INTRODUCTION

Previous studies have shown that alcohol affects different regions of the brain, which are associated with both physiological and behavioral effects. Alcohol is believed to exert its effects by affecting neuroendocrine systems and multiple neurotransmitter systems, including facilitation of GABA, dopamine, opiate and serotonin neurotransmission and by modulation of neuronal activation in social drinkers following alcohol intoxication (Calhoun et al., 2004a, Calhoun et al., 2004b; Van Horn et al., 2006; Gundersen et al., 2008). Alcohol is known to have vasoactive properties. However, the few published studies on how alcohol affects cerebral blood flow (CBF, [ml/100 g/min]) in social drinkers indicate that alcohol effects on vasoactive properties are dose dependent; suggesting that the CBF increases at low doses (Newlin et al., 1982; Mathew and Wilson, 1986; Sano et al., 1993; Tiihonen et al., 1994; Khalili-Mahani et al., 2011; Tolentino et al., 2011) and decreases at higher doses of alcohol in social drinkers (Mathew and Wilson, 1986). Long-term alcohol consumption has been shown to exhibit cerebral hypoperfusion in the sober state both globally and regionally in the thalamus, temporal, parietal and occipital cortices as well as in the thalamus (Melgaard et al., 1990; Erbas et al., 1992; Nicolas et al., 1993; Kuruoglu et al., 1996; Oishi et al., 1999; Christie et al., 2008).

Due to cerebral autoregulation, changes in regional CBF often co-occur with regional changes in the cerebral blood volume (CBV, [ml/100 g]). This is seen in several neurovascular disorders. For instance, in stroke patients an increase in CBV is seen when the cerebral perfusion pressure decreases to sustain circulation, i.e. CBF. But also in the healthy brain, there is a close to immediate hemodynamic response (increased CBF and increased CBV) due to local neuronal firing and the associated neurovascular coupling. The correlation between CBF and CBV is then considered fairly well-described by the Grubb’s relationship, CBV/\text{CBV}_0 = (\text{CBF}/\text{CBF}_0)^k$, where $k$ is Grubb’s constant and estimated to be 0.38 (Grubb et al., 1974).

Simultaneous measurements of CBF and CBV after acute alcohol intoxication in social drinkers have previously reported. Such measurements are important in basic human neuroscience research to elucidate and understand brain physiology in the presence of exogenous neuro-pharmaceutical manipulations.

MATERIALS AND METHODS

Participants

Eight healthy male volunteers, all with higher education, participated in the study (27 ± 4 years, 82 ± 12 kg). None of the participants were dependent on alcohol or other drugs, but consumed alcohol (between 3 and 5 beverages) in social settings about one to two times a month. In addition, none of the participants were dependent on nicotine, which is important to control for since nicotine and nicotine abstinence have...
been shown to affect CBF (Cruickshank et al., 1989). None of the participants had any known psychiatric or neurological disorders, and none were using any kind of medication at the time. Written consent was obtained from all participants, and the study was approved by the Regional Committee for Medical Research Ethics in Western Norway (REK Vest nr. 048.03).

Design
A within-subject design was used, with two separate MRI scanning sessions four weeks apart. Prior to the first scanning session, a soft-drink was consumed. Prior to the second scanning session, a beverage of alcohol, resulting in a blood-alcohol concentration (BAC) of ~0.08% (0.08 ± 0.01%), was consumed.

Recordings of breath alcohol concentrations (mg/l)
Breath alcohol concentrations (BrACs) were determined before and after the second scanning session, using the Evidenzer breath alcohol level recording equipment (Nanopulse, Inc., Sweden). BrAC measurements started 30 min after the alcoholic beverage was consumed, and continued every 5 min until the same BrAC was obtained in two successive measurements, or until the BrAC started to decrease. BrACs were then transformed to the more commonly reported BAC by multiplying the results with a factor if 2.0 (Jones, 2000).

Procedure
Participants were instructed not to drink alcohol 24 h before the MRI scanning. In addition they were instructed not to consume caffeine containing beverages four hours before the MRI scanning, as caffeine has vasoactive properties that could confound the results. To avoid slow alcohol absorption, the participants were asked not to eat a fatty meal two hours before the MRI scanning. Before being served the soft-drink, the participants filled out a questionnaire about handedness, age, bodyweight and educational level, about caffeine, nicotine and alcohol habits, and about drug abuse and medication. To ensure that the participants were not dependent on alcohol or other drugs, they were asked the following questions: Are you, or have you been dependent on alcohol? How often are you drinking alcohol? How many units of alcohol do you drink to feel alcohol intoxicated? In which setting are you usually drinking alcohol? Are you, or have you been dependent on any kind of drugs? Do you currently use any kind of medication? The participants were in addition informed about the exclusion criteria (whom alcohol dependence and drug abuse was one of them) prior to participating. None of the participants was excluded based on their answers in the questionnaire.

Both the soft-drink and the alcoholic beverage were consumed in a social setting (30 min) before entering the scanner. The alcoholic beverage contained 60% pure ethanol diluted with tonic water, orange juice, cranberry juice and lemonade. For reaching a theoretical BAC level of 0.08%, the ethanol content was 7.2%. Ethanol was replaced by a corresponding volume of tonic water in the soft-drink. The amount, which was consumed by each participant, was individually tailored to bodyweight. Immediately after the desired BrAC value was achieved, the participants entered the scanner for imaging.

Data acquisition
All images were acquired on a Symphony 1.5T MRI scanner system (Siemens, Germany). Three orthogonal, fast T2-weighted (T2w) turbo-spin-echo image series were acquired to assure that subsequent image slice positioning was according to the orientation of each participant’s corpus callosum. These 3D and 2D anatomical images (T1 and T2, respectively) were acquired to identify possible structural abnormalities. Finally, DSC-MRI was used to measure cerebral perfusion (CBF, CBV) using a gradient-echo echo planar imaging (EPI) sequence (TE/TR = 46 ms/1.31 s, slice thickness 5 mm, flip angle 90°, image matrix 128 × 128, number of slices 11, field of view 230 mm). The selected sampling rate (TR = 1.31 s) allowed only partial brain coverage (11 slices), exemplified in Fig. 1. The measurements were repeatedly acquired 100 times to monitor the passage of contrast through the brain in time.

During the DSC-MRI acquisition, the contrast agent Gadovist (Schering, Germany) was injected after 16 s using an automatic MR-compatible power injector (TomoJet MR, Maershame PLC, England, UK), with dose individually adjusted to body weight (0.1 ml/kg). Subsequent saline flushing (20 ml) was done with the same injection speed as was used for the contrast injection (5 ml/s).

Data analysis
The perfusion data were transferred to an offline computer and post-processed using the nordicICE software (NordicImagingLab Inc., Norway). Perfusion analysis was performed voxel-wise on the first pass bolus signal using classical tracer kinetics. An exponential relationship between the measured signal intensities, \( s_i(t) \), and the contrast agent concentration, \( c_i(t) \), in each image voxel, \( i \), (Rempp et al., 1994), \( c_i(t) \propto -\left(\ln s_i(t) - \ln s_i(0)\right) \), was assumed.
The twelve image volumes that were acquired before the contrast agent entered the brain $s_i(0)$, were averaged and used as baseline for the subsequent perfusion quantification. Using the remaining post-contrast images, $s_i(t)$, the contrast agent concentrations in each voxel, $i$, could be computed in time, $c_i(t)$, according to the formula given above. Absolute quantification DSC-MRI is challenging (Kiselev, 2005), however comparison between the participants is possible by deconvoluting the contrast agent concentrations in each imaged tissue voxel $c_i(t)$, by contrast agent concentration in an artery (the arterial input function) in each participant. This corrects for the amount of tracer and the injection speed of the contrast agent individually in each participant. The arterial input function was found by averaging the time course of the 10 voxels having the largest and steepest change in signal intensity in the most inferior image slice. To minimize unwanted experimenter effects when selecting the arterial input function, these voxels were automatically selected by the software. The arterial input function was then removed from the tissue voxels through deconvolution using a previous published circular singular value decomposition approach (Smith et al., 2004) and a fixed threshold of 0.2 to cut-off singular values. Finally, parametric maps were obtained for each participant through computation of the maximum height (CBF) and the area under the curve (CBV) of the deconvolved contrast agent concentration function (Rempp et al., 1994; Ostergaard et al., 1996) (Fig. 2).

Eighty-nine, equally sized regions of interest (ROIs), manually positioned on a pre-contrast image in one hemisphere (left), was defined in each individual. The positioned ROIs were reloaded in the pre-contrast images of the follow-up scanning, which was possible due to the anatomical landmark positioning of the acquired slices. Nevertheless, each ROI was visually inspected and adjusted manually in all individuals (2 × 8 subjects, 11 slices) to assure as homogenous tissue as possible within each ROI. On average, there were 45 ROIs (range 40–48) positioned in GM and 44 ROIs (range 41–49) positioned in WM in each participant.

**Statistical analysis**

For each ROI, the average value of CBF and CBV standard deviation (SD) and range of values were obtained. Two-class $k$-means clustering of the CBF values was used to classify the voxels as GM or white matter (WM) individually for each participant. Only those ROI locations were the classification was the same across all participants were analysed further. This allowed for a within-ROI-across-participants analysis, and thus made it possible to investigate regional differences in CBF and CBV. This reduced the total number of analysed ROIs to 64, i.e. 27 ROIs classified as GM and 37 ROIs classified as WM. No further multiple comparison corrections were included as the subsequent analysis was performed across participants rather than across ROIs.

Shapiro–Wilk was used to test for normality in the data. Paired $t$-tests were used for comparison in data (i.e. CBV and CBF after alcohol consumption and after soft drink consumption). Spearman rank correlation test was applied to analyse any correlation between ratio values of CBF and CBV values. The significance threshold was in all cases set to $P = 0.05$ (SPSS, Version 18, IBM Corporation, New York, NY, USA).

**RESULTS**

Average CBF was higher after alcohol consumption than after soft drink consumption, Table 1. The average CBF value was higher both in ROIs with GM tissue and in ROIs with WM tissue ($P < 0.0001$). The resulting ratio between CBF after alcohol consumption to CBF after soft drink consumption was $\approx 1.2$ averaged for all GM ROIs and for all WM ROIs, respectively, Table 1.

The average changes in CBV were significantly different before and after alcohol consumption when compared across WM ROIs ($P = 0.04$), but not across GM ROIs ($P = 0.72$), Table 1. However, there was in both cases a clear tendency towards an average increase in CBV after alcohol consumption in most ROIs. The resulting ratio between CBV after alcohol consumption to CBV after soft drink consumption was about unity when averaged in all GM ROIs and in all WM ROIs, Table 1.

When computing the ratio of the logarithm of CBV changes to the logarithm of CBF values (i.e. similarly to Grubb’s relation for neurovascular response to neuronal stimulation) (Grubb et al., 1974) of 0.18 and 0.39 were obtained in GM and WM, respectively, Table 1. The correlation of CBF and CBV changes due to alcohol consumption was tested in GM and WM. There was stronger correlation ($P < 0.001$) between alcohol-induced changes in CBF and CBV in WM (Spearman’s rho 0.698) than in GM.
This supports the above described logarithmic relations, Table 1.

In the most inferior slices, regional increases in CBF were seen in areas close to where the large brain-feeding blood vessels enter the brain and in the thalamus region. In more superior slices changes were most apparent in the frontal brain regions. The lowest CBF changes were seen in the regions in the occipital brain regions and in ROIs judged to be at the boundary of what is supplied by the anterior cerebri media, Fig. 3.

**DISCUSSION**

After alcohol consumption to a BAC of 0.08%, CBF values are typically increased in WM and GM. The changes in CBF are larger than changes in CBV. There were significant regional changes throughout the left hemisphere where all ROIs were placed.

Our results are seemingly in agreement with other studies in social drinkers (Newlin et al., 1982; Mathew and Wilson, 1986; Sano et al., 1993; Tiihonen et al., 1994; Khalili-Mahani et al., 2011; Tolentino et al., 2011), despite of the different BAC. The fact that there are regional neuro-vascular responses even after one acute dose of alcohol, suggest that alcohol affects the various brain regions differently. Further investigations are needed to determine in long term alcohol exposure whether certain brain regions then are more at risk for being damaged because of repeated exposure, or whether long-term exposure simply affects brain regions which are already in advance more vulnerable to alcohol.

In the current study it was seen that although CBF was typically higher after alcohol consumption, CBV did not...
change to the same extent. There was a higher correlation between simultaneous CBF and CBV in WM than in GM, possibly because of the slightly higher blood volumes measured in WM after alcohol consumption. Despite the small cohort, these initial findings could suggest that WM regions might be more affected by alcohol changes than the GM regions that were studied here. This could contribute to the understanding of WM changes seen in many of the more recent studies where diffusion tensor imaging has been applied (Pfefferbaum et al., 1997, 2001, 2009, 2010).

Calculating a logarithmic ratio between CBF and CBV similar to the one known as Grubb’s relation (Grubb et al., 1974), further supports that the vasculature in the WM might be relatively more affected than in the GM. Because of the strong linkage to cognition and studies thereof (fMRI), however, GM is typically more reported. Because the BOLD contrast relies on the vasculature being able to regionally increase both CBF and CBV care must be taken in fMRI studies were vasoactive drugs are used, i.e. in alcohol studies or pharmacological studies. This is also suggested by Luchtmann et al. (2010) who have previously investigated how the hemodynamic response function (HRF) is affected after alcohol consumption (BAC of 1.0%) in a within subject design. They found that alcohol prolonged the time course of the HRF both in the visual cortex and in the motor cortices, indicating an overall slow-down of neurovascular coupling in those areas, rather than a reduced neuronal activation. Based on that, they suggested measuring the participants drug-induced HRF changes in pharmacological fMRI studies to avoid misinterpretation of the results. According to a theoretically predicted flow-BOLD-dependence (Seifritz et al., 2000), an 11 to 12% increase in blood flow, as in the current study, would result in an alcohol-related BOLD signal reduction of ~15%.

Compared with methods used in previous studies on alcohol like positron emission tomography (PET) (Volkow et al., 1988), [99mTc] HMPAO SPECT (Tiihonen et al., 1994), the 133Xe inhalation technique (Newlin et al., 1982; Mathew and Wilson, 1986; Sano et al., 1993) and the arterial spin labelling (ASL) method (Khalili-Mahani et al., 2011; Tolentino et al., 2011), DSC-MRI has the advantage of higher spatial resolution and high image contrast both in GM and WM, in addition to the ability to estimate CBV which previously has not been investigated in relation to alcohol intoxication.

Technical limitations in this study include that not full brain coverage was achievable because of the need for high temporal sampling of the data (Fig. 1). The spatial coverage was further limited in that only a group of ROIs were compared across participants. It would have been interesting to have measured the blood pressure at the time of imaging or have had a more detailed knowledge about the effects of alcohol on cardiovascular functions. Finally, the number of participants is low, thus a within-participant design was used to partly compensate for this.

Participants in the study were homogeneous regarding gender (males), educational level (higher education), residential area, health status and age. This means that we only can generalize our results to a comparable population. It would have been interesting to investigate CBF and CBV in other populations, for instance in populations with different socio-economic status.

Information on dependency was generated by self-report. The participants in our study population reported not to be dependent on drugs, alcohol, nicotine or coffee. Self-reports constitutes a relatively simple method of obtaining information, but we cannot exclude report bias using self-reported data. On the other hand, there was nothing which indicated false reports in our study.

In conclusion, alcohol affects cerebral circulation in social drinkers. The stronger correlation between alcohol-induced changes in CBF and CBV in WM than in GM, suggests the vasculature in the WM to be relatively more affected than the GM. Further studies are needed to conclude whether this may explain alcohol induced damages in alcohol dependent individuals seen in WM regions. Moreover, regional increases in CBF after alcohol consumption must be taken into account when analysing fMRI results to avoid misinterpretations.

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REFERENCES


Kiselev VG. (2005) Transverse relaxation effect of MRI contrast relies on the vasculature being able to regionally increase both CBF and CBV care must be taken in fMRI studies were vasoactive drugs are used, i.e. in alcohol studies or pharmacological studies. This is also suggested by Luchtmann et al. (2010) who have previously investigated how the hemodynamic response function (HRF) is affected after alcohol consumption (BAC of 1.0%) in a within subject design. They found that alcohol prolonged the time course of the HRF both in the visual cortex and in the motor cortices, indicating an overall slow-down of neurovascular coupling in those areas, rather than a reduced neuronal activation. Based on that, they suggested measuring the participants drug-induced HRF changes in pharmacological fMRI studies to avoid misinterpretation of the results. According to a theoretically predicted flow-BOLD-dependence (Seifritz et al., 2000), an 11 to 12% increase in blood flow, as in the current study, would result in an alcohol-related BOLD signal reduction of ~15%.

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Effects of alcohol on cerebral circulation


