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Effect of acute and chronic alcohol consumption and withdrawal on rat visual evoked potentials: focus on ON/OFF responses

DOCTORAL DISSERTATION

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1. Dulinskas R, Buisas R, Vengeliene V, Ruksenas O. Effect of ethanol on the visual-evoked potential in rat: dynamics of ON and OFF responses. *Acta Neurobiol Exp (Wars).* (2017); 77(2):190-197.
2. Redas Dulinskas and Osvaldas Ruksenas. Modulation of responses to visual stimulus onset and offset by chronic alcohol consumption and withdrawal in the rat visual cortex and lateral geniculate nucleus. *Alcohol* (2020), 85: 101-110,

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Contents

Abbreviations

ADH	alcohol dehydrogenase
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	analysis of variance
BAC	blood alcohol concentration
cAMP	cyclic adenosine monophosphate
CAT	catalase
CB1	cannabinoid Receptor 1
CRF	corticotrophin-releasing factor
CYP2E1	cytochrome P450 2E1
DAMP	damage-associated molecular pattern
EEG	electroencephalography
ERP	event-related potentials
FASD	Fetal Alcohol Spectrum Disorders
FVEP	flash visual evoked potentials
GABA	gamma-aminobutyric acid
GBR	gamma-band response
GSH	glutathione
IP	intraperitoneal injection
ISI	interstimulus intervals
K	koniocellular streams
LGN	lateral geniculate nucleus
M	magnocellular streams
MDA	malondialdehyde
mGluR	metabotropic glutamate receptors
nAChR	nicotinic acetylcholine receptor
NMDA	n-methyl-d-aspartic acid
NPY	neuropeptide Y
ON	stimulus onset
OFF	stimulus offset
P	parvocellular streams
ROS	reactive oxidative species
SC	subcutaneous injection
VEP	visual evoked potentials
VC	visual cortex
5-HT	serotonin

1. Introduction

Ethanol (alcohol) is one of the most used and abusive drugs in the world. Ethanol use alters various aspects of information processing, cognitive function, and behavior that can increase the risk for alcohol-related accidents. Alcohol has a strong impact on the central nervous system function because ethanol is not a molecule with a single clear effect on a particular neurotransmitter system, but it may affect multiple stages of the neurotransmission cascade of most neurotransmitters. Moreover, the effect of ethanol depends on its consumption pattern. Acute alcohol consumption interferes with the transduction processes (Vengeliene et al. 2008; Little 1999), sensory information processing (J. L. Kenemans et al. 2010; Kunchulia et al. 2012; Skalka et al. 1986; Maurage et al. 2012; Chen et al. 2010; Johnston & Timney 2008; Yu et al. 2006; Donnelly & Miller 1995; Zhuang et al. 2012) and is related with the effect of ethanol to the neuronal elements, such as receptors. On the other hand, long-term alcohol consumption causes neuronal death, long term anatomical brain changes (Rintala et al. 1997; Thomas et al. 1998; Lukoyanov et al. 2000; Pfefferbaum et al. 1997; Crews 2006) and brain plasticity (Gutierrez & Heinemenn 1999; Steward et al. 1990; Tenkova 2003; Lukoyanov et al. 2000; Pinazo-Duran & Strömland 1994; Medina & Krahe 2008; Medina & Ramoa 2005). All these effects combined produce the withdrawal symptoms and the development of addiction.

The visual system is one of the most sensitive systems to the effects of ethanol. Various visual functions at different visual system levels are affected by acute alcohol intake such as spatial-frequency discrimination (Watten et al. 1998), depth perception (Hill & Toffolon 1990), visual acuity (Wilson & Mitchell 1983), dynamic visual acuity (Brown et al. 1975), contrast discrimination (Donnelly & Miller 1995; Pearson & Timney 1999), night-vision discrimination (Castro et al. 2014), lateral inhibition (Johnston & Timney 2008) and information processing (Tzambazis & Stough 2000). Long-term alcohol consumption causes neuronal death at the level of retina (Johnsen-Soriano *et al*. 2007; Sancho-Tello *et al*. 2008), optic nerve (Johnsen-Soriano et al. 2007), lateral geniculate nucleus (LGN) (Carmona *et al*. 1994) and visual cortex (VC) (Tenkova *et al*. 2003) and is related with disorders and modulation of sensory information processing at different levels in the visual system. Although previous studies have showed that acute or chronic alcohol consumption and withdrawal have a different impact to different visual stimulus processing stages at the level of retina and visual cortex, there are some unanswered questions. First, there is no information addressed to the

effect of chronic alcohol consumption and withdrawal on the function of LGN, and there is only one prior report that study which investigated acute alcohol effects to this nucleus. The LGN is a relay nucleus that transfers information from retina to the VC and is strongly modulated by descending projections from the VC (Wang *et al*. 2018). VC and LGN modulate each other functions. In order to understand the effect of alcohol to the visual system it is important to know how alcohol modulates different visual system levels. The second problem is minuteness of published studies. In chronic alcohol consumption or withdrawal studies, the alcohol consumption and/or withdrawal time are relatively short – up to one month of alcohol consumption (Bierley et al. 1980) and up to two week of withdrawal (Sokomba & Osuide 1985). That is leaving an open question on how long-term alcohol consumption (more than one month) and withdrawal (more than two weeks) changes the visual system function.

One of the methods used to characterize the functional effects of drugs and toxic substances on the visual system is visual evoked potentials (VEP). The individual VEP components reflect the various neural pathways that are activated by a stimulus. VEP is a tool with a great temporal resolution for investigating the function and pathophysiology of the visual system, including the visual cortex and visual pathway. VEP enables researchers to dissect and investigate processing of temporal information at the different processing stages (Hetzler & Bauer 2013; Fisch & Spehlmann 1999).

Perception of stimulus onset (ON) and offset (OFF) or stimulus duration is one of the fundamental functions in temporal information processing. General reaction time, which is composed from the sensory (processing of stimulus), central (decision making) and motor (execution of decision) phases (Posner 2005; Nicolas 1997), is shorter for the stimulus onset than offset (Di Lollo et al. 2000). More detailed studies revealed that stimuli (500 ms and more) generate two fully separated brain responses to stimulus onset (ON) and offset (OFF) and this has been observed in several sensory systems: visual (Bair et al. 2002; Wilson 1983), auditory (Hari et al. 1987; Qin et al. 2007; Yamashiro et al. 2009; Baba et al. 2016), and somatosensory (Spackman *et al*. 2006; Yamashiro *et al*. 2008). Also, these studies showed differences between responses to stimulus onset and offset in different modalities and some of them at different information processing stages, implying that processing of stimulus onset and offset could be based on different neuronal mechanisms and, correspondingly, could be differently modulated by biologically active compounds. Insufficient information processing in one of these responses might alter the reaction time and be

critically important for survival. In visual system it is still unclear the origin of the ON and OFF responses and which brain structures and information processing stages are responsible for the differences of the ON and OFF responses. Although previous studies have showed that acute and chronic alcohol consumption reduces amplitude and increases latency of response to flash stimulus and acute abstinence is related with hyper excitability of visual system, it is unknown how alcohol consumption modulates separated responses to stimulus onset and offset. Changes in the information processing chain in the ON or OFF responses might cause the changes in perception of stimulus parameters and alter the reaction time.

Aim

To study the effects of acute and chronic alcohol consumption and withdrawal on the rat visual cortex and lateral geniculate nucleus responses to visual stimulus onset and offset.

Objectives

- To investigate asymmetry of latency and amplitude between the ON and OFF responses in the visual cortex and lateral geniculate nucleus of alcohol-naive rat;
- To investigate the effect of acute alcohol consumption on the ON and OFF responses in the rat visual cortex;
- To investigate the effect of chronic alcohol consumption and withdrawal on the ON and OFF responses in rat visual cortex;
- To investigate the effect of chronic alcohol consumption and withdrawal on the ON and OFF responses in rat lateral geniculate nucleus.

Scientific novelty

Both parts of this study were designed to investigate the temporal information processing from the visual system and the effects that alcohol has on it. For the first time we studied separated responses, elicited by stimulus onset and offset, of the visual system and demonstrated that at the level of neuronal population the activation of the visual system during the visual stimulus onset is qualitatively different from that during the offset. The differences between these responses are noticeable at the cortical level in anesthetized and awake animals and are differently modulated by alcohol.

We showed that different stages of the temporal information processing in the visual system are differently modulated by acute and chronic alcohol consumption and withdrawal.

The first part of the study revealed that anesthesia alone or together with alcohol had a stronger effect on sensory responses to stimulus onset than offset. At cortical level sevoflurane anesthesia increased the latency from both stimuli, ON and OFF, responses in a similar manner, except for the last stage of stimulus processing: the latency of the last VEP component during OFF response was not affected by anesthesia or ethanol. Although, acute ethanol consumption reduced the amplitude of OFF, but not ON response.

The second part of the research showed that in awake freely moving rats long-term chronic alcohol consumption and abstinence had a strong long-term and, in some cases, irreversible impact on the visual information processing. Both of these conditions modulated only the last stage of stimulus onset processing at the level of VC, but not at the level of LGN. Response to the stimulus offset was more susceptible to the effect of alcohol consumption and/or abstinence and was modulated at both VC and LGN levels.

Together, these discoveries indicate that alcohol modulated the information processing chain at different stages, which can result in insufficient processing of parameters of visual stimuli and can lead to changes in perception of stimulus duration and intensity.

Defending statements

1. Amplitude and latency of visual evoked potentials (response) to visual stimulus onset was higher and shorter than responses to stimulus offset.
2. Biologically active compounds (ethanol and/or sevoflurane) modulated visual cortex responses to visual stimulus onset differently than to offset.
3. Chronic alcohol consumption and withdrawal modulated responses to stimulus onset at the level of visual cortex, but not lateral geniculate nucleus level.
4. Chronic alcohol consumption and withdrawal modulated responses to stimulus offset at visual cortex and lateral geniculate nucleus levels.
5. Responses to stimulus offset were more susceptible to the effect of chronic alcohol consumption and/or withdrawal as compared to responses to stimulus onset.

2. Literature overview

The time scale of processes in the brain changes from milliseconds to minutes. Communication between neurons occurs through electro-chemical signals within milliseconds. In order to record and analyze these signals researches have to use methods with a high temporal resolution. One of the methods to measure changes in neural activity at the millisecond level is electroencephalography (EEG) and event-related potentials (ERP). EEG is the recording of the spontaneous extracellular electrical field potentials generated by excitatory and inhibitory postsynaptic potentials on dendrites and neuronal cell bodies of the vertically orientated pyramidal neurons (Bucci & Galderisi 2011). Action potentials have higher amplitude but last too short time. Synaptic potentials have a longer duration (tens of milliseconds) and involve a larger membrane surface, this increases the probability to occur with a temporal overlap. Both of these features allow temporal and spatial summation of recorded potentials (Bucci & Galderisi 2011).

2.1 Visual evoked potentials

Changes in spontaneous activity of EEG triggered by stimuli and particularly by visual stimuli – are called VEP. Evoked potentials are generated by activation of specific neuronal population and is time-locked to a stimulus (Fisch & Spehlmann 1999).

VEP are a suitable tool for investigating the function and pathophysiology of visual systems, including the VC and visual pathway. An intact visual pathway, from the retina to the primary visual cortex, is needed to obtain a normal VEP. On the other hand, any factor, e.g. disease or biologically active compounds, that alters the central visual pathway function will affect the VEP response (Ridder & Nusinowitz 2006). VEP are a measure of physiological function, individual VEP components reflect neural pathways that are activated by a stimulus (Hetzler & Bauer 2013). VEP are usually described in terms of the amplitudes and latencies of their characteristic waves. Individual VEP are a small amplitude signals that are buried in noise, so responses to 100–200 stimuli are averaged and amplified to measure amplitude and latency of the peaks in waveform (Fisch & Spehlmann 1999). Latency is the delay between occurrence of the stimulus and the peak of component and represents temporal characteristic of information processing. While amplitude represents the neuronal activity or arousal of the structures involved in the generation of particular component and can be calculated by

two methods: peak-to-peak or baseline-to-peak, where baseline is an average of potential before stimulation (Hetzler et al. 2008).

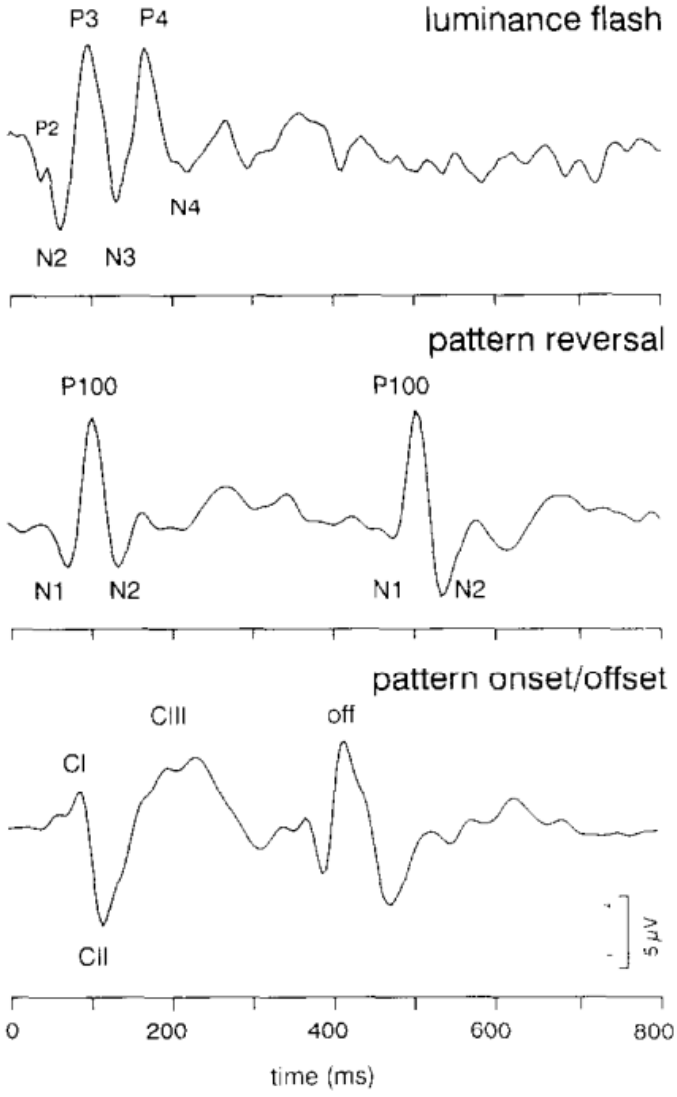


Fig. 2.1. Examples of three VEP types recorded from a human adult patient as response to flash (upper trace), pattern reversal (center trace) and pattern onset/offset (lower trace) stimulation (adapted from (Apkarian 1994)).

For VEP recordings three types of stimuli are usually used: pattern reversal, pattern onset/offset and flash (see Fig. 2.1). Pattern reversal stimulus consists of black and white checks or gratings which abruptly alternate. The responses to these stimuli consist of negative and positive peaks, that are

marked by polarity and mean latency. Stimuli for pattern onset/offset have similar parameters to those for pattern reversal and the VEP are recorded to the onset and offset of the pattern (Harding et al. 1995). These two types of stimuli are the preferred type for most clinical purposes, since occipital cortex is sensitive to the perception of edges, and a sharp-bordered checkerboard produces a strong and measurable response (Odom et al. 2010). But both of these methods require maintaining visual fixation to the center of the pattern.

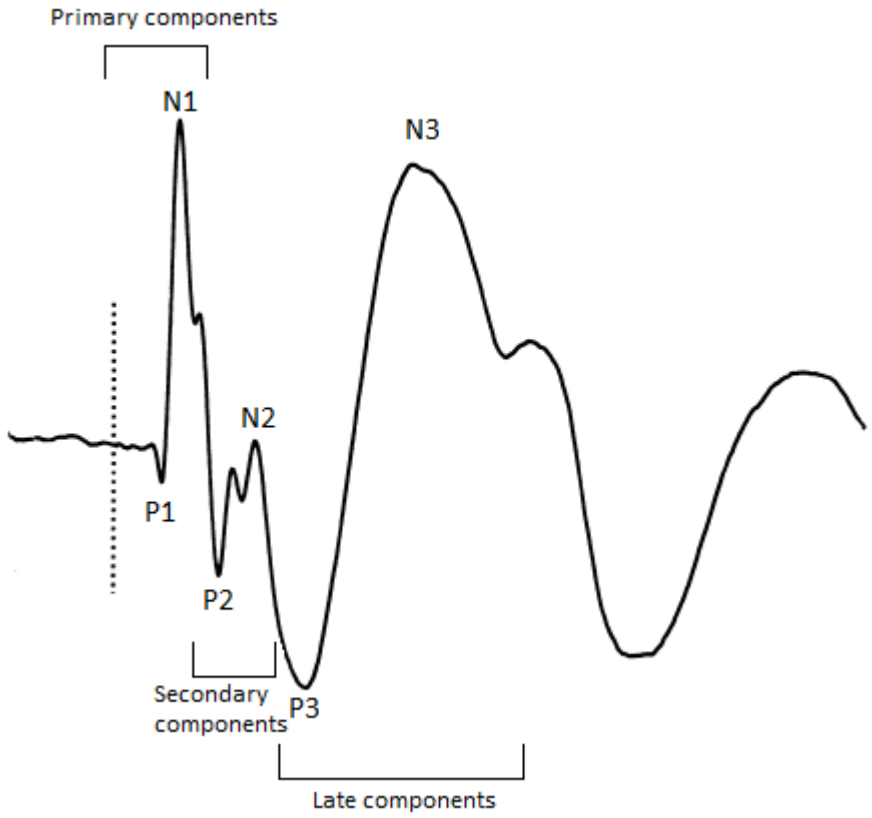


Fig. 2.2. Rat's visual cortex flash-evoked potential (FEP) waveform can be divided into primary (P1 and N1), secondary (P2 and N2) and late (P3 and N3) components. Individual FEP components are named by polarity and succession. P1, P2, and P3 are positive-going components, whereas N1, N2, and N3 are negative-going components. Dotted line represents onset of the evoking stimulus (adapted from (Hetzler & Bednarek 2001)).

Alternatively, stimulus can be flashing light in sequence or alternating intensities of light. Usually flash visual evoked potentials (FVEP) are induced by light flash (up to 5 ms) and require minimal co-operation of the participant

(no need to fix the gaze). The FVEP are useful when poor optical quality, poor cooperation or poor vision makes the use of pattern stimulation inappropriate (Odom et al. 2010) and commonly is used in vision research using freely moving animal models. In humans VEP are usually elicited by a flash that subtends a visual field by at least 20 degrees (Odom et al. 2010), but for studies using rats it is more appropriate to use a full-field stimulus, considering that rat photoreceptors are distributed more peripherally than in the human retina (You et al. 2011a). FVEP consist of complex series of negative and positive waves, the nomenclature of peaks is designated as negative and positive in a numeric sequence (see Figs 2.2 and 2.3b).

In general, the values of VEP components parameters depend on various physical and physiological factors: intensity (Creel et al. 1974) (Fig. 2.3A), contrast (Tobimatsu & Celesia 2006), size (Korth & Nguyen 1997), color (Rabin et al. 1994), type of stimulus (Plant et al. 1983; Aine et al. 1995), frequency of stimulation (Regan 1978), emotional content of the stimulus (Young et al. 2012), electrode placement (Guthkelch et al. 1987), type of electrodes (You et al. 2011a), scalp thickness, size of the pupil (Hawkes & Stow 1981), the location of the stimulus field, visual acuity (Regan 1978); state of dark adaptation, mental activity, fatigue (Young et al. 2012), attention (Aine et al. 1995), age (Allison et al. 1983; Celesla et al. 1987), sex (Stockard et al. 1979; Celesla et al. 1987), body temperature (Phurailatpam 2014). It is important to note that variations in the shape and timing of the rat's VEP waveform can be attributed to different anesthesia methods, rat strains and electrode configurations (You et al. 2011a) (Fig. 2.3b).

Currently visual evoked potential analysis is used for the diagnostics of diseases in humans and for preclinical research, e.g. fundamental research, drug testing. In studies involving animal models, it is important to know the origin of the VEP components. Although sources of VEP components are not well known, some studies provide data-based assumptions. Mouse study showed that the light-adapted VEP results from cone activity and the dark-adapted VEP results from rod activity. Moreover, if the visual signal is not transmitted through the optic nerve, no VEP will be recorded, even when electrical activity is produced in the retina. This was shown using Nob3 mutant mice for which signal transmission from photoreceptors to upstream cells has been disrupted. Thus, the VEP is not the result of the passive flow of electrical charge produced by retinal activity (Ridder & Nusinowitz 2006).

The cortical FVEPs of rats, similarly to humans, show positive and negative deflections, which are designated as the P1, N1, P2, N2, P3 and N3 components (You et al. 2011a) and can be divided into early, including

primary and secondary, and late components (Bigler 1977) (see Fig. 2.2). The primary components P1 and N1 are directly related to sensory information processing and affected by excitation of the primary VC via retino-geniculate fibers, secondary components result from superior colliculus, brain stem and diffuse thalamic projections. In general, early components (primary and secondary) of VEP are the result of retino-geniculo-striate activity (Eells & Wilkison 1989). Late components are associated with higher visual areas (Creel et al. 1974; Bigler 1977). It was shown that the latencies of the first two major early peaks (N1 and P2) are more reproducible compared to the late waves. The reason for the larger variability in the later VEP components could be its susceptibility to the activity of higher cortical levels, while the early waves are not affected (You et al. 2011a). The late components (two last components of VEP) are elicited by the first wave of photically evoked after-discharge bursts and represent the thalamo-cortical circuit (Shearer et al. 1976). This photically evoked after-discharge burst is produced in the LGN (Bigler & Eidelberg 1976).

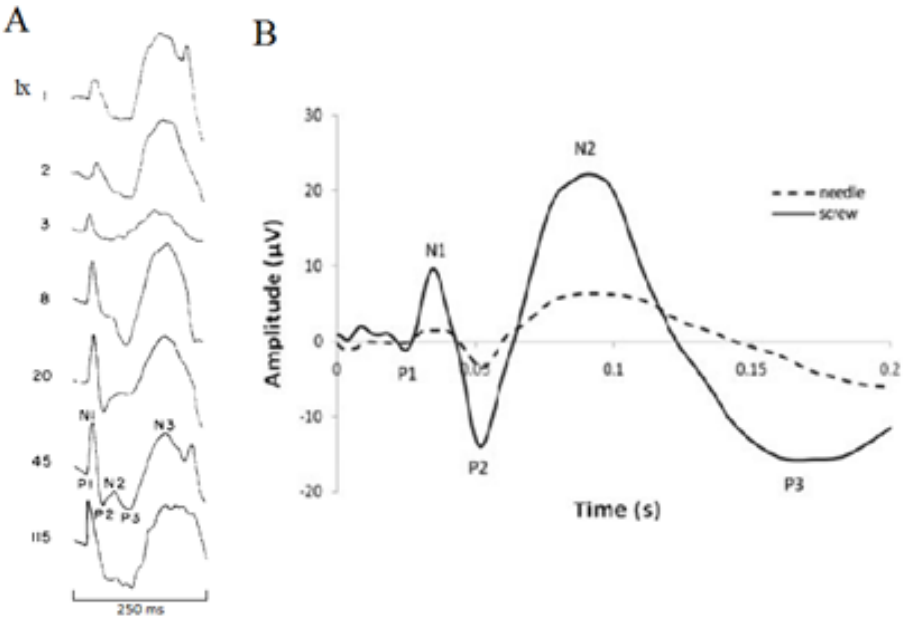


Fig. 2.3. Various factors modulate the parameters of VEP components: A) intensity of stimulus (adapted from (Creel et al. 1974)) and B) type of electrode (adapted from (You et al. 2011a)).

Although the exact sources of each VEP component from the rat are still debatable, published data gave us some information about generators of rat's VEP components. More specifically, N_{29} (P1) represents the geniculocortical synaptic processes in layer IV of the VC (Meeren et al. 1998). Latency delay of this component correlated to the demyelination in optic nerve and amplitude decrease correlated with axonal loss (You et al. 2011b). P_{46} (N1) represents inhibitory postsynaptic potentials on the pyramidal cells of cortical layers V and VI (Meeren et al. 1998). N_{63} (P2) reflects intracortical or subcortical input, but not direct dLGN, to the VC. P_{89} (N2), the last secondary component, results from connections between the superior colliculus, brain stem and diffuse thalamic projections (Creel et al. 1974). N_{143} (P3) represents secondary activation of cortical pyramidal cells (Meeren et al. 1998) and can be related to a sensitization-like phenomenon (Herr et al. 1991). P_{237} (N3) is the second wave in the after-discharge (Mwanza et al. 2008).

VEP also can be recorded in other structures of visual system such as superior colliculus or LGN. VEP components in these structures are named similarly to those of VC, by polarity and succession (see Fig. 2.4).

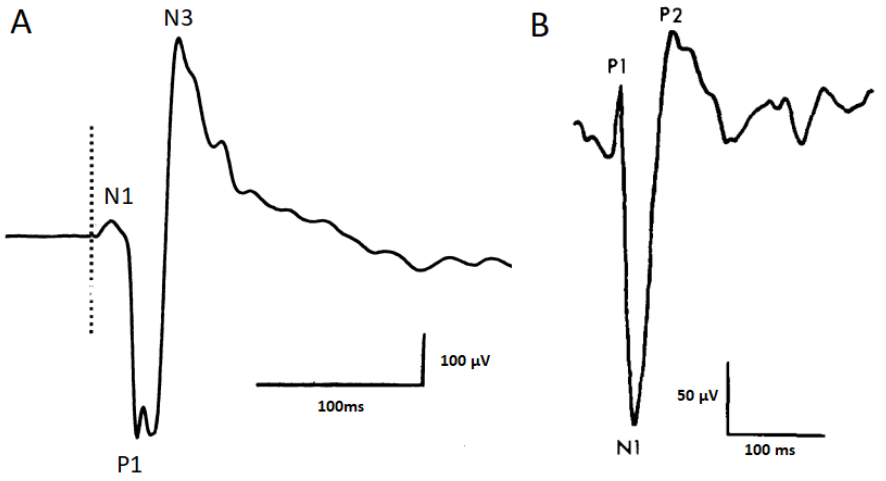


Fig. 2.4. Example of visual evoked potentials recorded in A) superior colliculus (adapted from (Hetzler & Bednarek 2001)) and B) lateral geniculate nucleus (adapted from Hetzler et al. 1983). Traces represent an average of 100 responses. Components marked by polarity and succession.

2.2 Responses to the stimulus onset and offset

Perception of stimulus onset and offset or duration is one of the fundamental functions in temporal information processing. Responses to stimulus onset and offset are crucial to organisms, living in a dynamic environment, as insufficient information processing in one of these responses might alter the reaction time and be critically important for survival.

2.2.1 Reaction time to the stimulus onset and offset

Almost twenty years ago researchers showed that the human reaction time to stimulus onset is shorter than to offset (Di Lollo et al. 2000). This phenomenon is called *onset advantage* (Di Lollo et al. 2000). Another study showed that offset reaction time was increased for attended compared to unattended stimuli, suggesting that visual attention enhances the activation of the parvocellular system and increases offset reaction time (Rolke et al. 2006). Similar onset advantage is noticed in visual motion detection, i.e. it takes longer to detect termination of motion than its start (Kreegipuu & Allik 2007). Onset advantage could be explained by a few theories. One theory is based on visible persistence, which implies that for a brief period of time the stimulus remains visible after the physical stimulus has been turned off (Briggs & Kinsbourne 1972). In this way visible persistence indirectly extends the phenomenal duration of the stimulus. In other words: in case of stimulus offset visible persistence delays the perception of the drop of stimulus intensity (Di Lollo et al. 2000).

Another theory claimed that the appearance of an object has different attentional consequences compared with the disappearance of stimulus, and that the stimulus onset reflects a more potent perceptual event since sudden appearance of an object requires the creation of a new visual object representation (Yantis & Jonides 1996). But in general reaction time is composed from the sensory (processing of stimulus), central (decision making) and motor (execution of decision) phases (Posner 2005; Nicolas 1997). Studies of reaction time are still looking for answers to the questions: which phase of reaction time and brain structures are responsible for different responses to stimulus onset and offset; how responses to stimulus onset and offset are modulated in our daily life.

2.2.2 Responses to stimulus onset and offset in somatosensory and auditory systems

More detailed studies, which eliminated motor phase of reaction time, showed that longer stimuli, 500 ms and greater, generate two fully separated brain responses to stimulus onset and offset. Separation of these two responses has been observed in several sensory systems: visual (Bair et al. 2002; Wilson 1983); auditory (Hari et al. 1987; Qin et al. 2007; Yamashiro et al. 2009; Baba et al. 2016) (Fig. 2.5); and somatosensory (Spackman *et al*. 2006; Yamashiro *et al*. 2008). Moreover, differences between ON and OFF responses are not unique to visual system, and they are demonstrated in other sensory systems as well.

Single cells study revealed two types of neurons in somatosensory cortex: those which responded only to stimulus change (onset and offset), named rapidly adapting neurons; and those which continued to respond to steady stimulation, named slowly adapting neurons (Sur et al. 1984). Investigation of somatosensory evoked potentials showed that in ON response latency of P100 and N140 components did not differ changing interstimulus intervals (ISI). In response to stimulus offset the latency from both components was longer for the longer ISI. Moreover, $P100_{off}$ was longer than the $P100_{on}$ (Yamashiro et al. 2008).

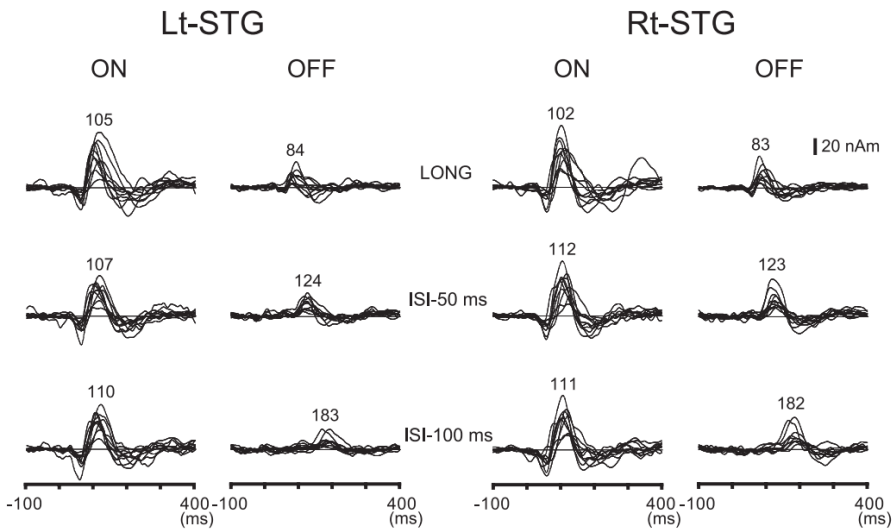


Fig. 2.5. Superimposed waveforms of the responses evoked by the onset and offset of auditory stimulus in the left and right superior temporal gyrus (STG)

of 10 subjects. ISI, interstimulus intervals. Adapted from Yamashiro et al. 2009.

In auditory cortex N1 is an automatic response to an auditory change (Yamashiro et al. 2009). The location of activity source and amplitude of this component were not different between ON and OFF responses, but latency of OFF N1 was determined by ISI (Hari et al. 1987; Yamashiro et al. 2009). Comparison of ON and OFF responses with short, 14-24 ms, ISI demonstrated that auditory $N1_{off}$ is shorter than the $N1_{on}$ (Noda et al. 1998). But when ISI is longer there is no difference in the latency of auditory N1 component elicited by stimulus onset and offset (Yamashiro et al. 2009). Also OFF responses in single cell showed similar peak latency, 19.5 vs. 21.5 ms, but lower peak firing, 20.4 vs 35.9 spikes/s, and longer half-decay time, 74.5 vs. 48.5 ms, compared with onset response (Qin et al. 2007). Thus, as it shown in previous studies, ON and OFF responses may vary between sensory systems and evoked potentials components.

2.2.3 Responses to stimulus onset and offset in vision system

Studies investigating the processing of stimuli onset and offset in the visual system provided information which contradicts the results of the reaction time studies. In general, the visual system is more sensitive to decrements than to increments in light intensity (Bowen et al. 1989). Moreover, it has been shown that in humans OFF responses have a more stable wave form than the ON responses, but stimulus intensity differently modulates individual peak latency: in the ON response variability of the latency is low when the stimulus intensity is high (20,000 lx), in the OFF response – when the stimulus intensity is low (10,000 lx) (Sato 2016). Single cell studies also gave an advantage to the offset response: macaque's LGN, V1 and V5 cells took longer to turn on (increase firing rate) than to turn off, and onset latency was more varied and depended on the stimulus as compared to the offset latency (Bair et al. 2002) (see Fig. 2.6). These studies suggest that the OFF response is less variable and the offset event acts as a more reliable timing cue to the visual system than the onset event (Tadin *et al*. 2010; Sato 2016). On the other hand, the amplitude (firing rate) of OFF response varied as a function of stimulus duration in one-third of the cat visual cortex cells, but not in the lateral geniculate nucleus, indicating that at the level of visual cortex OFF responses are able to code the duration of stimulus (Duysens et al. 1996).

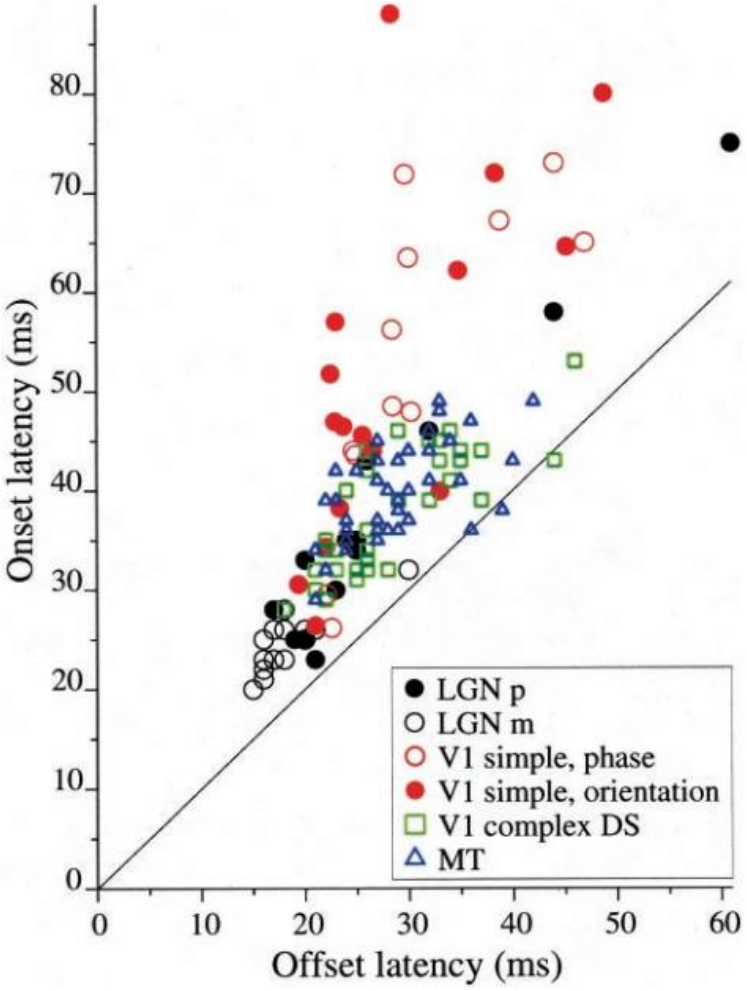


Fig. 2.6. A comparison of onset and offset latencies across cell types and stimulus categories in macaque monkeys. Nearly all points fell above the diagonal line of equality, indicating that onset latency is longer than offset latency. LGN p and LGN m represents cells of lateral geniculate nucleus parvocellular and magnocellular layers, V1 – cells of primary visual cortex, MT – cells of motor cortex. Adapted from Bair et al. 2002.

As mentioned above, stimulus duration is crucial for the separation of ON and OFF responses. It was noticed in 1970 that perceptual onset latency is independent of stimulus duration but offset latency is constant just for stimuli longer than 130 ms and becomes longer as the stimulus shortens (Efron 1970). Also, decreasing the stimulus duration from 1260 ms to 20 ms has no

effect on recognition of the stimulus onset, but decreases the perceptibility of stimulus offset (Wilson 1983). Using the evoked potential methodology and varying the stimulus duration allowed for the separation of components related to the onset and offset of illumination. The ERP study indicated that neither P1 nor N1 amplitudes nor latencies of gamma-band ON response (GBR) are affected by stimulus duration, 50, 150 and 250 ms. Meantime, the evoked gamma-band OFF response appears approximately 100 ms after stimulus offset and does not depend on stimulus duration. For the shortest stimulus duration evoked ON and OFF responses merge together, resulting in a larger evoked OFF response peak (Busch et al. 2004) (see Fig. 2.7). A more detailed study showed that the latencies of the earlier prominent components, *i*50 (intracortical feature at 50-80 ms after stimulus), P100, and N125, are not affected by stimulus duration and are therefore responses to stimulus onset. Only the P200 component peaks at a time that increases linearly with the stimulus duration and is thought to be related to the offset of illumination (Padnick & Linsenmeier 1999).

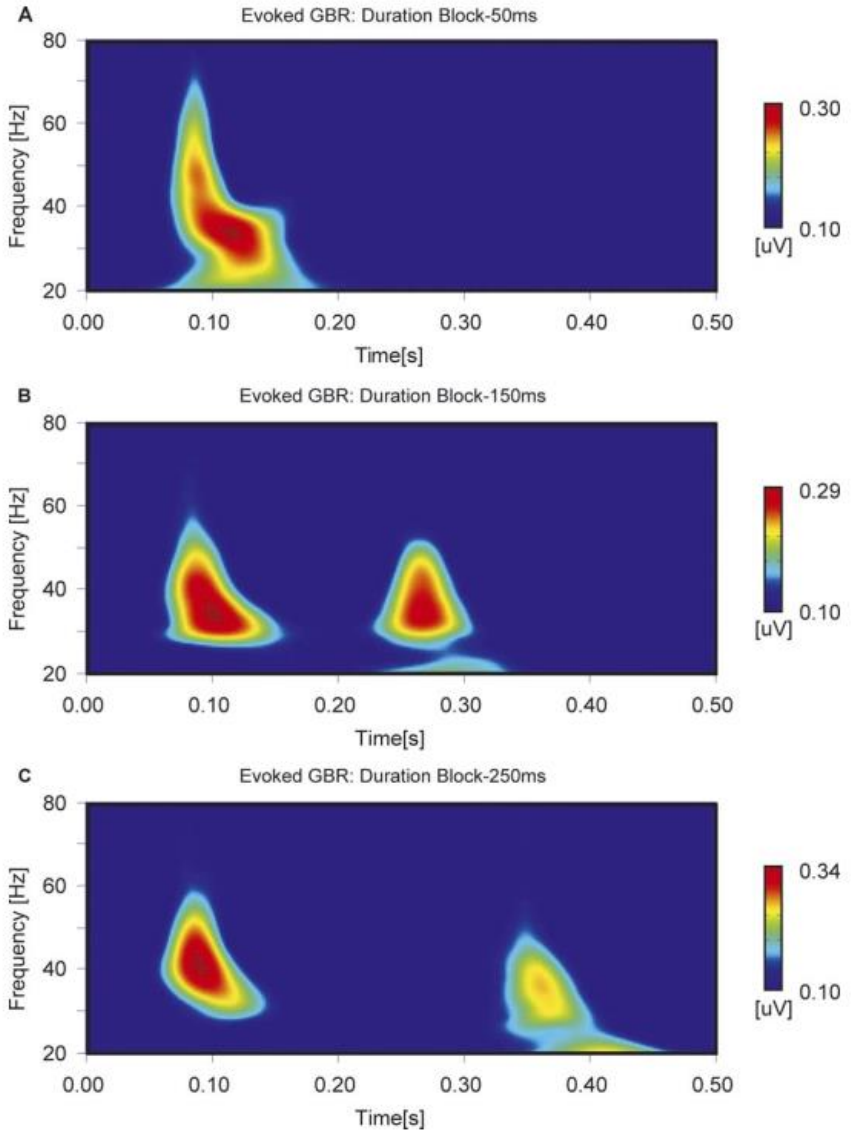


Fig. 2.7. Time – frequency plot for the 3 stimulus durations A) 50, B) 150 and C) 250 ms at electrode E34. ON and OFF responses merged together for the shortest stimulus duration, while in the other conditions OFF response clearly distinguishable. Adapted from Busch et al. 2004.

The onset-offset asymmetry could be explained by potentially different neuronal mechanisms involved in the generation of ON and OFF responses. Onset-offset asymmetries have been described in different animal species at different levels of the visual system. In flies, two anatomically

different pathways for light-on and light-off in the layers of the medulla were shown by Strother et al. (Strother et al. 2014). In mammals, visual signals are processed through the functionally separated ON and OFF channels, which do not interact before converging in the primary visual cortex (Schiller 1992). ON channel is sensitive to light incremental stimuli and signals lightness, whereas the OFF channel is sensitive to light decrement and signals darkness (Schiller 2010). Scientists suggest that the mammalian visual system has both ON and OFF channels to have equally sensitivity and rapid information transfer for decrement and increment of light stimuli and to facilitate high contrast sensitivity (Schiller et al. 1986). But do these morphologically and neurochemically distinct subsystems have the same temporal properties and can this be a reason for the differences in responses to stimulus onset and offset?

Several studies showed that the ON and OFF pathways are not simply mirror images of each other with opposite polarities in response to positive and negative contrast. In humans the asymmetry between the ON and OFF subsystems was revealed using the evoked potential method: OFF subsystems have finer spatial tuning and greater contrast gain than ON subsystems (Zemon et al. 1988). Moreover Viva et al. (2006) showed that white dots are processed faster than black dots by the human visual system, by about 3 ms and the explanation for this is that human ON channels are faster than OFF channels. This assumption is also supported by primate research. Primate retinal ON-center cells have larger receptive fields and faster responses compared to OFF-center cells, whereas OFF cells have more strongly rectifying responses that provide little information about contrast increments (Chichilnisky & Kalmar 2002). But other studies investigating ON and OFF pathways at separated visual system levels produced contradictory results. For example, in most ON/OFF cells from tiger salamander (cells that respond transiently to both light increment and light decrement) the latency of the first spike evoked by a negative contrast step is much shorter than that evoked by a positive contrast step of equal contrast (Burkhardt et al. 1998). More detailed study in mice showed that under photopic conditions the ON and OFF ganglion cells show similar temporal characteristics, but under scotopic conditions ON cells shift their tuning to low temporal frequencies, whereas OFF cells continue to respond to high frequencies (Pandarinath et al. 2010).

In the primate visual system, information is processed by few parallel channels. One is the above mentioned ON/OFF channel, others include the magnocellular (M), parvocellular (P) and koniocellular (K) streams (Schmolesky et al. 1998). These streams begin in the retina and proceed into

the visual cortex. Temporal differences of information processing in these streams could cause differences between ON and OFF responses. One of the studies showed that, in macaque, flash visual stimulus evoked onset latencies are shorter, 17 ms on average, in the LGN M layers than in the LGN P layers, and that the cortical cells innervated by the P stream are activated later than those innervated by the M stream (Schmolesky et al. 1998). On the other hand Bair et al. (2002) did not indicated that onset or offset latency is shorter in M layers than P layers, but in both of these layers cells took a longer time to turn on than to turn off.

Comparison of simple and complex cells in the cat visual cortex showed that complex cells exhibited greater similarity in peak latency between the onset and offset responses. For simple cells, the onset response has greater peak amplitude and signal-to-noise ratio than the offset response, and for complex cells, vice versa. For both types of cells, the amplitude of offset responses increases with stimulus duration while the onset response does not (Liang et al. 2008). In primates, both, simple and complex cells, have longer onset latency than offset latency. Also, difference between onset latency and offset latency is larger for simple than complex cell (Bair et al. 2002). So, all these studies indicate that there is a temporal asymmetry between ON and OFF pathways at each level of the visual system.

But some researches argue that identification of a response component with the onset or offset of illumination in VEP components does not imply that in one case only the ON pathway, i.e. ON-center cells, and in the other case only the OFF pathway generate these components. The pathways involved cannot be determined from VEP data, because ON and OFF cells both exhibit changes in firing at the onset and offset of illumination, and any change could give rise to a VEP component (Padnick & Linsenmeier 1999).

The above discussed studies demonstrate asymmetry of onset and offset responses in different modalities and some of them at different information processing stages. The majority of research on ON and OFF responses in visual system used single cell models, so these results are based on cell properties. Information about single neuron functioning is valuable, but in our everyday life, stimulus covering receptive fields of multiple cells and the brain works as a network. So, it is important to know how the signals are processed at the level of the neural population. In the visual system it is still unclear the origin of ON and OFF responses and which brain structures and information processing stages are responsible for differences on the ON and OFF responses.

2.3 Effect of acute and chronic alcohol consumption and withdrawal to the visual system

Ethanol is one of the most used and abusive drugs in the world. According to the World Health Organization during the year each inhabitant consumes on average 6.2 liters of alcohol (World Health Organisation 2014). Such a scale of alcohol consumption causes a number of health, social and economic problems. For example, the number of alcohol – related diseases and injuries is 139 million per year (World Health Organisation 2014). The economical consequences of alcohol consumption over the years for the European Union countries in 2002 cost was 125 billion euros, and for the United States in 2006 was 233.5 billion dollars (World Health Organisation 2014). Such a terrifying statistics is because alcohol is altering the function of almost all systems of the organism, first of all, the central nervous system. It interferes with the transduction processes (Vengeliene et al. 2008; Little 1999), anatomical brain changes (Rintala et al. 1997; Thomas et al. 1998; Lukoyanov et al. 2000; Pfefferbaum et al. 1997; Crews 2006), brain plasticity (Gutierrez & Heinemenn 1999; Steward et al. 1990; Tenkova 2003; Lukoyanov et al. 2000; Pinazo-Duran & Strömland 1994; Medina & Krahe 2008; Medina & Ramoa 2005), and sensory information processing (J. L. Kenemans et al. 2010; Kunchulia et al. 2012; Skalka et al. 1986; Maurage et al. 2012; Chen et al. 2010; Johnston & Timney 2008; Yu et al. 2006; Donnelly & Miller 1995; Zhuang et al. 2012).

2.3.1 Mechanisms of ethanol effects on the brain

In general, ethanol alters the brain, especially the visual system, by two different mechanisms, leading to different consequences. Acute alcohol consumption disrupts functioning of the brain for the short time and is more related with the effects of ethanol to the neuronal elements, like receptors. Meantime chronic alcohol consumption causes neuronal death and a long-term changes in the brain structures.

A single dose of alcohol interacts with a specific neuronal membrane proteins involved in signal transmission. Now it is known that ethanol directly or indirectly modulates activity of transcription factors in the nucleus, cytosolic signaling elements and membrane receptors (see Fig. 2.8) such as: voltage-sensitive sodium and calcium channels (Little 1999); n-methyl-d-aspartic acid (NMDA) (Carpenter-Hyland et al. 2004); gamma-aminobutyric acid (GABA) (Wallner et al. 2006; Vengeliene et al. 2008); nicotinic acetylcholine (nACh) (Vengeliene et al. 2008; Little 1999); serotonin (5-HT)

receptors (Vengeliene et al. 2008; Little 1999); cannabinoid Receptor 1 (CB1), neuropeptide Y (NPY); corticotrophin-releasing factor (CRF); protein kinase C and A; and cyclic adenosine monophosphate (cAMP) (Most et al. 2014; Erdozain & Callado 2014). After consumption, alcohol is absorbed into the blood stream and is distributed within body fluids at the same concentration in all the tissues, including nervous system. However, the above mentioned neuronal elements are not homogenously distributed through the brain structures and show dose-dependent modulation pattern. The result is that the effect of alcohol on the different brain structures or cell populations is not the same, as some are more vulnerable to the effect of alcohol than others, e.g. cortical areas are more vulnerable than subcortical structures.

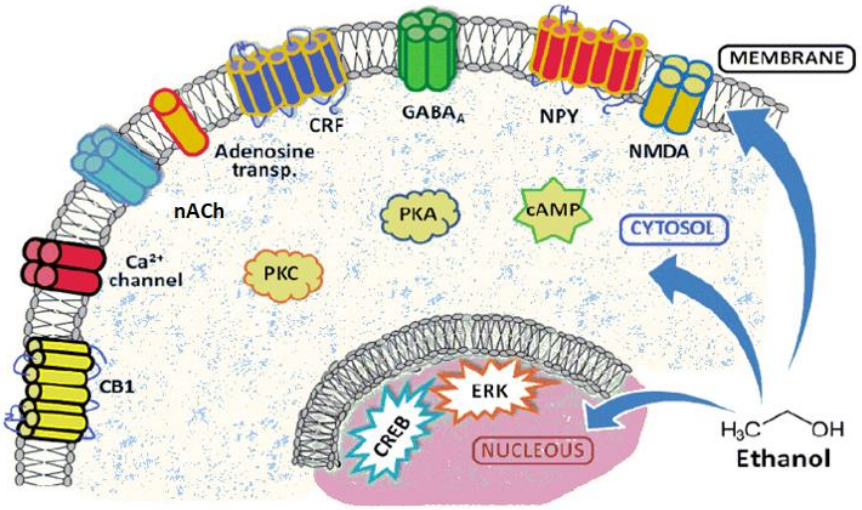
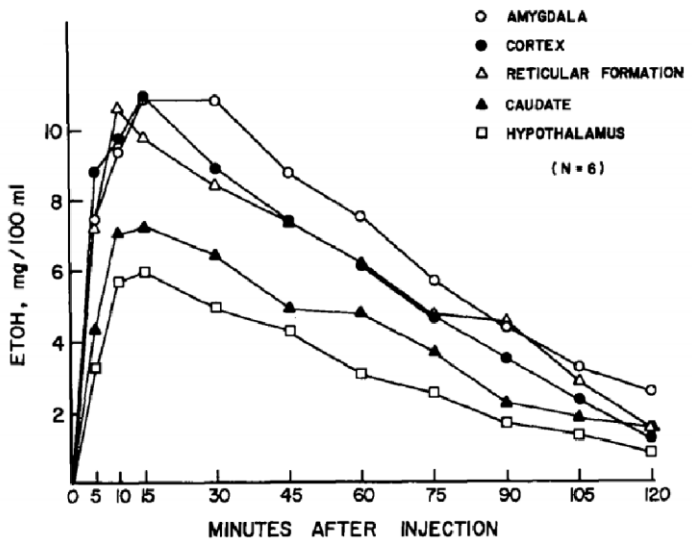


Fig. 2.8. Ethanol modulates activity of various cellular components: transcription factors in the nucleus, cytosolic signaling elements and membrane receptors. CREB: cAMP-responsive element binding protein, ERK: extracellular-signal-regulated kinase, PKC and PKA: protein kinase C and A, cAMP: cyclic adenosine monophosphate, NMDA: N-methyl-D-aspartic acid receptor, NPY: neuropeptide Y receptor, $GABA_A$: γ-amino-butyric acid receptor, CRF: corticotrophin-releasing factor receptor, nACh receptor, CB1: Cannabinoid Receptor 1. Adapted from Erdozain & Callado 2014.

The different effect of alcohol to different brain structures could be caused by several factors. The first reason could be an unequal distribution of ethanol throughout the brain. Studies have shown that ethanol does not

distribute equally to all parts of the rat brain after intraperitoneal injection of ethanol (Erickson 1976) (see Fig. 2.9 A). The second factor is the metabolism of ethanol. Although most of the ethanol is metabolized by the liver, some data shows measurable ethanol metabolism and acetaldehyde production in the brain (Zimatkin & Lindros 1996; Martínez et al. 2001; Wilson & Matschinsky 2020; Hipólito et al. 2007). The mammalian brain contains three enzyme systems for oxidizing ethanol to acetaldehyde: alcohol dehydrogenase (ADH); catalase (CAT); and cytochrome P450 2E1 (CYP2E1) (Martínez et al. 2001). These enzymes do not distribute equally in the brain (Brannan et al. 1981; Zimatkin & Lindros 1996; Martínez et al. 2001; Wilson & Matschinsky 2020; Hipólito et al. 2007). In particular, ADH1 and ADH4 mRNAs are detected in several cell types from the cerebellum, hippocampal formation and cerebral cortex (Martínez et al. 2001) (see Fig. 2.10). Brannan et al. (1981) indicated variations of CAT activity in 11 brain regions (see Fig. 2.9 B). Cytochrome P450 2E1 metabolizes ethanol to acetaldehyde and is inducible by chronic and acute ethanol exposure in brain (Hipólito et al. 2007). Cytochrome P450 2E1 was present in low basal amounts, concentrated primarily in evolutionarily older areas such as olfactory bulbs, olfactory cortex, hippocampus, cerebellum and brain stem (Hipólito et al. 2007). In general, the total amount of these three enzymes is low, but they are concentrated in small populations of the brain cells and could be a reason for different effect of ethanol to different brain structures (Wilson & Matschinsky 2020; Hipólito et al. 2007; Martínez et al. 2001).

A



B

Area	Mean activity (±S.E.M.)	No. of animals	No. of samples
Hypothalamus	3.95 (0.14)	7	38
Substantia nigra	3.38 (0.15)	7	31
Olfactory bulb	3.24 (0.21)	7	39
Cerebellar cortex	3.08 (0.12)	7	31
Corpus callosum	2.91 (0.16)	7	34
Septum	2.82 (0.04)	6	27
Hippocampus	2.57 (0.15)	6	32
Nucleus accumbens	2.44 (0.16)	7	33
Olfactory tubercle	2.22 (0.08)	3	16
Caudate-putamen	2.04 (0.08)	7	32
Frontal cortex	1.98 (0.13)	7	30

Fig. 2.9. Distribution and metabolism of ethanol in the brain. A) Perfusate ethanol levels (mg/100ml) observed for two hours in five different brain areas where the cannula was in contact with brain parenchyma (Adapted from 1976). B) Catalase activity in various brain regions (Adapted from 1981).

Region	Area	*ADH1*	*ADH4*
Cerebellum	Cortex		
	Inner granular cell layer	+	–
	Purkinje cell layer	+	+
	Outer molecular cell layer	–	–
	White matter	–	+
Inferior colliculus		–	–
Superior colliculus		–	–
Raphe nuclei		–	–
Hippocampal formation	Hippocampus (CA-fields)	+	+
	Dentate gyrus	+	+
	Hilus of dentate gyrus	+	+
	Subiculum	+	+
Thalamic nuclei		–	–
Cerebral cortex	Frontal	–	+
	Parietal	+	+
	Temporal	+	+
	Occipital	+	+
	Piriform	–	+
	White matter (corpus callosum)	–	–
Ventricular and vascular systems	Leptomeninges	+	+
	Ependymal epithelium	+	+
	Choroid plexus	+	+
	Vessels	+	+

Fig. 2.10. Distribution of ADH1 and ADH4 in adult rat CNS (Adapted from 2001).

Chronic alcohol consumption alters brain functioning through two pathways. Long term alcohol consumption, more than 4 weeks, leads to the increased expression of AMPA1 receptor subunit (Lewohl et al. 2000), reduced dopamine D2 receptors expression (Volkow et al. 2002), decreased dopamine and its metabolites in the striatum (Rothblat et al. 2001), and produced addiction to alcohol. But more importantly, alcohol induces neurotoxicity and neuronal cell death by stimulating reactive oxidative molecules (ROS), e.g. O_2^-, H_2O_2, NO. ROS are created by alcohol exposure as a natural by-product of alcohol metabolism, thus, creating an increase in oxidative stress and neuronal cell death (Alfonso-Loeches & Consuelo Guerri 2011; Chastain & Sarkar 2014) (see Fig. 2.11). Moreover, chronic alcohol consumption reduces the levels of antioxidants, and increases the stimulation of TGF-β1 apoptotic signaling (Chastain & Sarkar 2014). Also, alcohol

activates microglia, which release proinflammatory cytokines and ROS, exacerbating oxidative stress and neuroinflammation. All these events result in the increase of the proapoptotic proteins bad, bcl-xs and bak, the reduction of antiapoptotic protein levels, increased production of apoptotic enzyme caspase 3, increased neuronal death and increased phagocytosis of dead neurons by microglia (see Fig. 2.11) (Chastain & Sarkar 2014).

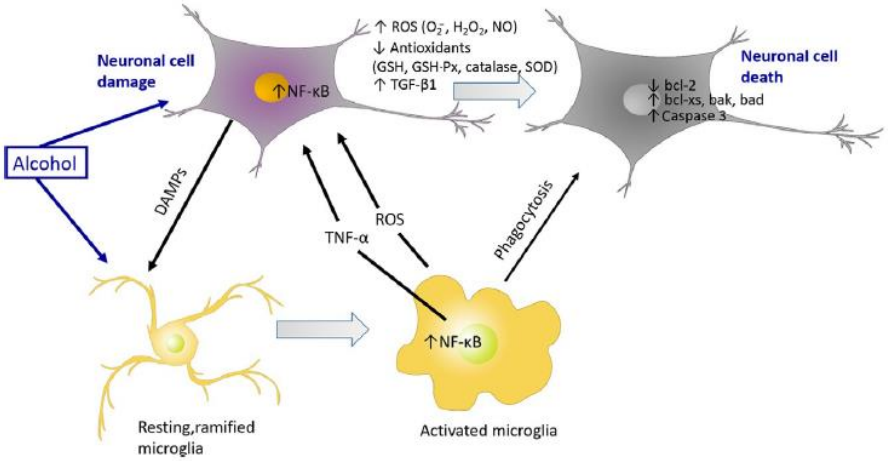


Fig. 2.11. General mechanisms by which alcohol induces neurotoxicity and neuronal cell death. Alcohol damages neurons, resulting in increased levels of reactive oxidative molecules (ROS) and stimulation of TGF-β1 apoptotic signaling, and decreased levels of antioxidants. Also, alcohol activates microglia directly and via damage-associated molecular patterns (DAMPs) resulting in increased microglial release of TNF-α and ROS, exacerbating oxidative stress and neuroinflammation. All these events result in TGF-β1 mediated increase of the proapoptotic proteins bad, bcl-xs and bak, reduced levels of the antiapoptotic protein bcl-2, increased production of the apoptotic enzyme caspase 3, increased neuronal cell death, and increased phagocytosis of dead neurons by microglia. Adapted from Chastain & Sarkar 2014.

2.3.2 Effect of acute alcohol consumption to the vision system

The physiological studies revealed effects of alcohol on various properties of the visual system reflected in changes on visual perception. Ethanol slows down information processing (Tzambazis & Stough 2000), decreases spatial-frequency discrimination (Watten et al. 1998), reduces contrast sensitivity in the magnocellular pathway and contrast gain in the parvocellular pathway (Zhuang et al. 2012), reduces depth perception (Hill & Toffolon 1990), visual

acuity (Wilson & Mitchell 1983), dynamic visual acuity (Brown et al. 1975), contrast discrimination (Donnelly & Miller 1995; Pearson & Timney 1999), night-vision discrimination capacity (Castro et al. 2014), impairs the rapid detection of unexpected changes (J. Kenemans et al. 2010) and reduces or eliminates lateral inhibition in retina (Johnston & Timney 2008). Moreover, in the visual cortex from cats, it was demonstrated a dose-depended effect of acute alcohol consumption that induces a decrease in the orientation selectivity and signal-to-noise ratio, suppression of spontaneous activity and shifts the preferred stimulus orientation and direction (Chen et al. 2010). A recent study showed that very low amount of ethanol, 1-50 mM, suppress excitatory synaptic transmission to layer 2/3 pyramidal neurons in rat visual cortex in a concentration-dependent manner (Luong et al. 2017). This is the first study that indicated the effect of low alcohol amount, from 1mM, which is equivalent to BAC of 0.005%, on synaptic transmission in the neocortex.

Prenatal alcohol consumption is an exceptional factor which has a strong impact to the visual system. Alcohol consumption during pregnancy can lead to a wide range of systemic defects and vision deficits in the offspring, varying from growth retardation, cognitive impairment to facial dysmorphism (Hiratsuka & Li 2001). These alterations are collectively referred to as Fetal Alcohol Spectrum Disorders (FASD). Children with FASD often have problems with visual processes (Pinazo-Duran & Strömland 1994). A lot of studies implicate visual system among the main targets of ethanol-induced teratogenic effects. Research performed on chick embryos showed retinal degeneration (pigment epithelium, inner plexiform layer and ganglion cells layer) and optic nerve hypoplasia in a dose-dependent manner (Tufan et al. 2007). Similarly, optic nerve hypoplasia and inhibition of photoreceptors, that was confirmed by reduction of a- and b-waves in electroretinogram, were noticed in the zebrafish model (Matsui et al. 2006). In mice pups reduced number of bipolar cells, smaller dendritic receptive field of horizontal cells, decreased amplitude of a- and b-waves in electroretinogram and deficit in contrast sensitivity were found in alcohol group compared with control group (Lantz et al. 2014; Deng et al. 2012). Moreover, it was shown that in rats and mice during synaptogenesis (at the third trimester of pregnancy) a single ethanol intoxication episode triggers apoptotic cell death of neurons at the lateral geniculate nucleus and visual cortex (Tenkova et al. 2003).

2.3.3 Effect of acute alcohol consumption to the visual evoked potentials

There are many studies on the effects of acute alcohol consumption on VEPs using short (up to few ms) light flashes, but none using long stimuli enabling separation of responses to stimulus onset and offset. One of the first experiments was made in 1972 by Begleiter et al. showing that effect of alcohol is stronger on visual cortex than on reticular formation (Begleiter et al. 1972). Later it was shown, that cortical structures are more susceptible to the depressant effects of alcohol than subcortical structures (Begleiter & Coltrera 1975). But these experiments did not reveal any effect of alcohol on the VEP late components. More detailed analysis showed that in general acute alcohol application decreased the amplitude of N_{29}, N_{39}, P_{89}, N_{143} and P_{237} components, but did not affect components P_{23} and N_{63}, and increased amplitude of component P_{46} (Hetzler et al. 1981; Hetzler & Ondracek 2007; Hetzler & Martin 2006; Hetzler et al. 2008; Hetzler & Bauer 2013). Moreover, the effects on the amplitudes of VEP components were dose- dependent. Early components were depressed only by alcohol dose of 2.5 g/kg, while late components were affected by 1.5 and 2.5 g/kg doses (Hetzler et al. 1983). Meanwhile, the effect of alcohol on the latency of VEP components is still questioned. It has been shown that alcohol increases the latency of components P_{22}, N_{29} and N_{53} (Hetzler & Bednarek 2001; Hetzler & Martin 2006), but these changes can be caused by hypothermia, since VEP latency can increase with decreasing animal temperature (Hetzler et al. 1988). Alcohol has a vasodilating effect which can result in hypothermia.

Acute alcohol consumption also altered the VEP components in LGN. Component P1 was depressed only by 2.5 g/kg alcohol dose, N1 by 1.5 and 2.5 g/kg doses and P2 by 1, 1.5 and 2.5 g/kg doses. Similarly, latency of component P1 was increased by the two largest doses of alcohol, while of component N1 only by 2.5 g/kg (Hetzler et al. 1983). These results were unexpected, because it is thought that cortical structures are more susceptible to depressant effect of alcohol than subcortical structures (Begleiter & Coltrera 1975; Begleiter et al. 1972).

The effect of acute alcohol consumption to VEP also was investigated in the visual cortex from humans. One study did not find any effect of alcohol on tested VEP components, BAC ranging from 0.05% to 0.15% (Skalka et al. 1986). But a more detailed study with alcohol dose ranging from 0.00 (placebo) to 0.72 g/kg total body weight showed that latencies of N2 and P3 displayed dose related increase with increasing alcohol level (Colrain et al.

1993). This study concluded that latency of the late, but not earlier, VEP components is sensitive to the effect of alcohol. Study of binge drinkers also demonstrated effect of alcohol on the late VEP components. Although parameters of the N2 component were not affected, P3b amplitude was larger in binge drinkers than controls (Crego et al. 2012). Another massive study (593 participants) showed slightly different results: binge drinking was associated with impairment at the perceptive level (P100/N1 and N170/P2), attention (N2b/P3a) and decision making (P3b) (Maurage et al. 2012).

2.3.4 Effect of chronic alcohol consumption to the visual system

Excessive use of alcohol leads to addiction and is associated with negative effects to the visual system. Multiple studies showed the relationship between chronic alcohol consumption and disorders of the visual system: cataract (Drews 1993), color vision deficiency (Mergler *et al*. 1988; Brasil *et al*. 2015), corneal arcus (Ewing & Rouse 1980) and age-related macular degeneration (Chong *et al*. 2008). In the visual system, chronic alcohol consumption causes disorders that have been attributed to nutritional deficits rather than a direct effect of ethanol. But histological studies showed that long-term ethanol consumption increases oxidative stress and causes neurons loss through all the main visual system levels: retina (Johnsen-Soriano *et al*. 2007; Sancho-Tello *et al*. 2008), optic nerve (Johnsen-Soriano et al. 2007), lateral geniculate nucleus (Carmona *et al*. 1994) and visual cortex (Tenkova *et al*. 2003). Moreover, chronic alcohol consumption and subsequent abstinence strongly modulate processing of sensory information at the different levels of the visual system.

2.3.5 Effect of chronic alcohol consumption and withdrawal to visual evoked potentials at the different levels of the visual system

Multiple studies explored changes of visual information processing caused by chronic alcohol consumption and demonstrated that both, alcohol intake and withdrawal modulate sensory information processing at the level of retina (Sancho-Tello et al. 2008) and visual cortex (Kjellstrom et al. 1994). It has been shown that in the retina long-term alcohol consumption increased latency of a- and b-waves and decreased amplitude of b-wave in the electroretinogram, indicating the impairment of rods and Muller cells function (Pawlosky *et al*. 2001; Sancho-Tello *et al*. 2008). These changes in parameters of electroretinogram were found together with decreased level of glutathione (GSH, intracellular antioxidant), and increased malondialdehyde (MDA, lipid peroxidation product) and antiapoptotic protein Bcl-2 (Sancho-Tello et al.

2008) and indicated functional changes in retina caused by oxidative stress. However, there is no information regarding the effect of alcohol consumption and withdrawal on the function of lateral geniculate nucleus, a relay nucleus which transfers information from retina to the VC and is also strongly modulated by descending projections from VC (Wang *et al*. 2018).

One of the first attempts to investigate the impact of alcohol withdrawal on VC revealed increased amplitude of visually evoked potentials early component during abstinence (Begleiter 1975). Few years later, it was demonstrated that during alcohol consumption the VEP amplitude was depressed, the latency increased (Bierley *et al*. 1980), and during the subsequent acute and protracted withdrawal VEP amplitude was enhanced (Sokomba and Osuide 1985). It is important to note that these studies were based on a relatively short-term alcohol exposure, 2-5 weeks. The study of Kjellstrom et al. (1994) examined the effect of ethanol on VEP in anesthetized rats that had been given ethanol for two months. The results from this study partly agreed with previous studies indicating that exposure to ethanol for two months reduced amplitude and increased latency of VEPs in the VC and these changes were partly restored after one week of abstinence. Functional changes in the VC were also demonstrated in alcohol dependent patients, namely prolonged latency and reduced amplitude of P100 (Cadaveira et al. 1991; Nazliel et al. 2007; Chan et al. 1986), N200 (Emmerson *et al*. 1987) and P300 (Biggins et al. 1995; Porjesz & Begleiter 2003; Rodriguez Holguin et al. 1999) components (see e.g. of reduced P300 amplitude in Fig. 2.12). The amplitude of P300 component is even considered as an electrophysiological marker of alcoholism risk (Prabhu et al. 2001; Porjesz et al. 1998; Hill & Steinhauer 1993). Moreover, fMRI studies showed decreased activation of the occipital cortex during visual stimulation (Bagga et al. 2014; Hermann et al. 2007).

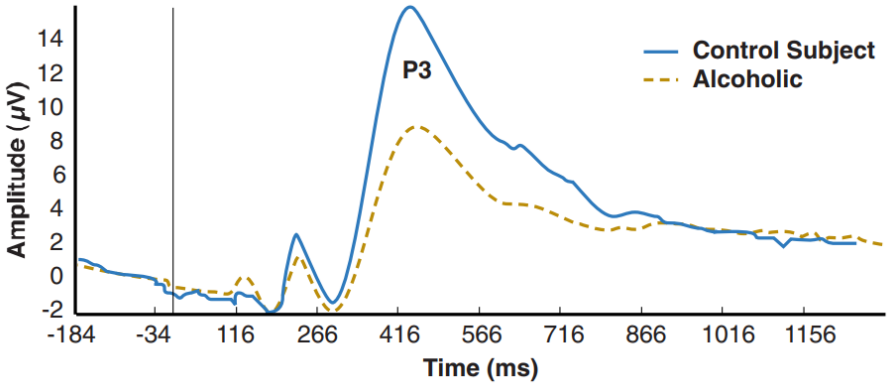


Fig. 2.12. Average event related potential waveform in response to a target

stimulus in a visual oddball task. Recorded at the midline central electrode in a group of alcoholics (yellow dashed line) and a group of control subjects (blue line). Note that the amplitude of P3 component is reduced in alcoholics compared with control subjects. Adapted from Porjesz & Begleiter 2003.

Although previous studies showed that acute, chronic alcohol consumption and withdrawal have a different impact to different visual stimulus processing stages at the level of retina and visual cortex, still there is a lack of detailed information about the effect of alcohol to the visual system. First, in the research of ethanol effect to the visual system, LGN has not been analyzed. There is no information addressed to the effect of chronic alcohol consumption and withdrawal on functioning of LGN and there is only one study which investigated acute alcohol effect to the rat LGN (Hetzler et al. 1983). The second problem is the lack of detailed, methodological approach in the studies. In chronic alcohol consumption or withdrawal studies most of the researchers focused just on one of the stages (drinking or withdrawal) and alcohol consumption and/or withdrawal times were relatively short, 2-5 weeks of alcohol consumption and less than 2 weeks of withdrawal. That is leaving an open question how long-term alcohol consumption, more than 1 month, and withdrawal, more than two weeks, change the function of the visual system. Finally, the effects of acute or chronic alcohol consumption and withdrawal on the ON/OFF stages of information processing in any modalities to the best of our knowledge have not been evaluated before.

3. Methods

In order to reach the goals two experiments were performed. The purpose of the first experiment was to investigate the effect of acute alcohol consumption to stimulus onset and offset at the level of visual cortex.

The second experiment was designed to study the effect of chronic alcohol consumption and withdrawal to the responses of stimulus onset and offset at the level of visual cortex ant lateral geniculate nucleus.

3.1 Experiment 1: Modulation of responses to visual stimulus onset and offset by acute alcohol consumption in the rat visual cortex

3.1.1 Animals

For the Experiment 1, ten two-months-old male Wistar rats (Vilnius University, Vilnius, Lithuania) were used. All animals were housed individually in standard rat cages under a 12/12-hour artificial light/dark cycle (lights on at 7:00 a.m.). Room temperature was kept constant (temperature: 22±1°C). Standard laboratory rat food (4RF21-GLP, Mucedola srl, Milan, Italy) and tap water were provided *ad libitum* throughout the experimental period. Body weights were measured weekly. All experimental procedures were approved by the State Food and Veterinary Service of the Republic of Lithuania.

3.1.2 Implantation of electrodes

Rats were anaesthetized with 5% sevoflurane and maintained anaesthetized with 3% sevoflurane during the entire surgery. The recording electrode (0-80 x 1/8 inch stainless steel screw) was placed above the visual cortex (coordinates AP: -7 mm; ML: ±3 mm). The grounding and the reference electrodes were placed above frontal cortex (coordinates AP: +2 mm, ML: ±2 mm). Dental cement (Prevest DenPro, Jammu, India) was used to ensure stability of electrodes and protection from the environment. During the whole surgery the animal temperature was maintained at 37 °C by using a temperature controller (ATC1000, WPI, Sarasota, USA). After the surgery rats were given daily Carprofen subcutaneous injection (SC) for pain relief (4 mg/kg, Rycarfa, KRKA, Novo mesto, Slovenia) for three days and antibiotics, Enrofloxacin (5 mg/kg, Vetoquinol Biowet, Gorzow Wielkopolski, Poland), for seven days (Lee-Parritz 2007). Rats were allowed to recover for two weeks before recordings of visually evoked potentials began.

3.1.3 Recordings of visually evoked potentials

All recordings were done in 3% sevoflurane anesthetized animals. Gas anesthesia was maintained by gas anesthesia system (Ugo Basile, Milan, Italy). VEPs were recorded for one hour under each of the conditions: baseline, intraperitoneal (IP) administration of 0.9% saline and IP administration of 2 g/kg of ethanol. Recordings under two conditions were made in a single day: baseline or saline following alcohol injection. The order of baseline and saline administration was randomized. Recording of evoked potentials started 2 min after saline/ethanol injections.

For VEP recordings stimuli were presented via an LCD monitor (SyncMaster P22370, Samsung); refresh time was 2 ms and the distance to the eye was 20 cm (stimulus covered all visual field). In order to protect rat eyes from drying, they were covered with transparent Lacripos gel (Ursapharm, Saarbrucken, Germany) (Geiger et al. 2008, Mostany and Portera-Cailliau, 2008). Visual stimulation was performed with the software VisStim 1.0. with 500 ms stimulus duration, 125 cd/m^2 intensity, 1 lx background illuminance and 0.25 Hz stimulation frequency was used. The VEP recordings started 100 ms before the application of stimulus and continued for 2900 ms after the stimulus onset. Stimuli were presented with an interval of 3.5 s. The one hour recording interval was divided into nine time windows of 400 s. In each time-window a visual stimulus was presented 100 times. Average VEPs calculated from 100 responses during each time window were used for data analysis.

Data collection was made with data acquisition system (Power 1401, CED, UK) using 1 kHz sampling rate. Evoked potentials were amplified (gain x1000) with a standard biopotential amplifier (Iso-DAM8A, WPI) using high (1 kHz), low (1 Hz) and notch (50 Hz) built in filters. Data analysis was performed using Signal 5.07 software (CED, UK).

3.1.4 Data analysis

VEP components were marked by their polarity (the positive peaks are noted P and the negative N) and by their number from the onset and the offset of the stimulus. The amplitude of component N1 (the first observed component) was measured using the baseline-to-peak method, while the amplitude of other components was measured using the peak-to-peak method. The baseline-to-peak amplitude was calculated as the difference between mean voltage of 100 ms before stimulus onset/ offset and the peak voltage. The peak-to-peak amplitude was calculated as the difference between two peak voltages (e.g. amplitude of component P2 is voltage difference between P2 and N1 peaks,

see Fig. 3.1A). Peak latencies were calculated relative to stimulus onset , i.e. 0 ms, and offset, i.e. 500 ms (see Fig. 3.1A).

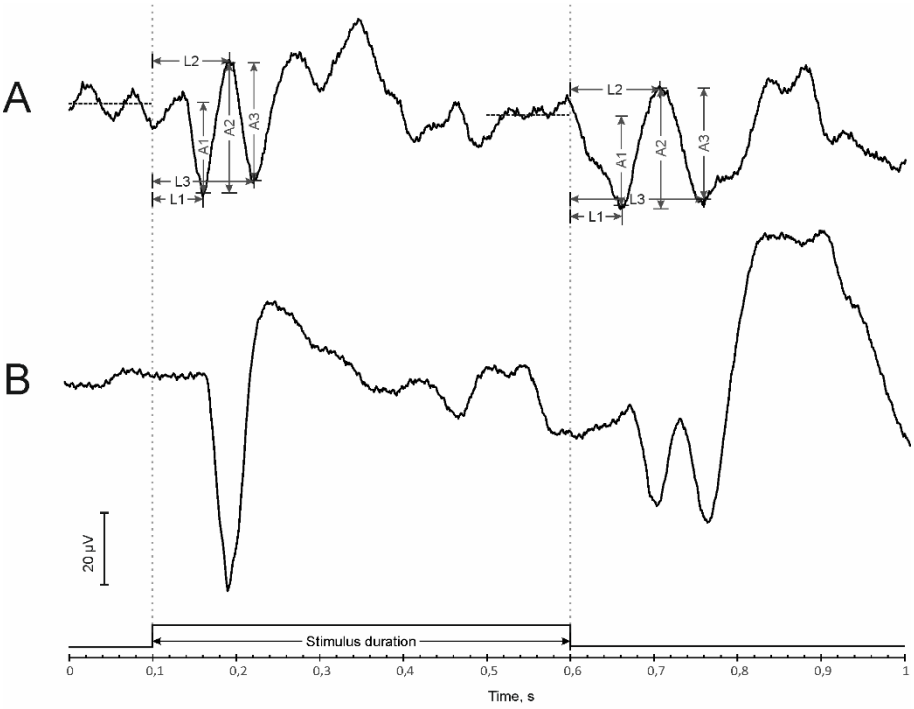


Fig. 3.1. Example of visually evoked potentials recorded after injection of either A) saline or B) 2 g/kg of ethanol during the 4th time bin. Arrows L1, L2 and L3 indicate latency of components N1, P2 and N2, respectively. Arrows A1, A2 and A3 indicate amplitude of components N1, P2 and N2, respectively. The amplitude of component P1 (A1) was measured using baseline-to-peak method, the amplitude of other components (A2, A3) using peak-to-peak method. The baseline-to-peak amplitude was calculated as the difference between mean voltage of 100 ms before stimulus onset/ offset (horizontal dotted lines) and the peak voltage. The peak-to-peak amplitude was calculated as the difference between two peak voltages. Peak latencies were calculated relative to stimulus onset and offset (vertical dotted lines).

Data were normally distributed as indicated by Shapiro – Wilk test. VEP recordings under baseline condition did not differ from recordings following saline administration (for all components $P>0.05$). Therefore, the effect of ethanol administration on latency and amplitude of VEP was assessed by comparing it with recordings after saline administration. Two-way repeated measures ANOVA was used for analysis of VEP amplitude and latency from the three most common peaks, N1, P2 and N2, with the factors: time and

treatment. Differences between latencies and amplitudes of ON and OFF responses for components N1, P2 and N2 under different treatment conditions were analyzed using the two-way repeated measures ANOVA with the factors: time and stimulus type (ON vs. OFF). Whenever significant differences were found, a post-hoc Student Newman-Keuls test was performed. The chosen level of significance was $P < 0.05$.

3.2 Experiment 2: Modulation of responses to visual stimulus onset and offset by chronic alcohol consumption and withdrawal in the rat visual cortex and lateral geniculate nucleus

3.2.1 Animals

Eighteen three-months-old male Wistar rats (Vilnius University, Vilnius, Lithuania) were used. Animal housing conditions were the same as in the first experiment. All experimental procedures were approved by the State Food and Veterinary Service of the Republic of Lithuania.

3.2.2 Implantation of electrodes

The procedure of electrode implantation was the same as in Eperiment 1, except one additional recording electrode was implanted. In this study the recording electrodes were chronically implanted in two brain regions: 0–80×1/8 inch stainless steel screw above the visual cortex (coordinates AP: −7 mm; ML: +3 mm), and single tip (2 µm) tungsten microelectrodes in lateral geniculate nucleus (coordinates AP: −4.56 mm; ML: -3.7 mm; DV: -4.8mm).

3.2.3 Ethanol consumption

Rats were divided into two separate groups. One group (alcohol group, n = 9) had access only to a 10% (v/v) ethanol solution for 8 weeks (drinking period), except for four hours before VEP recordings when ethanol solution was replaced by tap water. The ethanol solution was prepared from 96% ethanol (Vilniaus degtine, Vilnius, Lithuania) and tap water. After 8 weeks ethanol was changed to tap water (abstinence period) for 3 weeks. For the control group (n = 9) tap water was provided *ad libitum* throughout the both drinking and abstinence periods. During the experiment both groups of animals did not show loss of body weight.

3.2.4 VEP recordings

Electrophysiological recordings were done once per week, started one day before exposure to ethanol, continued during all 8 weeks of ethanol exposure

and were repeated 6 more times during the abstinence period: 12, 36 and 60 hours (acute withdrawal) and 4, 9 and 20 days (protracted withdrawal) after the initiation of withdrawal (see Fig. 3.2). All experiments were performed with freely moving animals. Pupil dilatator (Mydriacyl 10 mg/ml, Alcon-Couvreur N.V., Puurs, Belgium) was applied into both eyes before recording started, and the rat was dark-adapted for 15 min. During recordings, the animals were connected to the data acquisition setup via freely rotating wires.

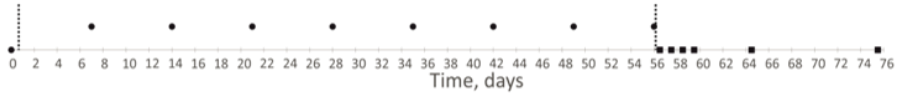


Fig. 3.2. Timeline of the visually evoked potentials recording. VEPs were recorded once a week, recordings started one day before alcohol application, continued following 8 weeks of ethanol exposure and 6 times during abstinence period: 12, 36 and 60 hours (acute withdrawal) and 4, 9 and 20 days (protracted withdrawal) after the initiation of withdrawal. Circles and squares indicate the time of recordings during "alcohol consumption" and "abstinence" stages, respectively. The beginning and the end of animal exposure to 10% ethanol solution are marked by vertical dotted lines. The elevated black circles indicate recordings under influence of chronic ethanol consumption.

For VEP recordings the rat cage was placed inside a square box with walls made from four 50 cm x 30 cm LED panels (King 24W, GTV) and stimulated with ambient illumination from LED panels. The distance between LED panels and transparent wall of the cage was 5 cm. The illuminance in the center (750 lx), corner (730 lx) and near the wall (750 lx) of the cage differed by less than 5%. Visual stimulation was controlled with the software Signal 5.07 (CED, UK). Stimulus luminance was set at 70 cd/m^2, duration of stimulus was 500 ms, background illuminance of 0 lx was maintained, and 0.25 Hz stimulation frequency was used. The VEP recordings started 100 ms before the presentation of the stimulus and continued for 2900 ms after the stimulus onset. The new stimulus onset followed the previous stimulus offset by 3.5 s. Average VEPs calculated from 100 responses were used for data analysis. Collection of electrophysiological data was performed as in the first experiment.

3.2.5 Data analysis

VEP components were labeled by their polarities and latency positions relative to each other. Subscript VC (visual cortex) and LGN (lateral geniculate nucleus) indicate the brain region of the recorded component. The amplitude of components was measured using the baseline-to-peak method, which was calculated as the difference between mean voltage of 100 ms before stimulus onset/offset and the peak voltage. Peak latencies were calculated relative to stimulus onset, i.e. 0 ms, and offset, i.e. 500 ms (see Fig. 3.3).

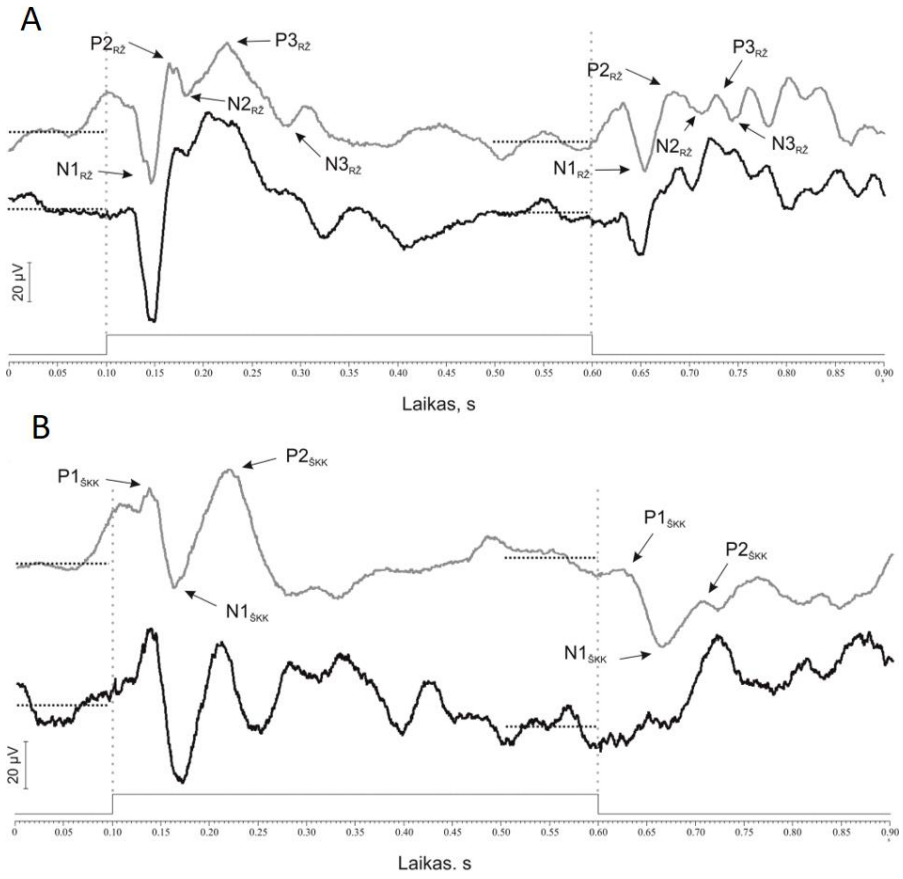


Fig. 3.3. Example of visually evoked potentials recorded in A) visual cortex and B) lateral geniculate nucleus in control (black line) and alcohol (grey line) rats after 8 weeks of chronic alcohol consumption. Arrows indicate components of visual cortex N1 $_{VC}$, P2 $_{VC}$, N2 $_{VC}$, P3 $_{VC}$, N3 $_{VC}$ and lateral geniculate nucleus P1 $_{LGN}$, N1 $_{LGN}$, P2 $_{LGN}$. The amplitude of components was

measured using baseline-to-peak method and calculated as the difference between the mean voltage at 100 ms before stimulus onset or offset (horizontal dotted lines) and the peak voltage. Peak latencies were calculated relative to the stimulus onset and offset (vertical dotted lines).

Data were normally distributed as indicated by Shapiro – Wilk test. Two-way ANOVA was used for analysis of VEP amplitude and latency of five components in visual cortex ($N1_{VC}$, $P2_{VC}$, $N2_{VC}$, $P3_{VC}$, $N3_{VC}$) and three components in lateral geniculate nucleus ($P1_{LGN}$, $N1_{LGN}$, $P2_{LGN}$), with factors: time and treatment. Differences between the ON and OFF responses under different treatment conditions were analyzed using two-way repeated measures ANOVA, with factors: time and stimulus type (ON *vs.* OFF). A *post hoc* Holm-Sidak test was performed if significant differences were found. The chosen level of significance was $P < 0.05$.

4. Results

4.1 Experiment 1: Modulation of responses to visual stimulus onset and offset by acute alcohol consumption in the rat visual cortex

Differences between ON and OFF responses were investigated by analyzing VEPs recorded after application of saline or ethanol (see Fig. 3.1, 4.1 and 4.2).

4.1.1 Latency

Data analysis revealed that the latency of component N1 increased over time during both ON (saline: from 48.44±6.75 ms to 62.11±9.57 ms; alcohol: from 71±4.8 ms to 89.56±9.09 ms) and OFF responses (from 48.56±5.04 ms to 70.33±4.53 ms; alcohol: from 74.33±6.78 ms to 95.67±7.41 ms) (factor time: $F_{8,242}$=7.6, P<0.001 and $F_{8,242}$=3.6, P<0.01 for ON and OFF responses respectively) (Fig. 4.1A, B)

The pattern of latency dynamics of component P2 was similar to that of component N1 – the latency of P2 increased from 90.11±2.69 ms to 110.33±6.03 ms in ON and from 89.22±2.63 ms to 127.33±10.44 ms in OFF response over the period of one hour (factor time: $F_{8,242}$=15.4, P<0.001 and $F_{8,242}$=3.8, P<0.001 for ON and OFF responses respectively) (Fig. 4.1C, D). After administration of ethanol latency increased from 109.89±7.84 ms to 144.77±13.35 ms in ON and from 126.89±7.81 ms to 138.11±8.76 ms in OFF responses.

The latency of component N2 also increased over time in both treatment conditions during ON response (from 128.78±8.38 ms to 169±6.62 ms after saline and from 185.44±11.05 ms to 208.56±16.55 ms after ethanol injection) (factor time: $F_{8,242}$=9.6, P<0.001) (see Fig. 4.1E). Unfortunately, latency of all above mentioned VEP components increased over one hour after saline injection. For this reason, it is not possible to evaluate the direct effect of ethanol on VEP parameters that changed over time in control condition.

Under saline condition the only unaffected component was OFF N2 latency. However, administration of ethanol also had no effect on the OFF N2 latency (see Fig. 4.1F).

Comparison of the latencies during ON and OFF responses has shown that the latency of components N1 and P2 during stimulus onset was not different from stimulus offset following either saline or ethanol injections (P>0.05). The latency of component N2 after saline injection changed over time and differed between ON and OFF responses (factor time × stimulus type: $F_{2,162}$=3.7, P<0.001). Post hoc analysis showed that, during the first four

time bins, the latency during OFF response was longer on average 29.72±6.05 ms than during ON response. Administration of 2 g/kg ethanol increased the latency during the response to stimulus onset compared to the response to stimulus offset (factor time × stimulus type: $F_{1,162}$=6.0, P<0.05).

These data revealed that over time gas anesthesia alone or combined with acute alcohol consumption increased latency of all three VEP components in ON responses and two first components (N1 and P2) of OFF responses. N2 of OFF responses was the only one unaffected component.

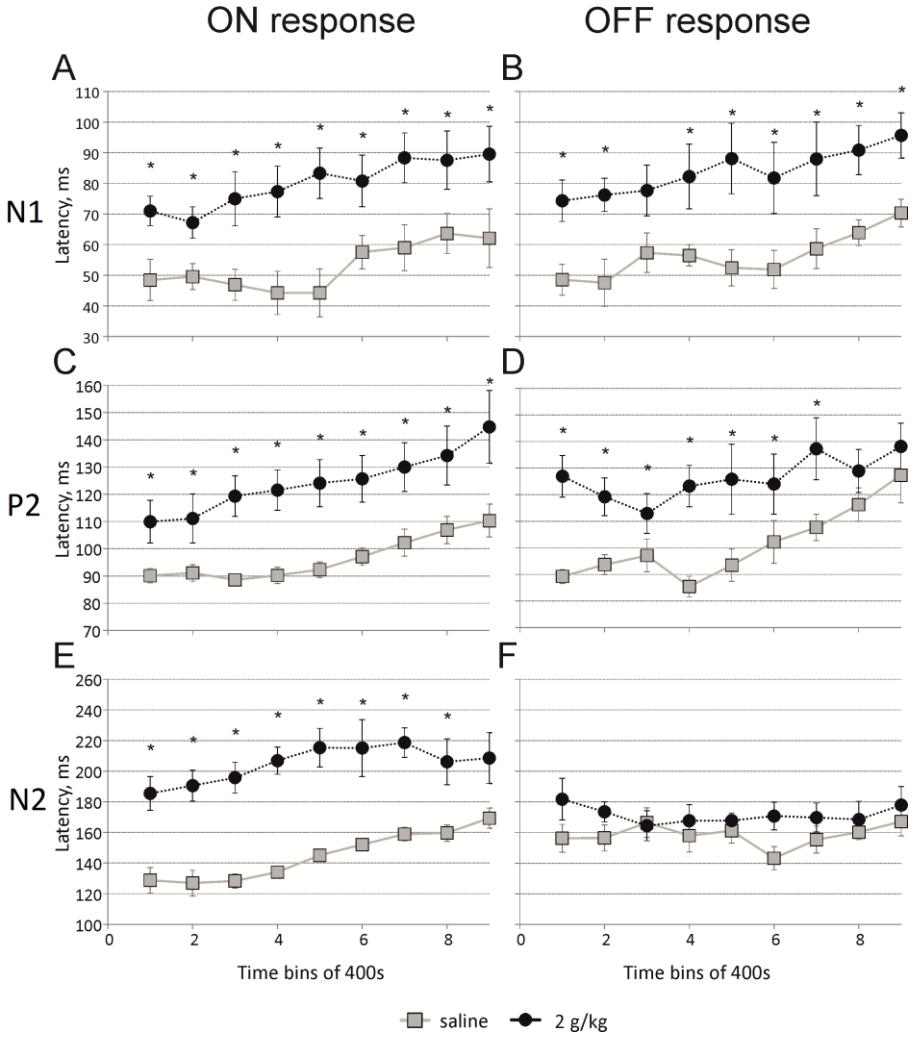


Fig. 4.1. Latency (ms) of VEP components N1 (A, B), P2 (C, D), and N2 (E, F) following injection of either saline (grey squares) or 2 g/kg of ethanol (black circles). One hour recording interval was divided into nine time bins of

400 s. In each time-window, visual stimulus of 500 ms duration was presented 100 times. Peak latencies were calculated relative to stimulus onset (A, C, E) and offset (B, D, F). The data is presented as the average VEPs calculated from 100 responses during each time window. * indicates significant difference between saline and 2 g/kg ethanol, $P<0.05$. Data are presented as mean ± S.E.M.

4.1.2 Amplitude

Analysis of amplitude data has shown that the amplitude of component N1, elicited by stimulus onset, did not change over time and was not affected by ethanol (Fig. 4.2A). The amplitude during OFF response changed over time (factor time: $F_{8,242}=2.1$, $P<0.05$), but there were no statistically significant changes induced by ethanol administration (Fig. 4.2B). The amplitude of component P2, elicited by stimulus onset and offset, did not depend on time or treatment condition (Fig. 4.2C, 4.2D).

The pattern of amplitude dynamics of component N2 is presented in Fig. 4.2E and 4.2F. No significant effect of ethanol administration was found during ON response. However, the amplitude decreased after ethanol administration during stimulus OFF response (factor treatment: $F_{2,242}=5.4$, $P<0.05$ and factor time × treatment interaction: $F_{16,242}=2.3$, $P<0.01$). Post hoc analysis showed that during the fourth and fifth time bins the amplitude was smaller by 14.19±4.33 μV and 11.83±6.33 μV after ethanol treatment than after saline treatment.

Comparison of ON and OFF responses showed that the amplitude of component N1 during stimulus onset and offset did not differ neither after administration of saline nor after administration of ethanol ($P>0.05$). Following saline administration, the amplitude of the P2 component was higher during ON response than during OFF response (factor stimulus type: $F_{1,161}=6.9$, $P<0.05$). Further analysis has shown that the amplitude was higher by 16.26±2.77 μV in ON than OFF response during the second to fourth time bins. However, administration of ethanol abolished the differences between ON and OFF responses. Finally, amplitude values of component N2 were lower by 21.91±2.37 μV during OFF response than during ON response following administration of saline (the second half of hour) (factor stimulus type: $F_{1,161}=12.34$, $P= 0.008$) and by 14.64±2.63 μV following ethanol administration (the first half of hour) (factor stimulus type $F_{1,161}=15.6$, $P<0.01$).

In summary, amplitude analysis showed that acute alcohol consumption reduced amplitude of N2 component in OFF response and

eliminated amplitude differences between ON and OFF responses in P2 component.

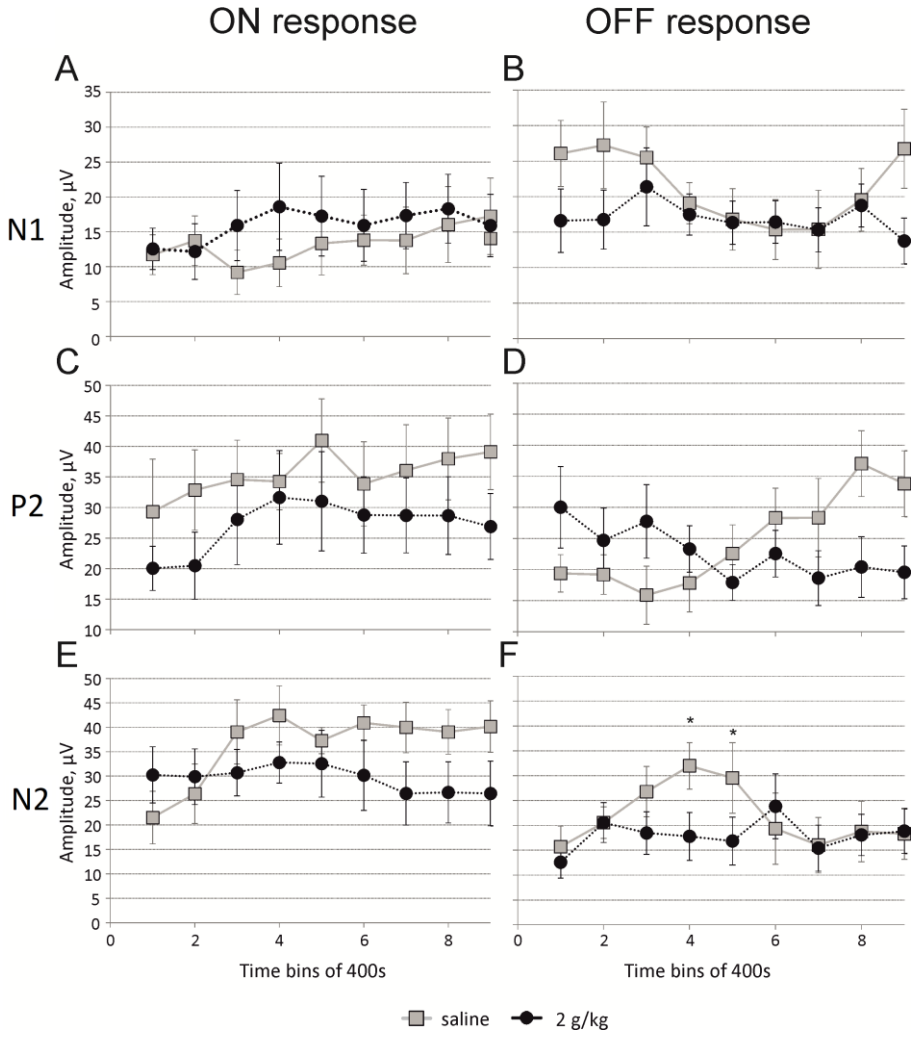


Fig. 4.2. Amplitude (µV) of VEP components N1 (A, B), P2 (C, D), and N2 (E, F) following injection of either saline (grey squares) or 2 g/kg of ethanol (black circles). One hour recording interval was divided into nine time bins of 400 s. In each time-window, visual stimulus of 500 ms duration was presented 100 times. Peak latencies were calculated relative to stimulus onset (A, C, E) and offset (B, D, F). The data is presented as the average VEPs calculated from 100 responses during each time window. * indicates significant difference between saline and 2 g/kg ethanol, $P<0.05$. Data are presented as mean ± S.E.M.

4.2 Experiment 2: Modulation of responses to visual stimulus onset and offset by chronic alcohol consumption and withdrawal in the rat visual cortex and lateral geniculate nucleus

Effect of chronic ethanol consumption and withdrawal on the ON and OFF responses from visual cortex and lateral geniculate nucleus were investigated by analyzing VEPs recorded during 8 weeks of alcohol consumption and 3 weeks of abstinence. In the present study rats consumed ethanol 5.2±0.7 g/kg per day. The results were grouped according to experimental conditions (alcohol consumption *vs.* abstinence) and further analyzed as alcohol consumption and abstinence stages (see Fig. 3.2). Our results demonstrated that alcohol consumption and withdrawal had different impact on the ON and OFF responses (Fig. 4.3, 4.4 and Tab. 1, 2).

Table 1. Summary of the effect of chronic alcohol consumption and abstinence on visual evoked potential elicited by stimulus onset and offset. The entries for each component show how the latency or amplitude of the component was altered by the long-term alcohol consumption or withdrawal in comparison to the control group in ON and OFF responses. An arrow pointing up indicates that value of the parameter is statistically significantly higher in alcohol consuming animal in comparison to the control group. An arrow pointing down indicates that value of parameter is statistically significantly lower in alcohol consuming animals in comparison to the control group. The minus sign indicates no significant changes. VC = visual cortex, LGN = lateral geniculate nucleus, ↑ = increase, ↓ = decrease, - = no change. For details of the analyses, see Results and Figs. 4.3 and 4.4.

Component	Parameter	Drinking		Abstinence	
		ON response	OFF response	ON response	OFF response
$N1_{VC}$	Latency	-	↑	-	↑
	Amplitude	-	-	↓	-
$P2_{VC}$	Latency	-	-	-	↑
	Amplitude	-	↓	-	↓
$N2_{VC}$	Latency	-	-	-	-
	Amplitude	-	-	-	-
$P3_{VC}$	Latency	-	↓	-	↓
	Amplitude	↑	-	-	↓
$N3_{VC}$	Latency	↑	-	↑	-

	Amplitude	↓	-	↓	-
$P1_{LGN}$	Latency	-	↓	↓	-
	Amplitude	-	-	-	-
$N1_{LGN}$	Latency	-	↓	-	-
	Amplitude	-	-	-	-
$P2_{LGN}$	Latency	-	-	-	-
	Amplitude	-	-	-	↑

Table 2. Summary of ON-OFF response asymmetry during alcohol consumption and abstinence periods in control and alcohol consuming rats. An arrow pointing up indicates that value of the parameter in the ON response is statistically significantly higher in comparison to the OFF response. An arrow pointing down indicates that value of the parameter in the ON response is statistically significantly lower in comparison to the OFF response. The minus sign indicates no significant changes. VC = visual cortex, LGN = lateral geniculate nucleus, ↑ = increase, ↓ = decrease, - = no difference. For details of the analyses, see Results and Figs 4.3 and 4.4.

Component	Parameter	Drinking		Abstinence	
		Alcoholic	Control	Alcoholic	Control
$N1_{VC}$	Latency	↓	-	↓	-
	Amplitude	↑	↑	↑	↑
$P2_{VC}$	Latency	-	-	↓	-
	Amplitude	↓	↓	-	↓
$N2_{VC}$	Latency	↓	↓	↓	↓
	Amplitude	-	-	-	-
$P3_{VC}$	Latency	-	↓	-	↓
	Amplitude	-	↑	-	-
$N3_{VC}$	Latency	-	-	-	↓
	Amplitude	↑	↑	↑	↑
$P1_{LGN}$	Latency	-	-	-	-
	Amplitude	-	-	-	-
$N1_{LGN}$	Latency	-	-	-	-
	Amplitude	↑	-	-	-
$P2_{LGN}$	Latency	-	-	-	-
	Amplitude	-	-	-	-

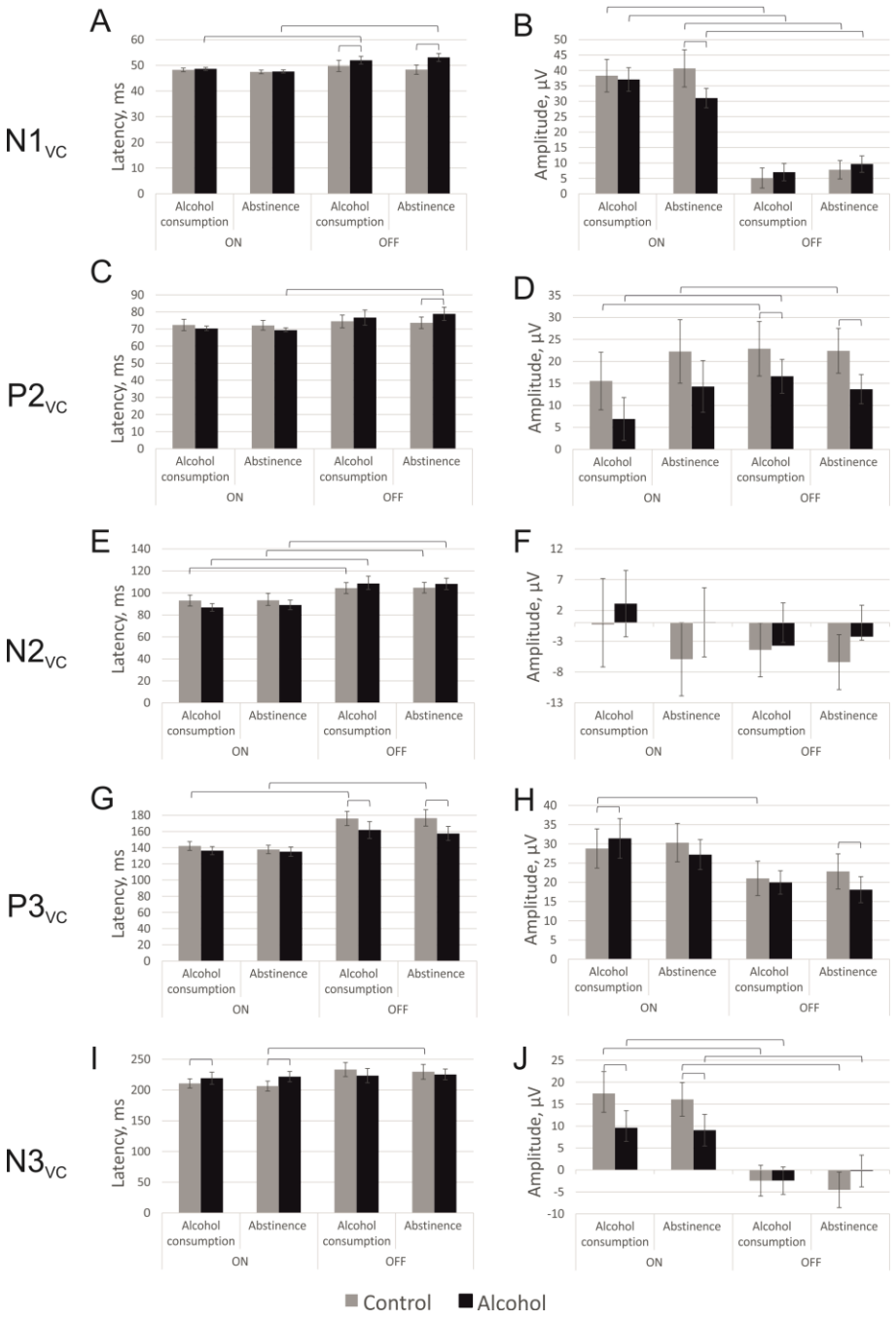


Fig. 4.3. Mean latency (A, C, E, G, I, ms) and amplitude (B, D, F, H, J, μV) of visual cortex VEP components – N1 VC, P2 VC, N2 VC, P3 VC, N3 VC - elicited by stimulus onset and offset, following chronic alcohol consumption and abstinence. Peak latencies were calculated relative to the stimulus onset and

offset. Peak amplitudes were calculated relative to the baseline (the average of 100 ms before onset/offset of stimulus). VEPs were calculated from 100 responses to each stimulus. The data presented as average; error bars indicate S.E.M. The horizontal brackets indicate significant differences between the groups of animals or type of the stimuli, P<0.05.

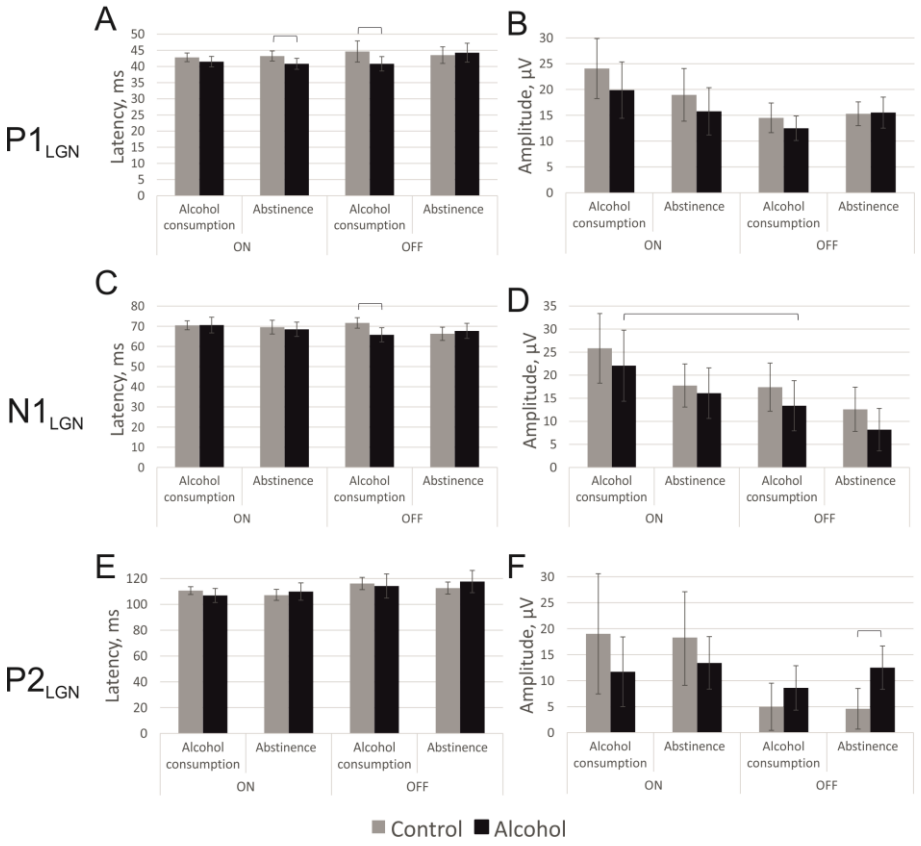


Fig. 4.4. Mean latency (A, C, E, ms) and amplitude (B, D, F, µV) of lateral geniculate nucleus VEP components - P1 $_{LGN}$, N2 $_{LGN}$, P2 $_{LGN}$ - elicited by stimulus onset and offset, following chronic alcohol consumption and abstinence. Peak latencies were calculated relative to stimulus the onset and offset. Peak amplitudes were calculated relative to the baseline (the average of 100 ms before onset/offset of stimulus). VEPs were calculated from 100 responses to each stimulus. The data presented as average; error bars indicate S.E.M. The horizontal brackets indicate significant differences between the groups of animals or type of stimuli, P<0.05.

4.2.1 Effect of chronic alcohol consumption

Summary of the effect of chronic alcohol consumption and abstinence on visual evoked potential elicited by stimulus onset and offset is shown in Table 1. Comparison of two groups (control and alcohol) of animals showed, that just two components of the ON response in the visual cortex were affected by chronic alcohol consumption: the amplitude of $P3_{VC}$ and the latency of $N3_{VC}$ components were higher on average by 2.62 μV and 8.43 ms (factor treatment: $F_{1,161}$=4.1, P<0.05 and $F_{1,161}$=8.8, P<0.01) and the amplitude of $N3_{VC}$ was lower on average by 7.82 μV (factor treatment: $F_{1,161}$=17.4, P<0.001) compared with the control group (Fig. 4.3H, 4.3I, 4.3J).

Components of the OFF response were more susceptible to alcohol consumption (five out of eight investigated components were affected). Chronic alcohol drinking reduced the amplitude of $P2_{VC}$ (16.60±3.84 μV vs. 22.87±6.2 μV) (factor treatment: $F_{1,161}$=6.2, P<0.05) (Fig. 4.3D), on average shortened the latency of $P3_{VC}$ by 14.19 ms, $P1_{LGN}$ by 3.79 ms, $N1_{LGN}$ by 5.88 ms (factor treatment: $F_{1,161}$=8.8, P<0.01; $F_{1,161}$=6.9, P<0.01; $F_{1,161}$=15, P<0.001, respectively) (Fig. 2G, 3A, 3C) and increased the latency of $N1_{VC}$ by 2.25 ms (factor treatment: $F_{1,161}$=6,01, P<0.05) (Fig. 4.3A).

4.2.2 Effect of withdrawal

During the period of abstinence in the alcohol drinking group, the amplitudes of $N1_{VC}$ (31.05±3.1 μV vs. 40.66±6.04 μV), $N3_{VC}$ (9.06±3.6 μV vs. 16.08±3.84 μV) and the latency of $P1_{LGN}$ (40.83±1.7 ms vs. 43.24±1.54 ms) (factor treatment: $F_{1,107}$=6.2, P=0.015; $F_{1,107}$=9.3, P<0.01; $F_{1,107}$=9.1, P<0.01, respectively) were smaller (Fig. 4.3B, 4.3J and 4.4A) and the latency of $N3_{VC}$ (221.68±8.53 ms vs. 206.37±8.05 ms) was slower (factor treatment: $F_{1,107}$=28.8, P<0.001) (Fig. 4.3I) in the ON response. More detailed analysis showed that $N3_{VC}$ latency was increased in ethanol drinking rats after 36 hours of abstinence by 16.51 ms and during abstinence days 4-20 by 20.64 ms, and $P1_{LGN}$ latency was reduced by 3.33 ms after 60 hours of withdrawal.

In the OFF response, during the period of abstinence the latency of $N1_{VC}$ (53.12±1.58 ms vs. 48.32±1.81 ms) (Fig. 4.3A), $P2_{VC}$ (78.79±3.89 ms vs. 73.62±3.37 ms) (Fig. 4.3C) and the amplitude of $P2_{LGN}$ (12.5±4.17 μV vs. 4.6±3.92 μV) (Fig. 4.4F) (factor treatment: $F_{1,107}$=22.9, P<0.001; $F_{1,107}$=5.6, P<0.05; $F_{1,107}$=10.8, P<0.001, respectively) were higher in alcohol group compared with control group. Furthermore, the amplitude of $P2_{VC}$ (factor treatment: $F_{1,107}$=11.5, P<0.001) (Fig. 4.3D) and both parameters of $P3_{VC}$ (factor treatment: for latency $F_{1,107}$=11.4, P<0.001, for amplitude $F_{1,107}$=4.0,

P<0.05) (Fig. 4.3G, 4.3H) were decreased by 4.43 μV, 19.06 ms and 4.73 μV, respectively. Moreover, the effect of withdrawal on $N1_{VC}$ latency was time-dependent: latency was higher during 12-60 hours by 5.60 ms and on the day 20 of abstinence by 5.08 ms.

4.2.3 Differences between the ON and OFF responses

Simplified summary of ON-OFF response asymmetry during alcohol consumption and abstinence periods in control and alcohol consuming rats is shown Table 2. Comparison of the ON and OFF responses in the control group showed a qualitative difference of some VEP components in the visual cortex, but not in the lateral geniculate nucleus. The amplitude of $N1_{VC}$ (ON 39.24±5.59 μV vs. OFF 6.18±3.17 μV) and $N3_{VC}$ was higher (ON 16.89±4.32 μV vs. OFF -3.27±3.73 μV) (factor stimulus: $F_{1,161}$=38.62, P<0.001; $F_{1,161}$=27.43, P<0.001, respectively) (Fig. 4.3B, 13J) and the amplitude of $P2_{VC}$ was lower (ON 18.21±6.89 μV vs. OFF 22.68±5.77 μV) (factor stimulus: $F_{1,161}$=30.63, P<0.001) (Fig. 4.3D) in the ON response than OFF response in control group animals. The latency analysis revealed more consistent results: the latency of $N2_{VC}$ (ON 93.06±5.16 ms vs. OFF 104.5±4.91 ms) and $P3_{VC}$ (ON 140.3±5.37 ms vs. OFF 176.21±9.34 ms) components was shorter (factor stimulus: $F_{1,161}$=14.72, P<0.01; $F_{1,161}$=13.28, P<0.01, respectively) in response, elicited by the stimulus onset (Fig. 4.3E, 4.3G).

Ethanol drinking animals showed the same pattern as the control animals of ON-OFF differences regarding the amplitudes of $N1_{VC}$ (for drinking ON 37.07±3.85 μV vs. OFF 7.07±2.78 μV, factor stimulus: $F_{1,161}$=82.46, P<0.001; for abstinence ON 31.05±3.14 μV vs. OFF 9.63±2.66 μV, factor stimulus: $F_{1,107}$=71.39, P<0.001) (Fig. 4.3B) and $N3_{VC}$ (for drinking ON 9.6±3.5 μV vs. OFF -2.42±3.13 μV, factor stimulus: $F_{1,161}$=19.01, P=0.002; for abstinence ON 9.06±3.6 μV vs. OFF -0.23±3.62 μV, factor stimulus: $F_{1,107}$=28.94, P<0.001) (Fig. 4.3J) components and the latency of $N2_{VC}$ component (for drinking ON 86.73±3.45 ms vs. OFF 108.51±6.01 ms, factor stimulus: $F_{1,161}$=14.93, P<0.01; for abstinence ON 89.01±4.37 ms vs. OFF 108.21±5.11 ms, factor stimulus: $F_{1,107}$=11.3, P<0.01) (Fig. 4.3E); however drinking had different effects on other measures.

During the time of ethanol consumption the latency of $N1_{VC}$ was shorter (ON 48.64±0.61 ms vs. OFF 51.98±1.5 ms; factor stimulus: $F_{1,161}$=11.98, P<0.01) (Fig. 4.3A) and the amplitude of $N1_{LGN}$ was higher (ON 22.08±7.7 μV vs. OFF 13.38±5.45 μV; factor stimulus: $F_{1,107}$=10.05, P<0.05) (Fig. 4.4D) in the ON response than in the OFF response. Difference in $N1_{VC}$

component occurred because ethanol consumption increased the latency of OFF, but not the ON response. Moreover, the latency difference of $P3_{VC}$ component between the ON and OFF responses was abolished due to effect of ethanol consumption on the OFF, but not the ON response (Fig. 4.3G).

Similarly, during the abstinence period differences between ON and OFF responses in alcohol drinking animals (in contrast to control animals) were not detected for the amplitude of $P2_{VC}$ and latency of $P3_{VC}$ (factor stimulus: $F_{1,107}$=10.05, P>0.05) (Fig. 4.3D, 4.3G). Also, ON response latency of $N1_{VC}$ (ON 47.63±0.6 ms vs. OFF 53.12±1.58 ms; factor stimulus: $F_{1,107}$=42.86, P<0.001) (Fig. 4.3A) and $P2_{VC}$ (ON 69.33±1.15 ms vs. OFF 78.79±3.89 ms; factor stimulus: $F_{1,107}$=11.18, P<0.05) (Fig. 4.3C) components appeared to be shorter (in alcohol drinking animals during the abstinence period that was not seen in the control animals. All these differences between parameters of the ON and OFF responses were due to the effect of withdrawal on OFF, but not on ON, response.

5. Discussion

The application of 500 ms stimulus enabled us to separate ON and OFF responses and study the effect of acute alcohol, chronic alcohol consumption and abstinence on these responses at cortical and subcortical levels. Our studies demonstrated that activation of the visual system during ON response to a 500 ms visual stimulus is qualitatively different from that during OFF response. These differences can be measured at cortical level of the visual system. Moreover, these responses are differently modulated by a depressant dose of acute ethanol, long term alcohol consumption and withdrawal. Our studies showed that, at the level of visual cortex, gas anesthesia alone and/or combined with acute alcohol consumption had strong, time dependent impact on the latency of VEP responses, and a depressant dose of ethanol did not changed the latency of the N2 component to visual stimuli offset. Long-term chronic alcohol consumption and abstinence have a strong long-term and, in some cases, irreversible impact on the visual information processing. These modulations at different stages of information processing chain can result in insufficient processing of parameters of visual stimuli and can lead to the changes in perception of stimulus duration and intensity.

5.1 Effect of acute alcohol administration

Although the results of the first experiment demonstrated that acute administration of ethanol caused a considerable increase in latency dynamics of nearly all VEP components at the onset and offset of 500 ms visual stimuli in the visual cortex from anesthetized male Wistar rat, we cannot affirm that these changes were caused by direct effect of ethanol. We have found that, with the exception of component N2, gas anesthesia affected latency dynamics of both ON and OFF responses in a similar manner. The latency of N2 during OFF response was not affected by saline or ethanol. The effect of ethanol on VEP amplitude was not pronounced, significant effect was only found for the amplitude of the OFF N2 component.

Previous research using FEP recordings has shown that the amplitude is sensitive to the effect of ethanol (Begleiter and Coltrera, 1975, Begleiter et al. 1972, Hetzler and Bednarek 2001, Hetzler et al. 1981, Hetzler et al. 1982, Hetzler et al. 1983, Hetzler et al. 1988). It is known that the amplitude of FEP is affected by both small (activating effect) and large (depressant effect) doses of ethanol. However, the results of studies in rats are ambiguous and suggest that the effect of ethanol is different on various FEP components: N_{29}, N_{39}, P_{88}, N_{139}, P_{234} were reduced by ethanol, P_{22}, N_{53}, N_{65} were not affected, and

the amplitude of the component P_{46} was increased (Hetzler and Bauer 2013, Hetzler and Bednarek 2001, Hetzler and Martin 2006). In contrast to the effect of ethanol on latency, the amplitude of late components was shown to be more affected by ethanol than the early components (Hetzler et al. 1981). Our findings agree with the results of the earlier research. No effect of ethanol on the amplitude of N1 was found in most studies (Hetzler and Bauer 2013, Hetzler and Martin 2006, Hetzler and Ondracek 2007, Hetzler et al. 1981, Hetzler et al. 2008), whereas the amplitude of the late component was reduced by ethanol, suggesting that the depressant doses of ethanol reduced the response to 500 ms visual stimulus offset in the cortical regions.

In our study, amplitude differences between the ON and OFF responses were seen already following saline application (Fig. 4.2), suggesting that the stimulus onset stimulate the visual system more than the stimulus offset. Indeed, the amplitude of components P2 and N2 during ON response was higher than that during OFF response, and acute administration of ethanol eliminated (in case of P2) or enhanced (in case of N2) this difference by affecting amplitude of ON response less than OFF response. An increase in the amplitude of the VEP component reflect either stronger sensory information processing or increased arousal in the visual system (Hetzler et al. 2008). Amplitude differences between ON and OFF responses of the P2 and N2 components show that, contrary to latency, processing of stimulus onset and offset differs at the cortical level of the visual system. Ethanol increased or reduced responsiveness of the visual system at the cortical level during OFF response and altered the asymmetry ON and OFF responses at the different stages of stimulus processing. These changes could be the reason of impaired processing of the visual stimulus, especially during its termination.

Our data showed that acute administration of ethanol in anesthetized rats elevated the latency dynamics of VEP components by more than 30 ms. This increase started 2 min after ethanol administration and remained during the entire one-hour recording time. Unfortunately, the similar increase in latency is visible after saline injection. Because of these changes in control group, our results cannot evaluate and confirm any direct effect of ethanol to the latency of VEP components. Most likely in our experiment anesthesia affected the overall latency of VEP components. Many studies showed that inhaled anesthetics reduce amplitude and increase latency in some of VEP components in a concentration-dependent manner (Ghita et al. 2013; Ito et al. 2014; Tanaka et al. 2020). It has been demonstrated that depending on the depth of sevoflurane anesthesia the latency of VEP peaks varied up to 15 ms (Ghita et al. 2013). Although in our experiment the level of gas anesthesia was

maintained stable (MAC 3%) through the whole experiment, the latency of most VEP components increased over one hour after saline injection. Similar pattern of increase in latency was visible after ethanol injection (second hour of experiment). Moreover, the latency value of the last time bin after saline injection was similar to the latency value of the first time bin after ethanol injection. It seems that through the 2 hours of VEP recording the depth of anesthesia increased over time and caused the increment of latency and ethanol did not had effect on that. Previous reports using awake freely moving rats demonstrated that the latency of flash evoked potential components increased 20 min after ethanol administration (Hetzler et al. 1981) and changes of latency components were less than 4 ms (Hetzler and Bednarek 2001, Hetzler and Martin 2006, Hetzler et al. 1981, Hetzler et al. 1988). Although the latency increased over time because of anesthesia, but we did not noticed effect of ethanol. The reason for that could be related with the specific anesthetic. Both ethanol and sevoflurane act on the NMDA and $GABA_A$ receptors (Nishikawa et al. 2005, Petrenko et al. 2014) and they are also metabolized by the same enzymes (Klotz and Ammon 1998). Moreover, results of other studies show that gas anesthesia have a stronger effect than ethanol, changes of latency more than 15 ms or less than 4 ms, respectively. Both of these reasons support the idea, that the effect of ethanol was masked by the stronger effect of sevoflurane.

ON and OFF response latency with and without ethanol did not differ in N1 and P2 components, which means that at the subcortical level processing of stimulus onset is similar to that of stimulus offset. This is in line with studies of other sensory systems, where no differences have been found in the latency of auditory N1 component elicited by stimulus onset and offset (Yamashiro et al. 2009). However, analysis of the N2 component showed that anesthesia and/or ethanol affected only the processing of stimulus onset. Following administration of saline, the latency of N2 ON response tended to be shorter, compared to the latency of N2 OFF response. After ethanol injection, during the second hour of anesthesia, latency of ON response increased, whereas the latency of OFF response was not affected. Therefore, it is possible that anesthesia alone and/or combined with ethanol interferes with the processing of the stimulus duration at the visual cortex level. Single cell recordings have shown that the stimulus onset latency varies and depends on the stimulus type which is not the case for the offset latency (Bair et al. 2002, Tadin et al. 2010). Our results extend these findings showing that the response to stimulus onset is more readily influenced by combination of anesthesia and acute administration of ethanol than the stimulus offset. If perception of stimulus

onset is affected without affecting its offset, it is likely that perception of the whole stimulus duration would become shorter. Hence, the brain will process stimulus onset with longer latency than in basal conditions, but if the stimulus termination remains unchanged, visual stimulus may be perceived as a shorter one. It was shown that changes in the processing of the stimulus onset and offset interfere with the reaction-time to the sensory input (Hari et al. 1987; Yamashiro et al. 2008; Yamashiro et al. 2009; Serviere et al. 1977). Our data confirm previous findings and extends them by suggesting that anesthesia alone or/and together with alcohol has a stronger effect on sensory response to stimulus onset than offset.

5.2 Effects of chronic alcohol consumption and abstinence

Our results demonstrated that only two components of the ON response were affected by chronic alcohol consumption, particularly we showed that $P3_{VC}$ amplitude and $N3_{VC}$ latency were increased and $N3_{VC}$ amplitude was reduced. Most of the previous studies showed that during ethanol intoxication amplitudes of both early (P1-N1, N1-P2) and late (P3-N3) components from the visual cortex were reduced (Begleiter 1975; Begleiter and Porjesz 1977; Bierley *et al*. 1980; Kjellstrom *et al*. 1994) and latencies of P2 increased (Bierley *et al*. 1980) as a result of neuronal death and damage of myelin coating (Johnsen-Soriano *et al*. 2007; Sancho-Tello *et al*. 2008); however, some studies showed increase of VEP amplitudes (Sokomba and Osuide 1985). This discrepancy in findings could partly be explained by the methodological differences: 1) all previous studies focused on effects of withdrawal and intoxication phases that were relatively short, up to one month; 2) in previous studies, the amount of ethanol consumed by rats was high, up to 15 g/kg per day; and 3) ethanol was delivered by intubation, liquid diet or vapor exposure, usually followed by the loss of body weight (Begleiter 1975; Kjellstrom *et al*. 1994).

The potential methodological influence is also supported by the different effects of abstinence in our study compared to previous reports. All earlier studies that investigated the effects of abstinence on FVEP demonstrated increment of the amplitude as a result of hyper excitability of the visual system, whereas latency remained unchanged (Begleiter 1975; Bierley *et al*. 1980; Sokomba and Osuide 1985; Kjellstrom *et al*. 1994). Usually, the elevated amplitude reached a peak in about 8-10 hours after withdrawal and stayed partly increased for at least one week (Bierley *et al*. 1980; Sokomba and Osuide 1985; Kjellstrom *et al*. 1994). However, our results showed decreased amplitude and increased latency in both ON and

OFF responses in the visual cortex. The reason for this could be the suboptimal time window selected for the recordings: during the abstinence phase our first recording was made 12 hours after the withdrawal. Therefore, the main excitation time may have been missed in our study, as other studies showed the first signs of abstinence 6-7 hours after withdrawal (Begleiter 1975; Begleiter and Porjesz 1977). Nevertheless, our data showed reduced latency of $P1_{LGN}$ in the ON responses, which indicate increased excitability of LGN relay cells.

In the ON response both parameters of $N3_{VC}$ component, which represents the second wave in the after-discharge processes (Mwanza et al. 2008), were affected by both ethanol consumption and withdrawal indicating that the last stage of visual information processing is the most sensitive. Components of the OFF response, on the contrary, were stronger modulated by ethanol: $N1_{VC}$, $P2_{VC}$ and $P3_{VC}$ were affected and the pattern of the effect on the components was the same during alcohol drinking and withdrawal stages. Two components of the OFF response, $N2_{VC}$ and $N3_{VC}$, from VC remained unaffected. Our previous study also showed resistance of the last component from OFF response to the effect of acute alcohol consumption (Dulinskas *et al*. 2017). These results might indicate that the component $N3_{VC}$ in the onset and offset has different origin. Moreover, alcohol consumption reduced latency of OFF components $P1_{LGN}$ and $N1_{LGN}$ (Fig. 4.4A and 4.4C). Although these changes on the LGN components were reversible (after withdrawal, latency of these components was comparable to the control animals), the timing of the visual stimulus offset processing during ethanol consumption stage is affected at the level of thalamus and cortex.

5.3 Asymmetry of stimulus onset – offset

The present study revealed that chronic alcohol consumption and abstinence enhanced and/or diminished differences between ON and OFF responses by affecting mostly the OFF response. This strong modulation of the onset-offset asymmetry was observed at the level of visual cortex, but not at the level of LGN. The onset-offset asymmetry could be explained by potentially different neuronal mechanisms involved in the generation of the ON and OFF responses. Onset-offset asymmetries were described in different animal species at different levels of the visual system. In flies, two anatomically different pathways for light-on and light-off in the layers of medulla were shown by Strother et al. (2014). In mammals, visual signals are processed through the functionally separated ON and OFF channels, which do not interact before converging in the primary visual cortex (Schiller 1992).

Primate retinal ON-center cells not only have larger receptive fields, but also faster responses compared to OFF-center cells, indicating specific mechanistic asymmetries in retinal ON and OFF circuits (Chichilnisky and Kalmar 2002). On the other hand, longer onset latency of LGN and visual cortex cells does not depend on a cell type (Bair *et al*. 2002). Furthermore, responses to stimulus onset and offset and their asymmetry are common in a large population of cortical neurons from different modalities (Noda *et al*. 1998; Yamashiro *et al*. 2008). These studies suggest that the OFF response is less variable (Tadin *et al*. 2010; Sato 2016) and the offset event acts as a more reliable timing cue than the onset event. For the first time, the present study demonstrated a direct qualitative difference between ON and OFF responses at the level of visual cortex, but not at the level of LGN. Also, our results showed temporal onset – offset asymmetry for $N2_{VC}$ and $P3_{VC}$ components indicating that input (except directly from LGN) to the visual cortex is processed faster during stimulus onset than offset. The higher amplitude of $N1_{VC}$, $N3_{VC}$ and lower amplitudes of $P2_{VC}$ components in the ON compared to the OFF responses, suggest that different number (or arousal) of neurons is involved in the intracortical signal transmission during stimulus onset and offset. Moreover, our data indicated that the modulation of the onset – offset asymmetry by ethanol consumption and withdrawal occurred through the effect on the OFF response. All together our results suggest that different neuronal mechanisms are involved in generation of the ON and OFF responses, but the OFF response is more sensitive to the effect of alcohol consumption and withdrawal.

Our results showed that chronic alcohol consumption affected just the OFF response at both LGN and VC levels. Chronic alcohol consumption alters brain functioning through two pathways: induces neurotoxicity and neuronal cell death (Alfonso-Loeches & Consuelo Guerri 2011; Chastain & Sarkar 2014); and causes neuroadaptations e.g. decreased GABA receptors expression, increased dopamine and NMDA (Most et al. 2014) and AMPA (Lewohl et al. 2000) receptors expression. One of the plausible reasons for stronger effect of alcohol consumption on the OFF response might be that alcohol affected the signal before it reached lateral geniculate nucleus i.e. at the level of retina.

Although rods and cones use glutamate to transmit signals, different types of bipolar cells have different neurotransmitter receptors: ON – center cells contain metabotropic glutamate receptors (mGluR6) (Slaughter & Miller 1983b; Slaughter & Miller 1983a) and some ionotropic AMPA receptors (Vardi et al. 1998), OFF – center bipolar cells have AMPA or kainate receptors (Devries & Schwartz 1999; Devries 2000; Peng et al. 1995; Nelson

et al. 2001). Studies of ON and OFF bipolar cells indicated that ethanol increases amplitude and reduces latency of the b-wave generated by ON bipolar cells and reduces the amplitude and increases latency of the d-wave generated by OFF bipolar cells in the retina (Bernhard & Knave 1973; Kuzeva et al. 2015). Some studies showed modulation of AMPA current by high concentration of ethanol in non-retinal neurons (Santerre et al. 2014; Marty & Spigelman 2012). Probably, the enhancing effect of ethanol on the ON response is through the inhibition of non-NMDA ionotropic glutamate receptors on horizontal cells, but not because of the effect on mGluR6; whereas the depressing effect of the OFF response is due to direct inhibition of non-NMDA ionotropic glutamate receptors on OFF bipolar cells (Kuzeva et al. 2015). It has been shown that in Purkinje neurons ethanol modulates electrophysiological responses through mGluRs (Netzeband & Gruol 1995). Although other studies stated that there is no evidence that mGluRs are altered by acute or chronic ethanol consumption (Chandler 2003). But it has been shown that in mesocorticolimbic areas chronic state of alcohol consumption upregulates expression of the AMPA receptors (Salling et al. 2014; Chandler et al. 1999). If it is the same in the visual system, chronic alcohol consumption could upregulate AMPA receptors in OFF – center bipolar cells and could have an influence on the OFF response in LGN and VC.

The mechanisms of ON and OFF responses are still debatable. Some research supports the theory that ON and OFF responses are defined by ON and OFF pathways originating from the inner plexiform layer of the retina (Chichilnisky & Kalmar 2002; Zemon et al. 1988). Whereas other studies support the idea that ON and OFF responses separate only at a cortical level (Padnick & Linsenmeier 1999; Liang et al. 2008; Bair et al. 2002). Our study showed that both late components of the ON response, P3 and N3, in visual cortex were affected by alcohol consumption. These components are elicited by the first wave of photically evoked after-discharge burst, which is produced in the lateral geniculate nucleus (Bigler & Eidelberg 1976), and represents the thalamo-cortical circuit (Shearer et al. 1976). Based on these findings, we believe that the ON response only the thalamo-cortical circuit is affected by alcohol consumption. Whereas early and late components of the OFF response were affected by both alcohol and abstinence. It is important to point out that the early components were affected more than the late components. It is known that early components, primary – P1 and N1, and secondary – P2 and N2, are result of retino-geniculo-striate activity (Eells & Wilkison 1989). Altogether, our data support the idea that differential effect of alcohol on the ON and OFF responses originates from different neuronal circuits.

One more factor that may have an impact on the asymmetry of the ON and OFF responses is the intensity of the stimulus. It is well known that intensity of the stimulus strongly modulates amplitude and latency of VEP components (Creel *et al*. 1974; Lopez *et al*. 2002). Moreover, it has been shown that in humans, stimulus intensity differently modulates individual peak latency: in the ON response variability of the latency is low when the stimulus intensity is high (20,000 lx), and in the OFF response when the stimulus intensity is low (10,000 lx) (Sato 2016). In order to protect the eyes of the animals from the damage we used a relatively low amount of light (750 lx). Most of the studies showed that amplitude increases and latency decreases of rat VEP components with increasing flash stimulus intensity (Tomita et al. 2009; Herr et al. 1991; Creel et al. 1974; Arena et al. 2017). But it is unclear how intensity level of our experiment affected the individual differences in the peak latency of the ON and OFF responses since rat and human visual systems are differently sensitive to light (Burn 2008).

In summary, this thesis demonstrates that acute, chronic alcohol consumption and/or withdrawal differently modulate visual information about stimulus onset and offset processing at visual cortex and lateral geniculate nucleus levels.

6. Conclusions

1. In the rat visual cortex the amplitude of VEP components $N1_{VC}$, $N3_{VC}$ are higher; and amplitude of $P2_{VC}$ and latency of $N2_{VC}$, $P3_{VC}$ are smaller during visual stimulus onset compared to visual stimulus offset.
2. At cortical level in sevoflurane anesthetized rat N2 latency is greatly increased in ON response than in OFF response; the amplitude in OFF response is more reduced by acute ethanol consumption than in ON response.
3. Long-term chronic alcohol consumption and withdrawal reduce the amplitude and increase the latency in the last stage of stimulus onset processing at the level of visual cortex and has no effect at the level of lateral geniculate nucleus.
4. Long-term chronic alcohol consumption and withdrawal modulate the response to the stimulus offset in the visual cortex (increase the latency of $N1_{VC}$, $P2_{VC}$ and reduce the latency of $P3_{VC}$ and the amplitude of $P2_{VC}$, $P3_{VC}$ components) and lateral geniculate nucleus (reduce the latency of $P1_{LGN}$, $N1_{LGN}$ and increase the amplitude of $P2_{LGN}$ components) levels.
5. At the visual cortex and lateral geniculate nucleus a higher number of parameters from the response to the stimulus offset are affected by chronic alcohol consumption and/or withdrawal as compared to stimulus onset.

7. References

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Publications

Publications on the thesis topic

- **Dulinskas R**, Buisas R, Vengeliene V, Ruksenas O. Effect of ethanol on the visual-evoked potential in rat: dynamics of ON and OFF responses. *Acta Neurobiol Exp (Wars).* (2017); 77(2):190-197.
- **Redas Dulinskas** and Osvaldas Ruksenas. Modulation of responses to visual stimulus onset and offset by chronic alcohol consumption and withdrawal in the rat visual cortex and lateral geniculate nucleus. *Alcohol* (2020), 85: 101-110.

Conferences on the thesis topic

- **R. Dulinskas**, O. Ruksenas „ Effect of nicotine on the rat lateral geniculate body and visual cortex "FENS Forum 2018", Berlin, Germany, July 07-11, 2018.
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Research Experience

- 2019.10.01-present: specialist at Experimetica UAB, Vilnius, Lithuania.
- 2013.07.01-2019.12.31: Technician at Vilnius University, Life Sciences Center, Department of Neurobiology and Biophysics.

Research Projects

- 2019.06.01-12.31: Junior Researcher position in the project: "Targeting the microbiota-gut-brain axis in Alzheimer's disease: the role of the endocannabinoid system".
- 2014.07.01-2016.12.31: Research associate position in the project: "Effect of chronic ethanol ingestion on regional neurophysiological

function of the brain", MIP-012/2014, Research Council of Lithuania.

Qualification improvement

- 2019.01.16-04.15 research intern at Okinawa Institute of Science and Technology, Okinawa, Japan.
- 2018.05.04-06 attended the Brain-Machine Interface symposium & workshop "Stepping into the Future – a Multi-Disciplinary Approach to Brain-Machine Interface", Vilnius, Lithuania
- 2018.02.28-03.03 Scientific visit at National Institute of Mental Health, Prague, Czech Republic. Visit was funded by LINO.
- 2018.02.19-23 attended the Winter School "Ethics and Neuroscience" (2,5 ECTS), Humboldt University of Berlin, Berlin, Germany.
- 2016.09.24-10.08 Completed the course „Sensory Ecology" (6 ECTS) Lund University, Lund, Sweden.
- 2014.09.01-2015.01.26 Completed the course "LABORATORY ANIMAL SCIENCE" held in Vilnius University, Faculty of Natural Sciences, and received certificate of FELASA C category for working with laboratory animals.
- 2014.04.01-06.31 ERASMUS practice at Werner Reichardt Institute for Integrative Neuroscience, University of Tübingen, Tübingen, Germany.

Awards

- LMT (Research Council of Lithuania) scholarship for academic results (2018).
- FENS-IBRO/PERC traveling grant for participating in the FENS Forum 2018 (2018).
- FENS-IBRO traveling grant to attend the Winter School "Ethics and Neuroscience" (2017).
- Scholarship from Vilnius University for academic results (2017).
- Master's degree finished with Magna Cum Laude (2015).

Publications

- **Redas Dulinskas** and Osvaldas Ruksenas "Modulation of responses to visual stimulus onset and offset by chronic alcohol consumption and withdrawal in the rat visual cortex and lateral geniculate nucleus." *Alcohol* (2020), 85: 101-110.
- **Redas Dulinskas**, Rokas Buisas, Valentina Vengeliene, and Osvaldas Ruksenas "Effect of ethanol on the visual-evoked potential in rat: dynamics of ON and OFF responses", *Acta Neurobiol Exp* (2017), 77: 190–197.

Conference: oral presantations

- Investigation of rat visual cortex evoked potentials to visual stimulus onset and offset. *Virtual instruments in biomedicine – 2013*, Klaipeda, Lithuania; May 24, 2013.
- Relationship between stimulus intensity and rat visual cortex evoked potentials to visual stimulus onset and offset. *Medical Physics in the Baltic States 2013*, Kaunas, Lithuania; October 10 – 12, 2013.
- Investigation of rat visual cortex evoked potentials to visual stimulus onset and offset. *XVIII Balt-LASA conference "Laboratory animals in research"*, Vilnius, Lithuania; December 4, 2014.
- Lamotrigino poveikio chroniškam alkoholio vartojimui tyrimas. *Toksinių įvairių organų pakenkimų diagnostikos ir gydymo aktualijos*, Kaunas, Lietuva, February 10, 2017.

Conference: poster presentations

- **R. Dulinskas**, O. Ruksenas „ Effect of nicotine on the rat lateral geniculate body and visual cortex", FENS Forum 2018, Berlin, Germany, July 07-11, 2018.
- **R. Dulinskas**, O. Ruksenas „Effect of nicotine on the rat visual cortex visually evoked potentials", Aspects of Neuroscience 2017, Warsaw, Poland, November 24-26, 2017.
- O. Ruksenas, **R. Dulinskas**, R. Buišas, V. Vengelienė "Effect of chronic ethanol ingestion on regional neurophysiological function of the brain", IUPS 38th Wolrd Congres, Rio de Janeiro, August 1-5, 2017.

- **R. Dulinskas**, O. Ruksenas „Alcohol differently affects proccesing of visual stimulus onset and offset“, Aspects of Neuroscience 2016, Warsaw, Poland, November 25-27, 2016.
- R. Buisas, **R. Dulinskas**, T. Ragauskas, O. Ruksenas ir V. Vengeliene „Effect of chronic ethanol ingestion on regional electrophysiological activity of the rat brain“, FENS Forum, Copenhagen, Denmark, July 2-6, 2016.
- **R. Dulinskas**, R. Buisas, V. Vengeliene ir O. Ruksenas „Effect of ethanol on the visual-evoked potentials: dynamics of ON and OFF responses“, FENS Forum, Copenhagen, Denmark, July 2-6, 2016.
- **R. Dulinskas**, R. Buisas, V. Vengeliene ir O. Ruksenas „Differential effect of alcohol on latency and amplitude of visually evoked potentials in rat visual cortex“. *European Society for Biomedical Research on Alcoholism*, Valencia, Spain, September 12–15, 2015.
- **R. Dulinskas**, R. Buisas, V. Vengeliene ir O. Ruksenas “Effect of alcohol on the rat visual cortex visually evoked potentials”, *FEPS*, Kaunas, Lietuva, August 26-29, 2015.
- **R. Dulinskas**, O. Ruksenas „Influence of stimulus intensity on the rat visual cortex visually evoked potentials to visual stimulus onset and offset“. *Aspects of Neuroscience*, Warsaw, Poland, November 14 - 16, 2014.
- **R. Dulinskas**, O. Ruksenas „Influence of stimulus duration on the rat visual cortex visually evoked potentials to visual stimulus onset and offset“. *V – ioji Lietuvos neuromokslų asociacijos konferencija*, Vilnius, Lithuania, December 6-7, 2013.

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