European Archives of Psychiatry and Clinical Neuroscience (2019) 269:285–294
https://doi.org/10.1007/s00406-018-0870-x

ORIGINAL PAPER

Grey matter structural differences in alcohol-dependent individuals with and without comorbid depression/anxiety—an MRI study

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Received: 14 March 2017 / Accepted: 15 January 2018 / Published online: 25 January 2018

Abstract

Although depression and anxiety disorders are common comorbid conditions in alcohol dependence, few structural brain imaging studies have compared alcohol-dependent subjects with and without such comorbidity. In the current study, brain scans of 35 alcohol-dependent with and 40 individuals without diagnosis of a comorbid ICD-10 depressive or anxiety disorder receiving detoxification inpatient treatment were evaluated. Thickness and volumes of automatically segmented neuroanatomical structures were measured in FreeSurfer. Furthermore, associations of brain structure with biological markers and clinical severity markers of alcohol dependence were assessed. Despite comparable addiction severity, the non-comorbid group had evidence of higher cytotoxic effects of alcohol use on hepatic and haematological markers, and showed significantly smaller volumes of total cerebral, and cerebellar grey matter. Similarly, they showed unexpected smaller hippocampal and nucleus accumbens volumes, and thinner frontal, temporal and occipital cortices. Smaller brain volumes correlated with increased markers of hepatic and haematological dysfunction, and with longer duration of alcohol dependence in the non-comorbid group. Evidence of higher biomarkers of alcohol use may be indicative of more severe alcohol dependence or higher vulnerability to ethanol toxicity in this group. Furthermore, psychopathology-related drug treatment, which occurred in 53% of the comorbid group over the recent years, or tissue inflammation may have a moderate effect on the grade of cerebral atrophy in alcohol-dependent patients. Longitudinal studies are needed to investigate this issue more fully.

Keywords Cortex · Subcortical volumes · Alcoholism · Liver enzymes · Erythrocyte measures

Introduction

Alcohol consumption, abuse and addiction are major health problems in European countries [1–3]. Harmful effects on the peripheral and central nervous system (CNS) have been reported in individuals with alcohol use disorders (AUD) [4] and associated brain atrophy has been described in numerous research studies [e.g. 5–9]. Structural CNS alterations observed in AUD patients include both white and grey matter in cortical and subcortical areas, comprising the frontal and temporal cortex, hippocampal–amygdala complex, cerebellum, insula and brainstem [e.g., 10–16]. Two recent meta-analyses identified smaller grey matter volumes most consistently in prefrontal cortical regions, the anterior cingulate, striatum, and insular cortex in AUD [17, 18]. Such brain atrophy has been correlated with lifetime duration and the amount of alcohol use [18, 19].

Furthermore, psychiatric comorbid conditions are a common phenomenon in AUD, also during short- and long-term abstinence [20, 21]. They have been reported

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00406-018-0870-x) contains supplementary material, which is available to authorized users.

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in 37% of AUD subjects [22] and up to 53.1% in alcohol-dependent subjects [23]. Among these, affective and anxiety disorders had by far the highest prevalence, with rates of 13.4–18.9 and 17.1–19.4% in AUD, respectively [22, 24]. Vice versa, alcohol misuse and addiction can be expected in more than a third of individuals with affective and anxiety disorders over the lifespan [22]. Altogether, with an existing AUD, or an affective or anxiety disorder, the risk of suffering from the other in the future appears to be increased from two to fourfold [25]. Different theories have emerged for this co-occurrence, including neurobiological and genetic influences as well as self-medication [22, 26, 27].

Despite the existence of neuronal network hypotheses on affective and anxiety disorders, structural imaging data in these disorders are still inconclusive and, in part, conflicting. In depression, three meta-analyses [28–30] reported smaller hippocampal volumes, in addition to smaller volumes in frontal areas of the brain, the striatum and the amygdala area [29, 31; for review, see 31]. Other studies found widespread brain atrophy in depressed patients in neo-cortical regions, whereas limbic damages could not be demonstrated [32]. Regarding anxiety disorders, recent meta-analytical studies and reviews on cortical thickness and volumes, especially in fronto-striato-thalamic circuits, have reported mixed findings of grey matter changes in social anxiety [33], panic disorder [34], and obsessive–compulsive disorder [35, 36].

Evidence from these neuroimaging studies suggests damage in similar structures in the corticolimbic and corticostriatal networks of the brain in affective and anxiety disorders and AUD [17, 18, 29, 33, 34], potentially explaining the concomitance of these disorders. These neurofunctional networks are associated with reward, behavioural inhibition, cognitive skills, motivation and emotional processing [37]. Disturbances in these integrative functions compromise the execution of appropriate behavioural responses to external and internal stimuli [14, 29]. Patients with a dual diagnosis of alcohol dependence and affective/anxiety disorder may exhibit amplified structural damage in these networks. For example, the central amygdala has been suggested to be an integrative structure for anxiety and AUD [38]. To date, there is a paucity of studies investigating brain structure of AUD subjects with and without comorbid depression and anxiety. In post-mortem brains, it has been shown that AUD patients with comorbid depressive symptoms showed reduced glial cell density in the dorsolateral prefrontal cortex compared to AUD without comorbidity and control subjects [39]. A neuroimaging study reported that alcohol-dependent subjects with psychiatric comorbidity, including mood, anxiety and externalising disorders, displayed significantly smaller volumes in distinct subcortical brain areas (hippocampus, amygdala and nucleus accumbens), compared to those without [40]. However, the two groups that did not differ in cranium size adjusted cortical grey matter volumes [41].

In the current literature, there is an additional lack of comparative data on the association of atrophy with hepatic or haematological markers in patients with and without comorbid depression/anxiety. It has been postulated that alcohol-induced neurodegeneration occurs during intoxication and is related to the neurotoxic effects of oxidative stress and pro-inflammatory proteins [42, 43; for a review, see 43]. Oxidative stress, in turn, has been associated with impaired liver function, which can complicate AUD associated brain atrophy [44]. One sensitive marker is the hepatic enzyme gamma-glutamyltransferase (GGT). Furthermore, increased levels of red blood cell indices, such as mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH), mark a limited oxygen transport to the brain. Elevated levels of such serum markers have been shown to relate to smaller grey matter volumes in AUD individuals [45, 46].

The present study was performed to test the hypothesis that alcohol-dependent patients with lifetime affective or/and anxiety disorder show lower cortical thickness in addition to smaller subcortical volumes in frontolimbic and frontostriatal networks, particularly the anterior cingulate, prefrontal cortex, striatum, and the hippocampal–amygdala complex, compared to alcohol-dependent subjects without such comorbidity. We further tested if this hypothesis is extendible to that AUD patients with comorbid depression/anxiety to find out if they have greater diffuse damage in the cortex of both hemispheres. We also investigated whether brain atrophy in both groups was associated with stronger deviations of AUD biological markers (hepatic and haematological) and with greater addiction severity, as indicated by longer duration of alcohol addiction and higher amount of alcohol intake prior to the respective detoxification treatment.

Methods

This study complies with the Declaration of Helsinki (2013) and was approved by the ethics committee of the University of Goettingen, Germany.

Patients

We included patients who had received inpatient treatment for alcohol dependence (ICD-10 F10.2, [47]) in the department of psychiatry and psychotherapy of the University of Goettingen between 2008 and 2012 and underwent a 3 T cranial MRI scan during the stay.

Of all 514 patients receiving inpatient treatment for alcohol dependence, 256 patients were excluded, because no MRI was done. Fifty-nine subjects received a 1.5 T MRI

and 74 had significantly altered scan parameters that would have made comparisons too imprecise and therefore, were not included.

Fifty patients were rejected from further investigation because of other comorbidities violating the protocol, including patients with an additional diagnosis of organic brain disorder or dementia (F0.x), hepatic encephalopathy (F10.5), epilepsy (G40.x), schizophrenia or related disorders (F20–29), and dependence on substances other than alcohol, except for tobacco (F17).

Seventy-five patients were finally included in the study. Two subgroups were established according to an additional diagnosis of either depressive disorder (ICD-10 F31.3-5; F32.0-9; F33.0-9; F43.2) and/or anxiety disorder (ICD-10 F40.x and 41.x), or no comorbidity. Of these 75 patients, 40 (53.3%) had the diagnosis of alcohol dependence (F10.2) without comorbidity (group AUD−) and 35 (46.7%) with affective and/or anxiety comorbidity (group AUD+). In the AUD+ group, 29 (82.9%) patients suffered from depressive disorder, 6 (17.1%) from adjustment disorder/ depressive episode, and 5 (14.3%) from an anxiety disorder. All patients with an anxiety disorder also suffered from comorbid depression. Of the five patients with an anxiety disorder, two had panic disorder, two had a social phobia and one specific phobia. There were five patients with an additional diagnosis of PTSD in the AUD+ group.

Demographic, clinical and biological variables

The following variables were extracted from the chart of each included individual: gender, age at the time of the MRI scan, duration of alcohol addiction in years, average alcohol consumption over the 3 months prior to admission in grams per day (*E* in g/day), number of lifetime alcohol detoxification treatments, comorbid psychiatric diagnoses, prescribed psychotropic medication over 12 months prior to admission, serum markers comprising liver function index gamma-glutamyltransferase (GGT; Goettingen reference values 12–64 U/l), and erythrocyte indices mean corpuscular volume (MCV; Goettingen reference values 81–95 fl) and mean corpuscular haemoglobin (MCH; Goettingen reference values 26–32 pg).

MRI scans

All included subjects received their 3 T MRI on the same device (Siemens Magnetom Trio) at the University of Goettingen. We acquired whole-brain scans using a sagittal T1-weighted MPRAGE (magnetization prepared rapid gradient echo) pulse sequence for efficient grey/white matter contrasts. Scan parameters were: repetition time (TR) = 2.0 or 2.3 s, echo time (TE) = 2.98 ms, voxel size = 1 × 1 × 1 mm, field of view (FOV) = 240 × 256 mm, flip angle = 9°, slice thickness = 1.1 mm, number of slices = 160. From the patients who underwent more than one eligible 3 T MRI scan over the investigation period, the most recent one was analysed. Data was evaluated for technical artefacts and structural abnormalities by an independent neuroradiologist.

MRI analysis

For the determination of morphometric group differences, images were analysed in FreeSurfer (version 5.1.0). The pipelines for automated analysis of cortical thickness and subcortical volumes have been described elsewhere [48, 49]. Thirty-four cortical regions of interest (ROI) were computed bilaterally [50]. Cortical thickness was calculated as the closest distance from the grey/white matter boundary to the grey matter/CSF boundary at each vertex on the tessellated surface [48]. Cortical thickness maps were smoothed at full width half-maximum (FWHM) at 10 mm.

Volumes of the following bilateral subcortical structures were extracted: caudate, putamen, pallidum, hippocampus, amygdala, thalamus, and nucleus accumbens. Additionally, measures of bilateral cerebral and cerebellar grey matter volumes were compared between the groups as described below.

Statistics

The statistical evaluation of demographic, clinical and biological data was done in IBM SPSS Statistics. Groups were compared using the independent *t* test or Mann–Whitney *U* test for interval variables (dependent on data distribution), and Fisher's exact test for categorical variables (see Table 1). The level of significance was set at $p \leq 0.05$.

FreeSurfer output of volumetric brain measures was also exported into SPSS. Based on multicollinearity between the overall grey matter volumes in cortex and cerebellum, a separate general linear model (GLM) was fit for each volumetric measure in both hemispheres as dependent variable, with patient group and gender as fixed factors, age (demeaned) and ICV as covariates, and a group-by-gender interaction term. To correct for multiple comparisons, false discovery rate (FDR) controlling procedures were applied [51]. Subcortical structures were analysed using a multivariate analysis of covariance (MANCOVA), including patient group and gender as fixed factors, age (demeaned) and intracranial volume (ICV) as covariates, and a group-by-gender interaction term. Volumes of the seven bilateral subcortical regions, including the caudate, putamen, pallidum, hippocampus, amygdala, thalamus, and nucleus accumbens, were included as dependent variables. Following a statistically significant multivariate test, we ran FDR adjusted univariate analyses of covariance for each structure. To assess potential confounding effects of drug treatment on brain volumes and biological

Table 1 Demographic, clinical and biological variables

	Group AUD− ($n=40$)	Group AUD+ ($n=35$)	Group differences
Gender, m/f	31/9	20/15	Fisher's exact $p=0.083$
Age in years	49 (24–69)	52 (24–73)	$t(73)=-1.1$, $p=0.289$
Illness duration in years	20 (1–50)	18 (2–41)	$U=208.5$, $p=0.772$
Alcohol amount in g/day	127 (42–429)	156 (18–336)	$U=339.5$, $p=0.105$
Number of treatments	1.5 (1–30)	2 (1–11)	$U=578.0$, $p=0.546$
GGT in U/l	142 (10–1340)	56 (12–406)	$U=444.5$, $p=0.007$
MCV in fl	97.4 (77–112)	94 (79–106)	$t(73)=2.3$, $p=0.023$
MCH in pg	32.8 (24–43)	31.7 (25–43)	$U=467.5$, $p=0.014$
Antidepressants, past year	39 no, 1 yes	14 no, 16 yes	Fisher's exact $p<0.001$

Groups: AUD−, non-comorbid alcohol-dependent individuals; AUD+, alcohol-dependent individuals with comorbid affective/anxiety disorder. All continuous variables are reported as median (range)

f female, *m* male, *GGT* gamma-glutamyl transferase, *MCV* mean corpuscular volume, *MCH* mean corpuscular haemoglobin

markers, we split the AUD+ group into patients who did ($n=16$) or did not ($n=14$) receive antidepressant drugs and reran the GLMs as specified above. An additional model was computed (without ICV) including the three biological markers, gamma-glutamyltransferase, mean corpuscular volume and mean corpuscular haemoglobin, as dependent variables.

Statistical group comparisons of cortical thickness data were performed with FreeSurfer's program QDEC, with age (demeaned) and gender included in the GLM. Statistical difference maps were corrected for multiple testing with FDR at $p \leq 0.05$.

Only brain measures that showed significant between-group differences were further examined for their relationship with biological (hepatic/haematological) markers, duration of illness, and average alcohol consumption among both patient groups. To achieve normal distribution, GGT data and overall cortical volumes of both hemispheres were log transformed. As normal data distribution is required for partial correlations, but transformation did not accommodate the following outliers, one data point was removed in average alcohol consumption in the AUD− group [characteristics: 52 years, male, $E=429$ g/day, deviation from mean by 3.7 × standard deviation (SD)], and one MCH outlier was removed from each patient group (AUD−: 52 years, male, MCH = 43 pg, deviation from mean by 3.4 × SD; AUD+: 62 years, female, MCH = 43.2 pg, deviation from mean by 4.1 × SD). Each outlier detection was confirmed by a median absolute deviation above threshold 3, as data were not normally distributed. Grey matter volumetric measures were regressed against ICV. Volumetric residuals and mean values of significant cortical thickness ROIs were entered into partial correlations, adjusted for age and gender (for correlations with duration of alcohol dependence, age was omitted). FDR controlling procedures were applied for each set of multiple comparisons [51].

Results

Demographic, clinical and biological variables

Table 1 presents an overview of demographic, clinical and biological variables in both study groups as well as their statistical comparison between groups. In the entire sample, mean duration of alcohol addiction was 19.3 years (SD = 13), in females 16 years (SD = 10, range 2–31) and in males 19 years (SD = 14, range 1–50). Of all patients, 44% underwent inpatient detoxification treatment for the first time. Twenty patients had one previous treatment, 16 patients had two to seven treatments, and two more than 10 (data were not available for 2 participants in each group). Thirty-eight of all subjects showed elevated gamma-glutamyltransferase (GGT) serum levels on admission exceeding 96 U/l, which is representative of values at least 50% over the upper reference value (64 U/l) of the Goettingen central laboratory, indicating abnormal liver function. Thirty-four subjects showed normal mean corpuscular volume (MCV), while 41 participants had a macrocytosis (MCV > 95 fl). Forty-nine of all subjects had normal mean corpuscular haemoglobin (MCH) serum levels, while 26 had elevated values (MCH > 33 pg).

In group AUD−, one subject received antidepressant drug treatment during a 12-month period prior to admission for reasons other than a depressive episode or an anxiety disorder. In group AUD+, 16 patients had antidepressant drug treatment over the past year (which was either SSNRI or SSRI treatment, exclusively) and 14 did not have any respective treatment, while this information was not recorded for five patients. When comparing AUD+ patients with and without antidepressant drug treatment in the past year, no significant group differences could be detected in total cortical grey matter volumes [left: $F(1, 24)=0.033$, $p=0.858$; right: $F(1, 24)=0.121$, $p=0.772$], cerebellar

grey matter volumes [left: $F(1, 24) = 0.001$, $p = 0.981$; right: $F(1, 24) = 0.037$, $p = 0.850$], the seven subcortical structures [Pillai's trace = 0.451, $F(14, 11) = 0.646$, $p = 0.781$], or biological markers [Pillai's trace = 0.104, $F(3, 22) = 0.851$, $p = 0.481$].

Morphometric data

Group comparisons of cortical thickness

Compared to group AUD−, significantly higher cortical thickness was detected in five clusters of the left hemisphere in group AUD+. Coordinates, size, z-statistic, and brain region of each cluster are provided in Table 2.

Group comparisons of overall grey matter and subcortical volumes

Group AUD+, compared to group AUD−, had significantly larger overall grey matter volumes in cortex [left: $F(1, 69) = 5.898$, $p = 0.018$; right: $F(1, 69) = 6.074$, $p = 0.016$] and cerebellum [left: $F(1, 69) = 10.010$, $p = 0.002$; right: $F(1, 69) = 10.084$, $p = 0.002$], (Table 3). A GLM including the seven bilateral subcortical structures found significant larger volumes in group AUD+ relative to group AUD− [Pillai's

Table 2 Clusters of greater cortical thickness in alcohol dependence with comorbid depression and/or anxiety compared to non-comorbid group

Cortex region	Talairach coordinates			Size (mm^2)	Number of vertices	*z* value	*p* value
	x	*y*	*z*				
Lateral occipital	−41	−82	−14	188	264	6.20	< 0.00001
	−22	−82	−8	84	86	4.07	< 0.00005
Medial orbitofrontal	−9	20	−18	38	76	4.71	< 0.00001
Middle temporal	−60	−19	−21	51	86	4.51	< 0.00001
Isthmus cingulate	−11	−51	29	9	22	4.03	< 0.0001

All significant clusters are located in the left hemisphere and are FDR-corrected. Cortical thickness is adjusted for age (demeaned) and gender

Table 3 Estimated marginal mean and group differences in overall cortical and cerebellar grey matter and subcortical structure volumes

Region	Hemisphere	Group AUD−		Group AUD+		Group differences			
		M	SE	*M*	SE	% diff	*p*	*q*	Cohen's *d*
Cortical grey	L	212,261	2711	220,915	2344	**4.1**	**0.018**	**0.018**	**0.56**
	R	212,947	2780	221,953	2403	**4.2**	**0.016**	**0.018**	**0.57**
Cerebellar grey	L	44,058	1028	48,333	889	**9.7**	**0.002**	**0.005**	**0.73**
	R	45,138	1047	49,509	905	**9.7**	**0.002**	**0.005**	**0.73**
Thalamus	L	5958	102	6147	88	3.2	0.163	0.285	0.32
	R	5998	107	6244	93	4.1	0.085	0.170	0.40
Caudate	L	3348	83	3588	72	7.2	0.031	0.106	0.51
	R	3548	81	3641	70	2.6	0.384	0.490	0.20
Putamen	L	5017	135	5377	117	7.2	0.045	0.106	0.47
	R	4867	140	4995	121	2.6	0.490	0.490	0.16
Pallidum	L	1491	40	1599	35	7.2	0.042	0.106	0.47
	R	1428	30	1457	26	2.0	0.461	0.490	0.17
Hippocampus	L	3363	84	3696	73	**9.9**	**0.004**	**0.024**	**0.69**
	R	3447	97	3847	84	**11.6**	**0.003**	**0.024**	**0.72**
Amygdala	L	1533	46	1579	40	3.0	0.449	0.490	0.18
	R	1582	48	1627	42	2.8	0.486	0.490	0.16
No. of accumbens	L	432	20	509	18	**17.8**	**0.005**	**0.024**	**0.66**
	R	560	18	586	16	4.6	0.280	0.436	0.25

Grey matter volumes are provided in mm^3, adjusted for age (demeaned), gender and intracranial volume. Groups: AUD−, non-comorbid alcohol-dependent individuals; AUD+, alcohol-dependent individuals with comorbid affective/anxiety disorder. Highlighted in bold are statistically significant results

M estimated marginal mean, *SE* standard error; *% diff.* volume differences in percent, *q* FDR adjusted *p* value, *L* left, *R* right

trace = 0.348, $F(14, 56) = 2.134$, $p = 0.023$], particularly in the left nucleus accumbens and bilateral hippocampus (Table 3).

Correlation analyses of morphometric data with clinical and biological variables

Statistical data for all correlational analyses are given in Online Resource 1.

Duration of alcohol dependence and cortical and subcortical measures

For group AUD− exclusively, negative correlations between brain volumes and duration of addiction were found for the bilateral cerebellum (left: $r = -0.606$, $p = 0.005$; right: $r = -0.625$, $p = 0.003$). This relationship was also found for the nucleus accumbens ($r = -0.503$, $p = 0.024$) and hippocampi (left: $r = -0.569$, $p = 0.009$; right: $r = -0.530$, $p = 0.016$).

Average daily consumption and cortical and subcortical measures

No significant correlations between average daily alcohol intake 3 months prior to admission and morphometric data could be observed.

Gamma-glutamyltransferase (GGT) and cortical and subcortical measures

In group AUD−, significant negative correlations between GGT levels and volumetric data were found for left hemisphere ($r = -0.526$, $p < 0.0001$; Fig. 1a) and right hemisphere ($r = -0.506$, $p = 0.001$; Fig. 1b) cortical volume. Correlations with individual subcortical structures did not survive FDR correction. In group AUD+, GGT levels showed a significant negative relationship with cerebellar volumes of the left ($r = -0.392$, $p = 0.012$; Fig. 1c) and right hemisphere ($r = -0.421$, $p = 0.007$; Fig. 1d).

Mean corpuscular volume (MCV) and cortical and subcortical measures

In group AUD−, MCV showed a negative correlation with mean thickness of the isthmus cingulate cortex ($r = -0.338$, $p = 0.019$) and the lateral occipital cortex ($r = -0.390$, $p = 0.008$) of the left hemisphere.

Mean corpuscular haemoglobin (MCH) and cortical and subcortical measures

In group AUD−, MCH showed a significant negative correlation with left hemisphere ($r = -0.381$, $p = 0.010$) and right hemisphere ($r = -0.363$, $p = 0.014$) cortical volume (see Fig. 2).

Discussion

Our main finding was unexpected; AUD patients with comorbid depression and/or anxiety (AUD+) showed larger volumes in bilateral cerebral and cerebellar grey matter, relative to non-comorbid patients (AUD−). Furthermore, they showed larger volumes in specific subcortical structures such as the left nucleus accumbens and the bilateral hippocampus. Similarly, the left hemisphere isthmus cingulate, lateral occipital, medial orbitofrontal, and middle temporal cortices showed clusters of greater thickness in the AUD+ group. Grey matter measures were negatively associated with duration of alcohol dependence, and levels of hepatic and haematological markers in the AUD− group. In AUD+ patients, such relationships could only be shown between GGT and cerebellar volumes.

These results reject our initial study hypothesis that comorbid alcohol addiction and affective/anxiety pathology would result in cumulative effects on smaller overall grey matter and subcortical volumes and lower cortical thickness in the corticolimbic and corticostriatal networks. Only two other neuroimaging studies compared alcohol-dependent individuals with and without a lifetime diagnosis of anxiety, mood or externalising disorders and reported smaller hippocampus, amygdala and nucleus accumbens volumes in comorbid patients, but no cortical volume differences [40, 41]. Notably, both studies assessed the same cohort of long-term abstinent alcoholics (mean = 6.3 years of abstinence) and suggested a recovery of subcortical brain volumes with sustained abstinence. Participants of the present study were actively enrolled in a detoxification treatment at the time of assessment and have abstained from alcohol for only a short term.

One possible explanation is that AUD− subjects are more vulnerable for neuronal or glial loss, while comorbid depressive and/or anxiety disorder might have a moderating effect on alcohol-related atrophy. Acute detoxification effects, including increased cell proliferation and neurogenesis in alcohol-affected and projection areas [43], might, therefore, be more pronounced in the AUD+ group. The observed group differences were most striking in corticolimbic brain areas involved in the processing of motor functions, memory, and reward; areas that animal models previously identified as most sensitive to alcohol degeneration [43].

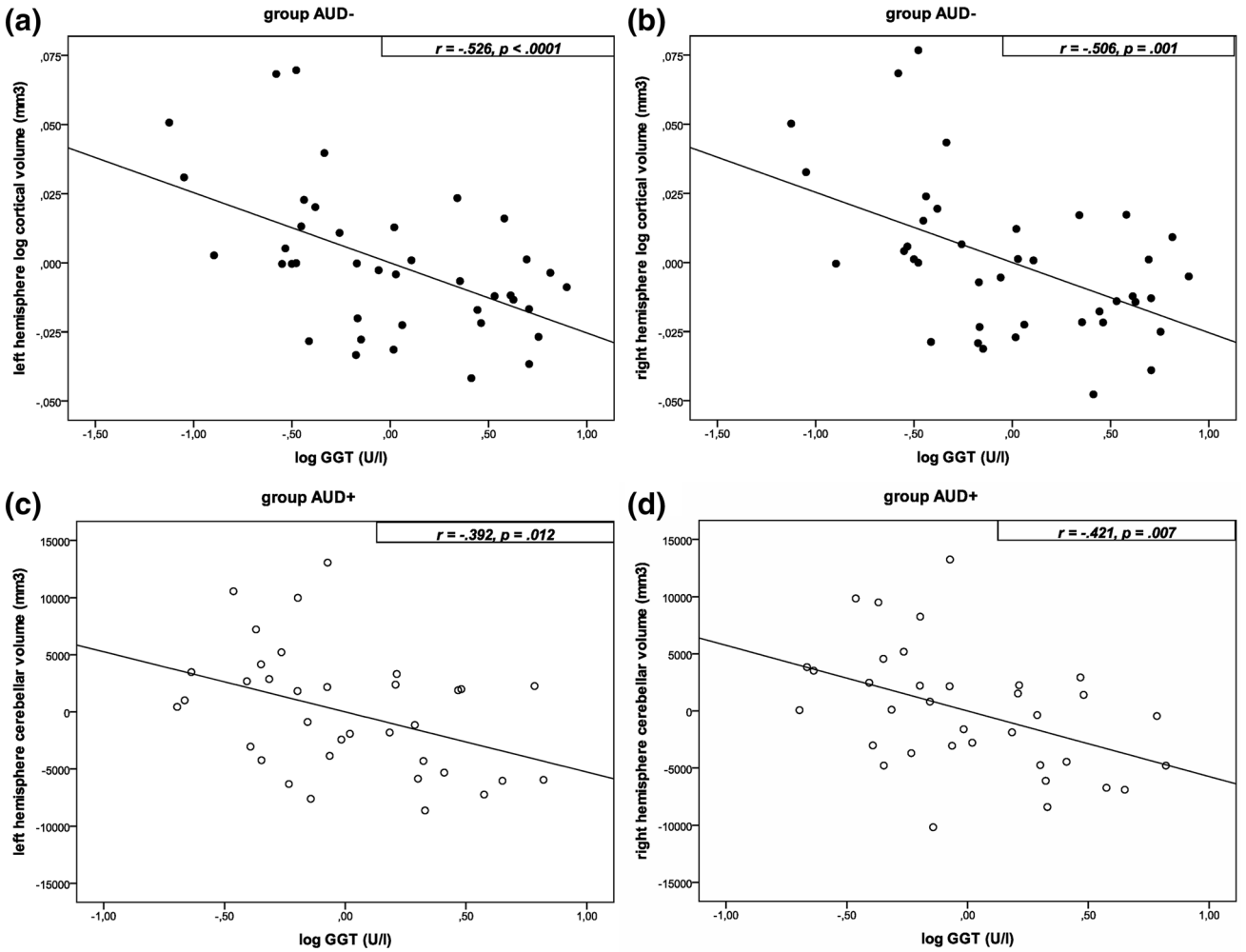

Fig. 1 Partial correlation between gamma-glutamyl transferase (GGT) and cortical volumes of **a** the left hemisphere and **b** right hemisphere in non-comorbid alcohol-dependent individuals (AUD−). GGT relationship with cerebellar volume of **c** the left hemisphere and **d** right hemisphere in alcohol-dependent individuals with comorbid depression and/or anxiety (AUD+). Correlations are adjusted for ICV, age and gender

Meanwhile, these brain areas, including the subcortical structures as well as orbitofrontal, cingulate and temporal cortices, are also major role players in depressive and anxiety disorders [28–36]. Hence, an alternative explanation may be that psychopathology-associated inflammatory processes in the AUD+ group result in grey matter volume gain and thereby mask alcohol-related neurodegeneration. Major depression has been discussed as a disorder of glial function and inflammation, and a swelling of astrocytes during depressive episodes has been described [52]. Central nervous pro-inflammatory effects and increased markers for oxidative stress have been found in both major depression [53] and AUD withdrawal [54]. However, a subgroup with depression and/or anxiety but without AUD has not been investigated and it cannot be clearly determined which phenomena have distinctively influenced our results.

Despite comparable clinical markers of addiction severity in both groups, participants in the AUD− group showed higher levels of investigated biological markers GGT, MCV and MCH, than the AUD+ group (see Table 1). In group AUD−, median GGT exceeded not only normal levels but also the cut-off point indicative of abnormal liver function by far. Yet, 46% of AUD+ patients (compared to 73% in group AUD−) showed GGT levels above normal, as defined by the referred central laboratory in Goettingen. Both alcohol-dependent groups showed a significant relationship between impaired liver function and smaller global grey matter volumes, specifically in the cortex in AUD− and the cerebellum in AUD+. This is in line with previous reports of GGT levels correlating with brain shrinkage in alcohol dependence, potentially due to a multifactorial increase in oxidative stress on brain cells [45, 46].

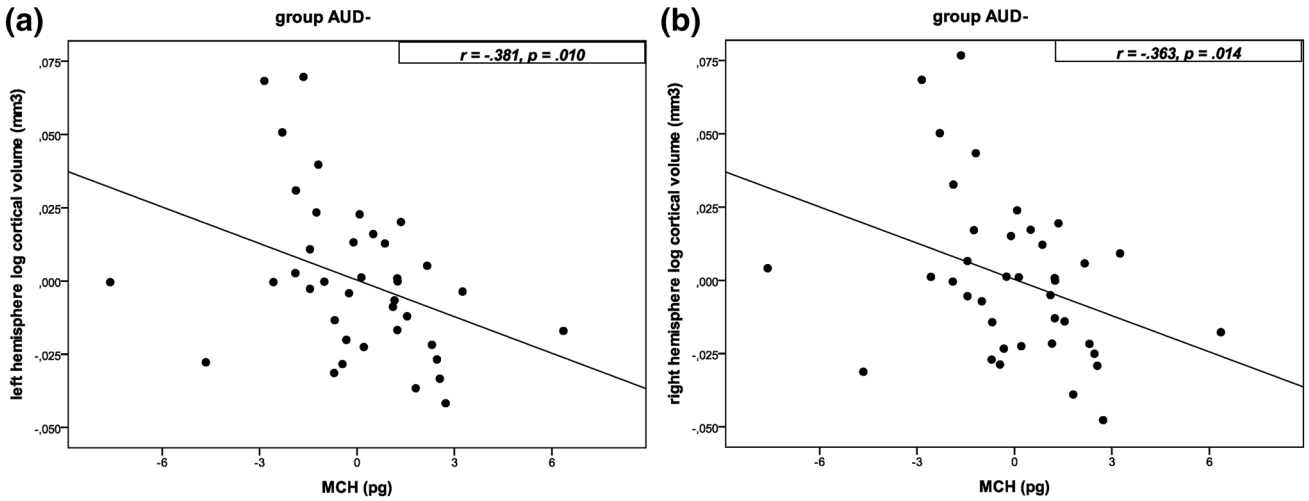

Fig. 2 Partial correlation between mean corpuscular haemoglobin (MCH) and cortical volumes of **a** the left hemisphere and **b** right hemisphere in non-comorbid alcohol-dependent individuals (AUD−), adjusted for ICV, age and gender

A significant negative relationship of red blood cell indices, MCV and MCH, with cortical thickness or volume was only found in the AUD− group. Elevated MCV levels together with increased amounts of haemoglobin per cell (MCH) may be indicators of macrocytic anaemia. Macrocytic anaemia can be caused by low levels of vitamin B12 and folic acid, both linked to chronic alcohol intake [55]. However, the AUD+ group of this study did not display such a relationship between red blood cell indices and brain atrophy. One possible explanation is that this patient group's nutritional status might differ from the AUD− group and therefore, prevent further alcohol-associated brain atrophy. But, this remains speculative as potential group differences in nutrition were not measured.

Besides the general impression that toxic effects by ethanol (on liver function, red blood cell indices, and grey matter) were significantly less pronounced in the AUD+ group, it appears of interest that no significant correlation existed between addiction severity and brain structure in these subjects. The less pronounced atrophy in AUD+ could possibly be related to the significantly higher prevalence of antidepressant drug treatment within the year prior to admission and the associated potential neuroprotective and neuroproliferative effects on astrocytes [52, 56, 57]. However, when comparing patients with psychiatric comorbidities who did or did not receive antidepressant drugs, no significant differences in overall brain volumetric measures, subcortical structure volumes, or serum markers were found. But, despite this lack of statistical significance, effects of medication cannot be ruled out completely [58]. Furthermore, due to the small subgroup ($n_{\text{treatment}} = 16$), a relationship to certain types of antidepressant drugs could not be drawn. Nor could a dose correlation be established due to the lack of information on dosage. However, it was not an objective of this study to elucidate the hypothesis that antidepressant drug treatment goes in line with less brain atrophy in alcohol-dependent subjects.

Further limitations should be emphasized. Cigarette smoking status was not known for our patients and therefore, possible confounding effects of nicotine on cortical thickness [59], subcortical volumes [60], and serum markers [46] could not be delineated. As measures of addiction severity are based on self-reports, these measures might be under or overestimated unequally in both study groups. AUD+ might have overestimated alcohol consumption, justifying their need for treatment [61]. Previous research has shown that comorbidity leads to increased use of treatment services in AUD [22]. Alternatively, the AUD− group might have been minimizing heavy drinking. There is some evidence for influencing factors, such as physical state, motivation, denial, cognitive deficits, potentially leading to a consumption underestimation [62]. Yet, other studies support the validity of self-report data regarding alcohol use [63]. Severity measures of comorbid depression and/or anxiety were not available, yet such variables may have differentiated results in AUD+ in more detail. Finally, a potential time lag between MRI scanning and laboratory tests may be of relevance. Usually, there was a delay of approximately 1 week between the two, so that correlations between atrophy and serum markers are not perfectly matched. Nonetheless, the assessment of a purely alcohol-dependent sample without further comorbid illicit substance use disorders is a strength of the present study.

In conclusion, alcohol-dependent subjects with a depressive and/or anxiety disorder showed greater cortical and subcortical grey matter volumes/thickness and lower levels

of distinct serum markers, compared to non-comorbid alcohol-dependent individuals. These findings and the lack of correlations with severity markers of alcohol addiction in the AUD+ group hint to a moderating effect of such comorbidity, which might be related to antidepressant drug treatment. Alternatively, greater grey matter volumes/thickness in AUD+ may result from inflammatory phenomena and astrogliosis in brain areas prone to structure changes in affective and anxiety disorders. Based on our haematological and hepatic findings, it further appears that alcohol-related tissue degeneration and consequent diminished oxygen transport to the brain can aggravate AUD effects on grey matter integrity. Further longitudinal work is needed to determine causal associations between alcohol dependence, depressive/anxiety disorders and brain atrophy over the course of disorder, treatment regimens and during abstinence.

Acknowledgements This collaboration and research project was supported by the EUSARNAD program of the "European Commission 7th Framework Program" (Marie Curie International Research Staff Exchange Scheme Program).

Compliance with ethical standards

Conflict of interest D. J. Stein has received research grants and/or consultancy honoraria from Abbott, ABMRF, Astrazeneca, Biocodex, Eli-Lilly, GlaxoSmithKline, Jazz Pharmaceuticals, Johnson & Johnson, Lundbeck, National Responsible Gambling Foundation, Novartis, Orion, Pfizer, Pharmacia, Roche, Servier, Solvay, Sumitomo, Sun, Takeda, Tikvah, and Wyeth. U. Havemann-Reinecke has received research grants and/or consultancy honoraria from CNMPB (Centre of nanomicroscopy and molecular biology of the Brain of the DFG, German research Federation), Astra Zeneca, Lundbeck, Lilly, Janssen. The other authors declare no conflict of interest with respect to subjects of the paper.

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