

Protocol

Studying semantics in the brain: the rapid stream stimulation paradigm

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Abstract

Event-related potentials (ERPs) provide information about the temporal course of cognitive processes in the brain. They have proved to be a valuable tool in order to explore semantic aspects of word processing. However, to date, research in this field has been mostly concerned with the study of post-lexical features by means of the N400-paradigm. We introduce here the rapid stream stimulation paradigm, in which stimuli reflecting different levels of linguistic information are presented to subjects at a high rate of stimulation. The present protocol shows in detail how this paradigm can be applied. The application of the rapid stream stimulation paradigm evokes the recognition potential (RP), an ERP component that peaks at around 260 ms after stimuli onset and seems to be reflecting lexical selection processes. Results of studies that revealed the sensibility of the RP to visual-semantic aspects and the location of its neural generators within basal extrastriate areas are reported. Although some research has been conducted with the rapid stream stimulation paradigm much remains still to be done. Some of the possibilities that this paradigm offers are further discussed. © 2001 Elsevier Science B.V. All rights reserved.

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1. Type of research

The rapid stream stimulation paradigm has shown to be helpful when studying processes related to semantic aspects of word comprehension by means of a component of the event-related potentials (ERPs), recognition potential (RP), that is obtained by applying this paradigm. The RP seems to index those aspects of semantic processing dealing with lexical selection, that is, the access to word meaning [6,12]. Thus, the protocol we will describe here constitutes a valuable tool to study the temporal course of issues related to lexical selection processes in the brain. The rapid stream stimulation has been already used in a

wide variety of studies dealing with semantic aspects of word processing. Some of these studies include semantic priming [25], word frequency [28], comparison of different vocabulary types [4] or the influence of imageability in word semantic processing [13] as we will show later. Even still, many of the possibilities that the rapid stream stimulation paradigm allows remain unexplored, especially in the clinical ambit. Therefore, it seems to be a promising tool for future research.

2. Time required

The application of the paradigm in the form we will describe here takes around 30 min. However, around 30 min should be added for those tasks concerning electrode placement, so a duration of 1 h is the estimated time for the whole experimental session.

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3. Materials

3.1. Stimuli elaboration

Stimuli were made in a PC (Pentium 200 MMX processor, 64 MB RAM, 4 GB hard disk) by using the Draw module of the STIM package (NeuroScan Inc.). Stimuli presented to subjects participating in these experiments reflect different levels of linguistic processing. The materials include different pools of stimuli.

- Usually, a pool of animal names is included as target words with the purpose of providing subjects with a task in order to maintain their attention during the application of the protocol.
- Different word types, that are selected according to the particular purposes of the study, that is, semantic content stimuli.
- Pseudowords, that is, stimuli following orthographic rules but devoid of meaning.
- Strings of random letters, that is, stimuli devoid of either semantic or orthographic content. These stimuli are created by randomizing the letters of the target words (animal names).
- Control stimuli, that is, stimuli devoid of any linguistic property at all. These stimuli are made by cutting target words in 'n' portions (n =number of letters that compose a word minus one) and replacing these portions according to the following rules: the first piece of the word is placed in the last position of the new stimulus, and vice versa; the penultimate portion is placed in the second position, and vice versa; and so on. Thus, every stimulus obtained this way has at least two complete letters but also clear identifiable non-letters (formed by the fusion of different letter fragments).
- Background stimuli. This kind of stimuli play a substantial role in the rapid stream stimulation paradigm as will be detailed bellow. This pool always includes the same control stimuli plus an additional set of stimuli made in the same way as controls, except that portions are replaced randomly.

Special care must be taken in order to equate stimuli length in both physical size and, in the case of target words, words and pseudowords, number of syllables. In the studies we will present in the Results stimuli were 1.3 cm in height and 3.5 cm in width. It is also important to match word frequency when experimental purposes require the comparison between different word types, since word frequency clearly affects RP latency [28]. Fig. 1 displays stimulus examples.

3.2. EEG recording equipment

Electroencephalographic data of the results shown in

HIENA	ANIMAL NAMES (TARGETS)
LLAVE	WORDS
KEMER	PSEUDOWORDS
ERJRA	RANDOM LETTERS
3ALUIPI	CONTROL
JOERIC	BACKGROUND

Fig. 1. Examples of the stimuli presented to subjects.

this protocol were recorded using an electrode cap (ElectroCap International) with tin electrodes. A total of 58 scalp locations were used: Fp1, Fpz, Fp2, AF3, AF4, F7, F5, F3, F1, Fz, F2, F4, F6, F8, FC5, FC3, FC1, FCz, FC2, FC4, FC6, T7, C5, C3, C1, Cz, C2, C4, C6, T8, TP7, CP5, CP3, CP1, CPz, CP2, CP4, CP6, TP8, P7, P5, P3, P1, Pz, P2, P4, P6, P8, PO7, PO3, PO1, POz, PO2, PO4, PO8, O1, Oz, and O2. These labels correspond to the revised 10/20 International System [1], plus two additional electrodes, PO1 and PO2 located halfway between POz and PO3 and between POz and PO4, respectively. All scalp electrodes, as well as one electrode at the left mastoid (M1), were referenced to one electrode at the right mastoid (M2). Also, an electrooculogram (EOG) was recorded from below versus above the left eye (vertical EOG) and the left versus right lateral orbital rim (horizontal EOG).

3.3. Stimulus presentation equipment

Stimuli were presented white-on-black on an NEC computer MultiSync monitor. A QBasic program was created in order to determine the order of stimulus presentation. This program was run and controlled by the Gentask module of the STIM package.

3.4. EEG data acquisition and analysis

Data were digitally recorded. Data acquisition was controlled by the Acquire module of the 4.0 version of the Scan package (NeuroScan Inc.). Two amplifiers (SynAmps, NeuroScan Inc.) were used for the registration of EEG and EOG. Data were stored in a PC (Pentium 200 MMX processor, 128 MB RAM, 8 GB hard disk).

EEG data were analyzed with the EDIT module of the 4.0 version of the Scan Package (NeuroScan Inc.).

Maps showing the topographic distribution were elaborated with the 3D Space module of the Scan Package (NeuroScan Inc.).

Neural generators were estimated by applying the Brain

Electric Source Analysis algorithm (BESA, MEGIS Software; [31]).

3.5. Statistical analyses

Data were statistically compared first by analysis of variance (ANOVA) using the SPSS 10.0 (SPSS Inc.) and if a significant difference was detected by the ANOVA, post-hoc analyses with the Bonferroni correction were applied.

3.6. Chemicals and reagents

None required, except Nuprep abrasive gel for skin cleaning (D.O. Weaver & Co) and Electro-Gel electroconductive gel (ElectroCap International Inc).

4. Detailed procedure

The rapid stream stimulation paradigm requires that stimuli are displayed at a high rate of presentation, with a stimulus onset asynchrony (SOA) of 257 ms, though this can be even shorter. Mostly, background stimuli are presented to subjects. Periodically (after either six or seven background stimuli, this number randomized), a test stimulus is presented. Test stimuli include target animal names, words, pseudowords, strings of random letters and controls in the experiments that will be presented in this paper. Stimulation is organized in sequences. Each sequence begins with six or seven background stimuli, followed by the first test stimulus. A random process determines the type of test stimulus that is presented with the constraint that no more than two of the same type occur in succession. A sequence ends with six or seven background stimuli following the presentation of the last test stimulus. Every sequence includes five of each type of test stimuli, together with the proportional amount of background stimuli. Sequence duration is around 50 s. An experimental session includes 16 sequences and every test stimulus is repeated four times along the session. However, special care is taken that a particular test stimulus is never presented twice within the same sequence [4,5,11–13]. The protocol is organized according to the following steps.

4.1. Electrode placement

The electrode cap, in addition to mastoids and EOG electrodes, is placed first. For more details concerning electrode placement see Ref. [19].

4.2. Instructions to subjects

The experiments were carried out on healthy subjects, all of them right handed according to the Oldfield questionnaire [17]. Subjects were paid for participating in the

studies according to a particular payment schedule (see below).

Subjects are told to press a button as rapidly as possible every time they detect a target stimulus, that is, an animal name. Subjects are also informed of the payment schedule. A response between 650 ms and 900 ms after target stimuli is considered as a hit and earns 5 units, whereas a response between 300 and 650 ms is considered a fast response that earns 10 units. False alarms and premature responses, those occurring 300 ms after target stimuli presentation, suffer from a 25-unit penalty. The points are exchanged for the corresponding amount of money at the end of the session.

Subjects are positioned on a comfortable chair with the eyes at 65 cm distance from the monitor screen. They are informed that at the beginning of each sequence a message will notify them to blink as much as they want in order to avoid blinking during stimuli presentation. Subjects are also told to fix their eyes in the center of the screen and not to move their head during sequence presentation. A button has to be pressed in order to start a sequence

Two practice sequences are allowed to every subject. Sixteen sequences are presented to every subject. Subjects are allowed to rest as much as they want between sequences. Feedback about their performance is provided to subjects at the end of every sequence.

4.3. EEG recording procedure

The EEG data are band-pass filtered between 0.3 Hz and 100 Hz and continuously digitized at a sampling rate of 250 Hz for the duration of each of the 16 task sequences. These data are stored on a computer hard disk.

4.4. Analysis of the EEG signal

The continuous recording is divided into epochs of 1024 ms duration, beginning from the onset of each test stimulus: animal names, words, pseudowords, strings of random letters, and control stimuli. Those epochs exceeding $\pm 65 \mu\text{V}$ in any electrode are automatically rejected. Additionally, epochs with eye movements or blinks are also eliminated after a visual inspection. ERP averages are categorized according to each type of stimulus. Only correct trials are considered for average purposes, so that epochs with false alarms or omissions are excluded. Those trials in which the reaction time is not between 300 and 900 ms are also excluded. Original M2-referenced data are algebraically re-referenced offline for the entire sample of cephalic electrodes according to the common average reference method [10]. RP latency is measured in the electrode showing the most negative peak in the interval ranging between 160 and 417 ms for every subject and in every type of test stimuli, with the exception of control stimuli, that do not show an RP response due to their lack of linguistic content. After determining the mean latency

of every type of stimulus, a time-window centered around the mean latency is considered for amplitude measuring purposes. The time window ranges ± 28 ms around the mean latency. Thereafter, a measurement of the area within this time-window is considered for statistical analyses and topographical maps.

The activity evoked by control stimuli is subtracted from the other test stimuli in order to enhance language factors reflected by the RP. These subtracted data are used for absolute grand-average waveform representation and topographical maps but not for statistical purposes.

4.5. Statistical analyses

Statistical analyses are conducted in raw data. Repeated measures ANOVAs with the purpose of comparing the activity evoked by each type of test stimulus are applied to a selected sample of electrodes when performing latency and amplitude comparisons (Fp1, Fp2, AF3, AF4, F5, F1, F2, F6, FC5, FC1, FC2, FC6, C5, C1, C2, C6, CP5, CP1, CP2, CP6, P5, P1, P2, P6, PO7, PO1, PO2, PO8, O1, and O2). Post-hoc analyses are performed in those particular electrodes showing the highest RP amplitude at each hemisphere (PO7 and PO8 electrodes).

4.6. Topographic distribution analyses

Topographic maps are elaborated for every stimulus type after subtracting activity evoked by controls. Topographical distribution of the RP activity is statistically compared across every type of stimulus by applying a profile analysis [15].

4.7. Source analyses

The source analysis for the RP is performed by applying the Brain Electrical Source Analysis algorithm (BESA; [31]).

5. Results

Fig. 2 displays waveforms corresponding to the activity evoked by animal names, words, pseudowords and strings of random letters after subtracting activity evoked by control stimuli. A negative response, the RP, peaking at around 260 ms is observed for every type of stimulus. This response is maximal for semantic content stimuli, animal names and words, and progressively diminishes for pseudowords and letter strings. The RP shows its highest amplitude values at PO7 and PO8 electrodes, for the left and the right hemispheres, respectively.

Fig. 3 illustrates the results of a further experiment that compared the semantic processing of open- and closed-class words by applying the rapid stream stimulation paradigm [4]. Open-class words are the main bearers of

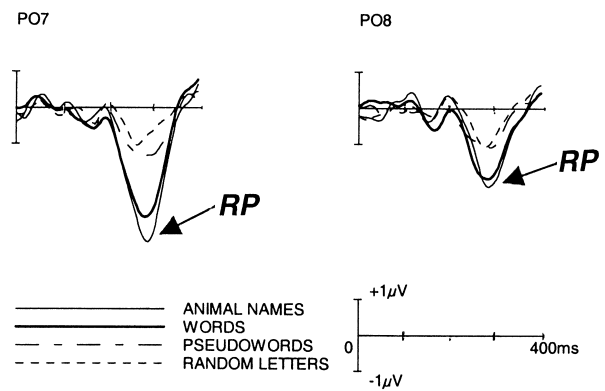


Fig. 2. Representative pattern for the recognition potential (RP) evoked by different types of stimuli after subtracting the activity evoked by control trials from each of the waveforms corresponding to animal names, words, pseudowords, and strings of random letters. Only data corresponding to PO7 and PO8 electrodes are represented since these are the electrodes that show the highest amplitudes at each hemisphere. Meaningful stimuli, that is, animal names and words, evoked higher amplitudes as compared to non-meaningful stimuli. However, there were no latency differences across types of stimuli. The latency was around 260 ms (from Ref. [11]).

meaning (nouns, verbs, . . .), whereas closed-class words mainly subserve syntactic functions (prepositions, conjunctions, . . .). Although the RP evoked by open- and closed-class words did not differ in the left hemisphere, there were amplitude differences in the right hemisphere. These results suggest that the semantic processing of closed-class words is left-lateralized, whereas brain regions from both hemispheres participate in the processing of open-class words.

Fig. 4 displays a topographical map of the RP distribution across the total array of cephalic electrodes. As the topographical distribution does not differ across type of

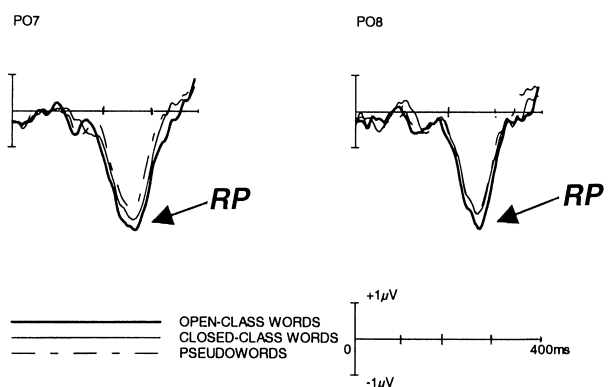


Fig. 3. Waveforms corresponding to open-class words, closed-class words and pseudowords after subtracting the activity evoked by control stimuli are shown. Again, a remarkable recognition potential (RP) can be appreciated for each type of stimulus. Despite a similar latency, amplitude differences between meaningful and non-meaningful stimuli are noticeable. Whereas the RP to open- and closed-class words did not differ at left-hemisphere regions, it differed at right-hemisphere areas. This suggests the existence of differences in lateralization in the semantic processing of open- compared to closed-class words (from Ref. [3]).

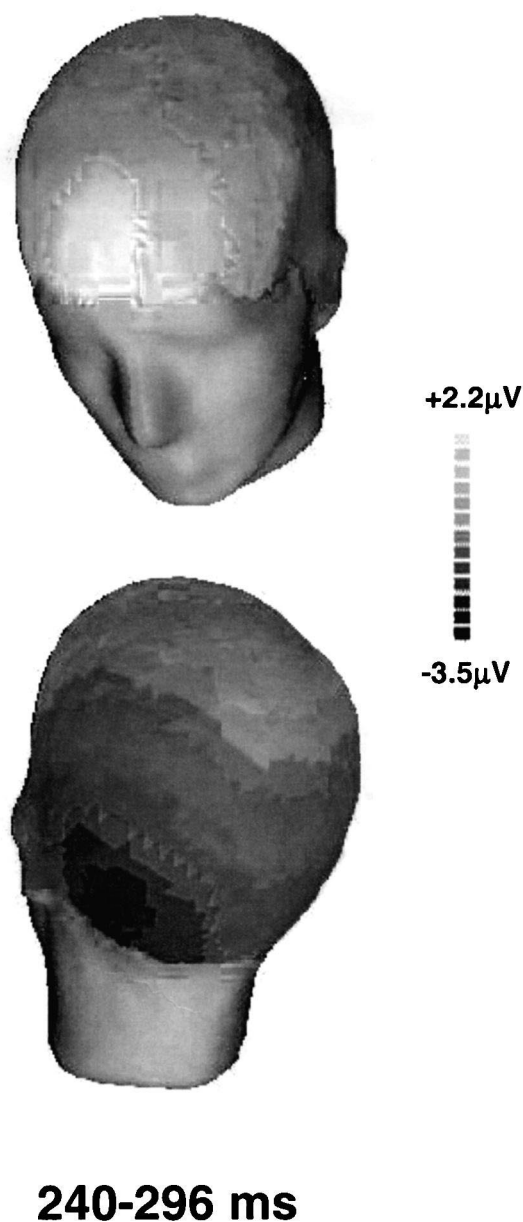


Fig. 4. A representative topographic map corresponding to the recognition potential (RP) evoked by words after subtracting activity to control trials. The topographic distribution of the (RP) basically consists in a left-lateralized parieto-occipital negativity. A lower positivity over frontal regions can also be observed.

stimulus [3,12,13] only data corresponding to words is represented. Activity to control stimuli has been subtracted. The RP shows a slight left-lateralized parieto-occipital distribution. A counterpart frontal activity is also observed.

Fig. 5 shows the results of dipole analyses after the application of the Brain Electrical Sources Analysis algorithm (BESA). The BESA was applied following the constraint of two dipoles being at mirror positions and presenting mirror orientations, according to the topographical distribution of the RP. Once again data corresponding

to word stimuli is represented. It can be observed that RP neural sources are located within the fusiform/lingual gyri, areas that are particularly implicated in the processing of visual-semantic information as recent neuroimaging studies have demonstrated [2,16,32].

6. Discussion

The rapid stream stimulation paradigm has proved to be a very valuable tool for evoking an ERP component, the RP, which is of great utility when approaching issues concerning semantic aspects of word processing. The rapid stream stimulation provides an alternative methodological approach to those that have been repeatedly used when studying semantics with ERPs. Such approaches traditionally concern sentences including a word that is semantically incongruent with the sentence context [8]. This leads to an ERP component, the so-called N400, that was firstly implicated in the access to word meaning. However, later research has identified processes reflected by the N400 as being related to post-lexical analyses [33]. The sensitivity of the RP to semantic aspects, together with its latency, that coincides with results from eye-movements studies suggesting that word meaning is extracted around 250 ms after word onset, makes the RP an appropriate candidate to be reflecting lexical selection processes (see Ref. [6] for a detailed discussion on this issue). Whatever the case, the application of the rapid stream stimulation procedure has been quite limited to the date. This situation opens many possibilities but also leaves some obscure points that demand further investigation.

6.1. Trouble-shooting

We will discuss now some of the points described in Section 4.

A high rate of stimuli presentation seems to be of the greatest importance when evoking RP, in order to attenuate other ERP components apart from RP. This might be a problematic issue when studying some particular populations such as infants, ancients or clinical populations. All research on RP conducted in our laboratory has used an SOA of 257 ms which is a rate of presentation of about 4 Hz. Different rates of presentation have been used by other groups (e.g. Ref. [22]), this rate of presentation being even quicker (10 Hz) than the one we use. Whatever the case, the effects of SOA manipulations on RP have not been deeply investigated and this question remains as a matter that deserves more attention. The same holds true for studying populations other than young healthy subjects. Regarding this question, previous observations in our laboratory suggest that schizophrenics can deal with the rapid stream stimulation paradigm, although their be-

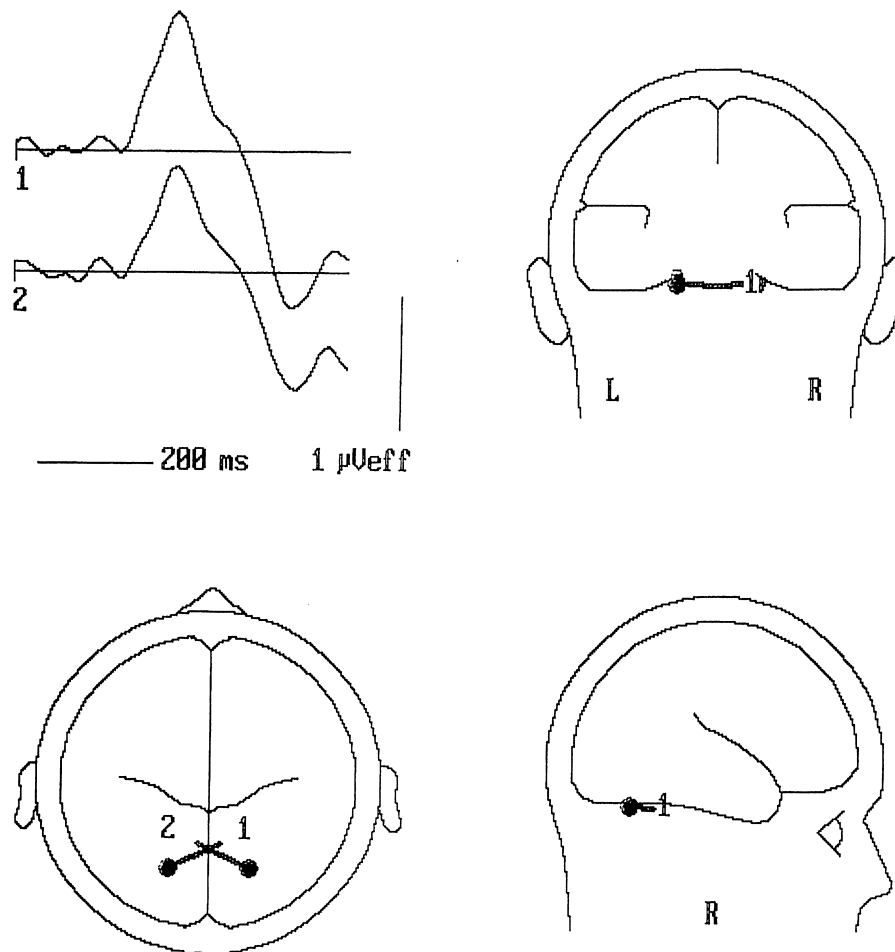


Fig. 5. Location of the dipoles for the recognition potential (RP) evoked by words after subtracting the activity to control stimuli. The time-varying source magnitude waveforms (top left) and positions (top right and bottom) of the two dipoles for the RP are shown. Dipole locations are shown as circles with a tail indicating the amount and direction of current flow. Dipole number is placed at the end of the tail. Dipole number 1 is located within the left hemisphere, whereas number 2 is within the right hemisphere. Best-fit solution for both dipole corresponds to a position within the lingual/fusiform gyri (from Ref. [11]). Copyright 2001 Society for Psychophysiological Research.

havioral performance is impaired compared to control subjects: schizophrenics make both more omissions and false alarms.

Another important point has to do with the organization of stimulation into sequences. This procedure has clear advantages when using the rapid stream stimulation paradigm. The high rate of stimulation forces subjects to make a special effort in order to maintain attention at high levels. Organizing stimulation into sequences of about 50 s duration greatly attenuates the emergence of fatigue effects and allows subjects to rest between sequences.

Instead of using a 58-channel electrode cap, the first studies on RP measured this component by means of a bipolar montage (e.g. Refs. [11,20,22]). The recording channel included an electrode placed midway between the inion and the Cz electrode, roughly corresponding to Pz electrode, referenced to an electrode placed on the inion. The usage of this montage does not allow to study either topography or neural generators of the RP. However, this

procedure has the advantage of a considerable reduction in the time dedicated to electrode placement. This might be convenient in some situations such as the clinical application of the protocol.

It is not necessary to instruct subjects to press a button every time they detect a target stimuli, although this is the most common procedure in the application of the rapid stream stimulation paradigm. Some studies have found RP responses even with passive viewing of images or keeping a count of the recognizable stimuli [22]. However, we think it is preferable to provide subjects with a task in order to maintain their attention.

The same situation holds for the payment schedule. Whereas this has been the most usual way of proceeding in RP research, this has not always been the case [13,22,23]. The use of a payment schedule, providing feedback of the performance immediately after every sequence is over, has an undoubted motivational role that helps subjects to maintain their attention close to the highest levels. How-

ever, no study has been conducted to date in order to explore the specific effects of the payment schedule on RP.

Although presenting stimulation in 50-s duration sequences greatly helps to attenuate eye-blinks, it is important to insist subjects avoid blinking as much as possible during stimuli presentation. Eye blinking might be very problematic in ERP methodology [19]. The same is true for eye movements, even though presenting stimuli at the center of the screen, as in the case of the rapid stream stimulation, prevents such movements. It should be noticed, nevertheless, that eye movements have shown to have little influence on RP [21].

Although the task itself is not difficult, the rapid rate of stimuli presentation makes practice essential in order to allow subjects to become familiarized with the high rate of stimulation. We have found two sequences to be enough for practice purposes for young healthy subjects. However, up to five practice sequences were allowed in other studies [22,27].

Presenting subjects with 16 sequences allows to present 80 test stimuli of each type during the experimental session, a number that is enough to get a high quality signal-to-noise ratio to obtain the RP even after rejecting some of the epochs because of eye movements or incorrect responses. Moreover, presenting subjects with eight sequences still produces an adequate signal-to-noise ratio and a good quality RP response [14]. This situation considerably reduces the time required for stimulation and might facilitate the application of the rapid stream stimulation paradigm to infants, ancient and clinical populations.

Recording EEG activity continuously instead of in an epoched mode seems preferable and this has always been used in RP research. Advantages of continuous registration concern the possibility of choosing different analysis time windows after the experiment and the facilitation of performing additional analyses (i.e. spectral power analysis) with different needs for the choice of time windows [7].

The normal procedure used in RP research consists in dividing continuous recording into epochs of 1024 ms duration [11,12,21,22]. This might seem a long epoch duration if we consider the time at which the RP occurs. Certainly, recent studies on RP have recorded epochs of 800 ms duration [29,30] and this procedure should be probably preferable for future research.

It might sound striking not to take baseline measurement previous to stimulus onset for average purposes. It should be noted that the filters used in RP studies involve frequencies that do not lead to a great distortion of the signal. Moreover, as RP is obtained by subtracting the activity evoked by control stimuli from that evoked by the other types of test stimuli, control activity is in fact taken as a baseline.

The common average reference method [10] has proved to be the best one in order to obtain a good and remarkable RP. Martín-Loeches et al. reached this conclusion in a

study in which this method was compared to several others, including mastoids average or Laplacian derivation (see Ref. [12], for a detailed description of results with these average methods).

Measurement of the RP amplitude in a 56-ms window is the habitual procedure in RP research. This might be a small time window, but it seems a reasonable time interval due to the fact that the RP is a short duration component with a remarkable peak.

Data provided by the application of the BESA algorithm should be taken with caution since this algorithm uses a non-realistic spherical head model. The finding that neural generators of the RP are located within the lingual/fusiform cortices seems, however, to be robust as has been reported in three studies [3,12,13]. Nevertheless, any approach related to the location of neural sources on the basis of EEG data has to deal with many trade-offs, the most problematic of them being the inverse problem with its infinite solutions (see Refs. [9,19] for a detailed description of problems concerning spatial resolution and EEG). Although more powerful tools have been developed, such as the low resolution brain electromagnetic tomography (LORETA; [18]) which assumes more realistic brain modeling, any conclusion on data provided by these methods must be carefully interpreted. The application of the rapid stream stimulation paradigm with other neuroimage methodologies, including PET and fMRI, seems both promising and necessary in order to clearly establish the neural generators of the RP.

6.2. Alternative and support protocols

Among the possibilities that the rapid stream stimulation paradigm and the RP offer, several seem of particular interest. Some of them concern the use of the rapid stimulation for presenting words in sentence contexts, the influence of syntactic constraints in those processes reflected by the RP, or age effects on RP. Other frames remain totally unexplored. This is the case of the application of the rapid stream stimulation paradigm in the auditory modality. Finally, the sensibility of the RP to reading skills in normal subjects [27] makes it a very promising tool in the study of reading disorders since no study to date has been conducted to explore such a question.

Nevertheless, a number of variations of the rapid stream stimulation paradigm have been used in other studies. These include the following.

1. A protocol contrasting the recognition potential with another ERP component, the P300 [21].
2. A protocol investigating how the quality of word images affects RP latency [24].
3. A protocol for studying the relationship between attention and word recognition [26].

4. A protocol for investigating word priming processes [25].
5. A protocol for investigating word difficulty and reading ability [27].
6. A protocol with the aim of studying word frequency effects [28,30].
7. A protocol for the investigation of processes concerning letter recognition [30].
8. A protocol that aims to explore the processing of orthographical aspects [29].
9. A protocol for investigating commonalities in the semantic processing of words and pictures [3,22].
10. A protocol for studying the effects of imageability in abstract and concrete words [13].
11. A protocol with the purpose of comparing the semantic processing of animals and tools categories [5].

7. Quick procedure

1. Place electrodes for EEG and EOG recordings.
2. Give task instructions and payment schedule to subjects.
3. Position subjects and tell them not to move their eyes during stimulation.
4. Subjects have to practice.
5. Present sequences to subjects according to the rapid stream stimulation paradigm. Give feedback of their performance after every sequence.
6. Record EEG during the experimental session. EEG is band-pass filtered between 0.3 Hz and 100 Hz, digitized at a sampling rate of 250 Hz and stored on a computer hard disk.
7. Averaging of epochs are separately performed off-line for every type of stimulus.
8. Subtract activity to controls from the one evoked by the other test stimuli for absolute grand-average waveforms and topographical maps representations.
9. Perform statistical analyses on raw data.
10. Explore the topographical distribution of the RP, both descriptively and statistically.
11. Estimation of neural sources.

8. Essential references

8.1. Original papers

Refs. [11–13,21,22,27].

8.2. Reviews

Ref. [6].

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