Olfactory Event-Related Potentials in Infants

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Objectives To evaluate olfactory event-related potentials (OERPs) as an objective measurement of olfactory function in infants.

Study design OERPs to phenylethyl alcohol were measured in 13 infants, between 23 and 41 days of age. The odor was delivered with a computer-controlled olfactometer. Recording electrodes were applied using the 10-20 system. Data from electrodes Fz, Cz, Pz, C3, and C4 were analyzed by MatLab’s Letswave toolbox (André Mouraux, Brussels, Belgium) using the canonical time-domain averaging as well as the time-frequency analyzing method.

Results Ten of 13 infants finished the recording session. We observed OERPs in 7 of these 10 infants. Recordings were best in electrodes Fz and Cz. The N1 peak was visible at 328 ms followed by P2 at 505 ms. In addition, the time-frequency analysis had an increase in low frequencies (4-7 Hz) around 550 ms after odor presentation.

Conclusions We were able to record OERPs in infants. The time-domain averaging as well as the time-frequency analysis was of value for data analysis. (J Pediatr 2014;165:372-5).

The sense of smell is of crucial importance for infants especially within the first weeks of their lives. Newborns and young infants use olfactory cues mainly for feeding and bonding with their mothers.1,2 Olfactory function develops early. Around 4-6 months postconception, the nostrils of the fetus open.1 Therefore, the fetus is exposed to odorous molecules within the amniotic fluid such as substances from the mothers’ diet,3 which may explain food preferences in early childhood.7 Several studies have shown that newborns have a well-functioning sense of smell.4-6 Infants are able to recognize and discriminate between odors.2,7 They are able to discriminate their mothers’ smell from others and can distinguish between human milk and formula.8 Congenital anosmia such as found in Kallmann syndrome is a relatively rare condition9; however, other causes such as choanal atresia and cerebral malformation might result in olfactory deficits,10 which might lead to feeding problems or impede mother–infant bonding.

Measurement of olfactory function relied on behavioral, autonomic, and facial responses of infants.3-5 Therefore the interpretation of the odor response is to some degree subjective. Olfactory event-related potentials (OERPs) objectively measure olfactory function in adults.11 One study has shown that the same method is applicable for children as young as 3 years of age.12,13 However, OERPs have not been measured in a systematic fashion in infants. Our aim was to measure OERPs in infants to establish an objective method for evaluating olfactory function. This might be of help in the clinical diagnosis of feeding, neurodevelopmental, and mental disorders.

Methods

Infants were recruited at the Obstetric and Pediatric services of Woman’s, Children’s, and Adolescent’s Department of Pedro Hispano Hospital in Matosinhos, Portugal. Thirteen families showed interest and participated with their infants in the study. Thus, the OERPs of 13 infants (6 female, 7 male) with an age range of 23-41 days were recorded. All infants were full-term, with birth weights ranging from 2620-4270 g and had normal Apgar scores. According to the parents, the infants were healthy at the time of the data collection. In addition, all infants were examined by a pediatrician to rule out flu-like symptoms, nasal congestion, and temporary respiratory problems. The study was explained to the families in great detail, and written informed consent was obtained. All aspects of the study were performed in accordance with the Declaration of Helsinki. The study protocol was approved by the local Ethics Committee of Pedro Hispano Hospital, Health Unit District of Matosinhos, Portugal.

For the comfort of the infants, they were held in their parents’ arms during setup and measurements. Parents were asked to stay quiet during the session and not to move the infant. If necessary, a pacifier was used to calm the infant during the session. The entire session took about 30 minutes (approximately

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EEG Electroencephalogram
OERP Olfactory event-related potential

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15 minutes for preparation and 15 minutes for data collection). During the setup, an electroencephalogram (EEG) cap was placed on the infant’s head and a small tube for odor presentation was inserted and fixed with Leukosilk (BSN medical GmbH, Hamburg Germany) in 1 nostril (Figure 1; available at www.jpeds.com). Placement of the tube took about 30 seconds, and all the infants were distressed by this procedure but they rapidly calmed down once the researcher stopped touching their nose.

To elicit OERPs, phenylethyl alcohol (rose like smell, Sigma, Deisenhofen, Germany), which is known to specifically activate the olfactory system with little or no trigeminal activation, was delivered in 3 different concentrations (10%, 30%, and 50% v/v using the Olfactometer OM2s [Burghart Instruments, Wedel, Germany]). The olfactometer provided a continuous airflow of 5 L/min with heated and humidified air (36.5°C, 80% relative humidity); odorous stimuli were embedded in this airstream preventing mechanical or thermal stimulation of the nasal mucosa. The stimulus duration was set to 200 ms with an average inter-stimulus interval of 17 seconds. Each odor concentration was presented 16 times in pseudo-randomized order.

Data were recorded using a QuickAmp amplifier linked to a BrainCap 32 channels and Vision Recorder 1.10 Software (Brain Products, Brainvision Inc Gilching, Germany). Before placing the BrainCap, the infant’s head was cleaned. The 32 electrodes were inserted in the respective position according to the 10-20 system. Data were recorded with a sampling rate of 250 Hz and an online band-pass filter (0.2-30 Hz) was applied.

### Statistical Analyses

All EEG processing and analyzing steps were carried out using Letwave 5 (André Mouraux, Brussels, Belgium). The EEG data were filtered offline with a band-pass filter of 0.1-15 Hz prior to segmentation of −0.5 to 1.5 seconds relative to stimulus onset. This was followed by a baseline correction with a reference interval of 500 ms before stimulus onset. Epochs containing artifacts attributable to blinking or movement were manually excluded following examination especially of electrodes Fp1 and Fp2. In addition, recordings exceeding −50/50 μV were excluded. Artifact free epochs were averaged for each infant. Because of the small number of epochs, the 3 odor concentrations were averaged together.

To obtain the signal to noise ratio, the peak-to-peak amplitude (N1 and P2) was calculated and divided by the mean of the 2 largest maxima and minima from the 500 ms prestimulus interval. In addition to this, the time-frequency analysis using the continuous Morlet wavelet transform as described by Huart et al was applied to analyze the EEG data. The time-frequency analysis was performed at the level of single trials. This method is the standard for analyzing OERPs in our laboratory and has proven to be superior to time-domain averaging in adults. Data were analyzed by means of SPSS 21.0 (SPSS Inc, Chicago, Illinois); t tests were used wherever appropriate. The level of significance was set at .05.

### Results

For this study, 13 infants and their parents volunteered for participation. The data of one infant was lost because of technical problems. The data recording was stopped for 2 infants because they cried and could not be calmed down. Therefore, the data of 10 infants were included in the final analysis. In 7 of the 10 infants, we identified clear OERPs. The OERPs were most prominently visible at electrodes Fz and Cz. The other electrodes C3, C4, and Pz did not show an OERP in all infants. OERPs were identified by different components and peaks (N1 and P2). Figure 2 (available at www.jpeds.com) shows the recording of 1 infant at electrode Cz. The OERP is clearly visible with a positive peak shortly after 500 ms. In addition, the time-frequency-analysis shows an increase in low frequencies (5-6 Hz) from 420-820 ms with a maximum at 5.35 Hz and 632 ms. The group average of the OERP wave is shown in Figure 3 (available at www.jpeds.com). When comparing electrodes Fz and Cz, no significant difference was found for N1 (t = 1.68, P = .14), P2 (t = 0.63, P = .55), and signal to noise ratio (t = 0.83, P = .44). At electrode Cz, N1 peaked on average at 328 ± 58 ms (range 248-424 ms) with a mean amplitude of −2.16 ± 3.10 μV (range −5.81 to 1.29 μV) and P2 at 505 ± 68 ms (range 408-608 ms) with a mean amplitude of 8.54 ± 5.95 μV (range −0.18 to 14.97 μV). The average signal to noise ratio was 2.27 ± 1.04 (range 1.07-3.65). The Table summarizes these findings.

### Discussion

We measured OERPs in infants as young as 23 days of age. Of the 10 infants tested, an OERP was detectable in 7. Considering that Lotsch and Hummel showed that even in normomiceinfants, OERPs are only detectable in about 70% of the cases, the present results appear to be in order. The sense of smell is well developed in infants. Most of the studies used subjective methods such as facial grimacing, head orientation, or movement of the extremities to evaluate the reaction of infants to odor stimulation. Only a few studies used objective measurements to evaluate the olfactory

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**Table. OERPs were recorded from electrodes Fz and Cz**

<table>
<thead>
<tr>
<th>Electrode</th>
<th>N1-latency (ms)</th>
<th>N1-amplitude (μV)</th>
<th>P2-latency (ms)</th>
<th>P2-amplitude (μV)</th>
<th>Signal-to-noise ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fz</td>
<td>320 ± 39</td>
<td>−0.50 ± 2.87</td>
<td>519 ± 78</td>
<td>7.37 ± 5.18</td>
<td>1.96 ± 0.92</td>
</tr>
<tr>
<td>Cz</td>
<td>328 ± 58</td>
<td>−2.16 ± 3.10</td>
<td>505 ± 68</td>
<td>8.54 ± 5.95</td>
<td>2.27 ± 1.04</td>
</tr>
</tbody>
</table>

The amplitudes, latencies, and signal-to-noise ratios are given for Fz and Cz.
function of infants.\textsuperscript{21-24} For example, Bartocci et al used near infrared spectroscopy to measure the cerebral blood oxygen level after odor stimulation.\textsuperscript{21} Cerebral blood oxygen level was increased after presentation of colostrum and vanilla odors compared with water as a control.\textsuperscript{22} These findings are in line with previous studies, which showed the ability of infants to discriminate between odors.\textsuperscript{1,20} The processing of olfactory information and discriminating of odors leads to a change in blood oxygen level, which can be measured by near infrared spectroscopy.\textsuperscript{21,22} This method has good spatial resolution, especially when applied to infants, because of their relatively thin skull.\textsuperscript{23} The temporal resolution of near infrared spectroscopy is rather slow compared with EEG, which is a disadvantage. Two studies have measured the frontal EEG asymmetry after odor presentation in infants.\textsuperscript{23,24} These investigations showed clearly that it is possible to measure EEG changes after odor presentation attributable to olfactory information, but also that further interpretation of the results is difficult and the major advantage of event-related potentials, the high temporal resolution, is not utilized with this method.

Olfactory processing changes with brain maturation.\textsuperscript{4,22} Newborns with asphyxia showed a more general activation after odor stimulation compared to healthy newborns.\textsuperscript{2} Therefore, it is believed that brain development and/or possible brain damage can be assessed by measuring odor processing in infants.\textsuperscript{4,22} In the current study, we present an objective method of evaluating olfactory processing in infants. A limitation of this study is the small sample size of 10 infants. Therefore, the data should be regarded as preliminary. In total, we obtained 48 recordings from each infant. In some infants, a large number of recordings had to be excluded because of artifacts. It has to be noted that to calm down the infants, pacifiers were used. This might have contributed to EEG artifacts. It was previously shown that a minimum of 8 recordings is necessary to obtain an OERP.\textsuperscript{3,6,27} For analyzing OERPs in adults, the time-frequency analysis has proven to be of advantage.\textsuperscript{17,18} In the time-domain averaging, we had an average signal to noise ratio of 2.27, which is low compared with previous studies in adults.\textsuperscript{27} For this reason, we included the time-frequency analysis in our study. In this study, we demonstrated the applicability of the time-frequency analysis in infants. Further studies are needed to evaluate whether the time-frequency analysis provides additional information as it does in adults. Huart et al obtained a frequency change after odor stimulation around 5 Hz.\textsuperscript{17} Results from our study are in line with this. We observed an increase in low frequencies around 4-7 Hz after odor stimulation.

We included only healthy infants. Further studies are planned to examine the difference in OERPs in infants with asphyxia compared to healthy infants. For example, we will evaluate the clinical relevance of measuring OERPs in infants.

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References

In 1964 Kearsley, Snider, and Eaton recognized “the magnitude of the challenge which disorders in childhood behavior presents to contemporary pediatrics,” and realized there was a deficit in techniques available to the general pediatrician to evaluate their young patients’ mental health. The only tools available at that time included personality inventories, doll play, and the structured interview, all of which were very open to personal interpretations instead of a uniform standard. To address this deficiency, a parent-completed questionnaire was developed, and this manuscript reported preliminary data and analysis. Using this approach appeared promising.

In the last 50 years, many standardized instruments have been developed to screen children’s development and behavioral concerns. It is of interest that many of these instruments continue to be based on parent report. However, the availability of these instruments has not resulted in comprehensive screening. In 2010, The American Academy of Pediatrics Task Force on Mental Health stated that screening with a validated tool is useful in identifying children with mental health problems.1,2 Unfortunately, they also found that fewer than 50% of children and adolescents receive psychosocial surveillance, and generally less than 1 of 3 children with a mental health problem is identified in the primary care setting. However, numerous studies have pointed to the feasibility of using brief validated mental health screening tools in the primary care settings. The tools have improved, but there is still much work to do in incorporating these tools into the health care provided children and youth.

The American Academy of Pediatrics Mental Health Initiatives has developed primary care tools for the general pediatrician. It provides a list of screening and assessment tools with their properties. It is recommended to review all the resources first, and then try out several tools, using a quality improvement strategy to determine the best instruments for a given clinical setting. Hopefully, this strategy will make accomplishing routine screening of children and youth for behavioral concerns more feasible.

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References

**Figure 1.** Setup for measuring OERPs. The feeding tube for odor delivery was inserted approximately 5 mm in one nostril and fixed to the nose with Leukosilk tape to prevent moving of the nose piece. The infants were held in their parents’ arms for more comfort.

**Figure 2.** A, OERP of a single infant. The graph shows changes in the low frequencies (4-6 Hz) around 400-700 ms after the stimulus (*stim*). The time-frequency analysis was performed on the single-trial. Please note the scaling of the y-axis (2-15 Hz). B, Frequency changes because of odor stimulation.
Figure 3. **A**, Group average of the OERP in the top part for all 7 subjects. The N1 and P2 peaks are nicely displayed. In addition, frequency changes occur around 4-7 Hz at 550 ms. The time-frequency analysis was performed in the single-trial. Please note the scaling of the y-axis (2-15 Hz). **B**, Frequency changes because of odor stimulation.