



## A VEP study in sleeping and awake one-month-old infants and its relation with social behavior

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### ABSTRACT

With the present study we aimed to analyze the relationship between infants' behavior and their visual evoked-potential (VEPs) response. Specifically, we want to verify differences regarding the VEP response in sleeping and awake infants and if an association between VEP components, in both groups, with neuro-behavioral outcome could be identified. To do so, thirty-two full-term and healthy infants, approximately 1-month of age, were assessed through a VEP unpatterned flashlight stimuli paradigm, offered in two different intensities, and were assessed using a neurobehavioral scale. However, only 18 infants have both assessments, and therefore, these is the total included in both analysis. Infants displayed a mature neuro-behavioral outcome, expected for their age. We observed that P2 and N3 components were present in both sleeping and awake infants. Differences between intensities were found regarding the P2 amplitude, but only in awake infants. Regression analysis showed that N3 amplitude predicted an adequate social interactive and internal regulatory behavior in infants who were awake during the stimuli presentation. Taking into account that social orientation and regulatory behaviors are fundamental keys for social-like behavior in 1-month-old infants, this study provides an important approach for assessing physiological biomarkers (VEPs) and its relation with social behavior, very early in postnatal development. Moreover, we evidence the importance of the infant's state when studying differences regarding visual threshold processing and its association with behavioral outcome.

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### 1. Introduction

From birth, infants respond differently to the surrounding environment by changing their state, being able to attend to distinct visual stimuli. This ability is especially noticeable in their preference for the human face, particularly their mother's, which is addressed by the infant's eye gaze and the imitation of face-like patterns (Johnson et al., 1991). Once this orientation behavior towards the environment is displayed very early, several authors have been interested in characterizing young infants according to their reactivity to external stimuli (Mikkola et al., 2007; Pihko et al., 2004; Ceroni et al., 2002). This reactivity can

be decoded through different behavioral characteristics (Rothbart, 2007; Calkins et al., 1996). For instance, Feldman and Eidelman (2006) showed that full-term newborns exhibit mature neuro-behavioral profiles emphasizing their state organization, motor maturity and higher orientation scores to both social and non-social stimuli, as well as more settled cognitive development and interactive behavior when assessed later.

Across the infancy period, developmental and behavioral changes are accompanied by brain alterations as the infant's response to the environmental stimuli changes in parallel to brain maturation mechanism (Huttenlocher, 2009). Different studies have used psychophysiological techniques, such as event-related potentials (ERP), to assess changes in the infant's brain activity that occur in response to a stimulus that is repeatedly presented. Particularly, the study of visual evoked potentials (VEPs) has been widely used to expand our knowledge about the different neurodevelopmental pathways in very young infants, allowing for further comprehension about the visual maturation and cortical function

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mechanisms, as well as visual sensory processing (McGlone et al., 2013).

Evidence from VEP studies has suggested that, at approximately 1 month of age, the presence of early VEP components, such as P2 or N3, play an important role as indicators of healthy brain development, as their presence is associated with visual processing and proper neural maturation of the visual cortex (McGlone et al., 2013; Kato and Watanabe, 2006; Benavente et al., 2005; Kraemer et al., 1999). Indeed, evidence from several studies has shown that the P2 and N3 are present in early ages, being characterized as the most robust components in sensory stimuli processing, translating a mature VEP neural development (McGlone et al., 2013). However, there seems to be little consensus regarding the VEP characteristics when assessed in awake or sleeping infants (Mercuri et al., 1995; Whyte et al., 1987). In fact, some studies have suggested differences regarding VEP components' latency and amplitude depending on the infant's alertness state, particularly reporting that awake infants display greater P2 amplitudes and shorter latencies (Benavente et al., 2005; Mercuri et al., 1995). In a study, conducted by Shepherd et al. (1999), with only a full-term infant, the authors have found differences regarding the N1 and P2 amplitude and peak latencies depending on the infant's behavioral state. Indeed, infants' state and its implications for development have been addressed, indicating that both sleeping and awake states seem essential for development and neural maturation mechanisms (Mento and Bisiacchi, 2012; Fifer et al., 2010).

More commonly, alterations in VEP morphology are linked to a typical developmental features that are mirrored in neurobehavioral changes, with implications for both cognitive and social domains (Liu et al., 2010; Kato and Watanabe, 2006; Tsuneishi et al., 1995). These physiological differences, when correlated with neurodevelopmental outcomes, may be used as physiological markers for the early identification of developmental pathways (Liu et al., 2010; Isler et al., 2007; Majnemer et al., 1990). Associating visual processing through a VEP assessment in very young infants may be a useful approach to identify abnormal developmental characteristics (Stanley et al., 2009), thereby, contributing to a better understanding about its implications in cognitive and behavior abnormalities (Kirk et al., 2013; Sampaio et al., 2008).

Therefore, with the present study, our objective was to identify VEP components in 1-month-old infants' response to an unpatterned flashlight visual stimulus offered in two different intensities in awake and sleeping infants. Additionally, we aimed to analyze if the VEP response can predict adjusted neurobehavioral outcomes. Taking into account previous studies, our hypothesis was that the VEP components could be identified in very young infants in the two intensities, with greater activation being displayed in response to the higher intensity stimulus. Moreover, we hypothesized that this response differed according to the infants' state (sleeping vs. awake infants), with this physiological response predicting mature neurobehavioral profiles with respect to their reactivity to both external (orienting/interactive characteristics) and internal stimuli (regulation characteristics).

## 2. Materials and methods

### 2.1. Participants

This study was reviewed and accepted by the ethical committee from Hospital Pedro Hispano in Matosinhos, Portugal. Mother/infant dyads were recruited at the Obstetric Department when the infant was born. Thirty-two healthy, full-term infants, aged 1-month-old, were assessed regarding their VEP response (17 [53%] sleeping and 15 [47%] awake). From this total, we lost 14 participants' neurobehavioral assessment due to different distress

**Table 1**  
Infant's health characteristics at the time of recruitment and collection.

	Participant's characteristics	
At recruitment time	Gestational age (mean weeks)	39
	Weight (mean kg)	3235
	Height (mean kg)	48.7
	Apgar score (10th min)	10
At collection time	Age (mean days)	33
	Total with VEPs	32
	Total with NBAS	18
	Total with VEPs and NBAS	18

presented at the moment of data collection (infant's behavioral distress, mothers' availability or even due to feeding routines). Therefore, overall, the total of infants having both the VEP and neurobehavioral assessments is 18 (10 girls and 8 boys; 9 in the sleep group and 9 in the awake group) – see Table 1.

For the state characterization we used the states concept developed and described by Brazelton and Nugent (Brazelton and Nugent, 1995). We considered as being in sleeping state those infants who presented eyes close, regular respiration and no or little spontaneous body movements (either in deep or active sleep). The awake infants were characterized as having bright look, directed to the stimuli, minimal motor activity and reactive to the stimuli. In this category, we also included infants that were irritable during the stimuli presentation.

### 2.2. VEP stimuli

White flashes were presented using the lamp of a Grass PS33-Plus Photic Stimulator (Astro-Med Inc., Warwick, USA), positioned at 50-cm distance from the infant. The stimulus was offered in two blocks of repeated flashes with the same frequency (2 Hz) separately, and each block with a different intensity, during 1 min. The stimulation intensity was set at 1 (0.09 J – intensity 1) and 2 (0.18 J – intensity 2) in the flash position (Odom et al., 2010). For each block presentation, the flash position was organized for the purpose of achieving different combinations and offered in a pseudo-randomized way (1 and 2; 2 and 1) so that we could control the presentation order effect.

### 2.3. VEP data recording and analysis

Electroencephalographic activity was recorded with a Quick-Amp™ system, with a 32-electrode Acticap™ System inserted in a cap with a frontopolar ground and average referenced. 32 recording electrodes were placed at Fp1, Fp2, F3, F4, Fz, F7, F8, FC1, FC2, FC5, FC6, T7, C3, Cz, C4, T8, TP9, CP1, CP2, CP5, CP6, TP10, P3, P4, Pz, P7, P8, PO9, O1, Oz, O2, PO10 in accordance with the international 10–20 system (Jasper, 1958) and electrode impedances were kept below 10 kΩ for all participants. EEG signals were continuously amplified, digitized at sample rate of 250 Hz and filtered on-line with a 0.01–100 Hz (12 dB/octave slope) band pass filter using a Quick-Amp™ system amplifier and Brain Vision Recorder software (Version 1.20). All EEG data was analyzed with Brain Vision Analyzer software (Version 2.0.1). The EEG was digitally filtered off-line with a 0.2–20 Hz band pass filter and 50 Hz notch filter. It was then corrected for ocular artifacts by the semiautomatic procedure in independent component analysis (ICA) (Jung et al., 2000) and segmented into epochs of 600 ms from 100 ms pre-stimulus to 500 ms post-stimulus. Next, baseline correction was applied and

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