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Assessment of nonlinear interactions in event-related potentials (ERPs) elicited by stimuli presented at short inter-stimulus intervals

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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.. However, when the temporal interval between two consecutive stimuli used to elicit ERPs is smaller than the latency of the main ERP peaks, i.e., when the stimuli are presented at a fast rate, overlaps between the ERPs elicited by successive stimuli may occur. These overlaps are usually dealt with by assuming that there is no nonlinear interaction between these responses, and thereby by performing algebraic waveform subtractions. Here we propose a straightforward approach to assess the presence of nonlinear interactions in a ERPs. This approach is a direct consequence of the concept of nonlinearity between two successive impulsive-like stimuli. By applying this approach to ERPs elicited by nociceptive cutaneous stimuli delivered at inter-stimulus intervals (ISIs) ranging from 250 ms to 2000 ms, we provide evidence that there are no nonlinear interactions between nociceptive ERPs, even at the shortest 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]. From a neurophysiological perspective, ERPs reflect synchronous changes of slow postsynaptic potentials occurring within a large number of similarly oriented cortical pyramidal neurons [3].

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. [4]-[5]), the most widely used approach to enhance their SNR is to average responses across-trials in the time domain, thus disclosing ERPs that are phase-locked to the stimuli [1]. For this reason, in most ERP experiments a large number of stimuli is presented.

In this case, when the inter-stimulus interval (ISI) is relatively long, i.e., the stimuli are presented at a slow rate (e.g. 0.5 Hz or less), the ERPs of successive stimuli do not overlap in time; therefore, simple across-trial averaging of ERPs does not pose any problems. However, in many cases short ISIs are necessary, e.g., where many stimuli are needed to obtain a reliable response [1] or interactions in the processing of successive stimuli are of interest. As a result, if the ISI is shorter than the latency of the last ERP deflection elicited by the preceding stimulus, overlap between successive ERPs and consequently distortion in the averaged ERPs may occur. In order to account for this, simple ERP subtraction [1] or more elaborate methods such as the adjacent response (Adjar) technique [6] have been proposed.

An implicit assumption in all the aforementioned approaches is that the interactions between two or more successive stimuli that are overlapping in time are linear, i.e., that the succeeding elicited ERP waveforms do not change whether additional stimuli have occurred shortly before them or not. The aim of the present paper is to examine this assumption by using somatosensory ERPs elicited by nociceptive laser stimulation presented in pairs at different ISIs ranging from 250 to 2000 ms. Our approach is straightforward and is a direct consequence of the definition of nonlinearity between successive impulsive-like stimuli. The results suggest that there are no significant interactions between successive stimuli, and that this kind of ERPs summate linearly. The proposed approach can be also extended to other recording modalities in similar studies.

II. METHODS

A. *Experimental methods*

Ten healthy volunteers (seven females and three males) aged from 22-50 years (29 ±8, mean ±SD) participated in the study. All participants gave written informed consent, and the local ethics committee approved the procedures. Noxious radiant-heat stimuli were generated by an infrared neodymium yttrium aluminium perovskite (Nd: YAP) laser with a wavelength of 1.34 μm (Electronical Engineering, Italy). 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. The laser pulse was transmitted via an optic fibre and focussed by lenses to a spot diameter of approximately 8 mm (50 mm^2) at the target site. The duration of the laser pulses was 4 ms and its energy was 3.5 J.

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 (ITI) ranging between 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-

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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. Therefore, the participants were not able to predict if and when a second laser pulse would follow the first stimulus of each trial. Using this presentation paradigm, S1 and S2 elicit ERPs of similar amplitude [8]. After each stimulus, the laser beam target was shifted by approximately 20 mm in a random direction, to avoid nociceptor fatigue and sensitization. The laser beam was controlled by a computer that used two servo-motors (HS-422; Hitec RCD, USA; angular speed, 60°/160 ms) to orient the laser beam along two perpendicular axes [9].

Participants wore protective goggles and acoustic isolation was ensured using earplugs and headphones. Both the laser beam and the controlling motors were completely screened from the view of the participants. The electroencephalogram (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. The electrocardiogram was recorded using two electrodes placed on the dorsal aspect of the left and right forearms. Signals were amplified and digitised using a sampling rate of 1,024 Hz and a precision of 12 bits, giving a resolution of 0.195 $\mu V\ digit^{-1}$ (System Plus; Micromed, Italy).

B. Data pre-processing

Data pre-processing was performed using Letswave (http://amouraux.webnode.com/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). Each epoch was baseline corrected using the time interval ranging from -0.5 to 0 s as reference, and bandpass filtered (1-30 Hz, fast Fourier transform filter). Electroculographic and electrocardiographic artifacts were subtracted using a validated method based on Independent Component Analysis [10]. In all datasets, Independent Components (ICs) related to eye movements had a large electrooculogram channel contribution and a frontal scalp distribution. Finally, epochs in which both laser stimuli were not perceived, epochs with pain ratings 2 standard deviations (SD) above or below the average of the condition, and epochs containing artifacts exceeding ±100 μV were rejected from further analysis. For each subject, epochs were averaged according to trial category (SINGLE, 250, 500, 1000, 2000), yielding five average waveforms.

C. Assessment of nonlinear interactions

Broadly speaking, a system is any entity that transforms an input variable into an output variable. A system can be described mathematically by a corresponding mapping *S*. This mapping may assume a variety of forms, depending on the system properties. A continuous- or discrete-time system is said to be linear if it satisfies the superposition principle, i.e. [11]:

$$S[a_1x_1(t)+a_2x_2(t)]=a_1S[x_1(t)]+a_2S[x_2(t)]\ ,\forall a_1,a_2\in\mathbb{R}$$

where $x_1(t)$ and $x_2(t)$ are any two input signals to the system and a_1 and a_2 are real constants. Any system that does not satisfy the above relation is termed nonlinear.

In the present case, consider the two input signals to be the single nociceptive laser stimuli S1 and S2 that are separated in time by the corresponding ISI and the elicited ERPs to be the corresponding outputs, i.e., $S[x_1(t)]$ and $S[x_2(t)]$ respectively. These two input signals $x_1(t)$ and $x_2(t)$ are identical; therefore, it is reasonable to assume that, when applied separately, they will elicit the same ERP response waveform, time-shifted by the respective ISI (see also Fig. 2 below). In this context, linearity (or equivalently no nonlinear interaction between the two stimuli) implies that the total ERP, when both stimuli are applied simultaneously, will be approximately equal to the linear addition between the ERPs elicited by each stimulus separately. On the other hand, if nonlinear interactions between the two stimuli occur, the total ERP when both stimuli are applied will be different from the linear addition between the single-stimulus ERPs. Schematically, this is shown in Fig. 1. In the hypothetic scenario presented in this figure, the second elicited ERP is smaller in magnitude than the first ERP (inhibitory modulation).

Based on the above, the detection of nonlinear interactions between successive stimuli was performed by comparing the measured ERP waveforms for all possible ISIs to the linear addition between the ERP elicited by each stimulus separately. In turn, this "reference" ERP was defined as the ERP waveform obtained when only one stimulus was applied (SINGLE data). As mentioned above, this implicitly assumes that the ERP waveform to a single stimulus remains approximately constant.

In order to examine this hypothesis, we first compared the following waveforms:

- The elicited ERPs from the SINGLE data
- The first elicited EPR from the ISI=1000 ms and ISI=2000 ms data and
- The second elicited ERP from the same data sets

Note that there is no overlap in time for the ERP responses for these two ISIs; therefore, we could isolate the ERP waveforms of interest. The comparison for both the first and second elicited ERP was done in order to examine whether there is modulation of the second ERP. Note that the ISI was varied randomly in the single-trial data in order to avoid this, as described in Experimental Methods.

After defining the reference ERP, we statistically compared the measured ERP data (which corresponds to the left-hand side term in (1)) with the linear addition between the two reference ERPs (right-hand side terms in (1)), whereby the second reference ERP waveform was shifted accordingly, for all four ISIs. It was hypothesized that the differences would be larger for the two smaller ISIs in case of nonlinear interactions, as interactions cannot occur for the two larger ISIs (no overlap). All the statistical ERP waveform comparisons were performed by analysis of variance (ANOVA) for each time point of the waveforms, with a subsequent consecutivity correction of 50 ms to account for multiple comparisons, as the number of points was around 1,000.

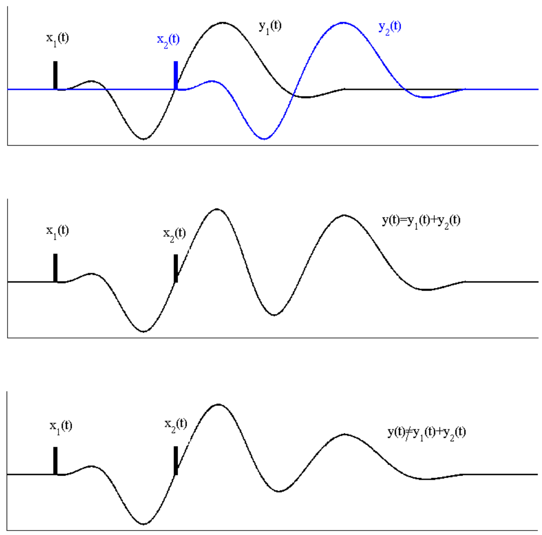

Fig. 1. Schematic representation of linear and nonlinear interactions between two successive impulsive-like stimuli. When applied separately, the two stimuli $x_1(t)$ and $x_2(t)$ elicit the output waveforms $y_1(t)$ and $y_2(t)$, respectively (top panel). When the two stimuli are applied in rapid succession 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). The ERPs elicited by the somatosensory stimuli used in the present report are similar, therefore we can assume that the elicited ERPs are approximately equal, i.e., $y_1(t)=y_2(t)$, as shown above (see also Fig. 2).

III. RESULTS

Fig. 2 shows the reference ERPs recorded at the vertex (electrode Cz). Specifically, we show the averaged reference ERPs obtained from the single stimulus data (SINGLE) and the ERPs elicited by the first and second stimuli from the 1000 and 2000 data (top panel), as well as the *p* values obtained from the ANOVA comparison between the five waveforms (bottom panel). The computed *p* values were high for all time lags. Therefore, the waveforms did not exhibit significant differences and, confirming that stimuli presented using this paradigm elicit ERP responses of approximately equal latency and amplitude. As a result, we can use the same reference ERP to compute the linear addition ERP of two consecutive stimuli (Fig. 2).

The comparison between the ERP waveforms obtained by linear addition and the measured ERP waveforms for ISI=250 ms and ISI=500 ms is shown in Figs. 3 and 4 respectively. The results of this comparison show that there were no significant differences between the two waveforms, thus indicating that there were no nonlinear interactions between successive stimuli presented at those ISIs. Note that, in these figures, the ERP responses where nonlinear interactions could be expected are those elicited by the second stimulus (denoted by the second vertical line). Importantly, the *p* values observed in the S2-ERP waveforms are comparable with those of the S1-ERP, which reflect variability of the ERP waveform, as they correspond to the difference between the reference ERP, obtained from the SINGLE data, and the first elicited ERP.

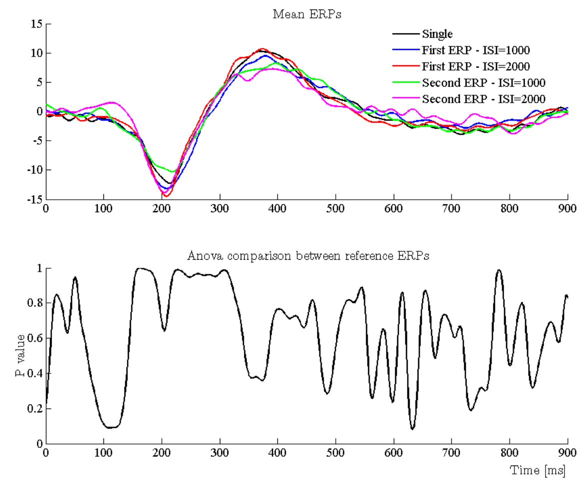

Fig. 2. Top panel: Reference ERP waveforms recorded at the vertex (electrode Cz) in response to somatosensory stimuli presented singularly (SINGLE condition), as well as in response to pairs of somatosensory stimuli (S1 and S2) presented at an ISI of 1000 ms and 2000 ms. At such ISIs overlap between the two responses is not expected. Bottom panel: Statistical comparison (repeated-measures ANOVA) between the five waveforms. No significant differences in waveform amplitude were observed, indicating the similarity between the ERPs elicited by somatosensory stimuli presented in those three conditions.

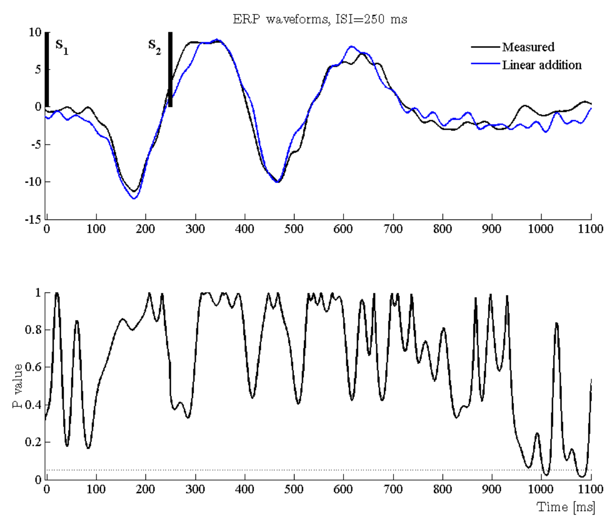

Fig. 3. Top panel: Averaged measured (black waveform) and linear addition (blue waveform) ERPs at ISI=250 ms). Bottom panel: Point-by-point paired statistical comparison between the two waveforms (bottom panel). The *p* values are generally high, suggesting that the waveforms are not significantly different (apart from a late segment around 800 ms after the second stimulus). The vertical lines (S1, S2) denote the onset of the two stimuli.

In order to provide further comparisons, we also show the measured and linear addition ERPs for the two remaining ISIs (1000 and 2000 ms). Since the duration of each ERP is less than 1000 ms, nonlinear interactions are not possible at these ISIs. Note that the *p* values at these longer ISIs (Fig. 5) are comparable to those obtained when comparing S2-ERP recorded at shorter ISIs, providing further evidence that there were no nonlinear interactions at the shortest ISIs (250 ms, 500 ms).

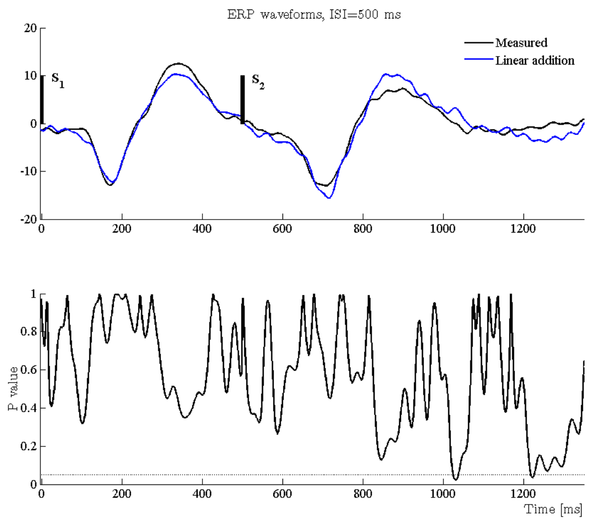

Fig. 4. Averaged measured and linear addition ERPs for ISI=500 ms (top panel) and statistical comparison between the waveforms (bottom panel). The P values are generally high, suggesting that the waveforms are not significantly different (apart from a late segment around 800 ms after the second stimulus as in Fig. 4). The vertical lines denote stimulus occurrence.

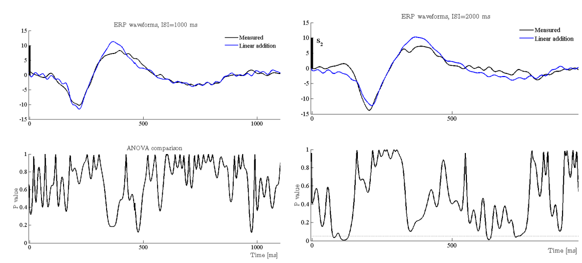

Fig. 5. Comparison between measured and linear addition ERPs for ISI=1000 ms (left panels) and ISI=2000 ms (right panels) – ERP segments after the second stimulus. Note that the P values are comparable with those of Figs. 4 and 4, despite the fact that there is no overlap.

IV. Discussion and Conclusions

We have presented a straightforward method to assess nonlinear interactions in the ERPs elicited by two consecutive sensory stimuli presented at short intervals, i.e. at intervals where overlap between successive ERPs occurs. Our approach is a direct consequence of the definition of nonlinearity and does not require an explicit description of the underlying system, as e.g. in [12]. It also takes advantage of the fact that the impulsive-like stimuli elicit approximately the same ERP waveform.

In a sense, the measured ERP response elicited by these stimuli approximates the "impulse response" of the underlying system, which is defined both for linear and nonlinear systems. However, whereas for linear systems the impulse response constitutes a complete description of the system dynamics, this is not the case for nonlinear systems.

Our results clearly indicate that there are no significant nonlinear interactions between successive stimuli for the somatosensory ERP data considered here. The measured ERPs were not significantly different from the linear addition between accordingly shifted reference ERPs, defined from single stimulus presentation data. This provides a way to identify objectively the reference ERP. The relative invariance of the ERP elicited by a single stimulus was assessed by comparing different reference ERPs obtained both from the single-stimulus trials and from the ISI=1000 ms and 2000 ms paired trials, whereby no overlap occurs. The computed level of significance was comparable between all ISIs, suggesting that there were no nonlinear interactions at the shorter ISIs (250 and 500 ms) - whereas nonlinear interactions are not possible for the two longer ISIs. Note also that these results were found to be consistent at other electrode locations. Since we are using waveforms averaged over single-trials, we should note that we are assessing nonlinearities in the phase-locked ERP components. The study of non phase-locked components using single-trial data and time-frequency methods is currently underway.The proposed approach can be applied to investigate the presence of nonlinear interactions in EEG responses elicited by stimuli belonging to different sensory modalities.

References

[1] S.J. Luck *An introduction to the event-related potential technique,* MIT Press, Cambridge MA, 2005.

[2] A. Mouraux and G.D. Iannetti "Across-trial averaging of event-related EEG responses and beyond," *Magn Reson Imaging* 26, pp. 1041-1054, 2008.

[3] P.L. Nunez and R. Srinivasan *Electric fields of the brain: The neurophysics of EEG*, Oxford University Press, Oxford United Kingdom 2006.

[4] W. Truccolo, K.H. Knuth, A. Shah, S.L. Bressler, C.E. Schroeder and M. Ding "Estimation of single-trial multicomponent ERPs: Differentially variable component analysis (dVCA)," *Biol Cybern* 89, pp. 426-438, 2003.

[5] L. Hu, A. Mouraux, Y. Hu and G.D. Iannetti "A novel approach for enhancing the signal-to-noise ratio and detecting automatically event-related potentials (ERPs) in single trials," *Neuroimage* vol. 50 (1), pp. 99-111, 2010.

[6] M.G. Woldorff "Distortion of ERP averages due to overlap from temporally adjacent ERPs: analysis and correction," *Psychophysiology* 30, pp. 98-119, 1993.

[7] G.D. Iannetti, L. Zambreanu and I. Tracey I "Similar nociceptive afferents mediate psychophysical and electrophysiological responses to heat stimulation of glabrous and hairy skin in humans," *J Physiol* 577, pp. 235-248, 2006.

[8] A. Mouraux, J.M. Guerit and L. Plaghki "Refractoriness cannot explain why C-fiber laser-evoked brain potentials are recorded only if concomitant Adelta-fiber activation is avoided," *Pain* vol. 112, pp. 16-26, 2004.

[9] M.C. Lee, A. Mouraux and G.D. Iannetti "Characterizing the cortical activity through which pain emerges from nociception," *J Neurosci* 29, pp. 7909-7916, 2009.

[10] T. P. Jung, S. Makeig, C. Humphries, T.W. Lee, M.J. McKeown, V. Iragui and T.J. Sejnowski "Removing electroencephalographic artifacts by blind source separation," *Psychophysiology* 37, pp. 163-178, 2000.

[11] A.W. Oppenheim and A.S. Willsky *Signals and Systems,* Prentice Hall International, Upper Saddle River NJ, 1997.

[12] G.D. Mitsis, A.S. French, U. Hoger, S. Courrellis and V.Z, Marmarelis "Principal dynamic mode analysis of action potential firing in a spider mechanoreceptor," *Biol Cybern* 96 (1), pp. 113-127, 2007.