light drinking controls: in dorsal anterior cingulate cortex, frontopolar area, dorsolateral prefrontal cortex, supplementary and premotor cortices, pars triangularis, superior and middle temporal gyrus, angular gyrus, entorhinal cortex and insular cortex. None of the regions displayed larger GM volumes in heavy-drinkers than in light-drinking controls. The results are presented in more detail in Fig. 2 and Table 2.

Table 2. Areas of reduced grey matter in heavy-drinking vs. light-drinking participants in voxel-based morphometry analysis

![Fig 2. Areas of reduced grey matter in heavy-drinking vs. light-drinking participants in voxel-based morphometry analysis, indicated by yellow color. The upper row indicates the results in anterior cingulate ROI and superior frontal gyrus ROI, whereas the lower row indicates the results in superior temporal gyrus ROI and insular ROI.](image)

Correlation of the GM volume and the cumulative AUDIT-C score

The results of correlation analyses with cumulative AUDIT-C scores are presented in detail in Table 2. In the ROI analyses, negative correlations were revealed in the same areas as the group differences. In the whole brain correlation analysis no areas survived the statistical threshold.

Discussion

The present prospective study evaluated GM volume in a group of young adults with a ten-year history of heavy alcohol use and compared it to age and gender matched light-drinking controls. Alcohol use was addressed using longitudinal data measured at three time points by the AUDIT-C score. Although the alcohol use in heavy drinking group was substantial, it did not meet the current
criteria for an alcohol use disorder. In this study, the GM volume was found to be smaller in heavy-drinking participants in anterior cingulate cortex, superior and middle frontal gyrus, prefrontal cortex, pars triangularis, superior and middle temporal gyrus and insula, as compared to light-drinking participants. Furthermore, the extent of volume loss in these areas correlated significantly with the cumulative amount of alcohol consumption as estimated by the AUDIT-C score.

Our results on smaller GM volume in anterior cingulate cortex, superior and middle frontal gyrus, prefrontal cortex, pars triangularis, superior and middle temporal gyrus and insula connected to drinking are in agreement with several earlier studies with adolescent participants who drank heavily but had no AUD diagnosis [19-20,22]. Statistically significant volumetric reductions of insula have previously been linked to alcohol and drug addictions but not to alcohol use at sub-diagnostic limits [7,32-34]. In our study, however, we observed reduced GM volumes also in the insula. In functional imaging studies, drug abusers have exhibited altered BOLD activity in insula and ACC and these alterations were associated with increased relapse propensity [35-36]. Insula has several functions, but its posterior part has been though mainly to be involved in detecting interoceptive cues and mediating them to cingulate cortex [37-39]. Smaller volumes of ACC and posterior insula have also been detected in conditions with chronic pain such as fibromyalgia, back pain and tension type headache [40-41], which could implicate that structural changes in posterior insula are connected to altered sensitivity to interoceptive messages. Two earlier studies have shown that brain damage involving insula has caused patients to lose the urge to smoke [42-43]. This would imply that the structural abnormalities of insula have an effect on the addiction, not the drug on the structure of insula. Reduced volume of the posterior insula could imply that the heavy-drinking adolescents have an altered sensitivity to interoceptive messages that would normally be emitted after a high level of intoxication and they may not experience any immediate withdrawal symptoms after a period of alcohol consumption [44]. This could be one explanation for the continuation of excessive drinking.

Some previous studies have found the GM volume of frontal and temporal lobes to decrease during follow-up of heavy-drinking adolescents [20-22]. Furthermore, a smaller GM volume of pars triangularis has been reported at baseline among adolescents who later began regular binge drinking [20] as well as an inverse correlation between alcohol consumption and GM volume in the ACC [19]. Our results corroborate these conclusions, as there was an inverse correlation between the amount of alcohol consumed and the frequency of drinking (measured indirectly by AUDIT-C) and GM volume in the frontal and temporal areas and ACC.

There are two possible explanations for the smaller GM volume. The neurotoxic effects of alcohol could have been responsible for the smaller GM. Alternatively, the heavy-drinkers may have had an atypical grey matter developmental trajectory and never reached peak cortical volume as compared to light-drinking participants [44]. Previous research supports the idea that the smaller GM volume of ACC is at least partly congenital [20,45]. On the other hand, the recently published study by Squeglia and her team discovered the grey matter volumes of frontal and temporal cortices to decrease more rapidly in heavy-drinking adolescents as compared to the non-drinking control subjects [21]. In contrast, abstinence from alcohol has been associated with increases in cortical GM volumes including those in the ACC and insula after 1–3 months of abstinence [34], which suggests that the volumetric changes are at least partly reversible.
Our observations are relevant from the brain developmental point of view. Frontal areas, including ACC, are among the last brain regions to develop, and the pruning process can continue until an individual is in her/his early thirties [46-47]. It has been hypothesized that this late maturation enhances the emotional, limbic system–driven responses of adolescents, predisposing them to risky behavior [46,48]. This developmental period has even been called a window of vulnerability [7]. In our study, the volume of the cingulate GM was smaller in the alcohol users, which could exert an effect on impulse control and error processing and thus could further predispose these individuals to harmful alcohol use in a vicious cycle. ACC has been implicated to have a role in error processing, cognitive functions and impulse control [49-50]. Altered function and structure of the pregenual ACC have also been linked to anxiety disorders [51-52]. The prevalence of diagnosed anxiety disorders did not significantly differ between the two groups in our study, but this cannot be interpreted to mean that there were no differences in the anxiety-handling capabilities of the heavy-drinking participants.

The inclusion criteria of the present study differ from most other studies with adolescent or young adult participants. First, the participants’ alcohol use was followed from adolescence to young adulthood at three time points over ten years. The youngest participants were only 13 years old at the first time point. Second, all alcohol-using participants were known to have consumed large amounts of alcohol regularly for at least ten years prior to the MRI assessment. Many previous studies have examined participants with a significantly shorter history of alcohol consumption [16,20,22-23]. Additionally, some studies included participants at risk who had initiated alcohol use during the longitudinal study, which means that their study subjects had had a history of alcohol use no longer than 2-3 years on average [20-22]. It is possible that the smaller volume of insula is connected to drinking that has lasted considerably longer, and therefore has not been detected in studies with shorter durations.

Another strength of the present study is that all subjects were also evaluated during a semi-structured psychiatric interview by a specialist in adolescent psychiatry, and the heavy-drinking and light-drinking control groups were balanced in terms of participants with a psychiatric diagnosis. Some of the previous volumetric studies have included participants with comorbid psychiatric diagnoses [16] or have not assessed the participants’ present psychiatric condition with a systematic psychiatric interview [22], a possible limitation when interpreting the results. Furthermore, only two participants of the heavy-drinking group in our study met the diagnostic criteria of DSM-IV alcohol abuse at time point 3 and none of the participants had a diagnosis of harmful use or dependence of drugs, in fact only five alcohol-using participants had smoked marijuana more than 6 times. Additionally, none of the participants had used marijuana in the past month. Some of the previous studies have recruited adolescent subjects with comorbid substance abuse, most often regular marijuana use [16,20], with reported effects on brain volumes [53].

In our study, however, smoking was much more common among the heavy alcohol users than in the light-drinking group. Even though smoking was added as a covariate in the statistical models, its synergic effects with alcohol causing possibly reductions in GM volume cannot be ruled out. Furthermore, smoking measured as pack years correlated positively with the AUDIT-C score among the participants. Nevertheless, the connection between the GM volume and alcohol use remained despite this correlation. The main limitation of the present study is the lack of longitudinal MRI information, which would have helped in distinguishing between causes
and consequences. Moreover, the alcohol consumption was measured at only three time points, each assessing the drinking from only the past 12 months. However, it has been shown that the alcohol using trajectories of adolescents remain fairly stable [54]. Lastly, another limitation is the lack of information of a family history of alcohol dependence.

Our study shows that young adults who have a history of excessive alcohol consumption that does not meet the current criteria for AUD exhibited structural changes in the frontal and temporal areas, ACC, and insula. Therefore, it seems that alcohol use that has begun early in adolescence exerts neurodevelopmental consequences even before the onset of a diagnostic alcohol-related disorder. These results support the critical comments by Kaminer and Winters [4] about the DSM-V criteria for teenage substance use disorder. They concluded that a serious limitation of the new DSM-V criteria is that they do identify reliably those adolescents with harmful alcohol consumption patterns. It would be very important to prevent and reduce both alcohol experimentation and use during the vulnerable years of social, psychic and biological development. One way to achieve this goal would be to provide reliable, evidence-based information to parents and teachers about the serious consequences of teenage alcohol drinking. Longitudinal studies are warranted to explore the possible reversibility of the observed volume loss in individuals reverting to abstinence.
References


3. SAMHSA. Results from the 2013 National Survey on Drug Use and Health. National Survey on Drug Use and Health (NSDUH).


Table 1. Demographic characteristics of the participants

<table>
<thead>
<tr>
<th></th>
<th>Heavy-drinking group</th>
<th>Light-drinking group</th>
<th>Statistical sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=35</td>
<td>n=27</td>
<td></td>
</tr>
<tr>
<td>Male/Female&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15/20</td>
<td>12/15</td>
<td>ns</td>
</tr>
<tr>
<td>Age at time point 3, mean (SD)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.9 (1.4)</td>
<td>24.6 (1.6)</td>
<td>ns</td>
</tr>
<tr>
<td>AUDIT-C (± SD)&lt;sup&gt;cd&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Time point 1</td>
<td>6.2 (1.7)</td>
<td>0.0 (0.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>• Time point 2</td>
<td>8.2 (0.9)</td>
<td>0.2 (0.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>• Time point 3</td>
<td>6.4 (1.9)</td>
<td>0.7 (0.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking, pack years (± SD)&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>2.4 (2.4)</td>
<td>0.01 (0.04)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lifetime marijuana use, n (%)&lt;sup&gt;ae&lt;/sup&gt;</td>
<td>5 (14.3)</td>
<td>0 (0.0)</td>
<td>ns</td>
</tr>
<tr>
<td>Education, n (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Without H.S. diploma</td>
<td>0 (0.0)</td>
<td>1 (3.7)</td>
<td>ns</td>
</tr>
<tr>
<td>• H.S. graduate without college education</td>
<td>2 (5.7)</td>
<td>3 (11.1)</td>
<td></td>
</tr>
<tr>
<td>• Some college education</td>
<td>31 (88.6)</td>
<td>23 (85.2)</td>
<td></td>
</tr>
<tr>
<td>• Degree from 4-year college or more</td>
<td>2 (5.7)</td>
<td>0 (0.0)</td>
<td></td>
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<tr>
<td>Psychiatric diagnosis, n (%)&lt;sup&gt;a&lt;/sup&gt;(Present; past)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Alcohol abuse</td>
<td>2 (5.7); 4 (11.4)</td>
<td>0 (0.0); 0 (0.0)</td>
<td>ns&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>• Anxiety disorders</td>
<td>5 (14.3); 5 (14.3)</td>
<td>3 (11.1); 4 (14.8)</td>
<td></td>
</tr>
<tr>
<td>• Mood disorders</td>
<td>2 (5.7); 8 (22.9)</td>
<td>2 (7.4); 6 (22.2)</td>
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<tr>
<td>• Personality disorders</td>
<td>3 (8.6); 0 (0.0)</td>
<td>2 (7.4); 0 (0.0)</td>
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<tr>
<td>• Eating disorders</td>
<td>0 (0.0); 3 (8.6)</td>
<td>0 (0.0); 2 (7.4)</td>
<td></td>
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</tbody>
</table>

Note: AUDIT-C = Alcohol Use Disorders Identification test, the first three questions, H.S. = high school, SD = Standard deviation
<sup>a</sup>Chi-squared test, <sup>b</sup>2-tailed independent samples t-test, <sup>c</sup>Mann-Whitney U-test, <sup>d</sup>Median (25<sup>th</sup> – 75<sup>th</sup> percentile), <sup>e</sup>Reported use of more than 6 times during lifetime, <sup>f</sup>When comparing the number of participants with at least one diagnosis of any of the diagnostic groups between the alcohol users and abstaining controls
## Table 2. Areas of reduced grey matter in heavy drinking vs. light drinking participants in voxel-based morphometry analysis

<table>
<thead>
<tr>
<th>Anatomical area</th>
<th>Coordinates (MNI)</th>
<th>Cluster size (voxels)</th>
<th>Peak T-value</th>
<th>Coordinates (MNI)</th>
<th>Cluster size (voxels)</th>
<th>Peak T-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heavy drinking vs. light-drinking participants</td>
<td>Negative correlation between GM volume and AUDIT-C score</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>subgenual and dorsal ACC, BA 25 and 32</td>
<td>2 42 16</td>
<td>2718</td>
<td>4.53</td>
<td>5 20 -9</td>
<td>709</td>
<td>4.34</td>
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<td></td>
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<td></td>
<td>2 44 16</td>
<td>4.30</td>
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<tr>
<td>dorsolateral PFC, BA 8</td>
<td>29 21 49</td>
<td>265</td>
<td>4.64</td>
<td>29 23 49</td>
<td>161</td>
<td>4.23</td>
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<tr>
<td></td>
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<td>4.50</td>
<td>27 23 48</td>
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<tr>
<td></td>
<td>0 15 51</td>
<td>110</td>
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<tr>
<td>dorsomedial PFC, BA 9</td>
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<td>superior frontal gyrus, supplementary motor area and prefrontal cortex, BA 6</td>
<td>-2 -12 66</td>
<td>353</td>
<td>4.84</td>
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Note: ACC = anterior cingulate cortex, BA = Brodmann area, PFC = prefrontal cortex
Figure 1. AUDIT-C scores of the participants at three time points, each line representing one participant. The lower set of lines depicts the light drinking participants, and the upper set of lines depicts the heavy drinking participants.
Fig 2. Areas of reduced grey matter in heavy-drinking vs. light-drinking participants in voxel-based morphometry analysis, indicated by yellow color. The upper row indicates the results in anterior cingulate ROI and superior frontal gyrus ROI, whereas the lower row indicates the results in superior temporal gyrus ROI and insular ROI.