Electrically Elicited Visual Evoked Potentials in Argus II Retinal Implant Wearers

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PURPOSE. We characterized electrically elicited visual evoked potentials (eVEPs) in Argus II retinal implant wearers.

METHODS. eVEPs were recorded in four subjects, and analyzed by determining amplitude and latency of the first two positive peaks (P₁ and P₂). Subjects provided subjective feedback by rating the brightness and size of the phosphenes. We established eVEP input-output relationships, eVEP variability between and within subjects, the effect of stimulating different areas of the retina, and the maximal pulse rate to record eVEPs reliably.

RESULTS. eVEP waveforms had low signal-to-noise ratios, requiring long recording times and substantial signal processing. Waveforms varied between subjects, but showed good reproducibility and consistent parameter dependence within subjects. P₂ amplitude overall was the most robust outcome measure and proved an accurate indicator of subjective threshold. Peak latencies showed small within-subject variability, yet their correlation with stimulus level and subjective rating were more variable than that of peak amplitudes. Pulse rates of up to 2/3 Hz resulted in reliable eVEP recordings. Perceived phosphene brightness declined over time, as reflected in P₁ amplitude, but not in P₂ amplitude or peak latencies. Stimulating-electrode location significantly affected P₁ and P₂ amplitude and latency, but not subjective percepts.

CONCLUSIONS. While recording times and signal processing are more demanding than for standard visually evoked potential (VEP) recordings, the eVEP has proven to be a reliable tool to verify retinal implant functionality. eVEPs correlated with various stimulus parameters and with perceptual ratings. In view of these findings, eVEPs may become an important tool in functional investigations of retinal prostheses. (ClinicalTrials.gov number NCT00407602.)

KEYWORDS: retinal implant, visual evoked potential, visual prosthesis, psychophysics

DOEL. Karakterisering van elektrisch opgeroepen visueel opgewekte potentialen (eVEPs) in Argus II retina prothese gebruikers.

METHODES. eVEP golfvormen werden in vier proefpersonen gemeten. De amplitude en latentie van de eerste twee positieve pieken (P₁ en P₂) werd gecorreleerd met stimulusnivo en subjectief percept. eVEP variabiliteit tussen en binnen subjecten werd bepaald, alswel de maximale pulsrepetitiesnelheid waarmee betrouwbaar eVEPs opgenomen konden worden. De proefpersonen gaven een indicatie van hun subjectieve percept door de helderheid en grootte van de fosfenen te schatten.

RESULTATEN. eVEP golfvormen hadden een lage signaal-ruis verhouding, wat lange opname-tijden en intensieve signaalbewerking nodig maakte. Golfvormen varieerden tussen proefpersonen, maar binnen proefpersonen waren ze goed reproduceerbaar. Pieklanties hadden een lage variabiliteit binnen een proefpersoon, maar desondanks waren de correlaties met stimulusnivo en subjectief percept ondergeschikt aan die van piekamplitude. P₂ amplitude was de meest betrouwbare uitkomstmaat en een goede schatter van de perceptieve drempel. Pulsrepetities tot 2/3 Hz resulteerde in betrouwbare eVEP metingen. Perceptie van fosfenen daalde over de tijd, wat werd weerspiegeld in de eVEP door dalende P₁ amplitudes. P₂ amplitude en pieklanties bleven echter stabiel. Stimulatie-electrodelokatie had een significant effect op P₁ en P₂ amplitudes en latenties, maar had geen effect op het subjectieve percept.

CONCLUSIE. Hoewel opnameduur en signaalbewerking arbeidsintensiever zijn vergeleken met normale VEP metingen, hebben wij aangetoond dat eVEPs een betrouwbare maat zijn voor het functioneren van retinaprotheses. eVEP's correleren met verschillende stimulus parameters en met het subjectieve percept. Wij verwachten dat eVEP's een belangrijk onderdeel kunnen vormen in functioneel onderzoek naar retina protheses.

The Argus II retinal prosthesis at present is the only visual prosthesis approved for commercial use; it received the CE Mark for use in Europe in 2011, and received FDA approval in 2013 in the United States. It is an epiretinal implant that stimulates the surviving neurons in the retina by means of an array of 6×10 electrodes.¹ The Argus II has been proven to improve visual performance in spatial motor,¹ and orientation and mobility tasks² in implanted subjects with end-stage retinitis pigmentosa.

Although subjective measures are critical to assess retinal implant functionality, objective measures of prosthetic vision also are essential. In cochlear implants, which have been approved clinically since 1984,³ built-in electronics and reverse telemetry have been developed to allow recording of auditory nerve responses to electric stimuli. These measures nowadays are used routinely to assess functionality of the implant intraoperatively,⁴ and can be applied for rehabilitation purposes.⁵ Measurement of electrically evoked responses in the visual system likewise may prove useful during surgery and rehabilitation. The visual equivalent of the auditory nerve response is the electrically elicited electroretinogram (eERG). However, reverse telemetry is not yet available in the Argus II system, complicating eERG recordings. Cortical responses recorded from the scalp, for example, electrically elicited visual evoked potentials (eVEPs), are, therefore, a promising alternative. An additional advantage of cortical over peripheral potentials is the fact that the former represent central nervous system activity (V1 in case of eVEPs) and may provide a more accurate indication of perception. However, in contrast to the eERG, eVEPs do not provide detailed information about retinal processing, such as which cell populations are being activated, or whether any synaptic transmission is involved. Despite this important limitation, quantitative analysis of the eVEP may provide useful insights regarding processing of electrical stimuli by the visual system following retinal degeneration.

eVEPs have been well documented in animal models of retinal,^{6–14} optic nerve,^{15–17} and cortical implants.¹⁸ Most of these reports were feasibility studies and made use of normally sighted animals, which may not be representative for human visual prosthesis users. A limited number of reports describe eVEPs in animal models of retinal degeneration,^{19–22} while to our knowledge only three reports exist that describe eVEPs obtained in humans with retinal degeneration. Two of these reports show eVEP waveforms obtained in Argus I subjects.^{22,23} Brelen et al. show eVEP waveforms from two subjects with an optic nerve implant and found the promising result that the eVEP threshold reflected perceptual threshold.²⁴ However, all of these studies are based mainly on sample waveforms, and not on a systematic quantitative study of the responses.

For eVEPs to gain clinical acceptance it is essential to characterize eVEP input-output characteristics quantitatively, establish the correlation between eVEPs and subjective percepts, determine the variability of eVEP recordings between and within subjects, and determine procedures to obtain the eVEP quickly and reliably. In our study we aimed to achieve these goals by characterizing eVEP responses in four Argus II-implanted subjects.

MATERIALS AND METHODS

Subjects

Four subjects implanted with an Argus II retinal prosthesis in the right eye participated in this study. They were enrolled in an ongoing safety/efficacy study sponsored by Second Sight Medical Products (SSMP, Sylmar, CA; available in the public

domain at <http://clinicaltrials.gov/ct2/show/NCT00407602>). All subjects had been diagnosed with end-stage retinitis pigmentosa, and had no residual visual function greater than bare-light perception. Three subjects had been implanted approximately 4.5 years before the experiment was initiated (subjects S2, S3, and S4), while one subject had the implant for 2.5 years (S5).

All experiments adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from each subject after explanation of the nature and possible consequences of evoked electrical response recording. This research was approved by the institutional review board of the Johns Hopkins Hospital.

Experimental Setup

eVEPs were measured using the Espion e² system (Diagnosys LLC, Westford, MA) operating at the maximum sampling rate of 5 kHz, with open filter settings (0–1000 Hz setting). The electrical stimulus artifact was used as an external trigger to start recording. A typical run consisted of 250 stimuli delivered at $\frac{1}{3}$ Hz (totaling 12.5 minutes recording time). Typically, 6 runs were performed in a single session. After each run, a short break (typically 2–4 minutes) was provided.

Recording was performed using gold cup electrodes (Grass Technologies, West Warwick, RI) placed on the scalp with conductive paste (Ten20; Weaver and Company, Aurora, CO). The active electrode was placed midline on V1, 1 cm above theinion, the reference was placed on the forehead, and the ground electrode was placed on the right wrist.

eVEPs were elicited with the prosthesis operating under direct computer control, rather than with camera input. To ensure maximal cortical responses, electrodes were stimulated synchronously, and typically all available electrodes were stimulated. The stimulation software we used set the same current amplitude for each electrode. Therefore, electrodes with lower thresholds could have conveyed brighter percepts, and the subjective threshold may have been determined mainly by those electrodes with low stimulus thresholds.

Stimuli consisted of biphasic, rectangular current pulses. Default stimulation parameter settings were as follows: Cathodic-phase first pulses, 450 μ s/phase, no interphase gap (IPG = 0), pulse rate $\frac{1}{3}$ Hz, and a stimulus level of approximately 2 times subjective threshold (as determined under default conditions). Effects of stimulus level (10–58 μ A per electrode, dependent on the subject), stimulus polarity (cathodic- versus anodic-first stimuli), IPG (0 or 1 ms), and pulse rate ($\frac{1}{6}$ to 2 Hz) were varied systematically, leaving all other parameters at default. Throughout each run all stimulus parameters were kept constant.

Because we stimulated all electrodes synchronously, the maximum possible current level was restricted by the implant electronics and depended on the number of available electrodes. S2 had 51 active electrodes, and the current limit was 58 μ A when all electrodes were stimulated synchronously. S3 and S5 had 55 and 56 active electrodes, respectively, and the current limit was 54 μ A. S4 had 33 electrodes available, but was not tested for current-level dependence due to time restrictions; only a sample waveform at moderate stimulus level was obtained. Given the electrode area of 0.03 mm² and typical pulse duration of 0.46 ms, the charge density at the highest tested current level was 0.08 mC/cm²; approximately an order of magnitude below the safe charge density limit of 1 mC/cm² for a Pt-Ir electrode surface, as defined in the safety regulations in the FDA-approved study protocol.

During each run, subjects were asked four times at regularly spaced intervals to provide subjective feedback by rating the brightness and size of the most recent phosphene (typically at

FIGURE 1. eVEP waveforms obtained at a stimulus level approximately 2 times subjective threshold at standard parameter settings (*thick lines*). eVEPs using anodic-first stimuli (*thin lines*) also are shown to illustrate the stimulus-polarity-dependent artifact at approximately 25 ms (shown for subject S3 in [B], *arrows*). eVEP waveforms were characterized by two positive peaks (P_1 and P_2) and two negative peaks (N_1 and N_2). N_1 and P_2 were robust peaks across subjects, and the most reliable eVEP amplitude was obtained using P_2 relative to N_1 . P_1 was robust in S5 only. P_1 amplitudes were also determined relative to N_1 . eVEP amplitudes and latencies for these waveforms are provided in Table 1.

3, 6, 9, and 12 minutes). Ratings were confined to integers from 0 to 10; “0” indicating “no percept” and “10” being the “brightest/largest phosphene they had ever seen.” Since eVEP amplitude is expected to depend on the product of the firing rate of V1 neurons (reflected by phosphene brightness), and the number of active neurons (phosphene size), brightness and size were multiplied and rescaled back to 0 to 10 by taking the square root of the product. The four ratings usually were averaged to obtain a whole-run rating.

Data Analysis

eVEP data were imported in a Matlab R2010a programming environment (Natick, MA). Data were contaminated by background noise, signal drift, movement artifacts, and electric stimulus artifacts. To reduce background noise, a level 6 stationary wavelet transformation (SWT)^{25–28} was performed using the “sym5” wavelet. After SWT the data had a spectral content, based on fast-Fourier transform analysis, similar to that when low-pass filtering the signal with a digital sixth order Butterworth filter with a cutoff-frequency of 50 Hz. The reason for using SWT was a superior reduction of stimulus artifacts.

Based on previous literature,²⁴ the first 400 ms of each epoch was analyzed. After SWT, the linear slope and mean value of each epoch were set to zero to eliminate signal drift. Noise was reduced by adaptive averaging of multiple epochs: First, only epochs with overall amplitude $< 50 \mu\text{V}$ were included. The amplitude limit for inclusion then was increased until at least 50% of epochs were accepted. If this procedure resulted in an insufficient signal-to-noise ratio (SNR) upon visual inspection, additional 250-epoch runs were performed in a next session. For S5, single 250-epoch runs were sufficient, while for the other subjects up to 5 runs had to be combined.

Averaged eVEPs were analyzed by visually determining positive and negative peaks. For peak designation we followed the ISCEV standard for flash VEPs. eVEPs are plotted with positive peaks up following earlier publications.^{22–24} eVEPs were characterized using the first two identifiable positive peaks (P_1 , P_2) and the first negative peak (N_1). Amplitudes of the positive peaks were determined relative to N_1 (Fig. 1), and latencies relative to stimulus onset ($t = 0$ ms).

Estimation of eVEP threshold was done by fitting the input-output relation with a straight line. The eVEP threshold response level was defined as 4-SD (i.e., the 99% confidence interval [CI_{99}]) of the mean waveform obtained just below

TABLE 1. Subjects Overview

	Thresh., mC	Imped., k Ω	Imped. Macula, k Ω	FST, dB	P ₁ Lat., ms	N ₁ Lat., ms	P ₂ Lat., ms	P ₁ Ampl., μ V	P ₂ Ampl., μ V
S2	0.7	14	17	+18	40	60	120	3	5
S3	0.4	7	6	-12	50	140	220	1	3
S4	0.4	19	16	+24	80	120	180	2	2
S5	0.3	13	13	+7	60	100	150	11	4

Implant parameters, residual light sensitivity, and eVEP characteristics per subject. The subjective electrical threshold (Thresh.) was determined by stimulating all electrodes at the same current level, and is expressed as the applied total charge to correct for the number of functional electrodes. Impedance is expressed as the mean across all individual functional electrodes (Imped.), and across those electrodes covering the macula (max. 4) based on fundus photos (Imped. macula). Residual light sensitivity was assessed by a standard flash test (Diagnosys full-field scotopic stimulus threshold [FST], 0 dB level: 3 Cd·s·m⁻²). eVEP peak (P₁ and P₂) latencies (lat.) and amplitudes (ampl.) were obtained at approximately 2 times subjective threshold (waveforms shown in Fig. 1).

FIGURE 2. Sample eVEP waveforms in 2 subjects S2 (A) and S5 (B) at various current levels. Recordings were performed at standard settings (*thick lines*). Where available, waveforms using anodic-first stimuli also are shown to illustrate stimulus artifact-related peaks (*thin lines*). Applied current levels and subjective threshold (thr) are indicated.

FIGURE 3. Effects of stimulus level on subjective rating (A–C), eVEP amplitude (D–F), and eVEP latency (G–I). Data were fitted by linear regression. Correlation coefficient (r^2), and P values based on F -tests (H_0 : slope = 0) are indicated (insets). If the fitted function was not significantly different from the no-effect level, no line was drawn ([G], P_1 data). eVEP data are shown for P_1 (solid symbols) and P_2 (open symbols). Outliers are indicated with triangles (A, H, I). When outliers were outside the y -axis limits, they are drawn at the highest level available (H, I). eVEP threshold amplitudes (D–F) were determined by calculating the x -intercept of the fitted function with the threshold amplitude $y = 4\text{-SD}$ (horizontal dashed line, see Materials and Methods). These P_1 and P_2 threshold stimulus levels were compared with the subjective threshold (vertical dashed line).

perceptual threshold. The stimulus level at the intersection of the fitted curve and the horizontal line $y = 4\text{-SD}$ yielded the eVEP threshold.

Statistical analyses were done with Prism 5.04 (GraphPad Software, San Diego, CA), and included correlation analyses using linear regression and F -tests on the slope (H_0 : slope = 0; i.e., a horizontal line), 2-tailed t -tests (paired or unpaired), and 1-way RM ANOVA. Effects were tested within subjects where possible. Significance level α was always 0.05.

RESULTS

eVEP Waveforms

Figure 1 shows sample eVEP waveforms obtained under default parameter settings. eVEPs were characterized by two positive peaks (P_1 and P_2) and a negative peak (N_1). We also identified an early component around 25 ms that most likely

represented a polarity-dependent stimulus artifact (see Discussion). The electrical stimulus artifact itself occurred between 0 and 1 ms (pulse width = 900 μs), and was removed effectively by SWT.

Table 1 shows that P_1 amplitude (P_1 amp.) varied by an order of a magnitude, while P_2 amplitude (P_2 amp.) was comparable between subjects. P_1 and P_2 latency (P_1 and P_2 lat.) varied by a factor 2 between subjects. Table 1 also shows subjective electrical threshold (Thresh.), mean impedance across all electrodes (Imped.), mean impedance in the macular region (Imped. macula), and residual light perception (FST) of the subjects.

Effects of Stimulus Level

In three subjects we obtained eVEP waveforms at stimulus levels from below subjective threshold up to the maximum safe current level (Fig. 2). All subjects showed a significant increase in subjective rating, and P_1 and P_2 amplitudes with

stimulus level (Figs. 3A–F, linear regression and F -tests, $P < 0.05$). P_1 and P_2 latencies decreased significantly with stimulus level in S3 and S5 (Figs. 3G–I, $P < 0.05$). In S5, P_2 was hardly visible at 11 and 15 μ A (Fig. 2B), and these data were disregarded (Fig. 3D). In S2, P_1 latency did not depend significantly on stimulus level, while P_2 latency unexpectedly increased with stimulus level (Fig. 3G).

To examine whether eVEP amplitude and latency reflected subjective percept, we correlated P_1 and P_2 amplitudes and latencies (Figs. 3D–I) with subjective ratings (Figs. 3A–C) using linear regression. For all subjects, P_1 and P_2 amplitudes increased significantly with rating (F -test, $P < 0.05$). Effects on latency were more variable: For S2, P_1 latency was not correlated significantly with rating ($P = 0.1$), while P_2 latency increased with rating ($P < 0.05$). For S3, there was a trend for P_1 and P_2 latency to decrease with rating ($P = 0.07$ in both cases), while in S5, P_1 and P_2 latency decreased significantly with rating ($P < 0.05$). N_1 latency showed a nonmonotonic dependence on stimulus level and rating in all subjects, and was not statistically analyzed.

We determined eVEP P_1 and P_2 thresholds by determining the stimulus amplitude at which the regression amplitude data dropped to the 4-SD level (horizontal dashed line in Figs. 3D–F). P_2 threshold approximated the subjective threshold better than P_1 threshold in all subjects (Table 2).

Effects of Stimulus Waveform

We recorded eVEPs using cathodic-first and anodic-first stimuli at various current levels in three subjects (for sample waveforms see Figs. 1 and 2). We had only a limited number of data points per subject and, therefore, the data were pooled, resulting in 11 anodic-first-cathodic-first stimulus pairs (S2, $n = 4$; S3, $n = 3$; S5, $n = 4$). To account for between-subject differences in absolute values, we normalized the data per subject (highest latency, amplitude, or rating recorded was set to 1). Since P_1 was robust only in S5 we analyzed P_2 . Subjective ratings were significantly larger using cathodic-first stimuli (mean difference 8%, 2-tailed t -test, $P < 0.01$), but no significant differences in either P_2 latency or amplitude were observed ($P > 0.05$; data not shown).

Effects of interphase gap (IPG 0 or 1 ms) were tested in a similar fashion by pooling and normalizing data from 2 subjects (S3 and S5, 3 stimulus levels per subject). Subjective rating was significantly higher at IPG = 1 ms (Fig. 4B, mean difference 17%, $P < 0.05$), and P_2 amplitudes were significantly larger (Fig. 4D, $P < 0.01$). No effect on P_2 latency was found ($P > 0.05$).

Effects of Pulse Rate

Pulse rate was varied in two subjects (S2 and S5). Pronounced effects on eVEP waveforms were observed (Fig. 5A), especially at rates of 1 Hz and above. S2 reported negligible subjective

TABLE 2. Subjective and Electrophysiological Threshold Levels, by Subject

Subject	Subjective Threshold, μ A	eVEP Threshold, μ A	
		P_1	P_2
S2	30	38	24
S3	15	40	21
S5	11	3	7

Subjective thresholds and interpolated eVEP thresholds obtained from the fitted eVEP input-output (IO) characteristics based on P_1 and P_2 amplitudes.

FIGURE 4. Effects of IPG in 2 subjects (S3 and S5). Sample eVEP waveforms are shown for S3 (A) and S5 (C) using pulses with IPG = 0 ms (thick lines) or IPG = 1 ms (dashed lines). Stimulus level was approximately 2 times subjective threshold. Normalized subjective ratings (B) and eVEP P_2 amplitudes (D) were significantly larger at IPG = 1 ms (2-tailed, paired t -tests, $P < 0.05$).

effects of pulse rate, except at 2 Hz, where only the first two (out of 250) stimuli were perceived (Fig. 5B). Pulse rate had no significant effect on subjective rating in S5 (Fig. 5C, linear regression, F -test, $P = 0.4$). Both subjects showed a significant reduction of eVEP amplitude with pulse rate (Figs. 5D, 5E; $P < 0.05$). P_2 latencies decreased significantly with increasing stimulus rate in both subjects (Figs. 5F, 5G; $P < 0.05$). P_1 latency was unaffected in S5 ($P > 0.05$; data not shown), and was not analyzed in S2 (P_1 too small).

Effects of Electrode Location

The effect of stimulating different sets of electrodes on eVEP waveforms (Fig. 6A) was tested in S5 using three conditions: stimulation of 3×3 electrodes overlaying the central macula based on fundus photos (C6–E8 on the electrode grid), stimulation of 9 peripheral electrodes (A1–B3, C1, C3, D1), and whole-array stimulation. The stimulus level was inversely proportional to the electrode number; when using 9 electrodes the current level was $56/9$ times that when stimulating the whole grid with 56 active electrodes (i.e., 137 μ A vs. 22 μ A). Although subjective ratings did not differ significantly across the three conditions (Fig. 6B, RM ANOVA, $P > 0.05$), macular stimulation resulted consistently in higher P_1 and P_2 amplitudes, and shorter latencies compared to peripheral stimulation (Figs. 6C–F, $P < 0.05$). Comparing whole-array with macular stimulation, the latter resulted in larger P_1 and P_2 amplitudes, and shorter P_1 latency ($P < 0.05$), but no effect on P_2 latency was found ($P > 0.05$). Peripheral stimulation resulted in very similar responses compared to stimulation of the whole array, except that P_1 amplitudes were significantly larger when stimulating all electrodes ($P < 0.05$).

FIGURE 5. Effects of stimulus rate in 2 subjects (S2 and S5). Sample eVEP waveforms at various pulse rates are shown for S5 (A). Subjective ratings (B, C), eVEP amplitude (D, E), and eVEP latency (F, G) as a function of pulse rate are shown for both subjects. Dependence of these parameters on pulse rate was tested with linear regression. Correlation coefficients (r^2) of the regression analysis and significance levels (P) based on F -tests on the slope are shown (insets).

Within-Subject eVEP Variability

eVEP data of S5 were well suited for analysis of within-subject variability, since single runs of 250 stimuli resulted in sufficient SNRs. Figures 7A to 7C show the available data from S5 across multiple sessions under default parameter settings, grouped according to run number. Ratings in the first run were higher compared to later runs (Fig. 7A, unpaired, 2-tailed t -test, $P < 0.001$). Likewise, P₁ amplitudes were significantly larger in the first run (Fig. 7B, $P < 0.01$). No significant effect of run number was found on P₂ amplitude (Fig. 7C, $P > 0.05$), or P₁ or P₂ latency ($P > 0.05$; data not shown). Overall, latencies showed a relatively small variability compared to amplitude measures.

In two sessions, four consecutive runs under identical, default settings were performed. In the first run, subjective ratings decreased significantly over time (Fig. 7D, linear regression and F -test, $P < 0.001$), while in later runs (runs 2–4) ratings did not change (shown for run 2 in Fig. 7E, $P > 0.05$). Individual eVEP amplitudes within each run were estimated from single epochs by determining P₁, N₁, and P₂ within a 20

ms range around the peaks identified in the mean waveform. Therefore, latencies could not be analyzed, because these were capped artificially. P₁ amplitudes decreased significantly over time in the first run (Fig. 7D, $P < 0.01$), but not in subsequent runs (shown for run 2 in Fig. 7E, $P > 0.05$). Correlation was weak ($r^2 \sim 0.01$) due to the low SNR of single waveforms. P₂ amplitude did not show a significant effect ($P > 0.05$; data not shown) in any of the runs.

DISCUSSION

eVEP Waveforms

Peak latencies obtained at stimulus levels 2 times above subjective threshold differed between subjects; P₁ ranged from 40 to 80 ms, N₁ from 60 to 140 ms, and P₂ from 120 to 220 ms (Table 1). Previous studies with Argus I subjects show the following eVEP peak latencies: N₁, 23 to 25 ms; P₁, 50 to 52 ms; N₂, 110 to 120 ms; and P₂, 220 to 240 ms.^{22,23} The N₁ peak reported in these studies

FIGURE 6. Effects of electrode location in S5. Sample eVEP waveforms using the whole array (*all*), the central macular 9 (*macular*), or the most peripheral 9 electrodes (*periph.*) are shown (A). Stimulus level was 22 μ A when stimulating the whole grid (56 active electrodes), and 137 μ A when stimulating 9 electrodes (proportionally corrected: 22 μ A $\times$ 56/9). Effects of electrode location on subjective rating (B), eVEP P₁ and P₂ amplitude (C, D) and latency (E, F) were tested for statistical significance with RM ANOVA and Tukey's post hoc test. Mean values (*lines*) and significance levels are indicated. * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

was identified by us as a stimulus artifact, because it reversed in polarity when using anodic-first stimuli (Figs. 1, 2). The other peak latencies correspond closely to ours (Table 1), as expected given the similarity between the Argus I and II devices. Their P₁ and P₂ amplitudes (relative to their N₂) were approximately 10 μ V. Our subjects generally had much smaller peak amplitudes (Table 1). However, VEP amplitudes are known to vary substantially between subjects, presumably due to differences in cortical layout, cranial thickness, and relative conductance levels²⁹ and, therefore, are not regarded as reliable parameters for between-subject comparisons.³⁰

Data from optic nerve-implanted subjects (stimulus levels up to two times subjective threshold) showed the following peaks: P₁, 75 ms; N₁, 200 ms; P₂, 250 ms.²⁴ Although their P₁ latencies lie within our P₁ range, their N₁ and P₂ are longer

than ours, which was not expected, since the optic nerve is located more centrally. Phosphenes were perceived centrally in their experiments,²⁴ as well as in our subjects (our subjects were implanted in the macular region) and, hence, similar optic nerve fibers will have been activated. However, latency differences may be related to the different nature of stimulation by optic nerve cuff electrodes; the percept of optic nerve stimulation is likely to be more diffuse than that of retinal stimulation,³¹ leading to more diffuse and, therefore possibly slower, cortical processing. In addition, while retinal neurons are not myelinated, the optic nerve is, and faster-conducting fibers (those with thicker myelin sheaths) are more likely to be activated,³² possibly resulting in altered cortical responses.

We were not able to find clear predictive factors between subjects that could explain differences in eVEP waveforms

FIGURE 7. eVEP and perceptual variability. Subjective percept ratings (A) and eVEP P₁ (B) and P₂ (C) amplitudes from 20 runs across 11 sessions were pooled. A significant part of total subjective and P₁ amplitude variability could be explained by between (A, B) and within (D, E) run variability. Subjective ratings and P₁ amplitudes were significantly higher in the first run compared to later runs (A, B), unpaired, 2-tailed *t*-test; both decreased significantly in the first run (D), linear regression, and *F*-test on the slope), but not in the second (E) or later runs. Significance levels ([A–C], ***P* < 0.01, ****P* < 0.001) and regression parameters ([D–E], correlation coefficient *r*² and *P* based on *F*-tests on the slopes) are indicated.

(Table 1). Low electrode impedances, low subjective thresholds, and high residual light sensitivity (suggesting better overall retinal condition) theoretically would be positive indicators for implant function, and were expected to result in high-amplitude eVEPs with low peak latencies. However, S5 had by far the largest P₁ amplitude, but this light sensitivity was only moderate, and impedances and subjective threshold were similar to S3, who had a negligible P₁. S2 had the largest P₂ amplitude, but had moderate light sensitivity, moderately high impedances, and the highest threshold. Similarly, peak latencies did not seem to relate to these parameters.

eVEP Input–Output Characteristics

Increased stimulus levels resulted in increased eVEP amplitudes in all subjects, and decreased latencies in 2 of 3 subjects, which is in accordance with the characteristics of the normal light-evoked VEP.³⁰ Amplitudes of evoked potentials in general increase with stimulus level due to recruitment of nerve cells and an increase in neuronal cell activity. Decreasing latencies at higher stimulus levels can be explained by assuming that neurons reach their activation thresholds quicker and/or fire more synchronously. However, S2 showed increasing peak latencies at higher stimulus levels. It generally is assumed that relatively short electrical pulses of low amplitude primarily activate retinal ganglion cells directly,^{33–36} while higher

stimulus levels may lead to recruitment of presynaptic elements, such as bipolar cells and residual photoreceptors. This activation of presynaptic cells can lead to a secondary volley of activity in ganglion cells up to 10 to 40 ms after the initial stimulus,^{37,38} in turn leading to delayed cortical activation.²² Such a recruitment of presynaptic cells in S2 theoretically could have contributed to increased eVEP peak latencies in S2 at higher stimulus levels (Fig. 5). However, attempting to explain retinal processing based on eVEP waveforms is speculative, because retinal processes are obscured in the eVEP due to visual processing in the thalamus (LGN) and the cortex. In addition, the origins of the different VEP peaks are not well understood. Although P₁ generally is attributed to V₁ activity, the origins of P₂ still are open to debate³⁰ and may represent activity in extrastriate areas, but it might as well be evoked by secondary V1 activity due to feedback from higher cortical areas.

The fact that our stimulation levels stayed well below the safe limit for the Pt-Ir surface (see Introduction) is relevant, since we never reached a response plateau in our subjects, and the input–output curves could be fitted with a simple linear equation (Fig. 3).

Since the correlation of peak latencies with stimulus level was relatively variable across subjects, and because P₂ threshold was a better predictor of subjective threshold than P₁ (Table 2), we concluded that eVEP P₂ amplitude is the most

robust measure of stimulus level and subjective percept, at least in this limited number of subjects.

Effects of IPG

Introduction of an IPG resulted in increased subjective ratings and larger P₂ amplitudes (Fig. 4). Although not significant at the eVEP level (but significant in our rating data), cathodic-first stimuli are slightly more effective than anodic-first stimuli in epiretinal implants,³⁹ and the effect of IPG in our study can be explained by assuming that the cathodic phase of a cathodic-first biphasic pulse is excitatory, while the following anodic phase has the opposite effect. Increasing IPG may allow the excitatory response to develop more fully, before being suppressed by the anodic phase.⁴⁰

eVEP Variability and Adaptation

P₁ and P₂ latency were robust measures across sessions, while amplitudes were more variable (Fig. 7). These findings are in line with the widely accepted idea that VEPs are analyzed more preferably in terms of latency than in amplitude.^{30,41} Nevertheless, in terms of defining input-output characteristics, we concluded that amplitudes are more robust than latency measures.

During the first run subjective percept was high, but declined over time. In subsequent runs, ratings were low and relatively constant (Fig. 7). Hence, our data indicated that within-session (between-run) differences can have substantial effects on the subjective percept. Perceptual “fading” was described recently by Pérez Fornos et al. in Argus II subjects.⁴² They report a decline in phosphene brightness within seconds after the start of stimulation, contrasting the fading we described in our subject, which progressed over a course of 12 minutes. The difference might be explained by different stimulation rates; they used 5 to 60 Hz, while we used 1/3 Hz.

In our data, fading was reflected in P₁ amplitudes, but not in P₂, indicating that the two peaks represent physiologically different processes.

Clinical Implications

We concluded that P₂ amplitude is a robust measure of visual cortical processing in retinal electrical stimulation, because it could be applied effectively to construct input-output characteristics, it significantly correlated with subjective percept, and it yielded fairly accurate predictions of subjective threshold. IPG significantly affected P₂ amplitude as well, but more subtle effects, such as that of stimulus polarity, were not reflected in the eVEP.

Each waveform required about 12 minutes of recording time (250 stimuli, 1/3 Hz). In addition, for S2, S3, and S4, multiple runs were needed to obtain eVEP waveforms of acceptable signal-to-noise ratio. In a clinical setting, recording times preferably would have to be reduced. We showed that eVEP waveforms could be recorded reliably at 2/3 Hz as well, effectively reducing recording time by half. If eVEPs are to be used for rehabilitation purposes, such as clinical device fitting, input-output characteristics might not need to be constructed with as many data points as in this study. Instead, based on our finding that P₂ amplitude linearly depends on stimulus level and percept, input-output characteristics might be approximated with just a few data points.

Central macular stimulation resulted in significantly higher eVEP amplitudes and shorter latencies when compared to more peripheral stimulation, while there was no significant effect on subjective ratings (Fig. 6). In addition, electrode impedances cannot explain the difference either, because

these were similar across all electrodes; for example, macular impedances closely resembled the mean impedance across all electrodes (Table 1). Increased amplitudes with macular stimulation can be explained, however, by the location of the cortical representation of the fovea close to the scalp, and by the relatively large area in V1 that is devoted to the central macula when compared to more eccentric retinal areas.³⁰ Hence, electrode location must be taken into account when using eVEPs in a clinical setting.

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