A mixed effects model framework for the assessment of nonlinear interactions in event-related potentials (ERPs) elicited by identical successive stimuli.

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Abstract— **The recording of brain event-related potentials (ERPs) is a widely used technique to investigate the neural basis of sensory perception and cognitive processing in humans. A commonly used assumption, when dealing with potentially overlapping ERPs elicited by successive stimuli with inter-stimulus interval (ISI) smaller than the latency of the ERPs, is that their interaction is linear. These overlaps are usually dealt by using averaged waveforms, mostly to enhance the signal-to-noise ratio (SNR) and performing algebraic waveform subtractions. In this paper, we examine the hypothesis of linear interactions by providing a statistical framework that examines the presence of nonlinear additive effects between overlapping ERPs elicited by successive stimuli with short ISIs. The statistical analysis is designed for single trial rather than averaged waveforms. The results suggest that there are no nonlinear additive effects due to the time overlap per se but that, for the range of ISIs examined, the second ERP is modulated by the presence of the first stimulus irrespective of whether there is time overlap or not. In other words, two ERPs that overlap in time can still be written as an addition of two ERPs, with the second ERP being different to the first. The modulation effect on the second ERP by the first stimulus varies for different ISIs.**

I. INTRODUCTION

Event-related potential (ERPs) consist in transient monophasic deflections in the human electroencephalogram (EEG), elicited by fast-rising sensory, motor or cognitive events [1], [2]. Because of their usually small magnitude compared to background EEG, the identification of ERPs relies on techniques that enhance their signal-to-noise ratio (SNR). Although approaches that allow estimating ERPs in single trials have been recently developed (e.g. [3],[4]), the most widely used approach to enhance their SNR is to average responses across-trials in the time domain [1]. For this reason, in most ERP experiments a large number of stimuli is presented.

There are many occasions where short inter-stimulus intervals (ISIs) are necessary; e.g., in order to obtain a reliable response [1]. This may cause overlap between successive ERPs and consequently distortion in their respective waveforms. In order to account for this, simple ERP subtraction [1] or more elaborate methods such as the adjacent response (Adjar) technique [5] have been proposed.

An implicit assumption in all the aforementioned approaches is that ERP generation is a *time-invariant* and *linear* process. This implies that the total ERP elicited by two (or more) successive stimuli is equal to the linear addition between the ERP waveforms that would have resulted if each stimulus was applied separately, irrespective of whether there is time-overlap or not between the successive ERPs. Whereas it is relatively straightforward to determine whether there exist nonlinear interactions between successive stimuli when there is no time overlap, i.e. when the ERP waveforms are distinct, this is not the case when there is overlap in time. Moreover, one may view the ERP generation process as a system with memory equal to the ERP duration. Therefore, an important question is whether this system behaves nonlinearly when multiple stimuli occuring at ISIs that are shorter than its memory.

In the above context, we present a statistical framework that can be used to examine the presence of nonlinear interactions between successive ERPs when there is possible time overlap, using the single-trial waveforms instead of the commonly used averaged ones [6]. We apply the proposed approach to somatosensory ERPs elicited by nociceptive laser stimulation presented in pairs at different ISIs ranging from 250 to 2000 ms, using EEG measurements. Our approach is a direct consequence of the definition of nonlinearity between successive impulsive-like stimuli. The results present strong evidence against the traditional assumption of linear interactions.

II. EXPERIMENTAL METHODS AND DATA

Eleven healthy volunteers aged from 22-50 years participated in the study. Noxious radiant-heat stimuli were generated by an infrared neodymium yttrium aluminium perovskite (YAP) laser with a wavelength of 1.34 μm. At this short wavelength, the skin is very transparent to the laser radiation and, consequently, the laser pulses activate directly nociceptive terminals in the most superficial skin layers [7]. Laser pulses were directed to the dorsum of the right hand and a He-Ne laser pointed to the area to be stimulated.

EEG data were collected in a single recording session, comprising ten blocks of stimulation. In each block 30 trials were presented, with an inter-trial interval ranging between

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15 and 18 seconds. In each trial, laser pulses were delivered to the dorsum of the right hand either as a single laser stimulus (SINGLE), or as a pair of laser stimuli (S1- S2) presented at an inter-stimulus interval (ISI) of 250, 500, 1000 or 2000 ms. The ISI was randomly varied across trials, and single-stimulus trials were intermixed with paired trials. The EEG was recorded using 30 Ag–AgCl electrodes placed on the scalp according to the International 10-20 system, using the nose as reference. To monitor ocular movements and eye blinks, electro–oculographic (EOG) signals were recorded from two surface electrodes, one placed over the lower eyelid, the other placed 1 cm lateral to the outer corner of the orbit.

Data pre-processing was performed using Letswave [2] and Matlab (The Mathworks Inc, USA). Continuous EEG recordings were segmented into epochs using a time window of 3.5 s (-0.5 to $+3$ s relative to the onset of the first stimulus). The sampling rate used was 1,024 Hz, thus each waveform was comprised of 3584 time points. Each epoch was baseline corrected using the time interval ranging from -0.5 to 0 s as reference, and bandpass filtered. Electroculographic and electrocardiographic artifacts were subtracted using a validated method based on Independent Component Analysis [8].

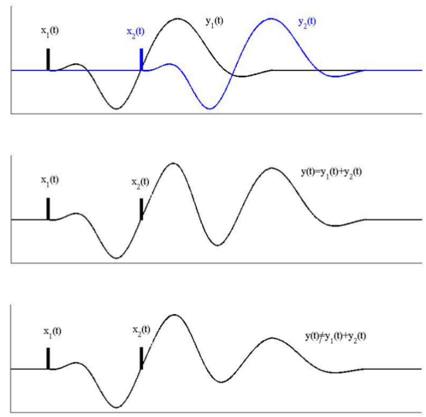

Fig. 1. Schematic representation of linear and nonlinear interactions between two successive impulsive-like stimuli. When applied separately, the two stimuli $x_1(t)$ and $x_2(t)$ elicit the output waveforms $y_1(t)$ and $y_2(t)$, respectively (top panel). When the two stimuli are applied in succession and there is no nonlinear interaction between them, the total output is simply the addition between $y_1(t)$ and $y_2(t)$ (middle panel). On the other hand, when nonlinear interaction between the two stimuli/responses occurs, the total output is not equal to $y_1(t)+y_2(t)$ (bottom panel).

III. METHODS

Broadly speaking, a system is any entity that transforms an input variable into an output variable. A continuous– or discrete–time system is said to be linear if it satisfies the superposition principle, i.e. [9]:

$$S[\alpha_1 x_1(t)+\alpha_2 x_2(t)] = \alpha_1 S[x_1(t)]+\alpha_2 S[x_2(t)], \quad \forall \alpha_1,\alpha_2 \in \Re \quad (1)$$

where S is the system mapping, $x_1(t)$ and $x_2(t)$ are any two input signals to the system and α_1 and α_2 are real constants (Fig. 1). In the present case, the two input signals are identical nociceptive laser stimuli that are separated by the corresponding ISI. Under the assumption of time–invariance and linearity, these stimuli elicit the same ERP waveform, time-shifted by the respective ISI. Thus, for identical impulsive stimuli, (1) can be written as follows:

$$S[x(t)+x(t-ISI)] = S[x(t)]+S[x(t-ISI)], \qquad (2)$$

where we have set $\alpha_1 = \alpha_2 = 1$, in accordance to our experimental design.

A. Statistical Methodology

Our tests are applied on a time point-by-time point basis, where the waveforms are time locked based on the time of a stimulus application. Let $y_{isk}(t)$ be the k^{th} single trial output waveform ($k = 1,\dots,n_{is}$) from the i^{th} individual ($i = 1,\dots,11$) that belongs to the s^{th} ISI category ($s = 0,\dots,4$) at time point $t = 1$. The subscripts for the ISI categories correspond to $s = 0$ (SINGLE), $s = 1$ (250ms), $s = 2$ (500ms), $s = 3$ (1000ms) and $s = 4$ (2000ms) while the number (n_{is}) of single trial ERPs varies for each subject and ISI category. A time locked averaged waveform is denoted by $y_{is\cdot}(t)$:

$$y_{is\cdot}(t) = \frac{1}{n_{is}}\sum_{k=1}^{n_{is}} y_{isk}(t) \qquad (3)$$

For the statistical analysis of the data, we design three testing schemes which are described in the next section. In these schemes, we do not use the ERP waveforms y_{isk} but rather, we form waveforms Y_{ijk} in order to perform appropriate comparisons between the groups $j = 1,\dots,J$ and assess the presence of nonlinear interactions.

Unlike the commonly used and relatively smooth averaged waveforms, the single-trial waveforms are extremely noisy. Thus the error variance of the model one chooses to employ for their analysis should have much larger error variance compared to models concerning the averaged responses. We choose to use Mixed Effects Models (MEM) since they allow the inclusion of multiple variance sources. The MEM framework allow us to incorporate random effects in the model. For our application this translates to decomposing the different variance components. Specifically, in addition to the error variance, we choose to include an additional variance term that reflects the variation between the individuals in our experiment in terms of their ERP waveforms.

The mixed effects model used in our analysis can be written as:

$$Y_{ijk}(t) = \mu(t)+\sum_{l=2}^{J}\beta_l(t)I_{[l=j]}+\zeta_i(t)+\varepsilon_{ijk}(t), \qquad (4)$$

$$\zeta_j(t) \overset{\text{i.i.d}}{\sim} N(0,\sigma_i^2(t))$$

$$\varepsilon_{ijk}(t) \overset{\text{i.i.d}}{\sim} N(0,\sigma_\varepsilon^2(t)).$$

The error term $\varepsilon_{ij}(t)$ is a zero-mean Gaussian noise and it is assumed IID across all subjects and groups. The term $\zeta_j(t)$ represents the between subject variance. This term essentially allows us to assume that each subject has its own error variance thus also allowing us to model discrepancies between subjects. $I_{[\cdot]}$ is an indicator function.

The statistical comparisons for the J groups are based on the coefficients $\beta_2,\ldots,\beta_J$. They are utilized for both the omnibus test (H_0 :All β equal) and for the post-hoc, pairwise, comparisons when the omnibus H_0 is rejected. Since the tests are applied on a time point–by–time point basis a multiple testing correction is applied the resulting p-values. Specifically, we use the Simes method [10], [11].

B. Assessment of Nonlinear Interactions

We design three testing schemes (TS) that aim to assess whether the interactions between ERPs elicited by successive stimuli are linear. This assessment is based on two principles: (i) Time invariance and (ii) linear addition of identical but time shifted ERPs. The three schemes (Table I) we use are designed to test whether these principles hold in our data. Note that TS2 is designed based on the results we obtain from TS1 and TS3 is designed based on the results of the previous two schemes.

TABLE I
TESTING SCHEMES

TS1: ERPs elicited from successive stimuli with no time overlap.
1) The ERPs from the SINGLE data $\{Y_{i1k}(t)=y_{i0k}(t)\}$, 2) The first elicited ERP from the ISI=1000ms data; $\{Y_{i2k}(t)=y_{i3k}(t)\}$, 3) The second elicited ERP from the ISI=1000ms data; $\{Y_{i3k}(t)=y_{i3k}(t+1024)\}$, 4) The first elicited ERP from the ISI=2000ms data; $\{Y_{i4k}(t)=y_{i4k}(t)\}$ and 5) The second elicited ERP from the ISI=2000ms data; $\{Y_{i5k}(t)=y_{i4k}(t+2048)\}$.
TS2: Comparison of ERPs elicited by the second stimulus
1) The ERPs from the ISI=250ms, starting from the application of the second stimulus after subtracting the respective averaged SINGLE waveform from each individual; $\{Y_{i1k}(t)=y_{i1k}(t+256)-y_{i0\cdot}(t+256)\}$, 2) Same as 1, only with data from the ISI=500ms; $\{Y_{i2k}(t)=y_{i2k}(t+512)-y_{i0\cdot}(t+512)\}$, 3) Same as 1, only with data from the ISI=1000ms; $\{Y_{i3k}(t)=y_{i3k}(t+1024)-y_{i0\cdot}(t+1024)\}$, 4) Same as 1, only with data from the ISI=2000ms; $\{Y_{i4k}(t)=y_{i4k}(t+2048)-y_{i0\cdot}(t+2048)\}$,
TS3: Assessment of linear addition by comparing observed and artificially created ERPs
1) The single trials ERPs from the ISI=ISI$_s$ data, where we use the section 0.75s after the application of the second stimulus; $Y_{i1k}(t+\mathrm{ISI}_s)=y_{isk}(t+\mathrm{ISI}_s)$, 2) The artificially created ERPs with ISI=ISI$_s$, using the averaged second stimulus waveform from the ISI=ISI$_{s'}$ data, added to the SINGLE ERPs; $Y_{i2k}(t+\mathrm{ISI}_s)=y_{i0k}(t+\mathrm{ISI}_s)+y_{is'\cdot}(t+\mathrm{ISI}_{s'})-y_{i0\cdot}(t+\mathrm{ISI}_{s'})$.

TS1 examines whether time invariance holds and whether any modulation occurs for successive ERPs with no time overlap that were elicited by identical stimuli. TS2 is formulated under the assumption that the first of the two successive stimuli elicit the same response for all ISIs in the experiment. Its purpose is to examine the hypothesis that the ERPs elicited by the second stimuli are the same, irrespective of the ISI. In TS3 we assume that modulation might have an impact on the ERPs elicited by the second stimuli. This scheme tests whether a waveform elicited by successive identical stimuli can still be written as an algebraic sum of two ERPs, with the second being different than the first, due to modulation. In other words, instead of (2), we have the expression:

$$S[x(t)+x(t-ISI)] = S_{UM}[x(t)] + S_M^{ISI}[x(t-ISI)], \quad (5)$$

where S_{UM} is the non-modulated ERP waveform while S_M^{ISI} is the modulated ERP waveform that depends on the specific ISI.

IV. RESULTS

The omnibus test in TS1 shows that there are significant differences between the five waveform groups. The time intervals that these differences are statistically significant, roughly correspond to the N1 and P2 peaks of the respective averaged ERP waveforms of those groups. Since the common practice is to simply use these modes for comparisons, our results, which take into account the entire bulges are more substantial and thus provide strong evidence that the five ERPs are different. The post-hoc comparisons (Fig. 2) provide a better insight to which of the ERPs from the successive stimuli trials are different than the ERPs from the single stimulus trials. There does not appear to be any difference between the SINGLE waveforms and the ERPs elicited by the first stimuli in either the ISI=1000ms or ISI=2000ms waveforms. However both ERPs that were elicited by the second stimulus appear to be significantly different compared to the ERPs from the SINGLE data, again mostly in the regions around the N1 and P2 peaks. From these results, it becomes apparent that when it comes to the ERPs elicited by the first stimulus, time invariance holds. However, due to modulation from the preceding stimulus, the ERPs elicited by the second stimulus are different from the first ERPs, even when there is no time overlap.

With time invariance for the first ERPs established, TS2 is used to compare the ERPs elicited by the second stimulus. The results from the omnibus test strongly indicate that at least one of the ERPs is different. Therefore modulation depends either on the ISI in general or on whether there is time overlap or not. The post-hoc tests (Fig. 3) that compare the four waveforms in pairs, reveal that the ERPs elicited by the second stimulus for the different ISIs are significantly different between each other. The N1 and P2 modes are always included in the regions that differ. Based on these results we can conclude that modulation depends on the ISI.

The results from TS3 reinforce our findings from the previous tests that compared the ERPs elicited by the second

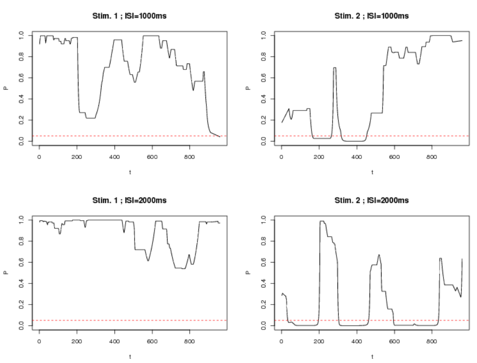

Fig. 2. Results (corrected p-values) from the TS1 post-hoc tests. The comparisons are between the ERP waveforms elicited by the first (Left) and second stimuli (Right) from the ISI=1000ms (Top) and ISI=2000ms (Bottom) trials to the ERP waveforms elicited by the single stimulus trial. Unlike the ERPs elicited by the first stimulus, the ones elicited by the second stimulus yield significant differences when compared to the single stimulus ERPs.

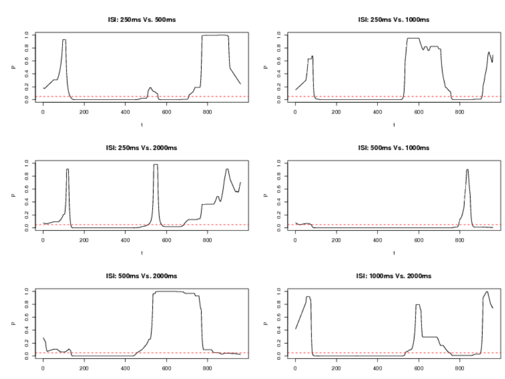

Fig. 3. P-values for the post-hoc pairwise comparisons of the four ERP waveform groups used in TS2. All comparisons yield statistically significant differences.

stimulus. The results (Fig. 4) show clearly that linear addition, as this is defined by equation (5), holds only when the averaged ERP that was used to create the artificial data comes from the data with the same ISI as the observed waveforms in our comparisons. This shows that modulation depends on ISI and (5) holds but the ERP waveform S_M^{ISI} depends on the ISI. Thus even though the interactions between ERPs elicited by successive stimuli are not linear in the traditional sense, the resulting waveform can still be written as a linear algebraic sum of two ERPs.

V. CONCLUSIONS

In this paper we have studied the presence of nonlinear interactions in the ERPs elicited by two successive sensory stimuli at short intervals, i.e. at intervals where overlap between successive ERPs may occur. In our study we use three testing schemes design to assess the assumptions of time invariance and linearity of the interactions between the elicited ERPs. Our results suggest that all ERPs that are elicited by the first stimuli are the same, i.e. these are time

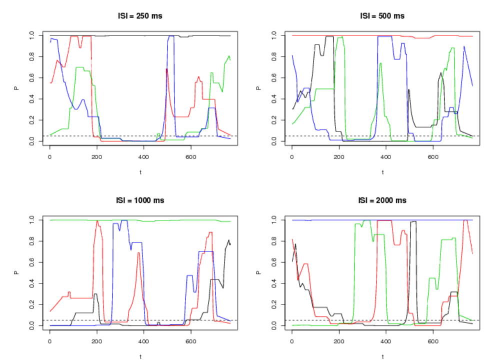

Fig. 4. Test results for the 16 tests in TS3. The colors indicate the dataset from which the averaged second ERP was obtained, in order to create the artificial waveforms. Black: ISI=250ms; Red: ISI=500ms; Green: ISI=1000ms and Blue: ISI=2000ms

invariant. However the ones elicited by the second stimuli are different, which implies a possible modulation effect. Moreover, this modulation depends on the ISI. Additionally, we show that the interactions between ERPs elicited by successive stimuli are linear in a more broader sense than the traditional definition. Specifically, if the effect of the ISI dependent modulation is known, then the interaction can still be considered a linear sum of an unmodulated and a modulated mappings.

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