Chemosensory event-related potentials in early blind humans

I. Cuevas1, P. Plaza1, P. Rombaux1, A. Mouraux1, J. Delbeke1, O. Collignon1, A. G. De Volder2 and L. Renier3

1Neural Rehabilitation Engineering Laboratory, Institute of Neuroscience, Université catholique de Louvain, Brussels, Belgium; 2Department of Otorhinolaryngology, Université catholique de Louvain, Cliniques Universitaires Saint-Luc, Brussels, Belgium; 3Readaptation Unit (READ), Institute of Neuroscience, Université catholique de Louvain, Brussels, Belgium

Key-words. Early blindness; chemosensory function; cross-modal plasticity; event-related potentials (ERPs); odour perception

Abstract. Chemosensory event-related potentials in early blind humans. While the effects of early visual deprivation on auditory and tactile functions have been widely studied, little is known about olfactory function in early blind subjects. The present study investigated the potential effect of early blindness on the electrophysiological correlates of passive odour perception. Event-related potentials (ERPs) were recorded in eight early blind humans and eight sighted controls matched for age, sex and handedness during olfactory stimulation with 2-phenyl ethyl alcohol and trigeminal stimulation with CO2. Latencies, amplitudes and topographical distributions were analysed. As expected, the olfactory and trigeminal ERP components showed normal latencies, amplitudes and topography in both groups. Olfactory stimuli generated responses of smaller amplitude than those observed in response to trigeminal stimulation. In addition, ERP analyses did not reveal any major difference in electrocortical responses in occipital areas in early blind and sighted subjects. These results suggest that passive olfactory and trigeminal stimulation elicit the same electrophysiological responses in both groups, confirming that the neurophysiological correlates of the cross-modal compensatory mechanisms in early blind subjects do not appear during passive olfactory and trigeminal perception.

Introduction

The study of early blind subjects provides an insight into the neuroplastic changes occurring after the de-afferentation of visual cortical areas. Early visual deprivation gives rise to a functional reorganisation of the brain and in particular the occipital cortex, a region traditionally thought to support visual functions in sighted subjects. Several studies have shown that the occipital cortex of early blind humans is recruited to process non-visual information such as sounds1 and tactile stimuli, including Braille.1 The cross-modal recruitment of de-afferented visual brain areas is thought to play a prominent role in the emergence of behavioural compensation in blind subjects.1,3 While auditory and tactile functions have been thoroughly investigated in blind subjects, little is known about olfactory abilities and the neural substrate of odour perception in this population. One may hypothesise that early blind individuals rely more on their olfactory sense than sighted subjects. For example, in the absence of vision, the sense of smell has an increased ecological value for the evaluation of food quality and the detection of other olfactory stimuli that supply information about the environment.5 Furthermore, the ability to focus on relevant olfactory stimuli may be essential for mobility and to identify persons and places. Behavioural studies have yielded divergent results about the performance of blind subjects during olfactory tasks.7-10 In addition, a neurophysiological study showed similar event-related potentials (amplitude and latency) during olfactory and trigeminal stimulation in blind persons and sighted controls.11

Financial support: This study was supported by the Belgian National Fund for Scientific Research (FNRS) grants #3.4505.04 and 3.4502.08. A.G. De Volder is a senior research associate and A. Mouraux, L. Renier and O. Collignon are postdoctoral researchers at the Belgian National Fund for Scientific Research (FNRS).

Isabel Cuevas received the Glaxosmithkline E.N.T. award “Fundamental Research” from the Royal Belgian Society for Ear, Nose and Throat, Head and Neck Surgery. Scientific Meeting of the Royal Belgian Society for Ear, Nose and Throat, Head and Neck Surgery, Palais des Académies, Brussels, Belgium, March 2009.
The apparent divergence between the results obtained in previous studies could be due to methodological differences such as the profiles of the tested blind subjects (e.g. the age of blindness onset). To address this issue, then, rigorous evaluations of olfactory function with standardised brain investigation techniques are clearly needed. The purpose of the present study was to investigate the effect of early blindness on electrophysiological activity during passive odour perception. In particular, we wanted to test whether any difference would be observed in the occipital cortex of early blind subjects in olfactory stimulation conditions. We examined the latencies, amplitudes and topographical distributions of olfactory and trigeminal event-related potentials (ERPs).

Materials and methods

Subjects
The study was carried out in eight early blind subjects (EB, range 20-55 years, mean ± SD: 37.4 ± 13.1) and eight sighted control participants (SC, range 20-53 years, mean ± SD: 36.5 ± 12.1), all males individually matched for age and handedness (7 right-handed and 1 ambidextrous in each group). We used a parametric test (student’s t-test) to compare the mean ages between both groups, obtaining a p-value > 0.05. The present study sample included males only to preclude any variability in the subject sample and to prevent the introduction of confounding factors. It has been shown that chemosensory ERPs elicited in women have a shorter latency and greater amplitude than in men. Furthermore, the menstrual cycle\(^{13}\) and oral contraceptives\(^{14}\) have been shown to influence olfactory performance and chemosensory ERPs.

No subject had any olfactory deficit (i.e. clinical hyposmia) as evaluated by the Sniffin’s Sticks test\(^{15}\). Threshold-Discrimination-Identification scores (TDI) were: mean ± SD: 36.75 ± 4.54 and 30.41 ± 2.43 in EB and SC, respectively (p = 0.09). EB subjects all had total blindness (absence of light perception) as a result of bilateral ocular or optic nerve lesions at birth or within the first two years of life. They had no history of normal vision and had no memories of visual experience. A summary of their characteristics is provided in Table 1. Except for their blindness, all the subjects were healthy, they had no recorded history of neurological or psychiatric problems, and they were well integrated socially. They provided written informed consent before the study. The protocol was approved by the Biomedical Ethics Committee of the school of Medicine of the Université catholique de Louvain.

Experimental procedure
Chemosensory event-related potentials
In one single session, event-related potentials (ERPs) were recorded in response to passive olfactory and trigeminal stimulation using a computer-controlled stimulator based on air-dilution olfactometry (OMS olfactometer, Burghart Medical Technology\(^{\text{®}},\) Wedel, Germany). With this stimulator, it is possible to deliver chemical stimuli without changing mechanical and thermal conditions in the nasal cavity. Stimuli reached the nose through a Teflon tube placed in one nostril, with the distal opening beyond the nasal valve, pointing towards the olfactory cleft. Subjects received a constant intranasal airflow (7.6-8.2 l/min), which was humidified (80% relative humidity) and warmed up to body temperature (36°C). The conditions were such that, after a short period of adaptation, the subjects became unaware of the constant airflow. Rose smell (2-phenyl ethyl alcohol) was used for olfactory stimulation and CO\(_2\) for trigeminal stimulation. The stimulation of the nasal fossa was obtained by embedding brief pulses of a 50% vol/vol concentration of 2-phenyl ethyl alcohol and CO\(_2\). Stimuli were presented monorurally with a stimulus duration of 200 ms and a stimulus rise time of <20 milliseconds.

Both stimuli were presented at least 20 times in a randomised sequence with an interstimulus interval of 30 seconds. Subjects were sitting in a well-ventilated room, which was dimly lit and acoustically shielded to minimise concomitant sensory stimulation. In addition, to avoid the possibility of auditory responses evoked by switching valves related to the presentation of the chemical stimulus, auditory masking was applied with white noise at 60-70 dB SPL through bilateral headphones. Subjects were asked to keep their eyes open and to breathe normally through their mouths during the recording session.

EEG recording
The EEG was recorded continuously at a sampling rate of 256 (band-pass filter 0.2-30 Hz) using a 32-channel amplifier (SAM 32 EP, Micromed, Mogliano Veneto, Italy). The recording was made
with 22 silver electrodes placed on the scalp in accordance with the international 10/20 system: Fp1, Fp2, F7, F3, Fz, F4, F8, T7, T3, Cz, C4, T8, P7, P3, Pz, P4, P8, POz, O1, O2, O9 and O10. An electrode placed on the forehead served as the common reference. In addition, two electrodes were placed on the left and right earlobes (A1, A2). Each recording lasted approximately 20 minutes.

EEG analysis
All offline signal-processing procedures were performed using a Let'swave EEG toolbox (http://amouraux.webnode.com/letswave). The EEG was filtered using a 0.2-10 Hz band-pass FFT filter, re-referenced to the left and right earlobes (A1A2), segmented into epochs ranging from -0.5 to +1.5 s relative to stimulus onset, and baseline corrected (reference interval: -0.5 to 0 s). Epochs contaminated by eye blinks (>50 µV in Fp1-Fp2 leads) or other large artifacts (e.g. high-frequency motor artifacts) were discarded. Average waveforms were then obtained for each subject and stimulus type (olfactory, trigeminal). Latencies and amplitudes of ERP peaks were estimated at electrodes Fz, Cz, and Pz, and the waveform was obtained by averaging POz, O1 and O2. A negative peak (N1) was identified as the most negative deflection occurring at Cz between 320 and 450 ms after stimulus onset for the olfactory ERP and between 250 and 500 ms for the trigeminal ERP. A positive peak (P2) was identified as the most positive deflection between 530 and 800 ms after stimulus onset for the olfactory ERP and between 400 and 800 ms after stimulus onset for the trigeminal ERP.

Statistical analysis
Olfactory and trigeminal ERPs
Latencies and baseline-to-peak amplitudes of each ERP peak component (N1 and P2) were submitted to a two-factor analysis of variance (ANOVA) for repeated measures using sensory modality (olfactory v. trigeminal) as a first factor, and the electrode site (Fz, Cz, Pz, and POz-O1-O2) as a second factor. This design was applied separately to N1 and P2 amplitude and latencies. In addition, multiple comparison data were analysed using a paired-sample t-test. The level of significance for all statistics was p<0.05. Furthermore, the scalp distributions of the two peaks were compared for the two conditions after normalisation. For each subject and condition, amplitudes were divided by the square root of the sum of the squared mean amplitudes from each of the 22 electrodes. This approach was chosen as a classical and widely used way of normalising electrophysiological data since, even in the method proposed by McCarthy and Wood, it is customary to correct for offsets from the origin. Mean-centring should then be used because it can also eliminate genuine topographical differences. A paired-sample t-test was then used to compare the normalised peak amplitudes estimated at each electrode location.

Results
Olfactory and trigeminal ERPs
Figure 1 shows grand average ERPs waveforms for each group, obtained from a selection of four
sites (Fz, Cz, Pz, and POz-O1-O2 average, re-referenced to A1A2). The scalp distributions of both N1 and P2 components are shown for the 22 electrodes in the topographical maps presented in Figure 2.

In both groups, the negative deflection for olfactory and trigeminal stimulation (N1) was larger at midline than at lateral recording sites. However, the potential was larger at Cz compared to the other sites at midline (frontal (Fz) and parietal (Pz)) recording sites. It was followed by a positive peak (P2), the amplitude of which was also larger at the Cz recording site (see Figure 1 and Table 2). ANOVA analysis revealed that the average amplitude of the N1 peak did not differ between groups ($F_{1,14} = 1.20; p = 0.29$) or between the type of stimulation ($F_{1,14} = 0.85; p = 0.36$) but differed between electrode sites ($F_{3,42} = 6.00; p = 0.001$). No interaction effect was observed between the group and sensory modalities, between the group and electrode sites, or between these three factors ($F_{1,14} = 1.41; p = 0.25, F_{3,42} = 0.56; p = 0.64, F_{3,42} = 0.579; p = 0.97$, respectively). The average amplitude of the P2 peak did not vary significantly between groups ($F_{1,14} = 2.45; p = 0.13$) or sensory modalities ($F_{1,14} = 3.77; p = 0.07$). However, it did vary significantly between the electrode sites ($F_{3,42} = 13.411; p < 0.001$). No interaction effect was observed between the group and sensory modalities or between the group and electrode sites, but there was an interaction effect between sensory modality and electrode site ($F_{1,14} = 1.25; p = 0.28, F_{3,42} = 0.36; p = 0.77, F_{3,42} = 90.084; p < 0.001$, respectively). At the central electrodes (Fz, Cz and Pz), early blind subjects tended to have larger N1 and P2 amplitudes for trigeminal stimulation than sighted subjects. However, post-hoc comparisons showed no difference in the amplitudes (paired-sample t-test, $p > 0.05$). Furthermore, at the posterior recording sites (POz-O1-O2), the mean amplitudes of the N1 and P2 peaks were the same for EB and sighted subjects in all stimulation conditions (in all cases, $p > 0.05$).

The average latency of the N1 peak did not vary between groups ($F_{1,14} = 0.18; p = 0.67$), between sensory modalities ($F_{1,14} = 0.56; p = 0.46$) or between electrode sites ($F_{3,42} = 1.23; p = 0.31$). No interaction effect was observed between the group and sensory modalities, between the group and electrode sites, or between sensory modality and electrode site ($F_{1,14} = 0.09; p = 0.77, F_{3,42} = 0.24; p = 0.86, F_{3,42} = 1.28; p = 0.29$).
**Olfactory function in early blindness**

![Olfactory stimulation and Trigeminal stimulation](image)

**Figure 2**
Scalp distribution of the N1 and P2 peaks for olfactory and trigeminal stimulation, after normalisation. Top: Early blind subjects. Bottom: Sighted control subjects. For each subject and condition, amplitudes (µV) were divided by the square root of the sum of the squared mean amplitudes from each of the 22 electrodes. The bar chart represents amplitude values (µV) in a graduated colour code from red (positive) to blue (negative).

respectively). The average latency of the P2 peak did not differ significantly between groups ($F_{1,14} = 0.33; p = 0.57$), between sensory modalities ($F_{1,14} = 3.61; p = 0.07$) or between electrode sites ($F_{1,12} = 1.838; p = 0.15$). No interaction effect was observed between the group and sensory modalities or between sensory modality and electrode site, but there was an interaction effect between the group and electrode sites ($F_{1,14} = 2.019; p = 0.17$, $F_{1,14} = 0.77; p = 0.51$, $F_{1,14} = 3.68; p = 0.01$, respectively). Group comparison using a paired-sample t-test confirmed that the latencies of N1 and P2 peaks following olfactory and trigeminal stimulation were similar in blind and sighted subjects (all p values >0.05, see Table 3).

**Discussion**

The sense of smell seems to play a less prominent role that vision and audition in humans. Blind subjects also mainly use audition and touch to acquire spatial and physical information about their environment. Nevertheless, in the absence of vision, olfaction may be particularly important in everyday life as it allows blind subjects to detect environmental hazards such as smoke, poisonous fumes, potential toxic substances or spilt food. Some blind subjects also report using olfaction, in addition to the other senses, to recognise objects and persons. This could further a degree of practice-related enhancement of the sense of smell. Behavioural compensations are generally thought to reflect practice-related perceptual functions and attentional strategies that are

| Table 2 |

<table>
<thead>
<tr>
<th></th>
<th>Olfactory N1</th>
<th>Olfactory P2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fz</td>
<td>Cz</td>
</tr>
<tr>
<td>Amplitude EB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude SC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trigeminal N1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude EB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude SC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>-4.801</td>
<td>-4.460</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD: standard deviation; EB: early blind subjects; SC: sighted controls.
relevant to the blind in everyday activities, rather than changes in sensory acuity.

There are very few data about the neural mechanisms of olfactory perception in EB subjects. Our results suggest there is no major difference between groups. This absence of group difference during passive olfactory and trigeminal stimulation in central activity distribution or in occipital areas concurs with a previous electrophysiological study. One possible explanation for this absence of group difference is that the occipital cortex of EB subjects is exclusively recruited when higher order perceptual processes are involved or during demanding tasks. Lending support to this idea, previous studies have shown that the occipital cortex of EB subjects was not significantly activated in the passive sweeping of Braille dots, passive listening, finger tapping and passive sensory electric stimulation or unattended listening to pitch changes. In addition, studies using transcranial magnetic stimulation (TMS) demonstrated that the occipital cortex of blind subjects affected higher order cognitive processes such as Braille reading but not simple perceptual tasks.

It is, however, worth noting that the latencies, amplitudes and topography of olfactory and trigeminal ERP components obtained in the present study were similar to those usually observed in sighted subjects. In accordance with previous studies, olfactory stimuli usually yield responses with smaller amplitudes than responses to trigeminal stimulation.

Methodological limitations

Given the relative small sample size, we cannot totally exclude the possibility of a group difference in the olfactory and/or trigeminal ERPs that could not be observed in the present study. However, the methodology used, as well as the fact that previous studies yielded similar results, justify a reasonable level of confidence about our data and conclusions.

Conclusion

The present study showed that the event-related potentials (ERPs) in response to olfactory and trigeminal passive stimulation seem to induce a similar electrophysiological response in EB and sighted subjects, including the response in occipital areas. The neurophysiological aspects of the cross-modal compensatory perceptual mechanisms for chemosensory perception in EB subjects are not manifest during passive olfactory and trigeminal perceptions. Additional investigation using experimental paradigms involving an active processing of olfactory stimuli in the EB are needed to explore further the potential role of the occipital cortex of EB subjects in the processing of odours. Despite its limitations, the present study is one of the rare attempts to evaluate olfactory function in EB subjects.

Acknowledgements

The authors gratefully thank all the volunteers for their participation to
the study. We wish to thank J. Frasnelli for helpful comments on a first version of the manuscript.

References


Laurent Renier
Neuro Rehabilitation Engineering Laboratory
Institute of Neuroscience
Université catholique de Louvain
Avenue Hippocrate 54
UCL-54-46
B-1200 Brussels, Belgium
Tel.: +32 2 764 5456,
Fax: +32 2 764 94 22
E-mail: laurent.renier@uclouvain.be