Nasal air-puff promotes default mode network activity in mechanically ventilated comatose patients: a non-invasive brain stimulation approach

Running Title: Nasal air-puff promotes default mode network activity

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Abstract

Objectives: Coma state and loss of consciousness are associated with decreased brain activity, including and especially gamma oscillations, which are involved in neural network integrity, as well as the default mode network (DMN). This condition can be aggravated by mechanical ventilation since nasal respiration, known to drive functional neural oscillations, is diminished. Hence, we proposed that rhythmic nasal air-puffing in mechanically ventilated comatose patients may promote brain activity and improve network connectivity.

Materials and Methods: We assessed the activity, complexity, and connectivity of the DMN using electroencephalography (EEG) in fifteen comatose patients (eight males) admitted to the intensive care unit due to opium poisoning before and during the application of nasal air-puff. Air-puffing into the nasal cavity was done using a nasal cannula via an electrical valve (open duration of 630ms) with a frequency of 0.2 Hz.

Results: Our analyses demonstrated that nasal air-puffing enhanced gamma power (30-40 Hz) oscillation in the DMN. Additionally, the coherency and synchrony between DMN regions were increased during nasal air-puffing. Recurrence quantification analysis (RQA) analysis revealed that global complexity and irregularity of EEG, which is typically seen during wakefulness and conscious state, were increased during rhythmic nasal air-puffing.

Conclusions: Rhythmic nasal air-puffing, as a non-invasive brain stimulation method, opens a new window into modifying the brain connectivity integration in comatose patients, which potential can influence their outcome by reducing the adverse effect of mechanical ventilation on brain activity.

Keywords: nasal air-puff, non-invasive brain stimulation, default mode network, coma state,

Conflict of interests: The authors declare that no competing interests exist.

Introduction

A coma is an unresponsiveness state characterised by a drastic reduction of brain activity, leading to the absence of consciousness, awareness, and arousal (1). Depending on underlying pathology and degree of consciousness, brain activity in disorders of consciousness (DOC), including coma, changes in multiple ways, such as oscillatory activity alterations, network interactions, and connectivity (2, 3). Organised oscillatory activities are critically involved in brain network communications during cognition and conscious processing, and their perturbation could be representative of impairment in intrinsic brain functions and decreased level of consciousness (4). The gamma-range activity, which has been suggested to correlate with conscious processing, awareness and arousal (5, 6), is disrupted in comatose patients (7). The activity of gamma oscillations, in particular brain regions of frontal, centroparital and temporal cortices, is potentially correlated with the level of consciousness (3, 8-10). Gamma power is reduced in DOC, such as unresponsive wakefulness syndrome and loss of consciousness (11, 12). Further, gamma activity has been proposed to drive the influx of information from lower to higher brain areas during conscious processes such as perception (13, 14). Connectivity across the brain regions at the gamma band is also pivotal for information integration (13, 15). The coherence between cortical or subcortical regions is increased at the gamma band during consciousness (14, 16, 17), contributing to the merging or binding of information (18).

Another aspect of brain activity that changes due to comas is the complex patterns of electroencephalography (EEG) (19, 20). Various complexity features are positively correlated with the consciousness level (11, 21-23). Therefore, non-linear analysis of EEG based on complexity and entropy (an index for irregularity) can provide a valuable tool to assess brain activity in patients with DOC (19, 24). The EEG dynamics complexity is suggested to discriminate brain state

between different levels of consciousness, such as sleep, wakefulness, anaesthesia, and coma (23, 25-27). Generally, complexity measures are higher during the awake state, in which the brain is more active than the asleep state and that the brain activity is decreased by anaesthetic agents (28). Notably, less complexity and irregularity of brain waves are observed in comatose patients (28, 29). Thus, the complexity measures of particular brain state EEG, such as resting-state, can be used in comatose patients to assess their brain activity.

Different brain networks are engaged in consciousness and information processing during wakefulness. Default mode network (DMN) activity is critically associated with consciousness (30, 31). The DMN consists of several brain areas, including the precuneus, medial-prefrontal cortex, and bilateral temporoparietal junction, which is more active at rest than attention-demanding functions (16). The function of DMN, regarded as an example of spontaneous brain activity, provides a baseline functional state of consciousness associated with internally oriented, complex cognitive processes (16, 17, 32). Additionally, the strength of connectivity among DMN regions is correlated with different levels of consciousness (32).The activity of DMN changes due to different neurological conditions, such as DOC, including coma (31-33). The DMN connectivity is reversibly disrupted in comatose patients and is suggested to be associated with clinical severity and outcome (17, 30, 31, 34).

On the other hand, endotracheal intubation and mechanical ventilation (MV) is another factor that may alter brain function (35-37). Due to impaired spontaneous breathing, comatose patients should receive MV, usually via an endotracheal tube (38). Although intubation and MV are lifesaving procedures that compensate for breathing impairment resulting from the coma, it is not without complications. Epidemiological studies reported that intubating comatose patients increase delirium incidence by 74-83% compared to 20-48% in non-intubated patients (39-41), and current

knowledge of potential contributing factors and strategies are not efficient control the problem (37, 42-44). Delirium in these patients is positively associated with cognitive impairments after discharge (45-47). Moreover, after recovery, long-term intubation in mechanically ventilated patients is linked with neurological impairment in critical care units, such as memory and cognitive decline (37, 48). Different explanations are introduced to justify the relationship between cognitive problems and MV (37, 49-51). Part of this cognitive decline may result from changes in neural activities of related networks (37), probably due to eliminated nasal airflow and olfactory receptors stimulation because of this endotracheal intubation (52).

Growing evidence shows that nasal breathing in mammals, including humans, generates oscillations that extent to distant brain areas, such as cortical and subcortical regions (53-56), and these activities diminish when breathing deviates from the nasal pathway (57). These neural oscillations are called respiration-entrained brain rhythms, which are global and phase-locked to nasal respiration (54). The respiration-entrained rhythms produced through the stimulation of olfactory sensory neurons (OSNs) by air passage can synchronise the activity of the piriform cortex and limbic regions and, significantly, affect cognitive functions such as memory retrieval (52, 57, 58). In addition, the respiratory cycle phase has been reported to entrain and modulate higher frequency bands such as gamma oscillations in cortical and subcortical regions (59-61).

Although eliminated nasal breathing has been demonstrated to disrupt respiratory-coupled neural oscillations, re-establishing nasal airflow can stimulate OSNs, and therefore, restore these neural activities (57). It has been demonstrated that respiration-coupled oscillations in the hippocampus of anaesthetised rats diminish when airway maintenance is restricted to tracheostomy and restore by rhythmic delivery of air puffs to nasal cavities (62). Applying nasal airflow can also alter the cortical activity in healthy individuals (63). It appears that applying air-puff into nasal cavities can

re-induce respiration-entrained oscillations and subsequently increase neural activities in resting-state networks, such as DMN.

Considering the bypassed route of respiration due to endotracheal intubation and the evidence that nasal breathing can drive oscillatory activities involved in brain network coordination, part of the decreased brain activity exhibited in comatose patients might be due to eliminated nasal airflow. Therefore, we hypothesised that MV with endotracheal intubation in comatose patients could worsen the resting-state activity during comas, and therefore, restoring the nasal airflow may alleviate the adverse effect of comatose condition on rhythmic brain activity. Hence, we conducted this study to evaluate the effect of nasal air-puffing on DMN reactivity and connectivity in comatose patients. We recorded and analysed the EEG from comatose patients with intubation and MV in two conditions of nasal air-puff (AP) and non-air-puff (nAP) to investigate the impact of nasal airflow on gamma frequency activity, complexity features, and connectivity across DMN regions.

Materials and methods

Subjects

Fifteen patients (seven females, with a median age of 30.0 years old, range: 25-38 years old), with initial Glasgow Coma Scale (GCS) score ranges between 3/15 to 8/15, were selected for the study (64). All patients were admitted to the intensive care unit (ICU) of Loghman Hospital, Tehran, Iran, due to opium poisoning. Demographic data and clinical characteristics of study subjects are summarised in the Table. They were sedated with fentanyl, midazolam, or a combination of both, based on their condition and treatment protocols. None of the patients had previous head trauma, coma status, or loss of consciousness. Written informed consent was provided by patients' next of

kin. The study was approved by the Ethics Committee at Tarbiat Modares University [IR.MODARES.REC.1397.036].

Non-invasive Air-puffing into the nasal cavity

Airflow was puffed into the nasal cavity (7-10 L/s; 1.1 bar) through a nasal cannula connected to a cylindrical tube following the previous study protocol but with a different frequency (63). We selected 0.2 Hz (i.e., 12 cycles per min, Open:Close, 1:2) according to regular respiration rate and breathing ratio of inspiration:expiration to adjust the rate of nasal air-puffing with the delivered ventilator respiratory rate avoiding a potential confounding factor. The air-puffing through the cannula was controlled by an electrical valve (BIODAC-Ev118, TRITA WaveGram Co., Tehran, Iran). We set up equipment as described for a control condition and started EEG acquisition from patients while the equipment was running, but without air-puff delivery. These baseline recordings were done before air-puff delivery and used to compare air-puff (AP) and no air-puff (nAP) conditions.

EEG Recording

Continuous EEG was recorded using 17 electrodes based on international 10/20 system (Fp1, Fp2, Fz, F3, F4, F7, F8, Cz, C3, C4, T5, T6, Pz P3, P4, O1, O2 (65); g.tec medical engineering GmbH, Graz, Austria). The ground and reference electrodes were attached to the left mastoid and the right earlobe, respectively. Raw EEG was sampled at 256 Hz with 16-bit resolution. Markers were placed at the beginning of non-air-puff (nAP) periods and air-puff (AP). Data were used to calculate 15 regions of interest (ROI) as the mean of channels in each ROI. Otherwise, the activity of the channels was averaged to generate each ROI data. The arrangements of the channels providing ROI's data is shown in Figure 1.

EEG analysis

EEG data were analysed using EEGLAB v2019 (66, 67) on MATLAB v2019 (MathWorks Inc., California, USA). Next, data were filtered using a 30–100 Hz bandpass filter with the linear finite impulse response. After artefacts rejection by visual inspection, the independent component analysis (ICA) technique was carried out using the EEGlab toolbox. Since patients in the ICU were connected to multiple electrical and mechanical equipment and delivered air-puff using an electrical valve suspected to induce artefact on the signal, the ICA was computed to find the source of component generating signals.

According to the assumptions of statistical properties, the ICA method can find the underlying components and sources mixed of oscillations (68). It, therefore, generated the power spectra and topographic plot showing the distribution of the component values across the scalp. Based on the artefact rejection guidance, we removed the components presenting mechanical noise and artifactual oscillations (ref). For example, as shown in Supplementary Figure S1, the heartbeat component with a clear signature, usually present in a single channel, was removed. Application of ICA resulted to removal of three ECG-like components from two patients in total (two during baseline and one during air-puff).

Following hospital safety protocols for patients' care in the ICU, we were allowed to record the signals for five minutes. Subsequently, after preprocessing, including noise cancellation, cleaning by eye and using ICA, which shortened signals, we used the remaining 60 seconds of the signals (60 seconds of baseline recording without air-puffing-which considered nAP, and 60 seconds of signals during air-puff which named AP condition).

Power of EEG signals was calculated during no air puff (baseline) and air puff using the pwelch function in MATLAB. To compare data, we carried out paired t-test analysis on brain oscillations from low gamma (30-50 Hz), middle (50-80 Hz), and high (80-100 Hz) with 0.5 Hz frequency resolution (69).

Network connectivity analysis

We investigated network properties within three distinct gamma frequency ranges: low: 30-50 Hz, middle: 50-80 Hz, and high gamma 80-100 Hz. In this case, we constructed a matrix: edges were defined as absolute values of Pearson's correlations applied to all pairwise electrode data with no lag. Next, the correlation matrix was thresholded from 0.6 to 0.95. The reason behind selecting a broad threshold is to compare highly correlated vs moderately correlated regions. The edges with a correlation value less than the threshold were removed. To measure graph parameters, we computed global efficacy (the mean inverse of the minimum number of edges that are needed to cross on the path from one node to another) and local efficacy (global efficiency in the subgraph that indicates neighbours of each node) (58, 70-72).

For further investigation, connectivity information was graphically rendered in a circular diagram displaying relationships between regions' pairs. Absolute values of Pearson's correlations were applied for all pairwise regions of DMN. Similar to the previous analysis, the connectogram was thresholded from 0.6 to 0.95 and connections that did not reach the threshold (i.e., 0.6) were removed. Number of connected regions were accounted within eight threshold bins ($r > 0.6$, $r > 0.65$, $r > 0.7$, $r > 0.75$, $r > 0.8$, $r > 0.85$, $r > 0.90$ and $r > 0.95$).

Following a time-domain analysis application to assess network connectivity, we measured cross-frequency spectra (coherence) of DMN regions pairwise. The coherence spectra were calculated

using the mscohere function in MATLAB that calculates the magnitude-squared coherence of two regions.

Global complexity analysis

Recurrence Quantification Analysis

We measured the EEG signals' complexity using the recurrence quantification analysis (RQA) technique (73, 74). RQA is a useful tool to study non-linear signals such as EEG (75, 76). This is computed using the visual aspects of the structure called recurrence plots (RPs), which are graphical representations of time series when pairwise states of a system are neighbours in phase space (77). To analyse the complexity, diagonally and vertically aligned recurrence points in the RP are measured, characterising the temporal interdependences between individual observations of the phase-space trajectory (78, 79). Further, to estimate the correct embedding dimension, the false nearest neighbour algorithm (FNN) was applied (80). The RP parameters are embedding dimensions calculated by the false nearest neighbour technique similar to the former report (80), obtained from the first local minimum in mutual information trace according to the protocol of previous studies (81). 10% threshold was selected for fixed amount neighbours computation (82). The neighbourhood was also considered the amount of nearest neighbour points. In order to observe the recurrences of states here, we compute the T × T matrix using the following formula:

$$R_{i.j} = \Theta\left(\varepsilon - \left|x_i - x_j\right|\right), i, j = 1, \ldots, T$$

Where Θ is the Heaviside function, ε is a predefined threshold, and $|\cdot|$ means absolute value (73). To conduct $RP_{(i,j)}$, we choose a black colour to plot the points if $R_{i,j} = 1$, in the recurrent case, and white colour otherwise. The white and black points can generate different lines (vertical, horizontal, and diagonal), representing the properties of the underlying dynamics. According to

the lines and structures encountered in an RP, we applied L-mean, L-max, determinism (DET), entropy (ENTR), trapping time (TT) and network transitivity.

L-mean: indicates the average time that two segments of the trajectory (of the EEG signals) are close to each other, calculated with the following formula.

$$L_{mean} = \frac{\sum_{l=l_{min}}^{N} l.P(l)}{\sum_{l=l_{min}}^{N} P(l)}$$

Results of the L-mean can be interpreted as the mean prediction time (83). So, values of the L-mean have an inverse relationship with the complexity of the EEG signals.

L-max: Another RQA we measured is the length of the longest diagonal line found in the RP called L-max (84).

$$L_{max} = max\ (\{l_i\}_{i=1}^{N_l}),$$

Where $N_l = \sum_{l \geq l_{min}} P(l)$ is the all-around diagonal lines. Similar to the L-mean, this also has an inverse relationship with complexity.

Determinism (DET) is defined as a diagonal line length in the RP that corresponds to the time system changes very similar to during another time. Otherwise, a segment of the phase space trajectory runs parallel and within an ε-tube of another phase space segment of the trajectory (85).

$$DET = \frac{\sum_{l \geq lmin} lP(l)}{\sum_{i,j} R_{i.j}}$$

Uncorrelated processes or weakly correlated with stochastic behaviour showed very short diagonals, while deterministic processes had longer diagonals with fewer single and isolated recurrence points. Otherwise, the ratio of recurrence points that form diagonal structures to all recurrence points is introduced as a measure for the system's determinism. Therefore, more level of complexity indicates less value of determinism.

Entropy (ENTR): This index measures the entropy of the diagonal line lengths and is calculated.

$$ENTR = -\sum_{l=l_{min}}^{N} p(l) lnp(l)$$

Where p(l) is the probability of occurring a diagonal line of length l. ln(.) is the natural logarithm, and L_{min} is the shortest length of the diagonal lines (86). As shown by previous studies, ENTR of a periodic signal exhibits higher than stochastic time-series.

Trapping time (TT): TT indicates the mean length of a vertical line, according to the line of length generated in RP (75).

$$TT = \frac{\sum_{v=v_{min}}^{N} vP(v)}{\sum_{v=v_{min}}^{N} P(v)}$$

TT estimates the meantime that the system will abide at a particular state or how long it is trapped in a certain state. Therefore, the value of TT inversely indicates the complexity.

Transitivity (Cl): This denotes the average probability of two neighbours among any state that they are also neighbours and is given by:

$$CI = \frac{\sum_{i,j,k=1}^{T} A_{jk} A_{i,j} A_{ik}}{\sum_{i,j,k=1}^{T} A_{i,j} A_{ik}}$$

CI is a global measure of the underlying attractive set's effective dimensionality (87). Then, when probability increase, predictivity increased, and complexity decreased.

Fractal dimension analysis

Higuchi's fractal dimension (HFD): HFD is directly showed the fractal dimension of time-series in which the original time series is defined as:

$$X_m^k = X(m), X(m+k), X(m+2k), \dots X(m + int(\frac{N-m}{k}) \times k$$

Where N is the total number of the sample in time series, m showed the initial time, and k denotes the interval time. Accordingly, the length of the curve is computed as follows:

$$L_m(k) = \frac{\sum_{i=1}^{int(\frac{N-m}{k})} |X(m+ik) - X(m+(i-1)k| \times (n-1)}{k \times int\left[\frac{N-m}{k}\right]}$$

In this study, 10 s duration of the EEG and Kmax=6 was taken. We finally computed the average of all windows (85, 88).

This algorithm is developed from the concept that the stochastic signals are more fractal-like with and higher length L(k) than periodic time series.

Katz's fractal dimension (KFD): This algorithm is developed to calculate fractal dimension and complexity, as the distance between two successive points is calculated. That distance can be considered as a measure of the complexity in time-series (89). maximum distance from the first point is measured as d that computed as following:

$$d = \max(|x1 - xj|)$$

Where j =2, 3, … N

Then a total length of the time series taken as

$$L = \sum_{(i=2)}^{N} X_i - X_{i-1}$$

The average distance of two successive points is

$$a = \frac{L}{N-1}$$

Finally, Katz's fractal dimension measured as

$$FD = \frac{ln_a^L}{ln_a^d}$$

The optimum threshold of 10% was selected. The neighbourhood was also considered the amount of nearest neighbour points. In order to reduce multichannel recordings to a single measure corresponding to complexity parameters, we calculated the global value. We first computed each

RQA parameter per DMN region; then, the global values were defined as a mean of one parameter for all regions. It, therefore, corresponds to the average RQA parameters values per DMN region.

Statistical analysis

We used GraphPad Prism v6.0 (GraphPad Software, USA) for statistical analysis. The normality assessment was done employing both Shapiro-Wilk and Kolmogorov-Smirnov tests. A parametric t-test and repeated measures ANOVA followed by post hoc Bonferroni tests were performed to assess the significance of the difference between groups. Pearson's correlation was performed based on the distribution of the data. For demographic analysis, all results have been shown with a mean (standard error of the mean; SEM) or median (25% and 75% quartile). P-values less than 0.05 were considered statistically significant.

Results

Demographic characteristics

The Table shows the demographic and clinical data collected from fifteen patients. The median of ICU hospitalisation was 11 (range 7-16) days, and the median of duration in coma state was 9 (range 6-15) days median of GCS was 3 (range 3-9) points.

The nasal air-puff increased power of gamma

To characterise the influence of nasal airflow on human brain oscillations, we analysed EEG data from 17 electrodes associated with the DMN. First, we analysed the power spectral density (PSD) for all regions in all frequencies (from 0.2 to 100-Hz) to investigate whether the effect of nasal air-puffing is global or limited to a specific frequency range. This analysis showed that the majority

of the differences is focused in the gamma oscillations (30-100 Hz) in the DMN (see Supplementary Figure S2 for comparison across frequencies 0.2-100 Hz and Figure 2 for 30-100 Hz). We applied Welch's periodogram function to assess the absolute value of power in each region of DMN with nasal AP versus without AP for better discrimination. The gamma (30-100 Hz) power was significantly increased at dACC, rACC, vmPFC, PCC and Insula at the left side, dACC, PCC, rACC and vmPFC at the right side, and finally dmPFC (Figure 2 and Supplementary Figure S2).

Connectivity of DMN increased during AP

The signals acquired from electrode recording sites are represented as nodes based on graph theory, and the edges denote the correlation coefficient between nodes (Figure 3). We quantified the topology of the graphs by measuring global and local efficacy within eight incremental thresholded bins. We observed the main effect of AP on higher thresholded calculation in the 30-50 Hz frequency range (see Videos 2 and 3 in the Supplementary for 50-80 and 80-100 Hz). This result means AP may drive a notable change in highly correlated electrodes (Figure 3).

The different frequency range of gamma from low to high has been assumed to reflect different brain functions (90). Hence, we investigated the relative impact of AP on different frequency ranges of gamma. We computed the correlation coefficient between brain regions across the low (30-50 Hz), middle (50-80 Hz), and high gamma (80-100 Hz). As shown in Figure 4A, AP increased the correlation of DMN pairwise regions (Supplementary Figure S3-S5 shows absolute values). Notably, this effect, provided by AP, increased concomitantly with the gamma frequency range. Instead of selecting the fixed point to compare highly correlated vs moderately correlated regions, we constructed a connectogram thresholded from >0.6 to >0.95 (Figure 4B indicates 30-

50 Hz range; see Supplementary Figure S6 for middle and high gamma frequency ranges). In this line, a repeated-measures ANOVA on the number of connections within eight correlation thresholds and frequency ranges revealed that high gamma (80-100 Hz) was more affected than low and middle gamma. Moreover, in the middle gamma band (50-80 Hz), number of the connections significantly increased compared to lower gamma (30-50 Hz; $F=20.5$, $p<0.0001$; Figure 4C). This suggests that AP has a more noticeable influence on high gamma than low gamma oscillation (for more details, see Supplementary Videos 1-3).

We further asked whether these phenomena were restricted to time-domain analysis. One way to answer this question is to calculate coherence, indicating cross-frequency spectra between signals (91). Consistent with our observations in time domain analysis, we found that AP significantly increased brain regions' coherence in DMN (Figure 5). Additionally, AP seems to have a different effect on the absolute value of coherence among low, middle, and high gamma oscillations (see Supplementary Figures S7-S9). As shown in Figure 5, during the air-puffing number of significantly heightened DMN regions compared to nAP, the p-value in 80-100 (Hz) was more significant than middle and low gamma subbands (50-80 and 30-50 respectively). In this respect, we note that AP induces more coherent interactions between DMN regions, particularly in higher gamma bands.

AP increased complexity EEG signals

To further explore EEG features reflecting a higher level of brain activity, we measured brain signals' complexity using RQA. This analysis constructs a 2D matrix according to this time series's recurrences of signal states (Figure 6). We then quantified the black points in the RP matrix, which indicates the similarity of two points in the signals' trajectory (for more details, see global

complexity analysis in the Method section). One way to evaluate RQA method complexity is to measure the longest (L_{max}) length and the average of diagonal lines (L_{mean}). In this case, we observed that both L_{max} and L_{mean} significantly decreased in AP, indicating a higher complexity level than the nAP state. We also established another approach using transitivity that indicates the average probability of two neighbours among any state of the neighbourhood. A significant reduction in transitivity (indicating higher complexity) during AP was observed. Our finding showed that EEG signals during the AP have a significantly higher global complexity. DET was also computed, indicating the ratio of recurrence points forming diagonal structures to all recurrence points. We found that AP reduces the ratio of diagonal structures as regularity parameters (i.e., more complex time series represents less ratio of diagonal lines). Furthermore, according to the result of ENTR that measures lengths of the diagonal line, AP is shown to reduce it, indicating further complexity compared to nAP. Finally, we calculated TT that estimates how long a system is trapped in a certain state. This analysis illustrated that AP causes DMN signal TT estimates not to abide at a particular state, i.e., increasing complexity.

Our analysis in Higuchi and Katz fractal dimension in line with RQA measures confirms that AP increases the DMN signals' complexity (Supplementary Figure S10).

Discussion

Our findings demonstrated that puffing air into the nasal cavity of mechanically ventilated comatose patients at a frequency range similar to regular respiration (i.e., 0.2 Hz) increases the activity of gamma oscillations (30-100 Hz) in DMN regions. Moreover, we identified that AP could affect connectivity between DMN regions in gamma frequency. This effect was more

remarkable in high-gamma frequencies. Finally, the EEG data showed that the gamma range's brain oscillation complexity increased when comatose patients received AP.

There is evidence suggesting that different modalities of external stimulation, such as visual, acoustic, nociceptive, and electrical current stimulation, can promote gamma activity in comatose patients (54, 92, 93). For example, Cavinato et al. reported electrophysiological changes following simple visual, acoustic, and noxious stimulations in parietal and frontoparietal regions within the gamma (30-50 Hz) band of healthy and minimally conscious subjects, but not in the vegetative state group (7). In this study, we suggested a novel method to increase the brain activity in mechanically ventilated comatose patients: patients had marked increase gamma oscillations (30-100 Hz) activity in the AP condition than the nAP condition. A more robust response was observed at higher frequencies.

Gamma activity has been proposed to play a fundamental role in information integration throughout the brain during wakefulness (13, 15, 94). There have been controversies regarding the necessity of gamma activity for consciousness (95, 96), suggesting that it may be more closely associated with selective attention rather than conscious experience (95). However, it has been shown that emerging from unconscious state to consciousness is associated with frontal-parietal coherence increment in high-gamma, emphasising the role of gamma in information transfer (96). Meanwhile, gamma power is lower in patients with consciousness disorders (11). In addition to power, the cortical oscillations connectivity in the gamma band is also associated with loss of consciousness (12, 97, 98). The brain connectivity in the gamma range provides a tool for determining brain states from awareness to deep coma in patients with consciousness disorder (7). Therefore, one key aspect of this study is that we suggested an innovative method to modulate the brain reactivity of comatose patients.

Another strength of our study is inaugurating the ability of nasal air-puffing on integrating DMN regions dynamics. Connectivity of different brain areas within DMN has been suggested as an indicator of the level of consciousness, which can help to differentiate minimally conscious patients from patients in vegetative and coma states (99). Multiple oscillatory processes have been linked to the DMN regions (100, 101). In this regard, the DMN activity is associated with an increase in gamma oscillation (101), related to awareness (102, 103). On the other hand, some earlier reports have suggested a disruption in DMN functional connectivity in comatose patients. For instance, Norton et al. observed that the connectivity between DMN regions in comatose patients who later emerged from coma can be detected, but such connectivity was absent in patients with irreversible coma (31).

In the present study, the number of correlated connections and connectivity patterns after stimulation with nasal air-puffing suggests a marked increase in global interaction among DMN regions in the gamma range. We presumed that the causative driver might be located in the olfactory sensory neurons (OSNs). Neural projections from OSNs are anatomically and functionally connected to areas comprising the DMN (54). Stimulating OSNs by airflow passage during natural nasal breathing generates oscillations that propagate throughout the brain called respiration-entrained rhythms (RRs) (54, 104). These oscillations are eliminated by olfactory bulb inhibition or when breathing root switches from nose to mouth (53, 57, 105). Consistently, it has been shown that the olfactory bulb stimulation can increase gamma activity in neocortical areas (106, 107). Taken together, we established a method in which nasal air-puffing increased the DMN connectivity. The olfactory system and OSN anatomical and functional association provide the possibility to stimulate the brain by a non-invasive approach like nasal air-puffing.

In line with past research, the coherence and time-domain correlation analyses showed that air-puffing-entrained high-frequency gamma is stronger than low-frequency gamma (80-100 Hz > 50-80 Hz > 30-50 Hz). Kay showed that high-gamma activity (65-100 Hz) rather than low-gamma (35-65 Hz) is correlated with the peak of inhalation (108). Zhong et al. also showed greater phase-locking of RR with high-gamma (80-120 Hz) compared to low-gamma (40-80 Hz) (109).

Moreover, we observed that brain oscillatory activities in comatose patients were more complex during nasal air-puffing. Complexity features analysis can be applied to differentiate the state of the brain (28, 29). For instance, EEG dynamic complexity is higher during wakefulness than anaesthesia (28). Previous studies also observed less complexity and irregularity of brain waves activity in comatose patients (28, 29, 110). Accordingly, our findings align with this evidence and support our hypothesis that nasal air-puffing seems to change brain signals into a more activated state.

As this study was designed for proof-of-concept regarding our hypothesis, and the results are preliminary, it faced several limitations. First, we performed this study in a cross-sectional manner. Therefore, our results should not be used to interpret outcomes of comatose patients under MR. To explore the long-term effects of our approach, longitudinal studies with larger sample sizes, more groups and thorough follow-ups both on cognitive and non-cognitive functionalities are needed. Second, we were allowed to experiment for a limited period, considering the patients' critical condition and ICU safety protocols. Since our results were promising, this approach should be applied for more extended periods and intervals to find their effect on the brain reactivity over a longer time scale. Third, we only enrolled patients in a coma due to opium toxicity; applying this approach on patients with other causes of non-traumatic coma can help to elucidate its generalizability and utility in this setting.

We provide several directions for future studies to elucidate the utility and also benefits of our approach. Firstly, our findings were observed only at the gamma range; these effects might depend on nasal air-puff features such as rate or duration of stimulation. Therefore, stimulation with a different strenght and frequency may provide a better understanding regarding its effect on other frequency bands. Secondly, the effects of nasal air-puffing stimulation should be investigated on the clinical outcome, particularly neurologic and cognitive function after coma and more extended periods after recovery. Although our results provide preliminary evidence for possible application of this method in clinical settings, further research is needed to clarify its effects and consequences on these patients deprived of nasal airflow. Moreover, more electrodes are needed to elucidate the accurate source localisation and connectivity across the sub-cortical regions and brain networks.

Conclusion

We investigated the possibility of modulating brain activity in comatose patients via sensory stimulation. We found that OSNs stimulation increases activity, connectivity, and complexity in multiple brain areas comprising DMN at the gamma band (30-100 Hz). Our findings enhance the application of a non-invasive and painless stimulation that can potentially counteract MR's adverse effects on cognition. Altogether, these effects can potentially lead to a better prognosis and faster recovery after a coma.

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Authorship statement

Morteza Salimi, Conceptualization, Data gathering, and analysis, Investigation, Visualisation and Writing; Amir-Homayoun Javadi, Data analysis, Visualisation, Writing and editing; Milad Nazari, Methodology, Data analysis; Sobhan Bamdad, Methodology, Data analysis; Farhad Tabasi, Writing - review and editing; Tannaz Parsazadegan, Data gathering; Fahime Ayene, Data gathering; Maede Karimian, Data gathering; Leila Gholami-Mahtaj, Visualization; Shahin Shadnia, Resources; Hamidreza Jamaati, Funding acquisition; Alireza Salimi, Supervision, Funding acquisition, Validation; Mohammad Reza Raoufy, Conceptualization, Methodology, Supervision, Project administration and Writing.

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Figure legends:

Figure 1. Electrode placement for EEG acquisition. (Left) Schematic arrangement of 17 electrodes corresponding to the default mode network (DMN). Each colour demonstrates one region of DMN, made by clustering of two or more electrodes. (Right) Raw signal sample from 17 electrodes. Blue and red represent brain waves during nAP and AP conditions, respectively; AP, air-puff; nAP, non-air-puff; dACC, dorsal anterior cingulate cortex; rACC, rostral anterior cingulate cortex; dmPFC, dorsomedial prefrontal cortex; vmPFC, ventromedial prefrontal cortex; PCC, posterior cingulate cortex.

Figure 2. The effect of nasal air-puff on power in the default mode network (DMN). Each plot demonstrates the comparison between mean spectral power in nAP (blue) and AP (red) groups at the gamma frequency range (30-100 Hz) in ten regions of DMN. Grey areas in plots display statistically significant differences between nAP and AP ($p < 0.05$), analysed by paired-sample t-tests. Error bars represent one SEM. PSD, power spectral density; AP, air-puff; nAP, non-air-puff; dACC, dorsal anterior cingulate cortex; rACC, rostral anterior cingulate cortex; dmPFC, dorsomedial prefrontal cortex; vmPFC, ventromedial prefrontal cortex; PCC, posterior cingulate cortex.

Figure 3. The effect of nasal air-puff on the connection between nodes. (A) The upper section shows a representative EEG epoch extracted to calculate graph theory parameters during nAP (blue) and AP (red). The middle section demonstrates functional connectivity matrices using the Pearson's correlations, in which each pixel indicates the correlation between the signal of pairwise channels. In the AP group, the correlation between channels was significantly increased, indicating

higher connectivity among pairwise regions. As the bottom section illustrates, following thresholding, the remaining edges constructed graphs based on graph measurements, which demonstrated higher connectivity between nodes in the AP group **(B)** network parameters, were compared in eight incremental thresholds. AP increased network connectivity parameters (global efficiency and local efficiency) compared to nAP. Data were analysed using paired-sample t-tests. Error bars represent one SEM.* p<0.05, **p<0.01, *** p<0.001, **** p<0.0001. AP, air-puff; nAP, non-air-puff; AU, arbitrary unit.

Figure 4. The effect of air-puff on connectivity between pairs of regions. (A) 2D histogram (cell plot) of cross-correlation differences between pairwise DMN regions of nAP and AP groups in three gamma subbands. Each cell represents the p-value from the comparison of a pair of regions between AP and nAP groups. Darker colours indicate a lower p-value (p<0.05, which is considered statistically significant, is shown in dark brown). These plots demonstrate that more cross-correlation between a pair of regions can occur in a higher gamma range (80-100 Hz > 50-80 Hz > 30-50 Hz). Data were analysed by paired-sample t-test. **(B)** Circular connectogram analysis using Pearson's correlation with eight incremental thresholds in 30-50 Hz frequency range. AP strongly increased the number of connections between different brain regions. See Supplementary Figures 6 for 50-80-Hz and 80-100-Hz. **(C)** Depict the number of connections within incremental thresholds. The number of connections in the AP group was higher in higher frequency ranges (80-100 Hz > 50-80 Hz > 30-50 Hz). Data were analysed using ANOVA followed by Bonferroni correction for multiple comparisons. †† p<0.01, ***, ### and † † † p<0.001, ****, #### and † † † † p<0.0001. Error bars represent one SEM. AP, air-puff; nAP, non-air-puff; L, left; R, right; AP, air-puff; nAP, non-air-puff; dACC, dorsal anterior cingulate cortex; rACC, rostral anterior

cingulate cortex; dmPFC, dorsomedial prefrontal cortex; vmPFC, ventromedial prefrontal cortex; PCC, posterior cingulate cortex; Thr, threshold

Figure 5. the effect of nasal air-puffing on the coherence of DMN connectivity. (Top) Visualisation of functional connectivity within three gamma subbands. The coherence between brain regions increases in higher gamma ranges after nasal air-puffing, compared to the nAP group (the coherence between regions is thresholded based on $p<0.05$). **(Bottom)** 2D histogram (cell plot) of coherence differences between pairwise DMN regions of nAP and AP groups in three gamma subbands. Each cell represents the p-value from the comparison of a pair of regions between AP and nAP groups. Darker colours indicate a lower p-value ($p<0.05$, which is considered statistically significant, is shown in dark brown). These plots demonstrate that more coherence can occur between a pair of regions in a higher gamma range (80-100-Hz > 50-80-Hz > 30-50-Hz). Data were analysed by paired-sample t-test. Error bars represent one SEM. AP, air-puff; nAP, non-air-puff; dACC, dorsal anterior cingulate cortex; rACC, rostral anterior cingulate cortex; dmPFC, dorsomedial prefrontal cortex; vmPFC, ventromedial prefrontal cortex; PCC, posterior cingulate cortex; Thr, threshold.

Figure 6. the effect of nasal air-puffing on EEG signal complexity. (Top) A representative example of the 2D matrix generated using the recurrence plot method with embedding dimension m=6 (calculated by the false nearest neighbours' algorithm), time delay t=9 (calculated by mutual information), and threshold r=10% (using a constant neighbour's value) in nAP and AP groups. **(Bottom)** EEG complexity values were averaged in all regions. AP strongly increased complexity parameters across all three gamma subbands (lower values of transitivity, L_{max}, L_{mean} recurrent

time, determinism, entropy and trapping time indicates more complexity of the signals). Data were analysed by paired-sample t-test. * $p<0.05$, **$p<0.01$, *** $p<0.001$ and **** $p<0.0001$. Error bars represent one SEM. AP: air-puff; nAP: non-air-puff; AU: arbitrary unit.

Figure S1. Independent component analysis example. ICA determines. (A) signal of one sample before (left) and after conducting the ICA **(B)** Topographic plot showing the component values across the scalp. **(C)** Decomposition of the signal in the trial of 420 (ms). (D) Power spectrum of the component. In this example, ICA detected non-cortical activity close to channel O2 channel.

Figure S2. Power spectral density in the default mode network (DMN). Panels display the mean spectral power of nAP (blue) and AP (red) among the regions of the DMN at 0.2 to 100 Hz. Line denotes mean, and shading area represents SEM. Grey areas display significant differences between nAP and AP. Data was analyzed by paired-sample t-tests; PSD: Power spectral density; AP: air-puffing; nAP: non-air-puff; dACC: dorsal anterior cingulate cortex; rACC: rostral anterior cingulate cortex; dmPFC: dorsomedial prefrontal cortex; vmPFC: ventromedial prefrontal cortex; PCC: posterior cingulate cortex.

Figure S3. Pairwise correlation between DMN areas in 30-50 Hz. Blue and red horizontal lines indicate the mean of correlation in nAP and AP, respectively. The Green area displays the significant difference between nAP and AP. Data were analysed by paired t-test. nAP: non-air-puff; AP: air-puffing; dACC: dorsal anterior cingulate cortex; rACC: rostral anterior cingulate

cortex; dmPFC: dorsomedial prefrontal cortex; vmPFC: ventromedial prefrontal cortex; PCC: posterior cingulate cortex. The shaded area represents one SEM.

Figure S4. Pairwise correlation between DMN areas in 50-80 Hz. Blue and red horizontal lines indicate the mean of correlation in nAP and AP, respectively. The Green area displays the significant difference between nAP and AP. Data were analysed using paired-sample t-tests. nAP: non-air-puff; AP: air-puffing; dACC: dorsal anterior cingulate cortex; rACC: rostral anterior cingulate cortex; dmPFC: dorsomedial prefrontal cortex; vmPFC: ventromedial prefrontal cortex; PCC: posterior cingulate cortex. Shaded areas represent one SEM.

Figure S5. Correlation between DMN pairwise in 80-100 Hz. Blue and red horizontal lines indicate the mean of correlation in nAP and AP, respectively. The Green area displays the significant difference between nAP and AP. Data were analysed using paired-sample t-tests. nAP: non-air-puff; AP: air-puffing; dACC: dorsal anterior cingulate cortex; rACC: rostral anterior cingulate cortex; dmPFC: dorsomedial prefrontal cortex; vmPFC: ventromedial prefrontal cortex; PCC: posterior cingulate cortex. The shaded area represents one SEM.

Figure S6. Circular connectogram analysis using Pearson's correlations with eight incremental thresholds in (left) 50-80 Hz and (right) 80-100 Hz frequency ranges.

Figure S7. Pairwise coherence between DMN areas in 30-50 Hz. Blue and red horizontal lines indicate the mean of coherence in nAP and AP, respectively. The Green area displays the significant difference between nAP and AP. Data were analysed using paired-sample t-tests. nAP:

non-air-puff; AP: air-puffing; dACC: dorsal anterior cingulate cortex; rACC: rostral anterior cingulate cortex; dmPFC: dorsomedial prefrontal cortex; vmPFC: ventromedial prefrontal cortex; PCC: posterior cingulate cortex. The shaded area represents one SEM.

Figure S8. Pairwise coherence between DMN areas in 50-80 Hz. Blue and red horizontal lines indicate the mean of coherence in nAP and AP, respectively. The Green area displays the significant difference between nAP and AP. Data were analysed using paired-sample t-tests. nAP: non-air-puff; AP: air-puffing; dACC: dorsal anterior cingulate cortex; rACC: rostral anterior cingulate cortex; dmPFC: dorsomedial prefrontal cortex; vmPFC: ventromedial prefrontal cortex; PCC: posterior cingulate cortex. The shaded area represents one SEM.

Figure S9. Pairwise coherence between DMN areas in 80-100 Hz. Blue and red horizontal lines indicate the mean of coherence in nAP and AP, respectively. The Green area displays the significant difference between nAP and AP. Data were analysed using paired-sample t-tests. nAP: non-air-puff; AP: air-puffing; dACC: dorsal anterior cingulate cortex; rACC: rostral anterior cingulate cortex; dmPFC: dorsomedial prefrontal cortex; vmPFC: ventromedial prefrontal cortex; PCC: posterior cingulate cortex. The shaded area represents one SEM.

Figure S10. Fractal dimension (FD) is computed as feature complexity. Katz and Higuchi's fractal dimension were calculated as an indicator of complexity according to the protocol of previous studies (Accardo et al. 1997). Here, 10 s duration with 75% overlap and KMax=2 for HFD was taken. We finally computed the average of all windows. Similar to our finding in RQA

parameters, FD indexes showed an enhancement of complexity during nasal air-puff. Data were analysed by paired-sample t-tests; AP: air-puffing; nAP: non-air-puff; AU, arbitrary unit.

Video 1. Interaction between network nodes within incremental thresholds (from r>0.95 to r>0.6) in 30-50 Hz. Circles represent correlated nodes (electrodes). Correlated edges are shown with blue lines for nAP and red for AP. nAP: non-air-puff; AP: air-puffing.

Video 2. Interaction between network nodes within incremental thresholds (from r>0.95 to r>0.6) in 50-80 Hz. Circles represent correlated nodes (electrodes). Correlated edges are shown with blue lines for nAP and red for AP. nAP: non-air-puff; AP: air-puffing.

Video 3. Interaction between network nodes within incremental thresholds (from r>0.95 to r>0.6) in 80-100 Hz. Circles represent correlated nodes (electrodes). Correlated edges are shown with blue lines for nAP and red for AP. nAP: non-air-puff; AP: air-puffing.