Slow-paced inspiration regularizes alpha phase dynamics in the human brain

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Abstract

The phase of low-frequency, rhythmic cortical activity is essential for organizing brain processes because it provides a recurrent temporal frame for information coding. However, the low-frequency cortical phase exhibits great flexibility in response to external influences. Given that brain rhythms have been found to track respiratory inputs, we hypothesized that slow breathing, commonly associated with mental regulation, could reorganize the relationship between these two rhythmic systems through the adjustment of the cortical phase to such a slow train of inputs. Based on simultaneous magnetoencephalography and respiratory measurements, we report that while participants performed paced breathing, slow relative to normal breathing modulated cortical phase activity in the alpha range across widespread brain areas. Such modulation effects were specifically locked to the middle of the inspiration stage and exhibited a well-structured pattern. At the single-subject level, the phase angles underlying the effects became more likely to be diametrically opposed across breaths, indicating unique and consistent phase adjustment to slow inspiratory inputs. Neither cardiac fluctuations nor breathing-unrelated task effects could account for the findings. We suggest that slow-paced inspiration could organize the cortical phase in a regularized phase pattern, revealing a rhythmic but dynamic neural network integrated with different neurophysiological systems through volitional control.

Keywords: breathing; MEG; oscillation; phase; alpha

Significance Statement

Breathing is more complicated than a simple gas exchange, as it is integrated with numerous cognitive and emotional functions. Controlled slow breathing has often been used to regulate mental processes. This magnetoencephalography study demonstrates that slow-paced relative to normal-paced inspiration could organize the timing of alpha rhythmic activities across breathing cycles in a structured manner over widespread brain areas. Our results reveal how a volitionally controlled change in respiratory behavior could systematically modulate cortical activity.

Introduction

The phase of rhythmic cortical activity, particularly in the theta (4-8 Hz) and alpha (8-14 Hz) ranges, is central to organizing brain processes (Sauseng and Klimesch 2008; Schroeder and Lakatos 2009; Thut et al. 2012; vanRullen 2016; Varela et al. 2001). The gating of neural transmission may be mediated by phase (Schroeder and Lakatos 2009; vanRullen 2016). In this view, cyclic phases reflect the fluctuations of cortical excitability such that events that coincide with the phases indexing high excitability are amplified and vice versa. Phase is also involved in mediating neural communication such that communication is facilitated when the phases of two neuronal assemblies are in synchrony (Sauseng and Klimesch 2008; Varela et al. 2001). All of these functions are construed as reflecting the common principle that rhythmic phases act as a temporal frame for information coding. One of the key features of such phase coding is its flexible dynamics. Accumulating evidence has shown that rhythmic phase activity can be adjusted according to bottom-up, stimulus-driven inputs (Luo and Poeppel 2007; Spaak et al. 2014) or top-down, expectation- or attention-related inputs (Lakatos et al. 2008; Stefanics et al. 2010). In general, these phenomena are manifested in terms of phase adjustment, as indexed by consistent, event-related phase clustering towards a certain direction, in response to a train of extrinsic and intrinsic inputs (Sauseng and Klimesch 2008; Thut et al. 2012; Voloh and Womelsdorf 2016).

Although the interaction between the brain and the physiological systems has been documented (Critchley and Garfinkel 2018; Varga and Heck 2017), it remains unclear

whether and how bodily inputs contribute to the shaping of cortical phase dynamics. Breathing is one of the vital rhythms of human life and constitutes one possible source that may influence ongoing, low-frequency phase dynamics. First, breathing may create rhythmic inputs to the human brain directly either via the mechanical or thermal sensation of airflow through the nasal cavity or via interoceptive signals from the respiratory system through the brainstem (Del Negro et al. 2018; Heck et al. 2017; Lorig 2007). In addition, although breathing occurs effortlessly and without thought, it is exquisitely coordinated with a multitude of cognitive and emotional functions (Arsenault et al. 2013; Flexman et al. 1974; Perl et al. 2019; Zelano et al. 2016). Furthermore, recent animal and human studies have reported that natural breathing can drive rhythmic brain activity across multiple brain areas, prominently in the olfactory cortex, the frontal cortex and the subcortical structure (Herrero et al. 2018; Ito et al. 2014; Tort et al. 2018; Zelano et al. 2016).

Notably, the pace of breathing is not fixed. In particular, slow breathing has been associated with mental calmness and is therefore thought to provide a health benefit (Boiten et al. 1994; Homma and Masaoka 2008; Kreibig 2010). Indeed, because breathing can be partly brought under voluntary control, conscious pacing of breathing at a slow rate is often embraced by meditative techniques to regulate cognitive and emotional states (Lutz et al. 2004; Paul et al. 2013; Zeidan et al. 2010). Therefore, given that brain and respiratory rhythms are highly correlated, we hypothesized that a slow breathing pace could reorganize the relationship between these two rhythmic systems. The process might be manifested as an adjustment of the

low-frequency cortical phase to such a slow train of respiratory inputs. Through simultaneous respiration and magnetoencephalography (MEG) measurements, the current study tested our hypothesis by instructing participants to breathe at a slow (0.125 Hz, below the normal range: 0.2 – 0.3 Hz (Barrett et al. 2010)) or a normal pace (0.25 Hz, within the normal range). By systematically manipulating the interplay between the brain and respiratory systems in a controlled manner, we investigated whether different breathing paces led to differential phase adjustment.

Materials and Methods

Participants. Fifteen right-handed participants without previous neurological or psychiatric history were enrolled in this study (12 males and 3 females, mean age ± SD = 26.27 ± 3.22 years, range = 22 – 32). All participants had normal or corrected-to-normal vision and provided written informed consent. All procedures were approved by the ethics committee of National Taiwan University. Because no prior similar study had been conducted, this number of participants was determined on the basis of previous parallel research on mind-body interaction (Critchley and Garfinkel 2018). Despite this, the effect size of the group-level result was large (Cohen's d for each point within the significant cluster: mean ± SD = 1.03 ± 0.25, range = 0.43 – 2.17), and the subsequent single-subject significant findings during slow breathing were consistently observed for every participant. Accordingly, we suggest that the robustness of the results can be justified.

Task. Participants performed paced breathing by following the cues ("|" or " - ") at the center of the screen, which indicated the onset of expiration or inspiration. The alternation rate between the two cues was 2 s or 4 s, resulting in a regular breathing rate of 0.25 (4 s/breath) or 0.125 Hz (8 s/breath) in the normal- and slow-breathing conditions, respectively. Each condition consisted of two runs, and each run lasted 5 min, with an approximately 1-min break between the runs. The cue types and the orders of the conditions were randomized across participants. Before each experiment, participants were acquainted with the procedure with a practice session and instructed to breathe through their noses because evidence has noted the importance of nasal breathing on cortical activity (Zelano et al. 2016). Their breathing conditions were monitored through a video camera located inside a magnetically shielded room.

Data recordings. MEG recordings were performed using a 306-channel whole-head MEG system (Elekta Neuromag TRIUX) with a sampling rate of 1000 Hz. Several physiological signals were simultaneously acquired. Eye-related activities were monitored via vertical and horizontal electrooculography (EOG). Electrocardiography (ECG) electrodes were placed over the chest close to the left and right clavicles. Respiratory activity was obtained via a respiratory belt positioned around the chest at the level of the armpits (respiratory transducer TSD201 BIOPAC system) and low-pass filtered (10 Hz) online. These signals were connected to and synchronized via the MEG acquisition system. All recorded data were subsequently analyzed using the FieldTrip toolbox (Oostenveld et al. 2011) in combination with MATLAB

(MathWorks) and R software (http://www.R-project.org). The data that support the findings of this study are available on request from the corresponding author.

Head movement. To ensure that the distinct patterns of chest movements between the inspiratory and respiratory phases did not propagate to the head and in turn bias the results, we continuously monitored the participants' head positions relative to the MEG sensors using a set of head localization coils placed at the nasion and the left and right ear canals. The results of the head movements, including the displacements and rotation angles of head positions along the x, y or z axes, were estimated using the Maxfilter software (Elekta Neuromag). Because the sampling resolution (1 Hz) of the continuous head positions was limited, the results of each head position parameter were averaged across the entire expiratory or inspiratory period. For every head-motion parameter, no significant difference was found between the inspiratory and expiratory periods in each condition or between the slow-breathing and normal-breathing conditions in each period (two-tailed paired t-test, all $t(14)s \leq 1.86$, all $ps \geq 0.09$).

Data preprocessing. The first 30 s of the data were discarded from the analysis to ensure steady breathing. Breathing cycles were estimated by detecting the intervals between two peaks of inspiration (i.e., one breath). Continuous MEG data were segmented according to the breathing cycles but extended from 1 s before to 1 s after the cycle period to avoid edge effects during spectral analysis. Trials contaminated

with muscular artifacts were visually identified and rejected. Eye movements, eye blinks, and cardiac artifacts were removed using independent component analysis implemented in the FieldTrip toolbox (3-4 components removed). To minimize the contributions of signal noise, we considered only the trials with durations less than 0.75 SD from the median. To ensure that participants followed the cues indicating inhalation and exhalation, we imposed an additional constraint by excluding the trials in which the mean inspiration and expiration onsets were more than 2 SD from the cues.

Respiratory and ECG analysis. To characterize breathing rates, we performed a fast Fourier transform analysis to calculate the power spectrum of the continuous respiratory signals. The dominant breathing frequency, as manifested by peak power, was determined in each participant. To ensure that the participants breathed in a normal manner without holding their breath during the slow-breathing condition, we computed the first derivative of the respiratory signal. If the breath was held, the respiratory signal fluctuated around a horizontal line, yielding multiple zero crossings in the curve derived from the calculated first derivative. However, for individual trials and participants, only three zero crossings were observed that were derived from two peaks of inspiration and one peak of expiration, indicating that participants did not significantly change their breathing behavior because of the slow pace. Heartbeat data (QRS complex) were extracted from the ECGs utilizing the Pan-Tompkins detection

algorithm (Pan and Tompkins 1985). Two participants were removed from the ECG analysis due to excessive noisy signals.

Time-frequency representations of respiration-locked MEG data. The instantaneous amplitude and phase at each sensor-time-frequency point were extracted using the Hilbert transform. Before the transform, the data were bandpass filtered (finite impulse response (FIR) filter, filter order dependent upon frequency band and data length (default setting in FieldTrip)) to create 13 frequency steps, with center frequencies from 2 to 14 Hz and bandwidths of 1 Hz.

Unity-based time normalization of respiration-locked MEG data. Given varying lengths of respiration-locked MEG trials within each condition and between conditions, we remapped the time course of each trial onto a common time scale that described the course of a breathing cycle to facilitate the subsequent phase-coherence computation and between-condition comparison. Unity-based time normalization was performed for the corresponding expiratory or inspiratory period of every trial within individual participants and conditions:

$$T_{normalized}(n,s,f) = \frac{T(n,s,f) - T_{\frac{EX}{IN}onset}(n,s,f)}{T_{\frac{EX}{IN}offset}(n,s,f) - T_{\frac{EX}{IN}onset}(n,s,f)},$$

where $T_{normalized}$ denotes a given normalized time point for each trial *n*, sensor *s*, and frequency *f*. T, $T_{EX/IN\ onset}$, and $T_{EX/IN\ offset}$ denote the original time value, the original time value during expiration or inspiration onset and the original time value during expiration or inspiration offset, respectively. As a result, the expiratory period

211 was scaled into the range [0 1], whereas the inspiratory period was scaled into the
212 range [1 2] after adding a constant value of one.
213
214 **Phase-coherence analysis.** To detect phase adjustment in brain signals, we
215 calculated inter-trial phase coherence (ITC), a classical approach that has been
216 commonly used (Tallon-Baudry et al. 1996). However, to mitigate sample-size bias
217 while comparing slow- and normal-breathing conditions, we computed the cosine
218 similarity version of phase coherence (ITC_{CS}) to quantify the concentration of phase
219 clustering across multiple repetitions of the trials locked to the breathing cycle. ITC_{CS}
220 represents the mean cosine of the angles of all phase pairs from any two trials θ_i, θ_j
221 (Chou and Hsu 2018). This metric produces almost exactly the same pattern of results
222 as the classical ITC analysis when the sample sizes in two given conditions are similar,
223 and it approximates the results of bootstrapping at a lower computational cost when
224 the sample sizes are different. For a given sensor *s*, frequency *f*, time *t*, and a total
225 number of trials *N*,

$$ITC_{cs}(s,f,t) = \frac{2}{N(N-1)} \sum_{i=1}^{N-1} \sum_{j=i+1}^{N} \cos(\theta_i - \theta_j).$$

226 An ITC_{CS} close to 1 reflects strong phase clustering (i.e., all trials exhibit the same
227 phase). A small or negative ITC_{CS} reflects low phase coherence, which indicates that
228 either the distribution of phase angles across trials is uniform or a proportion of the
229 phase vectors are distant from each other as two diametrically opposed vectors have a
230 similarity of -1.
231

Source localization. To localize the significant effects obtained from the sensor-level phase-coherence analyses, we used a linear constrained minimum variance (LCMV) algorithm (Bardouille and Ross 2008). We first coregistered the brain surface from participants' individual segmented MRIs with a single-shell head model. Montreal Neurological Institute (MNI)-aligned grids were then created in each subject's individual head space by warping each individual MRI to a template MRI in MNI coordinates and applying the inverse of this transformation matrix onto the template dipole grid. With this procedure, a consistent mapping of the spatial positions of grid points was achieved across participants. For each condition, the covariance matrix was derived from the bandpass-filtered raw signal using every data point in all the trials and the information from both the planar gradiometer and the magnetometer sensors. The lower and upper cut-off frequencies of this time-domain filter were 8 Hz and 14 Hz (FIR filter) to suppress the noise that was outside the frequency range of interest (see (Wutz et al. 2014) for a similar procedure). The LCMV beamformer was then applied to determine the weighting function that estimated the source activity on the basis of the covariance matrix. Next, we applied the weighting function to the Hilbert-transformed MEG data within the significant cluster window identified at the sensor level to calculate phase coherence in source space. This source activity was then projected onto the individual MNI-aligned grids. Anatomical structures corresponding to the localized sources were identified using the Automated Anatomical Labeling (AAL) brain atlas (Tzourio-Mazoyer et al. 2002). Source-level

comparisons were calculated using paired t-tests with Benjamini-Hochberg false discovery rate (FDR) correction.

Power analysis. To compensate for the 1/f decay in power, the choice of the baseline period for normalization is nontrivial, particularly in the current setting, because all the data points were task-related and there was no so-called pretrial period for defining a baseline. Therefore, Z-normalization using the entire trial period as a baseline was conducted after power was averaged across trials in each experimental condition for each participant. In the equation

$$P^z(s,f,t) = \frac{P(s,f,t) - \bar{P}(s,f)}{\sigma(s,f)},$$

P^z denotes the Z-normalized power activity for each sensor s, frequency f and time t; P denotes the original activity, and $\bar{P}$ and σ denote the mean power and the standard deviation, respectively, of all time points.

Cluster-based permutation test. To determine whether the data differed significantly between conditions, we conducted cluster-based permutation tests implemented in FieldTrip. This statistical test does not require specific assumptions about the shape of the population distribution, and it controls for the problem of multiple comparisons. In these tests, the conditional differences were quantified by means of paired t-tests for every sensor-time-frequency sample. The samples with t values exceeding the threshold ($p < 0.05$, two-tailed) were clustered in connected sets based on spatial, temporal or frequency adjacency. The cluster with the maximum sum of t values was

used as a test statistic. A distribution was then generated by randomly permuting the data across the conditions for each participant and recalculating the test statistic using a Monte Carlo estimate after repeating 1000 times. Finally, two-tailed *p*-values were determined by evaluating the proportion of the distribution resulting in a test T statistic larger than the observed T statistic.

Surrogate data. To confirm that our results were locked to the middle of the inspiration phase, we surrogated the slow-breathing data by adopting a cut-and-swap strategy to minimize the distortion of phase dynamics (Aru et al. 2015). Specifically, during the slow-breathing condition, we randomly selected a single time point and exchanged the resulting two sections of data in each MEG trial. Next, for every data point within the significant cluster window, a surrogate ITC_{CS} was computed and compared with the initial nonsurrogate ITC_{CS} during the normal-breathing condition to generate a new cluster t statistic (i.e., the sum of t values within the cluster window). This procedure was repeated 1000 times, resulting in a distribution of cluster t-statistics based on surrogate ITC_{CS} differences.

Results

Slow inspiration reduces cortical phase coherence. Fifteen participants followed the centered onscreen cues for when to start inhaling and exhaling and performed paced breathing at a slow (4 s each for expiration and inspiration or 8 s/breath, i.e., 0.125 Hz) or normal pace (2 s each for expiration and inspiration or 4 s/breath, i.e.,

0.25 Hz) for a total of 10 min each (Fig. 1a). Consistent with our task instruction, despite some variability, the breathing rate was 0.125 ± < 0.001 Hz (mean ± SD, collapsed across trials and participants) or 0.25 ± < 0.001 Hz for the slow-breathing or the normal-breathing condition, respectively. In addition, the duration of the slow or normal breathing cycle was 7.83 ± 0.71 s or 3.98 ± 0.16 s. Closer examination revealed that the duration of the expiration phase (slow-breathing: 4.22 ± 0.43 s; normal-breathing: 2.09 ± 0.14 s) was slightly longer than that of the inspiration phase (slow-breathing: 3.61 ± 0.38 s; normal-breathing: 1.89 ± 0.14 s) in both conditions. This observation reflects a common respiratory pattern in which expiration is passive and requires a longer time for exhalation (Lorig 2007). Despite this, the inspiration/expiration duration ratios were not significantly different between the two conditions (two-tailed paired t-test, $t(14) = 1.30$, $p = 0.22$).

To investigate whether cortical phase activity could be modulated by rhythmic breathing, MEG signals were epoched into trials after aligning them with each successive pair of peaks of inspiration (Fig. 1a; number of artifact-free trials, slow breathing: 52 ± 9; normal breathing: 101 ± 21). Time (the entire epoch)-frequency (2-14 Hz) representations of the MEG phase data were derived using the filter-Hilbert transform. Next, inter-trial phase coherence (ITC) was computed to examine the presence of cortical phase adjustment in response to slow- relative to normal-paced breathing because this commonly employed measure can quantify to what extent the phase data at a given sensor-time-frequency point are aligned in the same direction across the trials that are locked to the repetitions of breathing cycles (i.e., the degree

of event-related phase modulation as a result of repetitive inputs). Given that the slow- and normal-breathing conditions had different numbers of trials, cosine similarity was employed instead of the classical ITC analysis to compute phase coherence in each condition (Chou and Hsu 2018). Unlike ITC, this ITC_{CS} metric is robust to sample-size bias, and it computes the mean cosine angle of all phase pairs from a given trial set (Fig. 1d; see Materials and Methods for details).

Although the participants' breathing was paced, the breathing cycles inevitably contained periodic variation that resulted in variable lengths of respiration-locked MEG trials within individual participants in each condition (Fig. 1b). This factor may deteriorate the precision of the phase-coherence computation because the analysis represents the timing of phase activity across trials. Specifically, if the original time scale is used, the locus of a given time point for the computation would differ from trial to trial. In other words, the obtained ITC_{CS} value at a given time point would reflect the concentration of phase clustering across multiple repetitions of the trials locked to different time points along the course of the breathing cycle. To resolve this issue, for each condition and participant, we conducted unity-based time normalization by bringing the expiratory time points into the range [0 1] and the inspiratory time points into the range [1 2] for every trial within individual participants and conditions (Fig. 1c; see Materials and Methods for details) to facilitate the subsequent phase-coherence computation and between-condition comparison. It should be emphasized that the nature of respiration-locked MEG data or the associated respiratory behavior was not altered because this normalization

procedure simply transformed the varying trial lengths into a common time scale to consistently describe the course of a breathing cycle such that trials with longer durations had more MEG data samples to represent a breathing cycle (e.g., trial 1 in Fig. 1c), whereas trials with shorter durations had fewer data samples (e.g., trial 2 in Fig. 1c). Because of varying data samples across trials, we used the trial with the shortest duration as a new time frame because this new frame provides the time indices that could possibly exist in all the trials. For each of the remaining trials, a new set of time points was selected based on whether these points were closely aligned with this time frame (Fig. 1c, red highlights; mean difference between the selected time points and the time points in the new time frame: slow-breathing condition = 0.0001 a.u., collapsed across time points, trials and participants; normal-breathing condition = 0.0002 a.u.). As a result of this data-selection or "downsampling" procedure, for a given participant and condition, all trials had an equal number of time points that corresponded to almost equivalent timestamps along the breathing cycle; thus, ITC_{CS} that traced the temporal course of breaths could be properly estimated (slow breathing: number of time points per trial before selection ± SD = 3917 ± 354, collapsed across trials and participants; after selection = 2782 ± 626, collapsed across participants; normal breathing: before selection = 1991 ± 82; after selection = 1538 ± 162). After the computation of ITC_{CS} based on the selected time points (Fig. 1d), the data-selection procedure was performed again to ensure proper comparison of group-level ITC_{CS} between the slow- and normal-breathing conditions for every sensor-time-frequency MEG data point given that the number of ITC_{CS}

samples along the breathing cycle also varied across the conditions and participants (Fig. 1e & f, red highlights; mean difference between the selected time points and the time points in the new time frame: slow-breathing condition = 0.0002 a.u.; normal-breathing condition = 0.0003 a.u.; number of time points per trial after selection = 1131).

As shown in Fig. 2a, the respiration-locked ITC_{CS} values were significantly reduced during the slow-breathing condition compared with the normal-breathing condition (cluster-based permutation test to correct for multiple comparison, p = 0.008). The result occurred around the middle of the inspiration period (1.43-1.53 a.u.) at 8-14 Hz over the left frontal, temporal and occipital magnetometer sensors. Notably, due to the time-normalization and data-selection procedures, the obtained effect might initially seem to be transient. When projecting back to the original time scale, this significant time period actually spanned from approximately 5.54 to 5.92 s (collapsed across trials and participants) after expiration onset (time = 0 s) in the slow-breathing condition and from 2.81 to 3.01 s in the normal-breathing condition.

To identify the cortical regions that contributed to the effect described here, LCMV beamforming was performed to recompute ITC_{CS} in the source space based on the significant data points identified at the sensor level. Compared with normal breathing, a wide range of brain regions consistently reflected reduced ITC_{CS} during slow breathing, ranging from the bilateral occipital and parietal lobes to most of the areas of the temporal and frontal lobes (Fig. 2b, two-tailed paired t-test with Benjamini-Hochberg FDR correction, $t(14) \leq -2.15$, $p < 0.05$). A particularly strong

reduction (Fig. 2B, $p < 0.001$) was observed in the left superior parietal lobule (MNI coordinate of peak: x = -19, y = -80, z = 49), left precentral area (x = -54, y = 0, z = 32) extending to the inferior and middle frontal region, left superior orbital frontal gyrus (x = -18, y = 39, z = -20), right middle frontal gyrus (x = 50, y = 20, z = 40) and right inferior temporal gyrus (x = 50, y = -31, z = -25).

Reduced phase coherence could not be attributed to the potential caveats of the analyses. Our analysis approach might have biased the results. In particular, the trial lengths differed significantly between the slow- and normal-breathing conditions; therefore, the ITC_{CS} values in the slow-breathing condition were "downsampled" to some extent during the group-level data-selection step (Fig. 1f). To assess the impact of this process on estimating ITC_{CS} in the slow-breathing condition, we calculated and averaged the original ITC_{CS} values, which refer to the values obtained before group-level data selection within the significant cluster window. These original data from the slow-breathing condition were then contrasted with the initial data from the normal-breathing condition, which refer to the values obtained after group-level data selection and within the significant window. Still, reduced phase coherence was obtained (two-tailed paired t-test, $t(14) = -7.49$, $p < 0.001$).

To additionally ensure that the group-level data-selection step did not happen to select the data points with extreme values and, in turn, lead to a significant result, a resampling procedure was applied for the slow-breathing condition. In this procedure, each participant's original ITC_{CS} values (i.e., before group-level data selection) in the

significant cluster window were randomly selected without replacement to match the number of data points that were initially obtained (i.e., after group-level data selection). Next, we computed the mean for these samples. This procedure was repeated 1,000 times to generate a resampling distribution of ITC_{CS} that was derived from the initial sample size and represented the resampled means in the cluster window during slow breathing. For every participant, the initially obtained ITC_{CS} mean fell within the 95% confidence interval of the distribution (location: mean ± SD = 40.33% ± 13.32, range = 22.3 - 67.2%, above the lower bound), indicating that the group-level data-selection process did not substantially distort the results.

Because of the slight variability of the trial length in each condition, trial-level data selection should have little impact on the result (Fig. 1c). Nevertheless, a similar resampling procedure was conducted to ensure its validity. For this analysis, the original phases (i.e., before trial-level data selection) within the significant cluster window were resampled and averaged to generate a resampling distribution of the mean phase. For every participant, the initially obtained phase mean (i.e., after trial-level data selection) also fell within the confidence interval of the distribution (slow breathing: mean ± SD = 49.17% ± 6.50, range = 40.83 - 60.32%, collapsed across trials; normal breathing: 48.43% ± 4.74, 40.34 - 60.44%).

Reduced phase coherence is specifically locked to ongoing inspiration. To verify that reduced ITC_{CS} values were genuinely time locked to the middle of the inspiration phase, we shuffled the phase data from the slow-breathing condition to create

surrogate ITC_{CS} while the initial phase data from the normal-breathing condition remained untouched. Because ITC_{CS} represents the timing of phase activity over trials, the null hypothesis is that shifting the phase time series during the slow-breathing condition by a random amount (i.e., not locked to inspiration) would not affect its ITC_{CS} strength relative to that during the normal-breathing condition. For each MEG trial, we randomly selected a single time point of the phase data (Fig. 1b) and exchanged the resulting two sections of data so that the original phase information was retained with minimal distortion. After the ITC_{CS} computation, we recomputed the cluster t-statistics by contrasting surrogate ITC_{CS} from the slow-breathing condition with initial ITC_{CS} from the normal-breathing condition for individual data points within the significant cluster window. The entire analysis procedure was repeated 1000 times (Fig. 1b to 1f) such that a distribution of cluster t-statistics under the null hypothesis was generated. The initially obtained statistics were found to exceed all the surrogate ones (i.e., $p < 0.001$), thereby confirming that the observed effects were significantly locked to ongoing inspiration.

Reduced phase coherence is not accompanied by respiration-locked power, ERF or cardiac activity effects. Differences in the amplitude of event-related potentials/fields (ERPs/ERFs) or power may produce differing phase-coherence values that are independent of any actual change in the phase (van Diepen and Mazaheri 2018). Through a similar analysis pipeline including time normalization, trial-level and group-level data selection, raw MEG and single-trial normalized power

activities were computed for each condition and participant, respectively. No significant difference in power (Fig. 3a, cluster-based permutation test, $p = 0.57$) or ERF amplitude (Fig. 3b, $p = 1$) was found between the slow- and normal-breathing conditions. The same results were obtained even when the analyses were restricted in the reported sensor-time-frequency (two-tailed paired t-test on the mean data within the significant cluster window, $t(14) = -0.70$, $p = 0.50$) or sensor-time window ($t(14) = 1.11$, $p = 0.29$). The absence of a respiration-locked power difference further undermines the possibility that our results reflect a discrepancy in phase estimation due to differential power or that the results could be ascribed to differential task demands, as indexed by widespread alpha power differentiation (Fink et al. 2005; Jensen et al. 2002; Mahjoory et al. 2019).

The cardiovascular and respiratory systems strongly interact (Angelone and Coulter 1964). Therefore, cardiovascular input might contribute an indirect effect. We investigated whether there was a difference in cardiac activity between the two conditions and whether such a change could characterize the observed results. To account for variation in trial lengths across participants and conditions, we discretized each trial into 16 time bins with 8 bins each during inspiration and expiration, where bin 12 approximately corresponded to the timing of the reported phase-coherence result. Next, we counted the occurrence of QRS complexes within each time bin in simultaneously recorded ECG. The obtained results were expressed as the number of QRS complexes per second and averaged over trials for individual participants (Fig. 3c). Consistent with so-called respiratory sinus arrhythmia (Angelone and Coulter

1964) and previous reports that slow breathing is associated with increasing heart-rate fluctuation (Lehrer and Gevirtz 2014; Radaelli et al. 2004), QRS frequency significantly accelerated during ongoing inspiration and slowed during ongoing expiration in the slow-breathing condition (one-way repeated-measure ANOVA, $F(15,180) = 4.68$, $p < 0.001$) but not in the normal-breathing condition ($F(15,180) = 1.28$, $p = 0.22$). Despite the results, the time courses of QRS frequency were not significantly different between the two conditions (two-way repeated-measures ANOVA on interaction, $F(15,180) = 0.74$, $p = 0.74$), indicating that the patterns of the QRS complex could not fully characterize our findings.

Reduced phase coherence reflects increasing phase distance between the respiration-locked trials in the slow-breathing condition. Reduced phase coherence indices commonly indicate that phase angles are becoming randomly distributed across trials. However, ITC_{CS} may also decrease if large phase differences exist in some subsets of trial pairs because similarity measurements become more negative when the cosine of the angle between the two phases in these pairs becomes diametrically opposed (see Fig. 4 for details). To investigate the nature of the phase dynamics underlying our results, we examined the distribution of absolute phase differences in the significant sensor-time-frequency points. On the one hand, this examination reflects the intrinsic process during ITC_{CS} computation. On the other hand, because the exact pattern of the phase distribution at each data point differed greatly (Supplementary Fig. S1 (https://doi.org/10.5281/zenodo.3466135)), the

494 distribution of absolute phase differences (i.e., phase distance in each pair of phase
495 samples), which represents the phase composition in a relative fashion, was more
496 suitable than the phase distribution per se for detecting the converging pattern
497 revealed from the overall data within the significant cluster window (Supplementary
498 Fig. S2 (https://doi.org/10.5281/zenodo.3466135)).

499 For every significant sensor-time-frequency point, the absolute phase differences
500 between every two trials in all possible combinations were first calculated. In other
501 words, for this analysis, we followed the same ITC_{CS} computation (Fig. 1d) except
502 that the phase differences were not averaged but their absolute values were grouped
503 together. The results were then pooled together across all the data points for each
504 participant and condition. The final results were discretized into 20 bins according to
505 the phase difference, and in each bin, the relative probability of occurrence was
506 calculated. As shown in Fig. 5, slow breathing regularized the distribution of phase
507 differences in a consistent and specific manner. For every individual participant, there
508 was an increasing number of trial pairs whose phases were moving from each other
509 during the slow-breathing condition (t-test on the slope of the regression line, sub1:
510 $t(18) = 9.80$, $p < 0.001$, sub2: $t(18) = 9.58$, $p < 0.001$; see Supplementary Fig. S3
511 (https://doi.org/10.5281/zenodo.3466135) for the results from the rest of the
512 participants: $ts \geq 5.34$, $ps < 0.001$). However, during the normal-breathing condition,
513 the opposite pattern was found in 8 out of 15 participants (Fig. 5b, sub1: $t(18) =$
514 -16.88, $p < 0.001$; Fig. S3, $ts \leq -2.84$, $ps \leq 0.05$), while one followed the pattern
515 generally observed in the slow-breathing condition (Fig. S3, $t(18) = 4.42$, $p < 0.001$).

516 For the remaining 6 participants, the distribution did not significantly change
517 according to the phase difference (Fig. 5b, sub2: $t(18) = -1.50$, $p = 0.15$; Fig. S3, -1.5
518 $\geq ts \geq -1.76$, $0.15 \geq ps \geq 0.10$). Thus, for most of the participants, the
519 normal-breathing phases were either clustered around a similar direction or randomly
520 distributed and approaching uniform.

521

522 ## Discussion

523 The current study shows that cortical alpha phase activity can be modulated by
524 rhythmic inputs from controlled slow breathing. The findings extend and advance our
525 understanding of several aspects of the interaction between the respiratory system and
526 the brain. First, during ongoing slow breathing relative to normal breathing, phase
527 coherence was reduced over a wide range of brain areas, spanning most of the area of
528 the temporal and prefrontal lobes. This finding is consistent with prior animal and
529 human evidence that these two lobes are involved in respiration-entrained neural
530 activity (Herrero et al. 2018; Tort et al. 2018) and indicates that slow breathing could
531 affect phase dynamics in a large part of the brain. However, our results appear to be
532 locked to ongoing inspiration as opposed to expiration. This observation could be a
533 consequence of different mechanistic or evolutionary mechanisms involved in these
534 two stages of breathing. In contrast to inspiration, expiration is largely passive and
535 reflects the result of relaxation of the external intercostals and diaphragm (Lorig
536 2007), whereas inspiration is an active process and prepares the brain to receive
537 incoming sensory information (Corcoran et al. 2018). These two respiratory stages

might be differentially translated in the brain, irrespective of the underlying mechanism. Indeed, compared with expiration, ongoing inspiration is associated with pain perception (Arsenault et al. 2013), near-threshold stimuli detection (Flexman et al. 1974), fearful expression discrimination (Zelano et al. 2016), and visuospatial performance (Perl et al. 2019). Our finding thus provides a novel complement to the existing data and emphasizes the unique role of inspiration in cortical phase activity and potentially in accompanying cognitive functions.

Our further analyses go beyond the finding of reduced phase coherence and identify how slow breathing adjusts the pattern of phase dynamics that orchestrates the mechanism underlying the finding (see Supplementary Fig. S4 (https://doi.org/10.5281/zenodo.3466135) for the argument of noncircular inference). At the single-subject level, slow inspiration systematically organized the phase distribution such that phases became more likely to be diametrically opposed across the trials. In contrast, the phase distribution driven by normal inspiration tended to be unsettled, either clustering around a single direction or becoming randomized. Thus, the inherently coherent phase structure indicates the presence of consistent phase adjustment in response to slow-paced inspiratory inputs. Accordingly, the present results not only support our hypothesis that breathing dynamically shapes cortical phase activity but also imply that slow inspiration organizes the cortical phase in a rather regularized pattern. By virtue of this mechanism, we propose, rather speculatively, that the coding of existing or subsequent inputs could be suppressed during slow breathing because phase-mediated neural transmission or communication

might be either disrupted for preceding inputs or less susceptible to readjustment for subsequent inputs due to the regularized phase pattern imposed by slow inspiration. This idea echoes other phase-adjustment phenomena during extrinsic stimulation in which, during attention selection, for example, the oscillatory phase adjusted by attended stimuli prevents the coding of unattended stimuli (Schroeder and Lakatos 2009; Voloh and Womelsdorf 2016). Distinctively, we suggest that cortical phase adjustment can be internally governed based on volitional control of rhythmic inputs from a bodily source, namely, respiration, and not merely from extrinsic stimulation.

Our study also reveals a unique pattern of phase adjustment that has seldom been described. In contrast to prior reports, the adjusted phase does not cluster toward a certain direction; instead, the phase is adjusted in such a manner that the phase angles tend to be distant from each other across trials, resulting in reduced phase coherence. This finding highlights a shortcoming in commonly adopted phase-coherence analysis in which a high value is usually construed as reflecting consistent phase adjustment. Here, we show that phase adjustment does not necessarily accompany a high phase-coherence value if the shape of the phase distribution is not unimodal (Fig. 4). Alternatively, examining the distribution of absolute phase differences, as demonstrated in the current approach, might provide a window to gain better insights into the nature of phase coherence.

Although participants' breaths were paced throughout the study, different task-related effects might exist between the slow- and normal-breathing conditions that are not relevant to breathing per se. However, our results are unlikely to reflect

differential task demands because of a lack of widespread, task-dependent alpha power modulation between the conditions (Fink et al. 2005; Jensen et al. 2002; Mahjoory et al. 2019). Additionally, the participants did not seem to exhibit a notably increased degree of awareness of slow breaths compared with normal breaths as strong activation of interoception-related neural correlates – such as the anterior cingulate cortex and the insula (Herrero et al. 2018) – should be expected. Above all, these interpretations are not easily reconcilable with the specific role of ongoing inspiration in our results, and the latter interpretations are often associated with enhanced phase-coherence values (Park et al. 2018), which contradicts the present results. Nevertheless, partly due to technical constraints, cardiorespiratory parameters, such as blood pressure and diaphragmatic breathing, were not measured exhaustively. Thus, the effects of these additional parameters on the present findings remain to be determined. Moreover, future research needs to clarify whether the effect of slow breathing could be present during natural breathing when there is a lack of volitional control of breathing.

Respiration-entrained neural oscillations are thought to act as an integral part of rhythmic brain activity (Heck et al. 2017; Tort et al. 2018). Akin to this idea, by applying periodic perturbation through different breathing paces, the current study demonstrates that cortical phase dynamics can be systematically altered in response to such perturbation. This finding implies that the respiration-locked phase effects constitute a fundamental organizing principle of brain activity, thus uncovering a complex rhythmic neural network integrated with different neurophysiological

systems. It is interesting to note that slow breathing is associated with cognitive and emotional changes (Boiten et al. 1994; Homma and Masaoka 2008; Kreibig 2010). The overall results may shed light on how a volitionally controlled change in respiratory behavior will perturb the rhythmic brain-respiration network and ultimately regulate cognitive and emotional behavior.

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Figure captions

***Figure 1. Schematic illustration of the analysis pipeline**. **(a)** Participants performed paced breathing by following the cues "|" and " - " at the center of the screen, which*

indicated the onset of expiration and inspiration. The alternation rate between the two cues was either 2 s (normal breathing) or 4 s (slow breathing), resulting in 4 s/breath in the normal-breathing condition and 8 s/breath in the slow-breathing condition. The recorded MEG data at a given sensor were epoched into trials aligned to each successive pair of peaks of inspiration (corresponding to one breath) and extended from 1 s before to 1 s after the peaks to avoid edge effects during spectral analysis. ***(b)*** *For each trial, the respiration-locked MEG phase data were computed using the Hilbert transform after bandpass filtering the data to create 13 frequency steps between 2 and 14 Hz. Note that the sensor and frequency dimensions are not illustrated here.* ***(c)*** *Because of slightly variable MEG trial lengths within each participant and condition, the expiration- and inspiration-locked periods of each trial were normalized to the time ranges [0 1] and [1 2]. The dots represent individual normalized time points. For a given participant and condition, a new set of MEG time points in each trial was selected based on the time frame from the trial with the shortest duration (red highlight).* ***(d)*** *Phase coherence across respiration-locked trials (ITC_{CS}) was derived by computing the mean cosine angle of all phase pairs from two given trials at each selected data point.* ***(e, f)*** *Because of variable ITC_{CS} time points between participants and conditions, the same data-selection procedure was performed on the time domain for the group-level analysis using the time frame from the participant/condition with the shortest duration (red highlight). RESP, respiration; EX, expiration; IN, inspiration; S.B., slow breathing; N.B., normal breathing.*

Figure 2. ***Reduced phase coherence during slow breathing relative to normal breathing***. ***(a)*** *The left scalp topography expresses the t statistic (slow breathing vs. normal breathing) of the cluster of significant sensors (red dot; cluster-based permutation, p = 0.008) at peak 10 Hz and time 1.48 (a.u.). The middle panel expresses the time-frequency representation of the t statistic averaged from the significant sensors. The red brackets (8-14 Hz) highlight the frequency range of the cluster. The right panel shows the time course of averaged raw* ITC_{CS} *values from the significant sensor-frequency points. The red horizontal bar highlights the significant time period of the cluster. Notably, the expiratory/inspiratory time scales were normalized to the ranges [0 1]/[1 2]. In the original time scale, the significant time period corresponded to approximately 5.54-5.92 s (collapsed across trials and participants) after expiration onset (time = 0 s) in the slow-breathing condition and 2.81-3.01 s in the normal-breathing condition. Shaded regions indicate 95% confidence intervals. S.B., slow breathing; N.B., normal breathing; a.u., arbitrary unit.* ***(b)*** *The sources of reduced* ITC_{CS} *during slow relative to normal breathing, as localized by the LCMV beamformer, are located over the bilateral occipital, temporal, and parietal to frontal lobes (two-tailed paired t-test with FDR correction, p < 0.05; t values below an* α*-level of 0.05 are masked) and are prominent in the left superior parietal lobule, left precentral gyrus, left superior orbital frontal gyrus, right middle frontal gyrus and right inferior temporal gyrus (red circle; p < 0.001). To improve visualization of the left superior orbital frontal gyrus (right panel) and right inferior*

780 *temporal gyrus (left panel), the sagittal views are also illustrated (solid red circle; t*
781 *values below an α-level of 0.001 are masked).*
782
783 ***Figure 3. No significant difference in respiration-locked power ERF or cardiac***
784 ***activity during slow breathing relative to normal breathing. (a)*** *The scalp*
785 *topography expresses the t statistic (slow breathing vs. normal breathing) at 10 Hz*
786 *and time 1.48 (a.u.). The middle panel expresses the time-frequency representation of*
787 *the t statistic averaged from the sensors highlighted in white,* i.e., *previously reported*
788 *significant sensors. The right panel shows the time courses of averaged 8-14 Hz*
789 *power from the highlighted sensors. The expiratory/inspiratory period was*
790 *normalized to the ranges [0 1]/[1 2].* ***(b)*** *The scalp topography expresses the t statistic*
791 *(slow breathing vs. normal breathing) at time 1.48 (a.u.). The right panel shows the*
792 *time courses of averaged ERFs from the highlighted sensors.* ***(c)*** *The time courses of*
793 *the frequency of the QRS complex are illustrated. The expiratory/inspiratory period*
794 *was binned into the ranges [1 8]/[9 16]. All shaded regions indicate 95% confidence*
795 *intervals. S.B., slow breathing; N.B., normal breathing; a.u., arbitrary unit.*
796
797 ***Figure 4. Relations among ITC_{CS}, phase distribution, and distribution of absolute***
798 ***phase difference as illustrated using simulated phase data.*** *Data were generated by*
799 *randomly selecting 50 phase samples from –π/4 to π/4 (left panel), –π to π (middle*
800 *panel) or a mixture of 10 phases from 0 to π and 20 sample pairs with half of the*
801 *samples from 0 to π and the other half at the opposite angles (right panel). This data*
802 *generation procedure was repeated 1000 times. The top panel shows ITC_{CS} averaged*

803 *across repetitions. The circular and horizontal histograms depict the phase*
804 *distributions and the distributions of absolute phase differences (i.e., phase distance*
805 *between every two possible phase samples in a given data set) collapsed across*
806 *samples from all repetitions.*

807

808 ***Figure 5. Distinct phase patterns driven by slow and normal breathing as revealed***
809 ***from the distribution of absolute phase differences.*** *The horizontal histograms depict*
810 *the distributions of absolute phase differences collapsed across all significant data*
811 *points from two representative participants during the slow- (top panel) and*
812 *normal-breathing (bottom panel) conditions. Red lines represent the linear regression*
813 *lines. ***p < 0.001.*

814