SUPPLEMENTARY MATERIAL

Amblyopic deficit beyond the fovea: delayed and variable single-trial ERP response latencies, but unaltered amplitudes

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EXPERIMENT 1.

Results

Behavioral results

In the case of foveal presentation, accuracy was impaired, while reaction times (RT) increased in amblyopic vision compared to viewing with the fellow eye (Fig.1; eye: $F^r_{(1,14)}=47.45$, $p<0.0001$ and $F_{(1,14)}=22.05$, $p=0.0003$ for accuracy and RT, respectively), which was true for both *Br* and *Lo* stimuli (all eye × filtering: $F_{(1,14)}<1.85$, $p>0.20$). Nevertheless, filtering the faces had an additional effect in foveal vision further degrading accuracy in both eyes, which was due to the removal of higher frequencies including the characteristic frequencies for judging faces (filtering: $F^r_{(1,14)}=10.10$, $p=0.0067$). It tended to increase reaction times as well, however it failed to reach significance (filtering: $F_{(1,14)}=6.70$, $p=0.021$).

In the case of perifoveal presentation, however, accuracy did not differ between the two eyes (eye: $F_{(1,14)}=3.02$, $p=0.10$). Nevertheless, subjects were still significantly slower in responding when viewing with their amblyopic eye (eye: $F_{(1,14)}=21.57$, $p=0.0004$). Low-pass filtering resulted in a drop in accuracy (filtering: $F_{(1,14)}=24.53$, $p=0.0002$) and a slowing of RTs, similarly to that observed for foveal stimuli (filtering: $F_{(1,14)}=34.12$, $p<0.0001$). These effects were consistent across eyes and types of stimuli (all eye × filtering: $F_{(1,14)}<0.65$, $p>0.44$).

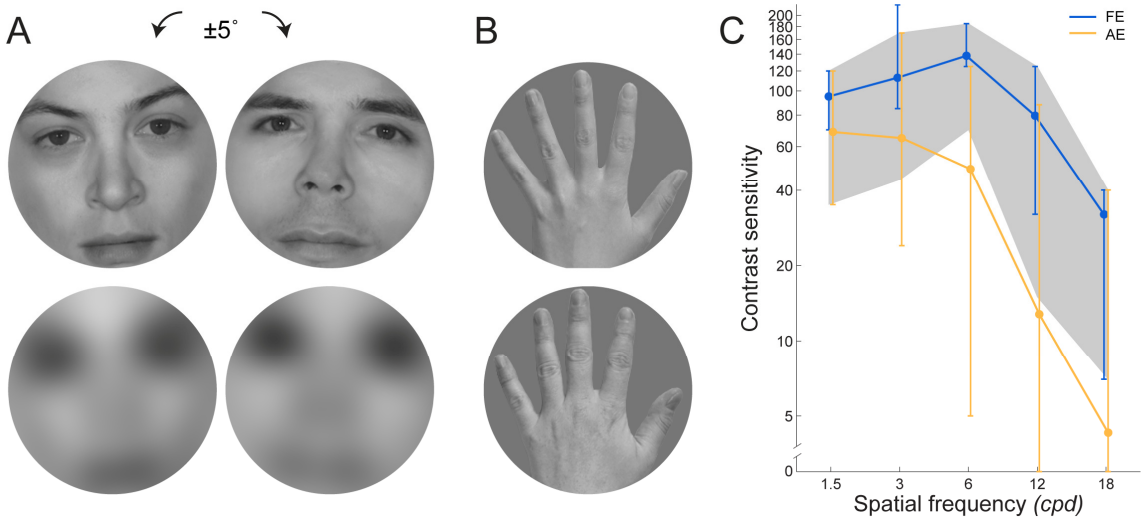

Figure S1. Exemplar stimuli from Experiment 1 (A) showing unfiltered stimuli with broad spatial frequency content and 1.5 cpd low-pass filtered stimuli rotated ±5° for the orientation categorization task. (B) Exemplar hand stimuli from Experiment 2, which were used in the face-hand categorization task along with unfiltered stimuli from Experiment 1. (C) Contrast sensitivity function of amblyopic patients participating in Experiment 1. Dots indicate the group average, while error bars show the lowest and highest sensitivity measure in the group. The highest spatial frequency that was within the normal range (grey shaded region) for all participants was 1.5 cpd. (FE: fellow eye, AE: amblyopic eye; N=15)

		Refraction		Visual Acuity (*logMAR VA*)		
Subject	**Age/Gender**	**RE**	**LE**	**RE**	**LE**	**Exp.**
Anisometropic						
GB	40/F	+3.5 +1.25 120°	-0.25 -0.25 132°	20/60 (*0.5*)	20/12.5 (*-0.2*)	Exp.1
HB	19/F	+1.0 +2.5 30°	plan	20/40 (*0.3*)	20/20 (*0*)	Exp.2
HK	31/F	-0.5	+0.5 +1.75 129°	20/12.5 (*-0.2*)	20/80 (*0.6*)	Exp.1
KG	32/M	-8.5 -3.5 178°	-3.0 -0.75 11°	20/200 (*1*)	20/12.5 (*-0.2*)	Exp.1+2
KF	24/F	-0.25 -0.5 135°	+3.75 +2.25 155°	20/16 (*-0.1*)	20/80 (*0.6*)	Exp.1+2
SE	52/F	+4.0 -6.0 15° add +2.0	+1.0 add +2.0	20/50 (*0.4*)	20/16 (*-0.1*)	Exp.2
SzB	24/M	+0.5	+3.5 +2.0 35°	20/20 (*0*)	20/80 (*0.6*)	Exp.2
VA	35/M	plan	+2.5	20/12.5 (*-0.2*)	20/60 (*0.5*)	Exp.1+2
Strabismic						
HA	28/F	-0.5	-0.5 132°	20/16 (*-0.1*)	20/40 (*0.3*)	Exp.1
KJ	21/M	+0.25	-0.25 -0.5 58°	20/80 (*0.6*)	20/10 (*-0.3*)	Exp.1
MCs	34/M	+1.5	+1.5	20/20 (*0*)	20/80 (*0.6*)	Exp.2
NB	18/M	+4.75 +1.25 86°	+5.5 +1.0 106°	20/12.5 (*-0.2*)	20/32 (*0.2*)	Exp.2
SzV	19/F	+0.5 -0.5 72°	+0.5	20/80 (*0.6*)	20/16 (*-0.1*)	Exp.1
VO	24/F	-4.25 -0.5 16°	-4.5 -0.75 176°	20/20 (*0*)	20/32 (*0.2*)	Exp.1
Strabismic-Anisometropic						
AA	37/F	+1.5 +1.75 91°	+2.5 +1.0 84°	20/20 (*0*)	20/40 (*0.3*)	Exp.2
BÁ	42/M	+3.0 +3.0 180° add +1.0	+4.0 +2.25 175° add +1.0	20/20 (*0*)	20/125 (*0.8*)	Exp.2
CsJ	33/F	+1.25 -1.5 53°	+0.25 +0.25 62°	20/100 (*0.7*)	20/20 (*0*)	Exp.1
DCs	39/F	+1.75	+3.5	20/12.5 (*-0.2*)	20/50 (*0.4*)	Exp.2
KCs	45/F	-1.5 -1.0 140°	+0.25 -1.75 19°	20/20 (*0*)	20/125 (*0.8*)	Exp.2
KV	34/F	+0.75 +0.25 22°	+3.0 +1.0 107°	20/20 (*0*)	20/125 (*0.8*)	Exp.1+2
KHZs	21/M	+1.5	+3.0 +0.5 75°	20/10 (*-0.3*)	20/60 (*0.5*)	Exp.1
SchA	38/M	+1.25 -1.25 11°	+0.5 +1.5 95°	20/12.5 (*-0.2*)	20/25 (*0.1*)	Exp.1
SI	22/M	+1.5 +1.25 100°	+2.75 +0.5 63°	20/40 (*0.3*)	20/12.5 (*-0.2*)	Exp.1
TK	23/M	+2.25 +1.0 177°	+3.75 +1.75 117°	20/16 (*-0.1*)	20/32 (*0.2*)	Exp.1+2

Table S1. Clinical details of amblyopic patients in Experiment 1 and 2. The rightmost column indicates the experiment the given subject took part in. Visual acuity is given both in Snellen fraction and in LogMAR units in parentheses. (RE: right eye, LE: left eye).

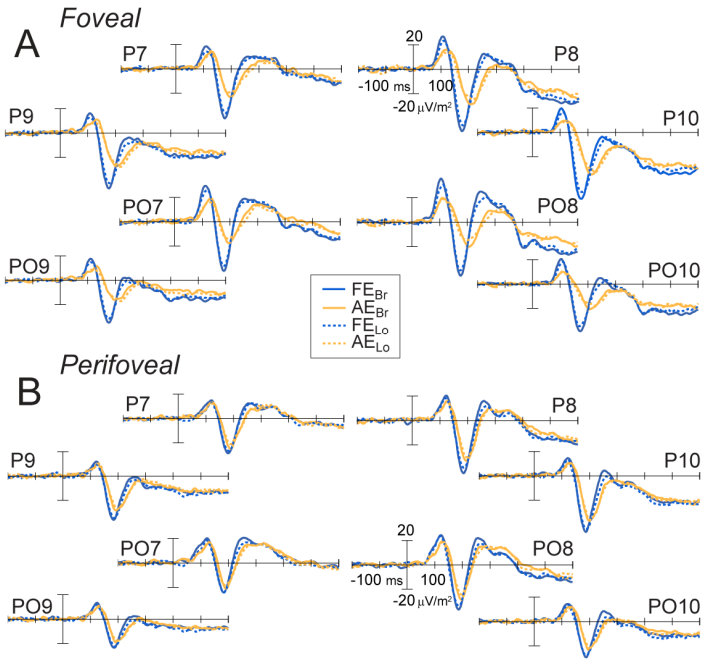

Figure S2. Averaged event-related potentials of amblyopic subjects from Experiment 1 for foveal (A) and perifoveal (B) presentation. Stimuli were matched in size according to the cortical magnification factor. Time courses from the amblyopic (AE) and fellow eye (FE) are shown in yellow and blue, respectively (Br: faces with broad spatial frequency content, solid lines; Lo: low-pass filtered face stimuli, dashed lines; N=15; negative is down).

EXPERIMENT 2.

Materials and Methods

Subjects

Fourteen amblyopic patients (mean ± SD age: 37 ± 10 years) gave their informed and written consent to participate in the study. None of them had any history of neurological or ophthalmologic diseases other than strabismus or/and anisometropia and all had best corrected visual acuity (Table S1.). None of them were under medication. Experiment 2 was also conducted on fourteen naive normal control subjects (seven females, mean ± SD age: 26 ± 4 years), medication free with no history of neurological or ophthalmologic diseases. All of them had normal or corrected-to-normal visual acuity and gave their informed and written consent to participate in the study, which was approved by the ethical committee of Semmelweis University and followed the tenets of the Declaration of Helsinki.

Eye dominance of normal subjects was determined using a variation of the Dolman method also known as the "hole-in-the-card test". The subject was given a CD with a small hole in the middle, instructed to hold it with both hands and then instructed to view a distant object (the experimenter nose) through the hole with both eyes open. The eye that the experimenter saw through the hole corresponded to the dominant eye of the subject. The procedure was repeated ten times to confirm dominance. The dominant eye of amblyopic patients corresponds to their non amblyopic eye.

Stimuli and Procedure

Stimuli consisted of four faces, chosen from the set of faces used in Experiment 1, and four hand photographs which were also covered with a circular mask. Stimuli were presented in two sizes following a study by Lerner et al[1]: 2 degrees in diameter for stimulation of the foveal region, while to stimulate the perifovea a 15-degree diameter stimuli were used with a 1.5-degree black disc placed on the fixation spot for better isolation from the foveal activation. Stimuli were presented centrally (viewing distance of 50cm) on a uniform black background.

Stimuli were displayed for 250 ms, and appeared in random order. Inter-trial interval was randomized between 500 and 900 ms, which was measured after button press. The fixation point was present trough out the trial. Subjects were tested in a dimly lit room where they were instructed to fixate the blue spot in the center of the monitor and to perform a two-alternative forced choice face-hand categorization task by pressing either the left or right mouse button. Testing was monocular, while the other eye was patched. Foveal and perifoveal stimuli were presented in different blocks making four types of blocks in total (Foveal – dominant / fellow eye, Foveal – nondominant / amblyopic eye, Perifoveal – dominant / fellow eye, Perifoveal – nondominant /

amblyopic eye). Block order was randomized with fellow and amblyopic eye alternating. There were a total of 96 trials for each block (i.e. stimulation) type, out of which 48 trials were face trials. In the current paper, we only consider these trials. Other experimental procedures were identical to Experiment 1.

Electrophysiological acquisition, processing and analysis

All acquisition and processing steps were identical to Experiment 1, except the high-pass filter was set to 0.5 Hz to eliminate slow baseline shifts as a result of sweating. Statistical analysis was performed on the latency and amplitude of averaged event-related responses (ERPs) by repeated-measures ANOVAs. There were two types of analysis: one contrasting the fellow and amblyopic eye of amblyopic observers (with *eye*, *side* and *electrode* (for amplitude only) as within-subject factors; the other comparing the dominant eyes for amblyopic and control observers (with *group* as between-subject factor, *side* and *electrode* (for amplitude only) as within-subject factors) separately for foveal and perifoveal stimulation. Post-hoc analyses and correction for unequal variances were done similarly as in Experiment 1. As many separate ANOVAs were conducted for analyzing the electrophysiological data, significance level was set to p=0.013 (~0.05/4 – four separate comparisons of: two positions × two independent measures) to control for the inflated type I error rate as a result of multiple comparisons.

Results

Large-field stimulation may mask perifoveal amblyopic deficit

Foveal stimulation. The results of Experiment 2. show strong amblyopic effects on the amplitude and latency of the P1 and N170 components of the event-related potentials in the case of foveal stimuli: reduced amplitudes (Fig. S3A; eye: $F^r_{(1,13)}$=9.08, p=0.0099 and $F_{(1,13)}$=25.95, p=0.0002 for components P1 and N170, respectively) as well as markedly delayed component latencies (eye: $F_{(1,13)}$=19.69, p=0.0007 and $F^r_{(1,13)}$=10.72, p=0.0060 for P1 and N170, respectively) obtained in the amblyopic compared with the fellow eye for both ERP components. In the case of the P1 component, this effect was modulated by the hemisphere the ERPs were measured over: the amplitude drop was significant over the left, while only a trend over the right hemisphere (eye × side: $F^r_{(1,13)}$=6.84, p=0.021, post-hoc: FE vs. AE p_{Left}=0.0002 and p_{Right}=0.039).

Large-field perifoveal stimulation. Averaged component amplitude and latency of P1 were not significantly affected by amblyopic viewing (Fig. S3B; eye: $F_{(1,13)}$=2.37, p=0.15 and $F_{(1,13)}$=1.03, p=0.33 for component amplitude and latency, respectively) but showed a non-significant reduction and increase, respectively over the left hemisphere as indicated by a trend in the eye × side interaction (eye × side: $F_{(1,13)}$=3.03, p=0.11 and $F_{(1,13)}$=4.50, p=0.054 for component amplitude and latency, respectively). Component N170 exhibited a slight but significant amblyopic effect similar to foveal stimulation in the case of latency (eye: $F_{(1,13)}$=39.37, p<0.0001), while the decrease in amplitude remained a non-significant trend (eye: $F^r_{(1,13)}$=7.33, p=0.018).

Taken together, our results show large and significant amblyopic impairments at the fovea and much smaller effects at the perifovea, which fail to reach significance for most measures, possibly due to the large area stimulated at the periphery. Our results at the perifovea are in agreement with those found by Lerner and colleagues using fMRI in a similar experiment, who also failed to find significant decrease in activation in the amblyopic compared with the fellow eye for large annular perifoveal stimuli.

Fellow eye ERPs do not differ significantly from control ERPs

Next, we were interested how closely the responses obtained from the fellow eye of amblyopic subjects approximate the ERPs of healthy subjects. In light of the null result concerning the comparison of component P1 of the fellow and amblyopic eyes of amblyopes under large-field perifoveal presentation, it

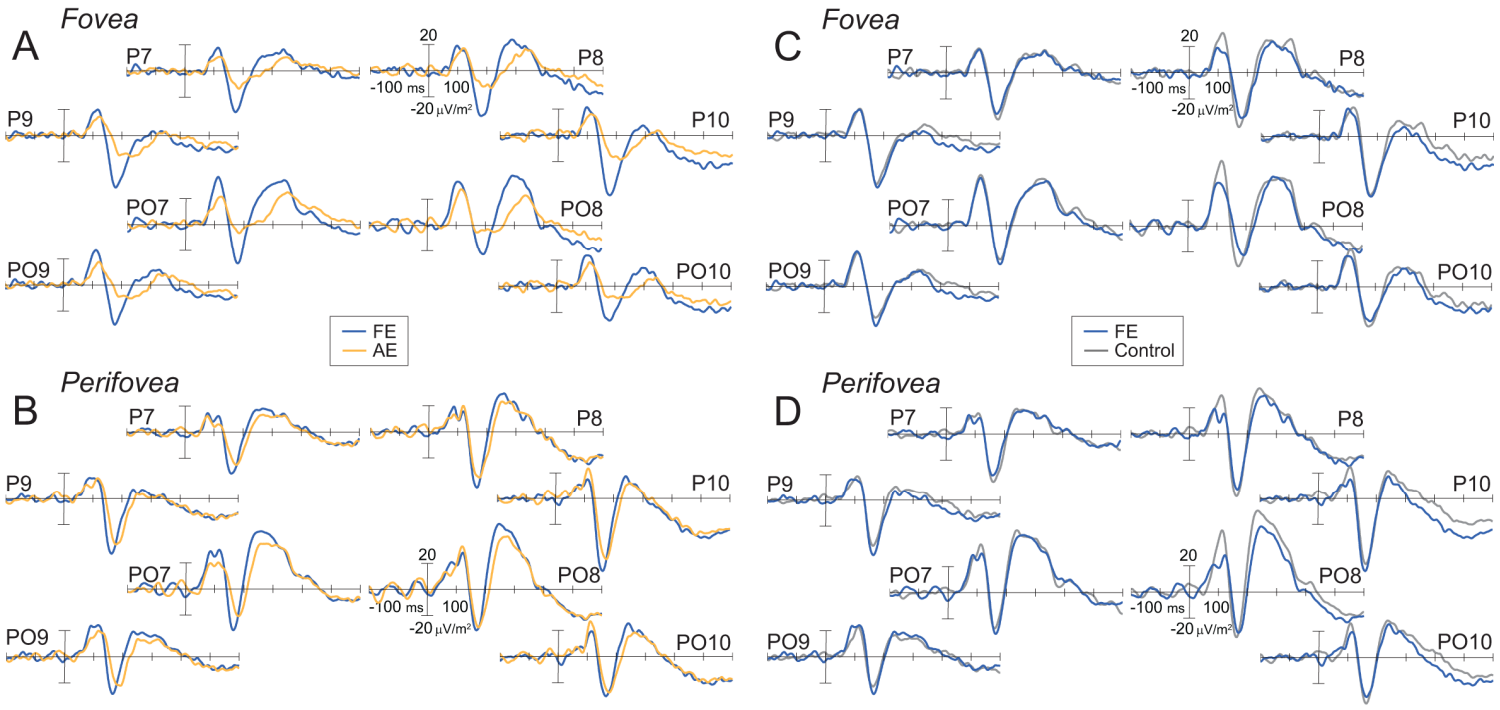

Figure S3. Averaged event-related potentials from Experiment 2. ERPs of amblyopic subjects for foveal (A) and large-field perifoveal (B) stimuli. Signal from the amblyopic eye (AE) is shown in yellow, while ERPs from the fellow eye (FE) are blue. Comparison between the averaged ERPs from the dominant eyes of amblyopic patients (blue) and control subjects (gray) for foveal (C) and large-field perifoveal (D) stimulation ($N_{amblyope}$=14, $N_{control}$=14, negative is down).

would be important to know whether the fellow eye can be considered normal in respect to the amplitude and latency of its evoked responses. Therefore, we conducted Experiment 2 on fourteen healthy control subjects and compared the ERPs obtained from their dominant eye to that of the fellow eye of the amblyopic subjects in a between-subject design. In addition, we compared their amblyopic eye to the control non-dominant eye for perifoveal stimulation.

Foveal stimulation. The results did not reveal any significant differences between the dominant eye of the two groups of subjects on the amplitude and latency of either ERP component in the case of foveal stimuli (Fig. S3C). There were no significant main effects of group or group × side interactions either for P1 (all $F_{(1,26)}<1.72$, $p>0.20$) or for N170 (all $F_{(1,26)}<1.66$, $p>0.21$).

Large-field perifoveal stimulation. Similarly to the results obtained in the foveal stimulation, we failed to find significant differences in the case of perifoveal stimulation as well (Fig. S3D). There were also no significant main effects of group or group × side interactions either for P1 (all $F_{(1,26)}<1.91$, $p>0.18$) or for N170 (all $F_{(1,26)}<1.40$, $p>0.24$).

In agreement with the within-subject analysis of amblyopic patients, ERPs obtained from the amblyopic eye did not differ from those of control subjects in the case of P1 latency and N170 amplitude (group: $F_{(1,26)}=0.06$, $p=0.81$ and $F_{(1,26)}=0.31$, $p=0.58$, respectively), where we also found no significant interocular differences between fellow and amblyopic eye. Similarly, N170 latency of the amblyopic eye was significantly longer than that of the non-dominant eyes of controls (group: $F_{(1,26)}=8.56$, $p=0.0071$) corresponding to the significant interocular difference found within amblyopic patients. However, amblyopic P1 amplitude also differed from controls (group: $F_{(1,26)}=4.71$, $p=0.039$), despite the fact that no significant interocular difference was found between the amblyopic and fellow eye in this respect. In

agreement with the results of Experiment 1, this also suggests that the amblyopic deficit in P1 amplitude extends beyond the fovea.

Thus, it can be concluded that the measure of averaged ERPs is insensitive to any difference in electrophysiological activity that might exist between the dominant eyes of amblyopes and normal subjects. This finding is also backed by VEP studies where no difference was found either in the latency and peak-to-peak amplitude of the P100 VEP[2] and the P50 PERG component or in retinocortical time (RCT) between the fellow eye of amblyopes and normal control subjects[3].

References:

1. Lerner Y, Hendler T, Malach R, et al. Selective fovea-related deprived activation in retinotopic and high-order visual cortex of human amblyopes. *Neuroimage*. 2006;33(1):169 – 179.

2. Moschos M, Margetis I, Tsapakis S, Panagakis G, Chatzistephanou I, Iliakis E. Multifocal visual evoked potentials in amblyopia due to anisometropia. *Clin Ophthalmol*. 2010;4:849 – 853.

3. Parisi V, Scarale ME, Balducci N, Fresina M, Campos EC. Electrophysiological detection of delayed postretinal neural conduction in human amblyopia. *Invest Ophthalmol Vis Sci*. 2010;51(10):5041 – 5048.