



# Bereitschaftspotential preceding spontaneous and voluntary eyelid blinks in normal individuals



Isabella Araujo Mota<sup>a,c,\*</sup>, Otávio Gomes Lins<sup>b,c</sup>

<sup>a</sup> Federal University of Paraíba, Brazil

<sup>b</sup> Federal University of Pernambuco – UFPE, Recife, PE, Brazil

<sup>c</sup> Postgraduate Program in Neuropsychiatry and Behavioral Sciences at the Federal University of Pernambuco – UFPE, Recife, PE, Brazil

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## HIGHLIGHTS

- A Bereitschaftspotential (BP) precedes voluntary eyelid blinking in healthy subjects.
- No BP precedes spontaneous blinking.

## ABSTRACT

**Objective:** To investigate the Bereitschaftspotential (BP) preceding spontaneous and voluntary eyelid blinks in normal subjects.

**Methods:** Eighteen healthy individuals (10 female) between 17 and 60 years (mean 35) were studied. The EEG was recorded from 11 scalp positions of the 10–20 international electrode placement system referenced to linked mastoids. The vertical electrooculogram (VEOG) was recorded from two electrodes positioned above and below the right eye. The ground electrode was placed in the right clavicle. The recordings were obtained during spontaneous and voluntary eyelid blinks. Two-second EEG segments before the onset of the blink potential (visually identified at the VEOG channel) were averaged and analyzed off-line. The statistical significance of differences was evaluated by repeated-measures analysis of variance with Geisser–Greenhouse correction for violation of sphericity and the Newman–Keuls test was used for post hoc comparisons.

**Results:** A BP starting around –1700 ms prior to the onset of voluntary blink was observed; it had average amplitude in the negative peak of 3.3  $\mu$ V. There was no BP preceding spontaneous blinking.

**Conclusion:** A BP precedes voluntary blinks but not spontaneous blinks.

**Significance:** This is the first study evaluating the BP preceding spontaneous and voluntary eye blinks.

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## 1. Introduction

The eyelid blink is affected in numerous neurological diseases, occurring with increasing frequency, as observed in dementia, hyperkinetic movement disorders, and schizophrenia, or its reduction, as in Parkinson's patients (Stevens, 1978; Chen et al., 2003; Bologna et al., 2009; Chan et al., 2010). In addition to the protective and moisturizing function of the cornea, the eyelid blink actively participates in saccadic eye movements, fixations, visual cognitive processing and emotional expressions (Delgado-García et al., 2002,

2003). Clinically, it occurs in the form of spontaneous or voluntary movements or reflex responses to sensory stimulation.

The physiology of spontaneous eye blinking is influenced by environmental factors, direct corneal afferent to the spinal trigeminal complex (Kaminer et al., 2011) and by hypo- or hyperdopaminergic modulation of basal ganglia (Taylor et al., 1999; Korosec et al., 2006; Colzato et al., 2008b; Ladas et al., 2014). During voluntary eye blinking, the premotor cortex activation occurs (Kato and Miyauchi, 2003) with subcortical dopaminergic modulation (Korosec et al., 2006; Agostino et al., 2008). The whole neural network and its facilitating and inhibiting factors, however, are not fully understood (Cruz et al., 2011).

The Bereitschaftspotential (BP) or readiness potential, initially reported by Kornhuber and Deecke in 1964, is a neurophysiological

\* Corresponding author at: BR 230, Km 10, Condomínio Villas do Atlântico, casa 3B, Intermares, Cabedelo, PB CEP: 58102-202, Brazil.

E-mail address: [isabella\\_mota@yahoo.com.br](mailto:isabella_mota@yahoo.com.br) (I.A. Mota).

tool that allows access to the premotor cortical neural pathways. This potential is obtained by back-averaging the electroencephalogram (EEG) time-locked to a repeated movement. This negative slow wave starts about one and a half to two seconds before the onset of the electromyographic (EMG) recording of the repeated movement and consists of an early component, related to motor planning and a late component, related to the execution of the movement. The early component would be an unconscious process whereas the late component would be conscious (Deecke et al., 1976; Deecke and Kornhuber, 1978; Deecke, 1987; Neshige et al., 1998; Caviness et al., 1998; Shibasaki and Hallet, 2006).

The early component has a symmetrical bilateral pattern, progressing through the cortex of the supplementary premotor area, supplementary motor area and lateral premotor cortex area, respectively. The late asymmetric component presents clear somatotopy in the contralateral motor cortex to the movement. The early component is influenced by the level of attention, learning, complexity, speed, strength, skill and movement imagination (Shibasaki and Hallet, 2006; Kranczioch et al., 2010).

This study aimed to investigate the presence of a BP preceding spontaneous and voluntary eyelid blinks in normal individuals.

## 2. Methods

### 2.1. Subjects

Eighteen healthy volunteers (ten women), between 17 and 60 years (mean age of 35 years) were included in the study. This study was approved by the Human Research Ethics Committee (HRECs) of the Health Sciences Center of UFPE. All patients signed an informed consent form prior to the start of this research.

### 2.2. Procedures

The individuals sat comfortably in a chair and they were told to look ahead and to avoid moving the muscles of the neck and face. The blinks were recorded under two conditions: spontaneous and voluntary. During the recording of spontaneous blinks the individuals watched a movie on a screen placed at eye level and about two meters away. For the recording of voluntary blinks the patient looked at a fixed point at the center of the screen, being told to blink as naturally as possible every five seconds. The blinking rhythm was trained with the patient before starting the recordings. The EEG was recorded from 11 EEG electrodes placed in positions F3, Fz, F4, C3, Cz, C4, P3, Pz, P4, O1, O2 of the international 10–20 system of electrode placement. Two linked electrodes placed in the mastoid (M1 and M2) and were used as reference. The vertical electrooculogram (VEOG) was recorded from two electrodes placed one centimeter above and below the right eye. The ground electrode was placed on the skin over the right clavicle.

The recordings were carried out by a Neuron-Spectrum NET (Neurosoft) polygraph. The filters used were 0.1 Hz (high-pass) and 35 Hz (low-pass). The impedances (10 Hz) were kept below 3 k $\Omega$ .

### 2.3. Data analysis

The blink potentials were visually identified as a large positive deflection at the VEOG channel and the EEG was back-averaged two seconds preceding the onset of the blink potential. Blinks which presented artifacts (movement, muscle contraction) at the preceding EEG were eliminated from the analysis. The averages were saved in text files and subsequently analyzed in a dedicated program written in MATLAB. This program read the text files, dis-

played them on the screen and performed the measurements to be used in the statistical analysis.

Initially the grand average obtained by the averaging the waves of all subjects were examined for defining the measurement points. These were established in  $-1800$  ms (prior to the beginning of the premotor potential in the grand average), considered as baseline,  $-500$  ms (immediately prior to a small change on the negative wave configuration at the central electrodes, considered as the beginning of the late component) and  $-50$  ms (immediately prior to the start of the blink potential). To minimize the effect of the background noise, the amplitudes were averaged in a segment of 100 ms centered on latency to be measured.

Statistical analysis of BP amplitudes was carried out only on C3, C4 and Cz electrodes. For the analysis, the statistical package STATISTICA version 10 (Stat Soft) was used. Inferential statistical analysis was performed by means of a repeated measure analysis of variance (rmANOVA), with Condition (spontaneous blinks vs. voluntary blinks), Time ( $-1800$  ms,  $-500$  ms,  $0$  ms) and electrode (C3, Cz, C4) as within-subject effects. The Geisser–Greenhouse correction was applied for violation of sphericity. For post hoc analysis, if required, the Newman–Keuls test was used. The critical  $p$ -value was set at 0.05.

## 3. Results

A total of 630 spontaneous blinks and 900 voluntary blinks were included in the analysis, a mean of 35 spontaneous blinks and 50 voluntary blinks per subject.

Fig. 1 plots the grand-mean waveforms of the spontaneous and voluntary blinks at all scalp positions. At voluntary blink condition (thick solid line), a negative potential of increasing amplitude is observed beginning around  $-1700$  ms before the onset of the blink potential. This potential has a broad field and it can be observed at all recorded sites, with larger amplitude in the central regions. These potentials are not observed in the grand-average waveforms preceding spontaneous blinks (dotted line).

Fig. 2 shows the analysis of the amplitudes of the potentials at C3, Cz and C4 measured at  $-1800$ ,  $-500$  and  $0$  ms preceding the onset of the blink potentials. The waveforms and measurement times are shown on the top of the figure. Amplitudes were averaged in a segment of 100 ms centered on latency to be measured (vertical gray shadows). The means and standard deviations of the amplitudes at each electrode, condition and time-point are illustrated at the bottom of the figure.

The ANOVA showed a significant interaction between CONDITION and LATENCY (Table 1, top). Post-hoc analysis (Neuman–Keuls) of this interaction showed that for the voluntary blink condition the amplitudes at  $-500$  ms and  $0$  ms were both significantly larger than the amplitudes at  $-1800$  ms (Table 1, bottom). The amplitudes at  $-500$  and  $0$  ms were not different. In the spontaneous blink condition, the amplitudes were not different among the times.

The ANOVA also showed a significant main effect for ELECTRODE (Fig. 2, top). The amplitudes at C3 and C4 were both larger than the amplitude at Cz, although the difference between C4 and Cz did not quite reach statistical significance (Neuman–Keuls,  $p = 0.0473$  for C3/Cz and  $p = 0.0538$  for C4/Cz).

## 4. Discussion

This research has shown that there is a pre-motor potential preceding voluntary blinks in normal individuals. This potential was not identified preceding spontaneous blinks. This negative slow wave starts about 1700 ms before the blinks and it is distributed widely on the scalp, with greater amplitude in the lateral central

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