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Automated single-trial measurement of amplitude and latency of laser-evoked potentials (LEPs) using multiple linear regression

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Abstract

Objective: Laser stimulation of Aδ-fibre nociceptors in the skin evokes nociceptive-specific brain responses (laser-evoked potentials, LEPs). The largest vertex complex (N2–P2) is widely used to assess nociceptive pathways in physiological and clinical studies. The aim of this study was to develop an automated method to measure amplitudes and latencies of the N2 and P2 peaks on a single-trial basis.

Methods: LEPs were recorded after Nd:YAP laser stimulation of the left hand dorsum in 7 normal volunteers. For each subject, a basis set of 4 regressors (the N2 and P2 waveforms and their respective temporal derivatives) was derived from the time-averaged data and regressed against every single-trial LEP response. This provided a separate quantitative estimate of amplitude and latency for the N2 and P2 components of each trial.

Results: All estimates of LEP parameters correlated significantly with the corresponding measurements performed by a human expert (N2 amplitude: $R^2=0.70$; P2 amplitude: $R^2=0.70$; N2 latency: $R^2=0.81$; P2 latency: $R^2=0.59$. All $P<0.0001$). Furthermore, regression analysis was able to extract an LEP response from a subset of the trials that had been classified by the human expert as without response.

Conclusions: This method provides a simple, fast and unbiased measurement of different components of single-trial LEP responses.

Significance: This method is particularly desirable in several experimental conditions (e.g. drug studies, correlations with experimental variables, simultaneous EEG/fMRI and low signal-to-noise ratio data) and in clinical practice. The described multiple linear regression approach can be easily implemented for measuring evoked potentials in other sensory modalities.

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1. Introduction

Brief radiant heat pulses, generated by laser stimulators, selectively excite thermal nociceptors in superficial skin layers and activate Aδ and C nociceptors (Bromm and Treede, 1983). The cortical brain responses evoked by standard laser stimuli (laser-evoked potentials, LEPs) are related to the activation of Aδ mechano-heat nociceptors, small-myelinated primary afferents, and spinothalamic tract neurons (Bromm and Treede, 1991; Treede et al., 1995). The largest LEP signal is a vertex negative–positive complex (N2–P2) probably generated by the bilateral operculoinsular areas and contralateral SI (N2 component), and by the cingulate gyrus (P2 component) (Bromm and Chen, 1995; Iannetti et al., 2003; Tarkka and Treede, 1993; Valeriani et al., 1996). These Aδ-related LEPs, investigated in physiological and clinical studies in patients with peripheral or central lesions (Bromm and Treede, 1991; Iannetti et al., 2001), are now considered the best tool for assessing function of central nociceptive pathways (Cruccu et al., 2004).

A positive relationship between the intensity of laser stimuli, the magnitude of perceived pain and the amplitude of the main N2–P2 scalp response has been confirmed by

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many different groups (Arendt-Nielsen, 1994; Beydoun et al., 1993; Bromm and Treede, 1991; Iannetti et al., 2005a). The latencies of the N2 and P2 components have also been found to vary with both stimulus intensity and stimulus duration (Iannetti et al., 2004; Ohara et al., 2004a). Furthermore, cognitive factors such as attention to the stimulus are known to influence both pain perception and amplitude and latency of LEP components (Legrain et al., 2002; Lorenz and Garcia-Larrea, 2003; Ohara et al., 2004b). Therefore, there is a potentially rich source of information available from analysis of EEG recordings of brain responses to laser stimulation on a single-trial basis.

Commonly, the EEG signal is assumed to be a linear combination of stimulus-evoked activity (both phase-locked and non-phase-locked to the stimulus onset), ongoing background activity, and noise. The standard analysis technique for extracting stimulus-evoked activity is to average the temporal signal across multiple trials (Dawson, 1951, 1954). Averaging the signal across n single-trials improves the signal-to-noise ratio by $\sqrt{n}$; this assumes independence between the signal and noise waveforms, a stationary signal waveform and random background noise. By averaging across a sufficiently large number of trials the responses phase-locked to the stimulus are disclosed.

However, standard averaging makes it impossible to detect physiologically relevant information non-phase-locked to the laser stimulus (Mouraux et al., 2003). In addition, it ignores the fact that LEPs are non-stationary and indeed trials may vary widely in both amplitude and latency (Iannetti et al., 2005a). In general, long-latency EPs (e.g. nociceptive-related responses, because of the slow conduction velocity of primary sensory neurons, or cognitive-related responses) commonly exhibit marked latency jitter and inconsistency of waveform (Iannetti et al., 2005a; Mocks et al., 1987; Turetsky et al., 1989). Consequently, standard averaging can result in a considerable decrease in amplitude, distortion of the waveform and inaccurate peak latency estimation due to signals being out of phase from trial to trial.

Amplitude and latency variability between trials may reflect important factors such as changes in subject performance or in subject state, possibly linked to fluctuations in expectation, attention, or task strategy (Haig et al., 1995). Therefore, alternative conceptualizations through better signal processing techniques are required to disclose the maximum biological information from single-trial responses.

Several methods for analysis of single-trial evoked potentials have been reported. A modified approach to conventional EP analysis was suggested by Woody (1967). It uses an iterative correlation procedure whereby the position of maximum cross-correlation between a template waveform and single trial data is used to estimate single trial latencies and perform a corrected standard average. Extensions of this work have included amplitude variability and placing estimation of single-trial parameters in a maximum likelihood framework (Jaskowski and Verleger, 1999; Lange et al., 1997; McGillam et al., 1985).

Purves and Boyd (1993) introduced time-shifted averaging of LEPs, whereby trials were manually measured and then corrected for latency jitter, allowing an average to be computed avoiding phase cancellation. This method was found to produce larger amplitude and more consistent waveforms than conventional averaging and the Woody filter methods.

Truccolo et al. (2003) used a Bayesian inference framework for estimating parameters of single-trial EPs. In this approach, single trials are modelled as linear combinations of ongoing activity and multicomponent waveforms phase-locked to the stimulus. Other approaches employed to analyse single-trial EEG responses include: time-frequency analysis using the wavelet transform, which has been used for both signal denoising (Bartnik et al., 1992; Quian Quiroga et al., 2001) and detection of laser (Mouraux and Plaghki, 2004) and auditory EPs (Bradley and Wilson, 2004; Demiralp et al., 1999); independent component analysis (ICA) (Bell and Sejnowski, 1995; Jung et al., 2001); and singular value decomposition (Wang et al., 2000).

In order to extract the maximal information from EP data (e.g. to study the effects of stimulus-related or cognitive variables), a simple tool for quantitative estimation of biologically useful EP parameters such as peak amplitudes and latencies of single-trial evoked potentials is desired. This paper describes the development and application of a simple and fast multiple linear regression procedure to obtain a numerical measure of the amplitude and latency of the N2 and P2 components of LEPs present in each single trial. The method does not aim to apply a statistical threshold for the presence or absence of a single-trial evoked response. Instead the output summary parameters are intended for use in further interpretation and analysis of the factors affecting stimulus-related evoked potentials as we illustrate herein. We compare automated measurements of single trial amplitude and latency of the N2 and P2 components to more conventional measurements obtained by standard across-trial averaging and single trial manual measurement.

2. Methods

2.1. Data collection and pre-processing

Seven healthy volunteers (five males and two females) participated in this study, mean age (±standard deviation) was 30.1±4.7 years. All participants gave their informed consent, and the local ethics committee approved the procedures.

Painful laser heat stimuli were delivered to skin of the dorsum of the left hand by an infrared neodymium yttrium aluminium perovskite (Nd:YAP) laser (Electronic Engineering, Florence, Italy; www.elengroup.com) with a

wavelength of 1.34 μm. A He–Ne guide laser pointed to the area to be stimulated. The diameter of the laser beam was set at approximately 8 mm (~50 mm^2). Previous experiments have shown that Nd:YAP laser pulses of high intensity (up to 2 J directed at a skin area of approximately 20 mm^2) were optimal to elicit painful pinprick sensation (Aδ input) and readily evoked LEPs after stimulation of different body districts, without inducing skin damage (Cruccu et al., 2003; Iannetti et al., 2004).

Subjects lay supine, wore protective goggles, and were asked to remain awake, relax their muscles and attend to the stimuli. Brain electrical activity was recorded using silver disc electrodes from Fz, Cz, Pz (versus linked earlobes), T3 and T4 (versus Fz) according to the international 10–20 system, at a sampling rate of 256 Hz. The electrode impedance was kept below 5 kΩ.

In order to monitor ocular movements and eye-blinks, and discard contaminated trials, electrooculographic (EOG) signals were simultaneously recorded with surface electrodes, with the active electrode over the mid-lower eyelid and the reference 1 cm lateral to the lateral corner of the orbit.

During the recording session, 4 blocks of 20 laser stimuli were delivered to each subject (80 laser stimuli in total). The 4 blocks were separated by approximately 5 min. Stimuli were delivered at 4 different stimulus intensities (20 stimuli of each intensity), in randomized order. The levels of laser stimulus intensity (range 1–4 J, steps of 0.5 J) were defined for each subject during a psychophysical session prior to the LEP recording, in order to cover the broadest range of perceived intensity on a numerical rating scale ranging from 0 to 10, where 0 was 'no pain' and 10 'worst pain imaginable'. Four seconds after each stimulus subjects were asked to rate verbally the pain intensity of the evoked sensation using the same scale.

In order to test for detection bias in the automated single-trial detection algorithm, 20 min of resting EEG data were collected from one of the subjects in a separate session, using the same recording parameters.

EEG data were imported and pre-processed using EEGLAB, an open-source toolbox running under the MATLAB environment (Delorme and Makeig, 2004). Continuous EEG data from the Cz channel was band-pass filtered between 1 and 30 Hz. EEG epochs containing the laser stimuli were then extracted using a window analysis time of 1500 ms (from 1000 ms pre-stimulus to 500 ms post-stimulus) and baseline corrected using the 1000 ms pre-stimulus data. The 0–500 ms post-stimulus window was considered to contain the signal of interest, and only this time interval was used for further analysis. Trials contaminated by artefacts due to eye-blinks were visually identified and removed from further analysis. Single-subject data where 15% or more of trials were corrupted by eye-blinks were corrected using an independent component analysis (ICA) algorithm (Jung et al., 2001). In all cases where this procedure was performed, individual eye movements could be seen in the IC removed. The IC removed also had a large EOG channel contribution and a frontal scalp map. After pre-processing, a total of 525 single-trials (across all the 7 subjects) remained for the automated measurement of N2 and P2 single-trial amplitudes and latencies using the method presented below. As the N2–P2 complex is maximal at the vertex, only data from the Cz channel were analysed.

2.2. Data analysis

The linear regression model can be written as follows

$$Y = \sum_{i=1}^{4} \boldsymbol{\beta}_i \mathbf{X}_i + \boldsymbol{\varepsilon} \quad (1)$$

where $\mathbf{Y}$ is an $\mathbf{m}\times\mathbf{n}$ matrix of data containing $\mathbf{m}$ rows of 500 ms EEG epochs (single-trial LEPs) recorded from a single subject, with $\mathbf{n}$ samples. $\mathbf{X}_i$, $i\in\{1,2,3,4\}$ are basis functions, length $\mathbf{n}$, used as regressors against the data $\mathbf{Y}$. $\boldsymbol{\beta}_i$, $i\in\{1,2,3,4\}$ are coefficients that describe the respective fit of $\mathbf{X}_i$ to the data $\mathbf{Y}$.

$\boldsymbol{\varepsilon}$ is the residual error in the model fitting. If a particular trial corresponds strongly to regressor $\mathbf{X}_1$ then the model will return a large, positive value of $\boldsymbol{\beta}_1$.

The selection of a suitable basis set to use as regressors in the model was crucial to maximize the ability of the model to detect a specific EP signal present in data without capturing unwanted EEG noise. To minimise this problem of 'overfitting' we decided to adopt a data-driven approach whereby for each subject the standard average was calculated and used to form the regressors for that subject.

N2 and P2 peaks of the Aδ-related LEPs are the results of the activity of different neural generators, the activity of which can be differentially modulated (e.g. Legrain et al., 2002). For this reason, we decided to model the N2 and P2 components separately, thus avoiding the assumption that all generators contributing to the LEP response covary linearly. Thus, for each subject the standard averaged signal was split between the N2 and the P2 peak, at the point where the signal had a voltage value = 0 (Fig. 1A). The standard averaged N2 and P2 components were then convolved with a Gaussian kernel ($\sigma = 4$ ms) in order to smooth the discontinuity arising at the zero crossing point. For each subject, the basis set of 4 regressors was formed from the standard averaged N2 and P2 waveforms and their respective temporal derivatives. A multiple linear regression of these basis vectors against each single trial epoch returns 4 coefficients representative of the amount of laser-evoked response (N2 and P2 components and their temporal derivatives) present in that epoch. The amplitude and latency of the N2 and P2 peaks can be measured for each trial from the fitted regressors. An example of a single-trial LEP recorded from channel Cz from a representative subject is illustrated in Fig. 1B. Manually detected N2 and P2 peaks are marked with vertical bars. The results of the

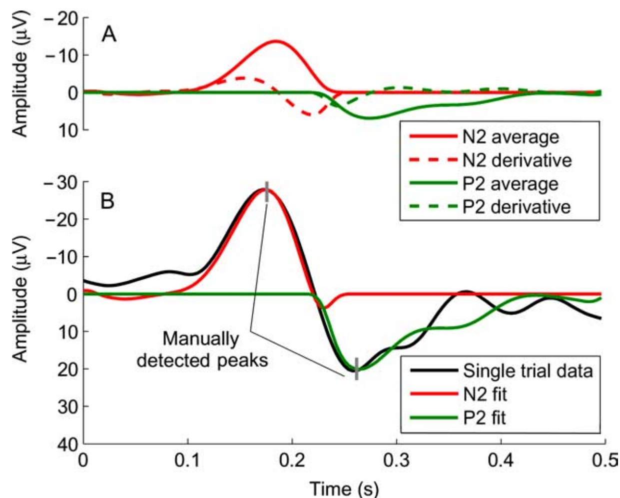


Fig. 1. (A) Example of the 4 regressors calculated from a representative subject for the N2 component (red) and P2 component (green). Solid line: standard average of that component calculated across all trials. Dashed line: temporal derivative of the component standard average. The multiple linear regression of these 4 basis vectors against each single trial epoch was used to model each single-trial LEP. (B) Representative example of a single-trial LEP (black) recorded from vertex electrode Cz (from the same dataset as A). The N2 and P2 peaks detected by a human expert were measured to obtain N2 and P2 amplitude and latency values. The automated fittings for the N2 and the P2 components are shown in red and in green, respectively.

automated fitting for the N2 component (red) and P2 component (green) of that trial are also plotted.

Using a simple basis set obtained from the data has an advantage over other possible alternative choices (e.g. gamma or cosine functions as regressors) for maintaining a strong linear relationship to the LEP data and minimising complexity, which could overfit EEG noise.

This method is analogous to that used to analyze functional magnetic resonance imaging (fMRI) data (Friston el al., 1998) whereby a design matrix consisting of two basis vectors, the haemodynamic response function and its temporal derivative is fitted to the data in a general linear model (GLM) framework. In the case of stimulus-evoked EEG responses, if a single-trial LEP is defined as a function of time $y(t)$ it can be modelled by the sum of the standard averaged N2 component $g(t)$ and P2 component $h(t)$ multiplied by scaling constants κ_1 and κ_2, respectively, giving Eq. (2):

$$y(t) = \kappa_1 g(t) + \kappa_2 h(t) \tag{2}$$

Having a scaling factor in the model for each LEP component in the data allows for their accurate and independent measurement. Because this simple model allows no flexibility to account for variation in the latency of the response, we introduce this flexibility using the Taylor expansion to derive an expression for an LEP component signal shifted in time by a seconds:

$$y(t + a) = y(t) + ay'(t) + \cdots \tag{3}$$

Substituting Eq. (2) into Eq. (3), and disregarding higher order terms, gives:

$$y(t + a) \approx \kappa_1 g(t) + a\kappa_1 g'(t) + \kappa_2 h(t) + a\kappa_2 h'(t) \tag{4}$$

For evoked potentials with latency jitter, incorporating the temporal derivative into the model allows an improved fit to the single trial data. This approach allows for temporal variation in the data and enables accurate measuring of single-trial latencies. Higher order temporal derivatives are not included as they would contribute negligible extra LEP information and only succeed in modelling higher frequency signals in the data, which are largely noise.

Multiple linear regression and statistical analysis were performed using MATLAB 6.5 (Mathworks Inc.). For each single-trial LEP, 4 coefficients reflecting how well each basis function described the data were calculated. In order to obtain biological measurements from the single-trial data, the ‘fit’ of the N2 and P2 component for each trial was calculated by multiplying the design matrix by the regression coefficients, hence for the **m**$^{\text{th}}$ trial of a single subject:

$$\text{N2 component fit}^m = \sum_{i=1}^{2} \beta_i^m X_i^m \tag{5}$$

$$\text{P2 component fit}^m = \sum_{i=3}^{4} \beta_i^m X_i^m \tag{6}$$

Eqs. (5) and (6) give the fitted waveform for each component, for each trial. Single-trial amplitudes for the N2 and P2 components were calculated by finding the maximum voltage peaks (according to the criterion that the stationary point of the N2 signal preceded that of the P2 signal) within a 200 ms time window centred on the latency of the N2 and P2 component in the standard average of each subject. Single-trial latencies of the N2 and P2 component peaks were obtained from the latencies of the corresponding maximum voltage peaks.

The single-trial results obtained with the multiple regression method were compared with more conventional measurements of average and single-trial LEPs. The most common approach is to compute the standard average across all the single trials of each experimental condition for each subject. This results in a waveform that represents the average brain response to that stimulus, from that subject. Summary EP parameters of N2 and P2 amplitude and latency obtained using this approach were manually measured from this waveform. Comparisons between standard averaged LEP measurements and the mean of automated single-trial measurements are presented in the Section 3.

N2 and P2 amplitude and latency values for every single trial were also manually measured by one of the authors (Giandomenico Iannetti) in a blinded manner. The fitted ‘automated’ single-trial values obtained by the method

outlined above were compared to the 'manually' measured single trial values by means of an error defined as

$$E_M = |M_{\text{manual}} - M_{\text{automated}}| \quad (7)$$

where M is either the amplitude or latency measurement of interest for each trial.

Single-trial manual measurements allowed us to assess the accuracy of the automated measurement. Manual measurement has hitherto been treated as a good approach for measuring LEP responses (Iannetti et al., 2005a; Purves and Boyd, 1993).

The multiple linear regression algorithm described in this work has been developed into a graphical user interface to run in the Matlab environment, and can be freely downloaded from www.fmrib.ox.ac.uk/~smayhew.

2.3. Detection bias

Multiple linear regression measurement of single-trial evoked potentials is proposed as an accurate and robust alternative to standard averaging and manual measurement. To demonstrate that obtaining regressors from the data does not introduce any bias into the analysis, the basis set displayed in Fig. 1A was regressed against a set of 1000 randomly-chosen, 500 ms epochs of resting EEG collected from one subject. During the data collection, the subject remained alert with eyes open in a quiet room and was not exposed to any external somatosensory stimulation. The automated algorithm was used to obtain single-trial amplitude measurements for these 1000 trials of resting EEG.

2.4. Cross validation

The ability of the automated algorithm to measure LEP parameters in single-trial epochs is strongly dependent on a linear relationship between single trials and the EP average used to form the regressors. It is well known that EPs show significant differences among the averages of subgroups of single trials (Glaser and Ruchkin, 1976; Shagass, 1972). In order to be of practical use for EP measurement, it is important that the results provided by the automated algorithm remain consistent for a range of different data set samples.

To test the suitability of the standard average and its temporal derivative as regressors for the automated single-trial measurement of each LEP component, a 5-fold cross validation was performed. This was done for each subject by using 4/5th of the single-trial data as a training set, from which the standard average and the temporal derivative were calculated for both N2 and P2 components and used as a basis set for a linear regression analysis of the remaining 1/5th of the trials. This was repeated 4 times (5 folds in total), using a different 4/5th and 1/5th of the data each time as training and test set, respectively, so that amplitude and latency measurements were obtained for each trial. For the N2 and P2 components of each single trial the error was defined as the absolute difference between the component amplitude measured automatically, using the regressors from the training set, and the component amplitude measured manually. The mean error in the combined N2–P2 amplitude was calculated for each fold and then across all the 5 folds for each subject (a schematic outline of this procedure is represented in Fig. 8A). A close correspondence between the error obtained with the 5-fold cross validation and the error obtained with the automated method (i.e. using all the trials to generate the regressors) would suggest that the chosen regressors are robust and appropriate for modelling all of the trials of an individual subject.

3. Results

3.1. Quality and intensity of sensation

Laser stimuli elicited a clear, pinprick sensation in all subjects, consistent with stimulation of Aδ-fibres. The mean pain intensity rating across all subjects increased with increasing stimulus intensity (Fig. 2). Pain ratings for each stimulus intensity were significantly different from the ratings for each of the other 3 intensities ($P<0.0001$, two-tailed paired t test).

3.2. Comparison between automated and standard-average LEP measurements

Figs. 3A and 3B show the correlation across all stimulus intensities between the amplitudes of the N2 and P2 components measured from the standard average of all subjects and those of the means of single-trial N2 and P2

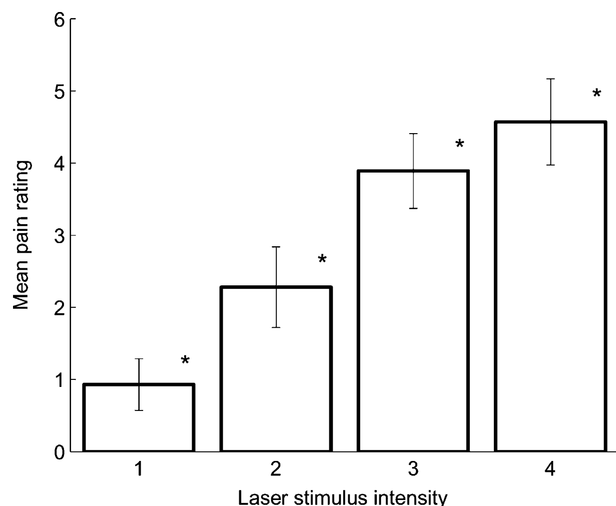


Fig. 2. Mean pain intensity ratings across all subjects for each of the 4 laser stimulus intensities. *Indicates a significant difference between consecutive pairs of ratings ($P<0.0001$, two-tailed paired t test). Error bars represent ± SEM across trials.

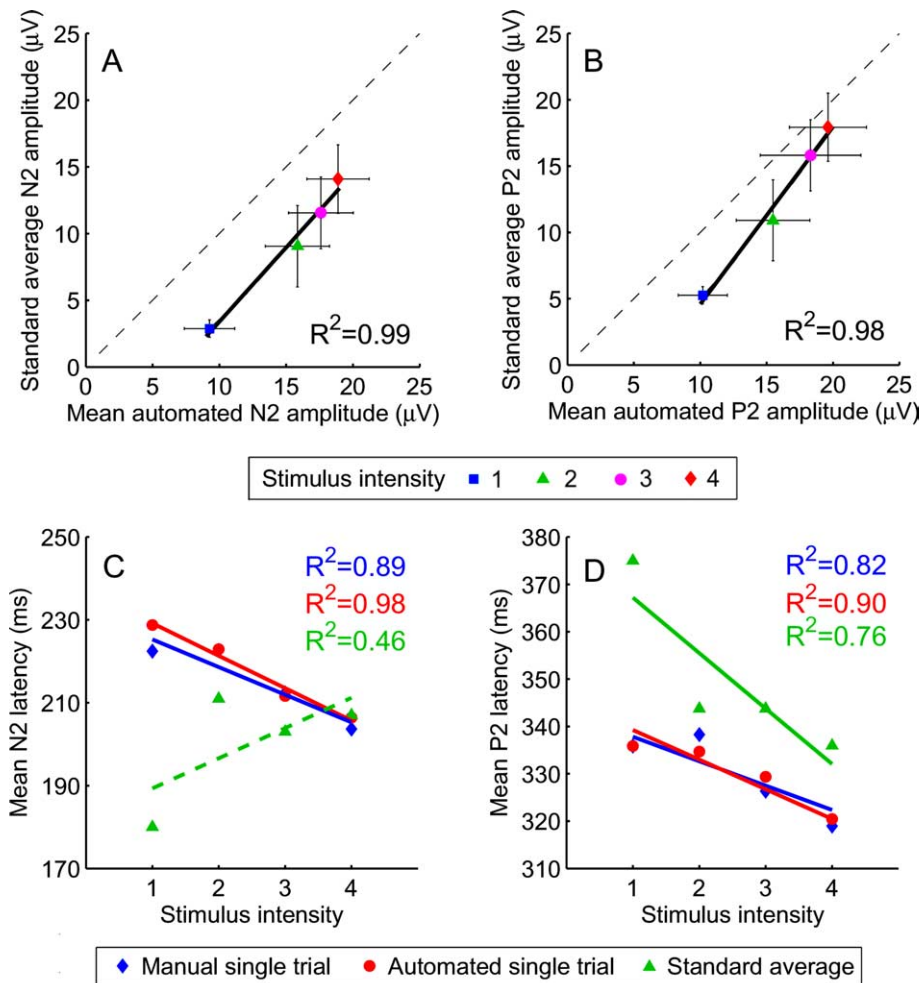


Fig. 3. (A and B), correlation between mean N2 and P2 amplitudes obtained by standard averaging and those obtained by automated single-trial measurement, for the 4 stimulus intensities used. Means were calculated using data from all subjects. Values obtained from the automated method for both components are larger than the corresponding standard average values ($P<0.01$, two-tailed paired *t* test) and show an excellent correlation at all stimulus intensities ($R^2=0.99$ and $R^2=0.98$). Error bars represent ±SEM across subjects. The dashed line is the identity line. (C and D), correlation between mean N2 and P2 latencies and stimulus intensity. Comparison of 3 alternative methods for measuring mean latency of the N2 or P2 component. Green: standard average; blue: manual single-trial measurement; red: automated single-trial measurement. Automated measurements agree closely with manual measurements for both N2 and P2 latency and have excellent correlation with stimulus intensity ($R^2=0.89$ and $R^2=0.82$, respectively). Solid lines represent best fit for significant correlation ($P<0.001$), dashed line represents non-significant best fit.

component amplitudes obtained by multiple linear regression from all subjects. Both the N2 and P2 amplitudes obtained by multiple linear regression showed an excellent correlation to ($R^2=0.99$ and 0.98, respectively) and were significantly larger ($P<0.001$, two-tailed paired *t* test) than the corresponding standard average measurements over all stimulus intensities. Figs. 3C and 3D show a comparison between the mean N2 and P2 latencies obtained from the standard average, by automated single-trial measurement and by manual single-trial measurement, for each stimulus intensity. As introduced in Eq. (7), measurement accuracy was assessed by comparing the estimated value, in this case either automated or standard average, against the corresponding value provide by manual measurement. Using this criterion, it is clear that automated measurement of the latency of both N2 and P2 components is more accurate than standard average measurements of latency. Automated measurements were also capable of detecting the decreasing trend in both N2 and P2 latency with increasing stimulus intensity.

Fig. 4 shows the correlation, for each subject, between automated and manual single-trial methods for measurement of the mean amplitude and mean latency of the N2 and P2 components. Although mean automated N2 and P2 amplitudes are consistently slightly smaller than the mean manual amplitudes, all LEP parameters obtained by multiple linear regression were significantly correlated to the corresponding values obtained manually (N2 latency: $R^2=0.99$, $P<0.0001$; P2 latency: $R^2=0.78$, $P<0.02$; N2 amplitude: $R^2=0.78$, $P<0.02$; P2 amplitude: $R^2=0.98$, $P<0.0001$).

It is of physiological interest to assess how automatically measured N2 and P2 amplitudes vary over a range of stimulus intensities compared to those measured

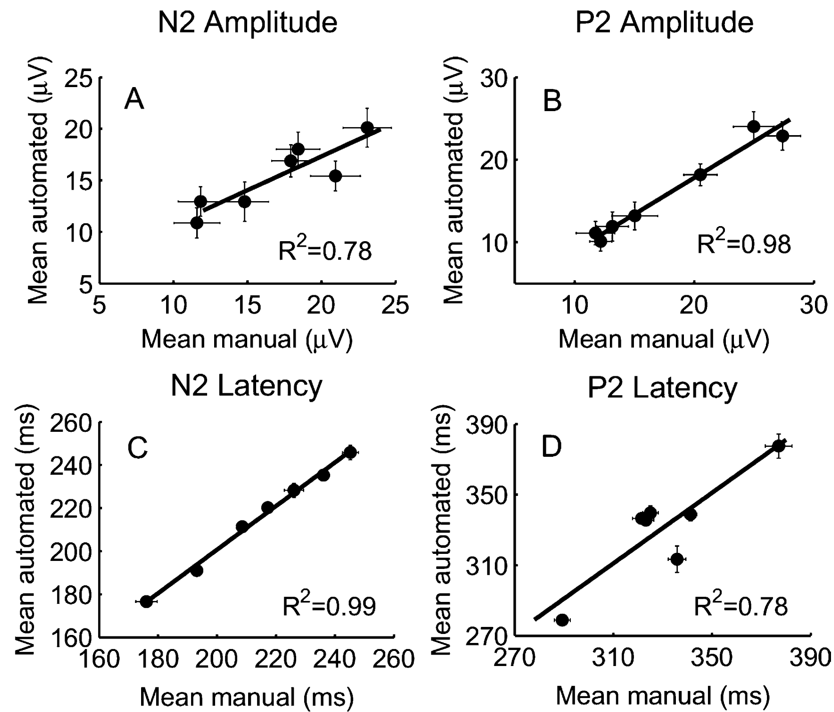


Fig. 4. Significant correlations found between mean single-trial LEP parameters measured using the automated and the manual method (N2 latency: $R^2=0.99$, $P<0.0001$; P2 latency: $R^2=0.78$, $P<0.02$; N2 amplitude: $R^2=0.78$, $P<0.02$; P2 amplitude: $R^2=0.98$, $P<0.0001$). Each point represents one subject. Error bars represent, for each subject, ±SEM across trials.

manually. For this purpose combined N2–P2 amplitudes measured automatically and manually, for each subject and stimulus intensity, are plotted in Fig. 5(A and B). In Fig. 5(A), the lines of best fit for both methods are parallel, indicating that the sensitivity of the automated amplitude measurement to changing stimulus intensity is consistently similar to that from manual measurement over all stimulus intensities. Fig. 5(B) plots the same data points as in Fig. 5(A), but data obtained from the same subject are connected by a line; this illustrates the excellent agreement between the automated measurements (dashed lines) and manual measurements (solid lines) for each subject.

3.3. Comparison between automated and manual single-trial LEP measurements

The correlations between automated and manual measurements for all single-trial LEP parameters were also significant (All $P<0.0001$; N2 latency, $R^2=0.81$; P2 latency, $R^2=0.56$; N2 amplitude, $R^2=0.70$; P2 amplitude, $R^2=0.70$) (Fig. 6). The multiple linear regression method was able to detect changes in LEP amplitude and latency caused by increasing stimulus intensity. Table 1 displays mean values of automated and manual measurements of N2 and P2 amplitudes and latencies for all 4 stimulus intensities.

In order to model the LEP latency jitter, the basis set for multiple linear regression contains the temporal derivative of the N2 and P2 waveforms obtained from the standard average, since it is desirable to have an accurate estimate of LEP values in trials with uncharacteristically long or short latency. Fig. 7 shows a single-trial LEP response and the corresponding automated fit for the N2 and P2 components. Despite the long-latency of this response (N2 = 270 ms; the mean N2 latency of the same subject was 214.9 ms), the automated method provided a good fit to the data.

Across all subjects there were 146 trials where an N2 LEP peak could not be detected manually (i.e. the manual measurement of the N2 amplitude was assumed to be zero) and 137 trials where a P2 LEP peak could not be detected manually (i.e. the manual measurement of the P2 amplitude was zero). The automatically measured single-trial N2 and P2 component amplitudes with manual measurement of zero were labelled as $N2_0$ and $P2_0$, respectively. The distributions of both $N2_0$ and $P2_0$ had means not significantly different from zero ($N2_0$ mean = 2.1 μV, SD = 11.2 μV, $n=146$; $P2_0$ mean = 0.9 μV, standard deviation = 10.5 μV, $n=137$). When the distributions of $N2_0$ and $P2_0$ were classified by stimulus intensity, only the trials collected during the strongest stimulation had a mean that was statistically different from zero ($P<0.04$, $N2_{0(\mathrm{stim}\ 4)}$ mean = 6.8 μV, $n=12$; $P<0.05$, $P2_{0(\mathrm{stim}\ 4)}$ mean = 6.3 μV, $n=11$), indicating that the automated analysis was able to detect biologically-relevant amplitudes, which were lost using the manual measurement.

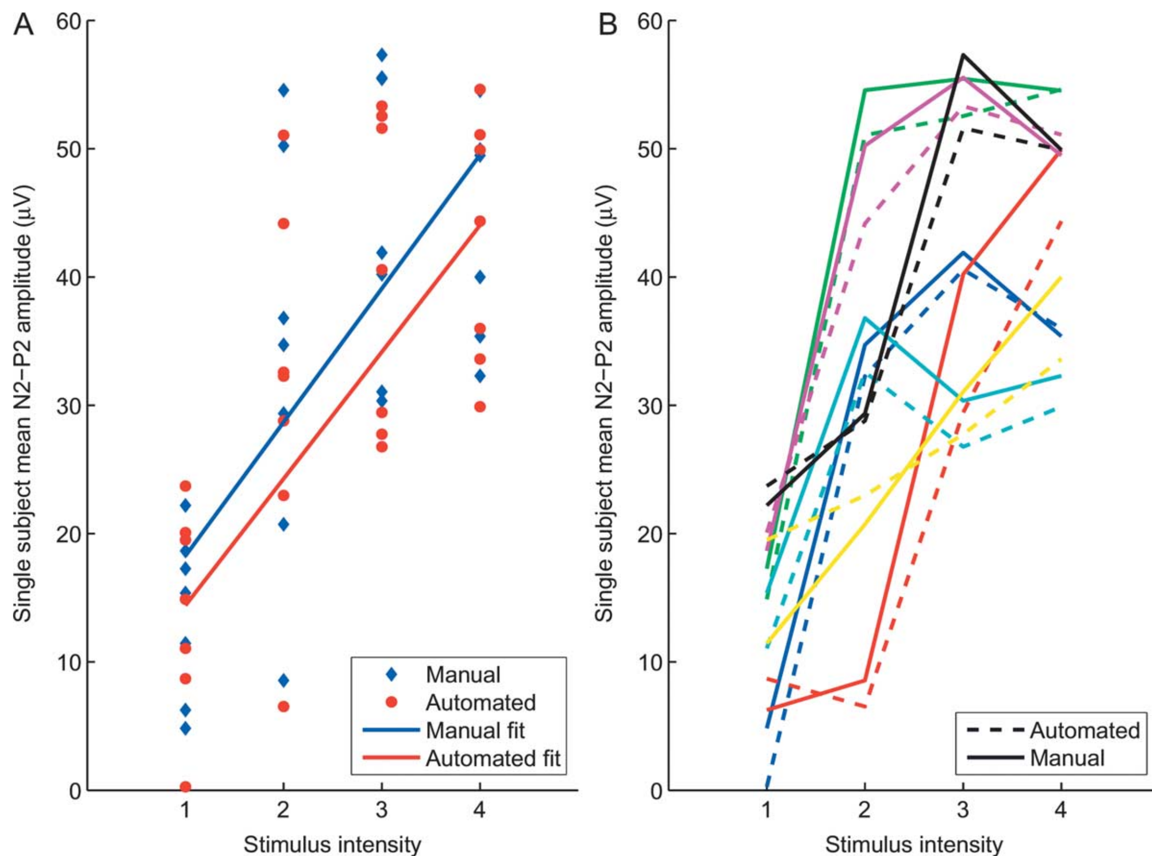


Fig. 5. (A) Comparison between LEP amplitude and stimulus intensity. Mean peak-to-peak N2–P2 amplitude was obtained from manual single-trial measurements (diamonds and blue line) and multiple linear regression (circles and red line). Each symbol represents the mean N2–P2 amplitude of one subject, for each stimulus intensity. Note that the lines of best fit for automated and manual data are parallel, showing a similar and consistent sensitivity to changes over all stimulus intensities. (B) Same data as in (A) but connected with lines to illustrate the close correspondence between automated (dashed) and manual (solid) measurements. Each individual subject is shown with a different coloured line.

3.4. Detection bias

The multiple linear regression performed on the 1000 trials randomly chosen from resting EEG yielded a mean (±SD) amplitude value of 0.45±21.3 μV for the N2 fit, and 0.6±25.1 μV for the P2 fit. Neither of these amplitude measurements was significantly different from zero ($P>0.40$, two-tailed paired *t* test). This result indicates that the automated analysis has a negligible bias towards LEP detection.

3.5. Cross validation

A comparison between the mean error of the 5-fold cross validation and the mean error of the standard automated method for each subject is shown in Fig. 8B. For all subjects, the difference between the automated and cross validation error was less than 1 μV. This was much smaller than the average automated error of 10.5 μV (standard deviation 2.3 μV), thus demonstrating that LEP amplitude measurements are not heavily dependent on the choice of the data used to calculate the regressors.

4. Discussion

We have developed a simple, automated method that, when applied to a dataset of laser-evoked brain responses, provides an independent, quantitative measure of the amplitude and latency of the N2 and P2 components in every single trial. This method is based on the multiple linear regression of a basis set, composed of the waveforms of the N2 and P2 components (obtained from the standard average of each subject) and their temporal derivatives, against each single trial, in order to model the amplitude and latency variability inherent in the LEP response. Single-trial N2 and P2 amplitudes and N2 and P2 latencies obtained by this method correlated well with corresponding values measured manually by a human expert.

4.1. Reliability of the automated LEP measurement

Stimulus-related brain potentials are widely employed in neuroscience as a marker of brain activity (Rugg and Coles, 1995). Potentials evoked by laser stimuli are now considered the best tool to assessing the function of nociceptive

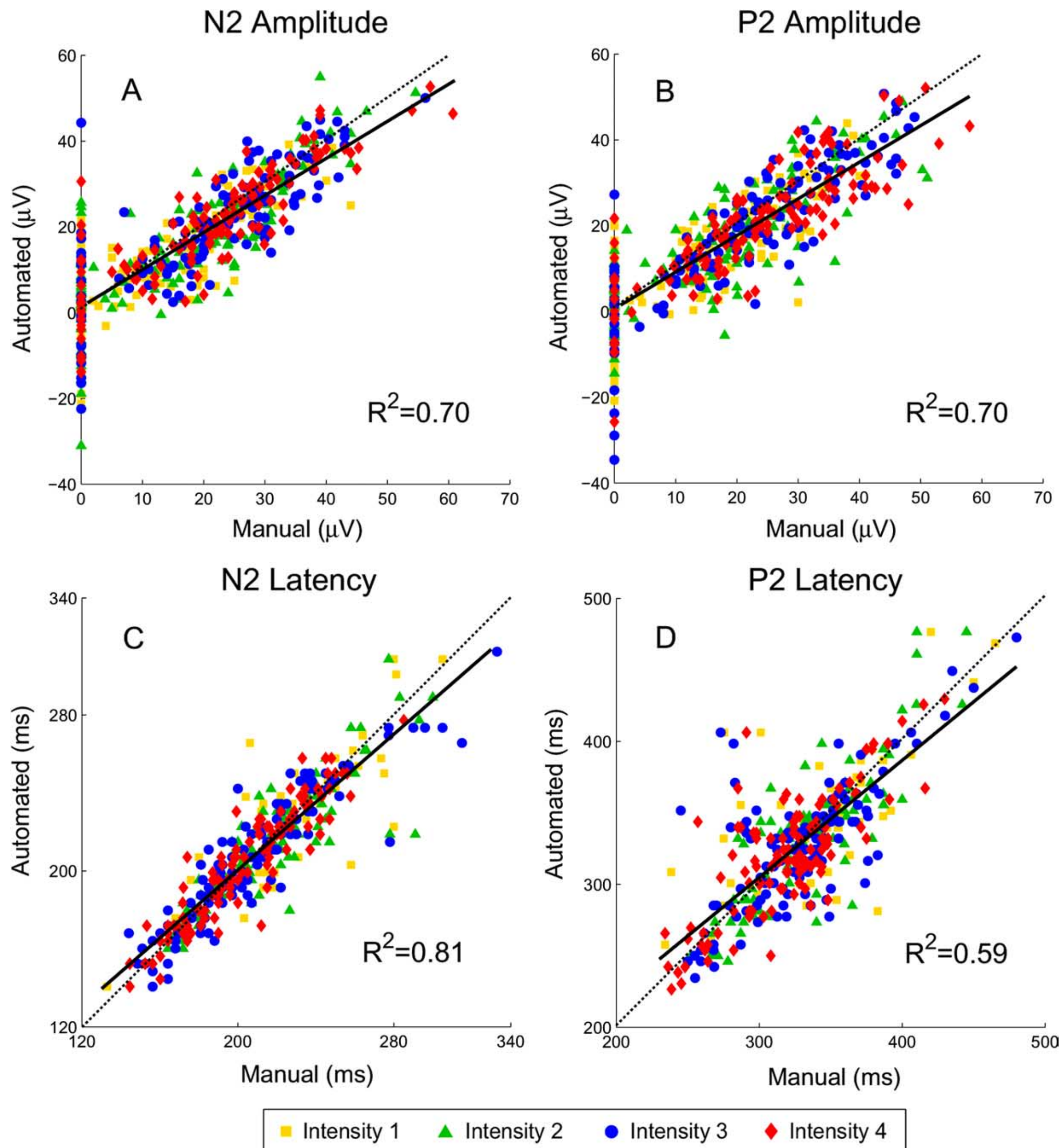


Fig. 6. Significant correlations found between single-trial LEP parameters measured manually and using the automated regression method (all $P<0.0001$; N2 latency, $R^2=0.81$; P2 latency, $R^2=0.56$; N2 amplitude, $R^2=0.70$; P2 amplitude, $R^2=0.70$). Each symbol represents one single trial. Symbols are colour-coded for the corresponding stimulus intensity. The black lines represent the best fit through all data points. Dashed lines represent the identity lines.

pathways (Cruccu et al., 2004). Because the N2 and P2 components of the vertex LEP complex exhibit a wide variability of latency and morphology (Arendt-Nielsen, 1990), standard time-locked averaging techniques may not provide a reliable estimate of its amplitude or latency, and a single-trial approach is highly desirable (Iannetti et al., 2005a; Purves and Boyd, 1993). The development of an automated method for measurement of single-trial evoked potential amplitude and latency was motivated by a desire to improve upon the conventional method of averaging, and provide a simple, fast and unbiased method of obtaining accurate information on single-trial variability. Therefore, the correlation between LEP parameters measured automatically and manually over all data ($n=525$ trials) was necessary to provide confidence in the method. As illustrated in Figs. 4A and 4B, and Figs. 6A and 6B, automated measurement provides excellent estimates of mean and single-trial amplitude of both N2 and P2 components.

Single-trial automated N2 latencies also show excellent correlation with single-trial manual measurements (Fig. 6C; $R^2=0.81$). In contrast, the correlation between automated and manual measurements of P2 latency was poorer than

Table 1
Changes in single-trial LEP parameters with stimulus intensity

Laser intensity	N2 component				P2 component			
	Amplitude (μV)		Latency (ms)		Amplitude (μV)		Latency (ms)	
	Automated	Manual	Automated	Manual	Automated	Manual	Automated	Manual
1	8.9±4.7	9.3±4.4	223±15	223±13	10.2±4.9	11.2±4.6	336±23	336±18
2	15.8±5.6*	16.8±5.3*	223±15*	220±11*	15.5±5.1*	16.8±5.2*	338±22*	335±16*
3	19.7±5.3*	21.2±4.9*	212±16*	209±14*	18.3±6*	21.0±5.3*	326±17*	329±16*
4	20.3±5.1*	21.2±5.2*	205±11*	205±11*	19.6±5.1*	22.8±5.2*	319±16*	320±15*

Single trial parameters were measured either by the automated multiple linear regression approach or manually by a human expert. Error is ±1 standard error in the mean (SEM) across subjects. * Represents a statistically significant difference ($P<0.01$, two-tailed paired t test) from the corresponding parameter at the intensity immediately lower.

that of the other explored LEP values (Fig. 6D; $R^2=0.59$). This finding may be explained by the greater temporal spread and greater inherent variability of the P2 component, as well as by the possible presence of later P3-like positive components (Lorenz and Garcia-Larrea, 2003).

The excellent correlation between the mean automated and standard averaged amplitudes of the N2 and P2 components across all subjects (Figs. 3A and 3B) is expected, since the standard average itself has been used as a regressor on the data. This result also indicates that using the temporal derivatives of the waveforms obtained from the standard average as additional regressors (to improve measurement of latency) does not compromise the accuracy of amplitude measurement. The single-trial LEP component amplitudes are primarily modelled by the standard average regressors (regression coefficient β_1 for N2, β_3 for P2), whilst the inclusion of the temporal derivative regressor (regression coefficient β_2 for N2, β_4 for P2) allows temporal flexibility and improved latency estimation. In single trial LEPs with latencies very different from the latencies measured from the standard average (of the same subject), the coefficients β_2 and β_4 are large, and contribute more to the 'fit' LEP generated using Eqs. (5) and (6) than in trials with latencies similar to those measured from the standard average. We have shown that the waveform of the 'fit' LEP is preserved in a representative trial, which is an outlier with respect to the latency distribution (Fig. 7).

We have observed an increase when the mean N2 and P2 amplitude is calculated from single-trial automated measurements, compared to measuring the component amplitude from the standard average (Fig. 3(A and B)). This amplitude increase is explained by the theoretical advantage of a single-trial approach in measuring the latency of a signal, which is out of phase from trial to trial and is in line with our previous reports (Iannetti et al., 2005a). A single-trial approach avoids the blurring of the signal across trials that results from calculating the standard average.

A positive relationship between the intensity of the laser stimulus and amplitude of the main N2–P2 scalp response has been repeatedly described (Arendt-Nielsen, 1994; Bromm and Treede, 1991). The automated method was found to be capable of detecting differences in the amplitude and latency of LEP components due to differences in stimulus intensity. Stronger laser stimuli elicited LEPs with larger N2 and P2 amplitudes across the whole range of intensities used (Figs. 5 and 6, Table 1). Automated measurements detected a stimulus intensity-dependent decrease in both N2 and P2 latency, which was confirmed by manual measurements (Fig. 3(C and D)). A reduction in N2 latency due to increasing the intensity of the laser stimulus has been already reported (Carmon et al., 1976; Kakigi et al., 1989), indicating that our automated method is capable of revealing this relatively subtle biological effect. In contrast, N2 latency measured from the standard average failed to detect this decreasing trend. Standard average measurements detect the decreasing trend in P2 latency but consistently overestimate the latency values compared to automated and manual measurements. This difference in latency is interesting, and suggests that the distribution of the temporal jitter is asymmetrical. Fig. 3(C and D) shows that latency jitter between trials makes measurements

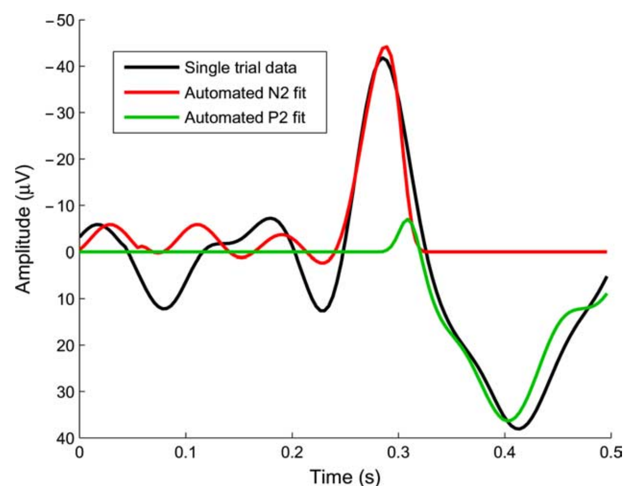
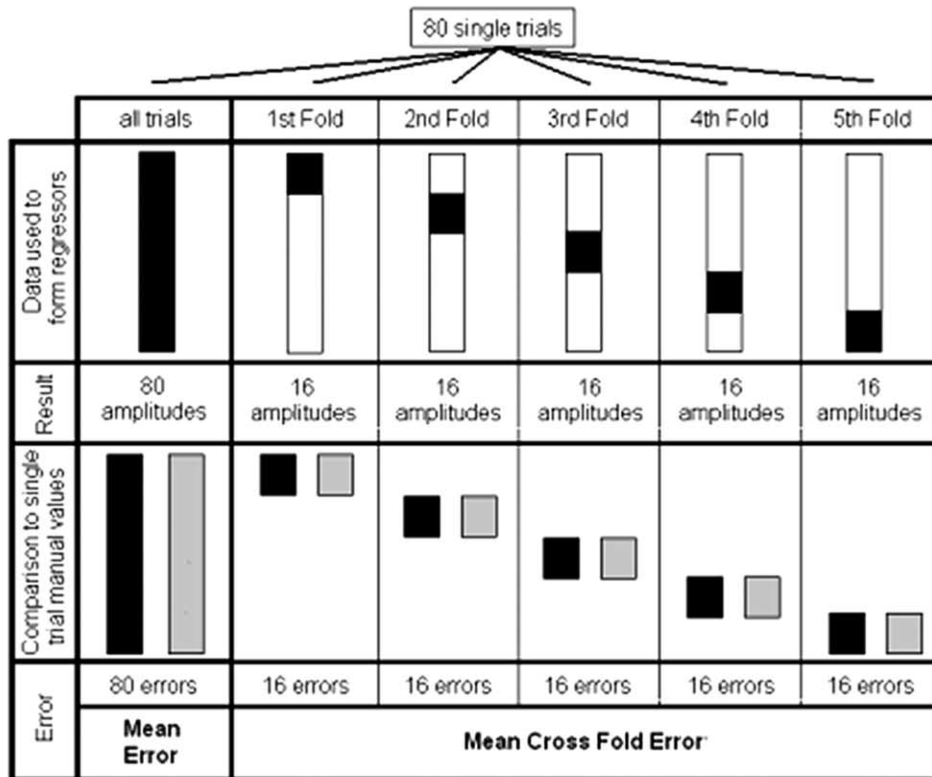


Fig. 7. Automated fit of the N2 (red) and P2 (green) components to one single-trial LEP (black) with long (>270 ms) N2 latency (the average N2 latency of the dataset was 214.9 ms). The good estimation of LEP amplitude and latency indicates the ability of the automated multiple linear regression approach to estimate LEP parameters in trials with significantly different latency from average.



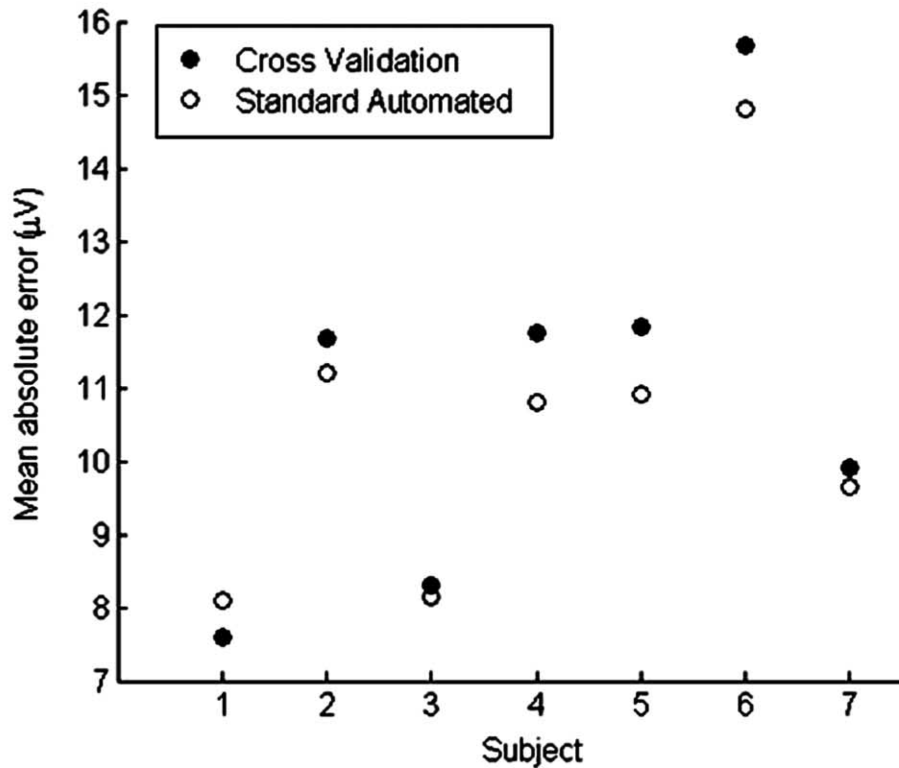


Fig. 8. Upper panel: scheme describing how the mean automated error and the 5-fold cross validation error is calculated for a single subject dataset containing 80 single trials. Left column: 80 automatically measured single trials are represented in black; the corresponding 80 manually measured single trials, used to calculate measurement error, are shown in grey. In the right columns, for each fold, the 4/5th of trials forming the training set are represented in white; the 1/5th of trials forming the test set are represented in black; the corresponding single trials manually measured are shown in grey. Lower panel: comparison between the error obtained with the 5-fold cross validation (closed circles) and the mean error obtained across all 80 trials, for each subject (open circles). Each error is defined as the absolute difference between the single-trial combined N2–P2 amplitude measured manually and by automated regression. The close similarity between the two errors supports the use of standard, across-trial averages as a regressor for automated analysis of single-trial LEPs.

obtained from the standard average a poor estimate of the latency of LEP components.

4.2. Advantages of automated LEP measurement

When a single-trial EP cannot be clearly detected manually, this can be for two reasons. Either no time-locked activity has been evoked by the laser stimulus, or the signal-to-noise ratio is low and the EP is swamped by noise. In both of these cases, human measurement cannot be performed with any certainty and that trial would be labelled by the researcher as 'no signal present'. The question of how to treat these trials that possibly contain small signals, which cannot be detected visually is important, and has yet to be satisfactorily addressed in the literature. This issue applies to both single-trial and standard averaged data, and it is especially relevant in studies examining the consequences of an experimental condition on EP parameters, for example the effect of an opiate agent on LEP amplitude (Beydoun et al., 1997). It is often the case that evoked responses are still visually detectable during administration of low doses of analgesic but when the dose of the drug increases, or low-intensity stimuli are applied, they become undetectable. Single trials (or standard averages) without an apparent response can be treated in two different ways: they can either be discarded from subsequent analyses, or treated as responses with LEP amplitude corresponding to zero. Discarding trials, besides constituting a loss of physiologically-relevant information, introduces the risk of biasing the study towards an underestimate of the experimental effect, by artificially selecting trials with a detectable response and thus increasing the average amplitude measurement. In contrast, assigning zero amplitude to these trials is equally unsatisfactory as it could introduce a bias in the opposite direction, i.e. an experimental effect could be overestimated since the possible contribution of a stimulus-evoked response, although not detectable, is lost. The automated method described in this paper addresses this problem by always assigning an amplitude and latency value to each trial.

Another experimental condition where automated detection of single-trial LEPs is highly desirable is the simultaneous collection of LEPs during fMRI. EEG data collected simultaneously with fMRI are affected by substantial artefacts due to the fMRI environment, and although specific algorithms to minimise these artefacts have been successfully developed, EP responses can still suffer from reduced signal-to-noise ratio, which compromises manual single-trial measurement (Iannetti et al., 2005b; Niazy et al., 2005).

Furthermore, we believe that the automated approach to single-trial measurement would be extremely useful in clinical practice. LEPs are widely used for evaluating the function of nociceptive pathways in patients (Treede et al., 2003). When central or peripheral lesions of these pathways are present, LEP components are delayed or reduced in amplitude. In addition signals recorded from patients usually have higher background noise than signals recorded from healthy volunteers. All these factors contribute in reducing the signal-to-noise ratio of the evoked response, thus suggesting the value of using the automated approach described in this paper.

It was not the purpose of this work to assess the statistical significance of an LEP in each single trial. Our aim was instead to provide a quantitative estimate of the amplitude and latency of the N2 and P2 component for each single-trial LEP, based on the linear relationship between a single trial and the regressors obtained from the standard average plus their temporal derivatives. The assigned amplitude will be small in the case of trials without a visually detectable response and occasionally large (with equal distribution of positive and negative amplitudes) for noisy trials. By allowing the amplitude of these trials to be positive or negative, the effect of noise peaks in the data is treated without bias, and in the resulting average of all fitted trial amplitudes, the contribution of the noise trials would tend to zero, while biological information may be detected. Thus, our automated method was able to extract significant amplitude information from single trials that had been rated 'no signal present' by manual measurement. Additional evidence of the unbiased nature of the method has been provided by automated analysis of a resting EEG dataset. The resting data contained no stimulus-evoked activity. Over 1000 single trials randomly defined from this resting dataset, the mean amplitude value measured by the automated method was negligible. Furthermore, the consistency of the automated measurement across a wide range of data set sizes has been demonstrated by the 5-fold cross validation analysis. This result supports the use of the standard average and its temporal derivative as a robust basis set for automated single-trial analysis. It also offers further evidence that using regressors formed from standard average introduces negligible bias to the measurement.

The automated multiple linear regression approach described in this paper can be theoretically applied to measurement of any kind of stimulus-evoked potentials if the single-trial waveforms appear similar in form to the standard, stimulus-locked average. Intensity-dependent changes in amplitude and latency of EPs have been reported in other sensory systems, such as auditory, visual and somatosensory (Beagley and Knight, 1967; Pratt et al., 1982; Rapin et al., 1966). Vertex potentials in the auditory and somatosensory modalities are similar to the LEP in that they consist of multiple waveform components, which have been shown to be generated by multiple, spatially distinct sources by dipolar modelling (Garcia-Larrea et al., 1995; Giard et al., 1994; Hari et al., 1980; Naatanen and Picton, 1987). An automated method capable of measuring independent waveforms in a single-trial is appropriate for analysis of such multiple component EPs. It has recently been suggested that the amplitude of the N2 and P2 components of the LEP are differentially modulated by

attention (Legrain et al., 2002). Furthermore, decreases in levels of subject vigilance and selective attention have also been reported to affect the amplitude of the EPs in all of these modalities (Bromm and Treede, 1991; Desmedt and Robertson, 1977; Hillyard et al., 1973). Our automated measurement would provide fast and accurate access to single-trial information concerning these modulating experimental factors.

Besides measurement sensitivity and accuracy, the automated method described for obtaining single trial information of evoked potentials has two other important advantages over conventional methods. Firstly, automated measurement offers considerable time saving compared to manual measurement of single trials, especially when dealing with large datasets or with EPs with multiple parameters of interest. Secondly, measuring EP parameters automatically is independent of individual human interpretation. Consequently, whereas the results from a manual analysis of a dataset would vary if performed by different researcher or by the same researcher in different days, the results obtained with an automated analysis are reproducible and comparable across experiments and laboratories.

In conclusion, we have developed a simple and fast automated method for measuring separately the amplitude and latency values of the N2 and P2 components of the vertex complex in single-trial LEPs. Our findings show that the obtained automated measurements are reliable, unbiased, and disclose biological information, which is lost when LEP values are measured manually. For EP data with low signal-to-noise ratio the described method provides a means of avoiding biasing and misinterpretation of results that can occur from a manual analysis.

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