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Effects of reading circumstances and acute alcohol consumption on cerebral circulation

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EFFECTS OF READING CIRCUMSTANCES AND ACUTE ALCOHOL CONSUMPTION ON CEREBRAL CIRCULATION

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Introduction

Investigation of cerebral blood flow regulation is of particular importance in neuroscience. The most important mechanisms of cerebral blood flow regulation are cerebral autoregulation, cerebral vasoreactivity and neurovascular coupling, for which transcranial Doppler (TCD) is an ideal examination method.

The effects of electronic reading devices (e-readers) and tablets on visual strain, reading and studying were investigated in several ophthalmological and pedagogical studies since these devices have been widely used nowadays. Reading is often used in neuroscience research to investigate the neurovascular coupling, although the effects of reading circumstances on the neurovascular coupling had not been studied yet. It is widely experienced that reading with direct light, that is, reading from a computer monitor or tablet, makes most people feel more tired than reading with indirect light (i.e., a newspaper or a paper book). However, to the best of our knowledge, neurovascular coupling evoked by reading with direct or indirect light has not been tested before. The question whether tiring effect of reading with direct light is caused through the negative visual effect or vascular component is also responsible for it, still needs to be replied. In addition, it is an important methodological question whether reading from a computer monitor with its own source of light and reading from paper with indirect light influences the neurovascular coupling.

Ethanol is one of the most commonly consumed, addictive, legal recreational drugs worldwide. In the majority of European countries, driving after drinking small-moderate amount of alcohol is legal, although there is no doubt that drivers under the influence of alcohol have higher accidental risk. The increased risk can mostly be explained by reduced attentional and cognitive capacities, as well as by the prolonged reaction time. Although several studies aimed to determine the neuronal and cerebrovascular effects of acute alcohol consumption, the effects of alcohol on cerebral blood flow regulation, including the cerebral vasoreactivity and the neurovascular coupling, have not been investigated yet.

Literature overview

Regulation of cerebral blood flow

Cerebral blood flow regulation is of great importance, as despite the small weight of the brain, it accounts for a large proportion of the cardiac output and total oxygen consumption of the body. Due to the high level of metabolic brain activity and the very low glycogen storage capacity, the brain requires constant and stable blood flow in order to maintain the appropriate neuronal function. However, in addition to the constant cerebral blood flow, the regional

cerebral blood flow has to be rapidly adjusted to the actual metabolic needs during neuronal activation. Regulation of cerebral blood flow is a complex and fine-tuned mechanism that depends on several local and systemic processes. The most important mechanisms of cerebral blood flow regulation are cerebral autoregulation, cerebral vasoreactivity and neurovascular coupling.

Cerebral autoregulation

Cerebral autoregulation is the response of cerebral blood vessels to the changes of cerebral perfusion pressure. Cerebral perfusion pressure is the difference between mean arterial blood pressure and intracranial pressure. Since under normal circumstances the intracranial pressure is nearly constant, cerebral perfusion pressure is mainly determined by the mean arterial blood pressure. As a result of changes in mean arterial pressure and cerebral perfusion pressure, cerebrovascular resistance also changes as part of the cerebral autoregulation resulting in a constant cerebral blood flow in a wide range of perfusion pressure. Consequently, the blood supply of the neurons is kept independent from systemic hemodynamic oscillations. Between the perfusion pressure limits of cerebral autoregulation, cerebral vasculature adopts to low arterial blood pressure by vasodilation and decreased cerebral vascular resistance, while high perfusion pressure will be compensated by vasoconstriction and increased cerebral vascular resistance. Beyond these limits, however, cerebral blood flow changes proportionally with the perfusion pressure.

Cerebral vasomotor reactivity

Cerebral vasoreactivity is defined as the vasodilation capacity of cerebral arterioles to external chemical stimuli, which enables the maintenance of cerebral perfusion, oxygen supply and acid-base balance despite changes of homeostasis. One of the most important regulators of cerebral blood flow is the change in the extracellular partial pressure of carbon dioxide ($PaCO_2$): hypercapnia results in vasodilation of the resistance vessels and increase in cerebral blood flow, while hypocapnia causes vasoconstriction and decrease in cerebral blood flow. As a result, breath holding and inhalation of 5% CO_2 results in an increase in cerebral blood flow, while hyperventilation induces decreased cerebral blood flow. In addition, decrease in extracellular pH (acidosis), hypoxia, decrease in the level of adenosine triphosphate, and increase in adenosine and potassium ion levels are also important factors influencing cerebral vascular tone and causing vasodilation.

Neurovascular coupling

Neurovascular coupling or functional hyperaemia refers to the mechanism that couples the transient neural activity to the subsequent change in regional cerebral blood flow. The neurovascular coupling is crucial for normal brain function, as it contributes to the increased

demand of energy and oxygen necessary for the functioning neurons, as well as to the removal of metabolic end products.

Regulation of cerebral vessel tone and cerebrovascular resistance

Changing of cerebrovascular resistance and cerebral arteriolar tone are the common endpoints of the mechanisms of cerebral blood flow regulation, i.e. the cerebral autoregulation, the cerebral vasoreactivity and the neurovascular coupling. Endothelium produces and releases potent vasoactive factors that regulate tone of underlying vascular muscle.

Cerebral autoregulation is dependent on myogenic responses of the resistance vessels, affected by metabolic and neurogenic factors. Bayliss effect is the basis of myogenic responses, which means that an increase in perfusion pressure activates mechanisms that increase myogenic tone and reduce vascular diameter. Conversely, in response to reductions in perfusion pressure, cerebral vessels respond with reduced myogenic tone and vasodilation. In addition, animal experimental data indicate that sympathetic stimulation has important effects on resistance of cerebral vessels during elevated arterial blood pressure.

During the process of cerebral vasomotor reactivity, the changes in vascular tone, caused by the alterations of $PaCO_2$ and brain extracellular pH, are mediated mostly by nitric oxide (NO), prostanoids, and intracellular calcium. Hypoxia mediates cerebral vessel tone by acidosis resulting from anaerobic metabolism, and release of vasodilator adenosine. Sensitivity to changes in blood gases can be detected throughout the whole cerebrovascular tree, with the most pronounced effect on the arterioles.

The mechanism of the neurovascular coupling has been investigated by several studies. Presently, there are three main, non-contradictory hypotheses about the involved mechanisms of neurovascular coupling: the first hypothesis assumed that activity-related shifts in ion contents and related changes in energy substrates influence the vasomotor tone. More advanced examination techniques with better time resolution revealed that the increase in blood flow follows the change in neuronal activity much more closely than the above mechanism would allow, and to a greater extent than the increase in metabolism would explain. Therefore, attention turned to the rich perivascular innervation of cerebral vessels: the second hypothesis assumed that special interneurons mediated the flow coupling by directly ending on arterioles. The third hypothesis addressed the role of astrocytes in neurovascular coupling, indicating that neurons, astrocytes and vascular cells (endothelium and smooth muscle cells) are closely related functionally. The term neurovascular unit was introduced to highlight the intimate functional relationships between these cells.

The mechanisms of cerebral blood flow regulation consist of complicated processes, however, knowing their many common pathways, it can be understood that these mechanisms discussed above act together and in a complex manner.

Examination of cerebral blood flow with transcranial Doppler ultrasound

Cerebral blood flow can be investigated by several methods. Transcranial Doppler ultrasound is a relatively cheap, non-invasive, repeatable bedside technique, which is suitable for monitoring cerebral blood flow and cerebrovascular hemodynamics continuously in real time. As a consequence, TCD is an ideal method for the examination of cerebral blood flow regulation mechanisms.

Functional ultrasound imaging is based on the Doppler technique. Pulsed-wave (PW) Doppler mode allows measurement of flow velocity and its direction in a region of interest. This is based on the principle of the Doppler effect. According to this principle, ultrasound waves emitted from the Doppler probe are reflected from moving red blood cells within the intracerebral vessels with different frequency depending on the flow velocity and direction. If the sources of the ultrasound and the observer approach, the frequency will be higher, however, if the source of the wave moves away from the observer, the frequency will be lower than the emitted frequency. The difference in the frequency between the emitted and reflected waves is referred to as the Doppler frequency shift, which can be used to calculate the blood flow velocity (v) by the following formula:

$$v = \frac{\Delta f \times c}{2 \times f_0 \times \cos\theta}$$

where Δf is the frequency shift, c is the speed of the ultrasound propagation (1540 m/s in brain tissue), f_0 is the transmitted ultrasound frequency (2 MHz in case of TCD examination) and θ is the angle between the ultrasound beam and the flow vector.

Flow velocities measured in a region of interest are displayed as the Doppler spectrum represented as a velocity over time curve. Because blood flow within the vessel is laminar, the Doppler signal obtained represents a mixture of different Doppler frequency shifts forming a spectral display of the distribution of the velocities of individual red blood cells. The Doppler curve is constituted by the maximal or the mean blood flow velocities of the Doppler spectrum, enveloping the curve and integrating the velocities below the curve. The shape of the Doppler curve is determined by the cardiac output, the peripheral vascular resistance, the diameter and length of the vessel, the elasticity of the vessel wall and the blood viscosity.

The Doppler curve represents the changes of blood flow velocity over a full cardiac cycle. The specific parameters obtained from this spectral analysis include peak systolic velocity and end diastolic velocity. Mean flow velocity is calculated by averaging the velocities below the curve. Peak systolic and mean flow velocity values measured in consecutive cardiac cycles are interpolated linearly. This interpolated data line can be used for data collection, allowing the measurement of blood flow velocities irrespective of the cardiac cycle. Using this

method, peak systolic flow velocity (PSV) and time-averaged mean flow velocity (TAMV) values are calculated and displayed by the TCD instrument. In addition, pulsatility index (PI) is also shown, which is equal to the difference between the peak systolic and end diastolic velocities divided by the mean velocity during the cardiac cycle. The index of resistance (RI), which is equal to the difference between the peak systolic and end diastolic velocities divided by the peak systolic velocity, is also calculated. The PI and RI values describe the regional peripheral resistance.

Insonation of the cerebral arteries is only possible through thinner regions of the skull, termed acoustic windows. Four main acoustic windows have been described: the transtemporal, the transorbital, the submandibular, and the suboccipital window. In functional TCD studies, the middle cerebral artery (MCA) and the posterior cerebral artery (PCA) are insonated through the transtemporal approach, which consists of an anterior, middle, and posterior window. The middle transtemporal window, located above the zygomatic arch and anterior to the ear, is used the most frequently. Lower frequency probes are necessary for TCD examinations, since higher frequency waves are not able to adequately penetrate through the skull.

Specific arteries of the circle of Willis are identified using the following criteria: relative direction of the probe within a specific acoustic window, depth of insonation, direction of blood flow relative to the probe, and the blood flow response to specific manoeuvres. Flow in the M1 segment of the MCA can be insonated through the transtemporal window at depths of 45 to 55 mm, the flow is directed towards the transducer. Since the M1 segment of the MCA runs laterally in the axial plane, a longer section of the vessel can be insonated. The PCA can also be insonated through the transtemporal window. In general, the PCA is found posterior and deep to the MCA, at a depth of 58 to 62 mm. Flow in the P1 (proximal) segment of the PCA is towards the probe and in the P2 (distal) segment of the PCA away from the probe. The PCA can be identified by eye closure and eye opening, because eye opening provokes elevated blood flow velocity. The normal spectral waveform of major intracranial arteries shows a sharp systolic upstroke and stepwise deceleration with high end-diastolic flow, indicating a low resistance flow pattern.

The limitations of TCD examination include that its spatial resolution is poor, and it is highly operator dependent, requiring detailed knowledge of cerebrovascular anatomy and its variations. The use of TCD can also be hampered by the 10 to 15% rate of inadequate acoustic windows.

Functional transcranial Doppler examinations

Examination of cerebral autoregulation

Examination of cerebral autoregulation with transcranial Doppler ultrasound consists of the continuous recording of cerebral blood flow velocity in the MCA bilaterally with beat-to-beat blood pressure and electrocardiography (ECG) monitoring. Static autoregulation is evaluated by a measurement of arterial blood pressure and cerebral blood flow velocity at baseline, followed by another steady-state measurement that is taken after the autoregulatory response to the manipulation of arterial blood pressure has been completed. If the blood flow is maintained at the baseline level, cerebral autoregulation is said to be intact. Dynamic autoregulation testing is more frequently used in clinical practice. With the dynamic approach, cerebral autoregulation can be evaluated during the response to a rapid change in blood pressure. Usually, a particular manoeuvre is applied to elicit the blood pressure change, including thigh cuffs release test (sudden release of a blood pressure cuff around one thigh), Valsalva manoeuvre, synchronized breathing and postural changes (tilt table test).

Examination of cerebral vasomotor reactivity

Cerebral vasomotor reactivity provides important information about the cerebral hemodynamic status and reflects cerebrovascular reserve capacity. The measurement of cerebral vasoreactivity by TCD consists of continuous monitoring of blood flow velocity in the MCA before and after the administration of a potent vasodilator stimulus, for instance the breath holding manoeuvre or inhalation of CO_2 (resulting in hypercapnia), administration of intravenous acetazolamide (inhibitor of carbonic anhydrase) or L-arginine (by NO release). Breath holding test is simple and applicable in clinical practice and research, but requires patient cooperation during the investigation. At the end of a deep inspiration, subjects are asked to hold their breath for a period of 40 s. Blood flow velocities in the MCAs are recorded before and after the breath holding period. BHI (breath holding index) is calculated by the following formula:

$$\text{BHI} = \left[\frac{\text{CBFV}_{\text{after BH}} - \text{CBFV}_{\text{before BH}}}{\text{CBFV}_{\text{before BH}}} \times 100 \right] / \text{breath holding time}$$

where $\text{CBFV}_{\text{before BH}}$ refers to cerebral blood flow velocity in the MCA before breath holding, and $\text{CBFV}_{\text{after BH}}$ refers to cerebral blood flow velocity in the MCA after breath holding.

In healthy volunteers, hypercapnia results in the elevation of cerebral blood flow, which is directly proportional to the increase in blood flow velocity in the MCA detected by TCD, assuming that the diameter of the artery remains unchanged, which was confirmed by previous studies. Accordingly, the increase of cerebral blood flow occurs through dilation of the cerebral resistance vessels. The dilation of the resistance vessels (mostly arterioles) results in decrease

of cerebrovascular resistance and increased blood flow velocity measured in the middle cerebral artery. Higher than 30% increase in cerebral blood flow velocity after breath holding is indicative of good vasomotor reactivity, based on studies in healthy subjects. It is important to note that hypercapnia affects the diameter of cerebral resistance vessels and cerebrovascular reserve capacity describes the vasodilation capacity of cerebral arterioles. Thus, if the resistance vessels become dilated due to an underlying process, a further vasodilator stimulus will not be able to enhance further vasodilation in the magnitude that is observed before.

Examination of the neurovascular coupling

Transcranial Doppler is an ideal method for the investigation of the neurovascular coupling, since the high temporal resolution of TCD permits the continuous measurement of cerebral blood flow velocity and analysis of the fast adaptation mechanisms of cerebral hemodynamics during functional changes in cerebral activity. Several stimuli can be applied, which result in the activation of cerebral cortex and related increase in blood flow velocity in the supplying artery proportional to the change in blood flow of the corresponding cerebral region. It is crucial to emphasize that transcranial Doppler is not suitable for the direct measurement of cerebral blood flow and the absolute blood flow velocity cannot be used as an indicator of cerebral blood flow. However, it was proved by several studies that the changes in flow velocity detected by TCD reliably correlate with changes in cerebral blood flow measured by SPECT (single photon emission computed tomography) and functional MRI (magnetic resonance imaging). The cerebral blood flow velocities are directly related to blood flow only if the diameter of the insonated vessel remains constant, which is obtained in case of functional TCD examinations.

The first and still the most common functional method is the examination of evoked flow velocity changes in the PCA during visual stimulation. The evoked flow velocity response triggered by neuronal activity highly depends on the applied stimulus, the site of measurement, and the proportion of activated neuronal pool to the entire brain tissue supplied by the examined vessel. Because the visual cortex is supplied almost exclusively by the P2 segment of the PCA, and this territory is not influenced by other stimuli (e.g. speaking, hearing, moving), measurement of visually evoked flow in the PCA is an ideal method for the examination of the neurovascular coupling. Several stimulation methods can be applied: white or coloured light and checkerboard pattern are simple, pictures or video movie, searching tasks and reading are complex visual stimuli.

The measurement of neuronal activity evoked blood flow changes can be hampered by other unrelated blood flow changes, namely the spontaneous fluctuations of blood flow due to heartbeat, breathing and other reasons. Two methods exist to circumvent the problem: the simultaneous measurement of blood flow velocity in two basal arteries and the calculation of

the relative regional perfusion increase, or application of averaging techniques. Using averaging techniques to increase the signal-to-noise ratio means that under stimulation conditions compared with the resting flow velocity level, the relative blood flow velocity changes can be calculated and averaged for several test repetitions. Regarding the PCA, 8 to 10 repetitions of resting and stimulation periods are convenient to increase the signal-to-noise ratio based on previous studies and our experiments. In addition to these methods, every effort must be done to establish very similar circumstances during functional TCD examinations.

The visually evoked flow velocity (VEF) response in the PCA shows a typical time course: with a short time delay at the beginning of the visual stimulation, cerebral blood flow velocity increases rapidly, overshoots, then stabilises at a constant but higher level than baseline (plateau). Using complex visual stimuli, the overshot appears between 8 to 12 seconds, whereas evoked flow velocity responses stabilise at around 10-20 seconds. To allow comparisons between volunteers and sets of experiments, absolute data must be transformed into relative changes of cerebral blood flow velocity in relation to baseline. Baseline is calculated from the blood flow velocities measured at the end of the resting phase. To analyse the visually evoked flow velocity response, the maximum increase of relative flow velocity changes can be calculated, which is the highest of the relative values obtained during the stimulation phase. Additional parameters such as latency (time elapsed from stimulus onset until the maximum increase), steepness of the increasing flow velocity curve and adaptation (decline of the response at the end of the stimulation phase relatively to the maximum increase), are also calculated.

Combined functional TCD and visual evoked potentials

An opportunity of the simultaneous investigation of the neuronal as well as vascular side of neurovascular coupling is necessary during the examination of cerebral blood flow regulation, since both the hampered neuronal activation and the disturbance of hemodynamic response can result in decreased cerebral blood flow response. Applying functional TCD examination together with simultaneous electroencephalography (EEG) recording, the relationship of the electric neuronal as well as vascular responses of the neurovascular coupling can be investigated. Regarding the functional homogeneity of the occipital cortex and that the PCA mainly supplies the visual cortex, visual evoked potential (VEP) and functional TCD together provide an ideal method to investigate the neuronal and the vascular components of the neurovascular coupling.

EEG can be used for determining visual evoked potentials. Evoked potentials are stereotyped alterations in electrical activity in the brain in response to sensory stimulation, which can be registered by surface electrodes on the scalp. Repetitive stimulation and averaging of registered curves are necessary for visualising the evoked potentials because their small size

makes impossible to differentiate them from the EEG waves. Latencies and amplitudes of the evoked potential waves are determined and evaluated, which let us to examine the functionality of the whole sensory pathway in the central nervous system (in case of VEP examination, the visual pathway). The visual pathway is stimulated by pattern display or reversal stimuli (checkerboard pattern reversal stimulation), and the evoked potentials are registered by surface electrodes positioned on the occipital scalp. The latency and amplitude of the first positive wave are evaluated in clinical practice. Since this wave is detected after the stimulus with a delay of approximately 100 ms in healthy individuals, it is called the P100 wave.

Theoretical background of our research topics

Effects of reading circumstances on neurovascular coupling

The effects of electronic reading devices (e-readers) and tablets on the human body were investigated in several ophthalmological and pedagogical studies since these devices have been widely used nowadays. Reading on LCD (liquid-crystal display) screen with its own source of light triggers higher subjective visual fatigue with respect to reading paper book with indirect light. Moreover, reading on the LCD leads to a larger decrease in the number of blinks, but higher rate of incomplete blinks compared to reading from paper. These negative visual effects might be imputable to the higher level of luminance emitted by the LCD screen, and they are also dependent on the applied display technology. The increasing number of digital reading devices in educational institutions results in pedagogical implications for reading speed, reading comprehension and memory. These studies indicate that reading texts on a computer screen leads to poorer reading comprehension than reading the same texts on paper. Behind this phenomenon, scrolling, decreasing memorability due to the lack of spatiotemporal markers, and the exhausting effect of reading with direct light should be considered.

The visually evoked flow in the posterior cerebral artery is influenced by the characteristic features of the visual stimulation applied. Visually evoked flow responses increase significantly with an increase in visual contrasts. Evoked response becomes more pronounced with an increasing complexity of the stimuli applied (e.g. white light, checkerboard pattern and colour video movie), whereas the adaptation to stimulation shows a significant decrement. Visually evoked flow is also influenced by the duration of stimuli: applying 5- and 10-second stimulus phases, the specific temporal pattern characteristic of responses for stimulation phases of 20 seconds or longer are missing. Brightness of the visual stimulus was proved to have a significant influence on the response amplitude, however, in another experiment, brightness did not appear to affect the visually evoked flow responses.

Reading is often used in neuroscience research to investigate the neurovascular coupling, although the effects of reading circumstances on the neurovascular coupling have not

been studied yet. It is widely experienced that reading with direct light, that is, reading from a computer monitor, makes most people feel more tired than reading with indirect light (i.e., a paper book) and several ophthalmological and pedagogical studies investigated the effects of reading with direct light on the human body. However, to the best of our knowledge, neurovascular coupling evoked by reading with direct or indirect light has not been tested before. The question whether tiring effect of reading with direct light is caused through the negative visual effect or vascular component is also responsible for it, still needs to be replied. In addition, it is an important methodological question whether reading from a computer monitor with its own source of light and reading from paper with indirect light influences the neurovascular coupling.

Effects of acute alcohol consumption on neuronal activity and cerebral circulation

Ethanol is one of the most commonly consumed, addictive, legal recreational drugs worldwide. In the majority of European countries, driving after drinking small-moderate amount of alcohol is legal, although the statement that drivers under the influence of alcohol have higher accidental risk is uncontroversial. Nevertheless, in most European countries, driving is legal under 0.5 g/L blood alcohol concentration (BAC), moreover, in the United Kingdom, 0.8 g/L is the maximum drink driving limit. The increased risk can mostly be explained by reduced attentional and cognitive capacities and prolonged reaction time. Several studies aimed to determine the effects of acute alcohol consumption on the human body, including researches investigating the background of reduced attention and prolonged reaction time. Regarding the effects of alcohol consumption on neuronal activity, depressive effects and delayed nerve conduction are well known. Moderate dose of alcohol was shown to cause deterioration of cognitive and motor performances and reduction of whole brain metabolism, which decrease was the most pronounced in the occipital cortex. Functional magnetic resonance imaging (MRI) examinations proved that the visually evoked occipital cortex activation is decreased after alcohol intake. These effects may explain the prolongation of VEP P100 wave latency.

Concerning the physiological effects of ethanol on cerebral blood flow, heterogeneous effects were described previously. High dose of ethanol (BAC > 2 g/L) caused vasoconstriction, however, lower doses were either vasoconstrictor, vasodilator, or ineffective on the cerebral arterioles of animal brain. The clue for this contradiction seems to be the metabolism of ethanol: whereas direct ethanol is vasoconstrictor, the main metabolite acetaldehyde and acetate have vasodilator effects. This hypothesis is supported by measurements on peripheral blood flow: oral alcohol has been shown to be a potent peripheral vasodilator, while intra-arterial infusion of alcohol had vasoconstrictor effects. In the human brain, the vasodilator metabolites of ethanol can dominate only at low to moderate ethanol doses, depending on the capacity of

alcohol dehydrogenase. These findings are supported by the results of human arterial spin labelling (ASL) MRI examinations showing that moderate doses of alcohol increase the global brain perfusion and cerebral blood flow. Transcranial Doppler examinations also showed that ethanol applied in moderate dose increased the blood flow velocities and decreased the pulsatility indices and the peripheral vascular resistance in the middle cerebral artery.

Regarding the effects of acute ethanol consumption on cerebral blood flow regulation, studies investigating the neurovascular coupling described an uncoupling mechanism after alcohol intake: instead of neuronal activity related cerebral blood flow increase, the direct enhancing effect of ethanol on the cerebral circulation was proved despite the negative effects on neuronal activity and whole brain metabolism. The neurovascular uncoupling, regarded as decreased glucose metabolism and increased cerebral blood flow may explain the neuroprotective effect of alcohol following traumatic brain injury: ethanol is associated with a marked attenuation of postinjury hyperglycolysis and simultaneously, the reduction in cerebral blood flow, typically seen after cortical injury, is less severe when ethanol is present. Beside these studies, the effects of alcohol on cerebral blood flow regulation, including the cerebral vasoreactivity and the neurovascular coupling, have not been investigated yet with transcranial Doppler ultrasound.

Aims

Our specific questions were the following:

1. The aim of our first study was to examine the cause of the tiring effect of reading with direct light. Therefore, we tested whether reading with direct light produces different vascular effects than reading with indirect light. We also intended to compare these two reading methods (to read with direct and indirect lights) from a methodological aspect.
   - Firstly, we compared the visually evoked blood flow responses in the posterior cerebral artery when reading from monitor with its own source of light or from paper with indirect light.
   - We also intended to study whether the visually evoked flow velocity responses are different AFTER reading from screen or from paper.
   - In addition, we also investigated the side difference in the flow response in the PCA evoked by reading, depending on the hemispheric dominance.
2. In our second investigation, we aimed to study whether a small-moderate blood alcohol content influences the neuronal activity, cerebral vasoreactivity, and neurovascular coupling.
   - In order to investigate the effect of ethanol on neuronal activity, visual evoked potential examination was performed.

– Furthermore, we intended to study the effects of acute alcohol consumption on cerebral vasoreactivity by analysing the breath holding index.
– Finally, we aimed to test the effects of acute ethanol intake on the neurovascular coupling, thus visually evoked flow velocity response during reading was measured in the PCA before and after alcohol consumption.

Methods

Subjects

We recruited 20 young healthy adults in the first, and 30 young healthy adults in the second study. The proportion of males and females was equal in both studies, and the age of participants was between 21 and 28 years. The study protocol included a detailed case history taking, physical examination, measurement of blood pressure, heart rate and body temperature, complete neurological and ophthalmological examination, carotid and vertebral artery duplex, and routine clinical laboratory tests. Subjects with cerebrovascular risk factors such as arterial hypertension, extreme obesity and diabetes mellitus, as well as alcohol dependency, history of neurological, coronary, pulmonary, hepatic or renal diseases, anaemia and malignant disease, were excluded. All subjects were right-handed in the first experiment. All volunteers had abstained from caffeine overnight before the study.

Transcranial Doppler (TCD) examination

Multidop T2 Doppler device (DWL, Singen, Germany) was applied for the TCD examinations. Two 2 MHz probes were mounted by an individually fitted headband over the temporal cranial window.

In our second study entitled “Effects of acute alcohol consumption on neuronal activity and cerebral vasomotor response”, the M1 segment of the MCA was insonated bilaterally at depths of 48 to 52 mm for measurement of vasoreactivity and breath holding index (BHI). Study subjects were asked to hold their breath after a deep inspiration for a period of 40 s. Systolic and mean blood flow velocities in the MCAs were recorded before (baseline), during, and after the breath holding period. Maximum time averaged mean flow velocity (TAMV) value measured within 10 s after the breath holding period was used for the analysis. BHI was calculated by dividing the percent increase in mean flow velocity by the duration of breath holding period (40 s). BHI values were provided as the ratio of percentage increase in blood flow velocity to breath holding time.

In both studies, PCA flow responses evoked by reading were investigated for the evaluation of neurovascular coupling. The P2 segment of the PCA was insonated on both sides at a depth of 58–62 mm. As a stimulation paradigm, we used an emotionally neutral text that the volunteers could read freely. The stimulation protocol consisted of 10 cycles with a resting phase of 20 s and a stimulation phase of 40 s for each cycle. In the resting phases, the subjects closed their eyes, in the stimulation phases, they were reading from paper or from screen.

Beat-to-beat intervals of cerebral blood flow velocity data were interpolated linearly with a "virtual" time resolution of 10 ms for averaging procedures, which allowed us to measure the blood flow velocities irrespective of the cardiac cycle. Within one person, flow velocity data of 10 cycles were averaged to increase the signal-to-noise ratio. Due to the data collection in every 10 ms, 100 data were received per second for each flow velocity parameter. Using this method, peak-systolic (PSV) and time averaged mean (TAMV) blood flow velocities as well as pulsatility indices (PI) were recorded in every 10 ms and averaged for every second. These averaged data were used for analysis and charting. To allow comparisons between volunteers, absolute data were transformed into relative changes of cerebral blood flow velocity in relation to baseline. Baseline was calculated from the blood flow velocity averaged for a time span of 5 s at the end of the resting phase, before the beginning of the stimulation phase. To analyse the maximum increase of relative flow velocity changes, the highest of the relative values obtained during the stimulation phase was taken from each subject. Additional parameters such as latency (time elapsed from stimulus onset until the maximum increase), steepness of the increasing flow velocity curve and adaptation (decline of the response at the end of the stimulation phase relatively to the maximum increase) were also calculated.

Visual evoked potential (VEP) examination

In our second study entitled "Effects of acute alcohol consumption on neuronal activity and cerebral vasomotor response", visual evoked potentials were recorded over the occipital cortex (Neuron-Spectrum-4/EPM, Neurosoft, Ivanovo, Russia). Subjects were seated in front of the screen of the monitor at 1 m distance. Refractive errors were corrected with contact lenses or glasses if needed. Binocular stimulation was applied, since we hypothesised that the effects of alcohol consumption on the visual pathway are similar on the two sides.

Visual evoked potentials were recorded by three scalp electrodes and a reference electrode. The reference electrode (Fz) was placed on the forehead below the hairline, in the midline, the ground electrode (Oz) was positioned at the vertex. The two active electrodes (O1 and O2) were placed on the occipital scalp overlying the calcarine fissure, which is 5 cm above the inion (external occipital protuberance) and 2 cm lateral from midline, since this is the closest location to primary visual cortex. Checkerboard pattern reversal stimulation with a temporal

frequency of 2 Hz was used for visual stimulation. At least 100 sequential visual stimuli were presented to generate an average waveform and reduce noise. Two stimulations were applied right after each other to test reproducibility of the responses. Latencies and amplitudes of the P100 waves were calculated and used for analysis.

Experimental design of "Effect of reading with direct or indirect light on the visually evoked flow response in the posterior cerebral artery" study

In order to minimise those factors that might influence the cerebral blood flow, we made every effort to establish very similar reading conditions during the experiments. The examinations were performed in a quiet, darkened room at about 23 °C while the subjects were sitting comfortably and reading silently the same emotionally neutral text. Subjects were reading from an LED (light-emitting diode)-backlit LCD screen with IPS (in-plane switching) technology of a first-generation Apple iPad® tablet with direct light or from an A4 printed paper with indirect light. The content of the text, the size, type, and font style of the letters, the contrast and text layout, the viewing angle, and the reading distance between the eyes and the screen or paper, as well as the external light were the same when the volunteers read from monitor or from paper. A custom-made stand was used to hold the tablet and the printed paper to ensure the same reading distance and viewing angle.

Flow velocity responses evoked by reading were measured 4 times, and each reading test lasted 10 minutes. Two sets of experiments were performed: first, flow responses evoked by reading from paper (VEF_paper) and by reading from the screen (VEF_screen) were measured in both PCAs in a random sequence. Second, flow responses evoked by reading from paper were tested after a 15-minute period of reading from a tablet screen (VEF_after_screen) as well as after 15-minute period of reading from paper (VEF_after_paper), also in a random order. A 10-minute pause was inserted after the first set of the experiment and between the two parts of the second set of the study, during which the volunteers relaxed with closed eyes.

Experimental design of "Effects of acute alcohol consumption on neuronal activity and cerebral vasomotor response" study

Every examination was performed before (control period) and after (test period) drinking alcohol. First, visual evoked potentials were recorded. Afterward, the TCD probes were mounted by an individually fitted headband over the temporal cranial window and cerebral vasoreactivity was investigated in both MCAs. Then neurovascular coupling was evaluated by visually evoked flow test in both PCAs.

After the first set of experiments (control period), alcohol (vodka, 37.5% alcohol content) was administered orally over a 10-min period. Volunteers were allowed to dilute the

alcoholic beverage with sugar-, and caffeine-free non-carbonated soft drinks up to a total volume of 200 mL. Vodka was chosen because of its purity as it contains few flavour compounds and consists essentially of ethanol and water. Our aim was to investigate the effects of mild-moderate drunkenness on cerebral circulation, therefore, the target blood alcohol concentration (BAC) was 0.8 g/L. In order to reach this concentration with low variance, we used the following formula for calculating the required amount of alcohol in grams (A):

$$A = BAC \times BW \times Wf$$

where BAC means the target blood alcohol concentration expressed in g/L, BW is the body weight in kg, and WF is the Widmark factor that is 0.68 in males and 0.55 in females.

During the post-alcohol test period, 30 and 60 min after the end of alcohol consumption, blood was drawn for the measurement of BAC. After the end of alcohol ingestion, the volunteers relaxed with closed eyes for 30 min. The second set of experiments (test period) was started 30 min after the end of alcohol administration. The experiments in the test period were performed in a reversed order compared to the control period: the visually evoked flow (VEF) test was followed by the breath holding test, and eventually VEP parameters were recorded. In order to compare the absolute flow velocity values in the PCAs, the TCD probes were not removed between the VEF tests in the control and in the test period. Blood pressure and heart rate were measured noninvasively at the beginning of the experiment and in every 5 min after alcohol consumption.

Statistical analysis

Data were expressed as means ± standard deviation (SD). Tests for normal distribution were performed. Relative flow velocity values in the MCAs, whereas absolute and relative flow velocity values in the PCAs were analysed. Peak-systolic (PSV) and time averaged mean (TAMV) blood flow velocities as well as pulsatility indices (PI) were used for the analysis.

Repeated measures analysis of variance (ANOVA) was applied to compare changes of visually evoked cerebral blood flow velocities in the PCAs. The results of repeated measures analysis of variance were shown by group main effect and group with time-of-measurement interaction. Group main effect showed whether there was a significant difference in flow velocities averaged over the 40 s active period during reading. The group with time-of-measurement interaction indicated whether the pattern of flow velocity changes over time was different in each experimental protocol. Non-significant interaction indicated that the pattern of flow velocity changes in the different experimental settings was parallel, while significant interaction referred to a different pattern.

Absolute baseline flow velocity values in the PCA, the maximum relative flow velocity increases, the latencies, the steepness values of the increasing slope and the adaptations at

different periods of the experiment, as well as blood pressure values, heart rates, BHI values, and VEP P100 amplitudes and latencies were compared by paired t-test.

In the first study entitled “Effect of reading with direct or indirect light on the visually evoked flow response in the posterior cerebral artery”, the flow data measured on the two sides were analysed separately, because we intended to study the side difference in the visually evoked flow response in the PCA evoked by reading depending on the hemispheric dominance. Therefore, maximum increase of relative flow velocity changes and the average of flow velocities in the second half of the stimulation phase measured on the left and on the right side were compared by two-sample t-test. In our second study entitled “Effects of acute alcohol consumption on neuronal activity and cerebral vasomotor response”, the flow data measured on the two sides were averaged, and the averaged data were used for analysis, since we hypothesised that the effects of alcohol consumption on the cerebral circulation are similar on the two sides.

A difference of $p \leq 0.05$ was considered statistically significant.

Results

Effect of reading with direct or indirect light on the visually evoked flow response in the posterior cerebral artery

The baseline absolute peak-systolic (PSV) and time averaged mean (TAMV) blood flow velocities in the PCAs in the different phases of the experiment were similar on both sides.

Comparison of visually evoked flow responses during reading from paper and reading from screen

Neither the group main effect ($p=0.32$ for the PSV and $p=0.55$ for the TAMV values), nor the group with time-of measurement interaction ($p=0.48$ for the PSV and $p=0.69$ for the TAMV values) was significant for the visually evoked relative flow velocity time courses between reading from paper (VEF_paper) or screen (VEF_screen), referring to a similar increase in relative flow velocities and a similar pattern of flow velocity changes during visual stimulation. On the left side, the adaptation of PSV was 3.3±1.9% for VEF_screen vs. 4.6±2.9% for VEF_paper ($p=0.07$), whereas the adaptation of TAMV was 4.7±2.8% for VEF_screen vs. 6.4±3.2% for VEF_paper ($p=0.05$) when reading from screen (VEF_screen) and reading from paper (VEF_paper), respectively. No significant difference was found in the adaptation values on the right side and in the other variables such as latency and steepness of the upslope on any side.

Comparison of visually evoked flow responses investigated following 15 minutes reading from screen and 15 minutes reading from paper

In this set of experiment, flow responses evoked by reading from paper were tested after a 15-minute period of reading from screen (VEF_after_screen) as well as after a 15-minute period of reading from paper (VEF_after_paper). There was no significant group main effect ($p=0.82$ for the PSV and $p=0.80$ for the TAMV values), or group with time-of measurement interaction ($p=0.85$ for the PSV and $p=0.94$ for the TAMV values) regarding VEF_after_screen or VEF_after_paper, referring to a similar increase in relative flow velocities and a similar pattern of flow velocity changes during visual stimulation. On the left side, VEF_after_screen latency was 13.9±3.2 seconds and VEF_after_paper latency was 12.5±3.8 seconds ($p=0.06$). The latency on the right side and the maximum increase of relative flow velocity, the adaptation values or the steepness of the upslope on any side were similar after reading from monitor or from paper.

Comparison of visually evoked flow velocity responses during reading on the right and on the left side

Comparing the relative evoked flow velocity curves on the right and on the left side, maximum increase of flow velocity changes were similar on the two sides at every phase of the experiment. However, in the second half of the stimulation phase (after flow adaptation), blood flow velocity values were greater on the left than on the right side (for VEF_screen, TAMV values 118.3±5.1% on the left side vs. 114.5±5.7% on the right side, $p=0.04$).

Effects of acute alcohol consumption on neuronal activity and cerebral vasomotor response

Alcohol consumption resulted in an alcohol concentration of 0.82±0.25 g/L measured 30 min and 0.94±0.15 g/L measured 60 min after drinking alcoholic beverage, indicating that the target 0.8 g/L BAC was reached during the test period and the measurements were performed in the 0,6-1,1 g/L BAC range. Blood pressure showed no significant changes following alcohol ingestion, while pulse rate increased significantly already 5 min after alcohol consumption and remained elevated (by approximately 8-10 beats per minute) until the end of the experiment.

Effects of alcohol on neuronal activity: VEP parameters

Parameters of VEP were within the normal range in all subjects. After alcohol ingestion, the latency of the VEP P100 wave increased (before alcohol 108.0±2.4 ms vs. after alcohol 110.8±3.4 ms, $p<0.01$), whereas amplitude of the VEP P100 wave decreased (before alcohol 9.7±3.2 μV vs. after alcohol 8.6±3.4 μV, $p=0.01$) compared to the control period.

Effects of alcohol on cerebral vasoreactivity: breath holding index parameters

The breath holding index was within the normal range in all subjects in the control period. The increment of blood flow velocity in the MCAs caused by 40 s of breath holding was lower after than before alcohol consumption (before alcohol 44.1±11.4 %/40s vs. after alcohol 34.9±14.3 %/40s, $p<0.01$).

Effects of alcohol on neurovascular coupling: visually evoked flow parameters

At first, baseline absolute flow velocity parameters measured in the PCAs before and after alcohol ingestion were compared. Blood flow velocities recorded for a time span of 5 s at the end of the resting phase were considered as baseline. Higher baseline absolute PSV (before alcohol 53.2±12.8 cm/s vs. after alcohol 55.8±13.0 cm/s, $p<0.01$) and TAMV (before alcohol 34.5±9.3 cm/s vs. after alcohol 37.4±9.8 cm/s, $p<0.01$), and lower PI (before alcohol 0.85±0.14 vs. after alcohol 0.76±0.14, $p<0.01$) were registered in the PCAs after than before alcohol consumption.

The visually evoked absolute flow velocity time courses between the control and test periods did not show significant group main effect ($p=0.30$ for the PSV and $p=0.15$ for the TAMV values). These data indicate that the increase in flow velocities during visual stimulation was not significantly different before and after alcohol consumption. The group with time of measurement interaction, however, was significant in both peak-systolic and mean flow velocity values ($p<0.01$), which means that the pattern of flow velocity changes was different before and after alcohol ingestion. The pulsatility indices were significantly lower after alcohol consumption not only in the resting phase but also during the stimulation phase compared to the corresponding values in the control period. Analysing the difference of the pulsatility index values during visual stimulation before and after alcohol consumption, repeated measures ANOVA revealed that both the group main effect ($p=0.03$) and the group with time-of-measurement interaction ($p<0.01$) were significant.

Then relative flow velocity values measured during the visual stimulation before and after alcohol ingestion were calculated in relation to the proper baseline values. The visually evoked relative flow velocity time courses between the control and test periods did not show significant group main effect ($p=0.85$ for the PSV and $p=0.29$ for the TAMV values). These data indicate that the relative flow velocities during visual stimulation were not significantly different before and after alcohol consumption. The group with time of measurement interaction, however, was significant in both peak-systolic and mean flow velocity values ($p<0.01$), which means that the pattern of flow velocity changes was different before and after alcohol ingestion. Analysis of different parameters of the relative flow velocity time courses showed that the maximum increase of relative flow velocity of TAMV values was lower, the

latency of both the PSV and TAMV values was longer, and the steepness of the increasing slope of both the PSV and TAMV values was smaller after than before alcohol consumption.

Discussion

The aim of our first study was to investigate the effects of reading with direct or indirect light on the evoked blood flow response in the posterior cerebral artery. Our results showed that reading from a computer screen with its own source of light and reading from paper with indirect light caused similar flow responses in the supplying artery of the visual cortex. Moreover, the flow velocity increase evoked by reading form paper was similar after a 15-minute period of reading from computer monitor and after a 15-minute period of reading from paper. Based on our experiments, reading with direct light did not cause either direct or indirect unfavourable effect on the neurovascular coupling in the occipital cortex. These data suggest that reading from screen was unlikely to produce different vascular effects that could explain the visual symptoms or the tiring and comprehension-inhibiting effects of reading with direct light. Considering previous findings, negative ophthalmological effects (e.g. decrease in the number of blinks and higher rate of incomplete blinks) are more probable than vascular effects that could be involved in the visual symptoms perceived when reading with direct light. Behind the negative effects on reading comprehension, scrolling and decreasing memorability when reading from screen should be considered. Our results also indicate that the two reading methods (i.e. reading from a computer screen vs. reading from paper) have the same effect on the visually evoked flow responses, thus data obtained by these two test procedures are comparable.

Although the neurovascular coupling did not appear to be influenced by reading on monitor, mild and nonsignificant differences of the flow response in the different experimental settings, even if not entirely consistent, were observed. Therefore, reading from screen may slightly influence certain parts of the visually evoked vascular response in the occipital cortex. Since we made every effort to establish very similar reading conditions when reading from screen and from paper, the main, if not the only difference between the two reading methods was that the screen had an internal source of light. Previous studies showed that the complexity and contrast of the visual pattern, monitor qualities and settings, including size, type and colour of the letters, as well as colour of the background may influence the vascular response in the occipital cortex. The light stimulus was shown to be responsible for 18%, while the letter and word recognition for an additional 8-10% increase in the PCA flow response induced by reading. Leaving the internal source of light as the only notable difference, the light stimulus provided by the monitor may had contributed to slightly higher flow values in the occipital

cortex in the second part of the stimulation phase and resulted in the nonsignificantly lower adaptation values.

In our first investigation, we also intended to study the side difference in the visually evoked flow response in the PCA evoked by reading. Higher blood flow velocity values were registered on the left than on the right side after adaptation in the second half of the stimulation phase. The cause of this phenomenon is apparently the fact that reading and comprehension mainly depend on the dominant hemisphere. As all of our volunteers were right-handed, their left hemisphere is assumed to be the dominant one, although hemispheric dominance was not investigated in our study. Reading triggers not only bilateral activation of the visual cortex, but also activates the cortical areas involved in the processing of written words, namely the visual word form area that is located in the temporooccipital and basal temporal regions of the dominant hemisphere. Since blood supply of these regions is provided by mainly the PCA, the side difference in the flow velocity increase evoked by reading could result from the more pronounced activation of the left than the right occipital and temporal structures.

As we concluded in the first study that reading from a computer screen and reading from paper have the same effect on the visually evoked flow responses, reading from screen was applied to visual stimulation in our second study. Our purpose was to study the impact of small-moderate dose of ethanol on neuronal activity and cerebral hemodynamics, including cerebral vasoreactivity and neurovascular coupling. The target 0.8 g/L BAC was reached, the measurements were performed in the 0,6-1,1 g/L BAC range. As this value is close to the BAC driving limit that applies in some European countries, our data represent the potential pathophysiological effects of this alcohol level.

Heterogeneous effects of low-to-moderate doses of ethanol on cardiovascular parameters were described previously. Studies in humans showed that acute alcohol consumption increased, did not affect, or decreased the blood pressure. Most of the experiments revealed that alcohol increased the pulse rate. Our results are in agreement with these observations: the blood pressure showed no significant changes, while the pulse rate elevated significantly following alcohol ingestion in our study.

Consistent with depressive effects of ethanol on central nervous system, we observed significant increase in the latency and decrease in the amplitude of VEP P100 wave under the effect of alcohol. In line with our findings, other authors pointed out earlier that alcohol ingestion was associated with prolongation of P100 wave latency. Moderate dose of alcohol consumption was reported to decrease the visually evoked occipital cortex activation and reduce the whole brain metabolism, which decrease was the most pronounced in the occipital cortex and may explain the changes in the VEP parameters.

Increase of baseline absolute flow velocities and decrease of the pulsatility index in the PCA after drinking alcohol suggest decreased cerebrovascular resistance indicating alcohol induced vasodilation in the cerebral resistance vessels. Similar changes in the MCA were already described after alcohol consumption. Since negative effects of alcohol was found on neuronal activity, alcohol induced increase in cerebral metabolism can be excluded in the background of vasodilation. Quite contrary, our data indicate the direct effect of ethanol on the cerebral vasculature which is congruent with the results of previous studies.

Our results showed that breath holding index, that is the hypercapnia-induced vasomotor response, decreased after ethanol consumption, indicating reduced cerebral vasoreactivity. The decrease in cerebral vasomotor response after alcohol consumption is probably due to the alcohol-induced dilation of cerebral microvessels. As alcohol caused a significant dilation of the cerebral resistance vessels, further vasodilator stimulus (breath holding manoeuvre) could only result in a smaller vasodilation, leading to lower breath holding induced flow velocity response.

Although significant vasodilation developed in the territory of the PCA after alcohol ingestion, indicated by the increase of baseline absolute flow velocities and decrease of the pulsatility index, additional increase of flow velocities and decrease of pulsatility indices during visual stimulation suggested further decrement of vascular resistance in the arterioles. As a consequence, the visual stimulation evoked increase in cerebral blood flow velocity and dilation of cerebral microvessels (i.e. the neurovascular coupling) are still present after acute alcohol consumption. Similar effects were assumed by previous studies using BOLD (blood oxygenation level dependent) functional MRI techniques, suggesting that the overall mechanisms of the neurovascular coupling that forms the BOLD signal are still acting well at a moderate ethanol level and the vasodilation capacity of the cerebral arterioles remains intact.

The neurovascular coupling as a complex process was affected by alcohol consumption. Although the group main effect of relative flow velocity changes during visual stimulation did not reveal significant difference before and after ethanol ingestion, the pattern of flow velocity changes was different. Moreover, the maximum increase of relative flow velocity was lower, the latency of reaching the maximum flow velocity value was longer, and the steepness of the increasing slope was smaller after than before alcohol consumption, indicating negative effects of ethanol on neurovascular coupling. Since regional cerebral blood flow changes are coupled with regional brain activation, disturbance of neurovascular coupling could be due to the reduced neuronal activity indicated by prolonged latency and decreased amplitude of VEP P100 wave observed after alcohol consumption. Further mechanism in the background of the smaller visually evoked flow changes could be the alcohol induced dilation of cerebral microvessels, which was indicated by an increase in absolute flow velocity values and a decrease in pulsatility

indices after alcohol ingestion. This cerebral vasodilation could interfere with the further dilation of the arterioles that is required for the neuronal activation induced flow response. Our results showed that low-moderate level of alcohol has a non-negligable effect on the neurovascular coupling, and may affect the cortical functions. Based on this conclusion, limits of alcohol level at which driving is allowed should be reconsidered.

New scientific findings

1. Reading from a computer monitor with its own source of light and reading from paper with indirect light cause almost identical flow responses in the PCA, thus data obtained by these two reading methods are similar and comparable.
2. A 15-minute period of reading from computer monitor or from paper induces similar PCA flow responses, indicating that the neurovascular coupling is not adversely influenced by a 15-minute period of reading from monitor.
3. Reading from screen with its own source of light is unlikely to produce different vascular effects that could be involved in the visual symptoms or tiring effects of reading from monitor with direct light.
4. Reading evokes higher PCA flow responses on the left than on the right side in right-handed individuals.
5. Acute ethyl alcohol consumption (BAC in the 0,6-1,1 g/L range) results in an increase in the latency and decrease in the amplitude of VEP P100 wave, referring to an inhibition of visually evoked occipital cortex activation.
6. Increase of baseline absolute flow velocities and decrease of the pulsatility index in the PCA after drinking small-moderate dose of ethyl alcohol suggest alcohol induced vasodilation in the cerebral resistance vessels.
7. Breath holding index (BHI) is decreased after ethanol ingestion indicating reduced cerebral vasoreactivity, which is probably due to the alcohol-induced dilation of cerebral microvessels.
8. Neurovascular coupling is compromised after acute ethanol consumption, which might be the consequence of the decreased neuronal activity and also of the alcohol induced cerebral vasodilation that may interfere with the further dilation of the arterioles, required for the neuronal activation induced flow response.

Summary

Introduction: Investigation of cerebral blood flow regulation is of particular importance in neuroscience, for which transcranial Doppler (TCD) is an ideal examination method. The aim of our first study was to test whether reading with direct light produced different visual evoked flow response (i.e. neurovascular coupling) compared to reading with indirect light, which could explain the visual fatigue experienced after reading from a computer display. In our second investigation, we aimed to study whether a small-moderate blood alcohol content, at which driving is legal in some countries (0.8 g/L), influences the neuronal activity, cerebral vasoreactivity, and neurovascular coupling.

Methods: Flow velocity responses evoked by reading from paper and from monitor were measured by transcranial Doppler sonography in both posterior cerebral arteries (PCAs) of young healthy adults. PCA flow response evoked by reading was also investigated after a 15-minute period of reading from monitor or paper. In our second experiment, neuronal and vascular effects of alcohol were investigated. Examination of visual evoked potential (VEP) was used to assess the neuronal activity, while TCD was applied to evaluate the cerebral vasoreactivity by breath holding test in both middle cerebral arteries, and to measure the visually evoked flow velocity response during reading in both PCAs. VEP and TCD examinations were performed before and after alcohol consumption.

Results: Reading from monitor with its own source of light and reading from paper with indirect light caused very similar PCA flow response, which did neither differ after a 15-minute reading from monitor or from paper. Higher PCA blood flow velocity values were evoked by reading on the left than on the right side in right-handed individuals. After alcohol consumption (BAC in the 0,6-1,1 g/L range) the VEP P100 wave latency increased, while the amplitude decreased. Resting absolute flow velocity values increased, whereas pulsatility indices in the PCA decreased after alcohol ingestion. Breath holding index in the MCA and the visually evoked maximum relative flow velocity increase in the PCA were decreased after alcohol consumption. The steepness of rise of the flow velocity curve was smaller, and latency of reaching the maximum increase was longer after than before alcohol consumption.

Conclusion: Reading with direct or indirect light produces similar flow response in the occipital cortex, thus reading from monitor does not cause unfavourable effect on the neurovascular coupling. Higher blood flow velocity values are evoked by reading in the PCA of the dominant hemisphere. Acute consumption of small-moderate dose of alcohol inhibits the neuronal activity and results in dilation of cerebral arterioles. Cerebral vasodilation may explain the decrease of cerebral vasoreactivity and might contribute to the disturbance of visually evoked flow response after alcohol consumption.

Publication list

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Candidate: Eszter Balogh
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List of publications related to the dissertation

1. **Balogh, E.**, Árokszállási, T., Körtefái, K., Nagy, V., Csiba, L., Oláh, L.: Effects of acute alcohol consumption on neuronal activity and cerebral vasomotor response.
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List of other publications

3. Árokszállási, T., **Balogh, E.**, Orbán-Kálmándi, R. A., Pásztor, M., Árokszállási, A., Nagy, E. B., Belán, I., May, Z., Csépány, T., Csiba, L., Bagoly, Z., Oláh, L.: Elevated Blood Alcohol Concentration Is Associated with Improved Clinical Outcomes of Intravenous Thrombolysis Treatment in Acute Ischemic Stroke Patients: A Retrospective Study.
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