Electrophysiological Answer to a Checkerboard Stimulus: A Pilot Study

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Abstract—Electroencephalography is a clinical signal that reveals the brain's electrical activity. In this category, the Visual Evoked Potentials (VEP) is one of the most frequent measures, especially when it is necessary to assess the maturity and function of the central visual system. The Electrodermal Activity (EDA) data gives information about skin conductance, and it is used to evaluate autonomic sympathetic reactions, often related to neuropsychological states. It could be used with all ages and in young subjects with healthy development or clinical practice with children with atypical development. In this paper, we propose an experimental setup based on checkerboard stimuli to assess the evolution of visual system development of preterm infants. This experimental protocol was applied to two female preterm born infants of 4 and 6 months of corrected age. The preliminary findings show that, as expected, the P100 latencies and amplitude are still different from those expected for adults and older children. However, the older infant presents results more similar to adults, corresponding to having a more mature visual system. Concerning EDA, it was observed that the older infant presents more responses to the stimulus, a higher level of skin conductance, and a shorter latency time than the younger infant, which is congruent with what is expected, given the maturation of the nervous system. The methodology used in this pilot application and the algorithm defined seem adequate to use in a longitudinal study to follow the evolution of preterm and term infants.

Keywords _ Electrophysiology; visual evoked potentials; electrodermal activity; preterm born.

I. INTRODUCTION

Electroencephalography is used in neuroscience to explore the electrical activity of living neurons. These cells communicate using electrical and chemical signals, and electroencephalographic techniques aim to measure this Mónica Baptista Central Lisbon University Hospital Center; Lisbon, Portugal monica.baptista@chlc.min-saude.pt

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activity non-invasively [1]. The electroencephalography signals have been measured since the XIX century [2], but optimization of signal processing techniques has helped uncover information that changed the way various diseases were diagnosed previously [3].

Visual Evoked Potentials (VEP) are massed electrical signals generated by occipital cortical areas in response to visual stimulation [4]. Specifically, the VEPs are utilized in research and clinical practice to characterize the function and integrity of the visual system, mainly optic nerve lesions, optic neuritis, and multiple sclerosis [5]. Those bioelectric signals generated in the striate and extrastriate cortex by retinal stimulation could be recorded from the scalp using electrodes [5] positioned in the specific Brodman Area 17, 18, and 19 [4] that can provide important diagnostic information regarding the functional integrity of the visual system [6].

Different stimuli are currently used to analyze the visual cortex response. These could be divided into two kinds of visual stimuli: unpatterned flashing lights and patterned stimuli. The most used pattern is a checkerboard with black and white squares [5]. In a pattern-reversal paradigm, the pattern is alternated without change in the luminance at a specific reversal rate [4]. Although the most used sample rate is 2 reversals per second [6], other taxa could be used with specific populations [7]-[10]. With that kind of stimuli, the typical waveform consists of N75, P100, and N135 peaks [6].

In traditional methodologies, the assessment is done in a specific location using fixed equipment. However, current clinical and research demands lead to developing portable devices, like g.Nautilus© [11] allows for a dependable data

recorder at the subjects' location. To assess is necessary to be stationed in a quiet room, guaranteed a typical ambient light level, correct positioning of the electrodes, and participants at least 70 cm from the stimulus [12]. To record the data, single electrodes could be used in occipital positions or a CAP where the electrodes are already positioned. This system's advantage is that the sensors' correct positioning is guaranteed. However, regardless of the system to position the sensors, they must be connected with the software that registers the signal and connects to the visual stimuli.

The Electrodermal Activity (EDA) signal is an electrical manifestation of the sympathetic innervation of the sweat glands. EDA has had a history in psychophysiological research (including emotional or cognitive stress) since the middle of XIX century. It is measured in a non-invasive skin surface mode, predominantly from plantar eccrine sweat glands [13].

In recent years, researchers began using EDA for pathophysiological applications like assessing fatigue, pain, sleepiness, exercise recovery, diagnosis of epilepsy, neuropathies, and depression, among others [14]. A recent study with newborns has shown that EDA parameters seem sensitive in detecting sympathetic regulation changes in early postnatal life. The EDA measure can represent an essential step towards a non-invasive early diagnosis of the pathological states linked to autonomic dysmaturation in newborns [15]. Although the skin biomarkers of preterm birth could be seriously altered [16], the EDA of that population has not been measured, associated with a visual stimulus, as far as we know.

Preterm born is a condition that has increased in the last few decades, and it is estimated by World Health Organization that 15 million infants are born too early every year. That represents more than 1 in 10 infants [17]. The consequences of preterm birth have a large spectrum of variability. Some infants without any permanent sequelae at 2 years old have the same motor development as full-term infants at the same age [18]. However, unfortunately, most infants suffer the consequences of being preterm born for a longer period [17] in ways that could be subtle or more apparent, with impacts on growing, playing, or learning [19]-[21]. Despite the knowledge about the changes in different areas of participation, the link between those and electrophysiological signals patterns and the early biomarker identification is not completely established. Accepting the electroencephalography response as a quantitative signal of nervous system function and knowing its selective use in clinical settings, our research group is motivated to deeper understand the VEP and EDA in the first months of life of preterm born infants. In order to answer the previously identified requirements for visual skills assessments [22] and enlarge the clinical and developmental follow-up evaluations that already exist, this paper aims to present an integrated experimental setup to

assess the evolution of visual system development in preterm infants. It also presents the results of a pilot application with two preterm infants.

To achieve these goals, the proceeding was organized to describe the materials and methods used, followed by results presentation and analysis, finishing with the conclusions and suggestions for further work.

II. MATERIALS AND METHODS

This section describes the experimental setup as well as the main procedures of data collection and analyses used.

The study was authorized by the Ethical Commission of Central Lisbon University Hospital Center with the official reference "Projeto INV14", and the parents gave written informed consent.

A. Experimental setup

As seen in Fig. 1, the experimental setup integrates the following equipment:

- Computer: all the equipment runs on a Dell Computer (Intel ® Core (TN) 2DUO, 4,00 GB) that projects the image for a LG flatron W1934s;
- E-Prime®: software used to create the visual stimuli, black/white checkerboard pattern;
- g.Nautilus®: serial number NB-2017.08.77 was used with software g.Recorder® version 5.16.00 and allows recording of the VEP. The connection between the g.Nautilus© and E-Prime was done using a parallel port;
- Biosignalsplux®: used with the opensignals® software to record EDA signals. The connection between biosignals and E-Prime was guaranteed by a socket that records the triggers related to the stimuli appearance.



Figure 1: Experimental setup

As previously referenced, the visual stimuli were created using the E-Prime® software. A pattern-reversal evoked potentials technique was chosen, creating a checkerboard pattern in which the colors of the squares (black and white) were inverted every 1 second for a total of 120 seconds. The choice of this pattern was based on the fact that it presents a lower variability in latency and amplitude between different measurements, which

facilitates data comparison [4]. Furthermore, the number of stimuli presented was thought to be comprehensive enough to allow several parts of the signal to be still used, considering that it would be almost impossible not to have many artifacts in the infants' signals. At the center of the checkerboard pattern, there is a red cross that serves as a fixation point to look at. Before and after the pattern is shown, warning messages about the start and end of signal acquisition are displayed to the participants. It is also important to note that the chosen inversion timing was inspired by previous studies implemented with preterm born infants [7][8]. Since the correct creation of VEP depends on the exact moment that the presentation of each stimulus is precisely marked, the connection between E-Prime® and the software that allows data collection (g.Nautilus®) was made. To accomplish this, E-Prime® has been parameterized to send a signal each time the checkerboard reversed (trigger pattern was moment). The parameterization was based on the instructions in the g.Recorder[©] - Parallel Port QuickStart script V5.16.00 referring to g.Nautilus® software. As for the acquisition rate, it was defined that it would use 250 Hz due to the equipment specifications.

B. Data collection procedures

The electrophysiological measurements were done with the infant on the parent's lap and using the infant's binocularity. Related to the VEP, the data collection was done using the g.Nautilus® software, whose equipment consists of a cap with 32 active channels. In the present study, the international 10/20 electrode placement system [6] was used, and the electrodes corresponding to PO7, PO8, Oz, Cz, Fz, and ear lobe, as reference electrode, are recorded. After placing the correct size cap, Sigma Gel was distributed over the electrodes to reduce the impedance, measured with the g.NEEDaccess® - Demo Client software. Considering that active electrodes were used, it was defined that the collection of the signals was ready to be done when the impedance of all the electrodes was less than 30 k Ω .

Regarding the EDA, the data collection was performed with Biosignalsplux[®]. To measure the electrodermal signal, an EDA sensor, including two channels was used, with two electrodes attached to it. The acquisition rate was defined at 500Hz, in order to collect as much information as possible. This methodological option was taken since this is an exploratory study.

On the computer, Opensignals[®], a software that allows the observation of the data collected with Biosignalsplux[®] in real time, was required. The data was acquired via Bluetooth communication and archived in ".txt" and ".h5" formats. Identical to the collection for VEP, for the study of EDA, the aforementioned visual stimulus created with E-Prime[©] was used.

Since the correct processing of the signal depends on marking the exact moment of the presentation of each stimulus, a connection was made between E-Prime and Opensignals in addition to the link between E-Prime and g.Nautilus already mentioned. For EDA, the connection between the two software programs was achieved using a socket, as suggested in the E-Prime Guide [23], and triggers were placed at the beginning and end of the stimulus presentation. For the further processing of the signal, this placement was taken into account and, with the use of the file saved by g.Nautilus®, it was possible to access the remaining triggers (obtained each time the checkerboard pattern was reversed). This parametrization was chosen since having the exact trigger moment for both connections (E-Prime[®]-Opensignals[®] and E-Prime[®]-g.Nautilus[®]) showed some incompatibilities.

C. Processing signals methodologies

For both VEP and EDA, signal processing was performed with MatLab® software.

For VEP, the signal processing started with removing the direct current component by subtracting its own average from each signal. Then, to improve the signal quality, it was decided to apply a filter prior to the average of the signal epochs corresponding to each stimulus. Based on previous studies using Butterworth filters [24] and filters with cut-off frequencies between 2.5 Hz [25] and 30 Hz, [26] the choice fell on:

- used the Filter Designer tool provided by MatLab® to design the filter;
- applied for a 4th order Butterworth bandpass filter with cut-off frequencies of 2 Hz and 30 Hz;
- applied the *filtfilt* function to prevent signal delay after the filter application.

Finally, the signal was divided into 120 epochs, each corresponding to a reversal of the checkerboard pattern. Therefore, for the entire evoked response to be visible, the cuts performed on the signal must be done a few milliseconds before and after the exact moment of the stimulus. In this way, the moments immediately prior to the application of the stimulus (baseline) are observed, as well as the total response to the stimulus that is prolonged in time. Thus, it was decided to cut the signal 100ms before and 700ms after viewing the pattern reversal. Consequently, these epochs were averaged by adding them and dividing them by the number of epochs.

As the result continued to present a lot of noise, the application of a low-pass filter was studied [27] and created again with the Filter Designer application and with the specification:

- applied with the *filtfilt* function;
- a 4th order low-pass Butterworth filter with a cut-off frequency of 10 Hz.

To make visualization of VEP formation more intuitive, an interface was created with the GUIDE tool provided by

MatLab®. From left to right, the first graphics on the interface show the signal obtained considering only the first 20 seconds, then considering 40 seconds and continuing to increase the time window until the final result. The remaining graphs show the evoked responses corresponding only to specific 20-epoch plots of the signal. The method described above was tested in adults, and the results showed that the algorithm developed effectively processed the VEP. However, for some children, it was found that the signal was still contaminated with artifacts. Knowing that the standard deviation can be used for artifact's detection [28] and testing threshold values, the method chosen was to eliminate the segments whose standard deviation was 0.6 times higher than the standard deviation of the signal segment, being this value heuristically found.

Since the EDA signal is composed of two different components, phasic and tonic [29], it was helpful to analyze them separately. This was performed using Ledalab©, a Matlab© toolbox that allows the decomposition of the signal and extraction of some parameters from the phasic component, which has information related to response to the stimulus. The signal processing began by reading the .txt file extracted from Opensignals® and the .hdf5 file from g.Nautilus®. The next step consisted of converting the raw data and downsampling the result data from 500 Hz to 100Hz to facilitate its analysis in Ledalab®. Next, a moving average filter, with a window of 50 sample points, was also applied to smooth the signal without losing relevant information. After this, Ledalab® was executed with two files: a file containing the converted values of EDA as input and the file with the trigger moments information, created with the g.Nautilus® software. The separation of the two components, phasic and tonic, was performed using the Discrete Decomposition Analysis [30], as it allows the individual study of each electrodermal response and presents a detailed time discretization. From this step, a graph like the one represented in Fig. 2 is obtained.



Figure 2: Result from non-negative deconvolution. It is possible to observe the separation between the tonic EDA, in grey, and the phasic EDA, in blue. The red lines represent the recorded trigger moments.

Afterward, parameters such as the number of responses, latency time, amplitude, area, and conductance level were extracted, only the responses in a time window of 0.5s to 1s from each stimulus (to extract specific responses to the stimuli before the next one occurs as mentioned previously the reversal rate is 1s) and with amplitude above 0.01 μS [31].

D. Sample characterization

The present pilot application was conducted with two preterm born infants (P.1 and P.2), followed by an outpatient development appointment of Dr. Alfredo da Costa Maternity. The two female infants in the pilot study did not have major lesions (periventricular haemorrhage grade III or plus and /or retinopathy of prematurity grade III or more in cranial ultrasound done at age equivalent at term). Participant n. 1 was 30 weeks and 6 days of gestational age, 1945g birth weight and 4 months corrected age at data collection time. Participate n. 2 was 24 weeks and 5 days of gestational age, 685g birth weight and 6 months corrected age at data collection time.

III. RESULTS PRESENTATION AND ANALYSES

In Fig 3, one can see an example of the electroencephalographic data recorded before, during and some time after the presentation of the stimuli. The epochs marked by a green bar were used to calculate the average and obtain the evoked potentials waveforms. The epochs marked by a red bar were removed since they were contaminated with artifacts. Given the results, we can conclude that, in both infants, most artifacts were found at the beginning and the end of the measurement, which could match the periods when they are still focusing on the stimulus and when they are tired of looking at it.



Figure 3: Selection of the signal epochs to use for processing (green epochs were included in the processing, and red epochs were excluded as artifacts)

The overview of the VEP results is presented in Fig. 4. Considering that P100 is one of the typical waveforms resulting from visual stimuli [6] in Table I, one can find the specific amplitude and latency of P100 obtained in PO8 electrode. In the following graphs, samples were converted to time for better understanding. As expected, latencies for the older child are lower, as her visual system is more mature [32]. It is also worth noting that the shape of the potentials for the two infants is "larger" than would be predicted for an adult, which is in agreement with the consulted literature [4] [7].



Figure 4: VEP of four (left) and a six-month (right) old infants with vertical lines to mark the stimulus presentation (black) and the P100 latency (orange)

TABLE I - LATENCY (MS) PER PARTICIPANT AND REGION

	PO8	CZ	<i>P07</i>	Oz	Mean
P.1 (4M)	240	236	240	248	241
P.2 (6M)	212	192	232	212	212

Figure 5 shows the VEP change (blue line) during sample collection for the 6-month-old infant using the GUIDE interface. It was found that beyond 80 stimuli presented the result did not vary appreciably. As for the various plots of 20 epochs, there were some differences in their shape, probably due to changes in the state of attention.



Figure 5: VEP changes during sample collection using GUIDE interface for a 6-month-old infant with vertical lines to mark the stimulus presentation (black) and the ideal P100 latency (blue). Each graph corresponds to adding 20 epochs to the signal processing from left to right.

The arousal state is one of the behavioral characteristics that could influence good-quality pattern VEP recordings [4]; this is why the present data were collected when infants were cooperative and calm. In that way is expected that negative components preceding and following the positivity appear at 2-4 months of age, and the waveform is adult-like by 68 months of age [10].

The methods previously described for the EDA were applied to the same preterm female infants for VEP. The

parameters extracted from Ledalab© for both infants can be seen in Table II. However, due to the lack of reference values for these parameters in infants using visual stimuli in the current literature, it is not feasible to draw compelling conclusions with just this pilot application.

TABLE II – PARAMETERS EXTRACTED FROM LEDALAB FOR BOTH PARTICIPANTS

	Number of responses	Amplitude (µS)	Area (nS ²)	Latency Time (s)	Skin conductance level (µS)
P.1 (4M)	29	8.68	48.87	0.79	6.39
P.2 (6M)	38	4.51	37.11	0.75	10.15

Still, these two pilot applications already show some evidence conforming to what was expected according to the results obtained for other types of stimulation. Namely, the older infant presents a higher number of responses to the stimuli, a lower amplitude [33], a lower latency time [34], and a higher skin conductance level [35] in comparison to the younger one. These are all related to the maturation of the nervous system and, consequently, greater reactivity to the stimuli.

Those preliminary results align with a previous study that proposes pattern VEP as a valuable technique for monitoring visual development in preterm infants using corrected age [7]. The continuity of the study allows the build of a database with healthy preterm born visual development that could be compared with typical development. The early data from VEP and EDA could probably function as a physiological marker to detect some fragility when development is not following a typical stream.

IV. CONCLUSION AND FUTURE WORK

The pilot application showed that this experimental setup could be used to study VEP and EDA in infants, helping compare recordings from different age groups.

The experiment and algorithm developed by the research group allow the processing of data collected with very young infants with low cooperation capacity. The results proved that one could find significant differences in premature infants with an age difference of 2 months. Moreover, this experiment has shown that visual stimuli affect visual and electrodermal responses that can be corresponded to the infants' age. Thus, the combined analysis may correlate more effectively with infant development. Although the reduced sample size may be a limitation, the results show that the procedures implemented apply to young participants. Furthermore, the algorithm developed allows for noise remotion and reliable data analysis.

After this experimental protocol and setup testing, the research team is prepared to integrate the data collected

with this electrophysiological setup with other clinical and developmental data. This way, it is possible to do a holistic assessment with longitudinal collections of signals in premature and full-term infants to compare the two groups and their evolution over time. In addition, this procedure allows the integration of data from statistically significant sample sizes in biomedical health records in children's healthcare systems.

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