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ORIGINAL PAPER

Unmasking Word Processing with ERPs: Two Novel Linear Techniques for the Estimation of Temporally Overlapped Waveforms

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Abstract Masked priming experiments are frequently used to study automatic aspects of word processing. Direct measures of such processing obtained with functional neuroimaging techniques (ERPs, fMRI, etc.) need to isolate the neural activation related to relevant events when they are rapidly followed by others (a situation found in other popular paradigms such as the attentional blink and repetition blindness). Here we examine the assumption of "simple insertion", which underlies the use of subtraction to isolate components of temporally overlapping waveforms. We propose two novel linear methods and illustrate how they extract temporal and spatial ERP components that the subtraction method fails to detect. We show this through the analysis of ERP data from a masked semantic priming procedure. The new techniques reveal activation generated by unconscious (masked) prime words as early as 100 ms and 200 ms post stimulus-onset; a pattern which simple subtraction fails to detect.

Keywords Event related potentials · Linear methods · Semantic priming · Consciousness

Introduction

The study of the neural basis of cognition relies to a great extent on the use of techniques that measure brain activation from human participants while they are engaged in cognitive tasks (Event Related Potentials, ERPs, functional magnetic resonance imaging, fMRI, magnetoencephalography, MEG). However, these imaging techniques are rarely able to record directly just the activity that is solely and unambiguously related to the cognitive operation of interest to the investigator. In general, further manipulations are needed to separate the relevant brain activation from the irrelevant; for instance, the simple subtraction of a set of control data from the experimental data. In this paper we examine the assumption of "simple insertion", which underlies the subtraction method, whereby a set of control data, C, are subtracted from the experimental data, E, to leave the brain activity generated by a relevant stimulus S ($S_i = E_i - C_i$, i being the time frame). More specifically, we study the very common situation in which the components (waveforms) of E are composed of temporally overlapping events. We derive alternatives to subtraction that allow us to test whether the key assumption actually holds for the data in question. If it does not, then the methods we propose produce a far superior reconstruction of the embedded data. Before deriving and testing these methods, we further motivate the need for them by giving a concrete example, viz. the problem of separating temporally overlapping ERP waveforms.

The method of ERPs is a central component in the toolkit of contemporary cognitive neuroscience. ERPs are

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derived from the scalp-recorded electroencephalogram (EEG) by averaging together short "epochs" time-locked to objective events, such as the presentation of stimuli constituting an experimental condition. The event-related activity thus derived typically manifests itself as a wave showing several components of different latencies, amplitudes and duration. In cognitive studies of stimulus-evoked potentials, the majority of these components occur in a time range of about 100–800 ms following the stimulus. For example, the visual N1 is the first negative component peaking around 150–160 ms post-stimulus onset that can last for tens of milliseconds. Due to the good temporal resolution of the ERP procedure, such components are fairly easy to identify when a low rate of stimulus presentation is used, e.g., in a priming procedure, when the onset of the prime precedes the target by 800 ms. In this case, the majority of components evoked by processing of the prime can be extracted without interference due to the onset of the following target. However, there are many procedures used in studies of attention and perception that, if used unmodified with ERPs, would impair the extraction of components due to a very high rate of stimulus presentation. For instance, in studies of the attentional blink (Kessler et al. 2005; Shapiro et al. 1994), the stimulus onset asynchrony can be as little as 80–100 ms. In studies using masked stimuli (e.g., in studies of unconscious semantic priming, Marcel 1983; Dehaene et al. 1998, or subliminal motor priming, Eimer and Schlagheken 1998), the stimulus-mask onset asynchrony may be only 16–30 ms. In other studies using rapid serial visual presentation (RSVP), such as repetition blindness, both stimuli and masks may immediately follow each other and last only 80 ms (Niedeggen et al. 2004). Under such circumstances, the brain activity associated with the stimulus of interest is combined with the brain activity evoked by stimuli immediately following in the sequence. The resulting ERP waveform is, therefore, a mixture of components evoked by the stimulus of interest (hereafter, the relevant stimulus) and those evoked by other stimuli in the sequence (hereafter, the irrelevant stimuli). Clearly, in order to study the waveform evoked by the relevant stimulus, it is necessary to separate it from the waveforms evoked by the irrelevant ones. A number of computational procedures have been proposed to perform this separation (briefly reviewed below), but the most common method is to use a subtraction technique, which we now discuss further.

In the subtraction method, additional trials must be run in which everything happens as in the experimental trials, except that the relevant stimulus itself does not occur (the "no-stimulus" condition). Thus, the no-stimulus average ERP will be an estimate of the brain activity when no relevant stimulus is presented; that is, it will contain the baseline activity and possibly some activity evoked by preceding and successive trials if the sequence is sufficiently fast. The no-stimulus ERP is then subtracted from the ERP of interest in an attempt to remove brain activity not linked to the relevant stimulus. The technique makes use of the well-known subtraction method (Donders 1968/1868), commonly used in reaction time studies (Egeth et al. 1972; Valdes et al. 2005), ERP (Brandt 2001), and fMRI research (Kouider et al. 2007; Petersen et al. 1988). In these latter fields, the no-stimulus condition is commonly a control or baseline condition, assumed to involve either no important modulation of brain activity (as in the "resting state" baseline) or the same brain processes as the experimental condition, minus the one we wish to study (the task-related baseline, Newman et al. 2001, for a critique).

The use of the subtraction method assumes the additivity of neural events: the brain activity evoked by the experimental condition is simply the arithmetic sum of the activity evoked by the control condition and that evoked by the process of interest (not implicated by the control condition). In other words, it is assumed that the observed waveform is the linear addition of two independent sources, an assumption also made by the independent component analysis (ICA) approach (Delorme and Makeig 2004). In formal terms, we can write:

$$\mathrm{E_i = S_i + C_i} \tag{1}$$

where $\mathrm{E_i}$ is the brain activity measured in the experimental condition at time i, $\mathrm{C_i}$ is that measured in the control condition, and $\mathrm{S_i}$, the unknown, is the brain activity we are actually interested in. In terms of our example study on unconscious semantic priming (see below), E is the actual ERP waveform produced by the prime followed by the mask, C is the ERP produced by the control, say the mask alone (without the prime) and S is the ERP we wish to extract, that produced by the masked prime (but without the interference caused by the mask). The unknown, S, can be easily derived as the sample-by-sample difference between E and C.

Note, however, the logic behind the subtraction in Eq. 1: neither C modulates S, nor S modulates C (Brandt 2001; Friston et al. 1996; Yao 2003, for critiques). Equation 1 can be seen as a particular case of the more general expression

$$\mathrm{E_i = S_i + b\,C_i} \tag{2}$$

where b measures the mutual influences of S and C (no pure insertion is assumed) and is believed to be constant across the experiment.

Below, we use these basic equations to derive two new methods of estimating the unknown waveform. To provide some relevant context, we briefly describe some existing approaches to this issue (see Talsma and Woldorff 2005, for a review), highlighting the difficulties that arise in applying these techniques to paradigms such as masked priming.

Estimation of Embedded Waveforms

Some of the techniques previously developed to solve the problem of blended ERP waveforms are difficult to apply in paradigms such as RSVP, simultaneous auditory-visual stimulation, or masked priming, since they require changes to the basic design that obviate the purpose of the experiment. To illustrate this, let us consider two of these techniques: (i) inter-stimulus interval jittering (Woldorff 1993) and (ii) control of the stimulus sequence. To make things concrete, we will consider their use in the context of a study on semantic priming with masked (unconscious) primes. Suppose that in our ideal design, on each trial we present the prime stimulus for 28 ms, immediately followed by a 70 ms mask, and that after 450 ms the target is displayed until the subject responds. We are interested in isolating the ERP waveform evoked by the masked prime, without contamination by the response to the mask.

Inter-stimulus interval jittering requires random changes to the interval between successive events in the trial. For example, we can vary the prime-mask onset asynchrony between, for example, 16 and 48 ms. However, it is likely that at longer asynchronies, the prime will be consciously perceived, and the ERP waveforms will be a mixture of seen and unseen objects,

The control of stimulus sequence can be done by fully randomizing and counter-balancing the order of events in the trial sequence. Thus we can randomize event order in the priming design, and display the prime after the mask (a forward masking procedure). However, because backward and forward masking mechanisms are likely to be different (Green et al. 2005; Keysers and Perrett 2002), the effect on the prime ERP would be different in the two cases, and hence the comparison between the two ERP waveforms would not be meaningful.

Two other approaches have been used to extract the contribution of the relevant stimuli to the blended ERP waveform: Post-experimental deconvolution (Woldorff 1993) and the Blind Source Separation (BSS) technique. Post-experimental deconvolution takes advantage of the known inter-stimulus interval distribution, which is convolved with the ERPs. However, it is computationally demanding, difficult to implement (Talsma and Woldorff 2005), and, as with the jittering technique, requires changes in the intervals between consecutive events in a trial. BSS, especially in the guise of independent components analysis (ICA), is an increasingly popular analysis technique, due to its ability to recover the components of the EEG activity (see Choi et al. 2005, for a technical review). For the blended waveform problem considered here, the ICA formulation would be similar to that of Eq. 2 (in matrix terms, we have $\mathbf{E} = \mathrm{w}\mathbf{S}$, where $\mathbf{S}$ is a number of unknown *brain sources*, and the whole system is undetermined). However, the problem we are tackling here is different, because we aim to recover an EEG signal combined with others, not the possible brain sources of each signal.

Below, we propose two solutions for separating two overlapping EEG waveforms when we have some knowledge of one—i.e. the control condition.

Two Novel Methods for Separating Overlapping EEG Waveforms

1. *A method based on regression*. First, we propose a way to use Eq. 2 instead of Eq. 1 to estimate the ERP activity evoked by the relevant stimuli and to avoid the problems related with the pure insertion assumption. As it stands, Eq. 2 is underdetermined, as it contains two unknowns (*b* and S), and just two knowns, C and E. To estimate the unknowns we need to make some simplifying assumptions. The main step is to find a good estimator for *b*, from which we can calculate *S* ($S = E - bC$). Note that Eq. 2 asserts a linear relationship between *C* and *E*, from which it follows that a good estimator for *b* is the regression coefficient, *p*, between C and E (see Appendix A).
2. *Parameter estimation using a smoothness assumption*. Recently, Yao (2003; Qiu et al. 2006) have proposed using a *spatial* smoothness assumption to solve Eq. 2. Hence, S and b are assumed to be approximately constant within a small scalp region. However, this assumption cannot be held in experiments with a small number of electrodes, or when the noise affecting each channel is different. Another way to solve the basic equation is based on the observation that many functions, including ERP waveforms, do not have abrupt changes from sample to sample when the sampling rate is high. Thus, we assume that S is smooth in a small time window, and, therefore, that the sum of squares of the first order differences in S, $\sum_i (S_i - S_i)^2$ ($j = i + 1, i$ being the time frame), is minimum (see Appendix A).

The two new linear methods are applied in a “real-world” experiment in which a semantic priming procedure is used for revealing the level of processing reached by words presented under the conscious threshold. We investigate unconscious semantic processing in two ways: the traditional indirect method and estimating the unknown ERP waveforms evoked by the masked words. In the semantic priming procedure, the processing of the unseen prime word is traditionally measured by comparing the response to targets preceded by semantically related masked primes with that to targets preceded by unrelated masked primes. Several control conditions are commonly included also: Related and unrelated conditions with visible primes as controls of the semantic relationship, and pseudoword targets as a control for the response biases. Therefore, six experimental conditions should be included in a traditional design on unconscious semantic processing: Three with invisible primes (related masked, unrelated masked and pseudowords masked) and another three with visible primes (related unmasked, unrelated unmasked and pseudoword unmasked). Semantic priming of seen words was studied for two further reasons. First, finding conscious semantic priming excludes the interpretation of null unconscious semantic priming as a failure in the manipulation of the prime-target semantic relationship. Second, it is unclear whether similar temporal course and topography can be observed in conscious and unconscious semantic processing, and whether they are partially supported by different brain areas (Ruz et al. 2003).

The two new linear methods we developed here need the ERP waveform of a control condition, since the masked prime ERP is considered to be a blend of the waveforms of two stimuli (the unseen prime word and the prime mask, in our application). The best control condition contains the mask alone, because our goal is to separate the unseen word waveform from that of the mask.

A Direct Measure of the Electrical Brain Activity Evoked by a Masked Prime Word

In this section we provide a "real-world" comparison in ERP research between the estimation methods we developed here and the subtraction method. The reported study involves the processing of masked words in an unconscious semantic processing procedure. In this procedure, a very short duration prime-word is displayed, immediately followed by a mask. After a blank interval, the target is presented. The usual behavioural method of determining whether the masked prime was processed is to look for differences in responses to the following (probe) targets (Henson 2003; Dehaene et al. 1998). However, this method, being indirect, may lack sensitivity leading to underestimates of the degree of processing received by the primes. Consequently, more direct approaches are desirable, which are able to measure responses to the prime itself. Brain activity evoked by the masked word (E in the discussion above) is a blend of activity evoked by the prime-word itself (S) and activity evoked by the mask. To extract S from E it is necessary to display the mask without the prime for a number of trials to produce the C waveform. We can then attempt to derive S using subtraction of C from E, and/or the estimation methods derived above. In the following study we use all three methods and compare the results.

Methods

Participants

The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All participants gave informed consent. Eighteen undergraduates (16 women) at the University of Granada, aged 19–27 years, participated for course credits. Subjects were all native speakers of Spanish, right-handed, and reported normal or corrected-to-normal vision.

Stimuli and Procedure

We selected a list of pairs of associatively related words from the Spanish word data base of Soto et al. (1982). The word pairs were controlled so as not to share low-level orthographic features, e.g., FALDA-VESTIDO (skirt-clothes), to avoid sub-lexical priming. A list of pseudo-words was created by changing one letter of the target words, e.g., the word VESTIDO (clothes) becomes VENTIDO. This procedure ensures the similarity of the low-level features of the target stimuli (e.g., on average, the number of letters in both words and pseudowords was 5.75).

A trial consisted of the following sequence of events: Fixation, Prime Word, Mask (or empty display), and Target Word. First, a centrally positioned plus sign (+) appeared for 500 ms to establish fixation, followed by the prime word for 27 ms. Then followed either a mask (GLPMZÑXQJ) in the masked conditions, or an empty display in the non-masked condition, for 67 ms. After a delay of 497 ms the target, a word or a pseudo-word, was presented for 27 ms, to which subjects made a lexical decision (word or non-word?). The subject's response was followed by a blank interval centred on 2,300 ms with a random jittering of ±700 ms. Prime-Mask onset asynchrony was determined according to the results of a preliminary study, in which we observed that d' (the discrimination index of the signal detection theory) for words vs. pseudo-words was not significantly different from zero below 40 ms. Subjects were not told that there would be prime words, but they were instructed to attend to the fixation sign in order to produce a reliable response. Responses were made with the left and right index fingers on a computer keyboard. One half of the subjects responded with a right key press for words and a left key press for non-words, while for the other half responses were reversed.

Six experimental conditions were defined according to the type of target (Related, Unrelated and Pseudoword targets) and whether a mask was present or not (Masked and Unmasked conditions). In the first condition the target word was preceded by a related masked prime. In the second, the target word was preceded by an unrelated masked prime. In the third, a target pseudoword was preceded by a masked word. In the next three conditions the targets were preceded by unmasked prime words. Targets that were in the related condition for half of the subjects served as unrelated targets for the other half of the subjects. The prime–target relationship was defined following the Soto et al. (1982) word base. Finally, in the control condition, target words were preceded by the mask but with no prime word present (Mask-only), making a total of seven conditions. The last condition was included for the purposes of the ERP analysis, to provide the average "no-stimulus" waveform. The conditions were presented in random order in four blocks containing 15 trials of each one of the seven conditions (105 trials per block for a total of 420 trials). Thus, each one of the 120 prime-target pairs (60 related and 60 unrelated) was displayed two times, one unmasked and the other one immediately followed by the mask. Also, each one of the 60 pseudoword targets was presented following both a masked and an unmasked prime. Another 60 new target words were used in the control condition. Stimuli were displayed in a VGA monitor with a refresh rate of 75 Hz.

ERP Measures

Brain electrical activity was recorded at a sampling rate of 250 Hz from 128 electrodes referred to the vertex lead (Cz) through a geodesic sensor net (EGI Inc.). Impedances in all channels were under 25 kOhms. We rejected trials with incorrect responses, electro-occulogram activity exceeding ±70 μV, voltages exceeding ±100 μV, transients exceeding ±50 μV, and reaction times (RTs) below 150 ms. ERPs were obtained for the remaining trials by averaging according to experimental condition over epochs defined with respect to the target onset (−800 to +1,000 ms). The ERP waveforms were digitally transformed to an average reference (Dien 1998; Marí-Beffa et al. 2007; Murray et al. 2008; Picton et al. 2000), band pass filtered (0.1–30 Hz), and corrected for baseline over a 200 ms window prior to the onset of the prime.

Behavioural Analysis

Semantic priming was analysed by submitting RTs and error rates to targets to two separate 2 (Masking: Unmasked vs. Masked) × 2 (Relatedness: Related vs. Unrelated) factorial within-subjects analyses of variance. Simple effects analyses were used for the detailed analysis of the 2 × 2 interactions, when significant. Repeated measures analysis of variance (related masked, unrelated masked and mask alone) was used for the analysis of the effects of the mask on the processing of the prime words. Post-hoc analysis was done using the Tukey test. The level of significance was fixed at $P < 0.05$ for all the analysis.

ERP Analysis

Although the focus in the current work is on the processing of the masked prime, we include an analysis of the target for the purpose of validation of the experiment. Most ERP analyses were dependent-samples *t*-tests made on a sample-by-sample basis. The significance threshold was set at $P < .025$, with the additional criterion that the threshold was reached for five consecutive samples (20 ms) (Picton et al. 2000) in at least five electrodes (e.g. Murray et al. 2008, for a similar criterion).

To examine the effect of the prime–target semantic relationship on the ERP waveforms, we computed two sets of dependent-samples *t*-tests. In the first set, sample-by-sample amplitude differences for targets preceded by unmasked related targets were compared to those of targets preceded by unmasked unrelated targets (the unmasked semantic priming effect). In the second set, we examined the amplitude differences between targets preceded by related masked and unrelated masked primes (the masked semantic priming effect).

To estimate the ERP waveform evoked by the masked primes we computed first the parameters of the two linear methods, p and d, which are the optimal estimators of b (Eq. 2). Then we derived S ($S = E - bC$), by subtracting the weighted control ERP (bC) from the ERP evoked by the word plus the mask (E). The computation of p and d (see Eqs. A1 and A2) was done electrode-by-electrode using a 100 ms time window (25 time samples), starting at 0 ms post-prime onset. This time window allows us to have the sample size needed to obtain accurate estimates of b (see, Kelley and Maxwell 2003, for a discussion on this topic). Note also that it is near to the sum of the Prime + Mask durations.[1] The estimated waveforms were submitted to single-sample *t*-test in a sample-by-sample way to determine at what time points the estimated amplitudes differed from zero. The brain sources of these waveforms were estimated using the sLORETA software (see Pascual-Marqui 2002).

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[1] It is possible, as noted by an anonymous reviewer, that p and d could change over time with the contribution of different brain sources. Variable p and d values where computed (Eqs. 3 and 4) in a sample-by-sample way using a 25 samples (100 ms) time window. Estimated waveforms for p and d (Eq. 2) were submitted to sample-by-sample single-sample *t*-tests. We have observed that the waveforms estimated from p or d showed differences in the same time windows and the same electrodes as when p and d are considered to be constant. Therefore, we adopted the simpler (constant) solutions to Eqs. 3 and 4.

Results

Behavioural Performance

Means and standard error of RTs and error rates for target responses are presented in Table 1. Both measures were analysed by separate 2 (Masking) × 2 (Relatedness) within-subjects analysis of variance. There were significant main effects of Masking and Relatedness on RTs ($F(1,17) = 8.26$, $P < 0.01$; and $F(1,17) = 24.37$, $P < 0.001$, respectively), but not on error rates. The Masking by Relatedness interaction was significant both for RTs ($F(1,17) = 10.46$, $P < 0.01$) and for errors ($F(1,7) = 6.74$, $P < 0.05$). Single effect analysis indicated that when the primes were not masked subjects performed better in the related than in the unrelated condition, being both faster ($F(1,17) = 26.96$, $P < 0.01$) and making fewer errors ($F(1,17) = 13.09$, $P < 0.01$). This semantic priming effect was observed in 17 out of 18 subjects, with a mean of 32 ms. However, no semantic priming was observed when primes were masked, either on reaction times (related 643 ms, unrelated 646 ms, $F(1,17) < 1$)) or on errors (related 5.6%, unrelated 5.6%, $F(1,17) < 1$). Hence the behavioural data clearly indicate that the presence of the mask interfered with the processing of the prime. However, there were behavioural indications that some processing of primes occurred in the masked conditions, as RTs to targets preceded by masked primes (related, 643 ms, unrelated, 646 ms) were 15 ms slower than those preceded by the mask alone (630 ms) ($F(2,34) = 8.34$, $P < 0.01$). The Tukey post-hoc test indicated that responses were faster for targets preceded by the mask alone than for those preceded by masked words. The delay might be due to activation of the whole lexicon produced by the unconscious prime (Posner et al. 1999).

ERP Results

Target Words: ERP Correlates of Semantic Priming and Word Processing

Conscious semantic priming was studied with a set of sample-by-sample repeated measures *t*-tests. The differences between unmasked unrelated and unmasked related targets appeared around 400 ms after target onset (at left hemisphere electrodes over frontal, central, temporal and parieto-occipital scalp regions, see Fig. 1). The peak latency of this component (around 400 ms), the smaller positivity for the related targets, and the central–parietal location suggest that it is an N400-like effect related to the semantic priming produced by non-masked primes (Kouider and Dehaene 2007; Marí-Beffa et al. 2005, 2007; Ruz et al. 2003). Earlier differences also appeared in a small number of electrodes. Similarly early results have been observed by other authors in the P1-N1 transition band (e.g. Rossell et al. 2003; Michel et al. 2004; Wirth et al. 2007), and considered to be an index of initial lexical processing. In contrast, no N400 effect was observed when targets preceded by related masked primes were compared to those preceded by masked unrelated ones (Fig. 1). However, some hints of a difference in processing of related primes were observed at frontal and parieto-occipital electrodes. Differences appeared in a negative component peaking at around 200 ms post-target onset (Fig. 1b). Ruz et al. (2003) suggested that this modulation of the N200 indicates that the brain mechanisms responsible for unconscious semantic priming are partially dissociable from those involved in conscious semantic priming.

As with the behavioural data, scant evidence of unconscious priming was found when we compared the ERP waves of target words preceded by related and unrelated masked primes (Fig. 1a, b). This null effect might indicate that no processing of the unconscious word has taken place at all. On the other hand, semantic processing of the prime could have occurred, but measuring activity during the probe (target) does not capture it because the mask has acted to disrupt or suppress this processing (cf. Marí-Beffa et al. 2005). To distinguish between these two alternatives we analysed the activity evoked by masked prime words, attempting to estimate the activity generated by the prime itself using the three linear methods described above.

ERPs to Prime Words

To make the relationship between this design and the foregoing (e.g., Eqs. 1 and 2) explicit, S is the (to-be-estimated) activity produced by the prime alone, C is the activity generated by the “no stimulus” condition, i.e., the

Table 1 Mean and standard error (SE) of reaction times and error percentages by target conditions

	Masked			Unmasked		
	RT (ms)	SE	% Errors	RT (ms)	SE	% Errors
Related	643	19.14	5.6	615	17.56	4.1
Unrelated	646	17.52	5.6	647	17.45	8.5
Non-word	809	27.49	13.8	791	27.39	12.3
Mask alone	630	17.7	5.1			
Size of priming	3		0	32		4.4

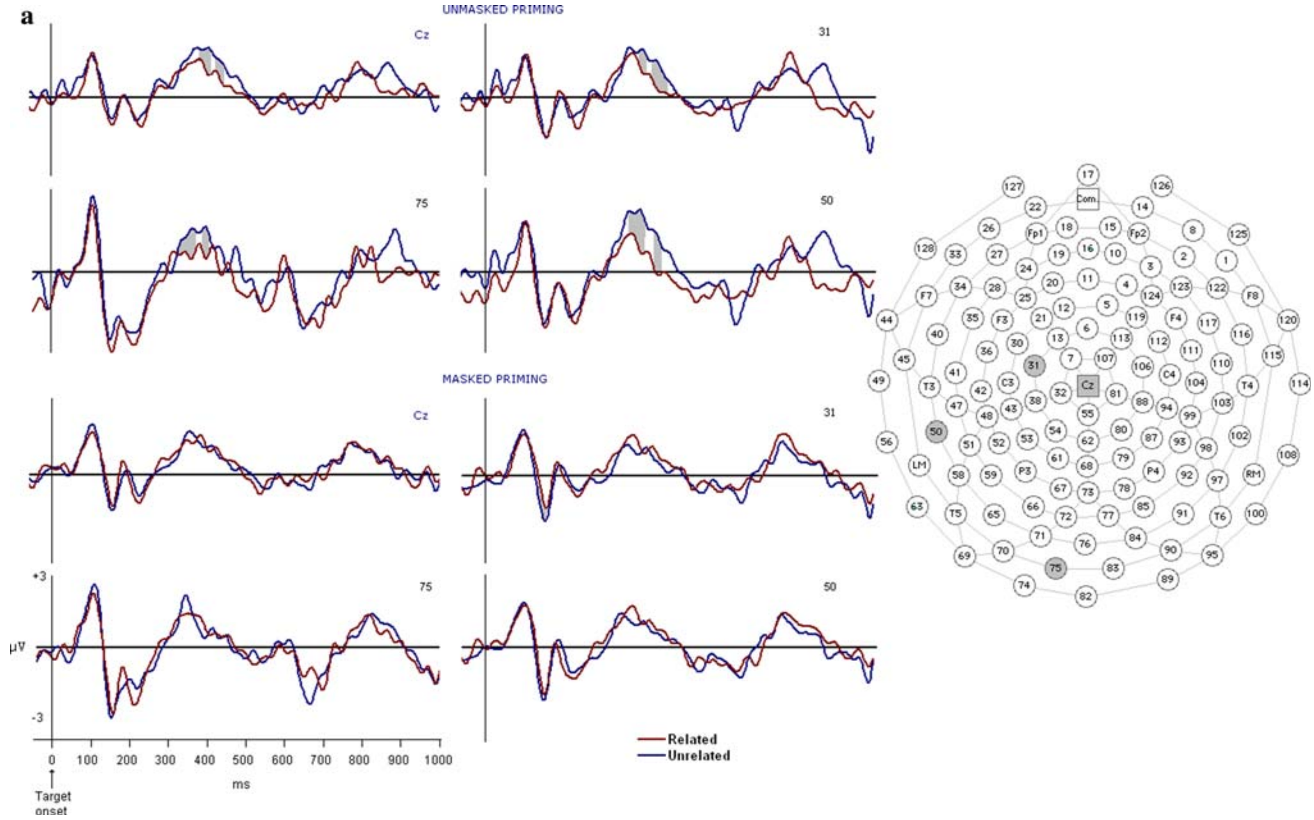

Fig. 1 Panel **a** *Left*: Example ERPs for targets from central, occipital and temporal electrodes. Note the unmasked N400 effect: lower amplitude of the related (brown) condition. Significant differences are shadowed in gray. *Right*: locations of electrodes in the EGI geodesic sensor net. Electrodes marked in gray are displayed in the left panel. Panel **b** Time-Electrode differences for masked and unmasked semantic priming (black marks show significantly lower amplitudes for related than for unrelated targets). Electrodes are arranged by number in the EGI system. *t*-test interpolated maps at times with significant differences for masked and unmasked priming. Significance values are color coded according to the scale shown on the right

mask on its own, and E is the activity generated by the combination of prime + mask. So, if the masked prime is not processed at all, then the whole activity registered from the masked condition (E) would be due to the mask (C), and therefore we should observe approximately zero levels of residual activity (S). Any activation significantly different from zero would mean that the unconscious prime has been processed to some degree.

The sample-by-sample single-sample *t*-test results for waveforms predicted from *p*, *d*, and the subtraction, are illustrated in Fig. 2. Using the two linear methods (*p* and *d*), the estimated waveforms differed significantly from 0 at around 100 ms and 200 ms (some earlier and later differences were also observed, especially in *p* waveforms; however, we restrict the discussion to the 100 and 200 ms time windows, as these are the intervals during which lexical access is believed to occur; see Hauk and Pulvermüller 2004; Fairhall et al. 2007; Sereno et al. 1998; Sereno and Rayner 2003). In stark contrast, the traditional subtraction method failed to identify significant differences in the 200 ms time window (Fig. 2). Importantly, sample-by-sample repeated measures *t*-tests failed to detect differences between ERP waveforms predicted from *p* and those predicted from *d*. Thus, the two linear methods converged both on when the differences occurred and on their topographic distribution at the scalp.

Prime words first showed significant processing at around 100 ms. Both methods located a positive spatial peak in this time windows centred over left parieto-temporal electrodes (Fig. 2). The next major difference occurred at around 200 ms (Fig. 2). Again, the two linear methods also showed notable agreement on the spatial distribution of the activity, exhibiting some positive peaks over left frontal scalp regions, accompanied by central and posterior negative peaks (Fig. 2). Source modelling of the *p* and *d* data (using sLORETA, see Pascual-Marqui et al. 1994; Pascual-Marqui 1999, 2002) indicated that the activity at 100 ms had a source in posterior regions, mainly in the occipital cortex. The later difference at 200 ms was also centred on posterior regions, but the

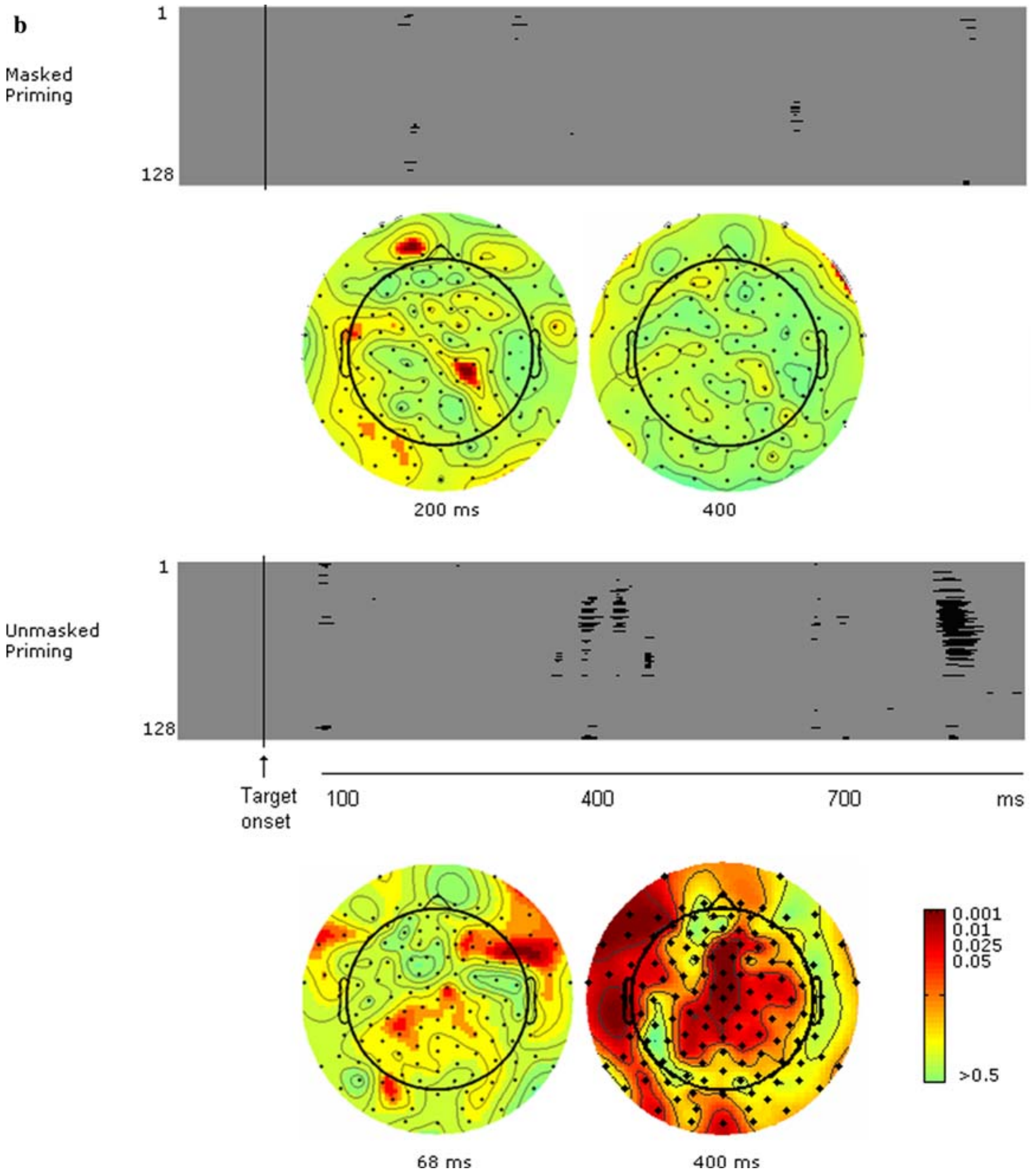

Fig. 1 continued

activity in early visual regions was now reduced, the peak differences being found more anteriorly in the left fusiform gyrus (Fig. 2). These data indicate that the prime word is being processed first as a visual stimulus, at around 100 ms, and then later specifically as a word at 200 ms. We would suggest that this difference reflects an inhibitory effect caused by the onset of the mask, perhaps related to a similar effect reported by Eimer and colleagues (Eimer and Schlagheken 1998; see also Jaśkowski et al. 2008).

Discussion

The main results obtained in this experiment are related to two major topics in the literature on ERPs and semantic processing. First, our behavioural and electrophysiological results indicated that a reliable N400 effect can be observed for visible primes, but not for unseen masked ones (Holcomb et al. 2005, although see, for example, Kiefer and Brendel 2006). In general, the ERP literature suggests that unseen stimuli can reach the lexical level of processing (Kouider

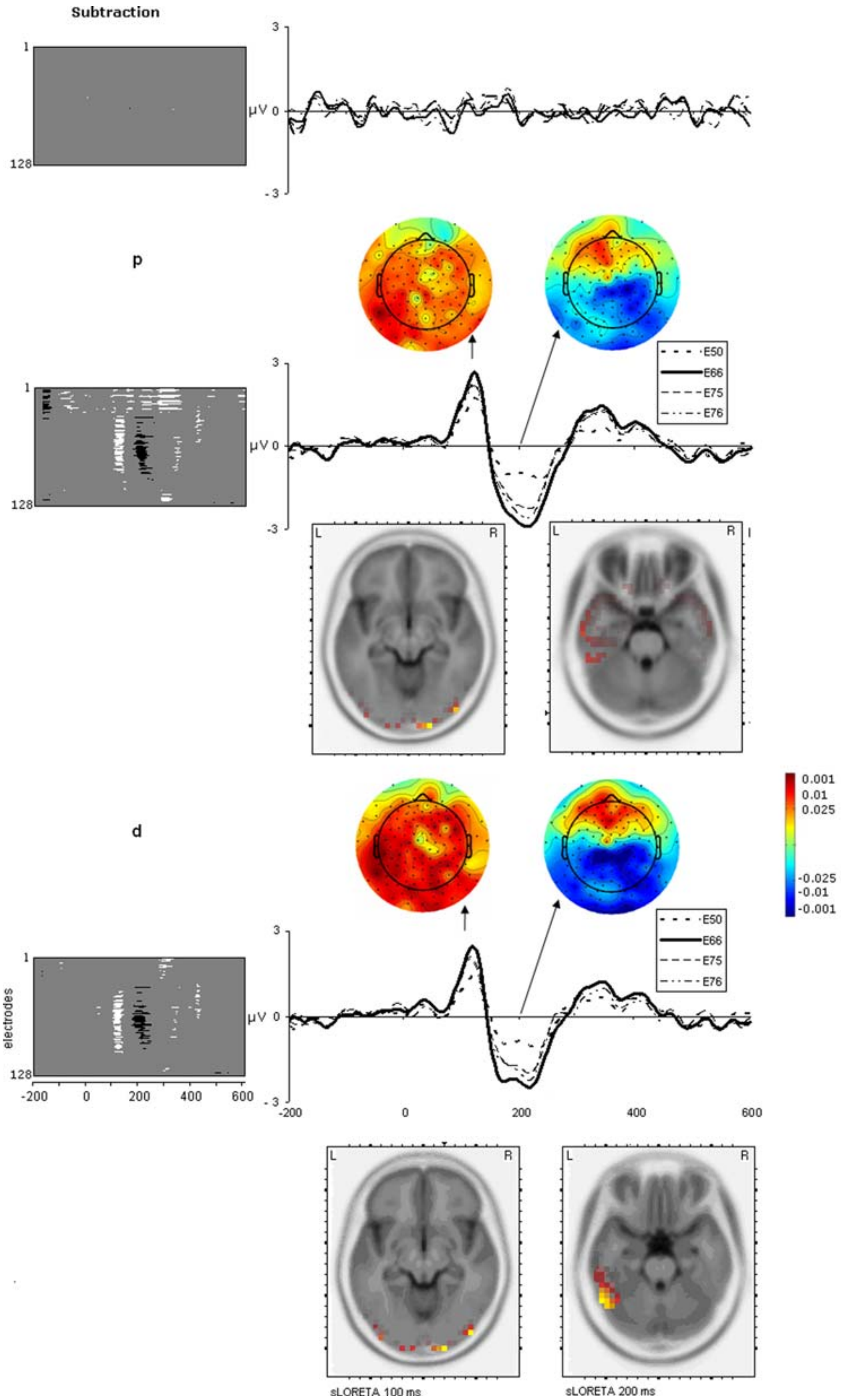

Fig. 2 *Left*: Time-electrode significant differences (repeated measures *t*-test, minimum $P < 0.025$, for five consecutive samples in at least five electrodes) for the unknown prime waveform, S, given by the estimators *p*, *d*, and the subtraction method. *Right*: ERPs for the masked word derived from subtraction and the two linear methods. Interpolated *t*-test maps for *d* and *p* at around 100 ms and 200 ms post-stimulus onset (EEGlab software). Significance values of *t*-tests are color coded according to the significance scale shown on the right. Points are the electrode locations of the EGIs dense array. Only the estimation methods *p* and *d* show significant peaks. Below: Brain sources estimated by sLORETA at 100 and 200 ms after prime onset, using ERPs estimated from *p* and *d*

and Dehaene 2007). However, almost all the evidence, especially with ERPs, is indirect, as the brain electrical activity evoked by the masked primes is considered to be a blend of the activity evoked by the mask and that evoked by the masked prime. Our second major result speaks to this issue. We have shown that the activity evoked by the masked word can be separated from the blended waveform using linear methods based on regression and smoothness assumptions. The results of these methods indicate that the masked word is being processed first as a visual stimulus, at around 100 ms, and then later specifically as a word at 200 ms. The topography and the probable brain generators of these differences (visual cortex and fusiform gyrus, respectively) strongly suggest that the first peak indicates the processing of low-level features of the masked prime, whereas the second peak indicates the lexical processing of the unseen word (Cohen et al. 2000; Ruz et al. 2003).

The above results indicate that the two novel linear techniques proposed here identified (and converged upon) a highly plausible spatio-temporal pattern of neural activity (congruent with recent evidence in written word processing; Fiebach et al. 2002; Kouider and Dehaene 2007; Hauk and Pulvermüller 2004; Posner et al. 1999; Marí-Beffa et al. 2005, 2007) which was missed by the subtraction method.

The linear methods presented here have proven to be able to detect the patterns of changes across time of an unknown source (S) by using the known changes in two time-series, one a control (C) and one considered to be a blend of the control and the unknown source (E). We have demonstrated the use of these techniques in an ERP study to uncover the processing of masked primes in a lexical/semantic priming procedure. The estimators found a highly plausible spatio-temporal pattern of activity which the use of the subtraction method missed entirely.

Though illustrated using ERPs, the methods are equally applicable in studies using other techniques of brain imaging, such as event-related fMRI or MEG, where overlapped brain activity is a by-product of the experimental design. Moreover, the linear methods are also applicable in experimental procedures when overlapping waveforms are expected. For example, in simultaneous auditory-visual presentations ERPs elicited by the auditory-alone and visual-alone stimuli are summed and contrasted to the ERP evoked by simultaneous presentation of both stimuli. Simple subtraction is used to disentangle the interaction between visual and auditory signals (Girard and Peronnet 1999; Molholm et al. 2002). Therefore, the simple subtraction assumed that the blended waveform is the arithmetic sum of the visual and auditory waveforms (see Eq. 1). We showed here that Eq. 2 can be a better way of disentangling this auditory-visual blended waveform.

As discussed in the introduction, there have been some previous attempts to provide alternatives to subtraction in disentangling overlapping waveforms (see Talsma and Woldorff 2005, for review). It is not our intention to compare the efficacy of our approach to these methods in detail here. However, we would note one major difference between our proposals and previous work, which is that these existing techniques all require changes to the basic trial sequence. For example, inter-stimulus interval jittering requires randomly changing the interval between successive stimuli in the trial, so that, in the case of masked priming, it would be necessary to vary the onset asynchrony between the prime and mask. As well as complicating the experimental design, the subsequent data analysis associated with these techniques is computationally demanding. It may be for such reasons that simple subtraction continues to be by far the most widely used method. The linear methods we have introduced allow the experiment to be run just as it would be when subtraction is to be used (and indeed these methods can be used to re-analyse data which have previously used subtraction). Other approaches, such as Blind Source Separation techniques (ICA), have the ability to recover the brain sources of EEG activity. A possible strategy for using ICA in the context analysed here would be to determine the brain sources of effects produced by masked words (i.e., word + mask) and those produced by the mask alone, and then to subtract the latter from the former. However, there is no clear way of deciding when two components are the same. Alternatively, ICA activities can be subtracted after estimating brain sources for all the experimental conditions. The problem is, however, that the component itself will remain unchanged after subtraction.

In summary, the common assumption that the experimental measure (E) is a non-weighted sum of sources (S and C) can lead to systematic errors in the estimation of the time course of brain activation using simple subtraction (Qiu et al. 2006; Yao 2003). The methods we propose here produce a demonstrably better estimate of the relevant signal, without adding a prohibitive computational load to data analysis. We would propose that as a minimum, a check on the value of *p* or *d* should be necessary before applying the subtraction technique.

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Appendix A

Regression Based Method

We obtain b from p as follows. First, we multiply both sides of Eq. 2 by C, and then sum across some time points (i) to give

$$\sum_i E_i C_i = \sum_i S_i C_i + b \sum C_i^2 \Rightarrow SP_{EC} = SP_{SC} + bSS_C,$$

where SP stands for sum of products, and SS stands for sum of squares. Finally, dividing by $\mathrm{SS_C}$ we have

$$p = \frac{SP_{EC}}{SS_C} = \frac{SP_{SC}}{SS_C} + b \tag{3}$$

The prediction error for S will be a function of the difference between the true value of *b* and that of its estimate, *p*, which depends on the strength of the relationship between C and S. To be precise, *p* is the exact estimator of *b* when SP_{SC} is zero; it is an overestimate of *b* when SP_{SC} is positive; and it is an underestimate of *b* when SP_{SC} is negative. Of course, the size of the estimation error depends on the quotient SP_{SC}/SS_C, which cannot be computed as S is unknown. Note, however, that the greater the relationship between the control and the experimental conditions, the bigger the expected estimation bias. This regression-based estimation differs from the use of covariance analysis. Although like regression covariance analysis uses one condition as a predictor of another, in most cases regression-coefficients in covariance analyses are estimated across subjects instead of across time-series in the same subjects.

Temporal Smoothness Method

We assume that the sum of squares of the first order differences in S, $\sum_i \left(S_i - S_j\right)^2$ ($j = i + 1$, i being the time frame), is minimum. Operating on this expression, taking partial derivatives with respect to *b*, and equating to zero, we get a second estimator for *b* which we will label *d*:

$$d = \frac{\sum \left(E_i - E_j\right)\left(C_i - C_j\right)}{\sum \left(C_i - C_j\right)^2} \tag{4}$$

which is the best estimator of *b* when changes in the waveform are smooth.

Estimation Errors of *p* and *d*

The computation of *p* and *d* was done using a 100 ms time window, starting at 0 ms post-prime onset. It is assumed that both parameters are constant. Under this assumption, we ran 13500 simulations aimed at estimating the standard error of the parameters *p* and *d* under a number of situations that can actually occur when two waveforms overlap. The simulations were done using three values of *b*: 0.8, 0.5 and 0.2. In each run, we computed the signal waveforms S and C as linear combinations of sinusoidal functions of several frequencies and amplitudes. Both were blended according to Eq. 2, using different values of *b* in each run. Moreover, in a simulation series, average amplitude was higher in S than in C, in another S was lower than C, and in the third, the amplitude average was equal in S than in C. Finally, the amplitude values were modulated by multiplying the S and C waveforms by either a Gaussian or a rectangular envelope. We summarize the results as average percentages of error (standard errors divided by the b parameter). For the *p* parameter, the percentages were 0.34, 1.38 and 4.56%, respectively, for 0.8, 0.5 and 0.2 *b* values. For *d* parameter, the percentages were 0.340, 1.38 and 4.68%, respectively, for 0.8, 0.5 and 0.2 *b* values.

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