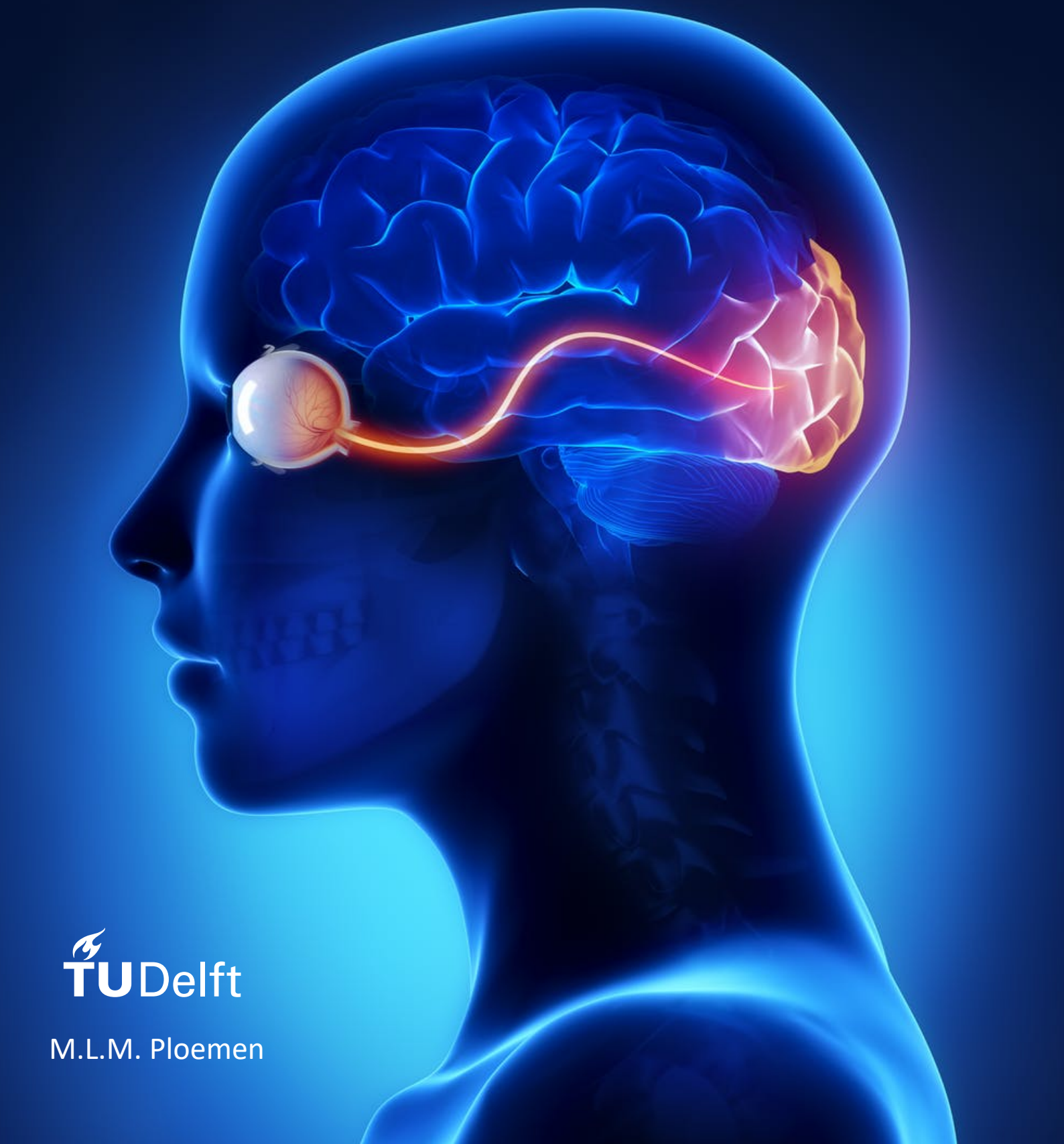
Rapid Acquisition of the Stimulus Response Relationship using Visual Evoked Potentials

Master Thesis



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RAPID ACQUISITION OF THE STIMULUS RESPONSE RELATIONSHIP USING VISUAL EVOKED POTENTIALS

By

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Contents

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Abstract

The flash visual evoked potential (FVEP) is an electrical potential recorded with electroencephalogram (EEG) at the occipital cortex. Current FVEP assessment may be elaborated with the acquisition of stimulus response (SR) relation of the visual system. A drawback of this adjustment is the time required for data acquisition. This study aimed to develop an optimal (rapid) stimulus paradigm for assessing the SR relationship using FVEP.

SR relationships were obtained using two different protocols in eight healthy participants; one considered 100 stimuli for 10 different intensity levels (standard technique), the other considered 1000 stimuli of varying intensity levels within a fixed intensity range (novel technique). Hypothetically the novel technique produces a similar SR relationship but requiring reduced number of stimuli.

However, the present study did not find a significant intensity dependency on the FVEP. Consequently, no mathematical model for the SR relation was fit to the data, and exploration of the novel technique was excluded. Instead, the present study investigated the minimal number of stimuli required to acquire a representative FVEP component. Secondly, the potential change of FVEP components during the experiment was investigated.

This study demonstrates that it is possible to acquire a reliable FVEP component with on average 54 stimuli (gross mean (39) + 1 SD (14)), independent of stimulus intensity. Additionally, this study demonstrated that the FVEP components change significantly ($p<0.05$) for most stimulus intensities when 1000 or more flashes are used; amplitudes increased with time for low intensity stimulation, while amplitudes reduced with time for high intensity stimulation.

Although the present study did not find an intensity dependency of the FVEP component, it demonstrated that stimulation time is an important parameter when acquiring SR relationships using FVEP. The stimulation time certainly influenced the SR relationship calculated in the present study. Further studies can and should reduce the number of stimuli to produce a representative SR relationship using FVEP.

Keywords: Electroencephalography (EEG), Flash Visual Evoked Potential (FVEP), Stimulus-Response (SR) Curve.

Introduction

Evoked potentials are electrical potentials measured at the cortex using electroencephalogram (EEG), that are time-locked to the onset of a stimulus [1]. Different types of sensory stimuli are used to evoke potentials in different parts of the brain to understand the physiology and pathophysiology of sensory systems. The visual system is studied by EEG recordings at the occipital cortex. The visual evoked potential (VEP) can be extracted using signal averaging of multiple evoked responses [1, 2].

Stimulation types used in VEP studies mostly include checkerboard patterns, images or flashes of light [1, 2]. The recorded response to each stimulation type has a specific waveform consisting of multiple positive and negative peaks [2, 3].

A wide variety of abnormalities in the VEP waveforms can be detected. For instance, prolonged latency of a peak or the absence of a peak. Hence, VEPs are used in early clinical diagnoses for several diseases; i.e. multiple sclerosis, ischemic optic neuropathy, traumatic brain injury, amblyopia, glaucoma and other neuropathies [3, 4].

Although the VEP method is a well-known test in clinic, the current approach is limited to a very small variety of stimuli. In fact, almost all clinics worldwide use standardized tests with only a small subset of specific parameters for the stimulation [2, 4]. For instance, the standard flash visual evoked potentials (FVEPs) are elicited monocular by a white light flash (3 cd s m^{-2}) which substance a visual field of at least 20^{o} at a presentation rate of 1 Hz [2].

The FVEP could potentially give more insight into the visual system's functioning state if the stimulation parameters would be varied. Presenting a single type of stimuli does not fully characterize the functioning of a neuronal system. In theory, presenting all possible stimuli and measuring their outputs is needed to fully characterize a neuron or neuronal system [5]. In practice however, it is not achievable to present all possible stimuli. Instead, a rich and dynamic stimulation paradigm can be presented and combined with mathematical tools to estimate a model [5-7]. Relating to FVEP method; measuring the function of the entire visual system using FVEP could be enriched by presenting a richer stimulation paradigm than current standard tests, i.e. using multiple colors, stimulus frequencies, stimulus intensities or stimulus durations.

Stimulation with multiple stimulus intensities (brightness of light) is used to acquire the stimulus-response (SR) relationship. Research has shown that increased stimulus intensity levels reduce the peak latencies in VEP [3, 8-10]. Hypothetically more intense flashes are becoming more important to the visual system, resulting in reduced latencies and increased amplitudes of the VEP. Acquisition of the SR relation using VEP can provide additional quantitative data of the visual system.

Possible abnormalities to the intensity dependency of FVEP have been studied in migraine, because patients with

this neurological disease experience hypersensitivity symptoms [11]. However, there has been no systematic study of the optimal stimulus paradigm for assessing the SR relationship using FVEP.

Assessment of neuronal responses as a function of stimulus intensity (SR relation) has already been done in other fields of neuroscience [12-14]. Usually, the SR relation is measured by plotting the neuronal response against a range of different stimulus intensity levels. Multiple outcomes for each stimulus intensity level are averaged, and a mathematical model is fit to the data to produce the SR curve.

A limitation of the traditional method is the time required to collect the data. It is important to realize that FVEP components can only be measured by averaging many responses, due to the bad signal-to-noise ratio (S/N ratio) in EEG recordings. According to the FVEP standard, the minimal number of stimuli per average should be at least 50 [2]. Consequently, adding a single intensity level to the standard protocol results in an addition of at least 50 stimuli, increasing acquisition time.

Time required to collect data with a new protocol should not increase, optimally decrease, compared to the current FVEP standard. Assessing the SR relation in the shortest possible time is practical and more comfortable for the participant. Besides, long stimulation of a checkerboard pattern (17-18 min) has been shown to result in considerable changes in most VEP components [15]. The source might be a well-known phenomenon of neural circuits, habituation.

It is possible to alter acquisition time by minimizing the number of stimulus intensity levels, minimizing the number of stimuli within each intensity level and minimizing the interstimulus interval (ISI). Minimizing ISI has physiological limits, as the response needs to be fully recovered before applying the next stimulus. When analyzing transient FVEPs stimulation frequency should not be more than 1 Hz; otherwise responses might overlap [3, 4]. Minimizing the number of stimuli within each intensity level depends on the S/N ratio; the number should be large enough to distinguish between the FVEP components and background noise accurately. When minimizing the stimulus intensity levels a trade-off must be made by presenting enough stimulus intensity levels to acquire a representative SR relationship, without increasing acquisition time dramatically.

To be valuable, a standardized protocol for assessing SR relation should be rapid and designed systematically. Assessment of the SR relation might be valuable to identify even more physiological abnormalities than the current standard or to answer specific clinical questions.

The objective of the present study was to develop a rapid acquisition protocol for the SR relationship of the visual system using FVEP. Two different protocols were used to acquire the SR relationships for FVEP amplitude and latency; one considered the golden standard and the other considered a novel technique.

Hypothetically the novel technique produces a similar SR relationship, requiring less acquisition time. Overall, FVEP amplitudes were hypothesized to increase (sigmoid curve), whereas latencies were hypothesized to decrease with increasing stimulation intensity.

However, no pronounced effects of intensity were observed in the present study. These results were unexpected. Therefore, no mathematical model was fit to the data, and exploration of the novel technique was excluded. Instead, to guide further research, the minimal number of stimuli required to acquire a representative FVEP component for each intensity level was determined. Lastly, the potential change of the FVEP during the experiment was examined.

Method

Participants

Healthy participants with a mean age of 25.2 $\pm$ 1.5 years were recruited for the study (*N*-8: Female-5). Participants were screened with a Migraine Screening Questionnaire (MS-Q) [16] and were excluded if they had any form of headache on more than 1 day per month. All participants were in self-reported good health, with medical histories free from neurological problems and currently no medication that could directly affect brain activity, and all gave informed consent. The approval of the Human Research Ethics Committee (HREC) TU Delft was obtained before the commencement of the study. The study was carried out in the Electroencephalography (EEG) laboratory within the Department of Biomedical Engineering at the Delft University of Technology.

Experimental setup

Participants were seated comfortably in a lightened room where they underwent EEG recording during visual flash stimulation, as depicted in Fig. **1**. The room light was maintained constant for all participants as there were no windows in the room. The flash stimulation was generated with binocular red-light LED goggles (Synergy Plinth; Medelec International, Pleasanton, CA, USA) at wavelength 654 nm. The goggles were placed directly over the participant's eyes where they were taped to the temples on both sides of the head. Light flash duration was 2 ms, and flash intensities were controlled via custom-written scripts in Matlab (R2020a; Mathworks, Natick, MA, USA).

Fig. 1 Experimental setup. Participant is comfortably seated with the goggles taped to the head. EEG is recorded during flash stimulation.

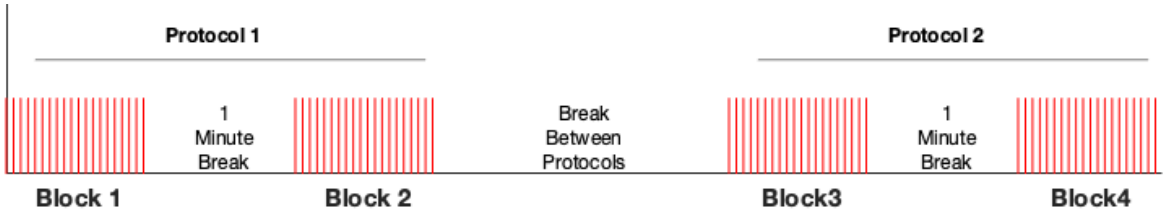

Fig. 2 Experimental timeline: two experimental protocols (with 1000 stimuli) were split by a one-minute break, resulting in four blocks of 500 stimuli (stimulation time per block ~8.3 min). Between the execution of protocols, a longer break of approx. 5 to 10 minutes. Order of the protocols was randomized between participants.

FVEPs were recorded with a high-density-EEG cap of 126 Ag-AgCl electrodes (WaveGuard; ANT, Enschede, The Netherlands) of which only 22 electrodes were used, mostly covering the occipital lobe (International 10-20 System, see Appendix B-1). A separate ground electrode was placed on the left mastoid, while all other electrodes on the cap remained unconnected. Data were recorded with a common reference and sampled at 1024Hz using a Refa system (TMSi, Oldenzaal, The Netherlands). Skin impedance was maintained below 5kΩ. Apart from antialiasing filters, no other filters were applied online. EEG data and trigger pulses at the start of each flash were simultaneously recorded for post-processing and stored on a computer for offline analyzes.

Experimental protocol

Participants were instructed to wash their hair with shampoo one day before the experiment, to reduce skin impedance. During the experiment participants were instructed to close their eyes, relax while sitting still, and avoid swallowing. First, the participants underwent 1.5-minute flash stimulation, similar to the experimental flashes, to get used to the unusual environment. All participants felt comfortable enough to proceed with the experiment.

In Fig. **2** the experimental timeline is illustrated. The experiment consisted of two experimental protocols of either 1000 flashes, called; Pre-set intensity- and Random intensity protocol. The maximum intensity was identical in both protocols, only the distribution of the intensities differed. For the participants, the protocols were indistinguishable from each other. All participants executed both protocols, but the order was randomized between participants. Halfway through each protocol, there was a one-minute break without stimulation, participants were allowed to make small movements while remaining seated but were not allowed to talk. Stimulation automatically resumed after the small break. Between the execution of protocols, there was a long break of approximately 5 to 10 minutes where participants were allowed to talk, drink water and stand upright. Participant preparation, familiarization minutes and testing with two protocols took approximately 1.5 hours.

The pre-set protocol can be seen as the current standard for the acquisition of SR relation. The pre-set intensity protocol consisted of 100 flashes for each of 10 different intensity levels (10intensity x100 flash = 1000 total) that were determined beforehand. The highest level had a light intensity of 2.64 log cd/m2 (438 lux), other intensities were set to 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% and 90% of that intensity. Flashes were presented pseudo-randomly on a pulse-by-pulse basis with one adjustment; for comfortability reasons, the flash scripts prevented two flashes >70% to be consecutive. The ISI was programmed randomly to be either 0.9, 1 or 1.1 seconds to counter anticipation.

The Random intensity protocol can be seen as a novel technique. It was developed to be less dependent on pre-selected intensity levels. It potentially produces a similar SR relation but requiring reduced number of stimuli. Besides, it could potentially be used online to acquire SR relations in the future.

The Random intensity protocol also contained 1000 flashes. However, the intensities of the flashes were pseudo-randomly selected from the 1% to 100% intensity range of 2.64 log cd/m2 (438 lux) resulting in stimulation with 100 different intensity levels (with on average 10 stimuli per intensity level). The ISI and comfortability corrections were the same as in the Pre-set protocol.

Data pre-processing and analysis

All data pre-processing and analyzes were performed offline using Matlab (R2020a; The Mathworks, Natick, MA, USA) with EEGLAB toolboxes [17]. After removing the DC-offset, the EEG data were band-pass filtered between 1 and 30 Hz using a windowed sinc FIR filter (cut-off frequencies (-6dB); 0.5 and 30.5 Hz, hamming, and estimated filter length 3381 taps). The signal was shifted by the filter's group delay to achieve zero-phase and re-referenced to Cz. An example of a pre-processed EEG recording is presented in Fig. **3** (top)**.**

Data were segmented in 900 ms epochs around the flash stimulus, including a 400 ms pre-stimulus interval, as depicted in Fig. **3** (middle). In all epochs, time zero was defined as the beginning of the flash. Mean baseline values were calculated from the -300 to -100 ms interval relative to stimulus onset and removed. Epochs with a peak-to-peak activity higher than 150 µV were automatically detected and removed (43 epochs were removed in total).

FVEP amplitudes can only be identified from EEG background noise if multiple responses are averaged. Averaging multiple responses gives a typical FVEP waveform, as depicted in Fig. **3** (bottom)**.** A positive peak in the latency interval of 125 to 165 ms post-stimulus was found to be most consistent and robust among participants (see Appendix A). This positive peak (P150) was also clearly present in a previous case study with this equipment (see Appendix A). Visual inspection of channels

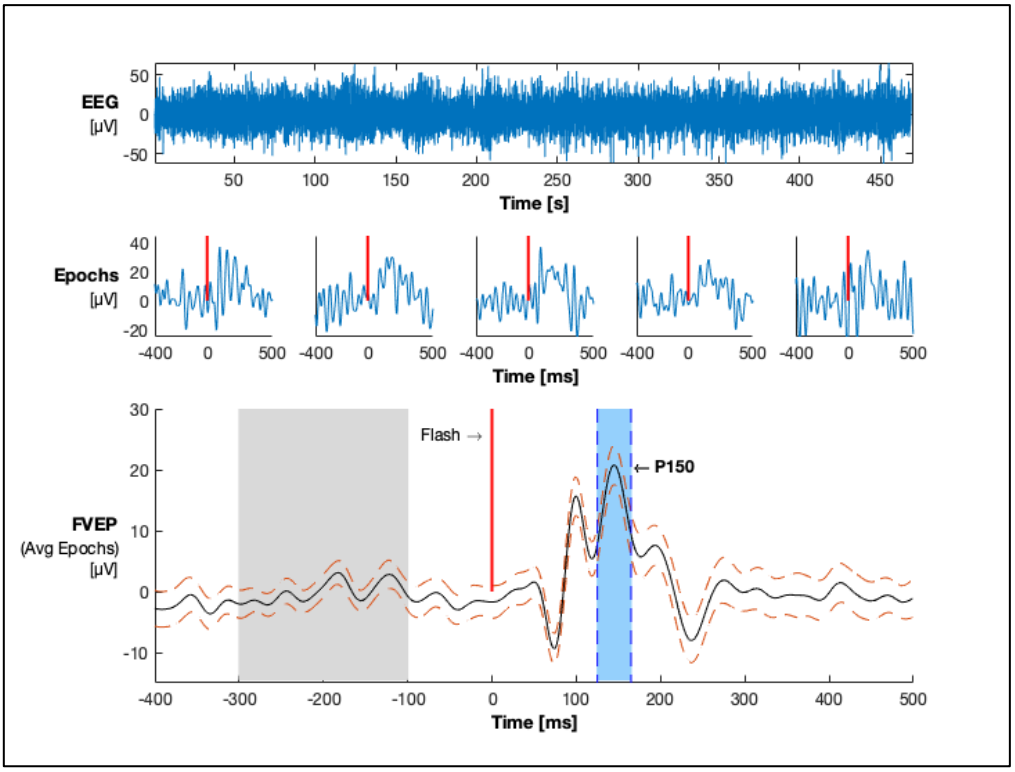

Fig. 3 Single participant data: **Top:** Example of a pre-processed EEG recording during the Pre-set intensity protocol. **Middle:** Five examples of segmented epochs from the EEG recording. Epochs were segmented (-400 to 500ms) around the flash (red vertical line on time point zero). **Bottom:** A representative example of the FVEP waveform to the highest stimulation intensity level (average of 100 epochs: solid black line) with 95% confidence interval (dashed orange lines). Red vertical line illustrated at the timepoint of the flash. Gray rectangle illustrating the period in which the baseline value is calculated. Light blue rectangle illustrating the interval of 125ms to 165ms in which the P150 peak is calculated.

revealed that Oz showed the most consistent P150 peaks with the highest amplitudes in both protocols (see Appendix B-2). Since most interests are in the occipital lobe, further analysis only focuses on P150 peak in the electrode Oz.

Effect intensity

Two different protocols were presented in an attempt to develop a rapid acquisition protocol for the SR relationship of the visual system using FVEP. However, the first inspection of the Pre-set protocol results revealed the absence of a sigmoid curve. This observation was unexpected and arose an important question; is there an effect of flash intensity on the occipital cortex response at all? As a consequence, modeling the SR relation and the exploration of the novel technique were excluded. Instead we will only investigate if flash intensity significantly affects the P150 component of the occipital cortex response.

To calculate P150 amplitude and latency values in the Pre-set protocol, the epochs were grouped to intensity level (10 total) and averaged (average of approx. 100 epochs), in all participants separately. Within the averaged epochs (FVEPs) the P150 peak could be calculated. The maximum amplitude in the interval 125 to 165 ms post-stimulus was defined as the P150 amplitude, calculated from the baseline level. The time in milliseconds from the start of the stimulus to the P150 peak (maximum in 125-165 ms interval) was defined as the P150 latency.

Since the SR relation in the Random intensity protocol did not depend on the distribution of intensities (see Appendix C), it could be analyzed similarly as the Pre-set protocol. The 100 different intensities of the Random intensity protocol were grouped to the 10 intensity levels of the Pre-set protocol. The intensity level of 10% represents all the epochs from intensities 1 to 10%, the intensity level of 20% represents all the epochs from intensities 11 to 20%, etc. The newly generated groups of epochs in the Random intensity protocol made the analyzes of both protocols similar, as the groups of epochs could be averaged. In the Random intensity protocol, the P150 amplitudes and latencies were determined separately for all participants as well.

In total, this resulted in 160 values (10intensity x2protocols x8number participants) for P150 amplitude and latency which were saved for statistical analysis.

Minimal number of stimuli required for a representative P150

In order to reduce acquisition time in further studies, we analyzed the minimal number of stimuli needed to calculate a representative P150 peak for each intensity separately. Hypothetically it might require fewer stimuli to acquire a reliable P150 peak with a high stimulus intensity level compared to a low stimulus intensity level. In other words, it is interesting to know how many responses need to be averaged to generate a reliable P150 peak for every intensity level separately, to subsequently identify if the

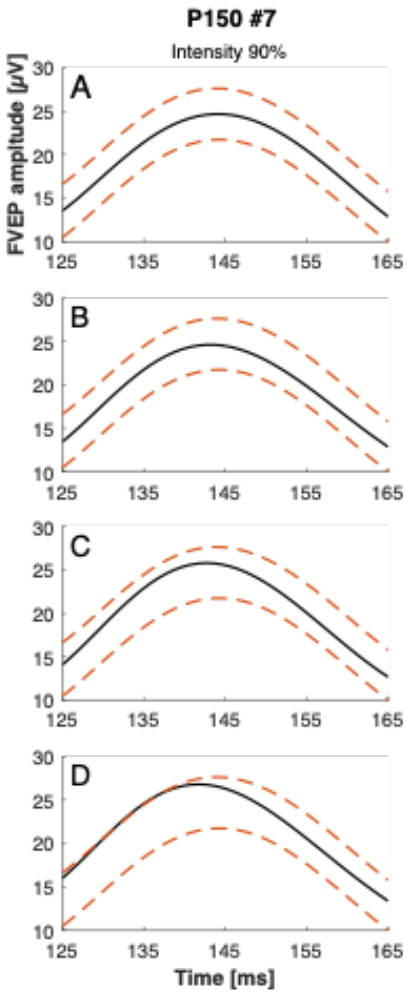

Fig. 4 A representative example of the data elimination process. **(A)** All 100 epochs with the intensity level of 90% are used to calculate the P150 peak in the latency interval of 125 to 165 ms (solid black line) with the 95% confidence interval of the peak (dashed orange lines). **(B-D)** 80, 54, and 44 epochs used for the calculation of the P150 peak (solid black line), superimposed with the 95% confidence interval of the initial peak (dashed orange lines).

number of stimuli needed to generate a representative P150 peak depends on intensity level.

For every participant, an iterative epoch elimination process was used to determine the minimal number of stimuli required to acquire a representative P150 peak in each intensity level separately, as depicted in Fig. **4**. Both protocols of 10 intensity levels were included in the analysis. For each participant, the average P150 peak (from 125 to 165 ms) was calculated for each intensity level in both protocols. This average (of approx. 100 responses) was defined to be the true peak. At each iteration, a random epoch was eliminated and the new average in the 125 to 165 ms interval was calculated. The minimal number of stimuli was defined at the second iteration where the new line in the 125 to 165 ms interval left the 95% confidence interval calculated from the initial peak. The elimination process resulted in 160 values (10intensity x8number participants x2protocol) for the number of stimuli needed to acquire a representative P150 peak that were saved for statistical analysis.

P150 temporal consistency

The absence of a sigmoid curve between stimulus intensity and occipital cortex response might be due to changes in the P150 component during the experiment. Hypothetically the experiment's length may have resulted in a habituation effect, mainly during the second trial. To test the temporal consistency of the P150 component, a block by a block comparison was executed.

We assessed the temporal consistency of the P150 component by comparing the mean P150 amplitude and latency of four consecutive blocks according to the experimental timeline (see Fig. **2**). Block 1 contains all the epochs before the one-minute break of the protocol that was executed first. Block 2 contains all the epochs after the one-minute break of the protocol that was executed first. Blocks 3 and 4 contain the epochs before (block 3) and after (block 4) the one-minute break of the protocol that was executed lastly. The analysis was executed for each participant and intensity level separately. Blocks contained at least 45 epochs. To avoid variability due to interindividual differences in P150 components, the P150 amplitude and latency were expressed as the P150 amplitude or latency change as a percentage of the mean of all blocks. Resulting in 320 values (10intensity x4blocks x8number participants) for P150 amplitude and latency that were saved for statistical analysis.

Statistical analysis

Statistical testing was conducted using SPSS v.25 (IBM Co, Armonk, NY, USA). All results were considered significant at an alpha of 0.05.

Effect intensity

To ensure valid statistical comparison across participants, P150 amplitudes were normalized to the mean P150 amplitude of a participant; calculated from all intensity levels in the protocol of interest. The normality of all variables (10intensity x2protocol) was evaluated using the Shapiro-Wilk test. The normality of all variables, for both latency and amplitude, could not be assumed. Two separate Friedman tests (for non-normally distributed variables) were conducted to evaluate the effect of stimulus intensity level on the P150 amplitude and latency.

Minimal number of stimuli required for a representative P150

A two-way repeated measures ANOVA with the factor's intensity (10 levels) and protocol (2 levels) was used to test the hypothesis that the acquisition of P150 peak with low intensity stimulation required more stimuli than stimulation with higher intensity levels.

P150 temporal consistency

One-way repeated measures ANOVAs for normally distributed variables and Friedman tests for non-normally distributed variables were used to evaluate the potential

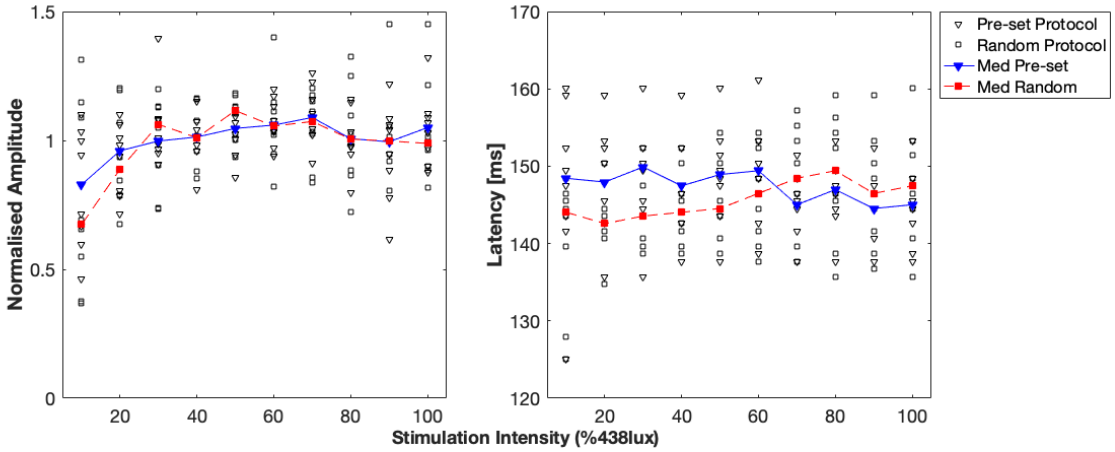

Fig. 5 Individual data (open symbols) of P150 component among each intensity level; triangles represents data from the Pre-set protocol and squares represents data from the Random protocol. **Left:** Normalized P150 amplitudes of all participants showing the great variability among participants in all intensity levels. Grouped data (median) showing a reduction of P150 amplitude with low intensity levels (most pronounced in Random protocol); however, this effect was not significant (Friedman test). **Right:** The P150 latency values showing less variability among participants, but a reduction of latency at the lowest intensity in two data points. Grouped data (median) showing a lack of effect of intensity on P150 latency.

difference in P150 amplitude or latency among four consecutive blocks. Post hoc tests with Bonferroni adjustments when normality could be assumed, and Wilcoxon signed-rank tests when normality could not be assumed were used to correct the level of significance.

Results

EEG responses to red light flash stimulation were measured in healthy volunteers. Two different protocols were executed to determine the SR relationship between stimulus intensity and occipital cortex response. All participants showed clear FVEP waveforms on the Oz electrode to the flash stimulation (see example in Fig. **2** (middle)).

Effect intensity

We assessed if stimulus intensity affects the P150 amplitude and latency. The P150 latency and normalized P150 amplitude values of all participants in both protocols are shown in Fig. **5**, with the median of every intensity for both protocols. Although lowest intensity levels (10% & 20%) seems to reduce P150 amplitude in some participants, grouped data showed no significant effect of stimulus intensity on the P150 amplitude or latency (Amplitude: $\chi2(19) = 24.657$, $p = 0.172$; Latency: $\chi2(19) = 21.811$, $p = 0.294$). These results suggest that stimulus intensity (or the protocol used) did not affect the P150 component significantly.

Minimal number of stimuli required for a representative P150

We assessed the minimal number of stimuli needed to achieve a representative P150 amplitude in every intensity level and protocol separately, by an eliminating epoch process. The minimum number of stimuli needed by the acquisition of the P150 peak in every intensity level is shown in Fig. **6**. The boxplot shows the 25 and 75 percentiles with median (black line) and maximum and minimal value (error bars). Table **1** summarizes the

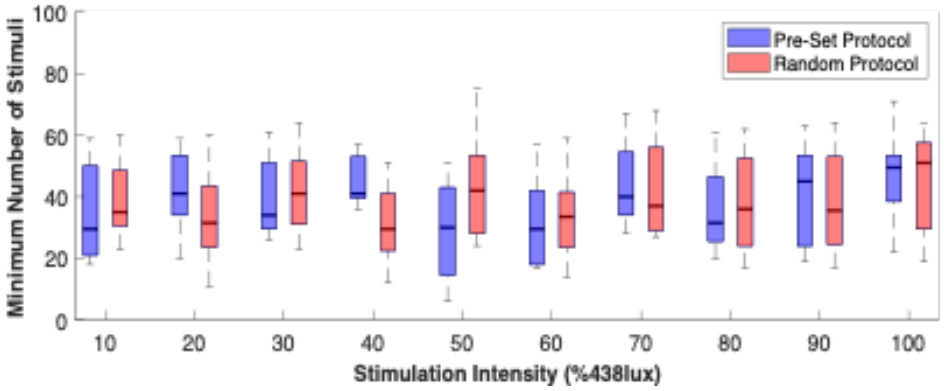

Fig. **6** Boxplot presentation of the minimal number of stimuli needed to asses a representative P150 peak for every intensity level in both protocols. The boxplot shows the 25 and 75 percentiles with median (black line) and maximum and minimal value (error bars).

descriptive statistics of every intensity in both protocols. The main and interaction effects were calculated using a two-way ANOVA with intensity (10 levels) and protocol (2 levels) as factors. Mauchly's test indicated that the assumption of sphericity had been violated. Thus, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon = 0.75$). Comparing the stimulus intensity levels and protocols revealed no significant difference between stimulus intensity levels ($F(3.327, 23.292) = 1.091$, $p = 0.377$), nor between protocols ($F(1,7) = 0.033$, $p = 0.860$), nor a significant interaction effect ($F(3.891, 27.237) = 0.962$, $p = 0.480$).

Table **1** Presentation (mean, SD) of the minimal number of stimuli needed to achieve reliable P150 peak according to the elimination process.

Intensity	Pre-set		Random	
% 438lux	mean	SD	mean	SD
10	35	17	39	13
20	42	13	34	15
30	40	14	42	14
40	45	8	31	13
50	29	17	43	17
60	32	15	34	14
70	44	14	42	16
80	36	14	38	16
90	41	17	38	17
100	47	14	45	17

P150 temporal consistency

To test the temporal consistency of the P150 component during the experiment, the P150 amplitudes and latency were compared in four subsequent blocks for each intensity separately. Table **2** summarizes the descriptive statistics and shows the statistical test used in every intensity for amplitude and latency. The table shows that P150 amplitude changed significantly in 8 of 10 intensity levels, and P150 latency changed significantly in 3 of the 10 intensity levels ($p<0.05$). Presentation of grouped data for P150 temporal consistency to stimulation with the intensity level of 10, 40, 70 and 100% is shown in Fig. **7**.

Discussion

The primary objective of this study was to develop a rapid acquisition protocol for the SR relationship of the visual system using FVEP. The present study did not find any significant difference in P150 amplitude and latency among ten different intensity levels. As a consequence, no mathematical model for the SR relation was fit to the data, and exploration of the novel technique was excluded. In order to reduce acquisition time in further studies, we analyzed the minimal number of stimuli needed to calculate a representative P150 peak for each intensity separately. Additionally, the temporal consistency of the P150 component of the occipital response was analyzed.

The present study demonstrates that it is possible to acquire a reliable P150 peak with on average 54 stimuli (gross mean (39) + 1 SD (14)), which does not depend on stimulus intensity. Additionally, P150 amplitude changed significantly ($p<0.05$) in 8 of 10 intensity levels, and P150 latency changed significantly in 3 of the 10 intensity levels (see Table **2**). Amplitudes increased with time for low intensity stimulation, while amplitudes reduced with time for high intensity stimulation.

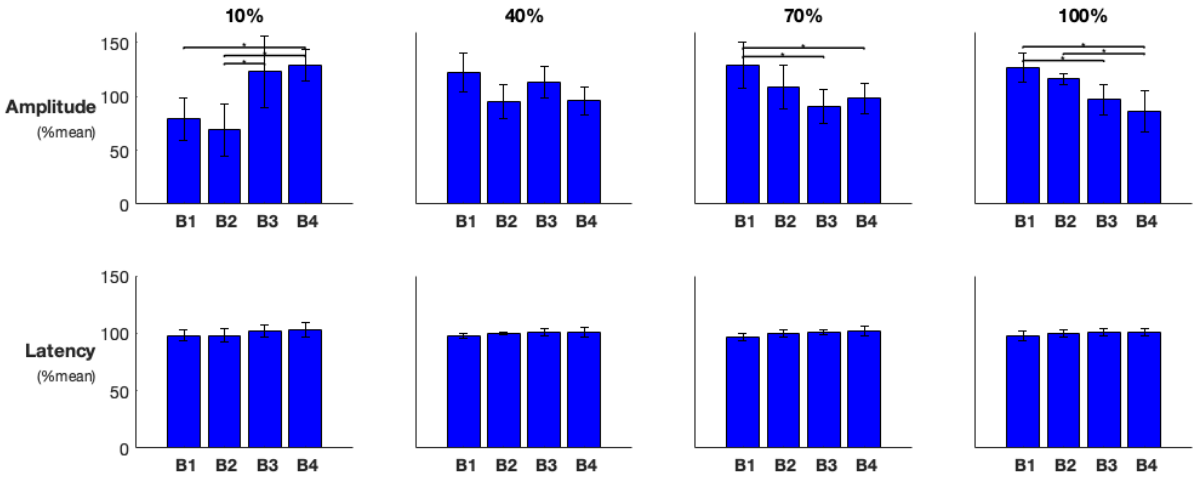

Fig. 7 Grouped P150 amplitude and latency data (bars) with the standard deviation (error bars) of the intensity levels 10, 40, 70 and 100 among four subsequent blocks. Amplitude and latency were normalized to the mean of the four blocks. **Top:** increased amplitudes in later blocks at the lowest intensity (10%), decreased amplitude with little recovery after the break between block 2 and 3 at intensity 40%, and a decreased amplitude at the higher intensities (70 and 100%) later blocks. **Bottom:** the P150 latency is fairly consistent with a small increase during the experiment. However, this was only significant in the intensity of 60% (not shown).

Table **2** Description (mean, SD) of the variation of the P150 peak (amplitude and latency) expressed in percentage of change relative to the mean across four consecutive blocks of stimulation.

P150	Intensity (%438lux)	B1 (mean, SD)		B2 (mean, SD)		B3 (mean, SD)		B4 (mean, SD)		Sig. p(a)	Post-Hoc p<0.05
Amplitude [% mean]	10	79	20	69	24	123	33	129	15	***0.001** +	B1vsB4, B2vsB3, B2vsB4
	20	98	13	88	19	111	14	103	20	0.141	
	30	110	15	90	20	97	21	103	15	0.249	
	40	115	17	89	15	106	14	90	12	***0.012**	
	50	114	14	93	13	97	11	96	15	***0.047**	
	60	111	18	108	7	91	19	91	7	***0.030**	B2vsB4
	70	121	20	102	19	85	15	92	13	***0.010** +	B1vsB3, B1vsB4
	80	123	16	108	14	78	16	91	17	***0.001** +	B1vsB3, B1vsB4, B2vsB4
	90	119	8	103	13	89	15	89	12	***0.001**	B1vsB2, B1vsB3, B1vsB4
	100	119	13	109	5	91	13	81	18	***0.000**	B1vsB3, B1vsB4, B2vsB4
Latency [% mean]	10	98	5	98	6	102	5	103	6	0.188	
	20	99	3	99	2	101	3	102	3	0.162	
	30	97	3	99	2	101	1	103	4	***0.040**	
	40	98	2	100	1	101	3	101	4	***0.032**	
	50	98	3	99	3	101	3	103	2	0.054	
	60	97	2	99	1	101	2	102	3	***0.005** +	B1vsB3, B1vsB4, B2vsB3
	70	97	3	100	3	101	2	102	4	0.126	
	80	98	3	100	3	101	2	101	3	0.324	
	90	98	3	100	3	100	4	102	3	0.138 +	
	100	98	4	100	3	101	3	101	3	0.383	

(*) Statistically significant p<0.05. One-way repeated measures ANOVA with Bonferroni post-hoc if normality was assumed
(+) Friedman test with Wilcoxon signed rank post-hoc tests when normality was not assumed.

Effect intensity

The relationship between stimulus intensity and VEP latency is well documented, demonstrating a reduction in latency with increased light intensities in most flash and checkerboard VEP studies [3, 8-10]. The latency reduction seems to indicate that brighter events are becoming more important to the visual system. For this reason, we hypothesized that brighter (i.e. more intense) flashes would decrease FVEP latency and increase amplitude, but neither effects were observed. The absence of an effect of intensity on P150 amplitude of the present study was in agreement with a relatively recent approach with a xenon flash tube [18], whereas stimulation with checkerboard pattern did show a reduction in VEP amplitudes when luminance filters were used [19]. Early research between the 1950s and 1980s used photo stimulator lamps to assess the effect of stimulus intensity on the FVEP. There was no consensus on the effect on FVEP amplitude, and it was suggested that the relation differed between individuals. However, as methodological standardization was missing (for review see [20]) this hypothesis remains plagued with concerns about validity. Interindividual differences in intensity dependency of P150 amplitude were not observed in the present study (see Appendix D), as will be discussed later. Note, the FVEP waveform strongly depends on the equipment used, resulting in significant differences in amplitude, latency and distributions of negative and positive peaks [21]. A huge number of neurons are involved in visual processing. Therefore, different stimulation parameters likely activate different groups of neurons. Thus, care must be taken when comparing results from different stimulation techniques.

The present study was a novel approach for acquiring the SR relationship for occipital cortex response as a function of stimulus intensity with a red-light LED goggle in humans. LED goggles have been used to study the effect of light intensity on visual processing in animals. Where amplitude amplifications were clearly visible in rats at higher intensity of stimulation [22]. Besides, the frog optic nerve peak signal, measured by a suction electrode, increased by increasing intensity which was most prominent in red compared to violet light [23]. Adverse to the animal studies, grouped data in the present study did not show a pronounced effect of stimulus intensity on the P150 component. However, as will be discussed, an important methodological parameter, total stimulation time, has likely influenced study results. Therefore, it is too

premature to draw any conclusions. Further research is required to validate if the SR function appears to be a sigmoid curve as seen in animals. Alternatively, if intensity does not give remarkable changes in occipital cortex response, as seen in this study, the intensity level of the current clinical FVEP standard could be reduced.

Stimulation time

The contradiction between study results from animal studies and the present study might be due to the stimulation time. Total stimulation time contained four blocks of approximately 8 minutes which may have influenced levels of arousal and attention. Response decrement resulting from repeated flash stimulation (habituation) is documented in adults after 3 minutes of stimulation [24-26]. Although stimulation in these studies contained a single intensity type, our stimulation time was a lot longer; a comparable effect has likely occurred. Since protocol order was randomized between participants, the calculated SR relations contained recordings that are likely influenced by factors as attention, habituation, fatigue and arousal.

Individual data, available in Appendix D-1, clearly illustrates this issue. Sigmoid curves for stimulus intensity and occipital cortex response (SR relation) appeared in the protocol that was executed first (trial 1) in almost all participants. Contrary, the individual SR relations in the second trial were flat or even decreased with increasing intensity in all participants except one.

In addition, the temporal consistency analysis (block comparison) of the P150 amplitude demonstrated that P150 amplitudes recorded after stimulation with low intensity increased significantly during the experiment. Contrary, recordings of high intensity stimulation decreased significantly during the experiment. Thus, the data seem to indicate that stimulation over time elicits an identical response, in terms of P150 amplitude, for all intensity levels.

Such an effect was not seen in P150 latency values. In trial one, latency values varied between participants were reduction, prolongation and equal latencies with increasing intensities are shown (available Appendix D-2). For some participants, the effects in the second trial were contrary to trial one. However, individual variability was too large to indicate a clear latency-stimulus intensity relation. Nevertheless, P150 amplitude values clearly demonstrated that the cortical excitability changed throughout the experiment, which certainly influenced the SR relationship of stimulus intensity and P150 amplitude found in the present study.

FVEP protocol

Above findings demonstrate that further research should decrease acquisition time dramatically. The intensity level of 90% showed a significant change in P150 amplitude between block 1 and 2. Therefore stimulation should not contain more than 250 stimuli in sequence. The present study demonstrated that acquisition time could be reduced by using 54 instead of 100 stimuli for each intensity level. Moreover, the number of intensity levels required might be lower. Adjustments to the present SR paradigm will lower the number of stimuli (i.e. acquisition time), which will be more suitable for testing the initial response.

An additional adjustment to the protocol that was used in the present study regards the intensity range. Clear FVEP waveforms were observed in all participants in the entire intensity range (10 to 100% of 438 lux). Optimally, a protocol for assessing the SR relationship would enclose an intensity interval from a lower limit where no neuronal activity is elicited (threshold) to an upper limit where saturation of neuronal activity has occurred. In practice, the ability to detect a threshold is determined by distinguishing between stimulus-driven activity and noise. Near threshold, lower stimulus-driven activity is expected, which further decreases the signal to EEG noise ratio, which will require additional (impractical) large number of (averaged) responses [4]. Even if sufficient numbers could be obtained, neurons themselves are rarely silent and show a certain ‘noise’ [27]. Thus, further research should lower the lowest intensity to detect the lowest intensity were the FVEP is measurable with practical number of responses. However, one should keep in mind that this will not be the physical threshold of neuronal activity. Regarding the upper limit of the intensity range, it is advised not to use higher intensity levels than used in the present study. It would not be comfortable for the participant and will likely not affect the response enormously.

The final adjustment to the present protocol regards the ISI. Although the three different ISIs used were large enough to achieve a ‘resting’ state of activity, differences between the ISIs might have been too small to prevent anticipation effects. Improvements can be made by longer ISIs additional to the current. Yet care must be taken when increasing the acquisition time.

Alternatively, the standard technique might be replaced by a novel technique. Instead of presenting at least 54 stimuli of a fixed number of stimulus intensity levels, stimulation can be done using varying intensity levels at a fixed intensity range (the Random protocol in this study). In that case, SR calculation cannot be done with the standard averaging method as there will not be 54 responses for each intensity level. However, a moving average technique can be used to overcome this issue. The moving averaging technique potentially produces a similar SR relationship but requiring less acquisition time. Further advantages are that the paradigm would be less dependent on pre-selected intensity levels. Also, it might be easier to optimize to an individual, and might be suitable for online acquisitions. Further research should explore if this novel paradigm is a reliable solution to reduce the SR relation acquisition time.

An alternative theory for the intensity dependency of sensory systems is that the brain is more sensitive to differential and not to absolute intensity changes. It may be that neurons in the retina are more likely to detect differences in luminance rather than the absolute luminance, comparable to the effect seen in an auditory evoked potential study [28]. They demonstrated this with

a novel paradigm that might be interesting to apply to visual functioning testing with the LED goggles. Nevertheless, in all likelihood, our protocol is not affected by this theory, as the background luminance was equal throughout the experiment and between participants, and the responses were fully recovered before the next stimulus was applied. Furthermore, the goggles stimulate a huge, almost complete, visual field with equal luminance.

In conclusion, the present protocol design can be improved by reducing the number of intensity levels, reducing the number of stimuli used for each intensity, reducing the lowest intensity level, and adding longer ISIs.

Variability concerns

When assessing visual system properties with the FVEP method, one should keep in mind the downside of this technique. The inter-individual variability of FVEP components is large, which increases the difficulty to find between-group effects. Yet, the inter-individual variability of intensity dependency on FVEP components is unknown. Hypothetically, normalized SR relation (curve) properties are less variable between individuals than the absolute FVEP components. Further research is required to test this hypothesis. Nevertheless, it is advised to still minimize factors that can increase the inter-and intra-individual variability of FVEPs when developing a protocol.

Factors such as age, gender, pupil size, drugs, color vision, auditory stimulation, frequency of stimulation, mono- or binocular stimulation, and equipment are all known to affect FVEP waveforms [3, 8, 29]. Notable is that the recording of FVEPs with closed eyes yields more reliable latencies and amplitudes than recording with open eyes. This is mainly due to the absence of eye blink artefacts and the absence of changes in gaze direction [8, 18]. Besides, monochromatic stimulation is preferred over multicolored stimulation because it reduces inter-and intra-individual variability [8, 30, 31]. It has been suggested that individuals may respond differently to different wavelengths, which depends on the type, number and distributions of cones on the retina of the eye. This might explain the reduction in variability by stimulation with monochromatic light because only a particular cone type is stimulated. Also, monochromatic light stimulation is known to produce a larger amplitude than does multicolored stimulation. Hence, monochromatic stimulation is favored to white (multicolored) stimulation because it might increase the S/N ratio of FVEP recordings [8, 29]. Moreover, care must be taken regarding the equipment used for flash stimulation. In most FVEP studies a photo stimulator lamp is used, newer technology has made it possible to generate flashes in a light-emitting diode (LED) goggles. The goggles can be placed directly over the eyes and therefore produce an increased field of stimulation. Besides, the effect of change in gaze direction is minimized by using LED goggles, thereby improving the FVEP recordings' consistency [32, 33].

The protocols in the present study were designed to minimize the effect of factors that can influence the FVEP recordings.

Applications

Due to the high number of factors that can increase the inter-and intra-variability, the introduction of a standard protocol in 2004 was crucial for the use of the FVEP method clinically [34, 35]. To be clinically relevant, acquisition of SR relationship must as well be made systematically with a standard protocol.

Although the large inter-variability is a major limitation, some advantages favor the FVEP method. FVEPs are adaptable, non-invasive, easy to reproduce, inexpensive, and easy to synchronize with the recording. Because of its ease, it could be a good tool to study the visual system in longitudinal studies, including home monitoring.

Present attempt to design a standard protocol for the SR relationship was not meant to replace current FVEP standard. It can be seen as an addition to the current process to give complementary information about the visual system. The protocol can provide quantitative data that potentially show abnormalities in various diseases that were not observed using the current standard. Since migraine sensitivity for light changes throughout the circle of attacks and no attacks, it is a good example of a disease where the acquisition of SR relationship might be relevant. Not only migraine but also Alzheimer, Autism and aging are promising areas for assessing SR relationships. Within these areas it might identify physiological abnormalities or answer specific disease related questions. In addition, due to its ease, it can track disease progression and provides data to support medical decision making.

Limitations

Given that the P150 component was most consistent across participants, the analyzes were limited to the P150 peak. At the same time, other FVEP components might provide additional knowledge about the effect of intensity. Analysis of the total response likely requires more work, since the distribution of peaks varies between individuals. Further studies might include analyzing all FVEP components by accurately measuring each peak's intensity dependency separately within a participant.

P150 component analyzes were limited to the Oz electrode because it showed the highest amplitudes in this channel. However, there is no assurance that the channel with the highest amplitudes is the most reliable and shows the most consistent intensity dependency. Furthermore, a difficulty arises in the identification of the P150 peak intensity dependency. The spatial resolution of EEG is poor. Therefore, current experiment did not allow for localizing FVEP components and the potential change in neuronal source with different intensity. Substantial research with alternative methods, like MRI, exists about the origin of the potential [3]. Although information about intensity dependency of the neuronal source might be relevant, techniques such as MRI are far more expensive and require more time than FVEP methods. Thus, the clinical utility will be limited.

Another limitation of this study is that the calculation of the minimal number of stimuli required for a reliable

P150 peak assumed that 100 stimuli represented the 'true' peak value. However, the present study demonstrated that the P150 peak changed throughout the experiment. With the 100 stimuli scattered throughout the experiment, validity concerns arise about the assumption of the 'true' peak. The reliability of the minimal number of stimuli found in the present study will require further research using 100 stimuli in total. Hence, it will not be influenced by temporal changes but is certainly large enough to decrease EEG noise.

Furthermore, the assumption was made that the LED goggles properties were linear; i.e. the different steps in intensity resulted in equal differentiation in luminance. Whereas different stimuli intensities could be subjectively distinguished from each other, precise absolute luminance levels were not obtained. This assumption can easily be tested with a Lux meter. However, this will not give precise information about the retina's illumination, since it depends on properties of the eyelid which will vary among individuals.

Finally, old research demonstrated different intensity dependency in children and elderly [36]. The present study only included young adults. Thus, subsequential research is needed to test the effect of age on the intensity dependency in recordings of responses to LED goggles.

Concluding remarks

The present approach to design a rapid acquisition protocol for assessing the SR relation was largely influenced by the experiment's stimulation time. The absence of sigmoid curves led to the exclusion of data modeling and exploration of a new paradigm. Although no pronounced intensity effects were observed, the first half of the data led us to suspect a sigmoid curve relation for the initial response as a function of stimulus intensity.

Even while the initial goal has not been achieved, the present study provided knowledge that can guide further research in developing a rapid acquisition paradigm. It demonstrated that stimulation time is an important parameter which can affect occipital response significantly. Besides, it demonstrated that fewer data could produce the same results. Possible adjustments were discussed to improve the experimental paradigm.

An optimal paradigm for assessing the SR relation using FVEP could be a relatively easy, quick and inexpensive tool that quantifies additional properties of the visual system.

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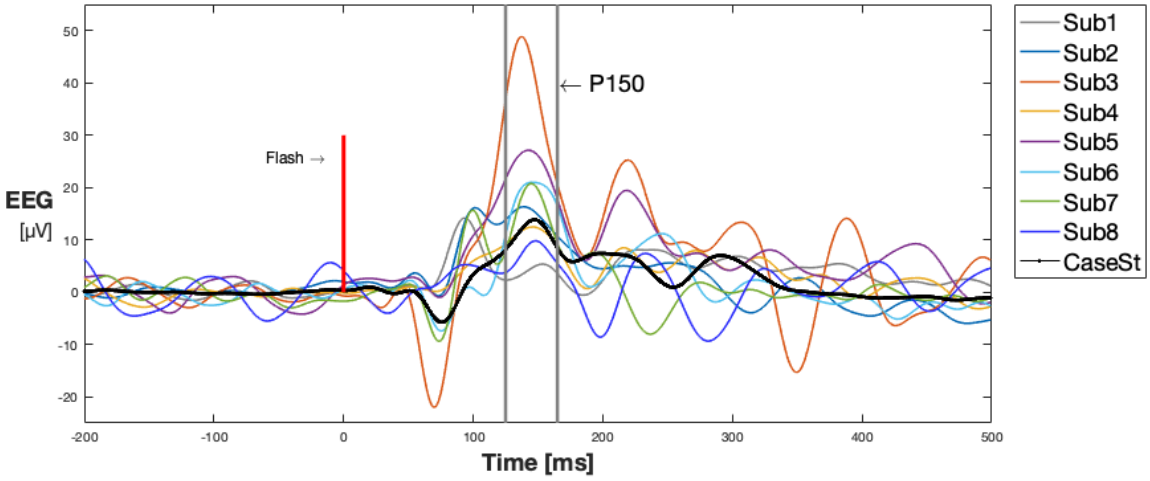
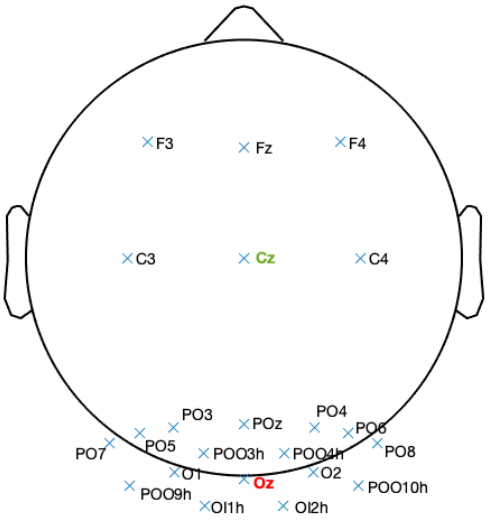
Appendix

A) WHY P150 PEAK?

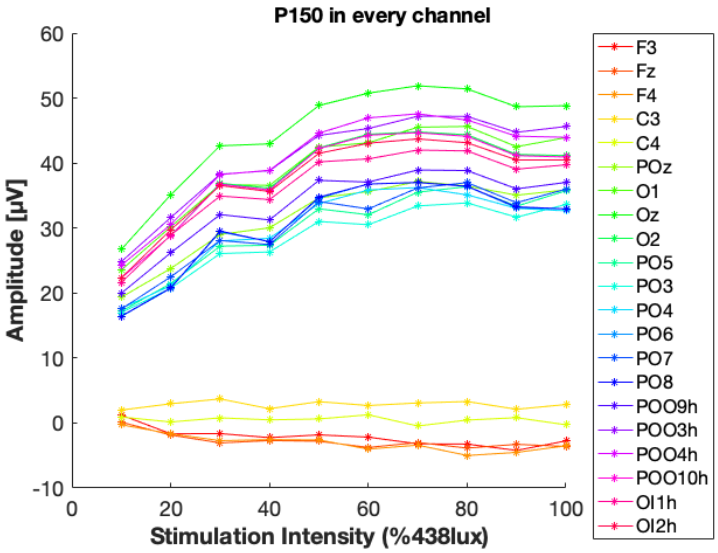


[A] Illustration of the mean FVEP response (average of 1000 stimuli including all intensity levels) recorded in the first trial. The individual data of all subjects are illustrated (colored lines) with data from a similar case study (black line). The most consisted peak among subjects is a positive peak (P150) in the latency interval 125 to 165 ms (gray vertical lines) after the onset of the stimulus (red vertical line). The present study only included this P150 peak in the analyzes.

B) WHY ELECTRODE OZ?

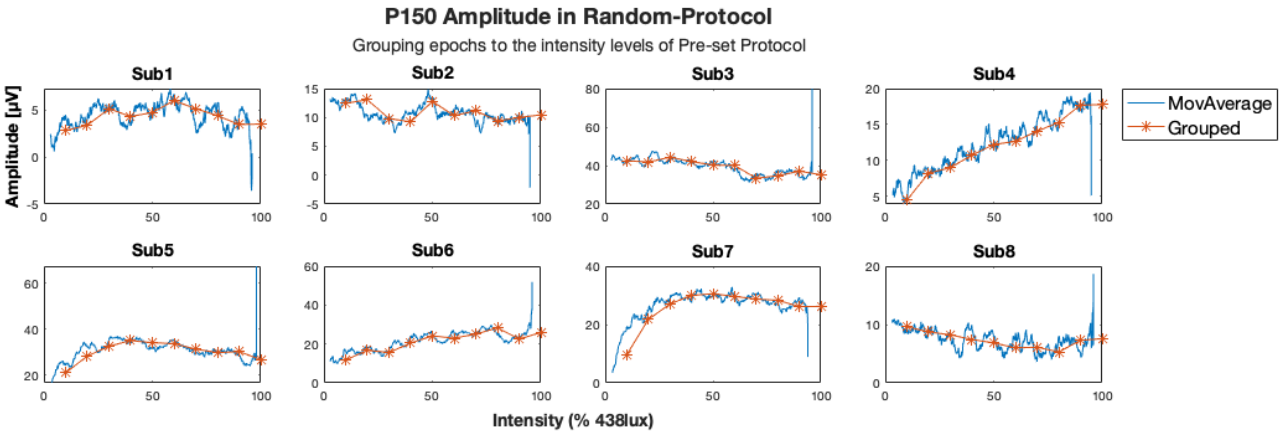


[B-1] Illustration of the channel locations used in the present study (blue markers); 22 electrodes were used (connected) of a high-density-EEG cap. The Cz electrode (green) was used as reference and the Oz electrode (red) was used in study analyzes.



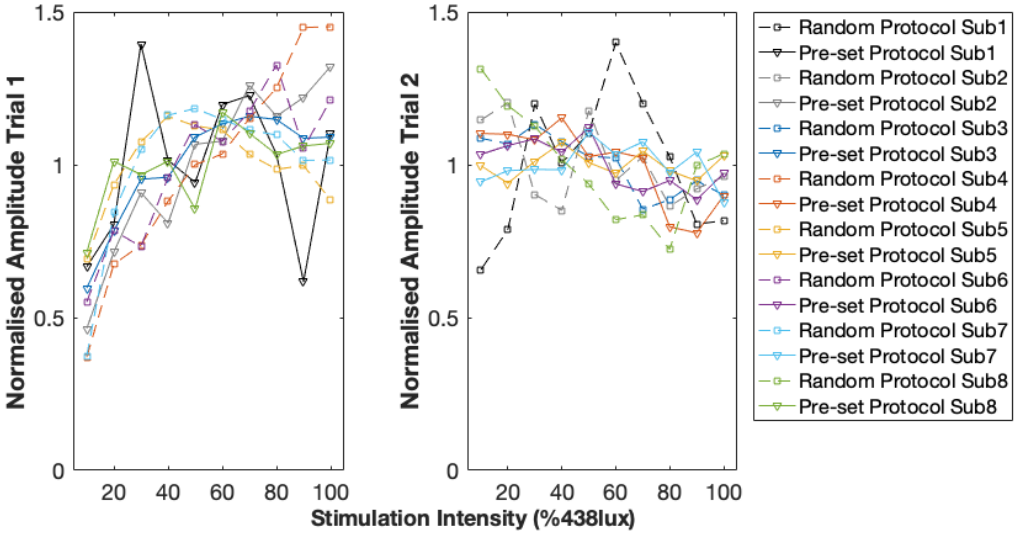
[B-2] A representative example of single subject data illustrating the P150 peak in all channels among the 10 different intensity levels. The response in the Oz electrode showed the highest amplitude in all intensity levels. The present study only included the Oz electrode in the analyzes.

C) GROUPING RANDOM PROTOCOL TO PRESET PROTOCOL INTENSITY LEVELS

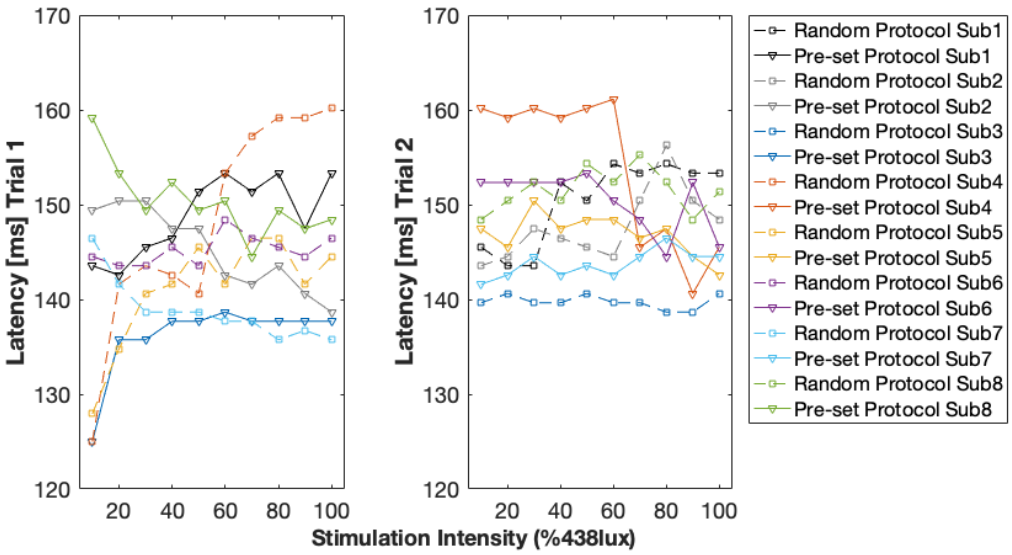


[C] Data from the Random intensity protocol: subplots represent a single subject. The illustration shows the intensity dependency of P150 amplitude calculated with the moving average technique (blue dots) compared to the calculation with the grouped epochs (orange asterisk). The moving average technique required three steps; first: all epochs were ordered by stimulus intensity, second: the P150 peak was calculated from the averaged 50 first epochs, and third: plotted against the mean intensity of that 50 epochs. Whereafter the process was repeated by the 2 to 51 epochs, 3 to 53, etc. P150 amplitudes calculated from the Grouped epochs were used in the present study analyzes; the intensity level of 10% represents all the epochs with intensity stimulation from 1 to 10% (on average 100 epochs), intensity level 20% represents all the epochs with intensity stimulation from 11 to 20%, etc. The illustration shows that the intensity dependency in the Random intensity protocol is similar among calculations.

D) INDIVIDUAL DATA OF TRIAL 1 AND TRIAL 2



[D-1] Illustration of all subject data of the SR relation (P150 amplitude vs stimulus intensity) in the protocol that was executed first (trial 1: left) and the protocol that was executed second (trial 2: right). **Left:** individual data of trial 1 shows increased P150 amplitude with increasing intensity in almost all subjects, except one. **Right:** individual data of trial 2 show a flat or even decreased relation between P150 amplitude and stimulation intensity. This illustration clearly shows that the P150 amplitudes were not consistent during the experiment.



[D-2] Illustration of all subject data of the SR relation (P150 latency vs stimulus intensity) in the protocol that was executed first (trial 1: left) and the protocol that was executed second (trial 2: right). **Left:** individual data of trial 1 shows that the SR relation for latency varied between subjects; were increasing, decreasing and flat relations are shown. **Right:** individual data of trial 2 were contrary to trial 1 for some subjects. Additionally, extreme latency reductions in the lowest intensity are not shown. However, individual variability is too large to indicate a clear latency SR relation.