Binocular summation evaluated by early and late visual evoked potentials

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The visual evoked potential technique makes it possible to explore the visual system with a high temporal resolution, thereby providing information about different phases of binocular integration. The aim of the study was to characterize the amplitude, latency and scalp distribution of pattern-reversal gratings visual evoked potentials related to binocular viewing. 12 adult participants without any pathology of the visual system were examined by electroencephalography (EEG), with three viewing conditions: the dominant eye, non-dominant eye, and both eyes. They were tested with reversed checkerboard patterns presented with a frequency of 1.02 Hz. The amplitudes of visual evoked potentials were calculated to detect the effect of binocular integration. The strongest binocular summation was observed on the N75 component. P100 component was also affected by binocular viewing, mainly above parieto-occipital cortex. In the later time window binocular summation was reflected in changes in amplitude of N145 component over the right parieto-occipital region. The obtained results suggest that binocular summation is not limited to occipital cortex as its influence was also observed above parieto-occipital regions. Changes in amplitude of N75 component looks to be a good predictor of binocular summation.

Keywords: binocular summation, binocular vision, visual evoked potentials.

1. Introduction

Binocular summation (BS) has been defined as a superiority of binocular over monocular visual performance [1]. The effects of BS depend on differences in the magnitude

of the quantitative value of the so-called BS factor as well as differences between various tests [2–6]. BS has been well studied in normal participants using indirect psychophysical tests [5, 7]. These tests commonly show an improvement in visual acuity (VA) under binocular viewing as compared to monocular viewing conditions [8–12]. Furthermore, binocular viewing leads to increased contrast sensitivity [8, 13–16], a perceived supra-threshold contrast [16, 17], and the visual field increases from 160° to 200° [18–21]. Finally, stereovision increases depth perception [22, 23].

The effect of binocular viewing has also been examined by recording visual evoked potentials (VEPs). Earlier studies [3, 24, 25] reported that VEP amplitudes in binocular vision are larger and/or the latency of the various components is shorter than with monocular vision. However, the amount of BS varies widely. For example, in the study of Di Summa *et al.* [3], an increase in VEP amplitudes (N75/P100 and P100/N145) with binocular vision was observed only when the comparison was made with the non-dominant eye, but not when the comparison was made with the dominant eye. Bagolini *et al.* [26] observed BS only when low contrast stimuli were used (3.2%). Other studies revealed that BS was more pronounced when stimulus contrast was larger [27, 28], when check sizes were smaller, and when mid-spatial frequencies were employed [2, 3]. In a more recent study by Matsumoto *et al.* [4], no significant BS was observed with a flash-VEP method when the amplitude and/or latency of the P2 and N2 components were compared. Similarly, no significant effects of BS were recorded in pattern-VEPs by Pineles *et al.* [5], where 36% of examined subjects with normal vision revealed BS, but 46% of the participants showed an opposite effect – inter-ocular suppression (IS – a smaller VEP signal with binocular viewing as compared to monocular viewing). In that study, the proportion of BS to IS was very similar in a group of strabismics where in general BS is usually not observed [29, 30]. These varying results may be due to the application of different stimulation methods to examine BS (*e.g.*, commonly used visual stimuli are strobe flash, flashing light-emitting diodes, transient and steady state pattern reversal and pattern onset/offset), but also a limited number of electrodes used for recording cortical responses. Usually only an occipital electrode (Oz) or in rare cases two additional electrodes (O1/2) were used. In addition, only the N75/P100 amplitude and latencies were usually compared between mono- and binocular viewing conditions. In rare cases [3, 31] the amplitude of P100/N145 was also calculated.

The development of a standard objective methodology to evaluate binocular vision would be helpful to diagnose and monitor the maturation of visual system in future studies as psychophysical tests are based on subjective responses and depend on variables such as the instructions given to patients, the level of attention, *etc*.

In the current study, we decided to employ a large number of electrodes, thereby avoiding the possibility to miss effects of BS. On the basis of earlier multiple electrodes analysis, three channels with the highest amount of BS were selected and were further analyzed statistically. Our study focused on various components (N75, P100, N145) that allowed us to establish the time course of BS.

2. Method

2.1. Participants

Twelve persons between 20 and 23 years of age participated (5 males and 9 females). For this study, we selected the homogenic group of young healthy participants, because the effect of binocular summation might depend on patient's age (it was proved that binocular summation is the strongest in infants, and much lower in adults [32]).

Each of the participants passed an ophthalmic ocular examination and a full optometric ocular test in Laboratory of Vision Science and Optometry (all of these patients were under care of ophthalmologists and optometrists, and did not have any ocular disease in the past). All participants had normal or corrected to normal visual acuity (20/20) and stereoacuity of at least 40″ (random-dot stereo butterfly, Stereo Optical, Chicago, IL). None of the participants reported any history of strabismus, inter-ocular suppression, eyestrain symptoms or ocular pathology. The study was approved by the local Ethics Committee of Adam Mickiewicz University and was performed in accordance with the Declaration of Helsinki.

2.2. Task and stimuli

Visual stimuli were presented on a cathode ray tube (CRT) screen with Presentation software (version 17.2) at a distance of 114 cm in front of the participant. Checker-board stimuli (a black-white reversal pattern with squares) were displayed with a frequency of 1.02 Hz in three visual conditions: 1) a monocular viewing condition with the dominant eye (DE); 2) a monocular viewing condition with the non-dominant eye (NDE); 3) a binocular viewing condition with both eyes (BE). Each stimulus was presented 120 times. Checker-board stimuli was 13.3° vertically and 17° horizontally. Standard sizes of stimuli were used: 15′ (size of the square) with high contrast (90%) to evoke strong visual potentials. The order of visual conditions (DE, NDE, BE) was counterbalanced among participants. A three-minute break was introduced after each block to allow for the development of light adaptation [33]. Participants were instructed to look at a small red square in the center of the screen, blink normally and keep their head and eyes still during the experiment.

2.3. Apparatus and EEG recording

During the experiment EEG was measured using a multichannel EEG system with 64 active electrodes (128 QuickAmp, Brain Products). Active electrodes were fixed to the scalp through a conventional rubber head-cap and then gel application was performed (Super-Visc High Viscosity Electrolyte-Gel – gel necessary for active electrodes). Electrodes were placed according to the extended International 10/20 system [34] (see Fig. 1). EEG signals were sampled at a rate of 250 Hz with a high pass filter of 0.015 Hz. An average reference built-in in the amplifier was used. The ground electrode was po-
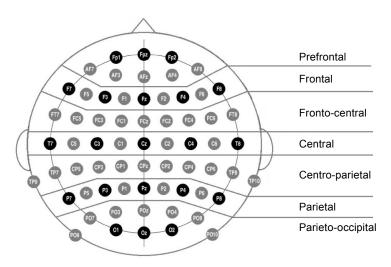
Fig. 1. Electrode positions and labels in the extended 10/20 system.

sitioned at AFz. To check for eye movements and blink artifacts, electrooculography was recorded bipolarly from above and below the right eye (vEOG) and horizontally from positions next to the outer rims of the eyes (hEOG). Together, EEG, EOG and task-related events such as stimulus onset were registered with Brain Vision Recorder (BrainProducts GmbH) installed on a separate acquisition computer. ActiCAP Control software (version 1.2.3.0) was used to allow impedance measurement of active electrodes. Electrode impedances were verified and kept below 5 kΩ.

3. Data processing

3.1. EEG and ERP parameters

Offline, EEG analyses were performed with Vision Analyzer 2.0.3 (Brain Products). The data were first filtered with a high cutoff frequency (70 Hz). Segments with blinks coinciding with stimulus presentation were rejected. Off-line data were analyzed for each subject at each electrode site from trials free of EOG artifact: after data segmentation (–980 until 980 ms stimulus onset), baseline correction (time window 100 ms before stimulus onset), ocular correction and artifact rejection, an average of each individual was completed. Next, after visual inspection, six channels with clear visual evoked potentials (VEPs) were selected for peak detection analyses: PO7, POz, PO8, O1, Oz, O2. The characteristics of three VEP components (N75, P100, N145) were estimated for each channel. The amplitude of N75 was measured from baseline to peak of N75 component. Others amplitudes were calculated, defined as the voltage difference between two adjacent negative and positive peaks, and the latency at which their components occurred after stimulus onset. BS was calculated as the ratio of the average amplitude when viewing with both eyes (AMP_{BE}) and the average amplitude of monocular viewing with the dominant eye (AMP_{DE}) – BS = AMP_{BE}/AMP_{DE}.

3.2. Statistical analyses

Statistical analyses were performed with Statistica 10 software (StatSoft), using ANOVAs with repeated measurements with 3 factors: 1) *peak* (3 levels: N75, P100, N145); 2) *electrode* (6 levels: PO7, POz, PO8, O1, Oz, O2); 3) *eye* (2 levels: DE, BE). To assess BS, amplitudes and latencies of VEPs were compared statistically between dominant eye, which has usually higher VEPs than non-dominant eye [35] and both eyes viewing. When necessary *p* value was corrected by Huynh and Feldt correction factors. Tukey's test was used as post hoc test. The value of BS for each channel and each peak was compared statistically with 1, by employing *t*-tests. BS was significant when BS-value was higher than 1, with $p < 0.050$.

4. Results

Amplitudes and latencies of the N75, P100, and N145 components are presented in Figs. 2 and 3. The effects of BS estimated with VEPs are displayed in Tables 1 and 2.
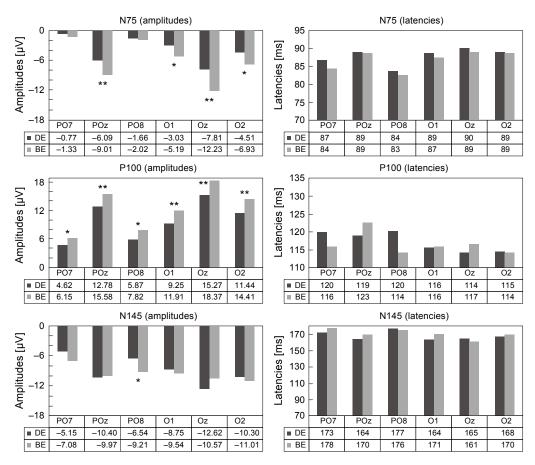

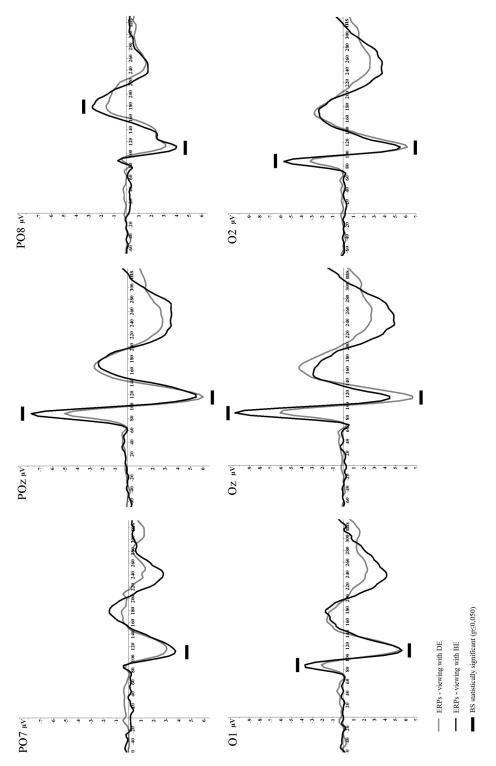Fig. 2. Amplitudes and latencies of N75, P100, N145 VEPs components; $p \leq 0.050$ (*); $p \leq 0.001$ (**).

Fig. 3. Event-related potentials (ERPs) at electrodes PO7, POz, PO8, O1, Oz and O2 after stimuli – viewing with DE (gray line) and BE (black line).

T a b l e 1. Comparison of mean values of the amplitude of the VEP components. AMP_{DE} – amplitude of the component when viewing with dominant eye, AMP_{BE} – amplitude of the component when viewing with both eyes; BS – binocular summation effect, $p \leq 0.050$ (*), $p \leq 0.001$ (**).

	Amplitudes [μV]								
	N75			P100			N145		
	AMP_{DE}	AMP_{BE}	BS	AMP_{DE}	AMP_{BE}	BS	AMP_{DE}	AMP_{BE}	BS
POz	−6.09	−9.01	1.5**	12.78	15.58	1.2**	−10.40	−9.97	1.0
PO7	−0.77	−1.33	1.7	4.62	6.15	1.3*	−5.15	−7.08	1.4
PO8	−1.66	−2.02	1.2	5.87	7.82	1.3*	−6.54	−9.21	1.4*
Oz	−7.81	−12.23	1.6**	15.27	18.37	1.2**	−12.62	−10.57	0.8
O1	−3.03	−5.19	1.7*	9.25	11.91	1.3**	−8.75	−9.54	1.1
O2	−4.51	−6.93	1.5*	11.44	14.41	1.3**	−10.30	−11.01	1.1

POz, PO7, PO8 – PO group; Oz, O1, O2 – O group.

T a b l e 2. Comparison of mean values of the latency of the VEP components. LAT_{DE} – the latency at which the components occur after stimulus presentation when viewing with dominant eye, LAT_{BE} – the latency at which the components occur after stimulus presentation when viewing with both eyes.

	Latencies [ms]					
	N75		P100		N145	
	LAT_{DE}	LAT_{BE}	LAT_{DE}	LAT_{BE}	LAT_{DE}	LAT_{BE}
POz	89	89	119	123	164	170
PO7	87	84	120	116	173	178
PO8	84	83	120	114	177	176
Oz	90	89	114	117	165	161
O1	89	87	116	116	164	171
O2	89	89	115	114	168	170

POz, PO7, PO8 – PO group; Oz, O1, O2 – O group.

In line with our expectations, the first visible negative VEP component (N75) had a peak latency of 88 ms, which was present on all selected channels. The second component (P100) had a positive polarity with a peak latency of about 118 ms, and the third component (N145) with a negative polarity, had a peak latency about 170 ms. As can be seen in Fig. 2, the amplitudes of all components were affected by binocular viewing, with the amplitude increasing in the case of binocular vision.

Statistical analyses showed that VEPs amplitude was dependent on the peak and eye (*peak × eye* interaction: $F(2,22) = 9.62$, $p < 0.001$, $\eta^2 = 0.47$) and varied for different electrodes (*peak × eye × electrode* interaction: $F(10,110) = 7.06$, $p < 0.001$, $\eta^2 = 0.39$). In order to explain these interactions, separate ANOVAs for each peak were performed.

The earliest VEP component – N75 – was strongly affected by binocular viewing, which was confirmed by a significant *eye × electrode* interaction ($F(2\ 55) = 8.02$, $p < 0.001$, $\eta^2 = 0.42$). Post hoc test showed that N75 amplitude was higher for BE than for DE on the O1, Oz, O2 and POz channels ($p < 0.009$). The *t*-test proved that BS effect was significantly higher than 1 on the occipital channels ($BS_{O1} = 1.7$, $BS_{Oz} = 1.6$, $BS_{O2} = 1.5$; $p < 0.012$) and on the POz electrode $BS_{POz} = 1.5$, $p = 0.005$).

The next VEP component – P100 – which is usually the most prominent, was also affected by binocular viewing (main effect of eye $F(1,11) = 11.28$, $p = 0.006$, $\eta^2 = 0.51$). Here, amplitude of the P100 for BE was higher than for DE on all channels from the occipital group (O1, Oz, O2, $p < 0.001$) but also on all electrodes from the parieto-occipital group (PO7, POz, PO8, $p < 0.042$). However, the effect of BS was lower than on the N75 component, but still statistically significant, which was shown by the *t*-test ($BS_{O1} = 1.3$, $BS_{Oz} = 1.2$, $BS_{O2} = 1.3$, $BS_{PO7} = 1.3$, $BS_{POz} = 1.2$, $BS_{PO8} = 1.3$; $p < 0.050$).

The third VEP component – N145 – was only partially affected by binocular viewing. The N145 component was significantly higher for BE compared to DE only on the PO8 channel, which was revealed by an *eye × electrode* interaction ($F(2,55) = 8.21$, $p < 0.001$, $\eta^2 = 0.43$) and post hoc test ($p = 0.002$). The effect on the PO8 electrode was confirmed also in *t*-test where BS_{PO8} was equal to 1.3 ($p = 0.014$).

Similar statistical analyses were performed on the latency parameter, but no significant influence of binocular viewing on the latencies of any VEP component on any analyzed channel was found ($p > 0.050$).

5. Discussion

The aim of the current study was to examine influence of binocular viewing on VEPs signal. Several methods have been proposed to test binocular competence in humans, using VEP method. Most often, amplitude of the P100 component was used as a diagnostic index of BS, based on the assumption that a binocular response should be higher than a monocular response [36]. Our results confirmed that VEPs amplitudes are dependent on binocular viewing and using optional channels configuration, it is possible to detect early as well as late phase of BS.

In line with our expectations, BS was most pronounced above occipital areas (O1/Oz/O2) but this mainly concerned the earliest VEP component (N75). When viewing with both eyes, the amplitude of N75 increased by 60%–70% (BS: 1.6–1.7) on the Oz and O1 channels. The value of BS was even higher than expected based on [37], which shows that the enhancement ratio should be lower than 1.4. The explanation can lie in neural generator of N75, probably located in primary cortex [38–42], and will be discussed later.

The largest VEP component – P100, was also affected by binocular viewing. BS was detected both on the occipital channels, as well as on the parieto-occipital electrodes. BS on the P100 was lower than on the N75 (BS: 1.2–1.3 *versus* 1.6–1.7, for P100 and N75 components, respectively). BS was also found on the ventral part of visual cortex (PO7, PO8), suggesting that neuronal generators of P100 are located not only in primary visual cortex, but also in extrastriate visual areas [43–45]. This point of view is consistent with the study on neural source generators [38–41, 46, 47].

The obtained results may explain why no BS was found in a number of studies. For example, Di Summa *et al.* [48] showed that an increase in P100 amplitude was demonstrated only for small checkboard sizes and only when compared with the eye of smaller

(worse) VEP signal. In another study [49] focusing on the influence of contrasts on BS in vernier acuity, significant summation was found for low contrast stimuli but negligible for high contrast stimuli. Estimation of BS in the fovea and peripheral visual field [50] found that BS in the periphery is lowered with decreasing stimulus size and is higher with increasing stimulus size. No increase in P100 was also observed in subjects with normal vision in the study by MIZOTA *et al.* [51] or SLOPER and COLLINS [52]: improvement in stereoacuity is accompanied by a fall in binocular enhancement of the P100 amplitude of the VEP to small checks and a reduction in the P100 latency. In our study, the strongest BS was observed not in the P100 time window, but on the N75 component. Importantly, this observation may indicate that the N75 is a better indicator of BS than the P100.

Later phases of BS were usually taken for granted in the literature, since N145 is a complex component reflecting processing on higher cortical levels. However, we found that BS may be detected also on the N145 component, but only on the channels located ventrally and specifically over the right hemisphere (PO8 channel).

As indicated above, in the majority of studies on BS, only the amplitude of P100 was measured and higher VEP amplitude for binocular than for monocular viewing was interpreted as a response of binocular cells of the primary visual cortex (BA17 or V1). However, more recent studies [39, 40, 47, 53, 54] suggested that the neural generators of the P100 component are not located in primary visual cortex, but rather in extrastriate cortex (middle occipital and fusiform gyrus). Thus, changes in the P100 component seem to reflect the integration of information from both eyes on a higher but not primary cortical level. However, the response from primary cortex seems to be reflected by changes in the N75 component, since source localization studies found neural generators of N75 (called also C1) in the calcarine sulcus [55–58].

Our data suggest that the increase in amplitude of the early N75 component may be a better predictor of BS than the amplitude of the P100. This result was in accordance with the study of HARTER *et al.* [59]. To better detect the dynamics of BS, we propose to use different than standard channels configuration: 1st measurement with O1/Oz/O2 channels, and the 2nd recording with PO7/POz/PO8 channels. The early N75 component over occipital electrodes likely reflects a response from primary visual cortex, while the later components (P100, N145) may reflect the integration of the signal from both eyes on a higher cortical level.

6. Conclusions

Multichannel EEG has demonstrated that BS occurs even in adult subjects and can be recorded by surface electrodes. However, to detect BS, not only P100, but also N75 component should be analysed. To investigate the early and late processing stages, it is necessary to use more than one electrode, positioned over occipital as well as lateral bank of the parieto-occipital cortex. The results obtained may provide important hints for further studies in which VEPs can be used as a diagnostic and monitoring method

in the instances of amblyopia or strabismus. Future studies are necessary on the larger group of people and should be also performed on different age groups.

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