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Clinical Significance of Olfactory Event-Related Potentials Related to Orthonasal and Retronasal Olfactory Testing

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Objective: The purpose of this study was to evaluate the likelihood of recording olfactory event-related potentials (OERPs) in patients with an olfactory dysfunction and to correlate the electrophysiological responses to orthonasal and retronasal olfactory testing.

Design/Materials and Methods: This was a prospective study of 65 patients with different origins of their olfactory loss. Orthonasal olfactory function was assessed with the "Sniffin' Sticks" test (orthonasal score; maximal score 48) and retronasal olfactory function with odorized powders presented intraorally (retronasal score; maximal score 20). The OERPs were obtained after presentation of 2-phenyl ethyl alcohol, the selected olfactory stimulus. Causes of olfactory dysfunction included postinfectious olfactory loss (n = 15), head trauma (n = 26), nasal polyposis (n = 15), and mixed causes (idiopathic, toxic, drug induced) (n = 9).

Results: Based on orthonasal testing, 32 and 33 patients were diagnosed with anosmia and hyposmia, respectively. Twenty-two patients from the hyposmic group demonstrated reliable OERPs. No OERPs were recorded in the anosmic group. Prevalence of OERPs in a cohort of patients with olfactory dysfunction was 33.8% (22 of 65). Median score (expressed as the percentage of the maximal score that could be obtained theoretically) in which OERPs were recorded was 50% (24 of 48) with orthonasal testing and 80% (16 of 20) with retronasal testing.

Conclusions: Patients with olfactory dysfunction usually demonstrate OERPs in one third of the cases. When olfactory dysfunction is in the range that separates normosmic subjects from anosmic patients, patients may have identifiable OERPs. Interpretation of both orthonasal and retronasal psychophysical olfactory testing should be supported by the recording of OERPs in a clinical setting.

Key Words: Smell, olfaction, olfactory event-related potential, orthonasal testing, retronasal testing.

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INTRODUCTION

Exploration of the olfactory system in patients with olfactory loss relies on the combination of a number of elements: the patient's personal history, clinical findings collected from otorhinolaryngological and neurological examinations, psychophysical assessment of olfactory performance, structural assessment of the olfactory apparatus with magnetic resonance imaging (MRI), and more recently, functional assessment of the olfactory system with chemosensory event-related potentials (CSERPs).[1] Orthonasal and retronasal psychophysical testing allows a semiobjective evaluation of the olfactory function. Orthonasal testing involves presenting different smells through the nose during inspiration or sniffing, or both. Retronasal testing involves presenting different odors through the mouth, the odorant molecules reaching the olfactory neuroepithelium through the retrovelopharyngeal pathway. Combining orthonasal and retronasal testing is critical because the different etiologies of olfactory dysfunction may differentially impair orthonasal or retronasal olfactory performances.[2]

The electrophysiological exploration of the olfactory system with the use of CSERPs has been proposed as a more objective diagnostic tool for evaluating olfactory function, and has been considered to be a complementary method to the more traditional psychophysical tests.[3,4] CSERPs may be elicited by a purely olfactory stimulus (often referred to as olfactory ERPs) or by a purely trigeminal stimulus (often referred to as somatosensory ERPs). Subjects with normal olfactory acuity usually elicit consistent and reproducible olfactory and somatosensory ERP components.[1,3,4]

The aim of the present study was to assess the prevalence of olfactory ERPs (OERPs) in a cohort of patients presenting with olfactory dysfunction resulting from different etiologies: postinfectious, posttraumatic, nasal

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polyposis, toxic, and idiopathic. The electrophysiological evaluation of olfactory function was correlated to orthonasal and retronasal psychophysical assessments that were concomitantly collected.

MATERIALS AND METHODS

We conducted the study at the Department of Otorhinolaryngology of the Cliniques Universitaires Saint-Luc (Brussels, Belgium). We collected all data of this prospective study between January 2005 and June 2006. We obtained informed consent after outlining the experimental paradigm. We followed the rules of the Ethics Committee of the Université Catholique de Louvain, which are in accordance with the principles of the revised Declaration of Helsinki.

Participants were all patients complaining of an olfactory dysfunction and presented themselves to the outpatient clinic. Sixty-five patients (31 men and 34 women) were included in the study. They were aged 51 ± 16 years (median age, 53 years). The youngest patient was 15 years old, while the oldest patient was 79 years old.

The different etiologies of olfactory dysfunction were classified into four different categories: postinfectious (n = 15); posttraumatic or head trauma (n = 26); nasal polyposis (n = 15); and an additional category (n = 9) that included idiopathic, toxic, and drug-related etiologies.

Experimental Procedures

We obtained psychophysical testing and OERPs for all 65 patients. Psychophysical and electrophysiological data were collected on the same day with a resting period of 1 hour separating the psychophysical testing session from the electrophysiological recording session. Orthonasal testing was always performed before retronasal testing, and OERP recording always followed psychophysical testing. Duration of the entire experiment was approximately 3 hours.

Psychophysical Olfactory Testing

Each patient underwent both orthonasal and retronasal psychophysical assessments.

To evaluate the orthonasal olfactory function, we used the standardized "Sniffin' Sticks."[5] Olfactory stimuli were presented birhinally. At first, we assessed odor perception threshold for n-butanol using stepwise dilutions in a series of 16 felt-tip pens. After that procedure, patients were asked to attempt discriminating one odor within a triplet of three different odorants. For each discrimination task, three felt-tip pens were presented, of which two contained the same concentration of one odorant and the third contained the target odorant. The target odorant was to be recognized in a series of 16 trials. Finally, a series of 16 odors were presented to the patients along with a choice list of four responses. For each patient, results of the testing of odor threshold, odor discrimination, and odor identification were used to compute the threshold-discrimination-identification (TDI) score, ranging from 0 to 48.[5] In healthy subjects, the TDI score at the 10th percentile was 24.9 in subjects younger than 15 years, 30.3 for ages 16 to 35 years, 27.3 for ages 36 to 55 years, and 19.6 for subjects older than 55 years.[5]

To evaluate the retronasal olfactory function, we used a previously standardized testing procedure, based on the presentation of odorized powders in the patient's mouth.[6,7] Twenty different odorous powders were applied to the midline of the tongue on a fenestrated plastic stick for a duration of 3 seconds. As with orthonasal identification testing, participants were asked to identify the presented odor from a list of four items (it should be noted that great care must be taken to avoid concomitantly stimulating the patient orthonasally by simply passing the powder at the level of the nares). Subjects were asked to block the nose and not to skew the tongue, such as to avoid decreasing the surface contact with taste receptors. After the presentation of each odorant powder, participants rinsed their mouth with clear water. In healthy subjects, the median retronasal testing score was 18 for subjects aged 36 to 55 years and 16 for subjects older than 55 years.

In the present study, the cutoff value defining hyposmia was arbitrarily set as an orthonasal score below the 10th percentile of the distribution of the scores obtained in healthy subjects matched for sex and ages or as a retronasal score below 16. The cutoff value defining anosmia was arbitrarily defined as an orthonasal score below 16.[5]

Olfactory Event-Related Potentials

Olfactory event-related brain potentials were recorded in response to the presentation of a brief and selective monorhinal olfactory stimulus. The stimulus was produced by a computer-controlled olfactory stimulator based on air-dilution olfactometry (Olfactometer OM2S; Burghart Medical Technology, Wedel, Germany).[8] The stimulator allowed delivering chemical stimuli without concomitantly altering the mechanical or thermal conditions of the nasal cavity. Stimuli reached the nasal cavity through a Teflon tube placed in one nostril (preferentially the right nostril), ending beyond the nasal valve, and pointing towards the olfactory cleft. The total flow rate was 8 L per minute. Temperature (36°C) and relative humidity (80%) were kept constant across trials. Stimulus rise-time was shorter than 20 milliseconds. Stimulus duration was 200 milliseconds; 2-phenyl ethyl alcohol (50% v/v) was used for olfactory stimulation.

The patients sat in a well-ventilated room. For the duration of the recording session, patients were asked to reduce their eye movements and eye blinks and to breathe through their mouth. Furthermore, to avoid the possible contamination of results by activity related to the noise produced by the valve-switching that occurs during presentation of the odorant stimulus, patients wore headphones playing a constant, binaural white noise of 60 to 70 dB SPL. Twenty stimuli (interstimulus interval: 30 seconds) were presented. Electroencephalogram (EEG) was recorded at 256 cps from three scalp midline electrode positions (F_Z, C_Z, and P_Z) using a SAM 32EP EEG amplifier and digitizer (Micromed, Mogliano Veneto, Italy). Linked earlobes (A_1A_2) were used as reference. Impedance was kept below 20 kOhm. Epochs extended from 500 millisecond before to 1,500 millisecond after stimulus onset. After baseline correction (reference interval: 500-0 ms), epochs were band-pass filtered (0.3–12 Hz FFT filter). Trials containing eye links and/or showing an activity higher than 50 μV were rejected before averaging. A minimum of 60% of artifact-free recording was considered as the limit allowing any further interpretation of the CSERPs (12 of 20 trials). Average waveforms were computed for each subject and electrode channel. All offline signal-processing procedures were performed with the LETS-WAVE EEG toolbox (Université Catholique de Louvain, Brussels, Belgium).[9]

CSERPs were considered present if the averaged waveforms demonstrated a negative-positive complex consisting of an initial negative peak (N1: latency: 290–490 ms, amplitude < -2 μV) followed by a positive peak (P2: latency: 460–820 ms, amplitude $> +2$ μV). Responses were independently analyzed by two different observers (P.R. and A.M.).

Statistical Analysis

Statistical analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL),[10] whereas the MedCalc program for medical statistics (MedCalc software, version 8.1; Mariakerke, Belgium) was used for the analysis of receiver operating characteristics

TABLE I.
Descriptive Statistics for Orthonasal and Retronasal Scores and Age.

Statistics Used	Orthonasal Score	Retronasal Score	Age
N	65	65	65
Mean	16.9	12.7	51.3
SD	7.2	3.6	16.1
Median	16	13	53.2
Minimum	6	5	14.9
Maximum	36	19	79.1
Deviation from normal distribution (K–S test)	$P = .427$	$P = .184$	$P = .786$

N = number; SD = standard deviation; K–S = Kolmogorov–Smirnov.

(ROC).[11] The normal distribution of psychophysical scores and patient age was assessed using the Kolmogorov–Smirnov test. Differences between the means of distributions were tested for significance using a paired-sample t test. The Pearson correlation was used to investigate associations between orthonasal and retronasal scores, and age.

The ROC curves were used to assess the performance of a diagnostic test in differentiating between the absence and presence of OERPs. Pairwise comparisons of the area under the ROC were performed. Higher areas under the ROC curve are associated with a better performance of the test in differentiating between the absence and presence of OERPs.

RESULTS

Descriptive statistics of orthonasal scores, retronasal scores, and patient age are outlined in Table I. Their distribution did not significantly differ from a normal distribution. Based on the psychophysical testing, thirty-three patients (50.7%) were classified as hyposmic and 32 (49.3%) as anosmic. Two patients were diagnosed as having hyposmia, although their orthonasal scores were in the normal range but their retronasal scores were below 16. A significant positive correlation between orthonasal scores and retronasal scores was found (see Table II). There was no significant correlation between orthonasal or retronasal scores and age.

The OERPs were identified in 22 of the 65 patients. Consequently, in the present cohort of patients, the prevalence of OERPs was 33.8%. The OERPs could not be identified in any of the 32 anosmic patients. Indeed, OERPs were only identified in hyposmic patients (22 of 33).

The scatterplot of orthonasal scores and retronasal scores and its relationship with the absence or presence of OERPs is shown in Figure 1. The distribution of orthonasal and retronasal scores dependent on the absence or presence of OERPs is illustrated in Figure 2. The median orthonasal score of patients from which OERPs could be recorded was 24 of 48 (mean score, 25.1) or 50% expressed as the maximal score that could be obtained theoretically.

The median retronasal score of patients from which OERPs could be recorded was 16 of 20 (mean score, 16) or 80% expressed as the maximal score that could be obtained theoretically.

The ROC curve, which assesses the performance of orthonasal and retronasal psychophysical testing by differentiating between the absence and presence of OERPs, is shown in Figure 3. When considering orthonasal scores, the area under the curve was 0.988 (95%, CI = 0.923–0.997). When considering retronasal scores, the area under the curve was 0.903 (95%, CI = 0.804–0.962). Both of these areas were significantly different from 0.500 ($P = .0001$). Thus, the orthonasal score is efficient in distinguishing between the absence and presence of OERPs, and the retronasal score does not warrant any additional information for this purpose.

DISCUSSION

Results of the present study may be summarized as follows: 1) Olfactory ERPs may be recorded in approximately one third of patients complaining of an olfactory dysfunction. 2) OERPs are never recorded in patients classified as anosmic using orthonasal psychophysical testing. 3) The turning point at which OERPs are most likely to disappear lies within the hyposmic range as assessed by orthonasal psychophysical testing. 4) In patients with olfactory dysfunction, OERPs most often are present if their retronasal psychophysical score lies within normal or subnormal values.

This study attempted to correlate and compare psychophysical and electrophysiological methods of assessing the olfactory function in patients complaining of impaired olfaction. Although basic psychophysical tests allow assessing olfactory performance in both healthy subjects and patients, more extensive psychophysical testing may allow differentiating anosmic and hyposmic patients from normosmic subjects.[5,7] The major disadvantages of the methods based on psychophysical testing are that they require the patient's collaboration and that, within a medicolegal context, their impact is limited by the possible biasing of results through malingering. Therefore, OERPs may constitute an interesting alternative method to assess the olfactory system and performances. Indeed, compared

TABLE II.
Correlations Between Orthonasal and Retronasal Scores and Ages.

Statistics Used	Orthonasal Score vs. Retronasal Score	Orthonasal Score vs. Age	Retronasal Score vs. Age
Pearson correlation	0.577*	−0.082	−0.065
Significance (2-tailed)	0.000	0.517	0.604
N	65	65	65

*Strong and significant correlations between both scores.
N = number.

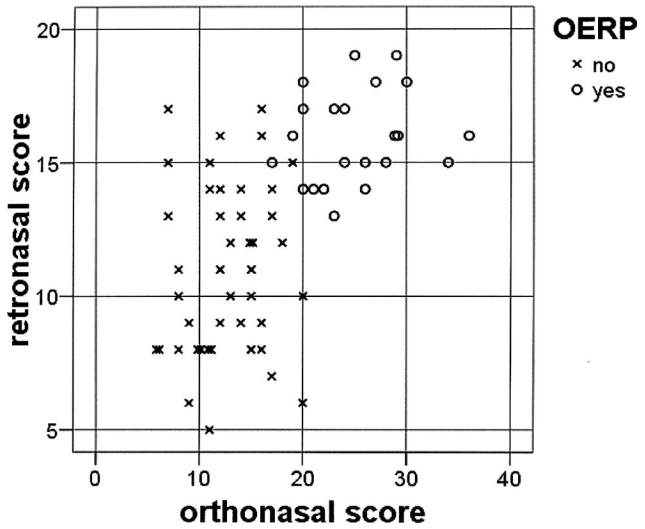

Fig. 1. Scatterplot showing distribution of both scores (orthonasal and retronasal) dependent on OERPs status.

with psychophysical methods, OERPs offer the advantage of not requiring full patient collaboration, and their objective nature may provide more impact within a medicolegal context. The major drawback of OERPs is that the procedure requires a stimulator capable of delivering a brief, synchronized, and selectively chemosensory stimulus. This technical requirement most probably explains why OERPs are not routinely used as a diagnostic tool for the evaluation of olfactory function.

In the clinical environment, clinicians relying on psychophysical olfactory testing may categorize olfactory function into three different categories: normosmic, hyposmic, or anosmic.[5] Indeed, using the "Sniffin' Sticks," the cutoff between hyposmic and anosmic patients is usually and arbitrarily defined as below 16 of 48, while the cutoff between normosmic and hyposmic is defined as a score below the 10th percentile of the distribution of the scores obtained in a control group of healthy subjects matched for sex and age.[4,5] In contrast, the interpretation of olfactory electrophysiological responses is dichotomous. Indeed, OERPs components are either present or absent. Nevertheless, it should be noted that a third category consisting of present yet altered (delayed latency, decreased amplitude) OERPs components could be defined, but normative data and its correlation with the degree of olfactory dysfunction is still lacking.[4,8] Combining the psychophysical and electrophysiological testing of olfactory function leads to six different outcome possibilities, which are illustrated in Table III. Follow-up studies should examine the prognostic value of the presence or absence of OERPs components in hyposmic patients. Indeed, one could postulate that the prognosis of hyposmic patients with preserved OERPs components could be better than that of hyposmic patients with absent OERPs components.

In a previous study, we demonstrated a significant correlation between orthonasal and retronasal scores and the N1P2 amplitudes of the OERPs in a cohort of 33 patients and 11 healthy subjects.[12] In another similar study that included both patients and healthy subjects, Lötsch and Hummel[4] compared the assessment of olfactory performance based either on psychophysical orthonasal testing or OERPs. Results of their study showed that the probability of recording identifiable OERPs becomes greater than 50% within the range of psychophysical olfactory performance scores that separate anosmia from normosmia.[4] The prevalence of OERPs in their cohort of patients complaining of olfactory dysfunction was 36.2% (29 of 80), a value quite similar to that obtained in the present study (33.8%; 22 of 65). Thus, our study may be viewed as confirmatory rather than novel based on a larger sample of patients, except for the data concerning retronasal scores.

Taken together, the converging results of both studies support the view that both psychophysical and electrophysiological methods of assessing olfactory function are reliable and reproducible. However, we did not record any OERPs in patients with anosmia, contrary to the Lötsch and Hummel study,[4] which demonstrated OERPs in 20% of their patients with functional anosmia (8 of 40). One could argue that the definition of the presence of OERPs is based in our study on electrophysiological criteria such as latencies and amplitudes of peaks and is thus more restricted than the definition

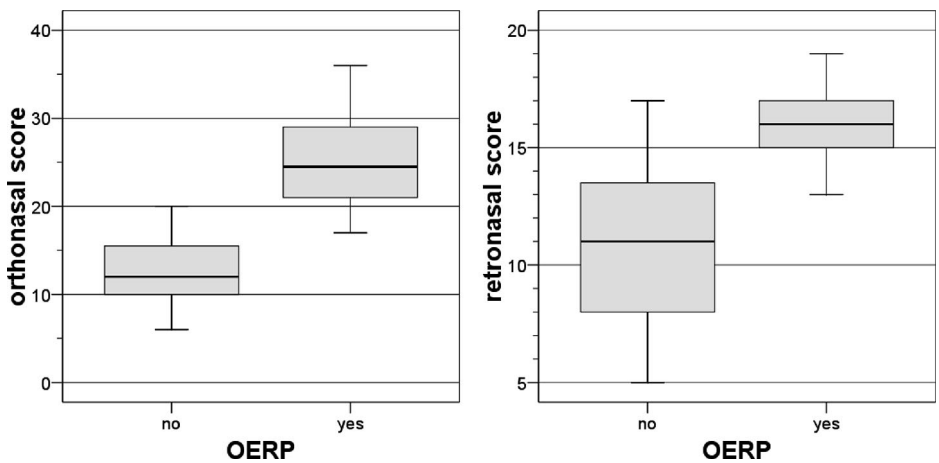

Fig. 2. Boxplot showing distribution of orthonasal scores and retronasal scores dependent on OERPs status.

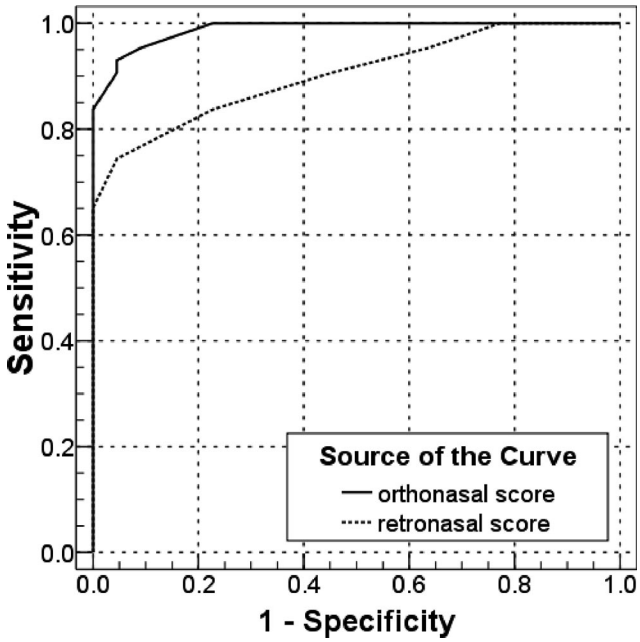

Fig. 3. The receiver operating characteristics (ROC) curve, assessing the performance of orthonasal and retronasal psychophysical testing when differentiating between the absence and presence of OERPs. Based on the orthonasal score, the area under the curve was 0.988 (95%, CI = 0.923–0.997). Based on the retronasal score, the area under the curve was 0.903 (95%, CI = 0.804–0.962).

used in the other study, in which the presence of OERPs was only based on the presence or absence of an identifiable response. Another explanation could be that the precise definition of anosmia ranges from complete anosmia (the patient cannot detect any olfactory sensation) to functional anosmia (ability to detect spurious odorous perception of certain flavors). In these two circumstances, even if the patients are below the arbitrary cutoff of anosmia, one could speculate that the relative percentage of these two kinds of patients is different when comparing the two studies.

In this study, OERPs were recorded in response to one single olfactory stimulus (we only used 2-phenyl ethyl alcohol). The performance and relevance of OERPs could be enhanced by recording OERPs elicited by a second olfactory stimulus, such as hydrogen sulfide (H_2S). However, one should also consider that the resulting increase of the duration of the acquisition procedure may not be feasible within a clinical setting (decreased patient collaboration). We speculate that with a second olfactory stimulus such as H_2S, the number of patients with detectable OERPs would not increase. Instead, perhaps some patients with no OERPs after the 2-phenyl ethyl alcohol stimulus would have an electrophysiological response after the H_2S stimulus. In fact, this has been demonstrated in only a limited number of healthy subjects and not in patients.[4]

Finally, we recorded OERPs in a cohort of patients with different origins of their olfactory loss. It should have been interesting to analyze the responses regarding the different etiologies of their olfactory loss. However, this comparison should have been based on paired groups for age and sex, which was not the case in our cohort of patients.

CONCLUSION

This study emphasizes the relationship between psychophysical and electrophysiological testing of olfactory function in patients complaining of a loss of smell. OERPs could be recorded in some of the hyposmic patients but in none of the anosmic patients. Combining psychophysical testing and OERPs recording may allow the clinician to perform an accurate diagnosis of olfactory dysfunction. Further studies are required to determine the prognostic value of the presence or absence of OERPs components in hyposmic patients.

TABLE III.
Clinical Assessment of Olfactory Function When Combining the Psychophysical and Electrophysiological Testing (OERPs).

Psychophysical Orthonasal Testing	OERPs	Conclusion
Normosmia	Present	Normal olfactory function
Normosmia	Absent	Possibly normal olfactory function, consider the possibility of a technical problem (e.g., EEG artifacts)
Hyposmia	Present	Decreased olfactory function (the presence of OERPs may be correlated with a good prognosis)
Hyposmia	Absent	Decreased olfactory function (the absence of OERPs may be correlated with a poor prognosis)
Anosmia	Present	Consider patient malingering
Anosmia	Absent	Severely altered olfactory function, poor prognosis

OERPs = olfactory event-related potentials; EEG = electroencephalogram.

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