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HIPPOCAMPAL VOLUME LOSS IN PATIENTS WITH ALCOHOLISM IS INFLUENCED BY THE CONSUMED TYPE OF ALCOHOLIC BEVERAGE

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Abstract — Aims: The individual extent of structural brain tissue changes in patients with alcohol dependence is influenced by genetic factors, gender, age and possibly a dose/duration-effect. Aim of the present study was to investigate different types of alcoholic beverages with regard to hippocampal volume loss in patients suffering from alcoholism. **Methods:** We included 52 patients with alcohol dependence and divided them according to their preferred type of beverage consumption (beer, wine, and spirits). Hippocampal volumes were determined using volumetric high-resolution MR imaging. **Results:** There was a significant difference in hippocampal volumes between patients consuming different beverages (ANOVA: $F = 7.454$; $df = 2$; $P = 0.0015$) with the smallest volumes in the wine group, followed by the spirits group. Furthermore, patients with a preferred spirits consumption showed significantly higher plasma homocysteine levels (ANOVA: $F = 3.39$; $df = 2$; $P = 0.042$). Linear regression analyses revealed an association of homocysteine and hippocampal volume only in the group of patients preferring spirits ($R^2 = 0.364$; $P = 0.008$). **Conclusions:** Homocysteine-mediated excitotoxicity may be an important pathophysiological mechanism in ethanol-related brain damage, particularly in patients consuming wine and spirits. The extent of brain atrophy in beer consuming patients seems to be more moderate.

INTRODUCTION

Excessive alcohol consumption leads to structural brain changes and impairment of cognitive function (de la Monte, 1988; Jernigan *et al*., 1991; Kril *et al*., 1997; Wilhelm *et al*., 2006). Chronic alcoholism results in global brain atrophy with typical points of predilection for neurotoxic effects (Mann *et al*., 2001): Alcohol-induced cortical and subcortical cerebral atrophy (de la Monte, 1988; Jernigan *et al*., 1991; Mann *et al*., 2001), atrophic changes in the cerebellum (Ferrer *et al*., 1984), enlargement of cerebrospinal fluid space (Fox *et al*., 1976; de la Monte, 1988), and hippocampal volume loss (Sullivan *et al*., 1995; Mann *et al*., 2001; Bleich *et al*., 2003b; Kurth *et al*., 2004) have been reported since modern imaging procedures such as magnetic resonance tomography make it possible to validate and quantify structural brain changes.

Recent research focused on pathophysiological mechanisms underlying these alcohol-induced brain tissue changes. Besides thiamine deficiency (Kril and Homewood, 1993) glutamate-mediated transmission appears to play a major role in ethanol-related brain damage (Dodd *et al*., 2000; Bleich *et al*., 2004; Harper and Matsumoto, 2005). Recently it was shown that the *N*-methyl-D-aspartate (NMDA) receptor agonist homocysteine is associated with hippocampal atrophy in patients with alcoholism (Bleich *et al*., 2003a).

Moreover, genetic factors such as apolipoprotein E genotype (Bleich *et al*., 2003c), gender (Hommer *et al*., 2001; Bleich *et al*., 2003c), age (Pfefferbaum *et al*., 1992), consumed amount of ethanol and duration of chronic alcohol consumption (Teichman *et al*., 1987) may determine the final and individual degree of alcohol-related brain damage.

The aim of this study was to investigate the influence of different types of alcoholic beverages on hippocampal volume in patients suffering from alcohol dependence.

MATERIALS AND METHODS

The present open and controlled investigation included 52 actively drinking patients (aged 29–67 years; 34 males, 18 females) with an established diagnosis of alcohol dependence according to the Diagnostic and Statistical Manual for Mental Disorders (DSM-IV) and 30 healthy controls (aged 26–64 years; 16 males, 14 females). The study was performed in the Department of Psychiatry and Psychotherapy of the University of Göttingen, Germany. The study was approved by the local Ethics Committee. Clinical diagnosis and examinations were made as described recently (Bleich *et al*., 2003c; Wilhelm *et al*., 2007). Patients were admitted for detoxification treatment and were taking no drugs before being enrolled in the study. Sociodemographic and personal data, such as known somatic illnesses, preceding withdrawals, lifetime drinking (calculated by estimating an average daily intake in kg, multiplied by 365 and by the years of drinking) in kilograms of ethanol and type of consumed alcoholic beverage were obtained in a standardized self-structured interview (Bleich *et al*., 2000b).

Patients showing signs of dementia (i.e., Korsakoff disease) or any other known illness possibly leading to brain tissue changes, and patients with any other substance abuse apart from nicotine dependence or body mass index (BMI) less than 17.5 (diagnosis of anorexia) were not included in the study. Patients with a history of repeated withdrawal episodes (>3) and/or previous withdrawal seizures were also excluded from the investigation to avoid confounding effects of repeated withdrawals on brain volume. All patients were right-handed and underwent MR imaging within 10 days after admission.

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Table 1. Demographic characteristics of the study population

	Beer Mean	SD	Wine Mean	SD	Spirits Mean	SD	Controls Mean	SD
N	19		15		18		30	
% Women[a]	21		67		22		47	
Age (years)	47.1	8.5	46.8	7.7	45.8	10.8	48.0	9.6
Right hippocampal volume (ml)	3.428	0.406	2.9	0.49	>2.987	0.471	3.936	0.408
Left hippocampal volume (ml)	3.309	0.416	2.795	0.444	2.858	0.454	3.776	0.397
Average hippocampal volume (ml)	3.368	0.393	2.848	0.462	2.922	0.455	3.856	0.358
Homocysteine (μmol)	19.1	6.4	24.5	14.4	31.1	18.9	11.6	2.6
Body mass index (kg/m^2)	24.3	3.2	24.4	2.6	25.1	4.4	23.8	2.5
Lifetime consumption of ethanol (kg)	1774.8	1894.0	800.8	530.0	1266.2	1025.4		
Years of drinking	13.3	5.1	12.6	2.9	13.3	6.5		

Demographic characteristics of the study sample; [a]percentage of female patients in the referring group of beer, wine or spirits consumers and the control group.

MR imaging volumetry

Cranial MR imagings were performed using a superconducting magnet at field strength of 1.5 T (1.5 Tesla Gyroscan ACS NT, Philips, Germany). The T1-weighted coronal images (FFE-sequence) were acquired by means of a 256 × 256 matrix with a repetition time of 24 ms and an echo time of 6 ms. Data was visualized using "Volume-Presentation-Software" on the Easy Vision Work Station.

The semiquantitative measurement of the hippocampal volumes has been described previously (Bleich *et al*., 2003c; Wilhelm *et al*., 2007). All measurements were performed by two operators independently (JW, SB) to allow for the determination of intraclass correlation (hippocampal volume: right: $r = 0.79$; left: $r = 0.86$) and interrater intraclass correlation coefficients (operator 1/operator 2; right: $r = 0.92/r = 0.90$; left $r = 0.89/r = 0.94$).

Statistical analysis

Variables did not deviate from normal distribution according to the Kolmorogov–Smirnov test. Categorical data were analyzed using χ^2 statistics. Group comparisons were performed using either *t*-tests or one-way analysis of variance (ANOVA) followed by Bonferroni's *post hoc* test. To compare the impact of different covariates and factors on hippocampal volume, we used a general linear model or multiple linear regression analyses after stratification for different factors. In these cases, R^2-values were corrected for the number of comparisons made. For all statistical tests we applied a significance level of $\alpha = 0.05$ (two-sided). All statistical analyses were done using the statistical software packages Statistical Package for the Social Sciences (SPSS) 14.0 for Windows (SPSS Inc., Chicago, IL) and GraphPad Prism 4.03 (GraphPad Software Inc., San Diego, CA).

RESULTS

The demographic characteristics of the study population are presented in Table 1.

The kind of preferred beverage had a significant impact on the plasma homocysteine levels (ANOVA: $F = 3.39$; $df = 2$; $P = 0.042$), with the highest homocysteine levels found in the group of patients consuming spirits compared to the beer-consuming group (Bonferroni's *post hoc* test: $P = 0.037$; Figure 1).

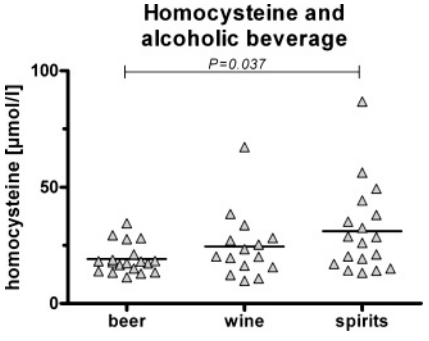

Fig. 1. Association between homocysteine and alcoholic beverages. Differences between groups tested with Bonferroni's *post hoc* test; significant difference regarding homocysteine serum levels between patients consuming beer or spirits ($P = 0.037$). Statistical details are summarized in the Results section.

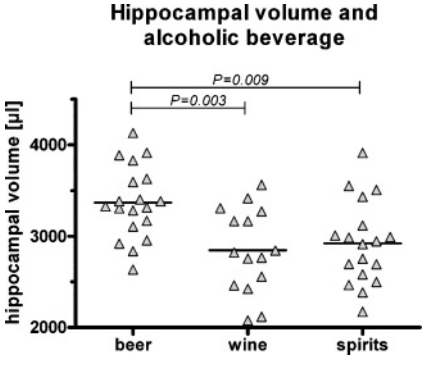

Fig. 2. Association between hippocampal volume and type of alcoholic beverage. Differences between groups tested with Bonferroni's *post hoc* test, significant difference regarding hippocampal volume between patients consuming beer or wine ($P = 0.003$) and between patients consuming beer or spirits ($P = 0.009$); no differences between wine and spirits drinkers. Statistical details are summarized in the Results section.

There was a significant difference in hippocampal volumes between patients preferring different types of beverages (ANOVA: $F = 7.454$; $df = 2$; $P = 0.0015$; *P*-values of the *post hoc* tests are provided in Figure 2) with the largest volumes in the beer group. To confirm these findings we repeated the analysis after correcting the hippocampal volume for the BMI (by division). Similar to the uncorrected data, we found a significant difference between patients preferring different

Table 2. Impact of different variables on hippocampal volume

	df	F	*P*-value	% Variance explained by the variable
Corrected model	7	10.260	0.000	0.620
Gender	1	12.411	0.001	0.220
Preferred beverage	2	5.341	0.008	0.195
Homocysteine (μmol/l)	1	8.313	0.006	0.159
BMI (kg/m^2)	1	5.746	0.021	0.116
Years of drinking	1	3.690	0.061	0.077
Age (years)	1	0.060	0.808	0.001

BMI, body mass index. Results of the general linear model. Statistical details are summarized in the Results section.

types of beverage, the differences being even more pronounced (ANOVA: $F = 8.334$; $df = 2$; $P < 0.001$). *Post hoc* analysis revealed differences between patients preferring beer and wine (Bonferroni: $P = 0.003$) and beer and spirits (Bonferroni: $P = 0.003$), respectively. No significant difference was observed between patients preferring wine and spirits.

To further analyze these results, we applied a general linear model with hippocampal volume as dependent variable and preferred beverage and gender as fixed factors and homocysteine levels, years of drinking, age, and BMI as covariates ($R^2_{corr} = 0.560$). As expected, gender had the largest influence on hippocampal volume, followed by the kind of preferred beverage and homocysteine levels. With the exception of "age" and "years of drinking," all other variables studied also had a significant impact on hippocampal volume (Table 2). No interaction between the fixed factors gender and preferred beverage was observed.

As these results suggest a potential causal connection between homocysteine and type of beverage in their impact on hippocampal volume, we performed linear regression analyses in subgroups divided according to the preferred beverage. The association between homocysteine and hippocampal volume ($R^2 = 0.282$; $P < 0.001$) was only present in the group of patients preferring spirits ($R^2 = 0.364$; $P = 0.008$), while there was no association in the beer group ($R^2 = 0.083$; $P = 0.23$). However, we found a trend in the wine group ($R^2 = 0.228$; $P = 0.071$).

DISCUSSION

To our knowledge, this is the first study investigating the impact of the type of preferred beverage on brain volume shrinkage in patients with alcohol dependence. Since our patients' sample contains a small number of female patients, a subgroup analysis differentiating the two genders did not seem appropriate. However, it is known that gender is one of the main factors influencing hippocampal volume. To account for this fact, we added gender as a fixed factor in the multivariate statistical analysis. As no interaction between gender and the preferred kind of alcoholic beverage was observed, we assume that gender differences in hippocampal volume were present equally in all three groups of the preferred alcoholic beverage.

The present results are in line with a recent investigation showing that homocysteine levels were highest in social drinkers consuming spirits compared to beer drinkers (Bleich *et al*., 2001).

Furthermore, a recent study investigating a more neuropsychological aspect in patients with alcoholism found that the extent of alcohol craving is modified by the type of consumed alcoholic beverage (Hillemacher *et al*., 2005).

As to our results, the kind of preferred beverage also influences plasma homocysteine levels a potential causal connection between homocysteine and type of beverage regarding their impact on hippocampal volume could be assumed. As described before, homocysteine mediates excitotoxicity and neurotoxicity via overstimulation of NMDA receptors (Lipton *et al*., 1997), oxidative stress (Outinen *et al*., 1998; Huang *et al*., 2001), activation of caspases, DNA damage, and mitochondrial dysfunction (Kruman *et al*., 2000). Clinically, a significant relationship between higher plasma homocysteine levels and brain atrophy in healthy elderly people (Sachdev *et al*., 2002), in elderly at risk of Alzheimer's disease (den Heijer *et al*., 2003), and in patients with alcoholism (Bleich *et al*., 2003a) has been observed.

We have found that the extent of brain atrophy in patients preferring beer was more moderate compared to patients consuming wine and spirits. Comparing the three subgroups in our investigation (beer, wine or spirits drinkers), patients preferring beer showed the highest lifetime consumption of ethanol. This is an interesting finding since patients preferring beer also showed the largest hippocampal volumes among patients with alcohol dependence, indicating that the dosage of ethanol alone does not determine the extent of brain atrophy.

Serum levels of homocysteine are influenced by dietary factors, especially B-vitamins and folate, which are involved in the homocysteine breakdown. Beer, in contrast to wine and spirits, is a rich source of B-vitamins and folate, which might explain the result of lowest homocysteine plasma levels in the beer-preferring subgroup of our patients' sample and consequently the moderate degree of brain volume reduction.

Similar to our findings, a recent investigation proposed that serum homocysteine increases after consumption of wine and spirits, but not after consumption of beer (van der Gaag *et al*., 2000). On the other hand, it has been suggested that variations in homocysteine levels observed in patients preferring different types of alcoholic beverages are confounded by the levels of blood alcohol, which also vary with regard to the consumption patterns (Bleich *et al*., 2000a). In this context it has been proposed that beer drinkers present much lower blood alcohol concentrations and consequently lower homocysteine serum levels compared to consumers of wine and spirits (Bleich *et al*., 2000a). Homocysteine levels decrease relatively rapidly and normalize within 3–5 days during the course of withdrawal (Bleich *et al*., 2000a). Some subjects of our patients' sample

had stopped drinking hours to a few days before admission. For patients with lower average blood alcohol concentrations this could have resulted in already decreased homocysteine levels, which may confound our results. To counteract this adverse effect, it would be necessary to analyze homocysteine levels and blood alcohol concentrations in actively drinking patients over a given time course and then perform the MRI measurements.

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