

Single Nostril Breathing Modulates EEG Microstate Dynamics, Cortical Source Activity, and Functional Connectivity in Healthy Adults

Kumar Abhishek

All India Institute of Medical Sciences

Sweta Sweta

All India Institute of Medical Sciences

Prashant Tayade

dr.prashanttayade@gmail.com

All India Institute of Medical Sciences

Suriya Prakash Muthukrishnan

All India Institute of Medical Sciences

Simran Kaur

All India Institute of Medical Sciences

Ratna Sharma

All India Institute of Medical Sciences

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Abstract

Pranayama, a yogic breathing practice, enables voluntary modulation of respiration and influences autonomic, cognitive, and affective processes. Single nostril breathing (SNB), comprising left nostril breathing (LNB) and right nostril breathing (RNB), has been proposed to modulate hemispheric activity and sympathovagal balance; however, its large-scale spatiotemporal brain dynamics remain poorly understood. This study investigated the effects of SNB on electroencephalographic (EEG) microstates, cortical source activity, and functional connectivity using high-density EEG. Twenty-two healthy right-handed male participants (20–35 years) underwent 128-channel EEG recording during spontaneous breathing (SB) and guided SNB performed at six breaths/min. EEG data were preprocessed using EEGLAB, followed by microstate segmentation in Cartool. Cortical source localization and source-space functional connectivity analyses were performed using standardized low-resolution electromagnetic tomography (sLORETA). Functional connectivity was assessed using lagged linear connectivity and lagged non-linear connectivity across multiple frequency bands. Six dominant EEG microstates were identified across conditions. Compared with SB, guided SNB showed significantly reduced mean duration, temporal coverage, and number of time frames of microstate maps 1 and 3, indicating faster transitions between transient brain states, which may reflect greater neural flexibility or reduced temporal stability, while microstate map 6 demonstrated increased global explained variance. Source localization revealed increased current density in the anterior cingulate cortex (ACC), insula, superior temporal gyrus, medial and inferior frontal gyri, superior frontal gyrus, precentral gyrus, and inferior parietal lobule during SNB. Functional connectivity analyses demonstrated frequency-specific reorganization of large-scale brain networks. LNB showed decreased theta-band connectivity between the superior temporal gyrus, insula, and inferior parietal lobule. RNB demonstrated decreased alpha- and beta-band connectivity among frontal, temporal, parietal, and cingulate regions. These findings indicate functional decoupling of salience, attentional, and executive control networks during SNB, providing neurophysiological evidence that guided slow unilateral breathing is associated with modulation of large-scale brain dynamics.

Introduction

The regulation of breathing is a readily accessible yet underexplored interface between voluntary control and autonomic physiology. Among yogic practices, *Pranayama* uses conscious regulation of respiration to influence physiological and psychological states. Ancient Indian traditions describe breath as the carrier of *prana* (vital life energy), linking controlled breathing with mental clarity, emotional stability, and bodily homeostasis, while contemporary evidence shows that it modulates autonomic balance, cortical excitability, emotional regulation, and large-scale brain network organization (Zaccaro et al., 2018; Novaes et al., 2020; Malhotra et al., 2022). Among its techniques, single nostril breathing (SNB) - including left nostril breathing (LNB) and right nostril breathing (RNB) - has attracted particular scientific interest because of its lateralized physiological and neural effects. Traditional yoga describes LNB as “cooling” and parasympathetic-dominant, whereas RNB is considered “activating” and sympathetic-enhancing (Sharma, 2022; Telles et al., 2017). Experimental and neurophysiological studies further

demonstrate that SNB modulates autonomic markers such as heart rate variability, galvanic skin response, oxygen consumption, and blood pressure, while differentially influencing hemispheric activity and cognitive functions, including attention, vigilance, executive function, and memory (Raghuraj et al., 2008; Telles et al., 2017; Block et al., 1989; Subramanya & Telles, 2009; Joshi et al., 2021).

Respiration has also emerged as a critical modulator of neural oscillations and large-scale brain dynamics. Studies using electroencephalography (EEG), functional magnetic resonance imaging (fMRI), and intracranial recordings have shown that breathing rhythms entrain cortical and limbic activity, particularly in regions associated with emotional processing, interoception, and cognitive control, including the insula, anterior cingulate cortex (ACC), hippocampus, and prefrontal cortex (Zelano et al., 2016; Herrero et al., 2018). Slow breathing practices have been associated with enhanced alpha and theta oscillatory activity, increased parasympathetic tone, and improved neural synchronization (Bhaskar et al., 2020; Zomorodi et al., 2019). Despite these findings, previous EEG studies investigating SNB have predominantly focused on spectral power analysis or hemispheric asymmetry, which provide relatively static measures of neural activity and do not adequately capture the rapidly changing dynamics of large-scale functional brain networks.

Brain activity is inherently dynamic, comprising transient patterns of coordinated neural activation across cortical regions. EEG microstate analysis captures these rapid state transitions by identifying quasi-stable scalp potential topographies lasting approximately 60–120 ms before shifting to another configuration (Lehmann et al., 1987; Michel & Koenig, 2018). These microstates are electrophysiological correlates of momentary global functional states and have been associated with canonical resting-state networks, including the default mode, salience, dorsal attention, and executive control networks (Britz et al., 2010; Khanna et al., 2015). Their temporal parameters - Global Explained Variance (GEV), Mean Duration (MD), Number of Time Frames (TF), Time Coverage (TC) - quantify neural stability, flexibility, and information processing efficiency (Van De Ville et al., 2010; Milz et al., 2016).

Recent evidence suggests that contemplative and breath-focused practices can alter microstate dynamics and reorganize large-scale neural networks associated with attention, interoception, and emotional regulation (Bréchet et al., 2021; Zanesco et al., 2024). Breath regulation has been shown to synchronize neural oscillations across cortical and subcortical regions, thereby facilitating top-down modulation of cognitive and autonomic processes (Zelano et al., 2016). Given the lateralized nature of SNB, its effects may extend beyond generalized respiratory entrainment and involve differential modulation of hemispheric specialization, salience processing, and attentional control networks. However, studies systematically examining the influence of SNB on EEG microstate organization and their underlying cortical generators remain limited.

In addition to transient microstate dynamics, the functional integration between distributed cortical regions is increasingly recognized as a critical mechanism underlying cognitive and autonomic regulation. Functional connectivity analysis provides insight into how anatomically distinct brain regions communicate and synchronize their activity over time. Source-space EEG connectivity methods,

particularly those based on lagged interactions, offer improved characterization of physiologically meaningful network interactions while minimizing spurious effects arising from volume conduction (Pascual-Marqui et al., 2011). Previous neuroimaging studies suggest that breathing-related modulation of frontal, temporal, insular, and parietal regions may support attentional regulation, sensory integration, and interoceptive awareness (Herrero et al., 2018; Vanutelli et al., 2024). However, the functional connectivity architecture underlying SNB-induced changes in large-scale brain dynamics remains poorly understood.

High-density EEG combined with source localization approaches provides a unique opportunity to investigate both the temporal and spatial organization of neural activity during controlled breathing. Standardized low-resolution brain electromagnetic tomography (sLORETA) enables estimation of intracortical current source density underlying EEG activity and allows identification of cortical regions contributing to specific microstate configurations. Integration of microstate analysis, cortical source localization, and functional connectivity measures may therefore provide a comprehensive understanding of how SNB reorganizes dynamic brain networks involved in attention, interoception, autonomic regulation, and cognitive control.

Accordingly, the present study employed 128-channel quantitative EEG to investigate the effects of SNB on EEG microstates, cortical source activity, and functional connectivity in healthy adults. Microstate segmentation was used to characterize transient large-scale brain states, used to estimate the probable cortical sources underlying these microstates, and source-space functional connectivity analyses were performed to examine frequency-specific interactions among significant cortical regions during SNB relative to spontaneous breathing (SB). Functional connectivity was evaluated using lagged linear connectivity and lagged non-linear connectivity across multiple frequency bands to capture complementary aspects of neural communication.

We hypothesized that SNB would primarily modulate the temporal dynamics of EEG microstates, reflecting altered neural flexibility and large-scale network stability, with concurrent frequency-specific changes in cortical activity and reorganization of functional connectivity, including decreased connectivity within salience, interoceptive, attentional, and executive networks, relative to spontaneous breathing. This multimodal electrophysiological approach aimed to elucidate the neural mechanisms through which controlled unilateral breathing reorganizes dynamic brain function and communication.

Materials and Methods

Participants

Twenty-two healthy male volunteers (Mean $\pm$ SD: 29.11 $\pm$ 4.51 years; 20–35 years) were recruited from undergraduate, postgraduate, and doctoral programs at the All India Institute of Medical Sciences (AIIMS), New Delhi, India. Sample size estimation was performed in consultation with the Department of Biostatistics, AIIMS, New Delhi. All participants were right-handed, as assessed using the Edinburgh

Handedness Inventory, and had normal or corrected-to-normal vision. Exclusion criteria included history of neurological, psychiatric, cardiovascular, or pulmonary disorders; current respiratory illness; smoking; regular yoga practice; sleep deprivation (< 6 h sleep); alcohol intake within 24 h; medications affecting CNS or autonomic function; and strenuous physical activity prior to testing. Participants were additionally instructed to avoid caffeine and other stimulants for at least 6 h before the session and to arrive with clean, product-free hair. Written informed consent was obtained from all participants prior to inclusion in the study. All procedures were conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee, AIIMS, New Delhi (Ref. No.: IEC-605/06.10.2023, RP-11/2023).

Experimental Protocol

Participants were familiarized with the SNB procedure through a standardized guided video and practice session prior to EEG recording. High-density EEG was recorded during five sequential conditions: spontaneous breathing-1 (SB1), left nostril breathing (LNB), spontaneous breathing-2 (SB2), right nostril breathing (RNB), and spontaneous breathing-3 (SB3) (Fig. 1). LNB and RNB breathing tasks were performed at a controlled rate of six breaths/min, guided visually for consistency. Guided SNB (i.e., LNB and RNB) and SB1 was therefore included in subsequent comparisons. Intermittent SB (SB1, SB2 and SB3) phases served as recovery periods. The order of LNB and RNB conditions was randomized to minimize order effects. Participants were seated comfortably in a dimly lit, electrically shielded, sound-attenuated room and instructed to remain relaxed, minimize movements, and avoid excessive blinking during recordings.

EEG Data Acquisition

EEG data were acquired using a 128-channel HydroCel Geodesic Sensor Net (HCGSN; Magstim Electrical Geodesics Inc., USA) connected to a Net Amps 300 amplifier. Recordings were obtained in an electrically shielded, sound-attenuated environment with electrode impedances maintained below 50 k Ω . Signals were sampled at 1000 Hz using Net Station software (version 5.4.2; EGI, USA), with Cz as the acquisition reference. Online hardware filtering included a 0.1–100 Hz band-pass filter and a 50 Hz notch filter. Channels were continuously monitored to ensure optimal signal quality throughout acquisition.

EEG Preprocessing

EEG preprocessing was performed in EEGLAB (Delorme & Makeig, 2004) using MATLAB (MathWorks, Natick, MA, USA). Continuous EEG data were downsampled to 500 Hz, denoised (50 Hz line noise removal), and band-pass filtered (1–40 Hz for microstate analysis and source localization; 1–100 Hz for functional connectivity). Data from each condition (SB1, LNB, and RNB) were segmented, visually inspected, and cleaned using the Clean Raw Data pipeline with Artifact Subspace Reconstruction (ASR). Noisy channels (≥ 200 μ V in $\geq 20\%$ of the recording) were identified, and Independent Component

Analysis (Infomax) was used to remove ocular, muscular, cardiac, and other non-neural artifacts. Bad channels were interpolated using spherical spline interpolation, and the data were re-referenced to the common average. Finally, a continuous 2-min artifact-minimized middle segment from each condition was extracted for microstate segmentation, cortical source localization, and functional connectivity analyses.

Microstate Analysis

Microstate analysis was performed using Cartool (v5.02; Functional Brain Mapping Lab, Geneva, Switzerland). Preprocessed EEG data were segmented into quasi-stable scalp topographies representing global brain states. Time points corresponding to local maxima of global field power were extracted to maximize signal-to-noise ratio, and subject-level microstate classes were identified using k-means clustering. The optimal number of microstates was determined using the cross-validation criterion to minimize residual variance and avoid overfitting. Individual microstate maps were subsequently clustered to derive group-level templates for SB1, LNB, and RNB conditions. Spatial correlation analysis was used to match individual microstates with group templates across participants. Microstate temporal dynamics were quantified using GEV, MD, TF, and TC, reflecting the contribution, stability, temporal occupancy, and occurrence of each microstate, respectively.

Source Localization

sLORETA was used to estimate the probable cortical sources underlying the EEG microstates. Source reconstruction was performed using the MNI152 template head model, with the solution space restricted to cortical gray matter and hippocampal regions (6,239 voxels; 5 mm spatial resolution). For each microstate class, voxel-wise current source density was computed from the preprocessed EEG data. Differences in source activity between experimental conditions (SB1, LNB, and RNB) were assessed using voxel-wise paired t-tests on log-transformed current density values. Statistical Non-Parametric Mapping with 5,000 random permutations was applied to control for multiple comparisons and non-normality. The resulting statistical maps identified cortical regions showing significant microstate-related differences in activation across conditions, with significant clusters anatomically labeled according to their MNI coordinates and corresponding Brodmann areas (BAs).

Functional Connectivity Analysis

Functional connectivity analysis was performed in source space to examine interactions between cortical regions underlying microstate dynamics during SNB, minimizing the effects of volume conduction and reference dependence. Preprocessed EEG data were reconstructed using sLORETA, and connectivity was computed using the LORETA-based connectivity module. Analysis was restricted to regions of interest (ROIs) showing significant source activity during microstate analysis, ensuring correspondence between microstate generators and connectivity patterns. Connectivity was assessed

using lagged linear connectivity and lagged non-linear connectivity to characterize complementary aspects of inter-regional interactions while reducing spurious effects arising from volume conduction. Connectivity estimates between ROI pairs were computed using high-density EEG recordings across delta (1–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), beta (13–30 Hz), gamma (30–100 Hz), and broadband (1–100 Hz) frequency bands. Group-level comparisons between SNB (LNB and RNB) and SB1 conditions were performed to identify frequency-specific alterations in cortical network interactions.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism (version 9.0) for microstate parameters and complementary statistical tools implemented within the respective analysis software for source localization and functional connectivity. For microstate, data normality of each microstate parameter was assessed using the Shapiro-Wilk test. Depending on the distribution of the data, differences in GEV, MD, TF, and TC across the three breathing conditions (SB1, LNB, and RNB) were evaluated using either one-way repeated-measures analysis of variance (RM-ANOVA) or the Friedman test. When the assumption of sphericity was violated, the Greenhouse–Geisser correction was applied to the RM-ANOVA. Pairwise post hoc comparisons were corrected for multiple testing using the Benjamini–Krieger–Yekutieli false discovery rate procedure. Results are reported as *F* statistics with Greenhouse–Geisser-adjusted degrees of freedom and partial eta squared (η^2) for RM-ANOVA, and as χ^2 statistics with Kendall's coefficient of concordance (*W*) for Friedman tests. Data are presented as mean $\pm$ standard deviation (SD) for normally distributed variables and as median (interquartile range [IQR]) for non-normally distributed variables. Statistical significance was set at $p < 0.05$. For source localization, Statistical Non-Parametric Mapping implemented in sLORETA was used to assess voxel-wise differences in cortical current density between conditions using permutation testing to correct for multiple comparisons. Functional connectivity differences between SNB (LNB and RNB) and SB1 conditions were evaluated using paired-sample *t*-tests across predefined frequency bands. Statistical significance was determined using non-parametric permutation testing with correction for multiple comparisons across ROI pairs.

Results

EEG Microstate Analysis

Six dominant scalp topographies were identified across conditions using the cross-validation criterion in the second k-means clustering analysis (Fig. 2A). These maps (Maps 1–6) represented the most stable EEG microstates observed during SNB and SB1, including (LNB and RNB). The polarity of scalp potentials is illustrated by the color scale, with red and blue denoting positive and negative voltages, respectively. Temporal characteristics of the microstates were quantified using GEV, MD, TF, and TC. Statistical outcomes are summarized in Table 1.

Between-Condition Comparison (SNB vs. SB1)

Repeated-measures analyses revealed significant modulation of microstate dynamics during SNB relative to SB1 (Fig. 2B-G). For Map 1, Friedman tests demonstrated significant effects of breathing condition on MD ($\chi^2(2) = 8.273, p = 0.016$, Kendall's $W = 0.188$) and TF ($\chi^2(2) = 9.091, p = 0.011$, Kendall's $W = 0.207$). Benjamini-Krieger-Yekutieli-corrected post hoc comparisons showed that MD was significantly reduced during both LNB ($p = 0.010$) and RNB ($p = 0.016$) relative to SB1, whereas TF was significantly reduced only during RNB ($p = 0.003$). For Map 3, RM-ANOVA revealed a significant effect of breathing condition on MD ($F(1.673, 35.14) = 5.439, p = 0.012, \eta p^2 = 0.206$), with Benjamini-Krieger-Yekutieli-corrected post hoc analyses indicating reduced MD during both LNB ($p = 0.023$) and RNB ($p = 0.010$) relative to SB1. Friedman tests further demonstrated significant effects on TF ($\chi^2(2) = 17.820, p < 0.001$, Kendall's $W = 0.405$) and TC ($\chi^2(2) = 8.455, p = 0.015$, Kendall's $W = 0.192$). Benjamini-Krieger-Yekutieli-corrected post hoc comparisons revealed significant reductions in TF during both LNB and RNB ($p < 0.001$) and decreased TC during LNB ($p = 0.024$) and RNB ($p = 0.007$) relative to SB1. Collectively, these findings indicate reduced temporal persistence, occurrence, and overall contribution of Map 3 during unilateral nostril breathing. In contrast, RM-ANOVA demonstrated a significant effect on the GEV of Map 6 ($F(1.818, 38.17) = 8.278, p = 0.001, \eta p^2 = 0.283$), with Benjamini-Krieger-Yekutieli-corrected post hoc analyses showing increased GEV during both LNB ($p = 0.004$) and RNB ($p = 0.003$) relative to SB1, indicating enhanced dominance and global contribution of this microstate class.

Within-Condition Comparison (LNB vs. RNB)

No statistically significant differences were observed between LNB and RNB across any microstate temporal parameters.

Cortical Source Localization

To estimate the cortical sources underlying microstates showing significant temporal differences across conditions, the corresponding microstate time points were extracted from preprocessed EEG data and analyzed using sLORETA. Cortical current source density distributions were computed, and statistically significant differences were identified using non-parametric mapping.

Between-Condition Comparison (SNB vs. SB1)

Source localization was performed for Map 1, Map 3, and Map 6, as these maps showed significant temporal alterations between SNB (LNB/RNB) and SB1. For Map 1, SNB (particularly LNB) showed significantly increased activation in the insula (BA 13) and cuneus (BA 18, BA 23), regions associated with interoceptive awareness and visual processing, respectively. For Map 3, SNB produced significantly increased activation in the middle frontal gyrus (BA 11), superior frontal gyrus (BA 11), inferior frontal gyrus (BA 47), ACC (BA 32, BA 24), inferior parietal lobule (BA 40), and superior temporal gyrus (BA 22). These regions collectively represent higher-order cognitive control and attentional networks. For Map 6, SNB was associated with significantly increased activation in the superior temporal gyrus (BA 22, BA 39, BA 13) and inferior parietal lobule (BA 40), regions involved in multimodal sensory integration and attentional processing.

The source plots and statistically significant regions are shown in Fig. 3A for Map 1 (LNB vs. SB1), Fig. 3B for Map 3 (LNB vs. SB1 and RNB vs. SB1, respectively), and Fig. 3C for Map 6 (LNB vs. SB1 and RNB vs. SB1, respectively). No statistically significant cortical source differences were observed for Map 1 in the RNB vs. SB1 comparison despite significant temporal parameter alterations, suggesting that these microstate dynamics may reflect distributed network interactions rather than focal cortical source changes.

Within-Condition Comparison (LNB vs. RNB)

No statistically significant differences in cortical source localization were observed between LNB and RNB.

Functional Connectivity Analysis

Functional connectivity analysis using sLORETA examined large-scale brain network interactions during SNB relative to SB1. Connectivity was assessed through lagged linear connectivity and lagged non-linear connectivity across multiple frequency bands. ROIs were selected from significant microstate source localization findings, enabling integration of connectivity, microstate, and cortical source results.

Lagged Linear Connectivity

Both SNB conditions showed significant alterations in lagged linear connectivity (Fig. 4A). During LNB relative to SB1, theta-band (4–8 Hz) connectivity was significantly decreased between the superior temporal gyrus and inferior parietal lobule, and between the superior temporal gyrus and insula, regions associated with auditory processing, interoceptive awareness, and attentional integration. RNB showed connectivity changes mainly in higher frequency bands. In the alpha band (8–13 Hz), decreased connectivity between the middle frontal gyrus and inferior parietal lobule suggested altered functional organization of the fronto-parietal network involved in cognitive control and attentional processes. In the beta band (13–30 Hz), widespread reductions in connectivity were observed between the superior temporal gyrus and ACC, superior temporal gyrus and superior frontal gyrus, precentral gyrus and inferior parietal lobule, and inferior parietal lobule and ACC, suggesting decreased sensorimotor integration and executive control during RNB.

Lagged Non-Linear Connectivity

Lagged non-linear connectivity revealed frequency-specific modulation, predominantly during RNB (Fig. 4B). In the alpha band (8–13 Hz), significantly decreased connectivity was observed between the superior frontal gyrus and inferior parietal lobule, middle frontal gyrus and inferior parietal lobule, and inferior parietal lobule and inferior frontal gyrus. These regions form core components of the fronto-parietal control network, indicating decreased non-linear integration supporting higher-order cognitive processing. In the beta band (13–30 Hz), significantly decreased connectivity was observed between the superior temporal gyrus and ACC, superior temporal gyrus and middle frontal gyrus, superior temporal gyrus and precentral gyrus, and middle frontal gyrus and inferior parietal lobule. These findings indicate

that RNB is associated with decreased complex non-linear interactions among temporal, frontal, and motor regions, suggesting reduced integration of cognitive and sensorimotor networks.

Discussion

SNB has long been practiced as a yogic breathing technique to improve attention, emotional regulation, and mental well-being. Previous neurophysiological studies have demonstrated that controlled breathing modulates autonomic activity, cortical oscillations, and large-scale brain networks. However, the rapid temporal dynamics underlying these neural changes remain poorly understood. EEG microstate analysis provides a useful framework to study such fast-changing brain states, as microstates represent transient, quasi-stable configurations of global brain activity lasting approximately 60–120 ms before transitioning into another state. These microstates are considered electrophysiological correlates of large-scale functional brain networks (Lehmann et al., 1987).

The present study investigated the effects of guided slow SNB (6 breaths/min) on EEG microstate dynamics, cortical source localization, and functional connectivity during LNB and RNB, compared with SB1. Significant alterations were observed in the temporal characteristics and cortical generators of Maps 1, 3, and 6, accompanied by frequency-specific reductions in functional connectivity involving fronto-parietal, temporo-parietal, insular, and cingulo-opercular networks. Together, these findings suggest that SNB induces coordinated reorganization of transient brain states and decreased large-scale neural interactions.

EEG Microstate Dynamics During SNB

Compared with SB1, SNB produced a significant reduction in the MD of Maps 1 and 3. In addition, Map 3 demonstrated reduced TF and TC, indicating decreased temporal persistence and predominance of this microstate during unilateral nostril breathing. Since microstate duration reflects the temporal stability of underlying neural networks, shorter durations are generally interpreted as greater neural flexibility and more rapid transitions between functional brain states (Lehmann et al., 1987; Khanna et al., 2015). Although shorter microstate duration is often interpreted as greater neural flexibility, alternative interpretations, including reduced temporal stability or increased state switching, should also be considered. Because these changes were observed in healthy participants during controlled breathing, they may be more consistent with adaptive modulation of neural dynamics than pathological instability. The reduced TF and TC of Map 3 further suggest decreased engagement of the underlying neural configuration, which has previously been associated with large-scale functional networks (Britz et al., 2010; Khanna et al., 2015; Michel & Koenig, 2018). Previous studies have associated reductions in microstate stability and temporal occupancy with altered attentional allocation and increased cognitive flexibility (Britz et al., 2010; Milz et al., 2016). These findings may therefore indicate redistribution of neural resources during SNB toward more adaptive and internally oriented processing states. In contrast, Map 6 showed significantly increased GEV during both LNB and RNB, reflecting greater predominance and contribution of this microstate during SNB. Increased GEV is considered indicative of stronger

engagement and synchronization of the corresponding functional network (Custo et al., 2017). The enhanced predominance of Map 6 therefore suggests increased engagement of a microstate that has previously been associated with attentional and sensory processing.

Importantly, these microstate alterations were paralleled by significant changes in functional connectivity. LNB was associated predominantly with decreased theta-band connectivity between the superior temporal gyrus, inferior parietal lobule, and insula. Theta oscillations are closely associated with attentional regulation, internally directed processing, and large-scale network integration (Khanna et al., 2015; Wang et al., 2016; Michel & Koenig, 2018). Decreased temporo-parietal and temporo-insular connectivity during LNB may reflect reduced functional integration between regions involved in interoceptive awareness and multisensory processing while preserving the cortical activation observed in source localization, consistent with the established role of the insula and temporo-parietal regions in integrating internal bodily signals and sensory information (Critchley et al., 2004; Craig, 2009; Herrero et al., 2018). In contrast, RNB demonstrated stronger modulation within alpha and beta frequency bands. Decreased alpha-band connectivity between frontal and parietal regions, along with decreased beta-band connectivity involving the superior temporal gyrus, ACC, superior frontal gyrus, precentral gyrus, and inferior parietal lobule, suggests reduced functional integration within fronto-parietal and sensorimotor network. Since alpha and beta oscillations are commonly linked to executive processing, attentional control, and active cognitive engagement, these findings suggest that RNB may reorganize executive and attentional networks by reducing functional coupling among frontal, parietal, temporal, and sensorimotor regions (Klimesch, 2012; Engel & Fries, 2010; Başar et al., 2001). Although source localization demonstrated increased activation in several cortical regions during SNB, functional connectivity analysis revealed decreased interactions among many of these same regions. These findings are not necessarily contradictory, as increased regional neuronal activity does not invariably require stronger inter-regional synchronization. Rather, this pattern may reflect greater functional segregation of cortical networks, whereby regional neuronal activation is enhanced while long-range synchronization among distributed regions is reduced, rather than indicating more efficient or selective neural processing. Importantly, reduced functional connectivity should not be interpreted as either beneficial or detrimental per se. Rather, it likely reflects altered large-scale network organization, which may arise through mechanisms such as functional segregation, reduced long-range synchronization, or task-specific network reconfiguration.

Taken together, these findings indicate that SNB induces frequency-specific reorganization of large-scale neural dynamics, characterized by reduced stability of certain microstates and increased dominance of others. Interestingly, Map 1 resembled canonical microstate Map D, which has been associated with the dorsal attention network (DAN) (Michel & Koenig, 2018). Reduced temporal stability of this map may therefore be consistent with altered attentional engagement during SNB. In contrast, the increased predominance of Map 6, together with decreased temporo-parietal and cingulo-opercular connectivity, may indicate altered integration of networks previously associated with attentional and sensory-processing networks during SNB (Custo et al., 2017; Dosenbach et al., 2008; Uddin, 2015).

Cortical Source Activity and Network Integration

Source localization analysis revealed significant alterations in the estimated cortical sources underlying Maps 1, 3, and 6 during SNB compared with SB1, indicating modulation of distributed neural systems involved in interoception, attentional control, executive processing, and sensory integration. For Map 1, SNB (particularly LNB) showed increased activation within the insula and cuneus. The insula is critically involved in interoceptive awareness and autonomic integration (Critchley et al., 2004), suggesting enhanced monitoring of internal bodily and respiratory states during SNB. This interpretation was considered alongside the observed decreased theta-band connectivity between the superior temporal gyrus and insula during LNB, suggesting that increased regional activation occurred despite reduced inter-regional functional coupling. Increased activation within posterior cortical regions, including the cuneus, may additionally reflect modulation of default mode network related processes associated with self-referential awareness and internally directed cognition (Raichle et al., 2001; Raichle, 2015; Fox et al., 2015). For Map 3, increased activation was observed within the middle frontal gyrus, superior frontal gyrus, inferior frontal gyrus, ACC, inferior parietal lobule, and superior temporal gyrus. These regions have been implicated in the fronto-parietal control and salience networks, which are involved in executive function, attentional regulation, and cognitive control (Seeley et al., 2007; Dosenbach et al., 2008; Menon & Uddin, 2010). Functional connectivity findings complemented these source localization results by demonstrating that increased regional cortical activation was accompanied by decreased inter-regional connectivity. This pattern suggests that SNB modulates local neuronal activity while simultaneously altering large-scale synchronization among distributed cortical regions. During RNB, decreased alpha- and beta-band connectivity among frontal, parietal, temporal, and cingulate regions suggested reduced communication within executive and attentional networks (Palva & Palva, 2007; Engel & Fries, 2010; Vincent et al., 2008). Increased ACC involvement is particularly noteworthy, given its role in autonomic regulation, salience detection, and conflict monitoring (Critchley et al., 2004; Botvinick et al., 2004; Menon & Uddin, 2010). The increased GEV of Map 6, together with decreased temporo-parietal connectivity, suggests that SNB increases the predominance of this microstate while reducing functional communication among distributed cortical regions. This pattern may reflect network reorganization characterized by enhanced local activity but reduced large-scale functional coupling (Britz et al., 2010; Custo et al., 2017; Michel & Koenig, 2018).

Overall, the convergence of microstate dynamics, cortical source localization, and functional connectivity findings indicates that SNB induces coordinated modulation of large-scale neural systems. Increased cortical activity within regions involved in interoception, attention, and executive processing occurred alongside decreased functional connectivity among distributed cortical networks, suggesting that unilateral nostril breathing modifies the balance between regional neural activation and large-scale network communication. LNB predominantly influenced temporo-insular and salience-related networks through reduced functional connectivity, whereas RNB was characterized by reduced fronto-parietal and sensorimotor connectivity, indicating distinct frequency-specific patterns of network reorganization during unilateral breathing.

Comparison Between LNB and RNB

No statistically significant differences were observed between LNB and RNB in microstate temporal parameters or cortical source localization, suggesting broadly comparable global neural organization across unilateral breathing conditions. Contrary to traditional hypotheses proposing pronounced lateralized autonomic and hemispheric effects of unilateral nostril breathing, both LNB and RNB produced broadly similar alterations in EEG microstate dynamics and cortical source activity. This suggests that, under the present experimental conditions, common neurophysiological effects associated with controlled unilateral breathing may predominate over nostril-specific lateralized effects. This finding is consistent with previous reports showing that microstate architecture remains relatively stable unless substantial cognitive or behavioural differences are introduced (Lehmann et al., 2005; Michel & Koenig, 2018).

Nevertheless, functional connectivity analysis revealed subtle frequency-dependent differences between the two breathing conditions. LNB predominantly showed decreased theta band connectivity involving insular and temporo-parietal regions associated with interoceptive awareness. In contrast, RNB preferentially showed decreased alpha- and beta-band connectivity within frontal, parietal, and sensorimotor networks. These findings suggest that LNB and RNB differentially reorganize large-scale cortical networks while maintaining the distinct frequency-specific patterns traditionally associated with unilateral nostril breathing. However, because both breathing conditions were performed at the same controlled respiratory rate, whereas spontaneous breathing was not, these findings should not be interpreted as reflecting nostril laterality alone.

The absence of significant differences in source localization between LNB and RNB may also reflect the limited spatial resolution inherent to sLORETA, which may reduce sensitivity for detecting subtle focal cortical differences (Pascual-Marqui, 2002; Grech et al., 2008; Michel & Brunet, 2019). Future studies using higher-resolution source localization methods or multimodal imaging approaches may help clarify finer distinctions between unilateral breathing conditions.

Overall, the present findings demonstrate that SNB modulates transient brain states, cortical activity, and reduces frequency-specific functional connectivity in a coordinated manner, indicating that unilateral nostril breathing reorganizes large-scale brain networks by altering regional activation and inter-regional communication. The integration of EEG microstate analysis, source localization, and functional connectivity provides important insight into the neurophysiological mechanisms underlying unilateral nostril breathing and supports the role of controlled breathing practices in modulating large-scale brain network organization.

Conclusion

In summary, this study demonstrates that SNB significantly modulates large-scale brain dynamics, as reflected by alterations in EEG microstate temporal properties, cortical source activity, and functional

connectivity patterns. Both LNB and RNB produced changes in microstates that have previously been associated with attentional, salience, and executive control networks, accompanied by increased activation in regions including the insula, ACC, frontal cortex, inferior parietal lobule, and superior temporal gyrus. Functional connectivity analysis further revealed frequency-specific reductions in cortical communication, with decreased theta-, alpha-, beta-band connectivity among frontal, temporal, parietal, and cingulate regions. Collectively, these findings suggest that guided SNB is associated with alterations in transient brain states by increasing activity within cortical regions involved in attention, salience, and interoception while simultaneously reducing functional connectivity among distributed brain networks. This pattern indicates a coordinated reorganization of large-scale neural communication during unilateral nostril breathing. However, the relatively small sample size, inclusion of only healthy young male participants, and cross-sectional design limit the generalizability and long-term interpretation of the findings. In addition, inherent limitations of EEG source localization and ROI-based connectivity analysis should be considered. An additional limitation is that unilateral nostril breathing was performed at a controlled rate of six breaths/min, whereas spontaneous breathing was performed at each participant's natural breathing rate. Consequently, the independent contributions of breathing rate and nostril laterality cannot be determined, and the observed neurophysiological changes may reflect the effects of slow breathing, unilateral nostril breathing, or an interaction between the two. Future studies incorporating larger and more diverse populations, longitudinal designs, multimodal neuroimaging, and cognitive-autonomic correlations may further clarify the neurophysiological significance of SNB.

Abbreviations

SNB, Single Nostril Breathing; LNB, Left Nostril Breathing; RNB, Right Nostril Breathing; EEG, Electroencephalography; SB, Spontaneous Breathing; sLORETA, Standardized Low-Resolution Electromagnetic Tomography; GEV, Global Explained Variance; MD, Mean Duration; TF, Number of Time Frames; TC, Time Coverage; ROI, Region of Interest; repeated-measures analysis of variance (RM-ANOVA); ACC, Anterior Cingulate Cortex; BA, Brodmann Area.

Declarations

Author Contributions

Kumar Abhishek: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing; **Sweta:** Methodology, Investigation, Data curation, Formal analysis, Validation, Visualization, Writing - original draft, Writing - review & editing; **Prashant Tayade:** Conceptualization, Methodology, Supervision, Project administration, Resources, Funding acquisition, Writing - review & editing; **Suriya Prakash Muthukrishnan:** Conceptualization, Supervision, Project administration, Resources, Funding acquisition, Writing - review & editing; **Simran Kaur:** Conceptualization, Supervision, Project administration, Resources, Funding acquisition, Writing -

review & editing; **Ratna Sharma:** Conceptualization, Supervision, Project administration, Resources, Funding acquisition, Writing - review & editing.

All authors contributed to the study conception and design, read and approved the final manuscript, and agree to be accountable for all aspects of the work.

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Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare that they have no competing interests.

Ethics Approval

The study was approved by the Institutional Ethics Committee of the All India Institute of Medical Sciences (AIIMS), New Delhi (Ref. No. IEC-605/06.10.2023, RP-11/2023). All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Consent to Participate

Informed written consent was obtained from all individual participants included in the study.

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Table 1

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Figures

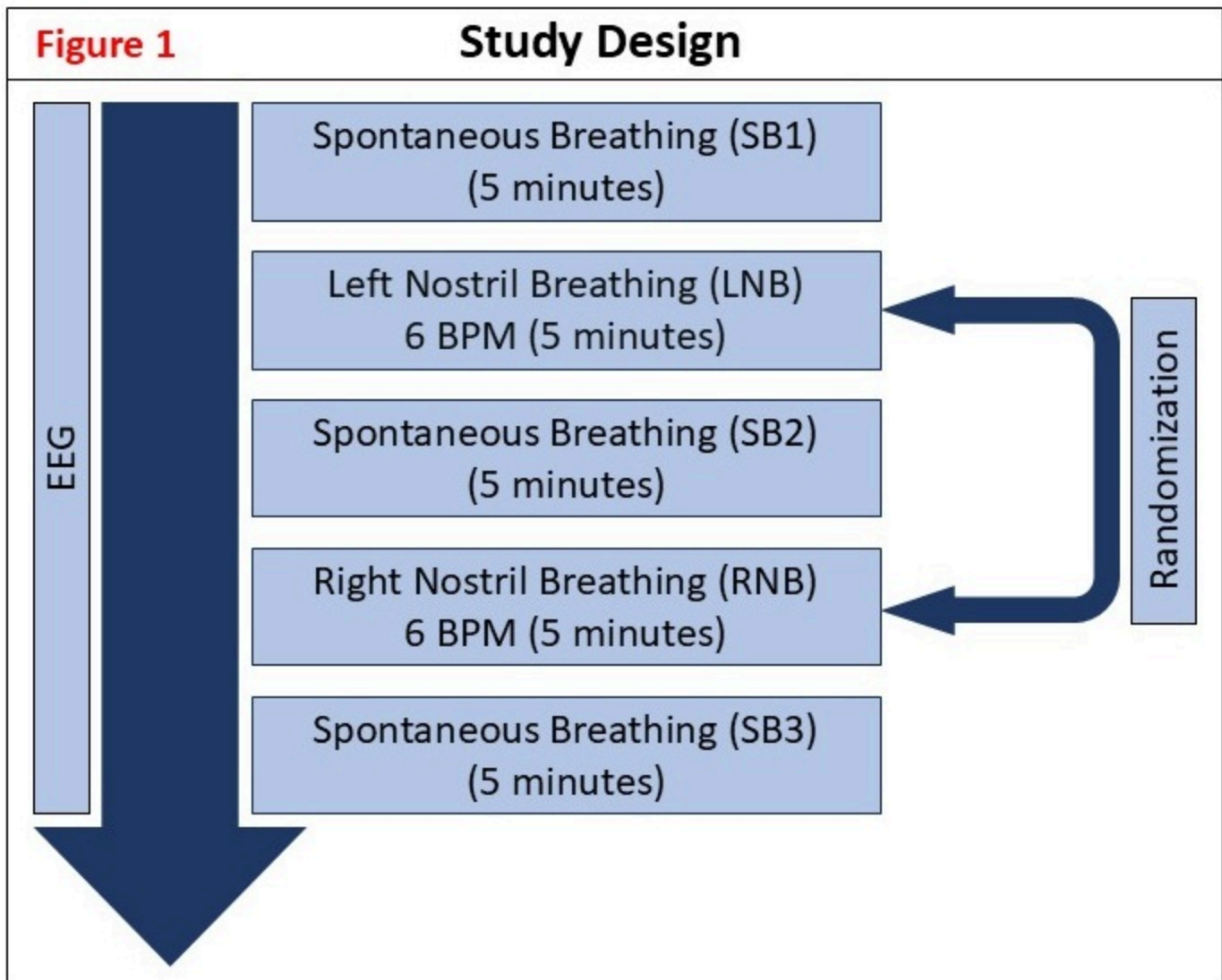


Figure 1

Study design

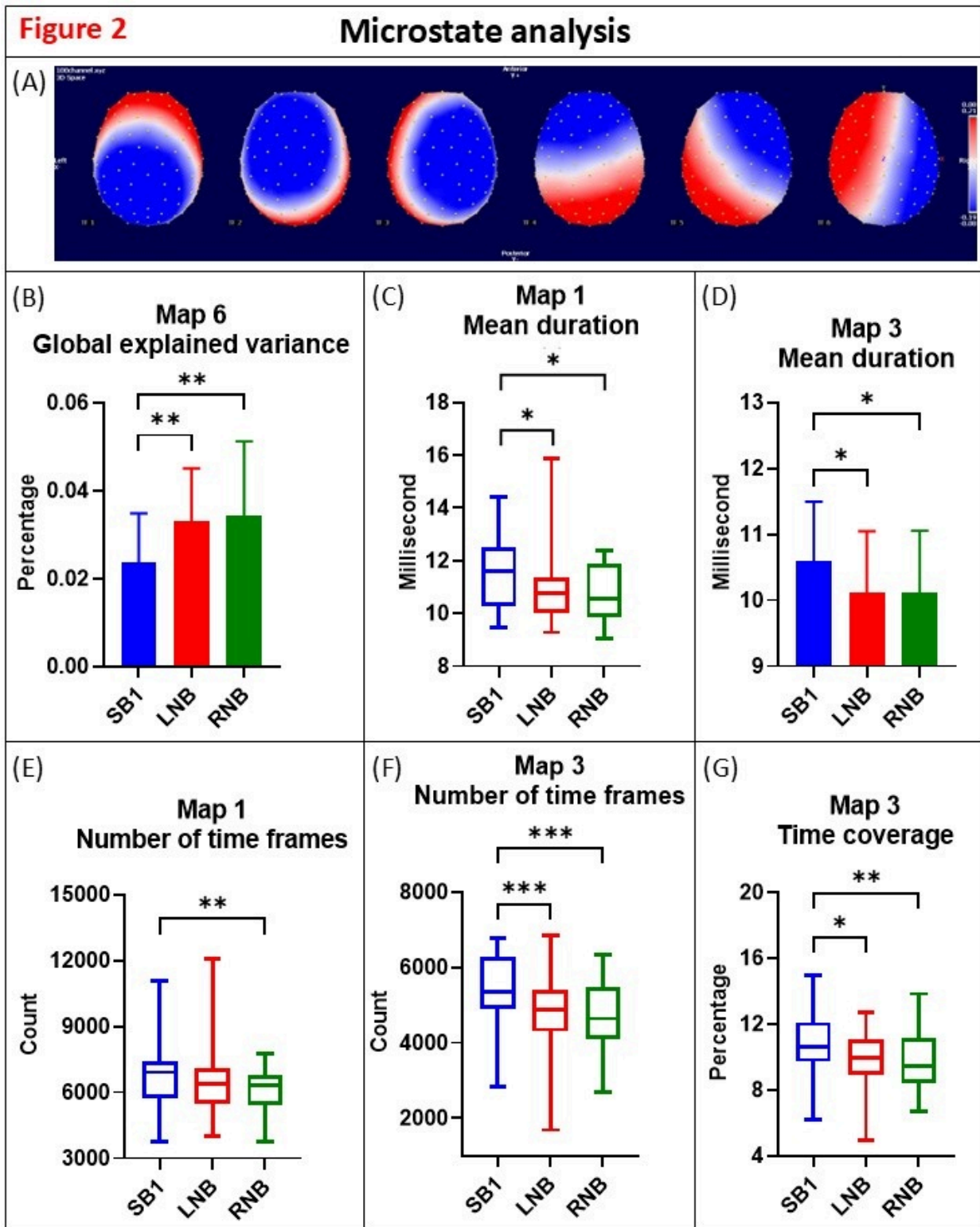


Figure 2

(A) Six scalp topographical template maps representing each condition of EEG microstates. (B-G) Microstate temporal dynamics that showed significant difference on statistical comparison between SNB (LNB/RNB) and SB1. The data is represented as Mean $\pm$ SD or median (IQR) based on the Gaussian distribution of the data; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

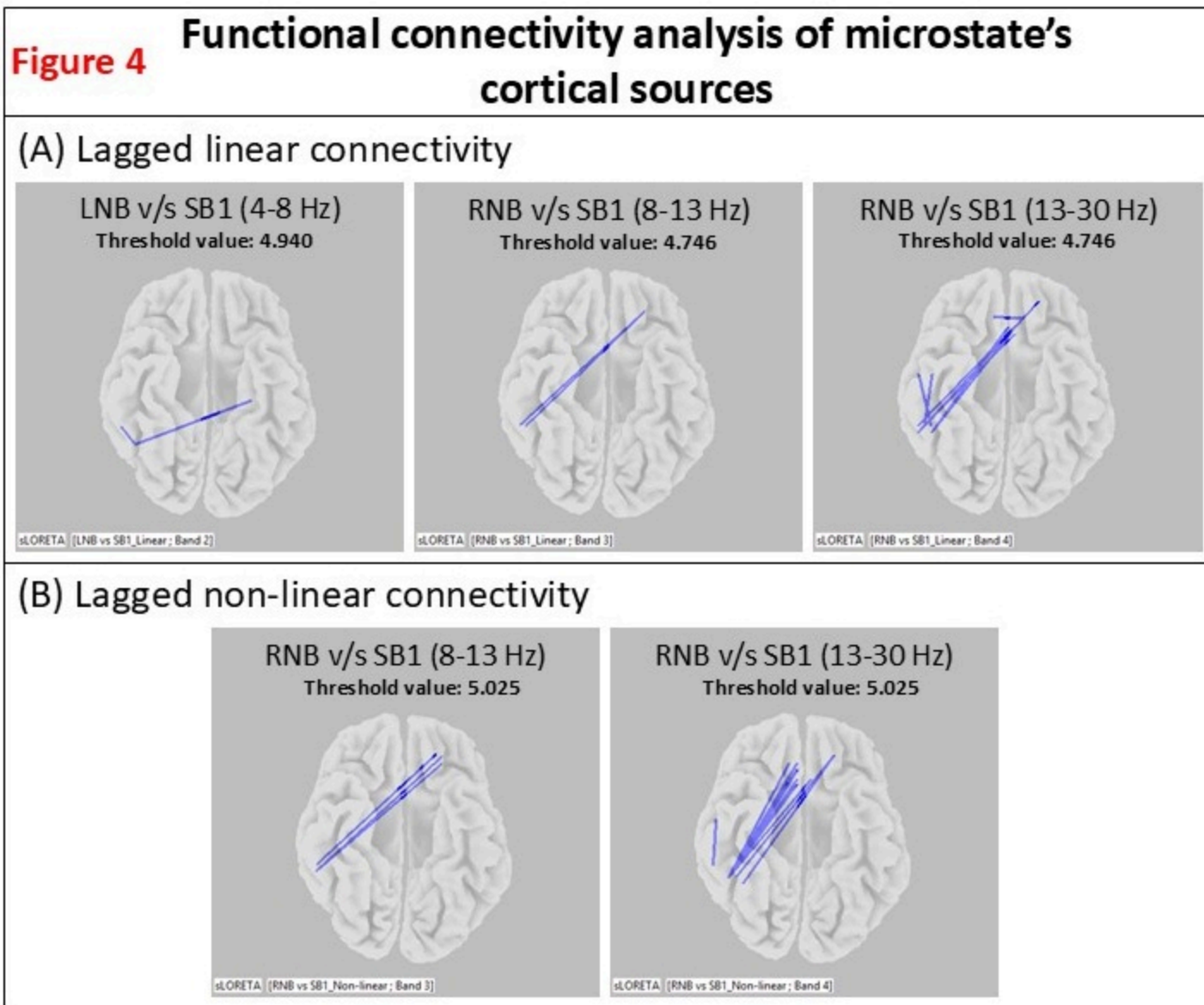


Figure 4

Functional connectivity analysis of microstate's cortical sources. (A) Lagged linear connectivity; (B) Lagged linear connectivity

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