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Olfactory event-related potentials (OERPs) and trigeminal event-related potentials (TERPs) – a pilot study in Czech participants with normal sense of smell

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Abstract
Introduction: In recent years, the evaluation of potential events related to olfactory events (OERPs) and trigeminal events (TERPs) has become increasingly important in the diagnosis of olfactory disorders. This technique is increasingly used in basic research and clinical practice to evaluate people suffering from olfactory disorders.
Purpose of the study: In a pilot project of the first investigations of OERPs and TERPs in the Czech Republic, we analyse the event-related potentials of the data of normosmic participants.
Methods: In the prospective study, 21 normosmic participants were enrolled for a 2-year period (5/2021–5/2023). OERPs/TERPs were recorded at the scalp vertex (electrode Pz/Cz). Odourants 2-phenylethanol/CO_2 were used to selectively activate *Nervus olfactorius/ Nervus trigeminus*. Brain responses to olfactory/trigeminal stimuli (EEG) were recorded in 21/18 normosmic subjects.
Results: In the statistical analysis of the olfactory interval N1-P2 (age, gender), we found no statistically significant differences. In the statistical analysis of the trigeminal interval N1-P2 (age, gender) we found statistically significant differences in amplitude by gender (male amplitudes were higher than female amplitudes, $p = 0.006$).
Conclusion: Our pilot data can function very well as an internal guide for ongoing and future olfactory research studies. Evaluation of the presence of OERPs appears to be an important parameter for the evaluation of olfactory disorders. The absence of OERPs is a strong indicator of the presence of olfactory dysfunction.

Keywords: Evaluation of smell; Objective olfactometry; Odourants; Olfactory event-related potentials; Trigeminal event-related potentials

Highlights:
- A prospective 2-year pilot study included 21 normosmic subjects.
- First investigation of potentials related to olfactory and trigeminal events in the Czech Republic.
- Pilot data can function very well as an internal guide for subsequent olfactory research.
- The presence of OERPs is an important parameter for the evaluation of olfactory disorders.
- The absence of OERPs is a strong indicator of the presence of olfactory dysfunction.

Introduction

In recent years, the evaluation of olfactory event-related potentials (OERPs) and trigeminal event-related potentials (TERPs) has become increasingly important in the diagnosis of smell disorders. This technique is used more frequently in research and clinical practice to assess people afflicted with smell disturbances (Červený et al., 2022; Hummel et al., 2023; Kobal and Hummel, 1994; Rombaux et al., 2009).

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Psychophysical olfactory testing currently plays a leading role in everyday clinical practice, but objective olfactory methods are needed whenever the cooperation of subjects in psychophysical testing is problematic. This may be the case, for example, in children, in persons with cognitive disorders or in the context of medico-legal examinations.

The clinical olfactometer delivers the stimuli necessary to elicit OERPs and TERPs. Event-related potentials (ERPs) are an electrophysiological measurement technique to evaluate changes in smell function, which is an objective estimate of the integrity of the olfactory pathways (Kobal and Hummel, 1994). The principle of the procedure is based on presenting the odourant with special equipment in the patient's nasal cavity, and the brain's reactions to olfactory/trigeminal stimuli are registered via electroencephalography (EEG).

The electric stimuli elicited by the odours of neurons is recognized in the olfactory tract.

The olfactory nerve is the first and shortest cranial nerve. It contains only afferent sensory nerve fibres. It is only a two-neurons pathway. The olfactory nerves originate from the cell bodies of bipolar olfactory neurons in the olfactory epithelium. Olfactory neurons give off projections towards the olfactory bulb, the central hub, and coordinator of olfactory transmission. From the olfactory bulbs, olfactory information reaches the primary olfactory cortex via the olfactory tract. The primary olfactory cortex interacts with a variety of cortical and limbic structures via sophisticated pathways (Červený et al., 2022; Kobal and Hummel, 1994).

The major benefit is the ability to react to the odorous substance and the direct evaluation of the preserved function of the olfactory nerve. It can also be helpful in detecting a patient's misdiagnosis (Červený et al., 2022; Lapid and Hummel, 2013; Rombaux et al., 2009). The olfactometer working principle is the application of an odorous substance into clean, odourless, non-polluted air, which is fed through a tube to the edge of the patient's nasal cavity. All internal parts of the equipment must be made of materials that avoid contamination by other odours.

It is also convenient to locate the olfactometer in a silent and well-ventilated space (Červený et al., 2022; Rombaux et al., 2009). To measure OERPs and TERPs, it is important to employ substances that selectively potentiate only one of them. Consequently, 2-phenylethanol, which selectively stimulates the olfactory nerve, and CO_2 which stimulates the trigeminal nerve, are utilized as odourants (Červený et al., 2022; Kobal and Hummel, 1994; Rombaux et al., 2009). The odorous substance is diluted in distilled water to form a suspension which is bubbled through the air to form the vapour phase of the odour.

This provides sufficient moisture to prevent the nasal mucosa from drying out during the experiment. It also maintains a constant temperature and prevents an uncomfortable and undesirable trigeminal reaction. In the period between the stimuli, a warm, odourless, moistened air stream is conducted into the nasal cavity.

When selecting a solvent, its physicochemical characteristics must be respected.

Varying pH levels or direct liquid-odour interactions may considerably alter the perception, and the outcome may not be valid (Červený et al., 2022; Kobal and Hummel, 1994; Rombaux et al., 2009).

The resulting vapour phase of the odour must then be adequately moistened (≥80%), warmed to near body temperature (36 °C), and supplied at a fixed flow rate (8 litres per minute). Dry, too hot/cold air or faster flow velocity excites the terminal fibres of the trigeminal nerve, and the result is a sum of signals from the trigeminal and olfactory fibres. This also appears when physiological changes in the air circulation in the nasal cavity occur. Consequently, the participant must breathe through the mouth during the entire experiment and the mixture is supplied only by the device. When the air is excessively dry or cold, the mucosa is congested. The appropriate study protocol is then selected in the computer program. The time durations of the individual stimuli, the intervals between stimuli, and the order in which the odourants and CO_2 are displayed can be configured. The goal is to select a sequence with minimal risk of habituation to the odourant (change in odours and CO_2), minimal stimulus duration to provide adequate outcomes, and of course minimal damage to the subject (Červený et al., 2022; Guo et al., 2021; Rombaux et al., 2009). The brain's reactance to the stimulus itself is determined via conventional EEG (Lötsch and Hummel, 2006; Pastorkova et al., 2023). Olfactory event-related potentials (OERPs) and trigeminal event-related potentials (TERPs) are composed of a negative peak N1 and a consequent positive peak P2. An additional important parameter is the evaluation of the N1-P2 interval (shown in Fig. 1) (Lötsch and Hummel, 2006; Pastorkova et al., 2023). For OERPs/TERPs analysis, the latency as well as the amplitude of the peaks N1, P2, and the interval N1-P2 are very important parameters to evaluate. The absence of OERPs is a robust predictor of the presence of olfactory dysfunction (Červený et al., 2022; Guo et al., 2021; Huart et al., 2012).

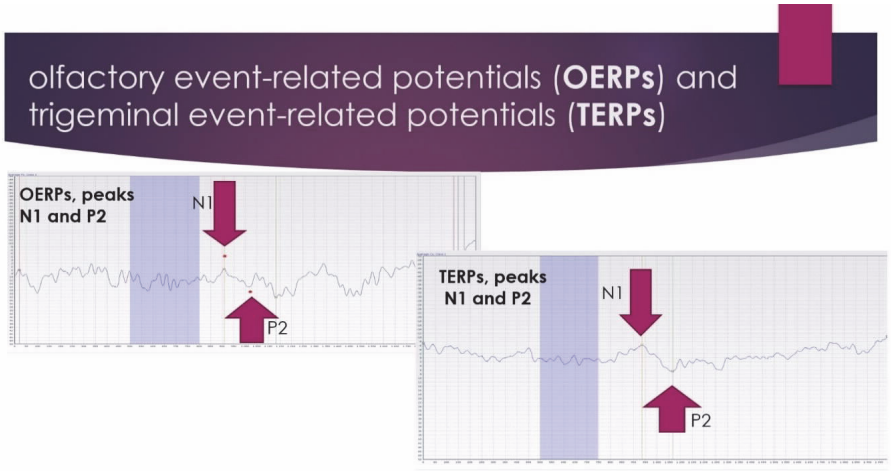

Fig. 1. Olfactory event-related potentials (OERPs) and Trigeminal event-related potentials (TERPs) and peaks N1 and P2 (from first author's archive)

This method of examining the sense of smell is beneficial, for example, in neurodegenerative diseases – Parkinson's disease, dementia, Alzheimer's disease, multiple sclerosis (Červený et al., 2022).

Furthermore, the use of this method is suitable for the investigation of olfactory loss in patients with chronic rhinosinusitis and nasal polyps, and in patients with pituitary adenomas and tumours in the olfactory region (Červený et al., 2022).

In a pilot project of the first investigations of OERPs and TERPs in the Czech Republic, we analysed the event-related potentials of the data of normosmic participants. OERPs are less biased than psychophysical smell tests. These pilot values will be the basis for further olfactory research studies.

Materials and methods

Our prospective study was approved by the Local Ethics Committee Military University Hospital Prague, approval numbers: 108/16-49/2021 (Project NU 22-09-00493), 108/16-24/2021 (Project MO 1012). In 2 years, 5/2021–5/2023, 21 participants with normal sense of smell were included. All participants signed an informed consent form. The group consisted of 21 subjects: 13 females, and 8 males. The mean age in the group was 42 years (range 22–84 years). We compared normative data of OERPs (21 probands) and TERPs (18 participants; three participants had non-evaluable TERPs curves).

We used an OL 024 clinical olfactometer Burghart, Germany (shown in Fig. 2). It provides the necessary stimuli to induce OERPs and TERPs. We stimulated patients with 2-phenylethanol and CO_2 using standard methods (Červený et al., 2022; Kobal and Hummel, 1994). Odourants were instilled into the left nostril. The resulting vapor phase of the odour was properly humidified ((≥80%), heated to a temperature close to body temperature (36 °C), and administered at a constant flow rate (8 litres/minute). During the detection process, the headphones generated 60 dB white noise. The patients sat in a comfortable, relaxed posture. They were asked to sit still without blinking or swallowing and to breathe through their mouths. The test was carried out in a well-ventilated room (Červený et al., 2022; Guo et al., 2021; Kobal and Hummel, 1994). We applied the 8-channel EEG system (order number OL 026; Burghart, Holm, Germany).

Methods of investigation: OERPs: OERPs recorded at the scalp vertex (EEG electrode Pz).

2-phenylethanol (50% v/v) was used to selectively activate olfactory afferents (2-phenylethanol also stimulates the trigeminal nerve) (Manescu et al., 2021). During the experiment, olfactory and trigeminal stimuli were presented separately. Each type of stimulus was repeated 20 times and lasted 250 milliseconds (ms). The interstimulus time interval between each stimulus was 10–20 seconds.

TERPs: TERPs recorded at the scalp vertex (EEG electrode Cz). Gaseous CO_2 (50% v/v) was used to selectively activate trigeminal afferents. During the experiment, olfactory and trigeminal stimuli were presented separately. Each type of stimulus was repeated 20 times and lasted 250 ms. Interstimulus time interval between each stimulus was 10–20 seconds.

Inclusion criteria were age over 18 years, subjects with normal sense of smell, with normal endoscopic intranasal finding, and the Sniffin' Sticks identification test is without pathological results.

Exclusion criteria were age below 18 years, patients with subjective olfactory dysfunction, patients after COVID-19, patients with Parkinson disease, Alzheimer disease and multiple sclerosis in anamnesis, and patients with pathological endoscopic intranasal finding.

Fig. 2. Objective olfactometer OL 024 Burghart and 8 – channel EEG system OL 026 Burghart – measurement of olfactory event-related potentials (OERPs) (from first author's archive)

Statistical analysis was performed using IBM SPSS Statistics (version 22.0; SPSS, IBM, Armonk, NY, USA). Data were analysed using descriptive statistics and Mann–Whitney *U* test; *p*-values equal to or less than 0.05 were considered significant.

Results

We present the pilot data of the OERPs and TERPs of Czech participants with normal sense of smell. Detailed OERPs results are presented in Table 1. Detailed TERPs results are presented in Table 2.

Here we present the measured values (mean) of the olfactory event-related potentials (OERPs). The wave N1 latency (Mean) is 394 ms and amplitude N1 is –4 microVolt (μV). The wave P2 latency (Mean) is 505 ms and amplitude P2 is 8 μV.

N1-P2 interval latency (Mean) is 113 ms and the amplitude N1-P2 interval is 12 μV.

We found no statistically significant differences by gender ($p = 0.916$).

The amplitude (by gender) N1-P2 is 14 μV in males and the amplitude N1-P2 is 10 μV in females. We found no statistically significant differences ($p = 0.089$) (Table 3, Fig. 3).

The amplitude N1-P2 (by age) is 12 μV (by age 18–35 years) and the amplitude N1-P2 is 11 μV (by age 36–84 years). We found no statistically significant differences ($p = 0.750$) (Table 4, Fig. 3).

Here we present the measured values (mean) of the Trigeminal event-related potentials (TERPs). The wave N1 latency (mean) is 312 ms and the amplitude N1 is –6 μV.

The wave P2 latency (mean) is 447 ms and the amplitude P2 is 10 μV.

Table 1. Olfactory event-related potentials (OERPs) results – healthy participants

OERP (*N* = 21)	Mean	Standard Deviation (SD)	Median	Minimum	Maximum
Wave N1 latency (ms)	393.81	77.42	410.00	250.00	549.00
Amplitude N1 (μV)	–3.95	8.13	–5.00	–23.00	19.00
Wave P2 latency (ms)	504.76	107.72	512.00	320.00	769.00
Amplitude P2 (μV)	7.62	6.38	6.00	–1.00	25.00
Interval N1-P2 latency (ms)	113.43	55.11	100.00	45.00	270.00
Amplitude N1-P2 (μV)	11.57	4.30	11.00	5.00	22.00

Table 2. Trigeminal event-related potentials (TERPs) results – healthy participants

TERP (*N* = 18)	Mean	Standard Deviation (SD)	Median	Minimum	Maximum
Wave N1 latency (ms)	312.28	79.51	284.00	225.00	513.00
Amplitude N1 (μV)	–5.94	7.19	–7.00	–16.00	15.00
Wave P2 latency (ms)	446.78	110.55	435.00	286.00	700.00
Amplitude P2 (μV)	9.67	8.01	8.00	–1.00	26.00
Interval N1-P2 latency (ms)	134.50	68.17	128.50	41.00	325.00
Amplitude N1-P2 (μV)	15.61	6.71	14.50	6.00	27.00

Table 3. Olfactory Amplitude N1-P2, statistical analysis (gender)

OERPs			Mean	Standard Deviation (SD)	Median	Minimum	Maximum	*p*-value
Amplitude N1-P2 (μV)	Gender	female	10.08	3.33	11.00	5.00	16.00	0.089
		male	14.00	4.78	14.00	8.00	22.00	

Table 4. Olfactory Amplitude N1-P2, statistical analysis (age)

OERPs			Mean	Standard Deviation (SD)	Median	Minimum	Maximum	*p*-value
Amplitude N1-P2 (μV)	Age (years)	≤35	11.88	3.44	11.50	6.00	17.00	0.750
		>35	11.38	4.87	11.00	5.00	22.00	

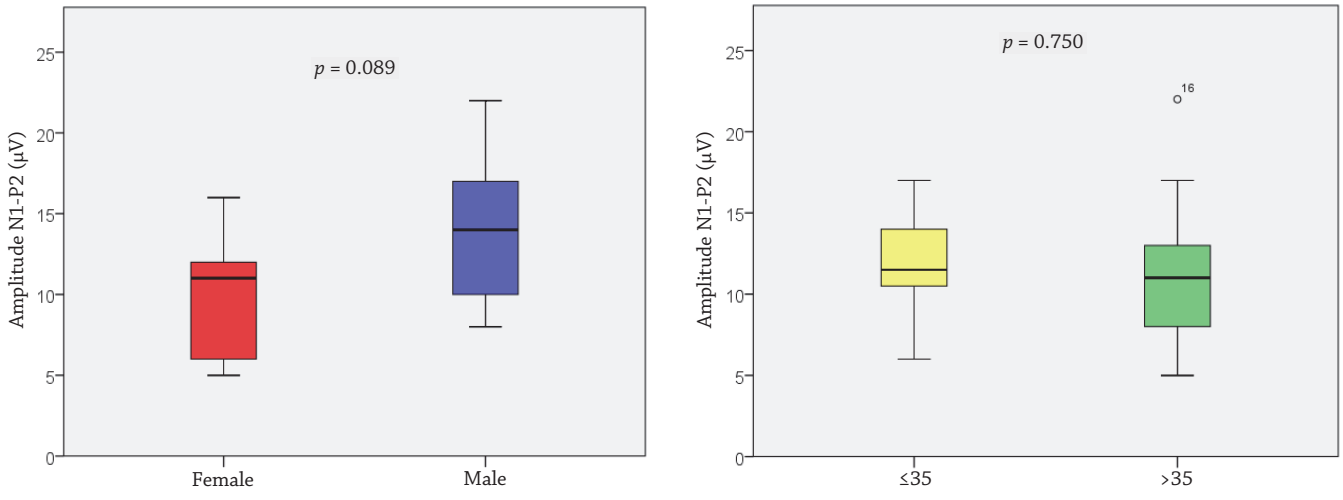

Fig 3. Olfactory Amplitude N1-P2, statistical analysis [gender (left) and age (right)]

N1-P2 interval latency (mean) is 135 ms. We found no statistically significant differences by gender (p = 0.659).

The amplitude N1-P2 interval (mean) is 16 μV.

The amplitude N1-P2 interval (mean) is 21 μV (by male) and the amplitude N1-P2 interval is 12 μV (by female). We found statistically significant differences in amplitude by gender (p = 0.006) (Table 5, Fig. 4).

The amplitude N1-P2 interval (mean by age) is 14 μV (by age 18–35 years) and the amplitude N1-P2 interval is 17 μV (by age 36–84 years). We found no statistically significant differences in amplitude by age (p = 0.536) (Table 6, Fig. 4).

Table 5. Trigeminal Amplitude N1-P2, statistical analysis (gender)

TERPs			Mean	Standard Deviation (SD)	Median	Minimum	Maximum	p-value
Amplitude N1-P2 (μV)	Gender	female	12.27	5.29	11.00	6.00	25.00	0.006
		male	20.86	5.30	22.00	13.00	27.00	

Table 6. Trigeminal Amplitude N1-P2, statistical analysis (age)

TERPs			Mean	Standard Deviation (SD)	Median	Minimum	Maximum	p-value
Amplitude N1-P2 (μV)	Age (years)	≤35	13.57	3.31	14.00	8.00	18.00	0.536
		>35	16.91	8.07	16.00	6.00	27.00	

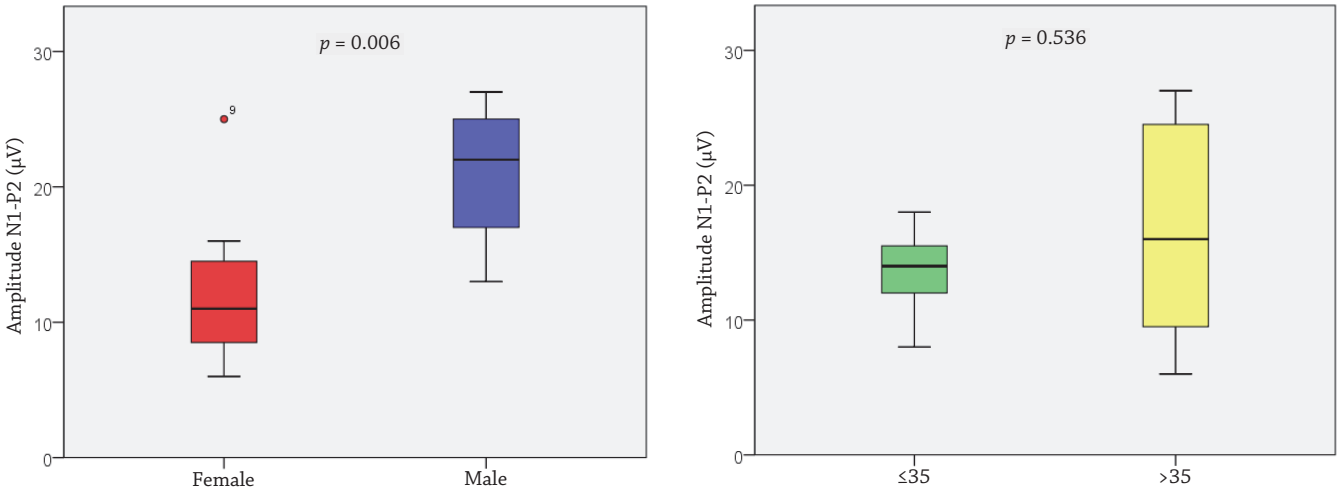

Fig. 4. Trigeminal Amplitude N1-P2, statistical analysis [gender (left) and age (right)]

Discussion

The validation of the first data of OERPs and TERPs in Czech normosmic participants is an important milestone in the management of olfactory assessment in the Czech Republic. The determination of these pilot values will be a starting point for us in future research projects related to objective olfactory investigation. In Table 7 and Table 8, we show a comparison of our mean values of N1 and P2 waves in OERPs and in TERPs with the same values in healthy subjects in published studies by Belgian, German, and Chinese authors (Guo et al., 2021; Huart et al., 2012; Lötsch and Hummel, 2006; Rombaux et al., 2006a, b; Stuck et al., 2006). Chinese authors (Guo et al., 2021) have reported that the first largest negative peak (OERPs) at 200–700 ms is considered to be N1, and the second positive peak P2 is measured at 300–800 ms. In their study, Rombaux et al. (2006a) considered the first largest negative peak (OERPs) at 320–450 ms (after stimulus) to be N1, and the second positive peak P2 is measured at 530–800 ms. Our peak values of N1 394 ms and P2 505 ms are both within the above ranges. The Chinese authors claimed that OERPs occur in 100% of healthy young people (Liu et al., 2008). OERPs were always equipotent in our group of 21 healthy probands, but TERPs were not equipotent in three probands. Age is known to strongly affect chemosensory function. Accordingly, OERPs latencies increase, and their amplitudes decrease with age (Rombaux et al., 2006a). An American study reported that young participants produced significantly higher amplitudes than older subjects for amplitudes N1-P2 (Murphy et al., 2000). Analysis of gender-related effects has shown that OERPs and TERPs generated in women subjects are, on average, shorter

in latency and of greater amplitude than in men (Rombaux et al., 2006a). Based on electrophysiological data obtained in a large sample size, the present results (German study) established an age-related loss of olfactory and trigeminal function, which appears to be almost linear. Furthermore, the current results emphasize that responses to chemosensory stimuli are related to sex (Stuck et al., 2006). In the statistical analysis of our olfactory and trigeminal latency N1-P2 (gender), we found no statistically significant differences. In the statistical analysis of our olfactory amplitude N1-P2 (age, gender) we found no statistically significant differences. In the statistical analysis of our trigeminal amplitude N1-P2 (age, gender), we found statistically significant differences by gender, with men reporting significantly higher amplitude than women. Chinese authors have reported that, in the near future, objective olfactometry will be the gold standard in olfactory investigation (Guo et al., 2021). In accordance with the Dresden researchers, we think this is a very optimistic perspective. We believe that psychophysical smell tests will remain the gold standard for a long time (Biswas et al., 2023). In our previous manuscript on the examination of olfaction in severe post-covid Guillain-Barré syndrome, we focused for the first time on the issue of objective olfactory examination and COVID-19 (Červený et al., 2022; Pastorkova et al., 2023).

Table 7. Olfactory event-related potentials (OERPs), healthy participants, wave N1, P2 latency

OERPs	Wave	Latency (ms)	Standard Deviation (SD)
Results of this study	N1	394	77
	P2	505	108
Huart et al. (2012)	N1	397	27
	P2	616	109
Guo et al. (2021)	N1	319	41
	P2	503	55
Rombaux et al. (2006a)	N1	372	
	P2	591	
Stuck et al. (2006) – age 18–35	N1	373	17
	P2	592	14

Table 8. Trigeminal event-related potentials (TERPs), healthy participants, wave N1, P2 latency

TERPs	Wave	Latency (ms)	Standard Deviation (SD)
Results of this study	N1	312	80
	P2	447	111
Huart et al. (2012)	N1	399	55
	P2	554	57
Guo et al. (2021)	N1	391	54
	P2	547	88
Rombaux et al. (2006a)	N1	402	
	P2	634	
Stuck et al. (2006) – age 18–35	N1	395	18
	P2	598	20

Our current ongoing lines of research on the sense of smell are on olfactory loss in patients after COVID-19 (Červený et al., 2022; Pastorkova et al., 2023) and research on olfactory loss in patients with Parkinson's disease and multiple sclerosis (Arpaia et al., 2022; Červený et al., 2022; Martinec Nováková et al., 2015). Our next line of research is the investigation of olfactory loss in patients with chronic rhinosinusitis and nasal polyps – correlation of the microbiome in nasal polyposis and chronic rhinitis with olfactory impairment (Biswas et al., 2023; Červený et al., 2022; Kovář et al., 2017; Vodicka et al., 2007).

In collaboration with neurosurgery, research on olfactory function in patients before/after endoscopic surgery for pituitary adenoma is being performed (Majovsky et al., 2019; Netuka et al., 2019).

Conclusion

Our pilot data of olfactory event-related potentials and trigeminal event-related potentials can function very well as an internal guide for our ongoing olfactory research studies. The evaluation of the presence of OERPs appears to be an important parameter for the evaluation of olfactory disorders. The absence of potentials related to olfactory events is a strong indicator of the presence of olfactory dysfunction. The examination of the olfactory sense should therefore not be neglected. Objective olfactometry appears to be a method with great potential; for example in neurodegenerative diseases, in chronic rhinosinusitis with nasal polyps, and in patients with tumours of the paranasal sinuses, olfactory region and pituitary region. Further olfactory research and data collection in practice could lead to its routine use across medical disciplines soon.

Contributions of authors

All authors contributed to the conception and design of the study. All authors have read and agreed to the published version of the manuscript. All authors gave their consent for publication.

Conceptualization, R.H., K.J, D.F. and J.A.; validation, R.H., L.V., E.M., K.J., K.M., D.K. and J.H.; investigation, R.H., K.J., E.A., and K.M.; resources, R.H., J.A., D.K., H.S. and D.F.; data curation, R.H., E.M., J.H., K.M., H.S. and D.K.; writing-original draft preparation, R.H. K.J., H.S., L.V., J.H. and D.K.; writing review and editing, R.H., K.J., E.M., K.M. and H.S.; visualization, R.H., E.A., K.J., L.V. and J.A.; supervision, R.H., D.F. and J.A.; project administration, R.H., L.V., E.A. and J.H.; funding acquisition, R.H., D.F., E.A., E.M. and J.A.

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Our prospective study was approved by the Ethics Committee of the Military University Hospital Prague – Reference Number 108/16-49/2021 (Project NU 22-09-00493) and 108/16-24/2021 (Project MO 1012). All patients signed an informed consent form.

Ethical aspects and conflict of interest
The authors have no conflict of interest to declare.

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