Journal of Visualized Experiments

How to Obtain Reliable Visual Event-Related Potentials in Newborns

--Manuscript Draft--

Article Type:	Methods Article - JoVE Produced Video
Manuscript Number:	JoVE60164R1
Full Title:	How to Obtain Reliable Visual Event-Related Potentials in Newborns
Keywords:	EEG; visual evoked potentials; sleep; newborn; preterm; active sleep; polysomnography
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Additional Information:	
Question	**Response**
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TITLE:

How to Obtain Reliable Visual Event-Related Potentials in Newborns

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KEYWORDS:

EEG, visual evoked potentials, sleep, newborn, preterm, active sleep, polysomnography

SUMMARY:

Several important points for obtaining high-quality reliable visual evoked potentials (VEPs) in newborns while minimizing variability and the risk of misleading prognoses are presented.

ABSTRACT:

The present study discusses the characteristics of visual event-related potentials (VEPs) and outlines methodological steps for obtaining reliable measurements in newborns. Obtaining high-quality, reliable VEPs is crucial for the early detection of abnormal development of the central nervous system in at-risk newborns, and for implementing successful early interventions. Recommendations are based on a previous study which showed that when post-conceptional age, polysomnography-identified sleep stages, and light-emitting diodes (LEDs) googles as the luminous source are controlled, no more than 4 repetitions of VEP averages are required to obtain replicable recordings, variability decreases, and reliable VEPs can be obtained. By controlling for these sources of variability and using statistical analyses, we were able to clearly and reliably identify the amplitude and latency of three main components (NII, PII and NIII) present in 100% of newborns (n = 20) during active sleep. Recording VEPs during awake states, quiet sleep and transitional sleep is not recommended because VEP morphology may differ significantly from one average to the next, leading to the risk of misleading clinical prognoses. Moreover, it is easier to obtain VEPs during active sleep because this state can be clearly and reliably identified at this stage of development, sleep cycles are short enough to allow measurements to be taken in a reasonable time, and the method does not require new o expensive equipment.

INTRODUCTION:

Early detection of abnormal development of the central nervous system in at-risk newborns is crucial for successful early interventions[1,2]. Visual event-related potentials (VEPs) provide a useful means of evaluating visual cortical status because they do not require patient cooperation, which is not possible in the first month of life, are objective, and are sensitive to structural and functional brain damage[3,4].

Though, some studies of newborns have shown that normal visual-evoked responses indicate adequate neural maturation of the cerebral cortex[4,5], and that this has often been studied in newborns to assess neurodevelopment and identify abnormal development of the visual pathways[4,5], the clinical use of VEPs has been limited by the variability observed in their morphology[4-7]. Therefore, it is important to obtain better, more reliable characterizations of VEPs in newborns.

One cause of the variability in VEP morphology is that earlier studies have mixed preterm and older babies (over one month)[8-10]. However, the most important source is the lack of attention paid to the infants' behavioral state while recording VEPs; namely, awake, quiet (QS), active (AS), or transitional sleep. QS and AS have either not been analyzed separately[5,11,12], or studies have relied exclusively on behavioral observation without using polysomnography to identify states[7,8]. *Tracé alternant,* which consists in bursts of high amplitude slow activity alternating with inter-burst intervals of minimal amplitudes is present in QS, but has not been taken into account when averaging VEPs. Some studies with newborns have measured VEPs by recording during wakefulness[13,14], but at this stage of development waking periods are brief and newborns are usually crying or moving, which makes it difficult to obtain high quality, reliable recordings.

Few studies have used light-emitting diodes (LEDs) googles[6,9] to elicit VEPs, though this light source generates more consistent recordings than the usual strobe flashes of white light[11,14,15], which are less reliable. Obtaining replicable VEPs in the same newborn is indispensable for clinical use[4], but another cause of variability is the low reproducibility of VEP morphology, likely due to the lack of control of physiological states and of the stimuli used to elicit VEPs. Given these conditions, the high variability of VEP morphology is hardly surprising.

A previous study conducted with 20 healthy full-term newborns that considered several sources of variability: post-conceptional age, polysomnographically-identified sleep states, LED googles to elicit VEPs, and measures of reproducibility between two VEP averages found that a clearer, more reliable VEP morphology can be obtained during active sleep. During this sleep stage all infants generated clear VEPs with higher correlations between two averages than in QS. Also, fewer VEP averages were required to obtain reproducibility[16].

Given the clinical usefulness of VEP studies to assess, as early as possible, the integrity of visual pathways, this study proposes a series of methodological steps designed to obtain reliable VEPs in preterm and older newborns, using LED goggles during AS unambiguously defined by

simultaneous polysomnography.

PROTOCOL:

This experimental protocol follows the principles of the World Medical Association's Helsinki Declaration (2013) and was approved by the Ethics Committee at the Children's and Women's Specialty Hospital, Querétaro, Mexico.

1. Preparation of the newborns

NOTE: The procedure followed is innocuous and painless, so there are no counter-indications for evaluating full-term and preterm newborns, once they are clinically stable.

1.1. Ensure two and half hour fasting and wakefulness before beginning the study, in neonates older than 40 weeks of postconceptional age.

1.2. Make sure that the baby's head be washed with neutral soap the day before the study. Thus, his/her hair will be clean and dry. Do not apply conditioners.

1.3. Allow the mother to start feeding the newborn 30 min before beginning the study. Allow him/her to burp and start the sleep wrapped in sheets. This will ensure that he/she sleeps easily and spontaneously.

1.4. Wash hands carefully before handling the neonate.

1.5. Use sanitary masks.

1.6. Gently wipe the scalp of the newborn with a cotton ball or gauze soaked in alcohol to remove residual dirt and superficial grease, before the neonate falls asleep.

1.7. Measure the distance between nasion and inion, and between both pre-auricular pits. Calculate 10% and 20% to ensure proper placement of the cranial electrodes according to the international 10-20 system of electrode placement.

1.8. Cover the newborn's entire head with a tubular elastic mesh for the correct attachment of the electroencephalography (EEG) and VEP electrodes. Leave the face fully free and exposed, as shown in **Figure 1**.

1.9. Mark on the mesh the location of the surface electrodes.

1.10. Use a swab to perfectly separate the newborn's hair at the sites where each electrode will be placed, and lightly rub the skin with abrasive gel for neurophysiological studies.

NOTE: Reschedule the study if the neonate takes more than 2 h to fall asleep.

2. Placement of the surface electrodes for EEG and VEP sleep recording

NOTE: Before beginning, set the values of the instrument's frequency filters using the specifications in **Table 1**. It is advisable to connect all electrodes to the EEG and VEP instruments before placing them on the newborn.

2.1. Place the elastic band sensor on the baby's chest to record thoracic respiratory expansion.

2.2. Place the individual surface disc electrodes (standard silver–silver chloride, or gold disc electrodes) with conductive paste through the mesh to fix them in the cranial locations established by the international 10-20 EEG system, adapted for newborns.

2.3. Locate the cranial electrodes for EEG at leads F3, F4, C3, C4, O1 and O2, or at least C3 and C4, referred to linked earlobes, to identify the stages of neonatal sleep.

2.4. Fix the surface disc electrodes to the skin with medical adhesive tape. To record ocular movements (EOG), place one electrode 1 cm above the external canthus of the left eye and place another 1 cm below the external canthus of the right eye, also referred to linked earlobes.

2.5. Similarly, attach the electrodes for surface electromyogram recording (EMG) on both sides of the chin, referenced against each other.

2.6. Use two channels of the VEP equipment with the following leads: Oz (-) vs. Fz (+), and Oz (-) vs. A1 (+); the ground electrode is to be placed on the right mastoid.

2.7. Set the analysis time for VEP registration in 600 ms.

NOTE: **Table 1** shows the filter settings used for recording sleep EEGs and VEPs.

2.8. Do not begin VEP recording until impedance values are below 5 kΩ.

3. Sleep recording

NOTE: VEPs are obtained while the newborn sleeps in hospital crib; the sleep stages are monitored simultaneously by polysomnography[17,18].

3.1. Prolong the EEG recording for 60−90 min or until AS is identified, to evaluate active (AS) and quiet sleep (QS) in newborns.

3.2. Begin EEG recording while carefully observing the characteristics of neonatal sleep, to identify the active sleep stage, during which VEPs will be recorded.

3.3. Identify neonatal sleep stages according to the criteria summarized in **Table 2**.

4. VEP recording

NOTE: VEPs are registered according to established standards[19,20].

4.1. Allow one minute of EEG recording without visual stimulation when the neonate begins well-defined active sleep.

4.2. Apply monocular light stimulation through handheld goggles with a LED matrix held manually 2 cm directly above each newborn's eyes.

4.3. Observe if the infant has his eyes closed during VEP registration in AS and note if this does not occur.

4.4. Begin the averaging of the VEPs in the equipment, presenting 20 to 40 luminous stimuli whose corresponding recordings are averaged to obtain an average curve or evoked response.

4.5. Observe the reproducibility of the recorded averages. At least two reproducible evoked potentials are recommended.

4.6. Visually recognize PII component of the VEPs during recording, since this peak is considered typical of neonatal VEPs. Identify the PII component as the maximal positive peak between 120 and 300 ms, preceded by a negative wave (NII) and followed by a maximal negativity between 200 and 400 ms, also called NIII.

4.7. Stop the averaging of the VEPs if the newborn moves excessively, wakes up, or changes to another sleep stage, distinct from AS. Renew recording once the AS stage reestablishes.

NOTE: This point is critical, because VEPs obtained during QS or transitional sleep are less reliable than in AS.

4.8. Finish registration after 2 averages with reproducible VEP are attained, or when 6 averages occur without a recognizable VEP. In the latter case, consider the result an absence of a replicable response.

5. Review and analysis of VEPs

NOTE: **Figure 2** shows the main components of neonatal VEPs and their measurements.

5.1. Evaluate the reproducibility of the VEPs by similar appearance and measurements between the two averaged curves.

NOTE: Some VEP recording systems offer a correlation measure between two averages.

5.2. Measure the absolute latencies of the NII, PII and NIII waves using the device's cursors. Absolute latency is the time in ms elapsed from the onset of stimulation to the maximal or minimal peak of each component.

5.3. Calculate the interpeak latencies in ms, including the differences between the absolute PII-NII, NII-NIII and PII-NIII latencies.

5.4. Measure peak-to-peak amplitudes in µV, for the NII-PII and PII-NIII components.

5.5. Compare the latency and amplitude values obtained to the normal, or expected, values estimated for a population of healthy, similar-age newborns.

REPRESENTATIVE RESULTS:

To detect adequate maturation in the function of the visual pathway it is essential to obtain the PII component of the VEP, which can be seen in both term and preterm infants. The simultaneous recording of VEPs with polysomnography during AS makes it possible to obtain typical VEPs.

Reliable VEP studies require obtaining reproducible average waveforms that will be indispensable for clinical use. **Figure 2** illustrates, in a healthy full-term newborn, a clear positivity around 200 ms, which is compatible with the PII component. NII, which corresponds to a preceding, negative small potential, is evident at about 130 ms. The NIII component follows PII as a negativity of approximately 300 ms.

Figure 3A shows three epochs of sleep EEGs, with the typical aspects of AS, QS and *tracé alternant.* **Figure 3B** shows the following: a typical VEP waveform with a clear PII in a full-term newborn; an immature response observed in preterm newborns that is normal at this age; and a non-replicable waveform, with waves that do not reproduce exactly the shape of the previous average, thus making it impossible to reliably measure the true latency or amplitude of the components. These VEPs were obtained in a 36 weeks preterm newborn with periventricular leukomalacia.

The application of these procedures allows obtaining reproducible VEPs, but the waveform may vary, according to the age of the newborn and the presence of risk factors. For example, in premature neonates, the form of the VEP can show an immature appearance with inverse polarity and maximum negative amplitudes, but these will change as the baby approaches the full-term age. It is important to identify these normal differences and their relation to the newborn's age, since the true condition of abnormality is given by the absence of reproducible responses or the interhemispheric asymmetry of the potentials obtained by monocular stimulation.

FIGURE AND TABLE LEGENDS:

Figure 1: Final placement of the surface electrodes to perform EEG and VEP recording in neonates. Observe the location of the cranial electrodes below the elastic mesh, and the immobilization of the newborns by wrapping them in blankets.

Figure 2: The NII, PII and NIII components of neonatal VEPs. Observe the measurements of the absolute latencies for NII, PII and NIII (solid arrows); the inter-peak intervals for NII-PII, PII-NIII and NII-NIII (dotted arrows); and the amplitude of the different components (dashed arrows).

Figure 3: Illustration of the EEG tracings in the sleep stages and the VEPs obtained. (**A**) Three 30-s periods of neonatal EEG with the main features of the stages of AS, QS and *tracé alternant*. (**B**) Three examples of VEPs waveform morphology in neonates, the first with 40 weeks of post-concepcional age, the second with 35 weeks, both are healthy newborns. Note in the second example the polarity inversion. The third is an example of a non-reproducible response, obtained in a 36 weeks newborn with periventricular leukomalacia.

Table 1: Instruments settings for recording neonatal EEG and VEP.

Table 2: Criteria applied for electroencephalographical detection of neonatal sleep stages.

DISCUSSION:

Three components of visual-evoked responses (NII, PII and NIII) were characterized in healthy, full-term newborns while doing stimulation with LED googles, and recorded during polygraphically-identified sleep states. The VEP morphology observed is consistent with previous results reported for fewer neonates[11,15]. The characterization of VEP responses was achieved by recording 20 healthy, full-term newborns at similar post-conceptional age[16]. This methodology allowed researchers to reduce the variability in VEP responses that has been reported previously when no control of age was applied so the study groups mixed preterm, newborns and several-months-old babies, no attention was paid to sleep states and white light strobe flash was used to elicit VEPs[7,10,11,15]. No significant differences were found in amplitude and latency between the VEPs obtained in QS and AS, but those recorded during the latter sleep stage are recommended because they can be obtained in 100% of newborns with fewer average repetitions required to obtain reproducibility. VEPs recorded during QS, in contrast cannot be obtained in all neonates, and require more repetitions[16].

VEP morphology during AS is more reliable because the reproducibility of the characteristics of these VEPs is significantly higher, as shown by the correlation between the two VEP averages. During QS, however, morphology can differ from one average to another because atypical morphologies are often seen, and lower correlations are obtained. As a result, the risk of arriving at misleading prognoses is higher[6,13].

For these reasons, recording VEPs during transitional and QS stages should be avoided. Although studying visual function in wakefulness is optimal in adults[21], in newborns cephalic movements to avoid luminous stimuli, blinking and electromyographic artifacts can all compromise VEP recording. The variability introduced during QS is probably due to *tracé*

alternant, which is present until 43-44 weeks of post-conceptional age[17,18]. VEP recording during AS, in contrast, is easier because the absolute power of EEG activity is lower in this sleep stage, and maximum relaxation due to muscular atonia helps avoid technical artifacts caused by movement. Finally, the use of handheld LED googles minimizes the variability introduced by eye movements and ambient light. To consult normal results of VEPs to handheld LEDs by age, see Taylor et al. and Tsuneishi and Casaer[9,22].

The methodology described herein has several advantages. First, there is no need for new or sophisticated equipment, just a polygraph synchronized with luminous stimuli or simultaneously recorded polysomnography during VEP recording. Second, it is easier to identify active sleep reliably than quiet sleep or awake states; AS is easily accessible since it occurs at the beginning of spontaneous neonatal sleep and occupies 50% of the time asleep at this age[17,18]. Third, this approach is not time-consuming because sleep cycles in this developmental stage are very short, lasting only around 40 minutes.

ACKNOWLEDGMENTS:
Engineer Héctor Belmont, Dr. Mónica Carlier, Dr. Yuria Cruz and Dr. María Elena Juárez collaborated in data collection. The authors thank Paul Kersey for revising English language use. The project was partially funded by PAPIIT grant IN2009/7 and CONACYT (National Council for Science and Technology, Mexico) grant 4971.

DISCLOSURES:
The authors have nothing to disclose.

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Figure

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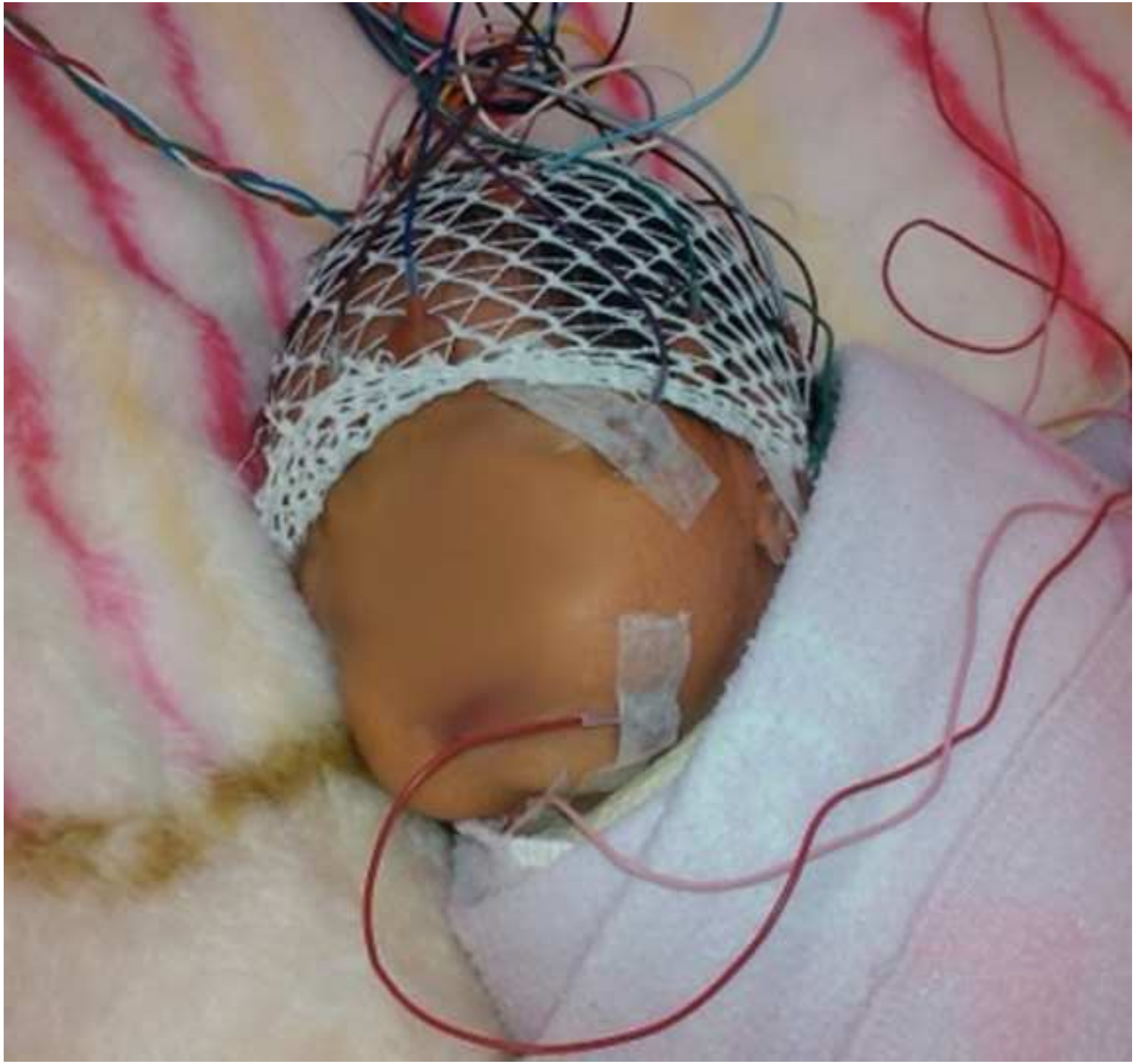

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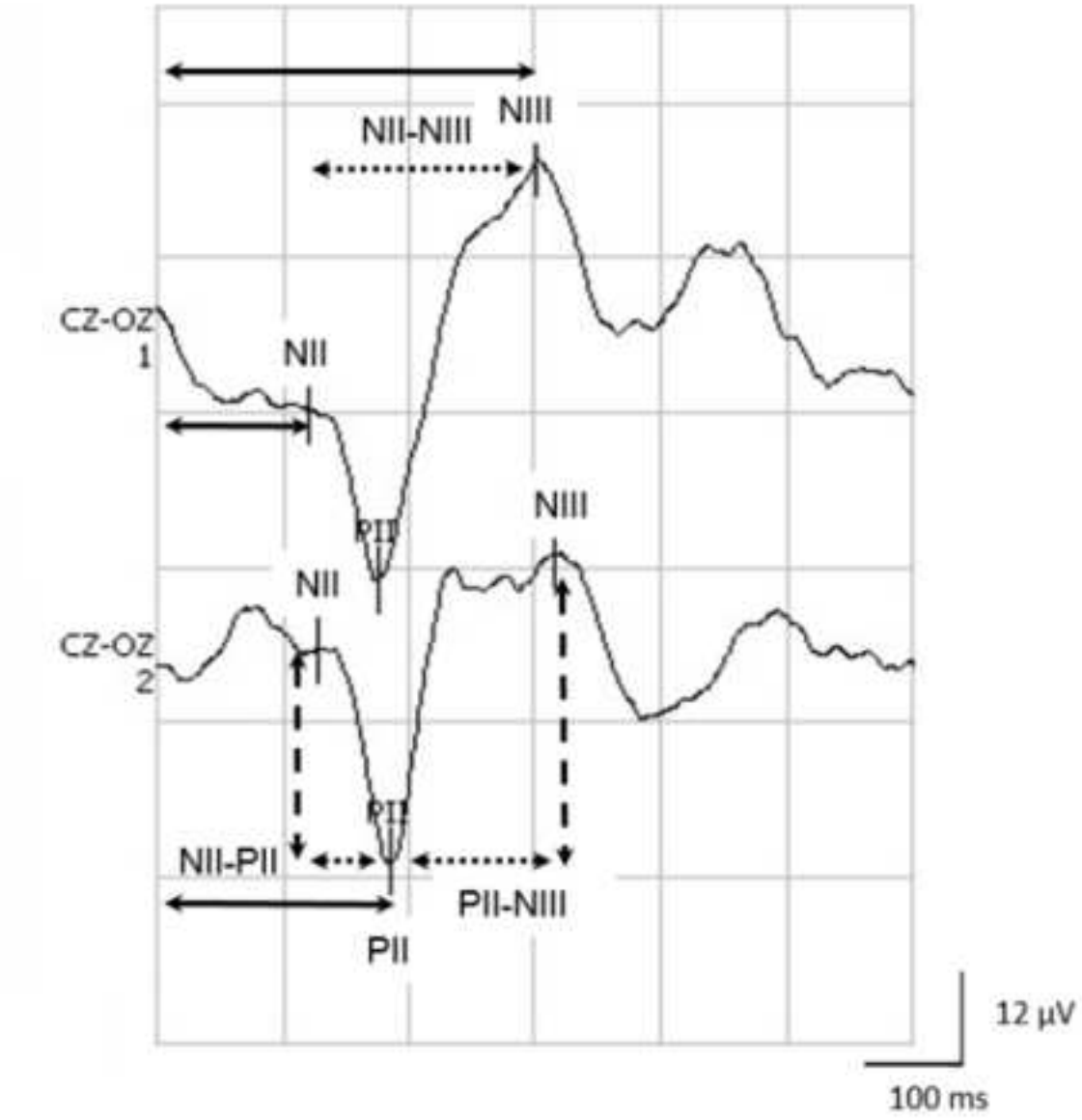

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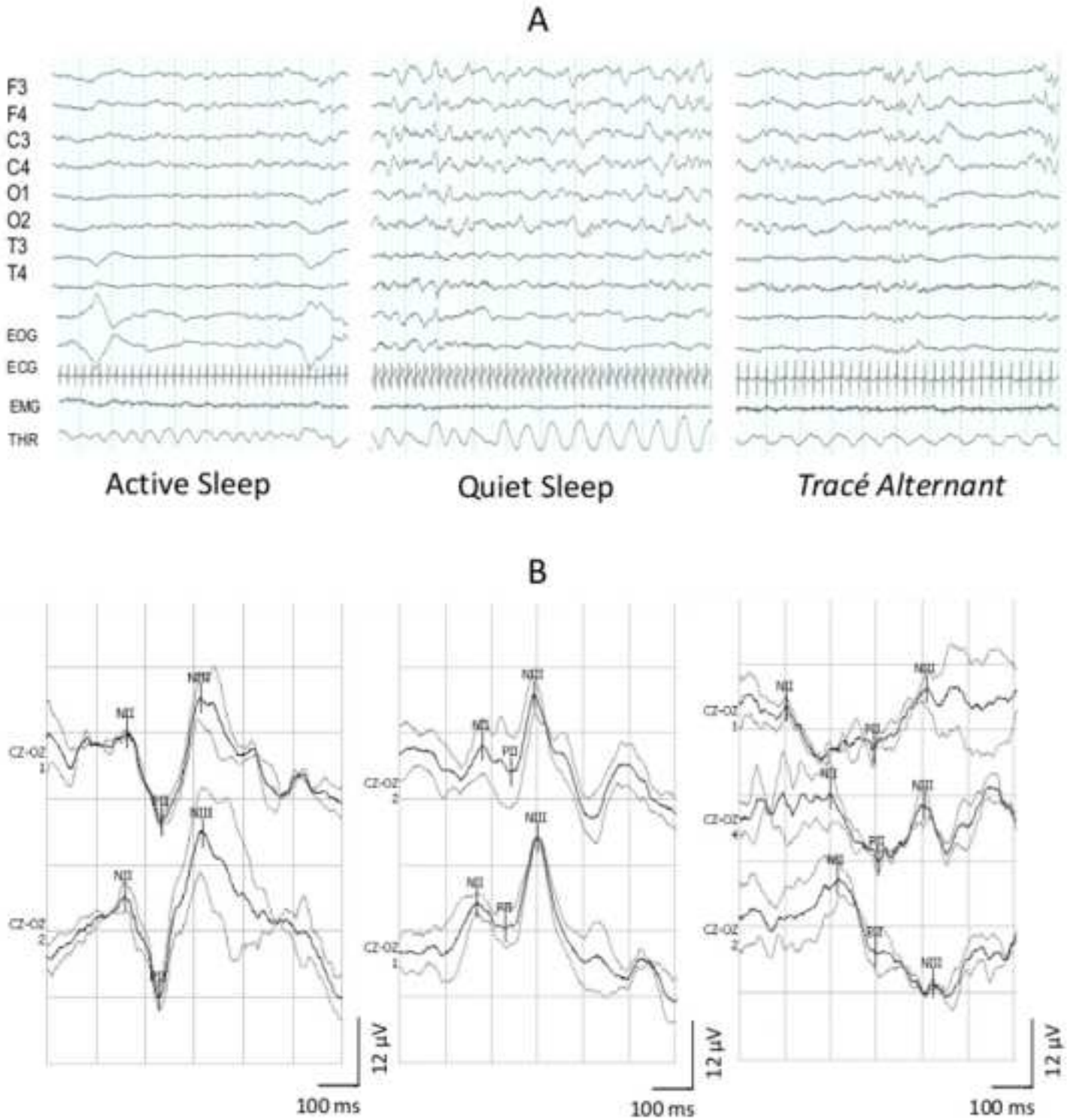

Table 1

EEG channels	Sensitivity	Low-frequency filter	High-frequency filter	Display time
Scalp leads (according to the international 10-20 system)		0.5 Hz	30 Hz	30 s
Electrooculogram (EOG)		0.5 Hz	30 Hz	30 s
Surface electromyogram (EMG)		1 Hz	100 Hz	30 s
Chest wall respiration (THR)		0.5 Hz	30 Hz	1 min
Electrocardiogram (ECG)		0.5 Hz	30 Hz	30 s
VEP Channels				
Cz-Oz	12 µ/div	1 Hz	100 Hz	600 ms
A1-Oz	12 µ/div	1 Hz	100 Hz	600 ms
Maximal averaging	100			
Stimulation	Monocular, one eye first, then the other			
Light	Red			
Intensity	Standard flash of 3 cd·s/m^2			
Stimulator type	Handheld LEDs google			
Frequency	1 Hz			
Duration	10 ms			

Notch filter (60 Hz)	Sampling rate
ON	200 Hz
ON	200 Hz
ON	200 Hz
ON	200 Hz
ON	200 Hz
ON	
ON	

Stage of the sleep-wake cycle	Predominant pattern in the EEG	Ocular movements	Surface chin electromyogram
Wakefulness	Frequent artifacts by movements. Irregular low-amplitude EEG+C23	Eyes open, blinking, transient closing when crying	Presence of large amplitudes
Active sleep (AS)	Irregular low-amplitude EEG	Eyes closed with conjugated and rapid eyes movements	Absent or at minimum levels throughout recording
Quiet sleep (QS)	Slow waves of large amplitude and *tracé alternant*	Eyes closed; absence of ocular movements	Muscle tone present is less than during wakefulness
Transitional sleep	3 traits of QS and 2 of AS are present in the same 30 seconds segment or vice versa		

Breathing
Irregular
Irregular
Regular
Regular or irregular

Name of Material/ Equipment	Company
Digital Electroencephalograph	Neuronic Mexicana, S
Evoked Potentials equipment	Neuronic Mexicana, S
Nuprep Gel	WEAVER and Compan
Ten20 Conductive Paste	WEAVER and Compan
Tubular elastic mesh bandage	Le Roy

	Catalog Number	Comments/Description
	Medicid 3E	Sleep electroencephalogram record
	Neuronic PE (N_N-SW-2.0)	Visual evoked potentials record
	ıy	Skin preparing abrasive gel (114 g)
	ıy	Neurodiagnostic electrode paste (228 g)
		Fixation of cranial surface electrodes, Siz

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Date:	April 25, 2019

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June 17, 2019

Ronald Myers, PhD, Editor
Senior Science Editor JoVE
1 Alewife Center, Suite 200, Cambridge, MA 02140

Dear Dr. Myers:

Please, find attached the resubmission of manuscript "How to obtain reliable visual event-related potentials in newborns", by Lourdes Cubero-Rego, et al., to be resubmitted as an original article to JOVE, for consideration of publication. Please, address all answers to Lourdes Cubero-Rego (lourdes.cubero@gmail.com), during the next month because the corresponding author (Dr. Josefina Ricardo-Garcell) will be in another country without internet.

Thank you very much for your valuable comments. We have considered and made the changes suggested by the editorial committee and the reviewers, as detailed below.

Editorial committee

1. "…Please submit each figure as a vector image file to ensure high resolution throughout production: (.svg, .eps, .ai). If submitting as a .tif or .psd, please ensure that the image is 1920 x 1080 pixels or 300 dpi. Additionally, please upload tables as .xlsx files." It has already done

2. Authors and affiliations: Please provide an email address for each author. They are provided. Page: 1 lines: 13, 15, 17

3. 3. Please include single line spacing between each numbered step or note in the protocol. It has already done.

4. "…Please revise the Protocol to contain only action items that direct the reader to do something (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "NOTE." It has already done

   Page 3 Lines: 94-100, 115, 214, 125,

   Page 4 Lines: 144, 145, 148, 151, 158-174.

5. "…Please include all safety procedures and use of hoods, etc.

   Page: 3 Lines: 101, 102

6. Please move the discussion about the protocol to the Discussion. Has already done

Reviewers' comments:

Reviewer #1:

1. "…For readers who may not be familiar with AS and QS, perhaps explaining the proportion of time a newborn spends in REM vs. non-REM sleep may be useful". It has already done, page 7, lines 265-267.
2. "…Preparation of newborns 1.1: We suggest more specific language in this section. Please state the maximum amount of time between feeding and study and also adding a sentence about burping the infant. It has already done, page 3, line 115.
3. "…If the authors have data or citations to further speak to the relationship between gestational age at study and VEP amplitude and latency, this information could also be helpful for clinicians and researchers. It has already done, page 6, lines: 261, 262.

Reviewer #2:

1. Line 94. Is the value 0.5 correct or it should be 5 or less? We agree with the pointing, the correct value is 5 or less. The correction is in page 4, line 139.
2. Line 122. Microporous tape should be medical adhesive tape. It has already done, page 3, line 129.
3. Lines 147-148. Please provide the light intensity of the stimulus and explain that is a stroboscopic stimulator. It has already done, page 3, line 115.
4. Line 179 - remove "of". It has already done, page 5 line 188.
5. Line 234 - "flashes were used". The LED goggles are also flash stimulator. You can find an updated explanation in reference #20. It has already done, page 6, line 242-243.
6. Line 245 - The authors state that recording VEPs during awake stage should be avoided. This is a very radical statement. Of course if you are assessing visual responses the better would be with the neonate awake with open eyes. You can find a comparison on different stimulators for flash VEP in adults on Podja_Wilczek et al, Doc Ophthalmol, 2018. It has already done, page 6, lines 253-256

We are very grateful for the time devoted to the consideration of this work.

Best regards,

Lourdes Cubero-Rego, PHD, on behalf of the authors.