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Research Paper

Quantitative assessment of olfactory dysfunction accurately detects asymptomatic COVID-19 carriers

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ABSTRACT

Background: COVID-19 threatens the global community because a large fraction of infected people are asymptomatic, yet can effectively transmit SARS-CoV-2. Finding and isolating these silent carriers is a crucial step in confining the spread of the disease. A sudden loss of the sense of smell has been self-reported by COVID-19 patients across different countries, consistent with expression of the molecular factors mediating SARS-CoV-2 uptake into human olfactory epithelial supporting cells. However, precise quantification of olfactory loss in asymptomatic COVID-19 carriers is missing to date.

Methods: To quantify olfactory functions in asymptomatic COVID-19 patients, we designed an olfactory-action meter that determines detectability indices at different odor concentrations and an olfactory matching accuracy score using monomolecular odors. The optimization of test parameters allowed us to reliably and accurately assess olfactory deficits in a patient within 20 minutes.

Findings: Measurement of detection indices at low concentrations revealed a 50% reduction in asymptomatic COVID-19 carriers. Further, patients with better detection scores showed significantly reduced olfactory matching accuracies compared to normal healthy subjects. Our quantification of olfactory loss, considering all parameters, identified 82% of the asymptomatic SARS-CoV-2 carriers with olfactory deficits. However, on subjective evaluation, only 15% of the patients noticed a compromised ability to smell.

Interpretation: Compromised olfactory fitness can serve as a strong basis for identifying asymptomatic COVID-19 patients. Detailed design specifications and protocols provided here should enable the development of a sensitive, fast, and economical screening strategy that can be administered to large populations to prevent the rapid spread of COVID-19.

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1. Introduction

The coronavirus disease 2019 (COVID-19) pandemic has claimed the lives of 0.86 million till date [1]. The rapidly spreading disease poses a serious threat to human life. Operative strategies to prevent the spread of disease have been implemented by the medical community along with policy makers and leaders [2]. However, identifying the large population of asymptomatic carriers has become a daunting challenge [3]. Reports from different countries suggest the onset of anosmia (loss of sense of smell) as a prevalent symptom for COVID-19 [4–9]. While an olfactory function test could facilitate the identification of asymptomatic carriers, precise quantitative characterization of the extent of olfactory loss in such individuals is missing [10,11].

How does COVID-19 infection cause anosmia? Olfactory sensation starts by binding of odorant molecules to odor receptors expressed on the dendritic cilia of olfactory sensory neurons (OSNs). OSNs convey odor related information to the first relay station, olfactory bulb and from there, information is further carried to higher brain centers to complete odor perception [12–16]. The novel coronavirus, SARS-CoV-2, enters the host cell by binding angiotensin-converting enzyme-2 (ACE2) receptors followed by priming of the viral spike protein by the Transmembrane Protease Serine 2 (TMPRSS2) [17]. Interestingly, single-cell sequencing studies reveal expression of ACE2 and TMPRSS2 in human olfactory epithelial supporting (sustentacular) cells, but not OSNs [18,19]. The reports of anosmia in COVID-19 patients, mostly revealed by self-reporting, can be attributed to

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Research in context

Evidence before this study

The existing literature reports loss of olfaction as one of the early and prevalent symptom for COVID-19 infection. This calls for an urgent need to establish if olfactory deficits can be used as a reliable biomarker for identifying asymptomatic COVID-19 carriers. Many studies also report neurological problems due to COVID-19 infection. Malfunctioning of the sensory periphery and/or higher centers in the olfactory pathway could potentially cause the reported olfactory loss. However, precise quantification of olfactory deficits at the detection thresholds and olfactory readouts reflecting cognitive impairments in asymptomatic patients are missing to date.

Added value of this study

In this study, we report a novel strategy to quantify olfactory deficits of varying severity in COVID-19 asymptomatic patients. Our findings using a custom-built olfactory-action meter offer a precise readout of olfactory fitness, combining olfactory detection abilities at threshold levels and olfactory matching skills reflecting cognitive functioning, to quantify olfactory deficits. The added advantage of our method lies in this combination, which mirrors deficits in olfactory perception caused by malfunction of sensory periphery and higher brain centers. While the subjective evaluation showed deficits only in 15% of the patients tested, quantification using our method detected 82% of asymptomatic carriers with olfactory deficits. This proves the sensitivity and reliability of the readouts reported here.

Implications of all the available evidence

Our results revealed higher detection thresholds for COVID-19 asymptomatic patients for all odorants tested. This implies that any method based on olfactory detection using precise odor pulses of varying concentrations could potentially be used to identify asymptomatic carriers with high success rates. Careful selection of olfactory stimulus factors would facilitate this process. As evidences are accumulating for neurological complications due to COVID-19, any readout for early diagnosis of the same would help better treatment strategies. The olfactory matching test reported in our study will help in detecting cognitive impairments associated with COVID-19 infection.

virus-induced impairments of the olfactory epithelium. Therefore, the extent of probable shutdown of OSN activity through the malfunctioning of supporting cells may vary depending on the severity of infection [20].

Identifying and isolating asymptomatic patients is critical for preventing the rapid spread of COVID-19. Considering the reports of smell dysfunctions and their predictability [21,22], we hypothesized that a multitude of olfactory dysfunctions could occur in asymptomatic carriers [9,23–26]. Assessing minor dysfunctions may not be possible with traditionally used methods due to the lack of precision in delivering stimuli to individuals [27,28]. Therefore, we custom-built an olfactory-action meter, and developed a quantitative and cost-effective olfactory function test to assess olfaction in COVID-19 asymptomatic individuals. Using our custom-written software, we were able to stipulate all stimuli parameters that facilitated the generation of well-controlled odor pulses of varying complexities (see Methods). Our apparatus, originally designed to measure olfactory decision-making abilities of normal healthy subjects and patients with smell dysfunctions, was modified to include safety precautions and we developed a novel paradigm to quantify olfaction in COVID-19 patients. This paradigm also enabled us to assess olfactory matching accuracies that could reflect neurological problems caused by malfunctioning of higher brain centers [29–31].

Re-design of olfactory function test parameters with normal healthy subjects helped us optimize evaluation routines that can be completed within 20 minutes in comparison to normal subjects, 82% of the asymptomatic patient population showed distinct olfactory dysfunctions. Therefore, this method can efficiently be used in clinics to screen and reliably find asymptomatic carriers. Moreover, the conditions established by our work enable the design of a simple screening test amenable to self-administration for highly sensitive, rapid, low-cost, and high-throughput screening of large populations.

2. Methods

2.1. Study population

Our study comprised of separate cohorts of healthy subjects (Table 1) and asymptomatic COVID-19 patients (Table 2). The healthy subjects recruited in this study were mostly from Indian Institute of Science Education and Research (IISER)-Pune residential campus without any symptoms at the time of olfactory function test.

Inclusion criteria for normal healthy subjects:

1. Only the subjects who were willing to be part of the study were included. All subjects signed a consent form to participate in the study.
2. No travel history prior to the test session.
3. No encounters with COVID-19 positive patients.
4. An active respiratory tract infection was not observed and all subjects did not have any nasal obstruction.
5. No symptoms of COVID-19 (fever, rhinorrhea, dry cough, sore throat, and dyspnea) were observed (Supplementary Table 1).

COVID-19 patients were defined as subjects with the confirmed infection for SARS-CoV-2 and were admitted to B. J. Government Medical College and Sassoon General Hospitals isolation wards for asymptomatic patients. The SARS-CoV-2 infection was confirmed by the real-time reverse-transcriptase polymerase chain reaction-based detection of the virus from nasal/nasopharyngeal/ throat/oropharyngeal swab. None of the patients we tested was clinically diagnosed with Parkinson's disease or any other neurological disorders that can cause smell dysfunctions at the time of olfactory function test. The detection protocol was approved by the Indian Council of Medical Research (ICMR). The patients who enrolled for the olfactory function test contracted the disease due to their unintentional interaction with COVID-19 patients in the locality of their residence or were health-care workers (clinicians and support staff) who contracted the disease on duty.

Inclusion/exclusion criteria for the asymptomatic COVID-19 patients:

1. The olfactory function test was performed only with the patients who were completely asymptomatic (Supplementary Table 1).
2. Only the patients with positive RT-PCR results for SARS-CoV-2 were included in the study.
3. Only the patients who were willing to be part of the study were included. All patients signed a consent form to participate in the study.
4. Patients with persistent COVID-19 symptoms were not included in the study.
5. One of the patient's data (BJMC P24) was excluded from data analysis as the patient was already tested negative for COVID-19 at the time of olfactory function testing. But the patient

Table 1
Readouts of olfactory functions measured from normal healthy subjects.

Median of detection indices measured for normal healthy subjects (%)	0.5	0.7	0.8	1		
Concentration tested (% v/v)	9.1	16.6	23.1	50	Normalized olfactory matching performance index	Olfactory deficits
Subject ID						
NS1	0.6	1	1	1	1.04	No
NS2	0.3	0.9	0.9	1	1.19	No
NS3	0.5	0.8	0.9	1	1.19	No
NS4	0.4	0.6	0.6	1	0.74	Yes
NS5	0.8	1	1	1	0.74	No
NS6	0.4	0.8	0.5	1	1.04	No
NS7	0.2	0.3	0.8	1	1.04	No
NS8	0.1	0.8	0.8	1		No
NS9	0.1	0.8	0.5	0.8	1.34	No
NS10	0.9	0.7	0.9	1	0.89	No
NS11	0.7	0.6	1	1	1.19	No
NS12	0.8	0.9	1	1	0.89	No
NS13	0.4	0.7	0.7	1	0.74	Yes
NS14	0.5	0.7	1	1	1.04	No
NS15	0.5	0.7	1	1	1.04	No
NS16	0.6	0.5	0.6	1	1.19	No
NS17	0.7	0.9	1	1	1.19	No
NS18	0.5	0.5	0.8	1	0.59	No
NS19	0.5	0.5	0.5	1	0.89	Yes
NS20	0.5	0.6	0.7	0.9	0.74	Yes
NS21	0.7	0.8	1	1	0.74	No
NS22	0.6	0.7	0.8	1	0.89	No
NS23	0.7	0.7	1	1	0.89	No
NS24	0.8	0.8	1	1	1.04	No
NS25	0.6	0.5	0.7	1	1.19	No
NS26	0.7	0.7	0.8	1	0.74	No
NS27	0.8	1	1	1	1.04	No
NS28	0.5	0.5	0.7	0.9	1.04	No
NS29	0.5	0.5	0.6	0.6	1.49	No
NS30	0.1	0.4	0.5	0.9	0.89	Yes
NS31	0.6	0.4	0.3	1	1.04	No
NS32	0.5	0.9	1	1	1.04	No
NS33	0.3	0.2	0.2	1	1.04	No
NS34	0.7	0.9	0.9	1	1.04	No
NS35	0.4	0.6	0.9	0.9	1.34	No
NS36	0.6	0.9	1	0.9	0.89	No
NS37	0.5	0.7	0.8	1	0.74	No

continued in the isolation ward due to the lack of an isolation facility at patient's residence.

6. Another patient, BJMC P4 did not want to participate in the whole test. Therefore, we evaluated the detection index at 50% (v/v) concentration. As the patient showed good detection (Please see Table 2 for details), we requested the patient to participate in the olfactory matching test; however, the patient disagreed. Hence, we were not able to include the patient's data in the final analysis.

In total, 37 normal healthy subjects and 34 COVID-19 patients participated in the study. To avoid potential sources of bias, olfactory function test was done only with the patients/normal healthy subjects who were willing to participate in the study and the paradigm was well-explained to all participants. The male/female ratio of the healthy subject and COVID-19 patients cohort were 1.642 and 1.615 respectively (Supplementary Table 1).

2.2. Ethics committee approval information

All experimental procedures and protocols used in this study were approved by the IISER Ethics Committee for Human Research (IECHR/Admin/2020/001), Biosafety Committee at IISER-Pune and the Ethics Committee at B. J. Government Medical College and Sassoon General Hospitals, Pune, India (BJGMC/IEC/Pharmac/ND-Dept 0420053−053).

2.3. Sample size determination

There are no reports available to date on precise quantification of olfactory detection thresholds and olfactory matching accuracies of asymptomatic COVID-19 patients for the array of monomolecular odors we report here. Therefore we hypothesized a minimum difference of 10% in the test readouts between the study groups. As per the power analysis, there should be more than 20 subjects in each study group if we expect to see a difference of 10%. However, the quantification of our detection indices showed a reduction of 38%−55% for ten odorants tested (See Results, Supplementary Table 2) and a 50% reduction for the combined data set for three concentrations tested at the detection threshold levels (See Results, Supplementary Table 2). This proves the statistical validity of the sample numbers we report here. We managed to collect the data from 37 normal healthy subjects over three weeks. We had 35 patients enrolled during the study period, that matches with the number of subjects enrolled for the control study. The consistency of our results and the statistically significant difference between the patient and normal subject cohorts reflects the reliability of our readouts.

2.4. Study location

All the instrumental standardization and the mini photo-ionization detector (miniPID) measurements were done at the Laboratory of Neural Circuits and Behavior (LNCB) at IISER Pune. The test sessions for the healthy subjects were done at the IISER Pune Biology

Table 2
Readouts of olfactory functions measured from asymptomatic COVID-19 patients.

Median of detection indices measured for normal healthy subjects (%)	0.5	0.7	0.8	1		
Concentration tested (% v/v)	9.1	16.6	23.1	50	Normalized olfactory matching performance index	Olfactory deficits
Patient ID						
BJMC P1	0.0	0.0		0.1		Yes
BJMC P2	0.0	0.0	0.0	0.0		Yes
BJMC P3	0.0	0.0	0.0	0.3		Yes
BJMC P4*				0.9		Not evaluated
BJMC P5	0.0	0.0	0.0	0.0		Yes
BJMC P6	0.0	0.0	0.0	0.4		Yes
BJMC P7	0.5	0.5	0.6	0.6	0.66	Yes
BJMC P8	0.4	0.6	0.6	0.4		Yes
BJMC P9	0.8	0.6	0.6	1.0	0.89	Yes
BJMC P10	0.4	0.4	0.6	0.7	0.44	Yes
BJMC P11	0.7	0.9	1.0	1.0	0.89	No
BJMC P12	0.0	0.3	0.3	0.5		Yes
BJMC P13				0.0		Yes
BJMC P14	0.1	0.1	0.2	1.0	0.44	Yes
BJMC P15	0.0	0.0	0.0	0.0		Yes
BJMC P16	0.0	0.2	0.1	0.8	1.12	Yes
BJMC P17	0.1	0.6	0.2	0.7	0.56	Yes
BJMC P18	0.6	0.6	1.0	1.0	1.30	No
BJMC P19	0.0	0.2	0.3	0.8	0.89	Yes
BJMC P20	0.8	0.8	0.7	0.8	0.89	Yes
BJMC P21	0.6	1.0	1.0	1.0	0.89	No
BJMC P22	0.1	0.2	0.3	0.1		Yes
BJMC P23	0.0	0.5	0.7	0.8	1.30	Yes
BJMC P25	0.0	0.0	0.0	0.0		Yes
BJMC P26	0.0	0.0	0.0	0.4		Yes
BJMC P27	0.4	0.6	0.5	0.7	0.66	Yes
BJMC P28	0.6	0.5	0.4	0.7	0.82	Yes
BJMC P29	0.4	0.3	0.8	1.0	1.32	No
BJMC P30	0.4	0.8	0.8	1.0	0.74	No
BJMC P31	0.8	0.7	0.7	0.9	0.89	Yes
BJMC P32	0.8	0.7	0.9	1.0	1.04	No
BJMC P33	0.0	0.1	0.2	0.5		Yes
BJMC P34	0.0	0.0	0.1	0.7	0.18	Yes
BJMC P35	0.0	0.0	0.0	0.8	0.56	Yes

* BJMC P4 did not want to participate in the whole test. Therefore, we evaluated only the detection at 50% (v/v) concentration.

Department in a location that matched the ambient environment of the COVID-19 ward at B. J. Government Medical College and Sassoon General Hospitals. This was specifically done to minimize the variability of odor profiles with varying temperature and humidity. The olfactory function test for the healthy subjects was carried out during the period 22nd April 2020 to 10th May 2020. To test the olfactory abilities of asymptomatic COVID-19 patients, the instrument was shifted to the COVID-19 ward at B. J. Government Medical College and Sassoon General Hospitals. Here, the study was performed during the period of 12th May 2020 to 21st May 2020. Overall, patients from two different wards were tested for their olfactory abilities during this period.

2.5. Olfactory-action meter design

The olfactory-action meter is a ten-channel olfactometer, which can deliver odors with high temporal precision. The instrument delivers odorized air through a glass funnel into an odor delivery unit through which subjects smell different odors. HEPA sterilized air is pumped in the olfactometer at a rate of 5 liters/min. Further, the air passes through an air filter, which traps background odor. From this filter, the deodorized sterile air is split into eleven channels using a metallic manifold. The air channels are connected to ten mini Mass Flow Controllers (Pneucleus Inc.) and one Main Mass Flow Controller (Pneucleus Inc.). The operation of these mass flow controllers (MFCs) is software-driven and the experimenter can control the volume of air passing through each of them. The output from the main MFC was bifurcated into ten channels using a battery of solenoid valves (one for each odor channel). The solenoid valves allowed us to have precise control over the clean air delivery timing. The outputs from each of the ten mini MFCs are connected to ten different odor reservoirs. The odor reservoirs were made up of 15 ml glass bottles with a glass cap with separate channels for the input of clean air (from mini MFC) and output for odorized air. Each reservoir was filled with 4 ml of pure monomolecular odorant. The odor vapors from the odor reservoirs travel through Tygon tubing and mixes with a stream of clean air (controlled by the main MFC) before entering the glass nozzle. The total odorized airflow is thus the sum of the volume of odor vapors and the volume of clean air. The volumetric concentration (%v/v) of the odor can therefore be defined as the ratio of the volume of odor vapors to the total volume of odorized air. By changing the ratio, we selected different concentration levels ranging from 9.1−50% (v/v) for each of the odors used. The output of the funnel was connected to a disposable odor delivery unit. The odor delivery unit was a 15 cm long tube with a suction outlet. The tube was fitted with four different layers of filters made from surgical mask grade material along its length. The suction output was guarded by a 0.2 μm PES filter (Whatman Uniflow). The odor delivery unit was UV sterilized before use and a new unit was used for each subject. This nullified the chances of cross-contamination between the subjects and also prevented contamination of the instrument. The PES filter of the odor delivery unit was attached to a vacuum pump operating at −450 mbar. The exhaust line was additionally fitted with two 0.2 μm HEPA filters and one 0.2 μm PES filter before the exhaust was released to a 60 cm long activated carbon filter. The vacuum was always functional except during the odor delivery, during which air traveled from the odor nozzle to the tip of the odor delivery unit. We placed an additional separating wall covering the instrument to prevent any physical contact of the patient with instrument. The tip of the odor delivery unit came out of a port made on the separating

wall. The height of the port was fixed by taking into consideration the average height of the Indian population. Further, all participants were asked to wear a surgical mask while performing the olfactory function tests. All normal healthy subjects and COVID-19 patients performed the olfactory function test wearing the mask. All these safety measures prevented any viral particle from entering into the system and made it suitable for use under clinical settings (See Results, Fig. 1A, and Supplementary Movie SM1).

2.6. Odors

The odors used in the study were monomolecular odorants, which are commonly used in human olfactory experiments [32,33]. Odors used were Hexanal, Isoamyl acetate, Octanal, 1,4-Cineole, (+)-Limonene, (-)-Limonene, Acetophenone, (-)-Carvone, Ethyl butyrate, and Eugenol. All these odors were procured from Sigma Aldrich and had ~99% purity. The odor profiles for each of the odors were measured using miniPID (Aurora Scientific) at 50% concentration (v/v). The measurements were done by placing the probe of the miniPID at the tip of the odor delivery unit. All the measurements were done by matching the parameters set for the olfactory function test.

2.7. Olfactory function test

The test session began by explaining the subjects about the study paradigm and asking them to read the consent form carefully. To facilitate better understanding, the forms were available in three different languages (English, Hindi, and Marathi). Once the subjects had proper understanding of the test, we began with the measurements of odor detection indices, followed by olfactory matching test.

2.8. Measurements of odor detection indices

In odor detection measurements, ten different odorants were delivered at different concentrations. For the normal healthy subjects, we continued the measurements until they could detect each odor consecutively for two different concentrations and the lower of two was taken as the threshold for a specific odorant. Their detection was also checked at 50% (v/v) concentration. On analyzing the odor detection threshold for this cohort, we observed that most of the participants could detect all odors within the lower three concentrations. The lower three concentrations were 9.1%, 16.6%, and 23.1% (v/v) of odorized air and the patient's detection abilities were probed at these concentrations. The odors were delivered in ascending order of concentration. All odors were sequentially delivered at a particular concentration level and then the concentration was increased to the next level. The sequence of odors changed with each concentration level.

The odors were delivered for 4 s with an inter-trial interval of 17.2 s. Before the odor delivery, there was a set preloading time of 3.2 s during which the odorized air traveled through the odor nozzle into the odor delivery unit. However, the odorized air was diverted into the suction line of the odor delivery unit. After the preloading time elapsed, the odorized air traveled to the tip of the odor delivery unit into the nose of the participant. The preloading time ensured a sharp odor pulse and minimized the delay in odor delivery. For the odors used in the study, we observed a delay of around 100−200 ms which was irrespective of the differences in the physicochemical properties of the odors. During the odor delivery, participants were expected to breathe normally and make an assessment of whether they could smell any odor. We categorically instructed the participants only to detect and not try to identify the odors delivered. To alert the participants for odor onset, a tone of 200 ms was played 1 s prior to the odor delivery. At the end of the odor delivery, the participants were expected to give a verbal response of 'YES' or 'NO' depending on whether they detected the odor or not. The experimenter noted down all the answers from each participant (Fig. 1A, Supplementary Movie SM1).

After delivering the odors at three different concentrations, the participants were asked to take a break for two minutes. Next, the concentration was increased to 50% (v/v) for all odors (highest concentration level tested with olfactory-action meter). The participants were again asked to continue detecting the odors. However, participants were not informed about the increasing odor concentrations to prevent having any preconceived responses towards the odorants of higher concentrations. At the end of the measurements, the detection threshold was measured separately for each odor and also the detection accuracy was calculated for each concentration level. Detection accuracy was measured by calculating the number of odors detected at that particular concentration over ten presented odors. For 38.2% of the patients (13 out of 34), we observed that the detection accuracy was $\leq$ 50% at a high concentration level (50% v/v). Out of these 13 patients, 10 patients agreed to further participate in the measurements of detection of neat odors. We continued scoring the detection accuracies by providing paper strips dipped in pure odorants. This additional step was not performed for the healthy subjects as all of the participants in the healthy subject cohort could detect more than five odors at the same concentration level.

To quantify the detection deficits in asymptomatic COVID-19 patients, we measured the median detection indices shown by healthy subjects and compared them with those measured from patients. Very stringent criteria of either having detection deficits at all four concentrations tested or detection deficits at two or more concentrations and reduced olfactory matching performance index were used to categorize patients as 'with olfactory deficits'.

2.9. Measurements of odor matching performance index

Participants with $\geq$60% detection accuracy at high concentration level (50% v/v) were asked to participate for olfactory matching measurements (1 out of 21 COVID-19 patients declined participation). The odor matching probed the discrimination abilities and working memory of the participants. Each session consisted of ten trials. The trial was initiated with a tone of 200 ms, and after 1 s, the first odor was delivered. The odor was delivered for 4 s and then, there was an inter-stimulus delay of 5 s after which the second odor was delivered for 4 s. In a trial, the two odors presented could either be same or different and the participants were expected to compare two odors delivered sequentially and assess if the odors were 'SAME' or 'DIFFERENT'. The participants were expected to give a verbal response after the second odor delivery. The response was registered using a response console by the experimenter and additionally, the responses were noted down.

Two odor pairs were used for olfactory matching measurements. One pair varied significantly in their response amplitudes when measured with miniPID (Hexanal vs. Acetophenone) while the other pair had similar response amplitudes (Isoamyl acetate vs. 1,4-Cineole) (Fig. 4A). The odors were delivered at 50% (v/v) concentration and the sequences of trials were randomized. The number of same and different trials varied randomly with each session. All participants from the healthy subject cohort performed in olfactory matching measurements with these two odor pairs. For the COVID-19 patient cohort, most of the patients performed the measurements with these two odor pairs. However, if they failed to detect any of these odorants at 50% (v/v), then that odorant was replaced with another odorant.

For analyzing the olfactory performance index, we calculated the mean performance accuracy of the normal healthy subject cohort. The performance accuracies shown by all participants (including normal subjects and patients) were then normalized to this mean value and the data is plotted as normalized matching accuracy (Fig. 3B).

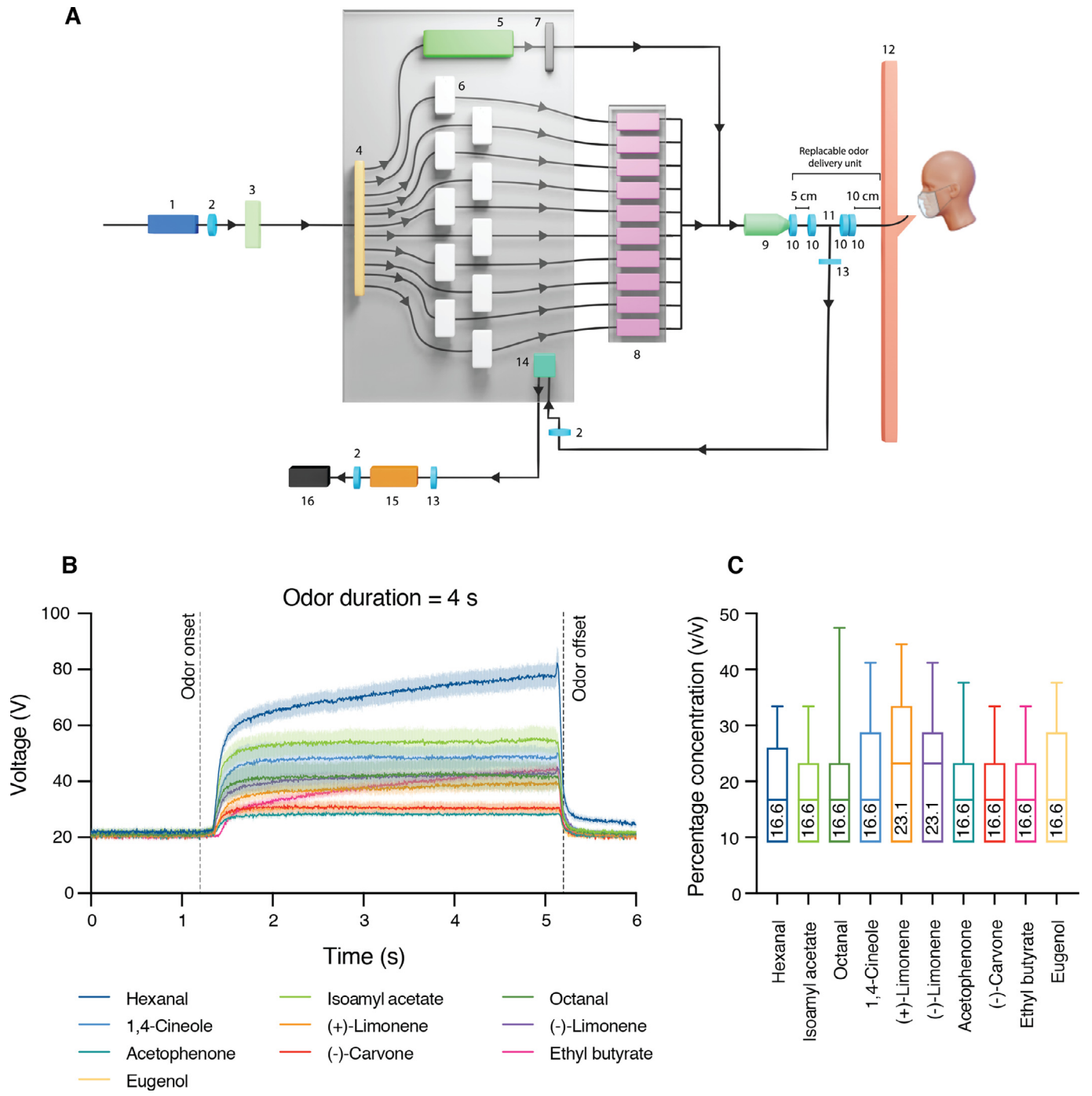

Fig. 1. Optimization of olfactory function testing parameters for COVID-19 patients.

A. Schematic representation of the olfactory-action meter. To ensure the utmost safety for usage in COVID-19 isolation wards, HEPA-filtered air was pumped in the instrument. The filtered air was bifurcated into eleven streams (into ten mini mass flow controllers (MFCs) and to the main MFC) using a manifold. The volumetric airflow was controlled using these MFCs by a custom-written software in LabWindows (National Instruments). The output from the main MFC was bifurcated into ten channels, which were controlled by using a battery of solenoid valves (one for each odor channel). The solenoid valves allowed us to have precise control over the clean air delivery timing. During the preloading phase (3.2 s), the air in each channel passes through the odor bottle and is mixed with a stream of clean air before entering in the odor nozzle. A suction (−450 millibar) placed outside the exit of the nozzle diverts air through a series of three 0.2 μm filters (one Whatman Uniflow and two HEPA filters) into the exhaust (activated carbon filter). The output towards the vacuum was guarded by 0.2 μm PES filter (Whatman Uniflow). To administer the test, suction is switched off and the odorized air travels through the odor delivery unit into the nose of the patients. All subjects are required to wear a surgical mask and the entire odor delivery unit is replaced for each test to avoid cross-contamination. Four layers of filters made from surgical mask grade material are placed along the length of the odor delivery unit to avoid instrument contamination. Further, a separating wall ensures that the patient doesn't come in physical contact with the instrument.

1. Air Pump (5 L/min). 2. 0.2 μm HEPA filter. 3. Air filter. 4. Manifold. 5. Main Mass flow controller (200 uccm). 6. Mini Mass flow controller (for each odor line, 20−200 uccm). 7. Solenoid valves. 8. Odor box containing ten odor bottles. 9. Glass odor nozzle. 10. Filter made from surgical mask material. 11. T joint (Replaceable odor delivery unit consists of 10, 11 and 13). 12. Separating wall. 13. 0.2 μm PES filter (Whatman uniflow). 14. Electromagnetic valve. 15. Vacuum pump (−450 mbar). 16. Carbon filter (60 cm in length).

B. Ten odorants of varying physical properties were selected for the olfactory function test. The kinetics of the odor pulses were measured for all 10 odorants using a mini-PID (Aurora Scientific). The use of vacuum during the preloading phase guaranteed precise delivery of odors with minimum delay (100 − 200 ms onset time) and allowed us to present subjects with a sharp odor pulse. Depending on the physicochemical properties of each odor, the amplitude and the rise time varied across different odors (PID amplitudes, Two-way ANOVA, $F[9,40] = 18.49$, $p<0.0001$). Traces were averaged across five trials. Data is represented as mean±SEM.

2.10. Calculation of olfactory function score (OFS)

To estimate the extent of olfactory deficits in the patients as compared to normal healthy subjects, we considered detection indices as well as olfactory matching performance index measured (Table 1 and Table 2). We gave equal weightage to the olfactory detection indices and olfactory matching performance index to calculate an olfactory function score. OFS was calculated by taking the average of detection indices measured for all four concentrations (9.1%, 16.6%, 23.1% and 50% v/v) and the normalized olfactory matching performance index measured. For patients who did not qualify for olfactory matching measurements, their OFS was calculated by taking the average of their detection indices measured at all concentrations. Separate analyses were performed to probe the effect of confounding variables such as age and gender (see Results, Supplementary Figure 1) and missing variables (see Results, Supplementary Figure 3).

2.11. Statistical analyses

All statistical analyses were done using GraphPad Prism 8, MATLAB 2017a and 2020a. We used Analysis of variance (ANOVA) and associated post-hoc tests, One-tailed, and Two-tailed unpaired t-tests, Receiver Operating Characteristic (ROC) analysis for sensitivity and specificity, linear regression analysis for confounding variables, box-plot analysis and Grubb's test for sensitivity of OFSs (found no outliers, see Results), analysis based on random sampling for missing variables and Correlation analysis of olfactory function test readouts (see Results and figure legends for details).

2.12. Role of funding

No study sponsor had any role in study design, data collection, analysis, or interpretation of the data; or in writing this manuscript or the decision to submit for publication. The corresponding author had full access to all study data and had final responsibility for the decision to submit for publication.

3. Results

3.1. Quantitative olfactory function test for COVID-19 patients

Establishing a diagnostic test for an infectious disease in clinical settings requires optimization of the time spent by each patient and prevention of cross-contamination between patients. To achieve this, we designed and custom-built an olfactory-action meter with a replaceable odor delivery unit by taking all safety precautions (Fig. 1A and Supplementary Movie SM1, see Methods). To identify parameters for a clinically relevant olfactory function test, we recorded olfactory responses from 37 normal healthy subjects (23 males and 14 females, for details of normal healthy subjects: Supplementary Table 1) towards different odorants. As different odorants evoke variable responses [32], we selected ten monomolecular odorants of varying volatilities. We performed PID measurements at 50% [v/v (volumetric concentration of the odorized air stream, see Methods)] concentration and the readouts reflect varying physical properties of the odorants (Fig. 1B, PID amplitudes, Two-way ANOVA, $F(9,40)=18.49$, $p<0.0001$).

We tested the detectability of normal healthy subjects towards ten different odorants of varying concentrations (9.1%, 16.6%, 23.1% and 50% v/v). We presented odorants from lower to higher concentrations and concluded their detection thresholds on detecting two consecutive concentrations successfully. The lower of these two concentrations was considered as the detection threshold for a subject. At a specific concentration, each of the odorants was presented with an inter-trial interval of 17.2 s. On testing the detectability, we found that majority of the healthy subjects could detect eight out of ten odorants at the second-lowest concentration (16.6% v/v) and the remaining two odorants were detected at the third-lowest concentration (23.1% v/v) (Fig. 1C and Table 1). These experiments identified the parameters for olfactory testing of COVID-19 patients.

3.2. Olfactory detection abilities are extremely compromised in asymptomatic COVID-19 patients

To assess the olfactory functions of asymptomatic COVID-19 patients, we first tested 34 patients (21 males and 13 females) admitted in two isolation wards for asymptomatic patients at B. J. Government Medical College and Sassoon General Hospitals, Pune, India. These patients include the ones identified through contact tracing due to their unintentional interaction with COVID-19 patients, healthcare workers and a small percentage with mild symptoms (For details of patients: Supplementary Table 1). They have been tested positive for COVID-19 (positive RT-PCR results of SARS-CoV-2 infection) and admitted to the isolation wards (see Methods). At the time of olfactory function test, none of these patients had any symptoms of fever, rhinorrhea, dry cough, sore throat and/or dyspnea (see Methods, Supplementary Table 1). Prior to carrying out the olfactory function test with a patient, we took the medical history and enquired about their ability to smell and taste food or any other substances around. We specifically asked if they could smell and taste the regular food items that the hospital provided as well as smell the other odorous substances they would have used. In response to these questions, 85% of the total patient population responded that they had no problems in detecting different smells. 91% of the total patients did not have issues with their taste.

At low concentrations for all odors, we find that asymptomatic COVID-19 patients display significantly reduced detection abilities compared to normal healthy subjects (Fig. 2A, pooled analysis for all ten odors, Two-way ANOVA, $p<0.0001$). The quantification of detection indices showed a reduction of 38% - 55% for ten odorants tested (Fig. 2A, comparison of area under curves [34], Supplementary Table 2) and a 50% reduction for the combined data set for three concentrations tested at the detection threshold levels (Fig. 2B, comparison of area under curves, Supplementary Table 2). To quantify the percentage of patients suffering from olfactory dysfunctions, we compared the pooled detection scores shown by normal healthy subjects and asymptomatic COVID-19 patients for all ten odorants (Fig. 2B, Two-way ANOVA, $F(1, 200) = 82.77$, $p<0.0001$). Comparison of detection scores shown by patients and normal healthy subjects showed that up to 81% of the asymptomatic patients had olfactory dysfunctions and failed in detecting odorants at low concentrations (Fig. 2C), and 65% (20 out of 31 patients) showed lower detection scores for all three concentrations tested (Table 2).

To investigate the extent of anosmia in these patients, we further evaluated their detection scores for higher concentrations. Normal healthy subjects showed detection accuracy of 96%, whereas asymptomatic patients showed significantly reduced detection accuracy of 61% (Fig. 2D, comparison between detection accuracies at 50% (v/v) concentration, Two-tailed t-test, $p<0.0001$, $t = 5.8$, df=69). For patients who failed to detect ≤ 5 odors at 50% (v/v) concentration, we further scored detection accuracies by providing paper strips dipped in pure odorants. Under such conditions, 82% of the asymptomatic

C. Detection thresholds shown by normal healthy subjects for all ten odorants. Healthy subjects could detect eight out of ten odors at the second-lowest value of 16.6 (% v/v) concentration. For the enantiomer pair of limonene, the detection threshold was found to be 23.1 (% v/v) concentration. The line within the box plot indicates the median detection value for the healthy subjects across different odorants. The whiskers indicate the highest detection thresholds observed in the healthy subjects for different odorants ($n = 37$ subjects).

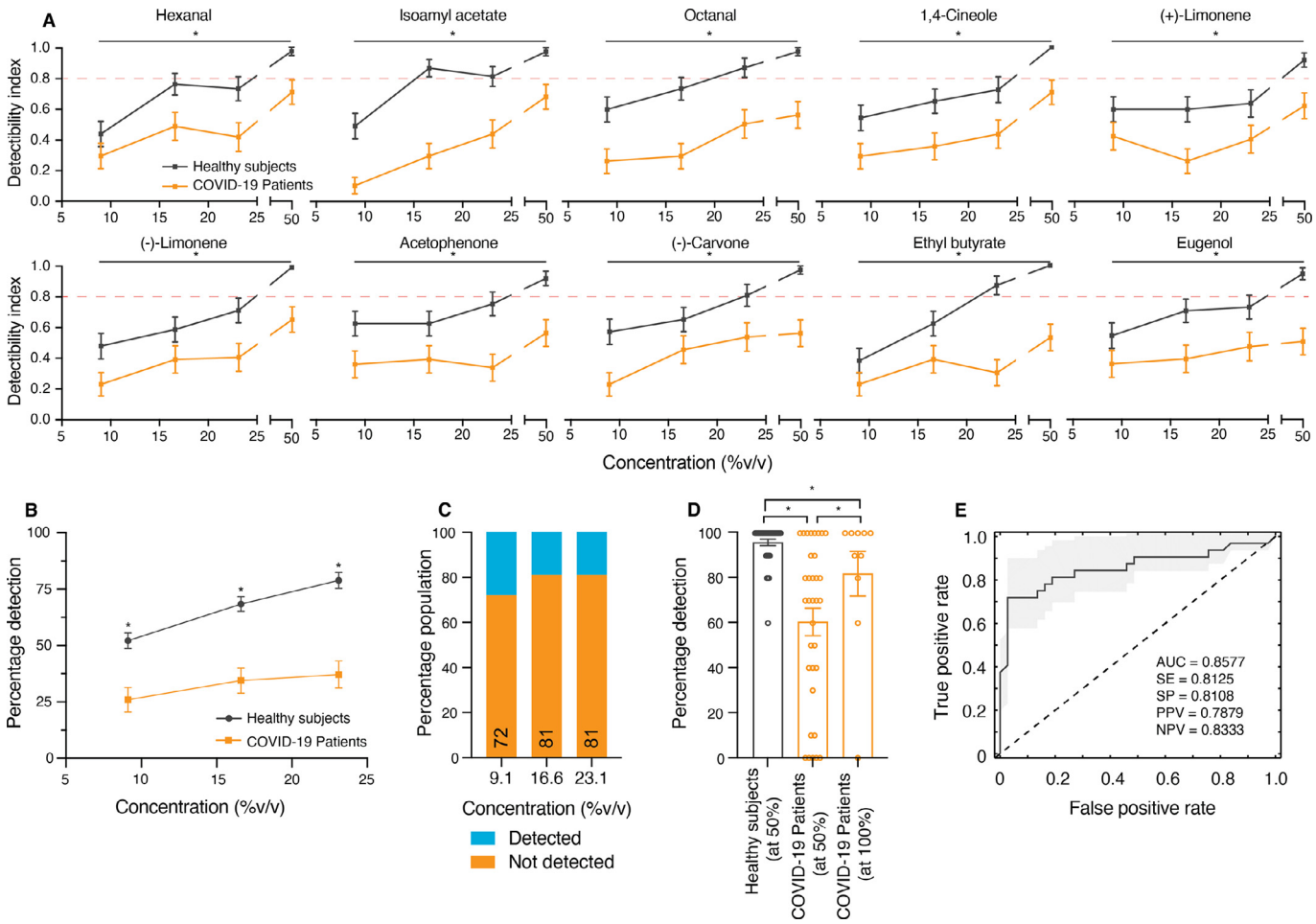

Fig. 2. Asymptomatic COVID-19 patients show severely compromised olfactory detection abilities.

A. Reduced olfactory detectability in asymptomatic COVID-19 patients for all odorants tested. Detectability index was measured by calculating the fraction of odors the subjects could detect at a given concentration. For all odorants tested, healthy subjects display higher detectability indices than asymptomatic COVID-19 patients. Two-way ANOVA: for Hexanal, $F(1, 260) = 20.72$, $p<0.0001$, for Isoamyl acetate, $F(1, 265) = 68.21$, $p<0.0001$, for Octanal, $F(1, 258) = 52.74$, $p<0.0001$, for 1,4-Cineole, $F(1, 257) = 26.2$, $p<0.0001$, for (+)-Limonene, $F(1, 258) = 20.62$, $p<0.0001$, for (-)-Limonene, $F(1, 260) = 26.84$, $p<0.0001$, for Acetophenone, $F(1, 260) = 31.64$, $p<0.0001$, for (-)-Carvone, $F(1, 259) = 30.82$, $p<0.0001$, for Ethyl butyrate, $F(1, 259) = 45.22$, $p<0.0001$, for Eugenol, $F(1, 261) = 28.45$, $p<0.0001$. Data is represented as mean±SEM.

B. Quantification of olfactory loss. Shown are the pooled detectability scores for all odorants for healthy subjects and asymptomatic patients (Two-way ANOVA, $F(1, 200) = 82.8$, $p<0.0001$). Data is represented as mean±SEM.

C. Majority of asymptomatic COVID-19 patients (>80%) show olfactory dysfunctions. Shown are the comparison between median percentage detection scores recorded for healthy subjects and asymptomatic patients at three concentration levels. Asymptomatic patients showed 72%, 81% and 81% reduction in the scores compared to normal healthy subjects.

D. Detectability at 50% (v/v) concentration and with a neat dose of pure odorants. On measuring the detectability at 50% (v/v) concentration, we observed that healthy subjects showed an average detection of 96% at 50% (v/v) concentration. However, when measured in the patient cohort, we observed greatly impaired detection of 61%. (Two-tailed *t*-test, $p<0.0001$, $t = 5.8$, df=69). Patients who were unable to detect at 50% (v/v) were then tested with a neat dose of pure odorants. For pure odorants, these patients showed a high detection of 82%. Data is represented as mean±SEM.

E. Receiver operating characteristic (ROC) analysis for predicting olfactory dysfunction using detection indices measured for different concentrations of various odorants in asymptomatic COVID-19 patients.

ROC analysis shows an AUC of 0.86, specificity of 0.81 and sensitivity of 0.81 for prediction based on detection indices measured from healthy subjects and asymptomatic COVID-19 patients. Values for Area Under Curve (AUC), Sensitivity (SE), Specificity (SP), Positive Predictive Value (PPV), Negative Predictive Value (NPV) are shown in the figure. 95% confidence interval bound is marked by the gray shaded area.

patient population displayed high detection accuracy (Fig. 2D, comparison between detection accuracies at 50% (v/v) concentration (n = 34 patients) and neat dose of pure odorants (n = 10 patients), One-tailed *t*-test, $p<0.05$, $t = 1.7$, df=42). ROC analysis with dataset of detection indices at four different concentrations (at 9.1%, 16.6%, 23.1% and 50% v/v) was performed. The area under curve (AUC), reflecting the accuracy of these measurements as diagnostic parameters, falls in a good predictive value of 0.86 (Fig. 2E). Further, the analysis showed good sensitivity and specificity above 80%. These results indicate that olfactory deficits in COVID-19 patients are mostly apparent with low odor concentrations and emphasize the necessity for sensitive and precise methods to reveal olfactory dysfunction in patients. Importantly, results from self-reporting or using less defined stimuli such as paper strips with neat/pure odorants (see methods, ~100%) must be interpreted with caution.

3.3. Asymptomatic COVID-19 patients show impaired olfactory matching skills

One of the entry points for SARS-CoV-2 virus is the non-neuronal supporting cells in the human olfactory epithelium [18,19]. While the extent of infection may decide the severity of olfactory loss in COVID-19 patients, the mechanism through which the virus possibly shuts down the OSN activity remains unknown. It is very unlikely that infection is restricted only to the sensory periphery. Recently published autopsy reports proved the presence of SARS-CoV-2 protein in

multiple organs, including the brain [35]. If the virus infects higher brain centers, depending on the severity of infection, consequences might be reflected in different behavioral readouts. Therefore, it is critical to probe if the cognitive skills involving higher brain centers beyond the periphery are also affected in patients.

Recently, we have developed an olfactory matching paradigm that reflects the discrimination abilities and working memory of participating subjects. We optimized parameters such as duration of stimuli, inter-stimulus interval and inter-trial interval using 265 normal healthy subjects (Bhowmik et al., unpublished data). We made use of these optimized parameters to study the olfactory matching abilities in COVID-19 patients. In this paradigm, the subject was provided with two consecutive stimuli, each with a duration of 4 s and separated by an inter-stimulus interval of 5 s. Following this, the subject was asked to report if the presented stimuli were same or different. Patients showing a percent detection index of 60 or above at 50% (v/v) concentration were requested to participate in olfactory matching measurements and we selected odorants based on their physical properties (Fig. 3A). On comparing with normal healthy subjects, asymptomatic COVID-19 patients showed impaired olfactory matching skills (Fig. 3B, comparison of normalized olfactory matching accuracies between normal healthy subjects and COVID-19 patients, Two-tailed *t*-test, $p = 0.015$, $t = 2.5$, df=54). This calls for further studies addressing how COVID-19 infection might affect the central nervous system function.

3.4. Severe olfactory dysfunctions in asymptomatic COVID-19 patients

Based on a cumulative analysis of behavioral readouts (detectability at 9.1%, 16.6%, 23.1% and 50% v/v, and normalized olfactory matching accuracy), we categorized patients as those with olfactory deficits based on whether they showed deficiency in detectability indices for all four concentrations tested or showed deficiency in detectability indices for two or more out of the four concentrations as well as in olfactory matching accuracy. Such analysis revealed that 82% of the patient population (27 out of 33) and 13% normal healthy subjects (5 out of 37) with olfactory deficits (Fig. 4A,B Tables 1 and 2). Further, we have calculated the sensitivity, specificity and accuracy considering the number of true positives (TP), false positives (FP), true negatives (TN) and false negatives as per this classification. This analysis showed a sensitivity of 82% [TP/(TP+FN)], specificity of 87% [TN/(TN+FP)] and accuracy of 85% [(TP+TN)/(TP+TN+FP+FN)]. Please note that one patient (BJMC P24) tested negative for COVID-19 at the time of olfactory function testing and was not considered. Another patient (BJMC P13) chose not to participate in the entire test. Therefore, we only evaluated detection indices at 50% (v/v) concentration and for pure odorants where the patient showed 0 and 0.6 detectability indices; hence categorized as a patient with olfactory deficit.

We further assigned equal weightage for all behavioral readouts to calculate an olfactory function score (OFS), calculated by averaging detectability indices and normalized olfactory matching performance index. Based on such analysis, asymptomatic COVID-19 patients showed significantly reduced OFSs compared to normal healthy subjects. Further, 85% of the patient population (28 out of 33) fell below the median scores shown by healthy subjects (Fig. 4C, Two-tailed unpaired *t*-test, $p<0.0001$, $t = 6.4$, df=68). ROC analysis with OFS values shows a good predictive AUC value of 0.83, indicating OFS as a good olfactory fitness predictor to diagnose COVID-19 (Fig. 4D). Further, the analysis shows a sensitivity of above 70% and specificity of above 90%. This signifies the robustness of our behavioral readouts and the methods employed for determining olfactory function across individuals.

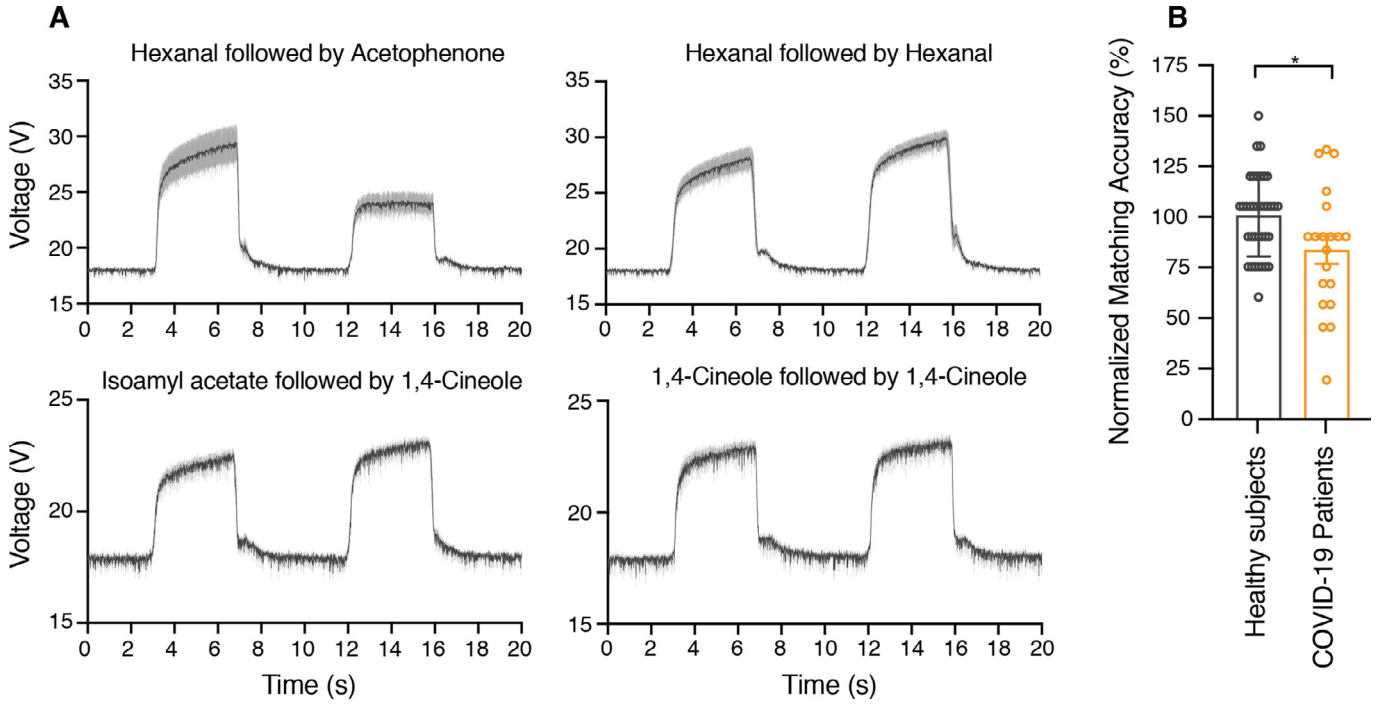

Fig. 3. Reduced olfactory matching accuracies shown by asymptomatic COVID-19 patients.

A. Precise odor delivery in an olfactory matching paradigm. For odor matching measurements, odor pairs were selected from the pool of detected odorants for each patient. One odor pair had a difference in the voltage amplitudes (Hexanal vs. Acetophenone) while the other odor pair had similar voltage amplitudes (Isoamyl acetate vs. 1,4-Cineole). For the olfactory matching paradigm, the odor delivery was for 4 s with an Inter-stimulus interval (ISI) of 5 s. For "same" odorant trials, an ISI of 5 s was sufficient to saturate the vapor phase in the odor bottles and the voltage amplitude of the second odorant matched with the first odorant. Representative traces of "same" and "different" trials are averaged over 4–5 trials and illustrated. Data is represented as mean±SEM.

B. Normalized odor matching accuracies. The odor matching accuracies were normalized to the mean accuracy shown by normal healthy subjects. COVID-19 patients showed significantly reduced odor matching accuracies compared to the normal healthy subjects (Two-tailed *t*-test, $p = 0.015$, $t = 2.5$, df=54). Data is represented as mean±SEM.

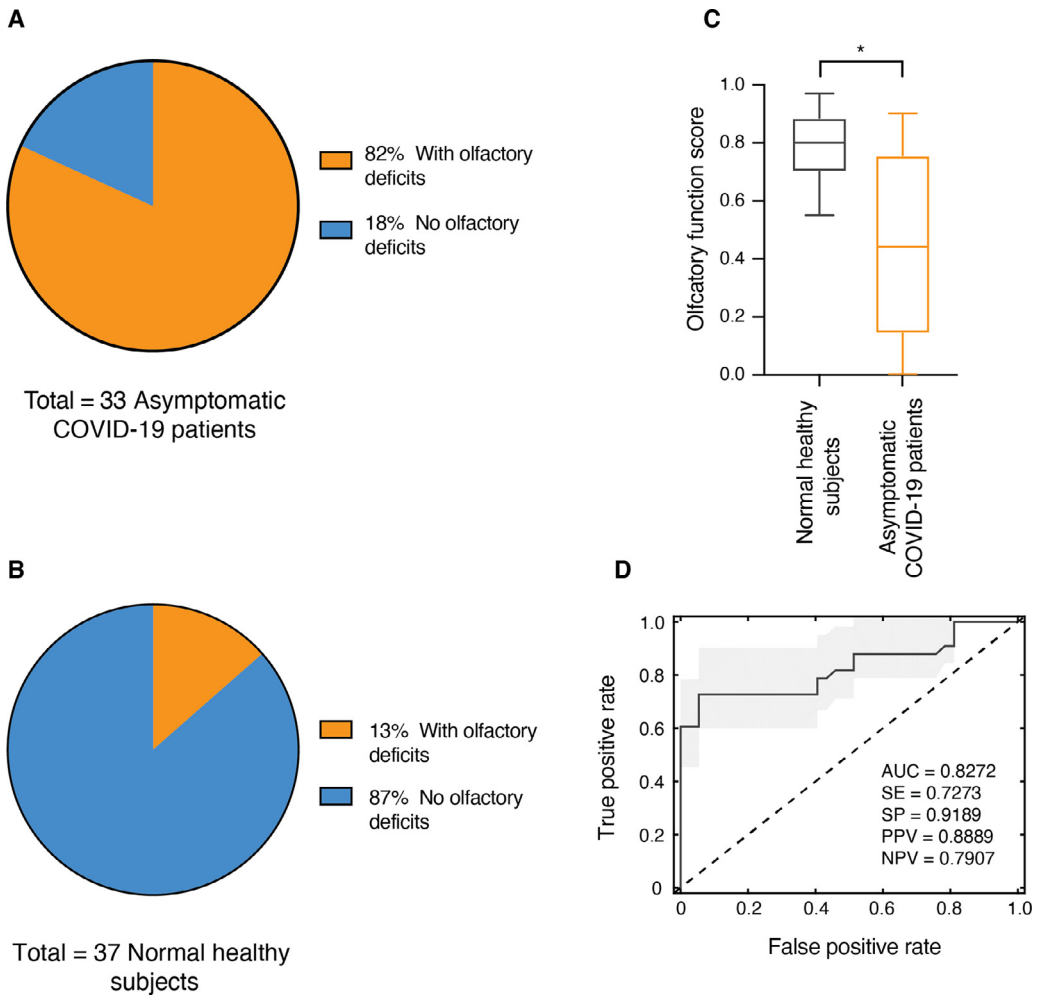

Fig. 4. Quantification of olfaction identifies asymptomatic COVID-19 carriers.

A. Asymptomatic COVID-19 patient population with olfactory deficits.

To quantify the percentage patient population with olfactory deficits, we compared their detectability indices at 9.1%, 16.6% 23.1% and 50% (v/v) concentrations and the normalized matching accuracies with that of shown by normal healthy subjects. If patients showed deficiency in detectability indices for all four concentrations tested or showed deficiency in detectability indices for two or more out of the four concentrations as well as in olfactory matching accuracy, they were classified as "with olfactory deficits". This criterion resulted in 82% of the asymptomatic patients with olfactory dysfunctions.

B. Majority of the healthy subjects are without any olfactory deficits.

The criterion set to classify subjects with or without olfactory deficits was applied to the normal healthy subject cohort. Even with strict criteria set, 87% of the healthy subject population did not show any olfactory deficits.

C. Olfactory function scores reflecting the olfactory loss in COVID-19 patients.

To establish a robust readout reflecting olfactory deficits, we calculated their olfactory function score by averaging detectability indices and normalized olfactory matching performance index shown by the individual patient. Asymptomatic COVID-19 patients showed significantly reduced olfactory function scores compared to normal healthy subjects (median for normal subjects=0.8, and median for patients=0.44, Two-tailed unpaired *t*-test, $p<0.0001$, $t = 6.4$, df=68).

D. ROC analysis for predicting olfactory deficits based on olfactory function scores measured for healthy subjects and asymptomatic COVID-19 patients.

ROC analysis shows an AUC of 0.83, specificity of 92% and sensitivity of 73% for the classifier based on olfactory function scores in detecting subjects with olfactory dysfunctions. Values for AUC, Sensitivity (SE), Specificity (SP), Positive Predictive Value (PPV), Negative Predictive Value (NPV) are shown in the figure. 95% confidence interval bound is marked by the gray shaded area.

To evaluate the limitations of the olfactory function score we propose here, we have analyzed different confounding factors. To avoid any bias shown by the subjects at the time of olfactory function test, we have tested only the subjects who were glad to participate in the study. We made sure that the paradigm was well explained to all participants. Moreover, none of the subjects had any prior experience of smell loss. To further probe the effect of confounding variables such as gender and age on test readouts, we analyzed the correlation between these variables and different test readouts, olfactory detection indices and OFSs. Our analyses showed no correlation between these confounding variables and test readouts (Supplementary Figure 1). This confirms the robustness of test readouts we report here.

To evaluate the sensitivity of OFSs, we have carried out a few different analyses. Firstly, we have checked for the outliers in our boxplot analysis. No points were found falling outside of 1.5 times the interquartile range, indicating no outliers in the dataset for both normal healthy subjects and patients. (Fig. 4C) [36]. Further, we have run the Grubb's test and found no outliers in the data set for normal healthy subjects and patients (Alpha = 0.05, Normal healthy subjects: Mean OFS = 0.794, SD = 0.1, Critical value of $Z = 3.002$, $G = 2.250$; Patients: Mean OFS = 0.426, SD = 0.32, Critical value of $Z = 2.952$, $G = 1.482$) [37]. As the asymptomatic COVID-19 patients showed variable detection indices at 50% concentration (v/v), we further evaluated the correlation between the detection index at 50% and the OFS. We see a good correlation between these parameters (Pearson Correlation coefficient $r = 0.8$). Further, the OFS and detection indices follow a logistic growth function ($R^2 = 0.8165$) (Supplementary Figure 2). This indicates that the OFS can be a reliable

readout for the olfactory fitness of the participants which could reflect problems both at the sensory periphery as well as higher centers.

The olfactory function score was calculated by averaging detectability indices and a normalized olfactory matching performance index. The olfactory matching test was carried out only if the subjects/patients had a detectability index of $\geq$0.6 at 50% (v/v) odor concentration. For the subjects/patients who did not qualify for the olfactory matching test, the OFS was calculated by averaging only the detectability indices. To probe if this missing readout biases the OFS measurements, we reanalyzed the OFS only for those patients (20 participants) who performed the olfactory matching test. To compare, we randomly selected OFS values from 20 healthy subjects. Even after this random sampling, the asymptomatic COVID-19 patient cohort showed significantly reduced olfactory function scores than normal healthy subjects. This result proves the robustness and sensitivity of the novel method employed for quantifying olfaction in asymptomatic COVID-19 patients (Supplementary Figure 3, Two-tailed unpaired *t*-test, $p<0.0044$, t = 3.031, df=38).

4. Discussion

In this article, we present a novel method to assess the olfactory fitness of asymptomatic COVID-19 patients diagnosed with SARS-CoV-2 infection. The olfactory function test we propose here challenges the detection abilities of the subjects at the threshold levels and probes olfactory matching skills. Therefore, the readouts reflect the damage caused at the sensory periphery level as well as higher olfactory centers due to virus infection. While our assessment using a custom-built olfactory-action meter detects 82% of asymptomatic patients with olfactory deficits, only 15% of these patients realized about their loss of sense of smell before participating in the olfactory function test. This discrepancy between self-report rate and quantitative assessment conceivably demonstrates the need to employ precise quantitative measures to detect olfactory deficits in asymptomatic carriers of COVID-19 [10,11,38].

Infectious diseases can be efficiently contained in a population if the symptoms presented are clear, well-defined and shown by the majority of patients. Anosmia is one such symptom reported for COVID-19 infection across different countries, but this has been arrived mostly by subjective assessments [4–9,21,22,39,40]. Strikingly, this symptom was observed in the absence of nasal obstruction or rhinorrhea associated with common upper respiratory tract viral infections [41,42]. Although severe, psychophysical readouts for olfactory dysfunctions among COVID-19 patients remain unexplored to date. Furthermore, the manifestation of such symptoms is remarkable since OSNs in the human olfactory epithelium are continuously replaced [43,44], and the SARS-CoV-2 virus appears to infect non-neuronal supporting cells in the olfactory epithelium but not OSNs [17–19]. Therefore, it is very likely that olfactory dysfunctions attributed to malfunction of OSNs will display a gradual onset, which our method is able to detect with high sensitivity and precision.

SARS-CoV-2 requires the co-expression of two genes, Angiotensin-Converting Enzyme-2 (ACE2) and Transmembrane Protease Serine 2 (TMPRSS2) receptors to gain entry into the host cells. While ACE2 mediates the binding of viral spike protein, TMPRSS2 facilitates the S protein priming. These two molecular factors are expressed in sustentacular cells of human olfactory epithelium but not on OSNs [18,19,45,46]. The effect of viral infection in sustentacular cells on the OSN's functioning remains largely unknown. However, in the case of asymptomatic carriers, where we do not observe a complete loss of olfaction, the elevated levels of inflammatory cytokines [47] or the possible ionic imbalances in the sustentacular cells could affect the firing activity of OSNs leading to olfactory deficits of varying severity [46,48].

To assess olfactory deficits with varying degrees of severity, precise methods with good control on stimulus properties are needed. In our study, the quantification using our custom-built olfactory-action meter picked olfactory dysfunctions in 82% of asymptomatic COVID-19 patients, who are potential carriers of infection. To overcome the limitations with the subjective evaluations where extreme cases of anosmia can be easily identified, few objective psychophysical methods have been tried using different strategies. These methods include identification of household objects [40], University of Pennsylvania smell identification tests [8] and n-butanol threshold tests [49]. These objective methods resulted in better detection accuracy of smell dysfunctions compared to subjective evaluations. Even with a total of 71 subjects, our results show a reduction of 50% in the detection abilities of asymptomatic COVID-19 patients (Fig. 2B). Along with the findings we report here, the above-mentioned studies emphasize the need for implementing sensitive olfactory tests as means to screen the population for asymptomatic carriers. While such a methodology for large-scale screening poses many practical problems, a possible economical alternative is to use odorant arrays with concentrations near detection thresholds. The possibility of increasing odor diversity using such methods may compensate for precision. Adopting such practices would allow screening large populations to identify asymptomatic COVID-19 carriers.

Recent findings from Diffusion Tensor Imaging studies in patients recovered from COVID-19 infection have revealed significant microstructural changes in different brain areas [50]. Although the neuroinvasive properties of SARS-CoV-2 is not clearly elucidated, the transient changes in the higher brain centers is quite alarming. Here, we report an olfactory matching task that involves detection, discrimination and holding the perceived information about the sensory stimuli. The asymptomatic patients who had a good detectability of different odorants at threshold concentrations were encouraged to participate for the olfactory matching test. Our results show significantly reduced matching performances by the patients compared to normal subjects. The reduced accuracies in olfactory matching test allude to the possible cognitive impairments in patients suffering from COVID-19. To conclude an efficient metric reflecting both sensory and cognitive impairments, we propose an olfactory function score that gives an equal weightage to all the parameters tested. The efficiency of using such a metric was determined by performing ROC analysis. The AUC value of 0.83 signifies OFS as a good predictor of olfactory deficits in asymptomatic COVID-19 patients. Further analysis proved that OFS is insensitive to missing variables and not confounded by age and gender of the population we tested. This shows the reliability of our method for accurate identification of asymptomatic carriers across different age groups.

To summarize, clinically relevant quantification using a custom-built olfactory-action meter reveals severe dysfunctions in olfaction among asymptomatic COVID-19 patients, which may have remained undetected with traditional methods due to lack of sensitivity. We, therefore, advise the global medical community to adopt more precise ways of quantifying the sense of smell to identify and isolate silent carriers of infection [10]. Our work provides the possibility of devising a sensitive, fast and economical screening strategy that can be self-administered by large populations.

Contributors

N.A.: Conceptualized the study; designed the experiments with inputs from other authors.

N.A. and A.B.: Collected and analyzed the data; wrote the manuscript with comments from other authors.

S.J., S.S. and S.N.: Helped and facilitated data collection from asymptomatic COVID-19 patients at BJGMC & SGH.

Data sharing statement

According to the informed consent, all the data involved is only used in this study and our institutions. Individual de-identified participant data that underlie the results reported in this article (tables, figures), the detailed protocols and methods will be made available upon request.

Declaration of Competing Interest

Indian Institute of Science Education and Research (IISER), Pune has submitted a provisional patent application for the olfactory-action meter with Dr. Nixon M. Abraham as the inventor (Patent application No: IN 202,021,035,482 dated 18/08/2020). Other authors declare that they have no known competing financial interest. All authors declare that they have no known personal relationships that could have appeared to influence the work reported in this paper. This paper has never been published elsewhere.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.eclinm.2020.100575.

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