Effects of spatial stimulus overlap in a visual P300-based brain-computer interface

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Highlights

- Stimuli overlap reduces performance, which should be considered in new proposals**.**
- The effect on performance is not directly proportional to the extent of overlap.
- Differences in performance are produced by modulation of the non-target stimuli.

Abstract. The rapid serial visual presentation (RSVP) paradigm seems to be one of the most appropriate for patients using P300-based brain-computer interface applications, since non-ocular movements are required. However, according to previous works, the use of different locations for each stimulus may improve performance. Thus, the aim of the present work is to explore how spatial overlap between stimuli influences performance in controlling a visual P300-based BCI. Nineteen participants were tested using four levels of overlap between two stimuli: 100%, 66.7%, 33.3% and 0%. Significant differences in accuracy were found between the 0% overlapped condition and all the other conditions, and between 33.3% and higher overlap (66.7% and 100%). These results can be explained due to a modulation in the non-target stimulus amplitude signal caused by the overlapping factor. In short, the stimulus overlap provokes a modulation in performance using a P300-based BCI; this should be considered in future BCI proposals in which an optimal surface exploitation is convenient and potential users have only residual ocular movement.

Keywords: brain-computer interface (BCI), P300, visual, stimuli, overlap, spatial.

Introduction

A brain-computer interface (BCI) is a technology that allows control over devices via the user's brain signal. This technology can be especially important for patients affected by certain diseases and/or lesions that affect their motor abilities (Wolpaw et al., 2002). The main applications developed with this technology include the so-called spellers: sets of symbols that can be chosen by the user for the purposes of communication (see (Rezeika et al., 2018) for an extended review of BCI spellers). Since spellers are the most frequently studied application, most of the literature cited in the present work will refer to these. The brain signal most commonly used by spellers is the P300 brain potential. This is a positive deflection in the amplitude of the signal, which is recorded via an electroencephalogram (EEG), and is generally registered in the parietal lobe of the cortex around 300 ms after the presentation of an uncommon target stimulus (Sutton et al., 1965). Thus, the user only needs to keep his/her attention on the target stimulus, evoking a P300 each time the stimulus appears. The system recognizes the P300 potential and selects the symbol related to that potential, and finally executes the command represented by that stimulus (e.g., typing a letter, deleting the last character or autocompleting a word).

The presentation paradigm most often used by researchers is the row-column paradigm (RCP), which was proposed in (Rezeika et al., 2018). In this paradigm, the stimuli are displayed using a matrix of symbols, and are flashed jointly in rows

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and columns (i.e., not individually). The target stimuli (which will produce the P300) are the corresponding flashes of the desired symbol. However, since the symbols are in different locations, this presentation paradigm requires that the users have some eye control; otherwise, the performance is severely affected (e.g., (Brunner et al., 2010; Treder and Blankertz, 2010)).

In addition to the RCP paradigm, there are other alternatives for the presentation of stimuli that do not require ocular mobility, i.e. alternatives that can be controlled under conditions of covert attention. The most commonly used paradigm for covert attention is the rapid serial visual presentation (RSVP) (see (Lees et al., 2018) for an extended review of RSVP). In this paradigm, the stimuli are serially presented, centred at the same position.

According to previous studies, the use of individual locations for each stimulus can support easier discrimination of stimuli, and thus an increase in performance when using a BCI speller (e.g., (Won et al., 2018; Fernández-Rodríguez et al., 2019a)). In (Fernández-Rodríguez et al., 2019a), it was observed that the RCP paradigm achieved better average performance than RSVP. However, in (Won et al., 2018), a pure RSVP paradigm gave worse results than a RSVP-based paradigm in which the stimuli were serially presented but at different locations, all of them slightly separated from the centre. In this second paradigm, the stimuli had an undetermined level of spatial overlap between them. These works may raise the question of how performance will evolve for different percentages of spatial stimulus overlap, rather than simply 100% or 0% as in RSVP or RCP, respectively. The overlap of these spatial stimuli may be a relevant factor in several applications. For example, spatial overlap may be suitable in cases where the stimuli must have a large size within a limited area (e.g., portable devices such as tablets or smartphones), or in order to exploit the residual ocular movements presented in some patients.

The issue of overlap cannot be easily approached from a practical perspective – that is, using a real P300-based speller with an adequate number of symbols – since it is not possible to present an interface in which the level of overlap between flashing stimuli would be constant (i.e., in which each stimulus overlaps the others to the same degree). Therefore, considering the novelty of the present study and to solve the problem of homogeneity of overlap between stimuli, a more theoretical approach has been chosen based on the use of an interface with only two stimuli.

Previous studies have shown the clear superiority of using pictures instead of letters under RCP (Fernández-Rodríguez et al., 2019a), although this improvement was not found under RSVP (Fernández-Rodríguez et al., 2019a). It may therefore be opportune to study how the performance changes due to the distance/overlap when using pictures as stimuli. In addition, the use of pictures may be more appropriate than familiar faces, for example (e.g., (Kaufmann et al., 2011; Li et al., 2015)), since the rectangular shapes of pictures allow for better control of the manipulated variable (i.e. the level of overlap between the stimuli).

Currently, the clinical BCI users described in the literature have residual gaze control (in fact, there are still no visual P300-based BCI publications for patients in a completely locked-in state (Bauer et al., 1979; Guger et al., 2017)). In view of this, it may be convenient to take advantage of these patients' residual capacity in order to establish a communication channel. However, these residual movements are sometimes insufficient to control a large speller matrix with separated stimuli, but may be sufficient to control an interface in which only small eye movements are required since the stimuli are presented with overlap. The previously cited works using pictures were carried out under overt attention (Fernández-Rodríguez et al., 2019a), and there is therefore enough evidence to develop adequate hypotheses. In addition, according to the review in (Rezeika et al., 2018), 84.4% of the selected P300-based BCI spellers were gaze-dependent, meaning that other proposals may benefit from the present work. Given all these reasons, a study of the effect of stimuli overlap may be convenient under conditions of overt attention.

In short, the aim of the present paper is to explore the effect produced under overt attention by different percentage overlaps (100%, 66.7%, 33.3% and 0%) on the *accuracy* and the event-related potential (ERP) waveforms. According to our hypothesis, the *accuracy* is expected to be significantly better for 0% than for 100% overlap; however, the effect produced by intermediate levels of overlap is unknown. Although the distance between stimuli may improve the performance, the amplitude difference between the target and non-target ERP signals may be reduced at some distance or level of overlap between stimuli, thus degrading the performance.

Methods

Participants

The experiment involved 19 participants (aged 22.42 ± 3.79, six male) who had normal or corrected-to-normal vision. Nine participants had previous experience controlling BCI systems and the rest did not. The study was approved by the Ethics Committee of the University of Malaga and met the ethical standards of the Helsinki Declaration. According to self-reports, none of the participants had any history of neurological or psychiatric illness or were taking any medication regularly. Participants received monetary remuneration of €5, and all of them provided written consent.

Data acquisition and signal processing

The EEG was recorded at a sample rate of 250 Hz using electrode positions Fz, Cz, Pz, Oz, P3, P4, PO7 and PO8, according to the 10/20 international system. All channels were referenced to TP8 and grounded to position AFz. Signals were amplified by an acti-CHamp amplifier (Brain Products GmbH, Munich, Germany). A band-pass filter at 0.1–9 Hz was used, and neither online nor offline artefact detection techniques were employed. All aspects of EEG data collection and processing were controlled by the BCI2000 system (Schalk et al., 2004). A stepwise linear discriminant analysis (SWLDA) of the data was performed by BCI2000 to obtain the weights for the P300 classifier and calculate the *accuracy*.

Spelling conditions

The software used to design the interfaces was the UMA-BCI Speller (Velasco-Álvarez et al., 2019), which serves as a user-friendly front-end for BCI2000. The present work involved four conditions based on the spatial overlap between two stimuli. The overlap level in the present work is defined as the percentage of the stimulus surface that is superposed by another stimulus, i.e., the common area that was shared by both stimuli. It is awarded that there was no temporal overlapping, so both stimuli were never presented at the same time. The four conditions used were: i) 100% overlap, ii) 66.7% overlap, iii) 33.3% overlap and iv) 0% overlap (Figure 1). The pictures used had a size of 4.87º × 4.87º (767 px × 767 px, 5.1 cm × 5.1 cm, 60 cm distance). Four paired picture sets were used, giving a total of eight different pictures. The pictures were obtained from the International Affective Picture System (IAPS) (Lang, P. J., Bradley, M. M., & Cuthbert, 2008). Pictures with the aforementioned proportions (which filled all the space and did not use black padding) and the lowest arousal score were chosen. Each picture was associated with a letter, where the letter selected for a specific picture was the first letter of the object shown in the picture in Spanish (e.g., L for *lámpara*). The purpose of this letter was to let the user to know which letter of the paired set of pictures should be chosen in a specific run during the calibration task. Above the pictures, there was a white rectangle indicating the letters to be selected by the user from left to right, being marked in black those already selected and in grey those not yet selected. The background of the interface was black. Due to copyright reasons, the pictures cannot be reproduced here. Therefore, the number corresponding to the IAPS codification will be used in order to know what pictures have been presented. The specific pictures selected according to the IAPS codification were (the corresponding object and letter are presented in parenthesis): i) set A, 7175 (lamp, L) and 7010 (basket, B); ii) set B, 7004 (spoon, C) and 7020 (fan, V); iii) set C, 7031 (shoes, Z) and 7110 (hammer, M); and iv) set D, 7950 (tissues, P) and 7080 (fork, T). The various overlap conditions were displayed on a 15.6-in (39.6 cm) screen at a distance of 60 cm and at a refresh rate of 60 Hz. A stimulus onset asynchrony (SOA) of 608 ms was used, and an inter-stimulus interval (ISI) of 416 ms, so that each stimulus was presented for 192 ms. The purpose of an extended ISI was to prevent a high appearance rate, which could cause a decrease in the P300 amplitude given the use of only two stimuli in the theoretical perspective of the present work (Duncan-Johnson and Donchin, 1977). A 2976 ms pause was used between each selection.

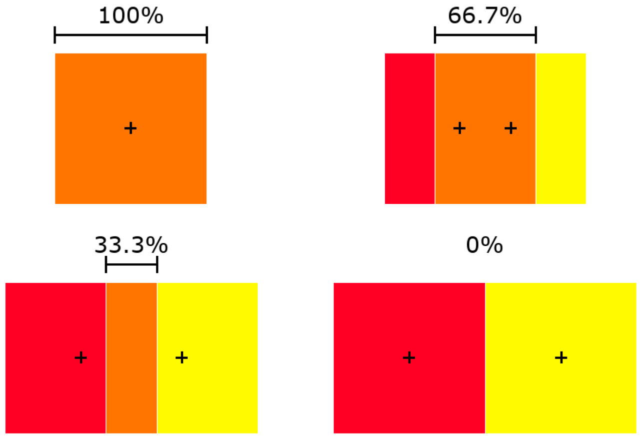

Fig. 1. Representation of the four overlapping conditions used in the present study. The red and yellow colours individually represent the two stimuli, while orange represents the overlapped area between the stimuli.

Although most spellers use more stimuli, only two were used in the present study in order to control the exact level of overlap between one stimulus and another. This control would not have been possible through the use of more than two stimuli. On the one hand, with three stimuli the level of overlap (using circular stimuli) could be controlled, but the location of the overlap region would have varied according to the illuminated stimulus with respect to the target one. On the other hand, with more than three stimuli, neither the overlapped surface nor the overlapped location could be controlled. Thus, only through the use of two stimuli, it can be guaranteed that the size and the location of the overlap remains constant. Likewise, an ISI that was longer than usual was applied to obtain a target stimulation frequency similar to that of a speller with a higher number of elements.

Procedure

The experiment was carried out in an isolated room in which only the participant was present at the time the task was performed, in order to allow the participant to concentrate without external distractions. It consisted of only one exercise, a calibration task to adapt the system to the user, and there was no online writing task in which the user actually controlled the interface. Consequently, the study was performed in only one session. Four overlap conditions were tested: 100%, 66.7%, 33.3% and 0% overlap. An intrasubject (also known as repeated measures) design was used, meaning that all users tested all four conditions. We defined a run as the action of selecting a target stimulus from the two shown in the interface.

During a run, each stimulus was presented five times. The stimulus presentation order was pseudo-random (BCI2000 never presented the same stimulus more than twice in a row) without reposition. The user was asked to mentally count the number of appearances of the target stimulus in order to ensure that his or her attention was on the task. From the first stimulus apparition, the time needed to select a stimulus was 5664 ms (10 stimulus presentations of 192 ms and 9 ISI of 416 ms). A group of 10 runs with alternate target stimuli—for example LBLBLBLB, which was used to select the commands associated with the *lámpara* (L, lamp, in Spanish) and *banasta* (B, basket, in Spanish), respectively—was defined as a block. These runs were carried out continuously, with a 2976 ms pause between runs. A short, user-dependent break was established between blocks.

The presented pictures and the overlap condition changed after every two blocks. Although the paired sets of pictures were always presented in the same order (A, B, C and D), the order of the overlap condition was selected pseudo-randomly for each participant, in order to prevent any unwanted effects such as learning or fatigue, and all conditions were equally distributed.

Evaluation

In order to explore the effect of the independent variable, i.e. the overlap, two dependent variables were analysed: the *accuracy* and the *amplitude* (µV) of the P300 ERP component.

Accuracy. The system classification *accuracy* (i.e. the number of correctly predicted selections divided by the total number of predicted selections) was obtained by applying a 4-fold cross validation method to assess the performance for the different conditions. The analysis consisted of a comparison between the *accuracies* obtained for different overlap conditions (100%, 66.7%, 33.3% and 0%) and sequences (from 1 to 5). The software used to carry out the statistical analysis was JASP (JASP and JASP Team, 2019).

Amplitude of the ERP waveform. In addition, the *amplitude* (µV) of the ERP waveform (from -200 to 800 ms) was studied in order to observe how the different overlap conditions affected the target and non-target stimuli. Two analyses were performed: i) a comparison between the *amplitudes* of the target and non-target stimuli signals for each condition and EEG channel; and ii) a comparison between conditions as a function of stimulus type (i.e. target and non-target stimulus) for each channel. The statistical analyses were carried out using EEGLAB (Delorme and Makeig, 2004).

The Holm correction method was applied to calculate the p value for multiple comparisons in order to avoid a type I error, that is, to avoid the null hypothesis being rejected when it was true. Likewise, Greenhouse-Geisser corrections were used for violation of the sphericity assumption.

It should be noted that the BCI2000's classifier was unable to obtain adequate weights for participant P1 using the 100% overlapped condition, and his/her performance therefore could not be reported.

Results

Performance analysis

A repeated two-way measure ANOVA (4 × 5) of the *overlap* (100%, 66.7%, 33.3% and 0%) and *sequence* (five sequences) factors was carried out. The dependent variable used was the *accuracy* (Figure 2). The analysis showed the main effects of the *overlap* ($F_{(3, 51)} = 35.48$, $p < .001$) and *sequence* ($F_{(2.53, 42.95)} = 35.82$, $p < .001$). Otherwise, the interaction effect between both factors (*overlap* × *sequence*) gave non-significant results ($F_{(5.31, 90.3)} = 2.01$, $p = .081$).

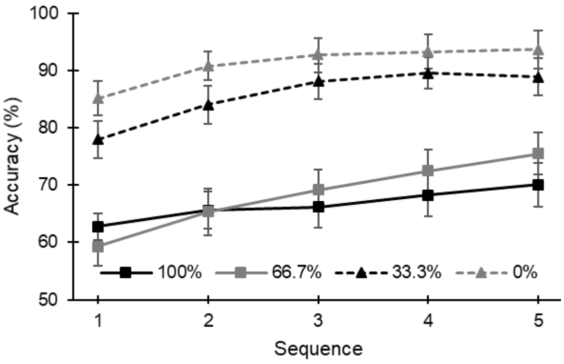

Fig. 2. Average accuracies (%, mean ± standard error) using a 4-fold cross validation method for the different overlap conditions as a function of the number of sequences in the calibration task.

Multiple comparisons of the *overlap* factor indicated the following significant differences: i) between 0% and the rest of the conditions, i.e., 33.3% ($p = .037$, $d = 0.615$), 66.7% ($p < .001$, $d = 1.977$) and 100% ($p < .001$, $d = 1.626$); and ii) between 33.3% and 66.7% ($p < .001$, $d = 1.869$) and 100% ($p < .001$, $d = 1.399$). There were no significant differences between 66.7% and 100% ($p = .525$, $d = 0.153$). On the other hand, multiple comparisons of the *sequence* factor showed significant differences ($p < .05$) between all sequences, with the exception of sequence 4 versus sequence 5 ($p = .163$, $d = -0.344$).

Event-related potential analysis

Figure 3 shows the ERP waveform and the results of the statistical analysis for the target and non-target stimuli for each condition and channel; the significant time intervals ($p < .05$) are presented with a grey background on the time axis of each plot. In most channels, significant differences between stimuli (target versus non-target) were found for conditions 33.3% and 0% overlap: for P300 component in the occipital region (Oz, PO7, PO8) and P4; for N200 in Cz; and for N400 in Fz. Otherwise, the 100% overlap condition only presented significant differences between stimuli in the channel Fz and CZ for the N200 component. No differences between target and non-target stimuli were found for 66.7% overlap condition. In those channels located in the occipital region (Oz, PO7 and PO8) for conditions 33.3% and 0% overlap, significant differences between target and non-target stimuli have been found around 600-800 ms after the apparition of the stimulus. These differences were not significant but also perceptible for the anterior region (Fz and Cz) for most conditions, but especially for 33.3% and 0% overlap. Moreover, channels Fz (condition 0% overlap), Cz (condition 100% overlap) and PO8 (conditions 33.3% and 0%) showed significant differences even before stimulus' apparition. These last results before stimulus onset or around 600-800 ms might be provoked by a refractory effect that will be detailed and discussed in the Discussion section.

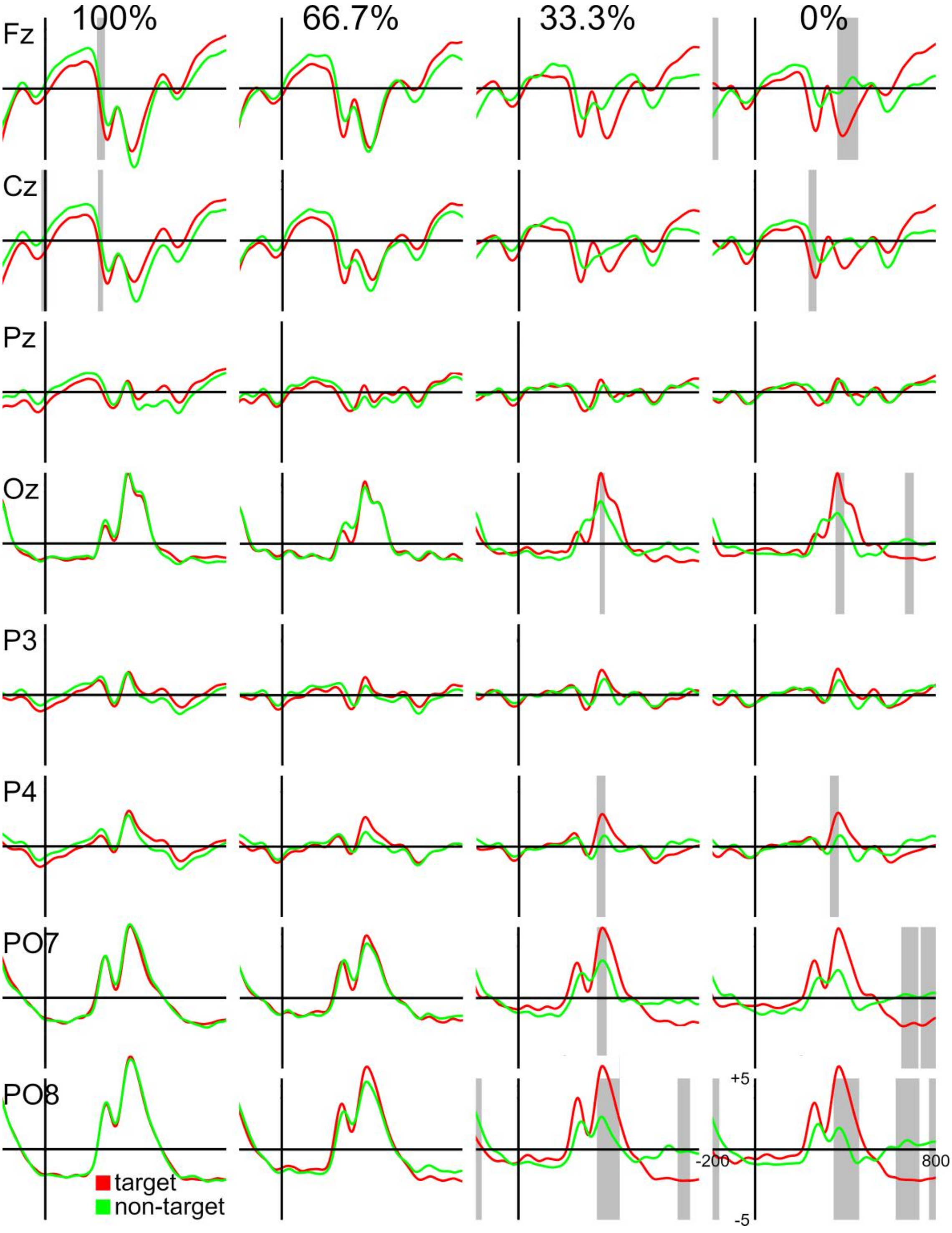

Fig. 3. Comparison of P300 amplitude (µV, 300–500 ms) between target and non-target stimuli for each condition (100%, 66.7%, 33.3% and 0% of overlap) and channel (Fz, Cz, Pz, Oz, P3, P4, PO7 and PO8). Significant intervals are denoted with a grey background on the time axis. The Holm correction method was applied.

Figure 4 presents the previous ERP waveforms, but in this case the statistical comparisons are carried out between conditions for the same stimulus (i.e., target or non-target) for each channel; again, the significant time intervals ($p < .05$) are presented on the time axis of each plot using a grey background. These analyses can therefore show whether the overlap effect can modify the amplitude of target and/or non-target stimuli.

The analyses indicate that the different ERP components of target stimuli are less modulated by the degree of overlap than the ERP components of the non-target stimuli. The non-target stimuli were strongly affected by the level of overlap: for N400 component in the anterior region (Fz and Cz), and for P300 component in the occipital region (Oz, PO7 and PO8) and in P4. The conditions showing the largest N400 and P300 potentials for non-target stimuli were 100% and 66.7% overlap. In addition, other unexpected significant differences have been found in time intervals around the stimulus onset (-200 to 200 ms, especially for target stimuli) and around 600-800 ms after stimulus onset (especially, for non-target stimuli). However, as mentioned before, these differences may be provoked by a refractory effect produced by the influence of the previous stimulus on the EEG signal of the current one. This fact will be explained in the Discussion section.

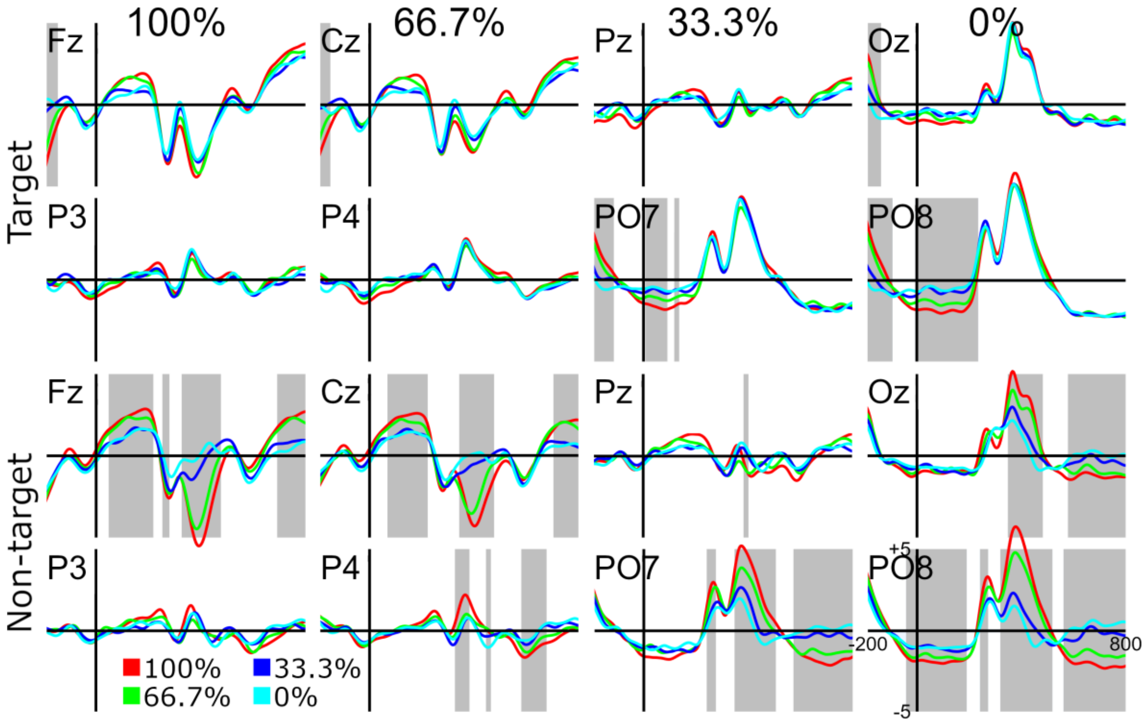

Fig. 4. Comparison of P300 amplitudes (μV, 300-500 ms) between target and non-target stimuli signals for each condition and channel. Significant intervals are denoted with a grey background on the time axis. The Holm correction method was applied.

Discussion

In general terms, the results presented here agree with those obtained in the works previously mentioned in the introduction section (Won et al., 2018; Fernández-Rodríguez et al., 2019a), in which an increasing spatial difference between flashing stimuli improves the performance. However, it should be noted that in (Won et al., 2018), only two RSVP conditions were studied (100% and an undetermined level of overlap, with a variable distance between stimuli), and in work by (Fernández-Rodríguez et al., 2019a), the compared paradigms were RSVP and RCP (100% and 0% with a variable distance between stimuli).

The results for performance show an interesting effect. Despite the differences between conditions being proportional (i.e., the difference in overlap between one condition and the next was equal to 33.3%), three groups were observed according to the obtained *accuracy*: i) the condition with 0% overlap, ii) the condition with 33.3% overlap, and iii) the conditions with 66.7% and 100% overlap. It should also be noted that despite the significant differences between the 0%

and 33.3% overlap conditions, the *accuracy* of the classifier was notably higher for conditions with an overlap level of less than 50%. That is, the changes in *accuracy* show that above a certain level of overlap (between 33.3% and 66.7%) the performance decreases strongly, and it would be advisable for future studies to investigate this threshold. However, the results of this research seem clear: all conditions with overlap (i.e., 33.3%, 66.7% and 100% overlap) gave significantly worse results than the condition without overlap (the 0% overlapping condition). It can therefore be established that whenever possible, and assuming that the user possesses sufficient ocular mobility, the stimuli should be separated. However, when this is not possible (for example, due to limited residual muscular movements, a small area on which to place the stimuli or a need to use larger stimuli), a certain level of overlap may be still adequate to allow a correct selection of commands. In addition, the available surface of mobile devices such as tablets or phones is quite limited, and the size of the stimuli has been shown to modify performance; more specifically, larger stimuli improve the performance (Kellicut-Jones and Sellers, 2018). Future studies could therefore study whether a certain level of overlap—at the cost of increasing the size of the stimuli—could improve performance; that is, to test whether the worsening produced by the overlap can be counteracted by increasing the size of the stimuli due to the surface area gained as a result of the overlap.

The main finding regarding the ERP signal was the modulation effect of overlapping over the N400 and P300 amplitudes for the non-target stimuli signal, while this effect was less relevant for the target stimuli (Figure 4). Additionally, it was observed that the conditions with the furthest flashing stimuli (0% and 33.3%) gave significant differences for P300, N200 or N400 potentials between target and non-target stimuli for most channels, in contrast to the other conditions (100% and 66.7%) (Figure 3). The obtained results therefore highlight that in interfaces where the stimuli are closely placed, it is not sufficient to present stimuli that increase the amplitude of the target stimuli; the key is to increase the differences between the ERP signals associated with the target and non-target stimuli. This effect could explain the results obtained by Fernández-Rodríguez (Fernández-Rodríguez et al., 2019a), where a novel set of flashing stimuli gave no improvement in either *accuracy* or *ITR* under RSVP (100% overlap) but only under RCP (0% overlap), since the amplitude of target and non-target stimuli were equally increased under RSVP.

In addition to the classic P300 component, a modulation of the N200 and N400 components has been shown in the present work. These negative components (N200 and N400) have been previously used by other BCI spellers (e.g., (Jin et al., 2012, 2015, 2017)). In the present work, while a clear P300 component has been shown for the posterior areas, the components N200 and N400 have predominated in the anterior region (Figure 3-4). This effect was previously reported in at least two recent BCI related works using pictures instead of the classical letters (Fernández-Rodríguez et al., 2019b, 2019a): while letters presented a predominance of P300 component around the scalp, the N200 and N400 components were found when the used flashing stimuli were pictures. This dissociation between pictures and letters in a BCI speller would require harder research to be explained. Outside the BCI field, N200 and N400 components has been also found in frontal regions using pictures (e.g., (Kutas et al., 1996; Garrison et al., 2017)).

Furthermore, the present work has obtained unexpected results for the anterior (Fz and Cz) and occipital (Oz, PO7 and PO8) region, in which significant differences have been found around stimulus onset (Figure 3-4). The use of a 50% of probability of (non)target stimulus appearance could provoke an increased refractory effect that was not consider initially. The initial problem about using only two stimuli is that a single stimulus had equal or more than 50% of probability to be followed by other stimulus of the different type (target or non-target) given BCI2000 does not allow to present the same stimulus more than twice in a row. It might cause that – using a SOA equal to 608 ms – the ERP waveform around the, for example, target stimulus onset is highly contaminated by the last ERP component of the non-target stimulus, and vice versa. This issue can be observed in Figure 3 for most channels and conditions, since the signal even before the stimulus onset was not as flat as desired. In addition, in figure 4, for some channels it can be observed how the time interval around 400 to 600 ms matches with the time interval equal to -200 to 0 ms of the opposite stimulus. Next, the possible origin of the refractory effect will be discussed.

On the one hand, in the anterior region, the refractory effect could be provoked by the appearance of a late positive potential (LPP) for non-targets stimuli that produced a positivity at the beginning of the target signal, instead of P100 or P200 components for the target stimulus. The data showed that the refractory effect for target stimulus was significantly higher for the conditions with the highest level of overlap (66.7% and 100%). This fits perfectly with the finding of a higher LPP for non-target in the conditions with the highest overlap, which elicited the refractory effect for the opposite stimuli, i.e., for the targets. The finding of this LPP has been previously reported in a work using the same type of pictures (Fernández-Rodríguez et al., 2019b), however, the hypothesis of the finding of potentials P100-P200 in frontal regions cannot be supported by other works based on a visual BCI. On the other hand, for the refractory effect in the occipital region, we cannot offer a clear explanation. In the occipital region, the amplitude of the target or non-target stimuli for different conditions were stabilized at different levels after the P300, and this stabilization continued for the first 200 ms of the opposite – next – stimulus. For future works it may be recommended to consider not only the use of a SOA with an adequate duration (as well as an adequate target-to-target interval and target-to-non-target interval (Martens et al., 2009)), but also the use of distractor stimuli in addition to the target and non-target stimuli as it was performed by a two commands tactile P300-based BCI with complete locked-in and locked-in patients (Guger et al., 2017).

Although the present study has used a paradigm quite different from the usual ones (mainly due to the use of only two stimuli), the performances obtained by the classifier – using a 4-fold cross validation – in all conditions have been acceptable, reaching over 90% with five sequences under the conditions 0% and 33.3% overlap. We also understand that the use of only two stimuli is a strong practical limitation. However, as justified in the introduction, this allows us to precisely control the different levels of overlap (regarding surface and location), which would not be possible with a larger number of stimuli. For example, in spellers with more stimuli, it would not be possible to associate a constant overlap with each stimulus, in terms of both the overlapped surface and the probability of being overlapped (the outer stimuli would have less overlap by others than the central stimuli). In addition, a binary P300-based BCI application would be convenient for patients with limited ocular movements if an overlap level of 0% or 33.3% is used.

We understand that the use of only two stimuli is a strong practical limitation. However, as justified in the introduction, this allows us to precisely control the different levels of overlap (regarding surface and location), which would not be possible with a larger number of stimuli. For example, in larger spellers, it would not possible to associate a constant overlap with each stimulus, both in terms of the overlapped surface and the probability of being overlapped since the location of the overlap would change (the outer stimuli would have less overlap by others than the central stimuli). In addition, a binary P300-based BCI application would be convenient for patients with limited ocular movements if an overlap level of 0% or 33.3% is used.

The present work represents an initial approach to studying the effect of overlap between visual stimuli in a P300-based BCI, which could be useful for spellers or other BCI applications. This study has shown how performance changes under four conditions with different levels of overlap. Specifically, the condition without overlap offered the best *accuracy*; between conditions with overlap, a level of 33.3% overlap offered a significantly higher *accuracy* than a level of 66.7% or 100%, while no differences were found between 66.7% and 100%. It seems that beyond a certain level of overlap, the performance is not reduced, as could be seen for overlap levels of 66.7% and 100%. Therefore, although the overlap levels were chosen proportionally (i.e., the overlap difference between each level was 33.3%), the influence on performance was not directly did not show that trend.

This work raises many questions that could be answered by future studies. It would be interesting to study this effect under conditions of covert attention, which might give totally different results for those conditions where the stimuli move away from the point of view of the user. Using the theoretical perspective of this study, it would also be interesting to explore the performance under intermediate overlap conditions (that is, between 33.3% and 66.7%) in order to observe the changes in the performance and P300 signal. Within this interval, there should be an inflection point at which the performance varies drastically. This study also establishes a basis for making the leap to testing these results using more practical interfaces, for example with a greater number of available symbols and with the possibility of a free writing task.

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