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Retinal conduction speed analysis reveals different origins of the P50 and N95 components of the (multifocal) pattern electroretinogram

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ABSTRACT

The pattern electroretinogram (PERG), an indicator of retinal ganglion cell (RGC) function, comprises a P50 and an N95 component. We addressed the question of whether the N95 originates, like the P50, from the RGC bodies or from the change of axon orientation at the optic nerve head (ONH). Thus, we recorded multifocal PERGs for 36 retinal locations in 21 participants. Second-order kernel responses were analyzed for the dependence of peak time topography on retinal fiber lengths to the ONH separately for the positive and negative excursions. We found that peak times were longer for macular [P1 (P50-like): 50 ms; N2 (N95-like): 76)] than for peripheral responses [P1: 43; N2: 66]. For the N2 another factor was necessary to explain the variability: The time difference (deltaT: N2 minus P1) was found to be proportional to fiber length from ganglion cell body to the ONH. We calculated retinal fiber length using an analytical function by Jansonius et al. (2009, 2012) and found that a linear model with factors eccentricity and fiber length explained 82% of the total N2 time variance ($p \ll 0.001$). The conduction speeds of the retinal axons were estimated from deltaT to range from 0.5 to 3.0 m/s for parafovea and periphery, respectively. The dependence of deltaT on the distance from ganglion cell body to the ONH suggests that the N2 originates at the ONH rather than at the ganglion cell body. While the multifocal N2 peaks earlier ($\approx$76 ms) than the non-multifocal PERG-N95 ($\approx$95 ms), considerations of high-pass filtering and frequency dependence of the mfPERG-N2 suggest that the source separation (P50 = ganglion cell body vs. N95 = ONH) also holds for the non-multifocal PERG.

1. Introduction

The pattern electroretinogram (PERG) is well established as a marker of retinal ganglion cell (RCG) function (Bach and Hoffmann, 2006; Maffei and Fiorentini, 1981). Consequently, it is a diagnostic aid e.g. for disorders of the optic nerves and macula (e.g., Holder, 2001) or detection of glaucoma (Bach and Hoffmann, 2008, 2006). It comprises two major components, the P50 (a positive excursion at approximately 50 ms) and the N95 (a negative excursion of more variable peak time around 95 ms in healthy subjects). These have been recognized to be differently affected by retinal pathology. There is evidence that PERG N95 originates with spiking activity of the RGCs, and that P50 largely arises in the RGCs or close by, with a contribution from non-spiking activity of the bipolar cells, resulting in post-synaptic potentials (Holder, 2001; Luo and Frishman, 2011; Viswanathan et al., 2000). Thus, the P50 likely relates to the spatial distribution and density of the underlying RGC bodies. Spiking activity occurs in the axon hillock and along the entire length of the retinal axon up to and including the optic nerve head, and N95 may therefore originate in different or multiple retinal locations. Considerations of local current flow as "seen" via the mass potential measurable at the cornea with reference to ipsilateral outer canthus (Bach et al., 2013) suggest the 90° fiber deflection at the ONH, leading to a mechanical – and consequently electrical – asymmetry, as an a-priori likely source of the N95. This is also the rationale of the ONH component that can be extracted from the multifocal ERG (Sutter and Bearse, 1999).

Ophthalmological pathologies have different effects on the P50 and N95 components of the PERG (Holder, 2001). The knowledge of the origin of these two components will consequently help to identify and understand the underlying retinal pathophysiology and damage mechanisms. One way to examine the retinal location of P50 and N95 origins is the localized activation of RGCs: The multifocal pattern ERG (mfPERG) technique allows the activity of the RGCs to be compared between discrete macular areas and has been studied by a few groups

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(Harrison et al., 2006; Herbik et al., 2014; Hoffmann and Flechner, 2008; Klistorner et al., 2000; Langrová et al., 2007; Monteiro et al., 2012; Stiefelmeyer et al., 2004). Its low signal-to-noise ratio can be markedly improved by slow stimulation (Hoffmann and Flechner, 2008); "slow" here means that pattern change does not occur at every frame, but on e.g. every 2nd or 4th frame. We here combined slow mfPERG with a recent computational model of the retinal nerve fiber paths, aiming to investigate the retinal origins of different mfPERG components, analogous to the P50 and N95 components of the conventional transient PERG.

2. Materials and methods

2.1. Participants

A total of 21 participants (median age: 57, range 21–78) with normal vision [visual acuity ≤0.1 logMAR, FrACT (Bach, 2007, 1996)] agreed to take part and gave their written informed consent after the nature of the experiment was explained in detail. The study design was approved by the Ethical Committee of the University of Magdeburg, Germany and procedures were performed in accordance to the ethical standards laid down in the 1964 declaration of Helsinki (World Medical Association, 2000). The participants wore refractive correction appropriate for the testing distance, if necessary.

2.2. Stimulation and recording

We used VERIS Science 5.1.12XScience (EDI: Electro-Diagnostic Imaging, Redwood City, CA, USA) for stimulation and recording. MfPERGs were recorded monocularly (12 left, 9 right eyes) with a DTL-like electrode (Dawson et al., 1979) referenced to the ipsilateral canthus. The signal was amplified by 100 k with a physiological amplifier (Grass Model 12, Astro-Med, Inc., West Warwick, RI, USA), band-pass filtered (low and high frequency cut-offs: 3 and 300 Hz), and digitised at 1200 Hz. Stimuli were presented at a frame rate of 75 Hz. Supported by a chin rest, the participants viewed the stimulus display, a circular dartboard-checkerboard pattern (mean luminance: 56 cd/m^2; contrast: 96%) covering 44° of visual angle, from a distance of 36 cm. The stimulus consisted of 36 elements which were arranged in 4 rings spanning the following eccentricity ranges: 0.0–3.6, 3.6–7.6, 7.6–14.3 and 14.3–22.7°. The central ring had 4 elements, ring 2 had 8, and ring 3 and 4 had 12 elements. Each single element comprised a 4 × 4 checkerboard. The 36 fields of this display were stimulated according to an m-sequence (length of 2^{14}–1), where a "1" caused reversal, a "0" left the local pattern unchanged. Fig. 1 shows at the left one frame of the multifocal stimulus, and on the right the resulting PERG traces (grand mean over the 21 participants ± SEM). Slow pattern-reversal stimulation was applied (Hoffmann and Flechner, 2008), i.e. each step lasted 2 frames (26.6 ms), resulting in an average reversal rate of 18.75 rps. This resulted in a total recording time of around 7 min per recording block. In total, 4 blocks were collected. Each recording block was subdivided into 32 overlapping segments, each lasting about 14 s, to allow the participants to blink and to facilitate steady fixation during the actual recording. Recording segments were occasionally contaminated by disturbances, e.g. caused by blinks, and consequently discarded online upon visual inspection and replaced by an acceptable repetition of the segment.

2.3. Analysis, PERG

Second order 1st-slice kernels were extracted using VERIS 5.01.12XScience (EDI, Inc., CA, USA) and exported for further analysis with IGOR 6.21 (WaveMetrics, Inc., OR, USA). As the polarity of the 2nd order kernels extracted with VERIS 5.01.12XScience is flipped with respect to conventional recordings (Fortune and Hood, 2003, p. 200; Sutter, 2001), the responses extracted with the 2nd order kernel were flipped back in relation to the software output for both the depiction of the traces and for the analysis. The traces for the four repetitions were averaged and digitally band-pass filtered from 3 to 35 Hz. Trace arrays recorded for the right eye were arranged to spatially correspond with left eye recordings to allow grand mean analysis of identical eccentricities. The grand mean was used for all further analyses, in Fig. 1B the grand mean traces are surrounded by ± SEMs, which are so small that they just seem to thicken the traces.

2.4. Analysis, path lengths and conduction times of retinal nerve fibers

Jansonius et al. (2012, 2009) [including the errata] published useful analytic formulas for the paths of the retinal nerve fibers. For the present problem, we implemented these formulas in R (R Development Core Team, 2014) and are making them available as "Data in Brief" (Bach and Hoffmann, submitted). We used these path morphologies to derive the fiber length leading from the respective retinal stimulation patch to the optic nerve had (Fig. 2, gray curves). A path length is calculated via the line integral; the formulas by Jansonius et al., however, cannot be analytically integrated. As an alternative, we numerically calculated the line integral using the trapezoidal rule (Wikipedia, 2017), also implemented in an R program ("Data in Brief", Bach and Hoffmann, submitted). Jansonius' formula is parameterized via the angle with which the fiber enters the ONH. This angle is defined from the center of the OHN (Fig. 2 left) in mathematical notation (90° = up). With manual iteration, we determined the angles that let fibers pass directly through the center of one of the 36 stimulation centers (yellow filled circles in Fig. 2). These are indicated as blue arcs in Fig. 2. Using these axon paths, we calculated fiber lengths (the line integrals), starting at the rim of the ONH, ending at the center of the stimulus

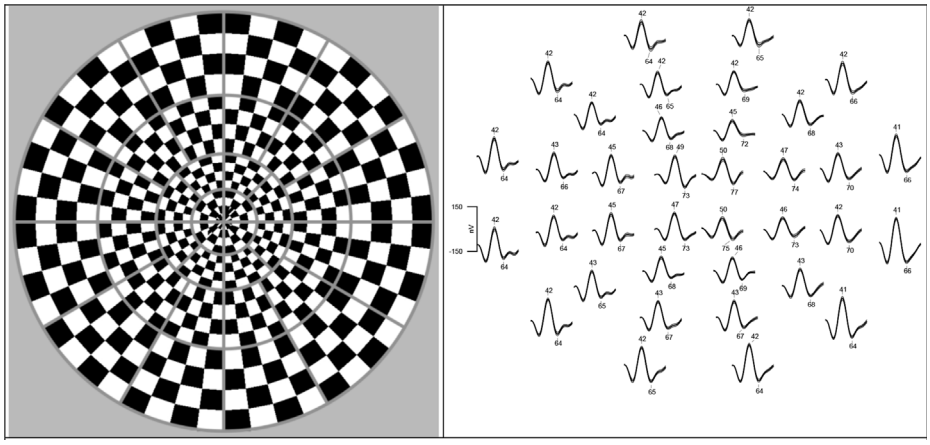

Fig. 1. Left: One stimulus phase. For each m-sequence step, a 4 × 4 element of the checkerboard was phase-reversed at around 18.75 reversals per second (see text). The gray lines are not visible in the actual stimulus display and added here to delineate the separate 36 contrast-reversing stimulus elements. Right: Grand mean 2nd-order kernel multifocal pattern electroretinograms from 21 normal participants depicted as if viewed through the left eye (field view) for all subjects. The SEM traces surrounding them (± SEM) are very small and consequently seem to thicken the traces. The small numbers indicate rounded peak times. A negative (N1) - positive (P1) - negative (N2) complex is clearly visible.