Olfactory Sniffing Signals Consciousness in Vegetative Patients

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Abstract

It is often difficult to determine state of consciousness following severe brain injury; is the patient vegetative, or perhaps minimally conscious¹? and if vegetative, will the patient recover? These diagnoses and prognoses are critical, as they determine therapeutic strategies such as pain management, and can underlie end-of-life decisions^{2,3}. And yet, troublingly, there is up to 40% error in determining state of consciousness in brain-injured patients^{4,5}. Olfaction relies on deep brain structures that are involved in basic mechanisms of arousal⁶, and therefore we hypothesized that it may serve as a biomarker for consciousness⁷. To test olfaction in brain injured patients we used a non-verbal non-task-dependent measure known as the sniff-response⁸⁻¹¹, namely automatic sniff modulations to account for odorant content. By precisely measuring odorant-dependent sniffing we gain a sensitive measure of olfactory function¹⁰⁻¹⁵. Here we applied this measure repeatedly over time in 43 severely brain-injured patients. We found that sniff-responses significantly discriminated between vegetative and minimally conscious states at the group level ($p < 0.0001$, effect-size $r = 0.63$). More remarkably, at the single patient level, if a vegetative patient had a sniff-response, this indicated future regaining of consciousness at 100% specificity. Finally, olfactory sniff-responses predicted long-term survival rates at 92% accuracy ($\chi^2 = 14.5$, $p = 0.0001$, Cramer's V effect size = 0.45). These results stress the primality of olfaction in functioning of the human brain, and provide for an accessible bedside tool that signals consciousness and recovery in brain-injured patients.

Sniff-responses can be sensory-driven, cognitively-driven, or both. Sensory-driven responses reflect automatic odorant-driven modifications in nasal airflow evident in humans^{8,10,11} and other animals^{9,16}. Sensory-driven sniff-responses have two levels: *Level 1*: odorant detection, namely a change in nasal airflow in response to odorant presence, and *Level 2*: odorant discrimination, namely a differential response for different odorants, such as reduced nasal airflow for unpleasant versus pleasant odorants^{8,12,13,17}. Cognitively-driven sniff-responses reflect situational understanding and/or learning. For example, in the current study patients were told that they will be presented with odorants. If a patient then modifies nasal airflow in response to an empty ("Blank") jar presented beneath their nose, this implies possible awareness of the jar, and/or possible learned anticipation of an odorant.

With these considerations in mind, we used pleasant (*shampoo*) and unpleasant (*rotten fish*) odorants to trigger sensory sniff-responses, and *Blank* presentations to trigger cognitive sniff-responses (Fig. 1a) in 43 brain-injured patients with disorders of consciousness (DoC) (Fig. 1, Table 1, Methods). Directly after each olfactory testing session, the patients' state of consciousness was assessed using standard clinical measures^{18,19} to determine between vegetative state/unresponsive wakefulness syndrome (VS/UWS) reflecting no signs of consciousness¹, or minimally conscious state (MCS) reflecting inconsistent but reproducible evidence of consciousness¹. The patients conducted a total of 146 sessions (1-12 sessions per patient, mean = 3.4 ± 3 , Table 1), with inter-session intervals ranging between 1 and 10 weeks (mean = 2.65 ± 1.7). These intervals allowed us longitudinal comparisons in 31 patients. Overall, 73 of the sessions were conducted in MCS (in 31 patients), and 73 sessions in VS/UWS (in 24 patients, 16 who transitioned from VS/UWS to MCS during the study).

Fig. 1: Measuring sniff-responses in brain-injured patients

a, Olfactory testing experimental design. Pleasant odorants (purple), unpleasant odorants (blue) and *Blank* (grey) were presented in random order, ~10 times each, for a duration of ~5 seconds. **b**, A trace of nasal respiration during a single trial, where 3 baseline respirations and 3 sniffs following unpleasant odorant presentation were recorded using a nasal cannula connected to a spirometer and amplifier. Dashed line denotes odorant onset and blue bar represents unpleasant odorant duration. This trace is genuine data from patient #37, and not an illustration. **c**, Example of a lacking sniff-responses in patient #28 in VS/UWS, (Normalized sniff volume pleasant = 1.00 ± 0.08 , 11 repetitions; Unpleasant = 0.94 ± 0.1 , 12 repetitions; Blank = 1.00 ± 0.09 , 11 repetitions). **d**, Example of an intact sniff-responses from patient #37 in MCS (Normalized sniff volume pleasant = 0.68 ± 0.22 , 7 repetitions; Unpleasant = 0.56 ± 0.19 , 7 repetitions; Blank = 0.84 ± 0.21 , 6 repetitions).

We first concentrated on the sensory-driven sniff-response. We compared normalized nasal inhalation volume (Fig. 1b, see Methods) of the first three nasal inhalations following *Unpleasant* and *Pleasant* odorants vs. respiratory baseline in MCS and VS/UWS sessions. Given the abnormal distribution of the data in both MCS and VS/UWS sessions (Shapiro-Wilk test: First sniff all W 's > 0.88, all p 's < 0.001, Second sniff all W 's > 0.66, all p 's < 0.01, Third

1 sniff all W's > 0.63 , all p 's < 0.04), and the greater variance in MCS than in VS/UWS sessions
 2 (Leven's test: First sniff $F(1,147) = 3.9$, $p = 0.05$, Second sniff $F(1,147) = 3.3$, $p = 0.07$, Third
 3 sniff $F(1,147) = 1.6$, $p = 0.21$), we used non-parametric tests, with Bonferroni correction for
 4 multiple comparisons. At the group level, we observed that whereas nasal inhalation volume
 5 was significantly reduced in response to odorants in MCS sessions, it was uninfluenced by
 6 odorants in VS/UWS sessions. More specifically, at the first sniff following odorant
 7 presentation, MCS session normalized sniff volume dropped from baseline ($=1$) to 0.89 ± 0.18
 8 normalized flow units (NFU) in response to a pleasant odorant (median = 0.92 NFU, $Z = 5.2$, p
 9 < 0.0001 , effect-size $r = 0.61$; Fig. 2a), and to 0.88 ± 0.2 NFU in response to an unpleasant
 10 odorant (median = 0.94 NFU, $Z = 4.7$, $p < 0.0001$, effect-size $r = 0.54$; Fig. 2b). This reflects a
 11 $\sim 10\%$ reduction in nasal airflow to account for odorant content in MCS sessions. We did not,
 12 however, observe a significant difference between pleasant and unpleasant odorants at the
 13 group level ($Z = 0.13$, $p = 0.90$, effect-size $r = 0.01$; Fig. 2a, 2b). In other words, MCS sessions
 14 contained *Level 1* (odorant detection) but not *Level 2* (odorant discrimination) sensory-driven
 15 sniff-responses in the first sniff following odorant presentation. In contrast, we observed no
 16 such group-level responses in VS/UWS sessions (normalized nasal inhalation volume, Pleasant
 17 $= 0.97 \pm 0.13$ NFU, median = 0.99 NFU, Unpleasant $= 0.97 \pm 0.12$ NFU, median = 0.98 NFU,
 18 difference from baseline, all $Z < 2.3$, all $p > 0.05$, Bonferroni corrected, all effect-size $r < 0.27$;
 19 Fig. 2a, 2b), and they were indeed significantly different from the MCS sessions (difference
 20 between groups in Pleasant: $Z = 3.3$, $p = 0.0009$, effect-size $r = 0.39$, Unpleasant: $Z = 2.8$, $p =$
 21 0.005 , effect-size $r = 0.33$; Fig. 2a, 2b). In other words, the *Level 1* sensory-driven sniff-
 22 response evident in MCS sessions significantly differentiated them as a group from VS/UWS
 23 sessions. Similar results were evident in the second sniff after odorant presentation, but not
 24 in the third sniff (given no difference between pleasant and unpleasant, we combine them
 25 here for brevity, but separate treatment can be seen in Extended Data Fig.1. Sniff 1: MCS:
 26 Odorants $= 0.89 \pm 0.18$, median = 0.95 NFU, difference from baseline, $Z = 5.35$, $p < 0.0001$,
 27 effect-size $r = 0.63$. VS/UWS: Odorants $= 0.97 \pm 0.11$, median = 0.98 NFU, difference from
 28 baseline, $Z = 1.86$, $p > 0.05$ Bonferroni corrected, effect-size $r = 0.22$, Fig. 2d. Sniff 2: MCS:
 29 Odorants $= 0.93 \pm 0.19$, median = 0.97 NFU, difference from baseline, $Z = 5.18$, $p < 0.0001$,
 30 effect-size $r = 0.61$. VS/UWS: Odorants $= 0.97 \pm 0.1$, median = 0.98 NFU, difference from
 31 baseline, $Z = 0.15$, > 0.05 Bonferroni corrected, effect-size $r = 0.15$. Fig. 2e. Sniff 3: MCS:
 32 Odorants $= 0.98 \pm 0.1$, median = 0.98 NFU, difference from baseline, $Z = 4.64$, $p > 0.05$

1 Bonferroni corrected, effect-size $r = 0.54$. VS/UWS: Odorants = 0.99 ± 0.11 , median = 0.99
 2 NFU, difference from baseline, $Z > 2.3$, $p > 0.05$ Bonferroni corrected, effect-size $r = 0.27$. Fig.
 3 2f). That altered sniffing persisted into the second sniff after each odorant presentation, but
 4 not to the third, indicates that this was a genuine transient odorant-driven response and not
 5 a state-change. Finally, we replicated this entire analysis, this time comparing the odorant-
 6 driven sniff-response to the first inhalation following *Blank* rather than to the respiratory
 7 baseline, and obtained similar results (See Extended Data Fig. 1). These results indicate
 8 sensory sniff-responses in MCS but not VS/UWS sessions.

9
 10 We further conducted two explorations of potential sources of variance in this result. First,
 11 odorants can activate both olfactory and/or trigeminal nerve-endings in the nose^{20,21}, and
 12 the odorant blends we used here may have had a mild trigeminal component. To estimate
 13 whether the effects we observed depended on trigeminal contribution, we added pure
 14 olfactory odorants in a subset of patients, and observed replication of the above effects
 15 (Extended Data Fig. 2). Second, about 60% of MCS sessions and 80% of VS/UWS sessions were
 16 conducted in patients with a tracheostomy. We note that olfaction can be partially relearned
 17 even following total laryngectomy²², yet tracheostomy in these patients significantly differs
 18 from total laryngectomy in that the nasal passage remains connected to the airway. Thus,
 19 although tracheostomy indeed significantly reduces nasal airflow, a measurable portion of
 20 nasal airflow remains. For example, we note that the raw data in Fig. 1c and 1d was obtained
 21 with a tracheostomy. To estimate impact of this on our findings, we compared results in
 22 patients with and without tracheostomy. We observed that although tracheostomy indeed
 23 significantly reduced total nasal airflow, it had no impact on sniff-responses (Extended Data
 24 Fig. 3). Considering these two verification analyses (Extended Data Fig. 2 and Fig. 3), we
 25 conclude that consistent with our hypothesis, the sensory component of the sniff-response
 26 indicates state of consciousness in DoC patients at the group session level.

27
 28 We next concentrated on the cognitively-driven component of the sniff-response. We
 29 compared normalized nasal inhalation volume of the first three inhalations following *Blank* in
 30 MCS and VS/UWS sessions. At the group level, we observed that whereas nasal inhalation
 31 volume was significantly reduced in response to *Blank* presentation in MCS sessions, it was
 32 uninfluenced by *Blank* presentation in VS/UWS sessions. More specifically, at the first sniff

1 following *Blank* presentation, MCS normalized sniff volume dropped from baseline (=1) to
 2 0.955 ± 0.13 NFU (median = 0.96 NFU, $Z = 3.25$, $p = 0.001$, effect-size $r = 0.38$; Fig. 2c). This
 3 reflects a ~5% reduction in nasal airflow in response to *Blank* in MCS sessions. In contrast, we
 4 observed no such response in VS/UWS sessions (*Blank* = 1.00 ± 0.15 NFU, median = 1.00 NFU,
 5 difference from baseline, $Z = 0.08$, $p = 0.94$, effect-size $r = 0.009$; Fig. 2c), and they were indeed
 6 significantly different from the MCS sessions (difference between groups: $Z = 2.31$, $p = 0.02$,
 7 effect-size $r = 0.27$; Fig. 2c). In other words, the cognitively-driven sniff-response evident in
 8 MCS sessions significantly differentiated them from VS/UWS sessions. This outcome was not
 9 evident in the second and third sniffs (MCS second sniff = 1.01 ± 0.21 NFU, median = 0.98
 10 NFU, third sniff = 1.03 ± 0.24 NFU, median = 0.98 NFU, difference from baseline, all $Z < 1.4$,
 11 all $p > 0.162$, all effect-size $r < 1.64$. VS/UWS second sniff = 0.99 ± 0.1 NFU, median = 10.99
 12 NFU, third sniff = 1.01 ± 0.12 NFU, median = 1.00 NFU, difference from baseline, all $Z < 1.3$,
 13 all $p > 0.19$, all effect-size $r < 0.15$; Extended Data Fig. 1f, 1i), again indicating that this group
 14 difference was a genuine transient task-driven response and not a state-change. In sum, we
 15 conclude that similar to the *Level 1* sensory-driven component of the sniff-response, the
 16 cognitively-driven component of the sniff-response also reflects state of consciousness in DoC
 17 patients at the group session level.

Fig. 2: The sniff-response reflects level of consciousness in DoC patients

Normalized sniff volume following **a**, pleasant odorants (purple), **b**, unpleasant odorants (blue) and **c**, Blank (grey), in VS/UWS (V) sessions (outline, left) and MCS (M) sessions (filled, right), in the first sniff following stimulus delivery. Each dot represents a session, flat violin plots show the distribution, the red line denotes the median, and the dashed horizontal line denotes the baseline value at 1 normalized flow units (NFU). The bar-graphs to the right of each distribution tabulate the same data, with error bars denoting standard error of the mean (SEM). The p-value beneath the distribution denotes its difference from baseline inhalation, i.e., the existence of a sniff-response. The p-values above the distributions denote the difference in sniff-response across groups. * = $p < 0.05$ corrected for multiple comparisons (see Methods). # < 0.05 uncorrected. **d**, The data from A and B combined. **e**, Same as D, but for the second, and

f, third sniff following odorant delivery. See Extended Data Fig. 1 for breakdown of pleasant/unpleasant.

Consistent with convention in longitudinal DoC studies, in the above we analysed data "by session" and not "by patient"^{23–25}. This is standard practice in this field, because consciousness fluctuates in DoC². Current Patient #4 provides an example for the reality underlying this approach. Patient #4 started the study with a sniff-response session in MCS, then deteriorated, conducting his following session in VS/UWS, only to later recover, and conduct a third and final sniff-response session again in MCS (today Patient #4 walks and talks). If we were to average the three Patient #4 sessions, this would not only be uninformative, but could in fact obscure any MCS-VS/UWS differences. All that said, whereas the group-wise session difference is telling for the basic link between olfactory processing and levels of consciousness, for this measure to gain clinical and not only basic science value, one would want it to be informative at the individual patient level as well. To make single-patient rather than group judgments we need to apply a sniff-response threshold, namely, an extent of change in nasal airflow that constitutes a sniff-response within an individual. Based on previous studies in healthy participants¹⁷, we designate both a sensory-driven *Level 1* sniff-response (odorant detection), and a cognitive sniff-response, at more than 15% change in normalized sniff volume between the event and baseline respiration, and/or a modulation in sniff volume reflecting a shift in standard deviation (SD) > 0.35 across trials. A sensory-driven *Level 2* sniff-response (odorant discrimination) is set at more than a 20% change in normalized sniff volume between pleasant and unpleasant odors (see Supplementary Materials). Using these previously published criteria¹⁷, we observe that 20 of 31 MCS patients had sniff-responses in at least one session (19 patients with *Level 1* sensory-driven sniff-responses, 13 of these with cognitively-driven sniff-responses, four of these with *Level 2* sensory-driven sniff-responses, and one with only a strong trend towards *Level 1* sensory-driven sniff-responses yet a significant cognitively-driven sniff-response). This implies sensitivity of 64.5% in determining MCS with this measure (Extended Data Fig. 4). The flip side of this sensitivity rate is that ~35% of MCS patients had no sniff-response, indicating that a lack of sniff-responses does not necessarily imply unconsciousness (more on this in the discussion).

We next turned to the VS/UWS patients. We observe that nine of 24 VS/UWS patients nevertheless had a sensory-driven *Level 1* sniff-response in at least one session. Of these, one patient had a *Level 2* sniff-response as well. Cognitively-driven sniff-responses were observed in nine VS/UWS patients, eight of which had a sensory-driven sniff-response (the ninth with only a trend). Taken together, whereas we failed to observe a sniff-response in VS/UWS sessions at the group level, 10 of 24 individual VS/UWS patients had sniff-responses in at least one session. To ask what are the implications of the sniff-responses we observed in these VS/UWS patients, we compared these results to the patient's later clinical progression over time. Remarkably, we observe that all 10 VS/UWS patients who had a sniff-response in one session or more, later transitioned into MCS. Thus, a sniff-response in VS/UWS indicated transition to MCS at 100% specificity (Fig. 3 a-d, Extended Data Fig. 5) and 62.5% sensitivity (10 out of 16 VS/UWS patients who transitioned; Fig. 3 a-d, Extended Data Fig. 5), suggesting that sniff-responses are informative for prognosis at the single patient level. Moreover, in four of these patients the sniff-response preceded any other sign of consciousness recovery by days up to months (2.5 months, ~2 months, ~1.5 months, 2 days). We note that the temporal gap between the detected sniff-response and other behavioural signs of conscious awareness also reflects the clinical testing schedule, so if tested more frequently, these temporal gaps may have been different.

Fig. 3: The sniff-response is prognostic for recovery of consciousness in DoC patients

The red lines denote previously published sniff-response threshold (more than 15% change in magnitude/0.35 SD): dots within the lines (white background) reflect sessions without a sniff-response, and dots beyond the lines (shaded background) reflect sessions with a sniff-response. **a-c**, Each dot is a VS/UWS session, where empty

dots represent sessions in later ‘recovered’ patients (transitioned to MCS) and filled dots represent sessions in patients who would not later recover consciousness during the study. **(a)** Pleasant odorant sessions (purple) **(b)** Unpleasant odorant sessions (blue) **(c)** Sessions with *Blank* (grey). **d.** Percentage of VS/UWS patients (not sessions) who later transitioned to MCS (left, ‘Recovered’) or remain unconscious (right, ‘Unrecovered’) with sniff-responses (white; ‘Recovered’: 62.5%; ‘Unrecovered’: 0%) and without sniff-responses (red; ‘Recovered’: 37.5%; ‘Unrecovered’: 100%) across all three conditions.

Last, we set out to test whether the sniff-response is informative for long-term outcome in DoC. We conducted a follow-up investigation at a common point in time about five years after injury of the first participating patient (average across patients = 37.4 ± 14.7 (range 17 – 64) months after injury). We observed that whereas patients who had a sniff-response shortly after injury mostly survived for years, patients who did not have a sniff-response after injury mostly failed to survive during this period. More specifically, only two of 24 patients (8.3%) who had a sniff-response following their injury, then did not survive (died at 5 and 7 months after injury). The remaining 22 of 24 patients (91.7%) with a sniff-response following injury, currently survive (current average 37.3 ± 14.1 months after injury). In contrast, 12 of 19 patients (63.2%) who did not have a sniff-response following injury, then did not survive (died within 17.5 ± 12.2 months from injury, median = 13 months; Fig. 4 a-d, and Extended Data Fig. 5). Thus, the sensitivity of the sniff-response in predicting survival at 37.3 ± 14.1 months after brain injury is a remarkable 91.7% (chi-square = 14.5, $p = 0.0001$, Cramer's V effect size = 0.45). We can appreciate that looking at Fig. 4 a-c, it is hard not to speculate that we drew the lines denoting sniff threshold to fit a discriminator for survival (...). Given such possible musing, we would like to very clearly reiterate and state that these sniff-threshold values were set (and used) well before we obtained this survival rate follow-up data. Finally, we also assessed functional independence in the 29 surviving patients using the Functional Independence Measure (FIM)²⁶. The FIM was independently obtained in clinical testing of these patients conducted 20.4 ± 11.5 months after injury. We observed a significant correlation whereby extent of sensory (but not cognitive) sniff-response predicted later level of independence in VS/UWS patients (Pleasant $r_{39}^{\text{Spearman}} = -0.49$, $p = 0.001$; Unpleasant $r_{39}^{\text{Spearman}} = -0.60$, $p < 0.0001$; Blank $r_{39}^{\text{Spearman}} = -0.20$, $p = 0.21$; Fig. 4 e-g, Extended Data Fig. 5; Note that the r values are negative because a larger sniff-response is reflected in lower post-

stimulus volume). We further note that this effect is carried by the 11 of 12 surviving VS/UWS patients who later transitioned to MCS. In MCS patients, however, no such association was observed (Pleasant $r_{62 \text{ Spearman}} = 0.30$; Unpleasant $r_{62 \text{ Spearman}} = 0.24$; Blank $r_{62 \text{ Spearman}} = 0.25$, all $p > 0.05$ Bonferroni corrected). This dissociation between MCS and VS/UWS (difference between correlations, Fisher: Pleasant $Z = 4.19$, $p < 0.001$; Unpleasant $Z = 4.64$, $p < 0.001$; Blank $Z = 2.27$, $p = 0.012$) in long-term predictive value for measures of functional independence suggests that the sniff-response may indeed tap into the very basic mechanisms of life and consciousness, but may not be equally informative for functionality beyond that basic level. Together, these findings suggest that the sniff-response can be used as an accessible bedside diagnostic and prognostic tool for level of consciousness and survival in DoC at the single patient level.

Fig. 4: The sniff-response is prognostic for long-term survival and recovery in DoC patients

The red lines denote sniff-response threshold (more than 15% change in magnitude/0.35 SD): dots within the lines reflect sessions without a sniff-response, and dots beyond the lines reflect sessions with a sniff-response. **a-c**, Each dot is a DoC

session (MCS and VS/UWS), where filled black dots represent sessions in later deceased patients and color dots represent sessions in surviving patients (37.3 ± 14.1 months after brain injury) (**a**) Pleasant odorant sessions (purple) (**b**) Unpleasant odorant sessions (blue) (**c**) Sessions with *Blank* (grey). **d**, Percentage of DoC patients (not sessions) with sniff-responses (left) that survived (white, 91.7%) or deceased (D) (red, 8.3%), and of DoC patients without sniff-responses that survived (red, 36.8%) or deceased (white, 63.2%). **e-g**, Relation between Functional Independence Measure (FIM) and normalized sniff volume. Each dot is a VS/UWS session in a surviving patient. (**e**) Pleasant odorant sessions (purple) (**f**) Unpleasant odorant sessions (blue) (**g**) Sessions with *Blank* (grey). r is Spearman correlation.

Olfaction is a primal mammalian sensory system²⁷, directly targeting the limbic brain without a thalamic relay⁶. In humans, this neuroanatomy combines with aspects of olfactory phenomenology and neurodynamics to assign olfaction a unique position in consciousness^{7,28–30}. Olfactory information is processed at several levels, and had we relied on higher-order olfactory processing, we would have likely not seen a response in DoC³¹. The olfactory sniff-response, however, is a very basic olfactory mechanism, persistent across species^{9,16}. In healthy intact humans, the olfactory sniff-response can persist even without conscious awareness in both wake¹⁴ and sleep^{12,32,33}. Nevertheless, such sniff-responses rely on intact olfactory neuroanatomy, and the current results imply that the integrity of this neuroanatomy is important for consciousness, and indeed for life itself. Whereas a powerful biomarker for consciousness in DoC has precedents³⁴, the added predictive power we observed for surviving three years and more after injury, is as far as we know, unprecedented. The very first and very last thing we do in life is to inhale, such inhalations can be modulated by smell, and this taps into the most basic processes of life.

Whereas the above relates our findings to basic research on the brain mechanisms of consciousness, our results also have very immediate clinical implications: Improvements in emergency medicine have increased survival-rates after brain injury, but paradoxically, have resulted in increasing numbers of survivors living with DoC^{35,36}. As previously noted, the rate of misdiagnosis in these patients is an alarming $\sim 40\%$ ^{4,5}. The sniff-response can provide an accessible and easy to use bedside tool that may significantly improve this outcome. This ease of use is an important feature, as it separates this approach from neuroimaging^{37,38} and

electrophysiology^{39,40}, that although powerful towards uncovering consciousness, are not always available to DoC patients, particularly in developing countries.

The sniff-response had 100% specificity for recovery of consciousness. All patients with a sniff-response ultimately had signs of consciousness, and all VS/UWS patients who remained unconscious did not have a sniff-response. This places the sniff-response with the top measures for estimating recovery of consciousness in DoC^{34,37-41}. In turn, sniff-response sensitivity at detecting consciousness in MCS patients was 64.5%, and in detecting transition from VS/UWS to MCS it was 62.5%. These sensitivity rates are better than most active command-following tests, and similar to other passive paradigms and resting state studies^{34,37-41}. This sensitivity rate, however, implies that ~35% of conscious DoC patients had no sniff-response. The absence of a sniff-response in conscious DoC patients may reflect a chronic or transient olfactory impairment, possibly related to their brain injury⁴², or an inability to execute the precisely timed motor act of sniffing despite intact olfaction⁴³. Relatedly, non-olfactory volitional nasal inhalations have been used in order to tap into the conscious brain of paralyzed individuals in the context of providing a means of communication⁴⁴, or a method for device control⁴⁵. Moreover, non-olfactory volitional nasal inhalation was also tested as a potential method for detection of consciousness in DoC, yet most DoC patients failed to inhale on command⁴⁶. Nevertheless, one MCS patient in that study was able to inhale on command, yet could not initiate any other motor movement, further indicating the primacy of this motor function. Together, we conclude that DoC patients may fail to inhale on command⁴⁶, but sniff in response to odorant presentation. This dissociation between volitional and odorant-induced sniffing, further indicates the fundamental role of the olfactory brain in basic mechanisms of arousal.

Finally, we would like to acknowledge several limitations of this study. First, in reflection of changes in clinical practice at the hospital where we conducted these studies, we have data using two different behavioural assessment protocols. Although we have in hand an additional third measure applied equally to all participants and suggesting that this is not a major concern (details in Methods), this nevertheless remains a limitation. Second, the clinical testing schedule dictated the temporal resolution of our estimation regarding the extent to which a sniff-response preceded transition from VS/UWS to MCS. Had the tests been more

1 frequent, we may have observed different timing for advance detection. Finally, and although
2 not a unique limitation of this study, we would like to acknowledge the following: We claim
3 that sniff-responses in VS/UWS predicted later recovery of consciousness. An alternative is
4 that these patients were already conscious, but misdiagnosed by the existing standard clinical
5 measures. The only way to unequivocally settle between these alternatives is by self-report
6 from patients who later transitioned into consciousness. If these patients provide
7 recollections from when they were deemed unconscious, this implies misdiagnosis. Indeed,
8 such instances reveal that in some cases where all available methods determined a lack of
9 conscious awareness, including fMRI and EEG, patients nevertheless recalled events from
10 their erroneously deemed unconscious state⁴⁷. Thus, this fundamental question remains
11 unanswered, and perhaps impossible to answer with currently available methods. We should
12 stress, however, that this does not take away in the least from the contribution of our
13 findings: whether a sniff-response in VS/UWS patients reflected misdiagnosis by standard
14 methods, or unique prognosis by this method, in both cases this constitutes signalling of
15 consciousness, with all its medical and ethical implications. We conclude that by combining
16 the unique properties of the sense of smell with longitudinal repeated examination of
17 patients over a period of months, we revealed that the olfactory sniff-response reflects state
18 of consciousness and signals recovery and long-term survival in DoC patients. These findings
19 reiterate that olfaction is one of the most basic processes in the human brain, and suggest
20 that the sniff-response can be used as an accessible bedside tool to improve diagnosis and
21 prognosis in the challenging condition of DoC.

1 Table 1: Patient information

Patient number	Consciousness state at enrolment	Age	Gender	Etiology	Time Since Onset (months)	Mean Inter-session interval (weeks)	Number of sessions	Number of sessions (all)
1	MCS	21	M	TBI	1	2.7	3	3
3	MCS	20	F	ABI	3.5	NaN	1	2
4	MCS	59	M	TBI	4	3.5	3	3
5	VS/UWS	26	F	TBI	7.5	3.1	3	3
6	VS/UWS	27	M	TBI	3	2.9	5	5
7	VS/UWS	23	M	TBI	1.5	1.7	9	9
8	VS/UWS	38	M	ABI	2	1.3	12	12
9	VS/UWS	19	M	ABI	2.5	1.4	10	10
11	VS/UWS	30	M	CVA	3	1.7	12	13
12	MCS	44	M	ABI	3.5	NaN	1	1
13	MCS	42	M	CVA	2.5	3.7	1	2
14	VS/UWS	57	M	TBI	2.5	1.1	9	11
16	VS/UWS	55	M	TBI	3.5	NaN	1	3
17	VS/UWS	42	M	TBI	5.5	NaN	1	3
18	MCS	34	M	TBI	9.5	3.6	1	3
19	MCS	21	M	TBI	7	6.4	3	5
20	VS/UWS	18	M	TBI	7	9.6	2	7
21	VS/UWS	60	M	TBI	3.5	1.0	2	3
22	VS/UWS	45	M	TBI	4	NaN	1	4
23	MCS	44	M	CVA	2.5	2.7	5	5
24	VS/UWS	66	M	TBI	6.5	2.4	3	4
25	MCS	38	M	TBI	2.5	3.2	3	3
26	MCS	70	M	TBI	1.5	NaN	1	1
27	MCS	22	M	TBI	3	NaN	1	3
28	VS/UWS	49	M	CVA	5	2.6	2	4
30	MCS	22	M	TBI	1	NaN	1	1
31	VS/UWS	64	M	TBI	2	2.6	4	2
32	VS/UWS	61	F	CVA	3.5	2.6	3	4
33	MCS	23	M	TBI	8	NaN	1	1
34	MCS	19	M	TBI	2.5	2.5	6	8
35	MCS	75	F	Infection	2.5	NaN	1	1
36	VS/UWS	68	F	TBI	2.5	1.4	3	3
37	VS/UWS	39	F	CVA	2	2.1	5	5
38	MCS	55	F	CVA	4.5	0.9	2	4
39	MCS	59	M	TBI	3	2.0	2	2
40	MCS	51	F	CVA	2.5	1.6	2	2
43	VS/UWS	27	M	TBI	1	2.2	2	3
44	MCS	48	M	TBI	5.5	2.3	2	2
45	VS/UWS	33	M	TBI	2	1.6	4	4
46	MCS	58	M	CVA	3.5	NaN	1	3
47	VS/UWS	48	M	ABI	6	NaN	1	2
48	MCS	69	M	CVA	3	2.0	7	8
49	MCS	45	M	TBI	10	4.0	4	4
Total							146	181

VS/UWS – Vegetative state/Unresponsive wakefulness syndrome; F – Female; M – Male; MCS – Minimally conscious state; TBI – Traumatic brain injury; ABI – Anoxic brain injury; CVA – cerebrovascular accident;

Reference

1. Giacino, J. T. *et al.* Comprehensive systematic review update summary: Disorders of consciousness: Report of the Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology; The American Congress of Rehabilitation Medicine; And the. *Neurology* **91**, 461–470 (2018).
2. Giacino, J. T., Fins, J. J., Laureys, S. & Schiff, N. D. Disorders of consciousness after acquired brain injury: The state of the science. *Nat. Rev. Neurol.* **10**, 99–114 (2014).
3. Thibaut, A., Schiff, N., Giacino, J., Laureys, S. & Gosseries, O. Therapeutic interventions in patients with prolonged disorders of consciousness. *Lancet Neurol.* **18**, 600–614 (2019).
4. Stender, J. *et al.* Diagnostic precision of PET imaging and functional MRI in disorders of consciousness: A clinical validation study. *Lancet* **384**, 514–522 (2014).
5. Schnakers, C. *et al.* Diagnostic accuracy of the vegetative and minimally conscious state: Clinical consensus versus standardized neurobehavioral assessment. *BMC Neurol.* **9**, 1–5 (2009).
6. Price, J. L. Olfactory System. *The Human Nervous System. Paxinos G* (1990).
7. Merrick, C., Godwin, C. A., Geisler, M. W. & Morsella, E. The olfactory system as the gateway to the neural correlates of consciousness. *Front. Psychol.* **4**, 1–15 (2014).
8. Mainland, J. & Sobel, N. The sniff is part of the olfactory percept. *Chem. Senses* **31**, 181–196 (2006).
9. Wachowiak, M. All in a sniff: olfaction as a model for active sensing. *Neuron* **71**, 962–73 (2011).
10. Frank, R. A., Dulay, M. F. & Gesteland, R. C. Assessment of the Sniff Magnitude Test as a clinical test of olfactory function. *Physiol. Behav.* **78**, 195–204 (2003).
11. Johnson, B. N., Mainland, J. D. & Sobel, N. Rapid olfactory processing implicates subcortical control of an olfactomotor system. *J. Neurophysiol.* **90**, 1084–1094 (2003).
12. Arzi, A. *et al.* Humans can learn new information during sleep. *Nat. Neurosci.* **15**, 1460–1465 (2012).
13. Rozenkrantz, L. *et al.* A Mechanistic Link between Olfaction and Autism Spectrum Disorder. *Curr. Biol.* **25**, 1904–1910 (2015).

14. Arzi, A., Rozenkrantz, L., Holtzman, Y., Secundo, L. & Sobel, N. Sniffing patterns uncover implicit memory for undetected odors. *Curr. Biol.* **24**, R263–R264 (2014).
15. Reden, J., Draf, C., Frank, R. A. & Hummel, T. Comparison of clinical tests of olfactory function. *Eur. Arch. Oto-Rhino-Laryngology* **273**, 927–931 (2016).
16. Rojas-Libano, D. & Kay, L. M. Interplay between Sniffing and Odorant Sorptive Properties in the Rat. *J. Neurosci.* **32**, 15577–15589 (2012).
17. Johnson, B. N., Russell, C., Khan, R. M. & Sobel, N. A comparison of methods for sniff measurement concurrent with olfactory tasks in humans. *Chem. Senses* **31**, 795–806 (2006).
18. Giacino, J. T., Ph, D., Kalmar, K. & Ph, D. COMA RECOVERY SCALE-REVISED ©2004 Administration and Scoring Guidelines. (2004).
19. Rappaport, M., Dougherty, A. M. & Kelting, D. L. Evaluation of coma and vegetative states. *Arch Phys Med Rehabil* **73**, 628–634 (1992).
20. Doty, R. L. *et al.* Intranasal trigeminal stimulation from odorous volatiles: psychometric responses from anosmic and normal humans. *Physiol. Behav.* **20**, 175–185 (1978).
21. Hummel, T. & Livermore, A. Intranasal chemosensory function of the trigeminal nerve and aspects of its relation to olfaction. *Int. Arch. Occup. Environ. Health* **75**, 305–313 (2002).
22. Moor, J. W., Rafferty, A. & Sood, S. Can laryngectomees smell? Considerations regarding olfactory rehabilitation following total laryngectomy. *J. Laryngol. Otol.* **124**, 361–365 (2010).
23. Bareham, C. A. *et al.* Longitudinal bedside assessments of brain networks in disorders of consciousness: Case reports from the field. *Front. Neurol.* **9**, (2018).
24. Wannez, S., Heine, L., Thonnard, M., Gosseries, O. & Laureys, S. The repetition of behavioral assessments in diagnosis of disorders of consciousness. *Ann. Neurol.* **81**, 883–889 (2017).
25. Bareham, C. A. *et al.* Longitudinal assessments highlight long-term behavioural recovery in disorders of consciousness. *Brain Commun.* (2019). doi:10.1093/braincomms/fcz017
26. Linacre, J. M., Heinemann, A. W., Wright, B. D., Granger, C. V. & Hamilton, B. B. The structure and stability of the functional independence measure. *Arch. Phys. Med.*

- 1 *Rehabil.* **75**, 127–132 (1994).
- 2 27. Rowe, T. B., Macrini, T. E. & Luo, Z.-X. Fossil Evidence on Origin of the Mammalian
3 Brain. *Science* (80-.). **332**, 955 LP-957 (2011).
- 4 28. Sela, L. & Sobel, N. Human olfaction: A constant state of change-blindness. *Exp. Brain*
5 *Res.* **205**, 13–29 (2010).
- 6 29. Keller, A. Attention and olfactory consciousness. *Front. Psychol.* **2**, 1–13 (2011).
- 7 30. Stevenson, R. J. Phenomenal and access consciousness in olfaction. *Conscious. Cogn.*
8 **18**, 1004–1017 (2009).
- 9 31. Heine, L. *et al.* Effects of preference and sensory modality on behavioural reaction in
10 patients with disorders of consciousness. *Brain Inj.* **31**, 1307–1311 (2017).
- 11 32. Arzi, A. *et al.* The influence of odorants on respiratory patterns in sleep. *Chem. Senses*
12 **35**, 31–40 (2009).
- 13 33. Arzi, A. *et al.* Olfactory aversive conditioning during sleep reduces cigarette-smoking
14 behavior. *J. Neurosci.* **34**, 5382–15393 (2014).
- 15 34. Noirhomme, Q., Brecheisen, R., Lesenfants, D., Antonopoulos, G. & Laureys, S. ‘Look
16 at my classifier’s result’: Disentangling unresponsive from (minimally) conscious
17 patients. *Neuroimage* **145**, 288–303 (2015).
- 18 35. Majdan, M. *et al.* Epidemiology of traumatic brain injuries in Europe: a cross-sectional
19 analysis. *Lancet Public Heal.* **1**, e76–e83 (2016).
- 20 36. Aidinoff, E. *et al.* Vegetative state outcomes improved over the last two decades.
21 *Brain Inj.* **32**, 297–302 (2018).
- 22 37. Monti, M. M. *et al.* Willful Modulation of Brain Activity in Disorders of Consciousness.
23 *N. Engl. J. Med.* **362**, 579–589 (2010).
- 24 38. Coleman, M. R. *et al.* Towards the routine use of brain imaging to aid the clinical
25 diagnosis of disorders of consciousness. *Brain* **132**, 2541–2552 (2009).
- 26 39. Napolitani, M. *et al.* Transcranial magnetic stimulation combined with high-density
27 EEG in altered states of consciousness. *Brain Inj.* **28**, 1180–1189 (2014).
- 28 40. Engemann, D. A. *et al.* Robust EEG-based cross-site and cross-protocol classification
29 of states of consciousness. *Brain* **141**, 3179–3192 (2018).
- 30 41. Bekinschtein, T. A. *et al.* Classical conditioning in the vegetative and minimally
31 conscious state. *Nat. Neurosci.* **12**, 1343–1349 (2009).
- 32 42. Green, P., Rohling, M. L., Iverson, G. L. & Gervais, R. O. Relationships between

- 1 olfactory discrimination and head injury severity. *Brain Inj.* **17**, 479–496 (2003).
- 2 43. Sobel, N. *et al.* An impairment in sniffing contributes to the olfactory impairment in
- 3 Parkinson's disease. *Proc. Natl. Acad. Sci.* **98**, 4154–4159 (2002).
- 4 44. Plotkin, A. *et al.* Sniffing enables communication and environmental control for the
- 5 severely disabled. *Proc. Natl. Acad. Sci.* **107**, 14413–14418 (2010).
- 6 45. Haviv, L. *et al.* Using a Sniff Controller to Self-Trigger Abdominal Functional Electrical
- 7 Stimulation for Assisted Coughing Following Cervical Spinal Cord Lesions. *IEEE Trans.*
- 8 *Neural Syst. Rehabil. Eng.* **25**, 1461–1471 (2017).
- 9 46. Charland-Verville, V. *et al.* Detection of response to command using voluntary control
- 10 of breathing in disorders of consciousness. *Front. Hum. Neurosci.* **8**, 1–5 (2014).
- 11 47. Owen, A. *Into the Gray Zone: A Neuroscientist Explores the Border Between Life and*
- 12 *Death.* (Simon and Schuster, 2017).
- 13 48. Borer-alafi, N., Sazbo, N. & Rn, C. K. O. Loewenstein communication scale for the
- 14 minimally responsive patient. **16**, (2002).
- 15 49. Seel, R. T. *et al.* Assessment scales for disorders of consciousness: Evidence-based
- 16 recommendations for clinical practice and research. *Arch. Phys. Med. Rehabil.* **91**,
- 17 1795–1813 (2010).
- 18 50. Rosenthal, R., Cooper, H. & Hedges, L. Parametric measures of effect size. *Handb.*
- 19 *Res. Synth.* **621**, 231–244 (1994).
- 20 51. Leech, N. L. & Onwuegbuzie, A. J. A Call for Greater Use of Nonparametric Statistics.
- 21 *Annu. Meet. Mid-South Educational Res. Assoc.* 1–24 (2002). doi:10.1016/1353-
- 22 8292(95)00002-4
- 23 52. Kim, H.-Y. Statistical notes for clinical researchers: Chi-squared test and Fisher's exact
- 24 test. *Restor. Dent. Endod.* **42**, 152 (2017).

Methods

Patients

Fifty patients (mean age 43.4 ± 17 years, 9 women) were recruited across a span of ~4 years at the Intensive Care and Rehabilitation of Consciousness Department at the Loewenstein rehabilitation hospital, Ra'anana, Israel. Patients were tested on multiple sessions (range 1-13, mean 3.8 ± 2.98 sessions, total 190) separated by days/weeks depending on their clinical and personal availability. Out of 190 olfactory testing sessions, 41 sessions were excluded due to lack of stable nasal respiration, and 3 sessions were excluded due to nasal inhalation volume larger than 3.5 SD of the group mean (see inclusion/exclusion criteria below), maintaining 146 sessions in 43 patients (Table 1). The study was approved by the ethics committee of Loewenstein rehabilitation hospital. Written informed consent was obtained from the patients' legal guardian.

Measurement of nasal airflow

We used methods we have applied extensively in the past^{11,13,17,44,45}. In brief, during the olfactory testing sessions, patients' nasal airflow was measured using a nasal cannula (1103, Teleflex medical) which carried a differential pressure wave to a spirometer (ML141, ADInstruments) that converted the pressure changes into a voltage sent to an instrumentation amplifier (PowerLab 16SP Monitoring System, ADInstruments), sampling at 1000 Hz using LabChart software (ADInstruments).

Odorants

We used two odorant mixtures (a pleasant "shampoo" and unpleasant "rotten fish", both from Sensale, Ramt Gan, Israel) presented to all patients, and two pure odorant molecules (the pleasant PhenylEthyl Alcohol (PEA), CAS #102-20-5, that smells like rose, and the slightly unpleasant decanoic acid, CAS #334-48-5, that smells like crayons, both from Sigma-Aldrich, Rehovot, Israel) presented to a subset of 31 patients (See Extended Data Fig. 2). These very same odorants were effective in generating sniff-responses in previous studies^{8,12,13,33}. The odorants were absorbed in a cotton pad and placed in a sniff-jar. A jar with a cotton pad alone served as *Blank*.

Procedure

At the beginning of each session the experimenter explained to the patient that odorants will be presented using sniff-jars, and that nasal respiration will be monitored during the session. This was repeated despite us having no indication that the patient heard or understood what was said. Next, the experimenter gently applied a nasal cannula to the patient's nostrils in order to record the patient's nasal respiration. If no nasal respiration was observed, the experimenter waited for a few minutes before observing again. If there were still no signs of nasal respiration, the experimenter changed the patient's body position if possible. If no nasal respiration was observed after position change, the olfactory testing session was terminated. Lack of nasal respiration in a given session did not exclude the patient from future sessions. If nasal respiration was detected, the olfactory testing session began. On each trial a jar with either a pleasant odorant, an unpleasant odorant, or *Blank*, was presented to the patient for ~5 seconds. The jar was placed under the nose of the patient (without touching the patient) at the exact end of an exhale so the patient would receive the odorant in the following inhale. Each odorant and *Blank* jar were presented ~10 times in random order as long as nasal respiration was evident. In rare cases, due to clinical needs or lack of stable nasal respiration, the session ended before administration of all 10 repetitions. Following each olfactory testing session, we obtained behavioural clinical evaluation of the patient's state of consciousness to determine whether the session was in VS/UWS or MCS (including emergence from MCS).

Behavioural clinical evaluation

After each olfactory testing session, evaluation of the patients' state of consciousness was assessed using the Coma Recovery Scale Revised (CRS-R)¹⁸ and/or the Coma-Near Coma scale (CNC)¹⁹. The CRS-R evaluates the presence or absence of responses to auditory, visual, motor, oromotor, communication and arousal function. CRS-R is quantitative with scores ranging from 0 (lowest level of consciousness) to 23 (highest level of consciousness), and also qualitative with 4 levels: coma, VS/UWS, MCS and emergence from MCS, with specific behaviours defining each level. The CNC evaluates the occurrence of responses to visual, auditory, command following, threat response, olfactory, tactile, pain, vocalization. The CNC is quantitative with scores ranging from 4 (lowest level of consciousness) to 0 (highest level of consciousness), and also qualitative with 5 levels: *extreme coma* (3.5-4), *marked coma* (2.9-3.49), *moderate coma* (2.01-2.89), *near coma* (0.9-2), *no coma* (0-0.89). We converted the

CNC qualitative levels based on Rappaport et al.,¹⁹ as follows: *extreme coma* and *marked coma* = VS/UWS; *moderate coma* and *near coma* = MCS; *No coma* = emergence from MCS. In addition, all patients were periodically (unrelated to our testing schedule) assessed using the Loewenstein Communication Scale (LCS)⁴⁸. LCS evaluates five hierarchical functions: mobility, respiration, visual responsiveness, auditory comprehension and linguistic skills (verbal or alternative). The LCS is quantitative with scores ranging from 0 to 100, where scores up to 20 are considered VS/UWS and scores above 20 are considered MCS. Evaluation of the state of consciousness obtained directly following the olfactory testing session are missing in three cases due to technical error and were estimated based on the nearest LCS (all 3 patients were in MCS). Follow-up functional independence of the patients was evaluated using the Functional independence measure (FIM)²⁶. The FIM is quantitative with scores ranging from 18 to 126.

Twenty-one patients were evaluated using CNC and LCS only, and the remaining patients' state of consciousness was evaluated using CRS-R, CNC, and LCS. In the cases where both CRS-R and CNC were used, level of consciousness was determined by the CRS-R⁴⁹. To estimate the impact of having CNC and LCS estimates only, we compared the classification of VS/UWS and MCS between CRS-R and CNC scales in the current dataset. We observed disagreement in 30 of 80 sessions with both scales (in 29 sessions CRS-R indicated on VS/UWS and the CNC suggested MCS; in one session the CRS-R indicated on MCS and the CNC suggested emergence from MCS). This could suggest that the CNC division to VS/UWS ('extreme coma', 'marked coma') and MCS ('moderate coma', 'near coma') by the scale subcategories might be too liberal. Out of the 21 patients assessed only with CNC scale following the olfactory session, 4 were in VS/UWS in all sessions, 12 were in MCS in all sessions, and 5 transitioned between VS/UWS and MCS across sessions. If indeed the CNC is too liberal, it is possible that some MCS patients were in fact VS/UWS. The consequences of such misclassification on the group level analysis means that we could have only underestimated the observed findings. Thus, we conclude that although such misclassification would be unfortunate, it would not weaken, but only strengthen our effects. A second possible consequence of the use of CNC scale is detection of recovery while no recovery occurred. Out of the 16 patients who transited from VS/UWS to MCS, 5 were assessed using the CNC but not with the CRS-R. Importantly, in all five patients the Loewenstein communication scale (LCS)⁴⁸ conducted independently by the

hospital team provided additional evidence for conscious awareness in all of the five patients, and supported the CNC behavioural assessment. Thus, the lower sensitivity of CNC versus CRS-R in this study may have underestimated the power of the results, but does not appear to inaccurately detect transition to MCS.

Airflow analysis

All raw nasal airflow data collected in this study, with its associated behavioural assessments, will be posted for download at <https://www.weizmann.ac.il/neurobiology/worg/materials.html> after acceptance for publication.

The ongoing respiration trace was filtered using LPF (10 Hz), followed by automatic division into inhales and exhales. Discrete inhales and exhales were identified based of hysteresis of up to 5mV or 5% of max-min values, the smallest of the two, and minimum duration of 250 msec. If the respiration variability was high, the hysteresis value was not constant for the whole session but was calculated using a sliding window of 30 seconds, based on the respiration variance. To account for changes in respiration pattern across a session and between sessions, each nasal inhalation following a stimulus was normalized by dividing the nasal inhalation volume following the stimulus by the baseline inhalation volume (average of three inhalations prior to odor administration; Fig. 1b). As DoC patients often breathe through a tracheostomy tube, we tested whether tracheostomy modulated the results; We found that magnitude although tracheostomy significantly reduce nasal inhalation (not normalized) and it does not modulate normalized sniff-responses (Extended Data Fig. 3).

Sniff-response threshold definition

At the single patient level, sensory-driven *Level 1* sniff-response and cognitively-driven sniff-response thresholds were defined based on previous studies in healthy participants¹⁷ as: (a) reduction in sniff-response magnitude of 15% or more in relation to baseline respiration (see Table S1), and/or (b) sniff-response standard deviation (SD) across all trials in the session larger than 0.35, based on variability within the dataset (twice the averaged SD in MCS sessions). *Level 2* sniff-response thresholds were defined as a) 20% difference in sniff-response magnitude between pleasant vs. unpleasant odorants and b) a reduction in

normalized nasal inhalation volume in relation to baseline (<1) for both odorants. More specifically, sniff-response magnitude threshold was based on previous work investigating methods of sniff measurements in healthy participants¹⁷. To define sniff-response threshold we calculated the change in sniff volume averaged across pleasant (PhenylEthyl Alcohol) and unpleasant (Valeric acid) odorants in relation to clean air in healthy participants¹⁷ (Extended Data Table 1). Sniff volume (integral) values used for the calculation were measured using the same method as in our study (nasal cannula) and were the following: sniff volume for pleasant odorants = 0.837, sniff volume for unpleasant odorants = 0.641, nasal inhalation volume for clear air = 0.86. Formula: $[(0.837 + 0.641)/2]/0.86 = 0.8593$ (~15% decrease).

Inclusion/ exclusion criteria

Sessions - A session was excluded from the group analysis if:

- 1) Nasal respiration was not evident or unstable, and therefore not enough trials were presented (41 sessions). Only sessions with more than 15 trials were included in the analysis (Seven patients had no nasal respiration or unstable nasal respiration, and therefore were not included in the analysis).
- 2) The averaged normalized nasal inhalation volume was larger than 3.5 SD of the group mean. One session from each of three different patients was excluded under this criterion. Notably, all three patients had a later score of at least 48 (48/62/84) on the Functional Independence Measure (FIM)²⁶ and at least 49 (49/55/57) on the LCS⁴⁸, indicating on emergence from MCS. This suggests that nasal inhalation could be informative of consciousness even in cases of potentially altered olfaction.

The above exclusion criteria retained 43 out of 50 (i.e., 14% patients excluded) DoC patients (Table 1), 146 out of 190 sessions (i.e., 23.1% sessions excluded), and 5,934 out of 6,106 trials (i.e., 2.82% trials excluded) in this study.

Trials – A trial was excluded from a session if:

- 1) Baseline inhalation was unstable, presenting monotonic decrease or increase of at least 40% change in peak between the 1st and 3rd baseline inhalation, and at least 25% change between 1st and 2nd, and between the 2nd and 3rd inhalations.
- 2) No sniff was detected within 6.5 seconds from odor presentation.

3) No baseline inhalation was detected - respiration was too flat or if two trials were too close in time and therefore there were only three or less inhalations between trials.

4) There was an extreme change in the inhalation volume between baseline and the following inhalations. To identify these rare cases (12 trials, 0.19% of all trials), we measured the averages and standard deviations of inhale volumes at baseline and following stimulus, and the coefficients of variation (CV, ratio of standard deviation to mean). A trial was excluded if all three conditions occurred: (A) The max of the two CVs was below 20%, (B) The percent signal change (PSC) of the two CVs was below 50%, and (C) The PSC of the two means was above 50%.

The above exclusion criteria retained 5,778 out of 5,934 trials remaining after session exclusion (i.e., 2.63% excluded) in this study.

Baseline inhalations - A baseline inhalation was excluded from the averaged baseline inhalation if:

- 1) The baseline inhale started more than 30 seconds before odor presentation.
- 2) The baseline inhale overlapped with sniff-response of previous trial.
- 3) Inhalation volume was 25% smaller or larger than the other two baseline inhalations in the trial. In this case, the baseline inhalation with the maximal difference in inhale volumes from the median volume was excluded.

The above exclusion criteria retained 16,300 out of 17,334 baseline inhalations (i.e., 5.96% excluded) in this study.

Sniffs – A sniff was excluded if:

- 1) The normalized sniff volume was ± 3.5 SD of the averaged sniff in the session.
- 2) The 1st sniff was excluded if no sniff was detected within 6.5 seconds from odor presentation (and then the trial was excluded as well), and each other sniff was excluded if not detected within 6.5 from previous sniff (and then the later sniffs were excluded as well). Note: in 8 patients, respiration rate was slower than typical and the threshold was extended (7.5 seconds in 3 patients, 8.5 seconds in 3 patients and 11 seconds in one patient).

The above exclusion criteria retained 16,999 out of 17,334 sniffs (i.e., 1.93% excluded) in this study.

Sensitivity and specificity

Sensitivity scores were calculated on the VS/UWS patients who remained VS/UWS and on patients' long-term survival rates. Specificity scores were calculated on the VS/UWS patients who transition to MCS and on the MCS patients³⁴.

Statistical analysis

Normalized nasal inhalation volume values were not normally distributed (Shapiro-Wilk test: First sniff all W 's > 0.88 , all p 's < 0.0007 , Second sniff all W 's > 0.66 , all p 's < 0.01 , Third sniff all W 's > 0.63 , all p 's < 0.04), and displayed greater variance in MCS than in VS/UWS sessions (Leven's test: First sniff $F(1,147) = 3.9$, $p = 0.05$, Second sniff $F(1,147) = 3.3$, $p = 0.07$, Third sniff $F(1,147) = 1.6$, $p = 0.21$). Thus, nonparametric tests were applied. For statistical analysis between MCS and VS/UWS sessions Wilcoxon *ranksum* tests were used, for statistical analysis within each group Wilcoxon *signrank* tests were used. Bonferroni correction for multiple comparison was applied for comparison of nasal inhalation volume between MCS and VS/UWS sessions and also within a group (two odors X three sniffs: $0.05/6 = 0.0083$). Nonparametric dependent samples effect size was calculated by the following formula $r = Z/\sqrt{n}$ ⁵⁰, where Z is the Wilcoxon signrank statistic and n is the sample size. The nonparametric independent samples effect size was estimated using cliff's delta⁵¹. Chi-square effect size was estimated using Cramer's V ⁵². Relation between the sniff-responses and Functional Independence Measure (FIM)²⁶ were assessed using Spearman correlations and were Bonferroni corrected for multiple comparisons [two states X three sniffs: $0.05/6 = 0.0083$]. Note: three sessions of two patients who presented outliers sniff-response value in later sessions implying impaired olfaction were excluded from the correlation analysis. When including these three sessions similar results were obtain (Pleasant $r_{42} = -0.27$, $p = 0.075$; Unpleasant $r_{42} = -0.55$, $p = 0.002$; Blank $r_{42} = -0.04$, $p = 0.80$).

References

48. Borer-alafi, N., Sazbo, N. & Rn, C. K. O. Loewenstein communication scale for the minimally responsive patient. **16**, (2002).

49. Seel, R. T. *et al.* Assessment scales for disorders of consciousness: Evidence-based recommendations for clinical practice and research. *Arch. Phys. Med. Rehabil.* **91**, 1795–1813 (2010).
50. Rosenthal, R., Cooper, H. & Hedges, L. Parametric measures of effect size. *Handb. Res. Synth.* **621**, 231–244 (1994).
51. Leech, N. L. & Onwuegbuzie, A. J. A Call for Greater Use of Nonparametric Statistics. *Annu. Meet. Mid-South Educational Res. Assoc.* 1–24 (2002). doi:10.1016/1353-8292(95)00002-4
52. Kim, H.-Y. Statistical notes for clinical researchers: Chi-squared test and Fisher’s exact test. *Restor. Dent. Endod.* **42**, 152 (2017).

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Author contributions

Conceptualization: AA, NS; Data collection: AA, LR, LG; Analysis: AA, LR, LG, NS; Funding Acquisition: AA, NS, YS; Investigation: AA, LR, DR, YH; Methodology: AA, LR, LG, NS; Project Administration: AA, LR, DR, YH, GC, TG, BK, AO, EA, YS, NS; Resources: AA, LR, LG, DR, YH, GC, TG, BK, AO, EA, YS, NS; Software: AA, LR, LG; Supervision: AA, TAB, TG, BK, AO, EA, YS, NS; Validation: AA, LR, LG; Visualization: AA, NS; Writing - original draft: AA, NS; Writing – Review & editing: AA, LR, LG, DR, YH, GC, TG, BK, AO, EA, YS, NS;

Author Information

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Competing interests

The findings of this manuscript are being used by the Weizmann Institute Office of Technology Licensing (Yeda) towards submission of a patent on a method for detection of consciousness.

Data availability

Correspondence and requests for materials should be addressed to Anat Arzi at anat.arzi@gmail.com or to Noam Sobel at noam.sobel@weizmann.ac.il

Code availability

All custom code created and used in this work is available upon request.

1 Extended Data

2
3 **Extended Data Fig. 1: The sniff-response reflects current level of consciousness in**
4 **DoC patients: Data by odorant and sniff**

Normalized sniff volume following pleasant odorants (purple), unpleasant odorants (blue) and Blank (grey), in VS/UWS (V) sessions (outline, left) and MCS (M) sessions (filled, right), in the first **a-c**, second **d-f**, and third **g-i**, sniff following stimulus delivery. Each dot represents a session, flat violin plots show the distribution, the red line denotes the median, and the dashed horizontal line denotes the baseline value at 1 normalized flow units (NFU). The bar-graphs to the right of each distribution tabulate the same data. The p-value beneath the distribution denotes its difference from baseline inhalation, i.e., the existence of a sniff-response. The p-values above the distributions denote the difference in sniff-response across groups. * = $p < 0.05$ corrected for multiple comparisons (see Methods). # < 0.05 uncorrected. (**d, e**) Similar results to the first sniff were evident in the second sniff after odorant presentation (MCS: Pleasant = 0.94 ± 0.20 NFU, median = 0.95 NFU; Unpleasant = 0.92 ± 0.21 NFU, median = 0.94 NFU, difference from baseline, all $Z > 3.86$, all $p < 0.0001$, all effect-size $r > 0.45$. VS/UWS: Pleasant = 0.97 ± 0.12 NFU, median = 0.98 NFU, Unpleasant = 0.97 ± 0.14 NFU, median = 0.98 NFU, difference from baseline, all $Z < 2.1$, all $p > 0.05$, Bonferroni corrected, all effect-size $r < 0.25$), (**g, i**) but not in the third sniff (MCS: Pleasant = 0.99 ± 0.11 NFU, median = 0.976 NFU, Unpleasant = 0.98 ± 0.14 NFU, median = 0.977 NFU, difference from baseline, all $Z < 1.4$, all $p > 0.16$, all effect-size $r < 0.14$. VS/UWS: Pleasant = 0.99 ± 0.14 NFU, median = 0.99 NFU, Unpleasant = 1.00 ± 0.14 NFU, median = 0.98 NFU, difference from baseline, all $Z < 0.97$, all $p > 0.33$, all effect-size $r < 0.11$). That altered sniffing persisted into the second sniff after each odorant presentation, but not to the third, indicates that this was a genuine transient odorant-driven response and not a state-change. In addition, we replicated this entire analysis, this time comparing the odorant-driven sniff-response to the first inhalation following Blank rather than to the respiratory baseline, and obtained similar results (**a, c**) First sniff: MCS Pleasant $Z = 2.266$, $p = 0.02$, effect-size $r = 0.2652$, MCS Unpleasant $Z = 1.93$, $p = 0.05$, effect-size $r = 0.23$. VS/UWS Pleasant $Z = 0.95$, $p = 0.34$, effect-size $r = 0.11$, VS/UWS Unpleasant $Z = 1.65$, $p = 0.1$, effect-size $r = 0.19$; (**d, f**) Second sniff: MCS Pleasant $Z = 2.27$, $p = 0.02$, effect-size $r = 0.2657$, MCS Unpleasant $Z = 2.76$, $p = 0.006$, effect-size $r = 0.32$. VS/UWS Pleasant $Z = 0.99$, $p = 0.32$, effect-size $r = 0.12$, VS/UWS Unpleasant $Z = 1.12$, $p = 0.26$, effect-size $r = 0.13$; (**g, i**) Third sniff: MCS Pleasant $Z = 0.17$, $p = 0.87$, effect-size $r = 0.02$, MCS Unpleasant $Z = 0.53$, $p = 0.60$, effect-size $r = 0.06$. VS/UWS Pleasant $Z = 1.41$, $p = 0.17$, effect-size $r = 0.16$, VS/UWS Unpleasant $Z = 0.98$, $p = 0.33$, effect-size $r = 0.11$).

Extended Data Fig. 2: The sniff-response to pure olfactory odorants in DoC patients

Normalized sniff volume following ‘pure olfactant’ pleasant odorants (PhenylEthyl Alcohol (PEA); purple) and ‘pure olfactant’ unpleasant odorants (decanoic acid; blue) in VS/UWS (V) sessions (outline; left) and in MCS (M) sessions (filled; right) in the **a**,

b, first, **c,d**, second and **e,f**, third sniff following odorants delivery. Each dot represents a session, flat violin plots show the distribution, the red line denotes the median, and the dashed horizontal line denotes the baseline value at 1 normalized flow units (NFU). The bar-graphs to the right of each distribution tabulate the same data, with error bars denotes standard errors of the mean (SEM). The p-value beneath the distribution denotes its difference from baseline inhalation, i.e., the existence of a sniff-response. * < 0.05 uncorrected. For the unpleasant “pure olfactant”, similar to the “mild trigeminal” unpleasant odorant, normalized sniff volume significantly dropped from baseline in all three sniffs in MCS sessions (**b**) First sniff: 0.960 ± 0.21 NFU, median = 0.928, $Z = 3.92$, $p = 0.0001$, $r = 0.46$; (**d**) Second sniff: 0.9359 ± 0.15 NFU, median = 0.95, $Z = 2.98$, $p = 0.003$, $r = 0.36$; (**f**) Third sniff: 0.96 ± 0.12 NFU, median = 0.968, $Z = 2.62$, $p = 0.009$, $r = 0.31$), however not for VS/UWS sessions (**b**) First sniff: 0.96 ± 0.15 NFU, median = 0.98, $Z = 1.39$, $p = 0.17$, $r = 0.16$; (**d**) Second sniff: 0.95 ± 0.17 NFU, median = 0.98, $Z = 2.3$, $p = 0.02$, $r = 0.26$; (**f**) Third sniff: 0.97 ± 0.18 NFU, median = 0.98, $Z = 1.3$, $p = 0.19$, $r = 0.15$). For the pleasant “pure olfactant”, MCS normalized sniff volume dropped from baseline in the first two sniffs (**a**) First sniff: 0.94 ± 0.25 NFU, median = 0.94, $Z = 3.48$, $p = 0.0005$, $r = 0.41$; (**c**) Second sniff: 0.95 ± 0.15 NFU, median = 0.94, $Z = 2.67$, $p = 0.008$, $r = 0.31$), and (**e**) was back to baseline in the third sniff (0.99 ± 0.12 NFU, median = 0.99, $Z = 1.05$, $p = 0.29$, $r = 0.12$). In VS/UWS sessions, normalized sniff volume significantly dropped from baseline in the second and third sniff (**a**) First sniff: 0.96 ± 0.13 NFU, median = 0.98, $Z = 2.55$, $p = 0.01$, $r = 0.30$; (**c**) Second sniff: 0.93 ± 0.14 NFU, median = 0.95, $Z = 3.4$, $p = 0.006$, $r = 0.40$; (**e**) Third sniff: 0.94 ± 0.14 NFU, median = 0.96, $Z = 3.03$, $p = 0.003$, $r = 0.35$), reflecting a sniff-responses in VS/WUS patients who later transition to MCS, but not in those remained unconscious. This “pure olfactant” sensory-driven sniff-response suggests that olfactory stimulation and not simply trigeminal activation modulated sniff volume in DoC patients.

Extended Data Fig. 3: The sniff-response is similar with and without Tracheostomy

Normalized sniff volume following pleasant odorants (purple), unpleasant odorants (blue), in MCS sessions with (W; left) and without (O; right) Tracheostomy in the **a, b,**

first, **c, d**, second and **e, f**, third sniff following odorants delivery. Each dot represents a session, flat violin plots show the distribution, the red line denotes the median, and the dashed horizontal line denotes the baseline value at 1 normalized flow units (NFU). The bar-graphs to the right of each distribution tabulate the same data, with error bars denotes standard errors of the mean (SEM). The p-value beneath the distribution denotes its difference from baseline inhalation, i.e., the existence of a sniff-response. * $p < 0.05$.

DoC patients often breathe through a tracheostomy tube, and this was the case in 44 of the 73 MCS sessions (60.3%) and 61 of the 73 VS/UWS sessions (83.6%) reported in this manuscript. We note that some level of olfaction, albeit reduced, remains even following total laryngectomy²². Total laryngectomy, however, typically entails zero nasal airflow, and these patients can learn to smell using a form of air gulping. Tracheostomy in these DoC patients, in contrast to total laryngectomy, retained a portion of nasal airflow, as evidenced in our recordings. This also reflects clinical practice at this rehabilitation facility, where the standard of care is to deflate tracheostomy balloons immediately upon admission from intensive care (so as to minimize pressure-ulcers in the respiratory path), and to replace the tracheostomy with a balloonless tracheostomy as early as possible. Nevertheless, to address this issue, we first tested whether tracheostomy modulated the magnitude of nasal inhalation (not normalized) in MCS sessions where sniff-responses were observed at the group level. Unsurprisingly, we observed 3-fold greater nasal inhalation volume in MCS sessions without tracheostomy compared to sessions with tracheostomy (without = 0.07 ± 0.044 , with = 0.02 ± 0.016 , $Z = 5.8$ $p < 0.0001$, Cliff's delta effect size = 0.8). We note, however, that the sniff-response is individually determined using individual baseline nasal airflow for normalization. Thus, even very low total levels of flow may produce equal size sniff-responses. To estimate this, we next asked whether the normalized sniff-response was impacted by tracheostomy. To test this, we examined the normalized sniff-response in MCS sessions in which patients were tested with and without tracheostomy, and found no difference between the groups. More specifically, a sensory-driven sniff-response was evident in the first and second sniff with tracheostomy (all $Z > 2.3$, all $p < 0.02$, all $r > 0.35$) and without tracheostomy (all $Z > 2.93$, all $p < 0.003$, all $r > 0.54$). Notably, there were no significant differences in normalized sniff-response magnitude between MCS sessions with and without tracheostomy (all $z < 1.7$, all $p > 0.09$, all $r < 0.31$). These findings imply that although tracheostomy decreased nasal airflow it did not bias the normalized sniff-response.

1 The robust results in the MCS sessions imply that the difference observed in
 2 normalized sniff-response between MCS and VS/UWS sessions could not be explained
 3 merely by different prevalence of tracheostomy between the MCS and VS/UWS groups
 4 (62% vs 84% respectively).

Extended Data Fig. 4: The sniff-response in MCS sessions

The red lines denote sniff-response threshold (more than 15% change in magnitude/0.35 SD): dots within the lines (white background) reflect sessions without a sniff-response, and dots beyond the lines (shaded background) reflect sessions with a sniff-response. **a-c**, Each dot is a MCS session. **(a)** Pleasant odorant sessions (purple) **(b)** Unpleasant odorant sessions (blue) **(c)** Sessions with *Blank* (grey). **d**, Percentage of MCS patients (not sessions) with sniff-responses (white, 64.5%) and without sniff-responses (red, 35.5%) across all three conditions.

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Extended Data Fig. 5: The sniff-response was prognostic for recovery of consciousness and long-term survival in DoC: Data by patient rather than by session

The red lines denote the threshold of a sniff-response (more than 15% change in magnitude/0.35 SD). **a-c**, Each dot is a session with the strongest sniff-response of a MCS patient. **(a)** Pleasant odorant (purple) **(b)** Unpleasant odorant (blue) **(c)** *Blank* (grey). **d**, Percentage of MCS patients with sniff-responses (white, 64.5%) and without sniff-responses (red, 35.5%) across all three conditions. **e-g**, Each dot is a session with the strongest sniff-response of a VS/UWS patient, where empty dots later ‘recovered’ patients (transitioned to MCS) and filled dots represent patients who would not later recover and remain unconscious. **(e)** Pleasant odorant (purple) **(f)** Unpleasant odorant (blue) **(g)** *Blank* (grey). **h**, Percentage of VS/UWS patients who later transitioned to MCS (left, ‘Recovered’) and remain unconscious (right, ‘Unrecovered’) with sniff-responses (white; ‘Recovered’: 62.5%; ‘Unrecovered’: 0%) and without sniff-responses (red; ‘Recovered’: 37.5%; ‘Unrecovered’: 100%) across all three conditions. **i-k**, Each dot is a DoC patient (MCS and VS/UWS), where filled black dots represent later deceased patients and color dots represent patients surviving during the study (37.3 ± 14.1 months after brain injury) **(i)** Pleasant odorant (purple) **(j)** Unpleasant odorant (blue) **(k)** *Blank* (grey). **l**, Percentage of DoC patients with sniff-responses (left) that survived (white, 91.7%) and deceased (D) (red, 8.3%), and of DoC patients without sniff-responses that survived (white, 36.8%) and deceased (red, 63.2%) during the study. **m-o**, Relation between Functional Independence Measure (FIM) and normalized sniff volume. Each dot is a VS/UWS patient surviving during the study. **(m)** Pleasant odorant (purple) **(n)** Unpleasant odorant (blue) **(o)** *Blank* (grey). r represents Spearman correlation.

Table 1 Consolidated data from ANOVA tests

		VAL	AIR	PEA	F	P	SD bootstrap
Perceived intensity rating		0.673 ± 0.042	0.382 ± 0.026	0.437 ± 0.027	38.9009	4.60 × 10 ⁻⁹	N/A
Perceived pleasantness rating		0.327 ± 0.039	0.565 ± 0.043	0.530 ± 0.044	22.2698	1.20 × 10 ⁻⁶	N/A
Thorax	Integral	0.147 ± 0.157	0.153 ± 0.187	0.173 ± 0.184	0.1126	0.8939	0.3696
Thorax	Max value	0.110 ± 0.113	0.122 ± 0.130	0.111 ± 0.126	0.0612	0.9408	0.3584
Thorax	Mean value	0.072 ± 0.057	0.080 ± 0.068	0.068 ± 0.064	0.2027	0.8176	0.38
Abdomen	Integral	0.250 ± 0.153	0.323 ± 0.191	0.312 ± 0.193	0.8112	0.4538	0.5832
Abdomen	Max value	0.170 ± 0.113	0.217 ± 0.126	0.197 ± 0.127	1.6662	0.206	0.9734
Abdomen	Mean value	0.089 ± 0.061	0.123 ± 0.071	0.118 ± 0.069	3.53	0.0419	1.561
Combo	Integral	0.412 ± 0.085	0.506 ± 0.118	0.491 ± 0.121	1.4131	0.2591	0.9741
Combo	Max value	0.325 ± 0.062	0.388 ± 0.075	0.371 ± 0.069	1.7482	0.1914	1.2377
Combo	Mean value	0.161 ± 0.035	0.202 ± 0.044	0.178 ± 0.041	2.006	0.1522	1.1469
Pneumotac	Integral	0.849 ± 0.086	1.063 ± 0.117	1.034 ± 0.123	4.9335	0.014	1.362
Pneumotac	Max value	0.581 ± 0.035	0.618 ± 0.032	0.589 ± 0.036	1.6461	0.2097	1.1594
Pneumotac	Mean value	0.340 ± 0.019	0.392 ± 0.022	0.368 ± 0.025	4.0558	0.0276	1.6895
Cannula	Integral	0.641 ± 0.081	0.860 ± 0.100	0.837 ± 0.105	8.1849	0.0015	2.0606
Cannula	Max value	0.488 ± 0.035	0.542 ± 0.027	0.521 ± 0.031	4.3826	0.0214	2.1415
Cannula	Mean value	0.256 ± 0.021	0.324 ± 0.023	0.300 ± 0.020	7.3643	0.0025	2.6243
Temperature	Integral	-0.538 ± 0.070	-0.722 ± 0.095	-0.706 ± 0.091	7.3139	0.0026	2.0964
Temperature	Max value	-0.431 ± 0.033	-0.538 ± 0.033	-0.494 ± 0.031	12.1081	0.0001	3.4898
Temperature	Mean value	-0.204 ± 0.018	0.258 ± 0.019	-0.243 ± 0.018	8.8795	0.0009	3.1173

The measurement technique and summary variables vary by row. The group mean value and standard error for each stimulus condition (e.g., valeric acid, air, and phenethyl alcohol) are shown in the respectively headed columns (e.g., VAL, AIR, and PEA). The *F* and *P* values for each test are shown in the associated columns (e.g., *F* and *P*). Finally, the standard deviation (SD) from the associated bootstrap distribution is shown in the right-most column (e.g., SD bootstrap). N/A = not applicable.

Extended Data Table 1: Original table from Johnson et al., 2006

Sniff-response thresholds were obtained from a previous study. To define sniff-response threshold we calculated the change in sniff volume averaged across pleasant (PhenylEthyl Alcohol) and unpleasant (Valeric acid) odorants in relation to clean air in healthy participants¹⁷ (Extended Data Table 1). Sniff volume (integral) values used for the calculation were measured using the same method as in our study (nasal cannula) and were the following: sniff volume for pleasant odorants = 0.837, sniff volume for unpleasant odorants = 0.641, nasal inhalation volume for clear air = 0.86. Formula: $[(0.837 + 0.641)/2]/0.86 = 0.8593$ (~15% decrease). We would like to again clearly state that we were using these exact threshold values well before we obtained the follow-up survival data.