Analysis of Nociceptive Evoked Potentials during Multi-Stimulus Experiments using Linear Mixed Models

B. van den Berg[1] and J. R. Buitenweg[1]

***Abstract*— Neural processing of sensory stimuli can be studied using EEG by estimation of the evoked potential using the averages of large sets of trials. However, it is not always possible to include all stimulus parameters in a conventional analysis, since this would lead to an insufficient amount of trials to obtain the evoked potential by averaging. Linear mixed models use dependencies within the data to combine information from all data for the estimation of the evoked potential. In this work, it is shown that in multi-stimulus EEG data the quality of an evoked potential estimate can be improved by using a linear mixed model. Furthermore, the linear mixed model effectively deals with correlation between parameters in the data and reveals the influence of individual stimulus parameters.**

I. INTRODUCTION

To study neural processing of sensory stimuli using EEG, the evoked potential (EP) must be estimated using sufficient amounts of trials. To identify important parameters of stimulus processing, it is required to apply stimuli with multiple properties. However, experiments to gather the required data on human subjects cannot take too long and the amount of stimuli is limited, which is problematic for the acquisition of sufficient trials. Often, stimulus selection methods are used for a more efficient probing of the stimulus parameter space. However, this results in different amounts of trials per stimulus property. Since the variance of the estimated EP depends on the amount of acquired trials, analysis of those trials using conventional averaging is impeded. This could be overcome by using an analysis method which is robust for variations in the amount of acquired trials. Such a method is provided by a linear mixed model (LMM), which deals with those variations by using dependencies within the data. This means that a lower amount of trials is required to accurately estimate the effect of stimulus parameters with respect to averaging.

Recently, we have used EPs to study neural processing of single and double pulse nociceptive electrical stimuli around the detection threshold, which is defined as the stimulus amplitude at which 50% of the stimuli are detected. For optimal estimation of the probability that a stimulus is detected roughly equal amounts of detected and undetected stimulus-response pairs have to be acquired. To keep stimulus amplitudes around the detection threshold, we developed a method for simultaneous tracking of nociceptive detection thresholds (NDTs) for multiple types of stimuli [1]. A single detection threshold is tracked by an adaptive randomized stimulus sequence which automatically varies the stimulus amplitude with respect to the amount of detected and undetected stimuli. Detection thresholds for multiple stimulus types are tracked by randomly interchanging the stimulus types (*single-pulse* and *double-pulse*) during stimulation, which is shown in Figure 1. Because NDTs change over time due to habituation of the nociceptive system, a wide variety of stimulus amplitudes is used throughout the experiment. Because of this variety, the data does not include equal amounts of trials per stimulus amplitude. This leads to a poor estimation of the signal by averaging, which is shown in Figure 2. To extract and analyze the brain activity during detected and undetected stimuli, a more efficient method than averaging is required.

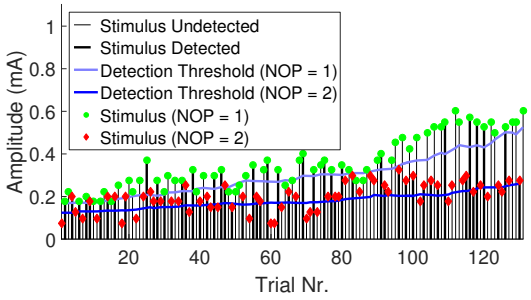

Fig. 1. Simultaneous tracking of NDTs for multiple stimulus types, with a varying number of pulses (NOP), by randomized stimulation around the nociceptive detection threshold [1]. In this case, stimuli with a single pulse (NOP = 1) and a double pulse with 10 ms inter-pulse interval (NOP = 2) were used.

A tool which successfully accounts for the effects of multiple stimulus parameters simultaneously is the linear model (LM). Regression using a LM has the benefit over averaging that it allows for a large number of repeated measures without using many subjects, deals more efficiently with missing data and is flexible in modeling covariates and correlation structures. Although LMs are a popular statistical tool in fMRI research, they have been used by few researchers for EEG analysis, of which some interesting examples include [2] and [3].

Recently Vossen et al. [4] used linear mixed models (LMMs) in EEG analysis to account for between-subject variations and habituation. A major difference with LMs is that LMMs attempt to model the distribution of random effects in the data, enabling subject-level and group-level intercepts and slopes. This provides a convenient way of modeling the dependence of EEG data within one subject

[1]B. van den Berg and J. R. Buitenweg are with the Biomedical Signals and Systems group at the MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, 7500 AE Enschede, The Netherlands.

Correspondence: b.van.den.berg@utwente.nl

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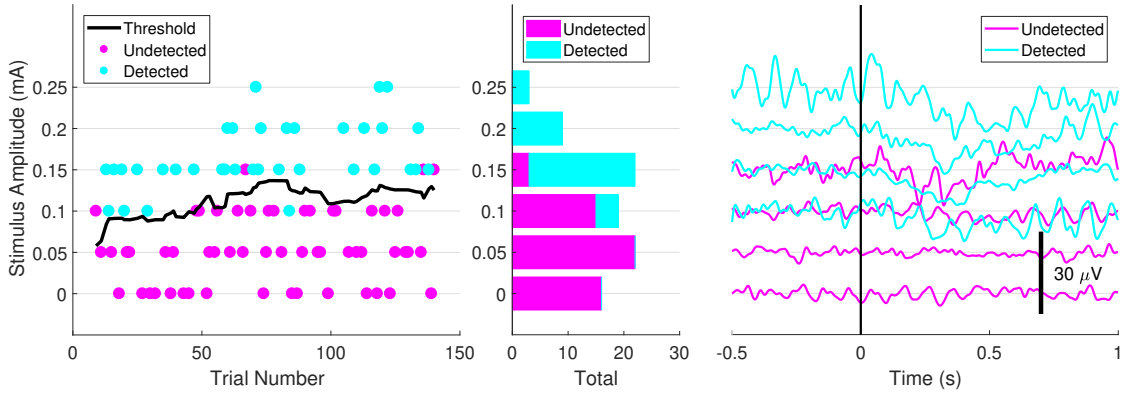

Fig. 2. Summary of data acquired from one of the subjects. The figure on the left shows the amplitudes of all detected and undetected double-pulse stimuli with respect to the NDT. The histogram in the middle shows that at most 25 times the same stimulus was used. This leads to a poor estimation of the EP by averaging, which is shown in the figure on the right.

or one group. Considering their efficiency in dealing with high-dimensional data, trial-to-trial variability and between-subject variations, they provide an ideal tool for analysis of multivariate EEG data. In addition, they provide means to measure the influence of within-subject and between-subject variations simultaneously, which is useful in clinical studies.

In this work, it is demonstrated that a LMM enables the analysis of variations within EEG data with respect to stimulus intensity and stimulus properties, such as the variation of the EEG signals obtained during NDT tracking experiments. It will be shown that a LMM effectively reduces the amount of background activity, and can be used to measure and test relations between stimulus parameters, psychophysical responses and nociceptive EPs.

II. METHOD

A. Experiment

Single and double-pulse stimuli are applied to twelve healthy subjects (5 male, 7 female) via intra-epidermal electrocutaneous stimulation. Nociceptive detection thresholds are tracked by randomized application of stimuli around the detection threshold [1]. A vector of 5 stimulus amplitudes with a step size of 0.025 mA is initialized, of which one amplitude is chosen randomly for the next stimulus of that type. During the experiment, all amplitudes in this vector are increased or decreased depending on the previous response of the subject. In total 171 ± 24 trials were recorded for every stimulus type from each subject, with a variable amplitude. Figure 1 shows an example of the paradigm. The institution's ethical review board approved all experimental procedures involving human subjects.

B. EEG Data Recording and Pre-processing

EEG data was recorded continuously with a sampling rate of 1024 Hz at 64 Ag/AgCl electrodes placed on the scalp according to the international 10-20 system using a TMSi REFA amplifier. In this work, data from the Cz channel is analyzed. Signals are pre-processed using FieldTrip [5], a Matlab toolbox for scientific EEG and MEG analysis. Contamination of the EEG by eye-blinks or movements is corrected using an independent component analysis algorithm [6]. Trials for EP analysis are extracted from the EEG using a window ranging from 0.5 s before until 1.0 s after the stimulus, bandpass filtered from 0.1 to 40 Hz and baseline corrected using the interval ranging from -0.5 s to 0 s relative to stimulus onset. EEG data is downsampled to 200 Hz to increase computational speed.

C. Model Formulation

The statistical model should ideally include all relevant experimental parameters. However, the total amount of model parameters should be restricted to prevent overfitting. In this case, the detection of a stimulus (D) can be expected to be of major influence on the EP. Furthermore, another part of the activity might be directly related to the intensity of the pulse. Both pulses (P1 and P2) can cause an independent increase of brain activity. Brain activity can decrease over time with respect to the number of received stimuli (TRL) due to habituation. Additionally, effect sizes might be dependent on the subject. The LMM that is used to describe those modulations and random effects during the j-th trial of the i-th subject at time τ is shown in equation 1.

$$\begin{aligned} y_{ij}(\tau) = {} & \beta_{Int.}(\tau) + \beta_{P1}(\tau)x_{P1,ij} + \beta_{P2}(\tau)x_{P2,ij} + \beta_{D}(\tau)x_{D,ij} \\ & + \beta_{TRL}(\tau)x_{TRL,ij} + u_{Int.,i}(\tau) + u_{P1,i}(\tau)x_{P1,ij} \\ & + u_{P2,i}(\tau)x_{P2,ij} + u_{D,i}(\tau)x_{D,ij} + u_{TRL,i}(\tau)x_{TRL,ij} \\ & + \eta_{ij}(\tau) \end{aligned} \tag{1}$$

Where:

- The single-channel EEG signal in one trial is $y_{ij}(\tau)$
- The stimulus parameters are $x_{P1/P2/D/TRL,ij}(\tau)$
- The general model intercept is $\beta_{Int.}(\tau)$
- The general model slopes with respect to stimulus parameters are $\beta_{P1/P2/D/TRL}(\tau)$
- The subject-specific model intercept is $u_{Int.,i}(\tau)$
- The subject-specific model slopes with respect to stimulus parameters are $u_{P1/P2/D/TRL,i}(\tau)$
- The model residual is $\eta_{ij}(\tau)$

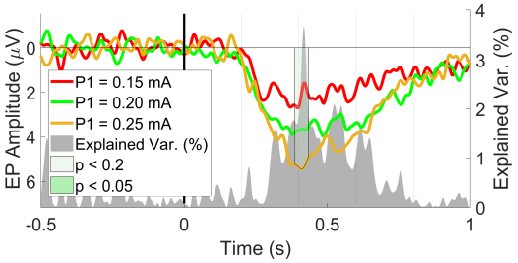

Fig. 3. Grand averages of the EEG signal at Cz, pooled with respect to amplitude values with more than 300 trials. Significance is computed using cluster-based permutation testing [7].

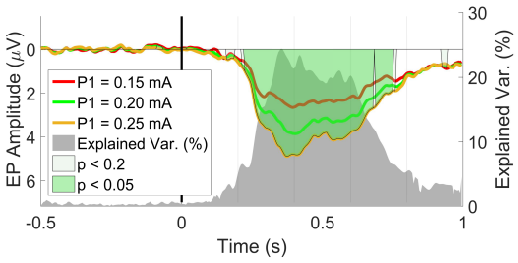

Fig. 4. Average model fit for amplitude values with more than 300 trials at Cz, and the percentage of explained variance of the average model fit. Significance was computed by a Wald t-test of the model coefficient.

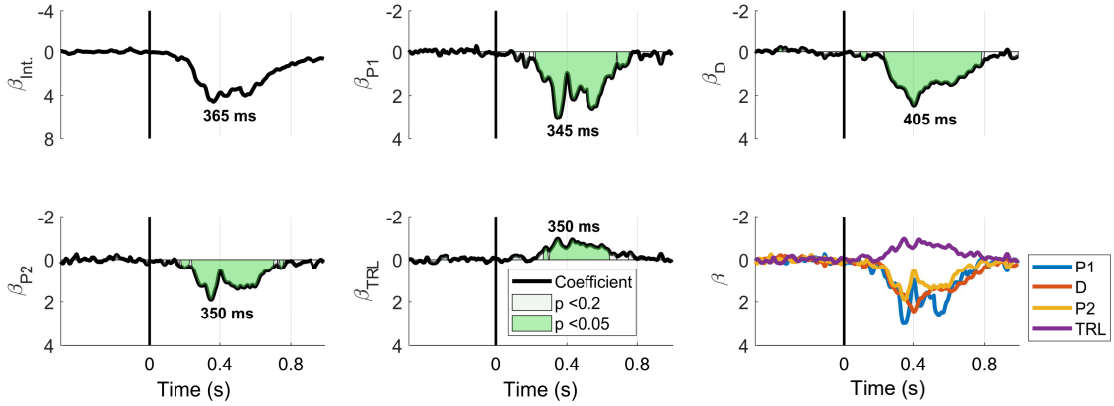

Fig. 5. The model coefficients and their significance based on a Wald t-test at Cz. All coefficients are significant during the post-stimulus interval. The influence of the first pulse and second pulse is computed by the coefficients β_{P1} and β_{P2}. The influence of stimulus detection and the number of received stimuli is computed by the coefficients β_D and β_{TRL}.

D. Analysis and Statistical Testing

The model variables are centered and scaled based on their mean and standard deviation. Next, model coefficients are estimated for every point in time by optimization of the restricted maximum likelihood using Matlab (The MathWorks Inc., version 2015b). To verify model validity, the model residuals are assessed for normality along the entire EP interval. Significance of the model coefficients is tested against the null-hypothesis using a Wald t-test. To reduce the chance of false significance due to retesting, the requirement is imposed that a coefficient should be significant ($p < 0.05$) for at least 4 subsequent time points. Furthermore, the residual is checked for normality along the entire interval.

III. Results

A. Reduction of Background Activity

Figure 2 shows that averaging data for every amplitude and stimulus type per subject results in estimated EPs where post-stimulus activity is difficult to distinguish from pre-stimulus activity due to the high amount of background activity. One way to obtain information about how the EP varies with respect to the stimulus amplitude is by pooling the data with respect to the amplitude and average over considerably larger sets of trials. Figure 3 shows EP waveforms computed by averaging over trials pooled for the three stimulus amplitudes with the largest number of trials. Although the estimated EPs show a clear variation with respect to stimulus amplitude, the pre-stimulus period shows that our estimate still contains a considerable amount of background activity. Figure 4 shows the average fit of a LMM on the same data. In this figure, the pre-stimulus period shows clearly less background activity.

For both figures, the percentage of explained variance was computed by dividing the variance of the model fit by the total variance on each point in time. In the case of averaging, the average was considered the model fit. A comparison between the amount of explained variance in Figure 3 and Figure 4 shows that the data from all trials using a LMM increases the amount of explained variance. A comparison between the significance returned by cluster-based permutation testing [7] of the contrast (left) and the significance of the model coefficient (right), shows that testing the model coefficient results in a higher and more sustained significance of the effect of stimulus amplitude.

B. Influence of Stimulus Parameters

Model coefficients are shown in Figure 5. All coefficients show a significant modulation of the EP. The coefficients can be used to predict the variation of the EP with respect to the variation of a single parameter. In Figure 6 the variation of EP with respect to the pulse amplitudes and stimulus

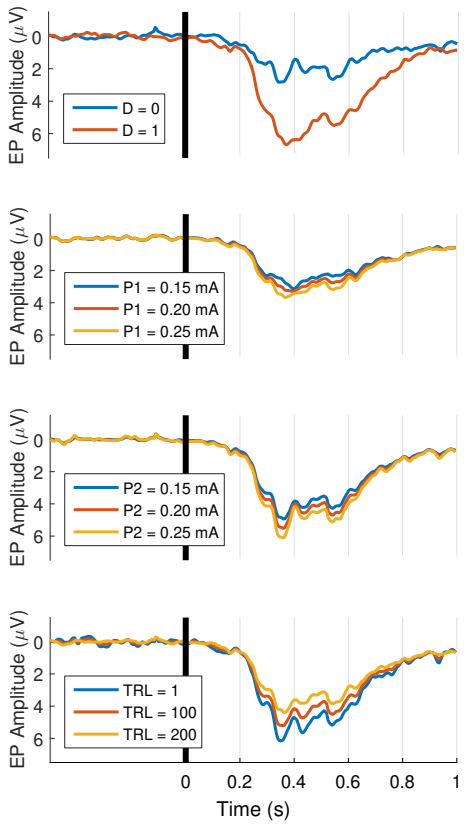

Fig. 6. The effect of variation of stimulus detection (D), the pulse amplitude (P1 and P2) and the amount of received stimuli (TRL) with respect to the model intercept at Cz.

detection is predicted using the model. The Figures 3 and 4 both show a strong modulation by the stimulus amplitude. However, the prediction of the linear mixed model in Figure 6 mostly varies with respect to stimulus detection.

IV. Discussion

A. Reduction of Background Activity

Figure 5 shows that evoked potentials which are estimated using a linear mixed model include less background activity and therefore provide a more accurate estimate of the stimulus-related electrophysiological activity. Furthermore, using a LMM increases the percentage of explained variance with respect to averaging by using a larger amount of trials. This was successfully demonstrated in Figures 3 and 4, where an increase of the explained variance from 3.6% to 24.4% can be observed around 0.4 s. The significance returned by a Wald t-test of the model coefficient shows a higher and more sustained significance of the effect of stimulus amplitude than cluster-based permutation testing. This demonstrates that for a multi-stimulus experiment a Wald t-test of the model coefficient is a more efficient statistical test than cluster-based permutation testing of the contrast.

B. Influence of Stimulus Parameters

The model shows significant modulation of the EP by all factors. As can be expected based on neurophysiology, the coefficient of the first pulse modulates an earlier part of the EP than the coefficient of the second pulse and the coefficient of stimulus detection. A major part of the EP waveform is significantly modulated by the amount of received stimuli: due to habituation the EP will be lower with respect to stimuli of the same amplitude at the end of the experiment. While conventional averaging would not have enabled analysis of the influence of stimulus amplitude and the amount of received stimuli, the LMM successfully accounts for those effects.

Since stimulus detection is correlated with the pulse amplitude (i.e. a higher pulse amplitude results in an increased detection probability), the variation in the average EP in Figures 3 and 4 is likely confounded by stimulus detection. The model prediction in Figure 6 shows that the observed variation of the EP is mainly caused by stimulus detection, while changes in pulse amplitudes only result in minor changes of the EP. Conventional averaging might not have revealed these relations between stimulus parameters and the EP, since inclusion of all potential confounders in the analysis would not be possible due to a lack of trials.

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