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Original research article

Olfactory event-related potentials (OERPs) and trigeminal event-related potentials (TERPs) in subjects after Covid-19 infection: single-center prospective study

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Abstract
Objectives: Olfactory dysfunction (OD) is a common symptom associated with Covid-19. During the Covid-19 pandemic, the importance of psychophysical olfactory tests and electrophysiological olfactory assessment increased. The purpose of the study was to analyze the psychophysical olfactory tests and the post-covid curves of olfactory event-related potentials (OERPs) and trigeminal event-related potentials (TERPs).
Methods: The prospective study included 98 subjects (62 females / 36 males). The average age was 42 years (range 21–84 years). Group I (n = 77) contained participants who had been infected with Covid-19. They were enrolled in the study at least 1 year after Covid-19. Group II (n = 21) was the healthy normosmic control group.
Results: In Group I, the OERPs of 18% participants were absent. Patients in Group I were statistically more likely to have an absence of OERPs (p = 0.036) than subjects in Group II. We did not detect a statistical difference in amplitudes and latencies of the OERPs between Group I and Group II. In Group I, N1 latency of the TERPs was significantly longer (p = 0.002) than in Group II. The amplitude of the N1-P2 interval of the TERPs was significantly lower (p = 0.025) in Group I than in Group II. According to the psychophysical Sniffin stick identification test, hyposmia was detected in 39% in Group I versus 0% in the control Group II.
Conclusion: OD is a common post-covid symptom. The presence of OERPs is a significant prognostic factor for olfactory function after Covid 19. We detected a lower percentage of absence of OERPs after Covid-19 compared to the previously published studies of post-viral OD and post-infectious OD. For TERPs, we detected a longer N1 latency and a lower amplitude for the N1-P2 interval after Covid-19. OERPs and TERPs can be considered valid biomarkers to evaluate the progress of post-covid OD.

Keywords: Chemosensory functions; Covid-19; OERPs; Olfactory dysfunction; Post-covid; Smell; TERPs

Highlights:
- Prospective olfactory study with 98 subjects in Group I (post-covid) and Group II (healthy probands).
- We analyzed the psychophysical olfactory tests and electrophysiological olfactory tests.
- In post-covid Group I, the OERPs of 18% participants were absent.
- According to the psychophysical olfactory tests, post-covid hyposmia was detected in 39%.
- The presence of OERPs is a significant prognostic factor for olfactory function after Covid-19.

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Introduction

Smell disorder is a severe clinical problem that has affected millions of people globally, particularly during the Covid-19 pandemic. Persistent olfactory dysfunction (OD) following loss of olfaction associated with Covid-19 infection is a dominant characteristic of long Covid-19 and the most often cited post-covid symptom (Boscolo-Rizzo et al., 2024; Invitto et al., 2023; Pastorkova et al., 2023; Tervo et al., 2024). The proper nomenclature based on the position paper on olfactory disturbance (2023) is "COVID-19-associated post-infectious olfactory dysfunction (C19OD)" (Whitcroft et al., 2023).

Viral illnesses can induce long-term olfactory loss, most likely due to a protective neuronal reaction to stop the intracranial propagation of the virus. The virus may target not only the olfactory neuroepithelium but also the central nervous system, including areas that encode olfactory and gustatory afferents (Boscolo-Rizzo et al., 2024; Invitto et al., 2023; Pastorkova et al., 2023; Tervo et al., 2024). It has been suggested that the post-covid loss of smell is caused by viral damage to the olfactory nerve during its entry through the Angiotensin converting enzyme-2 (ACE2) receptor and transmembrane serine protease 2 (Červený et al., 2022). SARS-CoV-2 is a single-stranded RNA virus with a spike of glycoproteins (protein S) (Najafloo et al., 2021). C19OD has an acute beginning, and its duration is relatively short with a high level of spontaneous recovery (Jung et al., 2023). The incidence of self-reported olfactory dysfunction was 44%–65% after Covid-19 (Ohla et al., 2022). Recovery of olfaction appears to proceed over 3 years after initial Covid-19 infection. The persistent post-covid olfactory damage is predicted to be 16% (Boscolo-Rizzo et al., 2024). Systematic testing using psychophysical olfactory tests showed that initially reduced olfactory performance was re-established in 95% of the patients after 1 year. These patients are frequently assessed as completely recovered based on subjective assessment, but objective testing commonly identifies residual deficits (Hummel et al., 2023).

Qualitative post-covid smell impairments have been observed to affect mood, enjoyment of eating, decrease the recognition of danger, and affect health and social life (Burges Watson et al., 2021; Červený et al., 2022). Psychophysical olfactory tests are the gold standard in everyday clinical practice. During the Covid-19 pandemic, the significance of not only psychophysical smell tests but also objective olfactory methods grew. Electrophysiological olfactory tests are important in cases where the cooperation of participants in psychophysical olfactory tests is difficult and complicated. This may be the case in children, subjects with cognitive disorders, or in the context of medico-legal examinations. The olfactory and trigeminal systems are deeply interlinked (Boscolo-Rizzo et al., 2024; Holy et al., 2023; Invitto et al., 2023; Pastorkova et al., 2023; Ren et al., 2012; Tervo et al., 2024). Olfactory event-related potentials (OERPs) and trigeminal event-related potentials (TERPs) are electrophysiological techniques that provide the evaluation of changes in olfactory and trigeminal function, which is an objective assessment of the integrity of olfactory pathways (Červený et al., 2022; Holy et al., 2023; Invitto et al., 2023). OERPs may be regarded as valuable biomarkers for evaluating the progress of smell dysfunction (Guo et al., 2021a, b). The activity of OERPs can be helpful to validate the presence of olfactory function (Luke et al., 2022). The absence of OERPs is a robust predictor of the presence of OD (Holy et al., 2023).

The chemoreceptors of the intranasal trigeminal system induce high sensitivity of the nasal mucosa to painful stimulation via nociceptors, avoid inhalation of possibly life-threatening substances by suppressing inhalation, and give rise to feelings such as pricking, tingling, coldness, and other chemical-derived sensations (Ren et al., 2012).

For OERPs/TERPs, the N1, P2 waves and the N1-P2 interval are estimated. The first largest negative peak (N1) is assessed at 200–700 milliseconds (ms) and the second positive peak (P2) is assessed at 300–800 ms (Rombaux et al., 2006, 2012). The aim of this prospective study was to analyze the post-covid curves of olfactory event-related potentials and trigeminal event-related potentials and also psychofysical identification Sniffin' Sticks tests. These results were compared with data from healthy normosmic participants.

Materials and methods

Our prospective single-center study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethics committee of the Military University Hospital Prague, reference number: 108/16-24/2021 (project MO 1012, Ministry of Defence of the Czech Republic).

In the period 12/2020–2/2024, 98 subjects were included in the study. All participants signed an informed consent. The subjects consisted of 62 females and 36 males. The average age was 42 years (range 21–84 years). The results of the post-covid curves of olfactory event-related potentials (OERPs) and trigeminal event-related potentials (TERPs) were statistically processed.

The participants were divided into two main groups. Group I (n = 77, post-covid Group) had suffered from Covid-19. Group I was further divided into two subgroups Group Ia and Group Ib. Group Ia consisted of normosmic post-covid subjects – with score results of identification Sniffin' Sticks olfactory test of 12–16 points. Group Ib consisted of OD subjects (C19OD) – with score results of identification Sniffin' Sticks olfactory test was equal to or below 11 points.

Group II (n = 21, normosmic healthy probands) was the control group containing healthy normosmic subjects (NCs) who had not Covid-19 and achieved an identification Sniffin' Sticks olfactory test score of 12–16 points.

For the investigation, we used a clinical olfactometer OL 024 Burghart, Germany. The clinical olfactometer gives precisely defined odor stimuli that are necessary to evoke OERPs and TERPs. An 8-channel EEG system (OL 026; Burghart, Holm, Germany) was used to record the responses.

OERPs were recorded at the top of the head (EEG, electrode Pz). 2-phenylethanol (50% v/v) was used to selectively activate the olfactory afferents. TERPs were recorded at the top of the head (EEG, electrode Cz). CO_2 gas (50% v/v) was used to selectively activate trigeminal afferents. During the experiment, olfactory and trigeminal stimuli were presented separately. Each stimulus type was repeated 20 times and lasted 250 ms. The interstimulus time interval between each stimulus was 10–20 seconds (Červený et al., 2022; Holy et al., 2023). The participants underwent a psychophysical test of odorant identification Sniffin Sticks with a possible maximum score of 16 points, normative data were stated for the Czech population (Kovář et al., 2017; Vodička et al., 2011).

The inclusion and exclusion criteria

The inclusion criteria of Group I was: 18 years of age or older and post-Covid-19 infection (confirmed by PCR (polymerase chain reaction) test), subjects with normal endoscopic intranasal finding. Subject enrollment in the study occurred at a minimum of 12 months after the Covid-19 infection.

The inclusion criteria of Group II was: 18 years of age, subjects without a history of Covid-19 infection and a normal sense of smell, with normal endoscopic intranasal finding, and a Sniffin Sticks identification test without pathological results.

The exclusion criteria of Group I was: less than 18 years of age and without a history of Covid-19 infection, subjects with Parkinson's disease, Alzheimer's disease, Multiple sclerosis in anamnesis, and participants with pathological endoscopic intranasal finding.

The exclusion criteria of Group II was: under 18 years of age and had been infected with Covid-19 infect. Participants with subjective olfactory dysfunction, subjects with Parkinson's disease, Alzheimer's disease, multiple sclerosis in anamnesis, and participants with pathological endoscopic intranasal finding.

The results of these curves of OERPs and TERPs were then statistically processed. Statistical analysis was performed using IBM SPSS Statistics (version 22.0; SPSS, IBM, Armonk, NY, USA). Data were analyzed using descriptive statistics and Mann–Whitney *U* test; Fisher exact test; Chi-Square test; *p*-values equal to or less than 0.05 were considered significant.

The study protocol is shown in the Guidelines Flow Diagram (see Fig. 1).

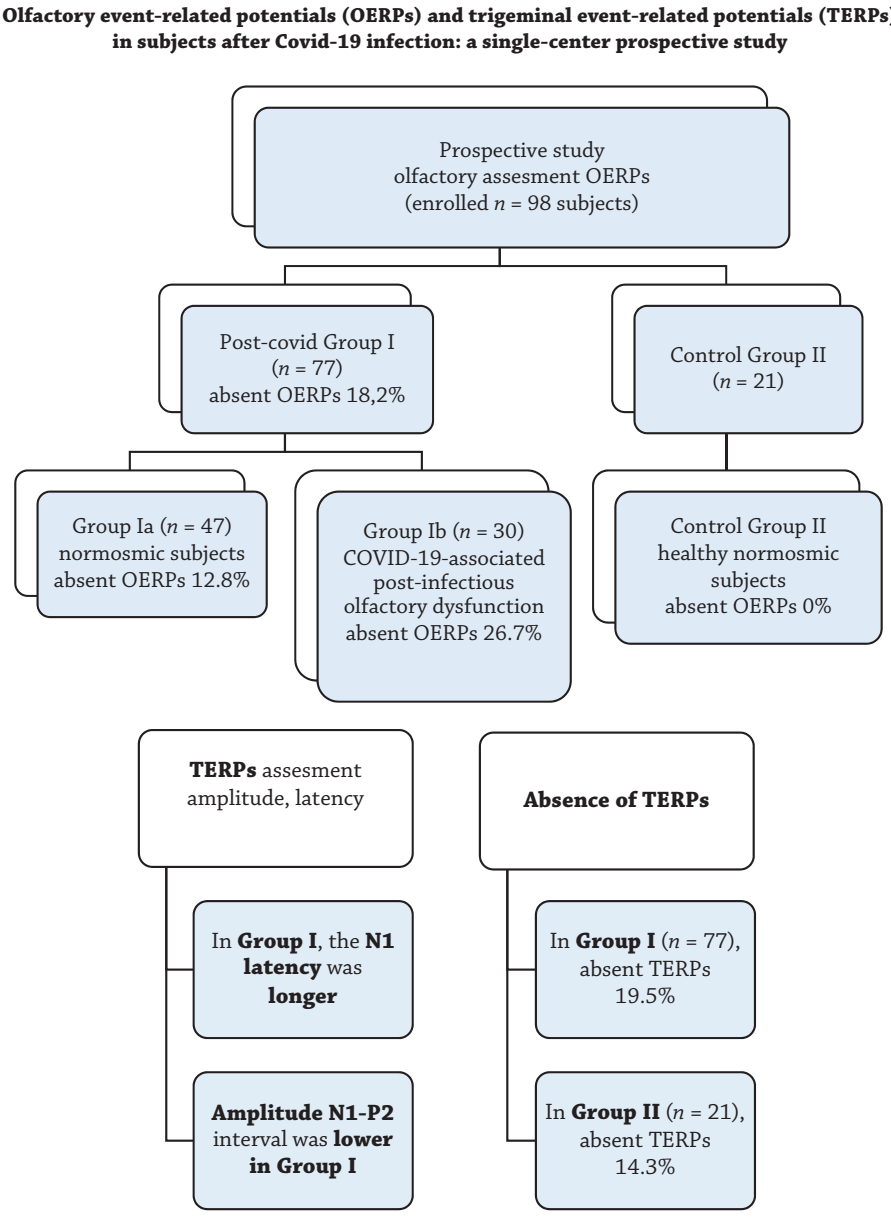

Fig. 1. Guidelines Flow Diagram

Results

We present data on OERPs, TERPs, and the psychophysical Sniffin stick identification test in participants after Covid-19 infection. These results were compared with a group of healthy normosmic participants. We compared the important parameter of whether olfactory event-related potentials (OERPs) are present or absent in Group I and Group II (see Table 1).

Table 1. Comparison of the absence of OERPs in post-covid Group I and healthy normosmic probands in Group II

		OERPs		Total	*p*-Value
		Present	Absent		
Group I	Count	63	14	77	**0.036**
	%	81.8	18.2	100.0	
Group II	Count	21	0	21	
	%	100.0	0.0	100.0	

We did not detect a statistical difference (Mann–Whitney *U* test) in amplitudes and latencies of the OERPs curves between participants in Group I and Group II who exhibited present OERPs. The latencies and amplitudes for N1, P2 waves, and the N1-P2 interval were then analyzed (see Table 3).

In Group I, the absence of TERPs was detected in 15 participants (19,5%). In Group II, the absence of TERPs was detected in 3 participants (14,3%). Statistical analysis of the latencies and amplitudes of the TERPs showed a statistically significant difference ($p = 0.002$) in the latency of the N1 wave. In post-covid Group I, the N1 latency was significantly longer than in the NCs Group II (see Table 4).

In the analysis of latencies and amplitudes of the N1-P2 interval for OERPs and TERPs, there was a statistically significant difference ($p = 0.025$) between the amplitude values of TERPs between Group I and Group II. The amplitude value of the N1-P2 interval was lower in the post-covid Group I than in the NCs Group II (see Table 5).

The OERPs were absent in 14 (18,2%) participants in Group I. In the post-covid Group Ia, the incidence of absent OERPs in normosmic subjects was 12.8%. In the post-covid Group Ib (C19OD), the incidence of absent OERPs in subjects with olfactory dysfunction was 26.7% (see Table 2). Patients in Group I were statistically more likely to have an absence of OERPs ($p = 0.036$) than patients in Group II (see Table 1).

Table 2. Post-covid Group I – analysis of the presence/absence of OERPs in relation to subjective psychophysical olfactory evaluation using the Sniffin' sticks identification test

Post-covid Group I ($n = 77$)		OERPs		Total	*p*-Value
		Present	Absent		
Group Ia (Normosmia)	Count	41	6	47	0.141
	%	87.2	12.8	100.0	
Group Ib [Olfactory dysfunction (C19OD)]	Count	22	8	30	
	%	73.3	26.7	100.0	

Table 3. OERPs – analysis of latencies and amplitudes of N1, P2 waves

OERPs	Group II – Healthy subjects			Post-covid Group I			*p*-Value
	Mean	Median	Valid *N*	Mean	Median	Valid *N*	
Amplitude N1 μV	–3.95	–5.00	21	–5.29	–5.00	63	0.791
Amplitude P2 μV	7.62	6.00	21	5.86	5.00	63	0.393
Latency N1 ms	393.81	410.00	21	412.03	411.00	63	0.470
Latency P2 ms	504.76	512.00	21	524.49	511.00	63	0.624

Table 4. TERPs – analysis of latencies and amplitudes of N1, P2 waves

TERPs	Group II – Healthy subjects			Post-covid Group I			*p*-Value
	Mean	Median	Valid *N*	Mean	Median	Valid *N*	
Amplitude N1 μV	–5.94	–7.00	18	–6.11	–6.00	62	0.583
Amplitude P2 μV	9.67	8.00	18	5.50	5.00	62	0.056
Latency N1 ms	312.28	284.00	18	381.26	384.50	62	**0.002**
Latency P2 ms	446.78	435.00	18	492.23	501.50	62	0.111

Table 5. Analysis of latencies and amplitudes of the OERPs and TERPs of the N1-P2 interval

	Group II – Healthy subjects			Post-covid Group I			*p*-Value
	Mean	Median	Valid *N*	Mean	Median	Valid *N*	
OERPs_latency_N1_P2	113.43	100.00	21	112.46	100.00	63	0.808
OERPs_amplitude_N1_P2	11.57	11.00	21	11.14	10.00	63	0.390
TERPs_latency_N1_P2	134.50	128.50	18	110.97	94.50	62	0.143
TERPs_amplitude_N1_P2	15.61	14.50	18	11.61	10.00	62	**0.025**

Table 6 presents the results of the psychophysical Sniffin stick identification tests. In post-covid Group I, hyposmia was detected in 39% versus 0% in the NCs Group II.

Table 6. Psychophysical olfactory test – Sniffin' stick identification test in post-covid Group Ia, Ib, and Group II with healthy probands

Sniffin' stick identification test	Normosmia	Hyposmia
	12–16 points	0–11 points
Post-covid Group I *N* = 77	Group Ia 47 (61%)	Group Ib 30 (39%)
Group II healthy probands *N* = 21	21 (100%)	0 (0%)

Discussion

Our study focused on the analysis of post-covid OERPs/ TERPs. These are used not only in olfactory research, but also in the diagnosis of olfactory disorders (Guo et al., 2021a, b). The position paper on olfactory dysfunction (2023) reported that EEG-based olfactory testing can be useful for medico-legal purposes (Whitcroft et al., 2023). For COVID-19-associated post-infectious olfactory dysfunction (C19OD), very few studies on OERPs/ TERPs have been published. Some studies have shown that the presence of OERPs is one of the indicators of good prognosis of olfactory function in patients with post-viral OD. Consequently, it was hypothesized that the parameters of OERPs waves could also serve as predictors of olfactory recovery (Rombaux et al., 2010). The presence of OERPs could be used as a prognostic indicator in post-infectious OD patients (Guo et al., 2021a, b). Liu et al. (2016, 2022) indicated that the comparisons of the prevalence of abnormalities in OERPs did not show significant differences between post-viral OD and non-post-viral OD groups. OERPs can still be observed in patients with hyposmia but are less likely to be normal in anosmia.

The literature has reported that the amplitudes of N1 and P2 in OERPs were significantly lower in patients with post-infectious OD (Liu et al., 2016; Rombaux et al., 2010, 2012). Another study indicated that the latencies of N1 and P2 in OERPs were prolonged in patients with post-infectious OD (Guo et al., 2021a, b). In our study, we did not detect a statistical difference in latencies and amplitudes of the OERPs curves in the post-covid Group and NCs Group.

Rombaux et al. (2010) reported that OERPs were found in only 33% of post-viral OD patients (evaluated between 4–18 months after infection). Guo et al. (2021a, b) showed that the presence of OERPs in post-infectious OD was 52%. In our study, we predicted that the post-covid Group was statistically more likely predicted to exhibit the absence of OERPs than the NCs Group. Overall, OERPs were detected in 82% of post-covid patients. In the post-covid normosmic Group, OERPs were found in 87%. In the post-covid C19OD Group with a pathological result of the psychophysical Sniffin' sticks identification olfactory test the curves OERPs were present in 73%.

We recently published a case series of two normosmic patients with post-covid Guillain–Barré syndrome who had a very severe course of the disease. No olfactory disturbance was detected, and psychophysical Sniffin sticks identification olfactory tests were normal. The OERPs were present (Pastorkova et al., 2023). In contrast, in this study, post-covid hyposmia was detected in 39% according to the Sniffin' sticks identification tests. The American authors reported that 55% of the enrolled subjects in the study who underwent Covid-19 were found to have olfactory dysfunction. This was verified by psychophysical olfactory tests (Tervo et al., 2024).

Chinese researchers reported that the presence of TERPs in the post-viral infection OD group was 64% (Liu et al., 2022). However, in our study the presence of TERPs in the post-covid OD Group I was 80.5%. Ren et al. (2012) presented that in the post-viral infection OD group, the latencies for N1 were between 180 and 856 ms, for P2 between 260 and 1004 ms.

In our study, the average value of the latency for N1 was 381 ms and for P2 it was 492 ms. In previous cross-sectional observational studies, it was observed that the latency of TERPs in post-viral infection OD subjects became longer and the amplitude was smaller (Liu et al., 2016, 2022; Ren et al., 2012). The latencies of the N1 wave of TERPs showed significant differences among normosmic healthy subjects Group (NCs) versus the post-viral infection OD Group, with the NCs Group demonstrating the shortest latencies, and the post-viral infection OD Group showing the longest. The NCs Group had a significantly higher amplitude than the Group with post-viral infection OD subjects (Ren et al., 2012). In our study, we are in accordance with the Chinese authors Liu et al. (2022). In post-covid Group I, the latency of the N1 wave (TERPs) was significantly longer than in control Group II.

The amplitude value of the N1-P2 interval was lower in the post-covid OD Group compared to the NCs Group. The amplitude of the N1 wave of TERPs was not statistically different in the NCs Group and the post-covid OD Group. TERPs signals significantly correlated with the Sniffin' sticks score and the deficiency of TERPs. The OD patients had neurophysiological deficits in trigeminal function. The absence of TERPs or lower amplitude in N1 waves are the important characteristics of patients with olfactory diseases (Liu et al., 2016, 2022). In our study, the absence of TERPs in the NCs Group was 14,3% and in the post-covid OD Group was 19.5%.

Conclusion

OD is a common symptom associated with Covid-19. In our study, we focused on the analysis of OERPs and TERPs curves in participants with a history of Covid-19 infection. We detected a lower percentage of absent OERPs in participants after Covid-19 infection compared to the previously published studies of post-viral OD and post-infectious OD. In the post-covid Group Ib (C19OD), the absence of OERPs was observed in 27%. For post-covid TERPs, we identified a longer N1 latency and a lower amplitude for the N1-P2 interval, compared to healthy normosmic probands. Post-covid hyposmia was detected in 39% according to the Sniffin sticks identification tests. Psychophysical olfactory tests are still the gold standard, but OERPs and TERPs could be considered valid biomarkers to evaluate the course of post-covid OD.

Authors' contribution

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., P.D., D.Ka., K.M.* and *J.A.*; validation, *R.H., L.V., D. Ko., K.J., N.P., D.Ka., S.K.* and *J.H.*;

investigation, *R.H., K.J., N.P., L.V., O.V., E.A.* and *K.M.*; resources, *R.H., K.J., D.Ko., J.H.* and *J.A.*; data curation, *R.H., J.V., J.H., E.A., N.P., K.M., P.D., S.K.* and *D.Ko.*; writing original draft, *R.H. K.J., O.V., L.V., S.K., J.V., P.D.* and *D.Ka.*; writing review and editing, *R.H., D. Ka., K.M., S.K., J.V., E.A.* and *J.A.*; visualization, *R.H., P.D., L.V., O.V., N.P., E.A.* and *J.V.*; supervision, *R.H., J.V., D. Ko, D.Ka., J.H.* and *J.A.*; project administration, *R.H., L.V., O.V., K.J.* and *J.H.*; funding acquisition, *R.H., K.J.* and *J.A.*

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

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