Title: Resting-State Functional Connectivity during Controlled Respiratory Cycles using Functional Magnetic Resonance Imaging

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Abstract

This study aimed to assess the effect of controlled mouth breathing during the resting-state using functional magnetic resonance imaging (fMRI). Eleven subjects participated in this experiment in which the controlled “Nose” and “Mouth” breathings of 6 seconds respiratory cycle were performed with a visual cue at 3T MRI. Voxel-wise seed-to-voxel maps and whole-brain region of interest (ROI)-to-ROI connectome maps were analyzed in both “Nose>Mouth” and “Mouth>Nose” contrasts. As the result, there were more connection pairs in the “Mouth” breathing condition, that is, 14 seeds and 14 connecting pairs in the “Mouth>Nose” contrast, compared to 7 seeds and 4 connecting pairs in the “Nose>Mouth” contrast (false discovery rate (FDR) of \( P < 0.05 \)). The present study demonstrated that mouth breathing with controlled respiratory cycles could significantly induce alterations in functional connectivity in the resting-state network, suggesting that it can differently affect the resting brain function; in particular, the brain can hardly rest during mouth breathing, as opposed to conventional nasal breathing.

Keywords: Breathing, Mouth breathing, Controlled respiration, Resting-state fMRI, Functional connectivity, ROI-to-ROI analysis
Introduction

Humans generally breathe through the nasal respiratory pathway, but mouth breathing is inevitable in certain circumstances such as nasal congestion. Many studies have shown that abnormal respiration via the mouth has various adverse effects on the body and brain (Chlif et al., 2009; Harari et al., 2010; Jefferson, 2010; Lessa et al., 2005; Lin & Lin, 2012). There are several reasons for the functional inefficiency of mouth breathing compared to nasal breathing. First of all, our mouth cannot obstruct viruses and germs, while the nasal passages play an important role in filtering the air before it enters the lungs. Furthermore, in mouth breathing, oxygen absorption decreases due to the large amount of air exhaled, which leaves insufficient time for oxygen absorption by the lungs. In contrast, the smaller air pathways of the nose provide greater resistance to the air flow; thus, when one breathes through the nose, the lung has more time to extract oxygen, resulting in a 10–20% increase in oxygen uptake (Cottle, 1972, 1980). Additional deleterious effects of mouth breathing include low academic or arithmetic achievement in children who breathe with their mouth, along with deficiencies in working memory, reading comprehension, and learning skills (Kuroishi et al., 2015).

Since the introduction of a new functional technique by Dr. Ogawa et al in the 1990s (Ogawa et al., 1990), a number of studies have examined the effect of respiration through nose breathing on brain function using blood oxygenation level–dependent (BOLD) functional magnetic resonance imaging (fMRI) (Gozal et al., 1995, 1996; Harper et al., 1998). Some studies have compared the effects of nasal and mouth
breathing using electroencephalography (EEG) (Bell et al., 1998; Lee et al., 2019) and near-infrared spectroscopy (Sano et al., 2013). Studies to examine the respiratory mechanism in the human brain have typically used the task-based imaging modality.

A neuroimaging-based assessing method for resting-state human brain function was recently introduced. Increased neuronal activity has been noted in the default mode network (DMN) during the resting-state rather than during tasks (Gusnard et al., 2001; Raichle et al., 2001). This network includes the prefrontal cortex (PFC) and posterior cingulate cortex (PCC) medially, and the parietal and temporal cortices laterally. Functional connectivity (FC) analysis is typically performed using the DMN seeds or region of interest (ROI)-based correlation of fMRI BOLD signals throughout the brain during the resting-state (Fransson & Marrelec, 2008). A resting-state fMRI study has investigated the relationship between respiratory motion and BOLD signals using low-frequency components analysis in a time series (Birn et al., 2006). However, most resting-state fMRI experiments were conducted only in the nasal breathing condition (Fransson & Marrelec, 2008; Greicius et al., 2003; Gusnard et al., 2001; Raichle et al., 2001; van de Ven et al., 2004; Wu et al., 2014).

It is known that mouth breathing could make one susceptible to mandibular and vertical craniofacial growth, and adversely affects various cognitive functions. Based on previous studies, our hypothesis is that the resting-state FC of mouth breathing with controlled respiratory cycles can be identified and distinguished from that of normal nasal breathing because of the detrimental influence of mouth breathing on brain function. In this study, we aimed to examine the resting-state FC of the two breathing modes to investigate the unclear but undeniable effects of mouth breathing on DMN.
Materials and Methods

Subjects and data acquisition

Eleven healthy young subjects (seven men and four women; mean age, 33.27 ± 4.76 years) participated in this experiment and written consent was provided after receiving an explanation of the study. The study protocol was complied with the Declaration of Helsinki and approved by the institutional review board (GDIRB2013-23). The exclusion criteria included (1) history of neurological or psychiatric diseases, and (2) any respiratory disorders. The experiment was conducted using a 3T magnetic resonance imaging (MRI) (Siemens Verio, Erlangen, Germany) with a 12-channel radio-frequency head matrix coil. All participants were given a pair of earplugs, and underwent two MRI imaging sequences. At first, T1-weighted anatomical imaging sequence of three-dimensional magnetization–prepared rapid acquisition gradient echo (MP-RAGE) was acquired for the anatomical reference. In the second, BOLD fMRI sequence of two-dimensional echo planar imaging (EPI) was obtained for the functional imaging with a repetition time (TR) of 3000 ms, echo time (TE) of 30 ms, imaging resolution of 3×3×3 mm³, imaging slices of 46 to cover the whole brain (138 mm in z-axis), and flip angle (FA) of 90°.

Each participant breathed only through their mouth or nose depending on the visual cue presented through a beam projector at the beginning of each session. During the session, the color of a cross placed in the center of the screen (red and blue, corresponding to inhalation and exhalation, respectively) was changed every 3 sec, and the subjects were asked to maintain a constant breathing cycle (i.e., approximately 0.3
Hz) following the cross color. This protocol followed that used in a previous study, in which subjects were asked to keep their eyes open and fixated on the cross, and the results showed the highest reliability during examination of the within-network connections as well as the DMN, the attention network, and auditory connectivity (Patriat et al., 2013). Subjects were instructed not to move their head as much as possible and stay awake throughout the data acquisition process. Each subject bit a cylindrical plastic bar to ensure that the mouth remained open and to prevent systematic motion artifacts that could unexpectedly increase upon the changing of breathing modes during the two resting-state sessions (“Nose” and “Mouth” breathing conditions). The order of the sessions was randomly assigned between subjects. Each session included the collection of 102 dynamic data volumes for about 5 min.

**Data processing and statistical analysis**

A MATLAB-based CONN FC-toolbox software (www.nitrc.org/projects/conn) was used for preprocessing, denoising and statistical analysis. For preprocessing, we discarded the first two volumes for MRI signal stabilization, and then we realigned the other functional volumes to the first volume as a reference for head motion compensation. And they were co-registered to the structural volume, segmented the grey and white matters and cerebrospinal fluid (CSF) regions, normalized to the EPI template of 2×2×2 mm³, and smoothed with a Gaussian kernel of 8 mm full width half maximum. For denoising BOLD signals, white matter and CSF were considered as additional confounds and linear regression was used for this denoising process. And then band-pass filtering of 0.008 to 0.09 Hz was performed to remove the subject’s estimated motion parameters and other artificial effects.
For statistical analysis, the strength and significance of ROI pairs within all subjects’ data in the “Nose” and “Mouth” breathing conditions were calculated by CONN toolbox. To reduce the skewness of the distribution of connectivity values caused by motion and/or physiological noise sources, linear detrending was applied, which made the histogram of mean BOLD signals approximately centered and normalized for the regression processing after temporal preprocessing. To measure the level of linear association of the BOLD time series, a bivariate correlation was used to conduct the first-level analysis, in which the effect size is the correlation coefficient (Whitfield-Gabrieli & Nieto-Castanon, 2012).

In the CONN toolbox, 163 ROIs consist of the followings: 1) an atlas for cortical and subcortical regions from FMRIB Software Library (FSL) Harvard-Oxford Atlas and cerebellar regions from the automated anatomical labeling (AAL) atlas (atlas.nii), and 2) an atlas for networks (networks.nii) including medial PFC (MPFC), PCC, right lateral parietal (RLP), and left lateral parietal (LLP) regions. In total, 132 ROIs are obtained from atlas.nii, and 31 ROIs in 8 networks from networks.nii (Whitfield-Gabrieli & Nieto-Castanon, 2012).

First, the PCC among the DMN hubs was selected to construct a seed-to-voxel FC map in each “Nose” and “Mouth” breathing condition for the second level analysis. Note that the PCC was selected since it usually plays the role of a hub seed for resting-state fMRI seed-to-voxel FC analysis (Whitfield-Gabrieli & Nieto-Castanon, 2012). The maps are obtained at a false discovery rate (FDR)-corrected height threshold of \( P < 0.05 \), and a FDR-corrected cluster-sized extent threshold of \( P < 0.05 \), with 1000 simulations for non-parametric statistics. Second, the ROI-to-ROI FC was computed in the entire brain to show the correlation between all ROI seeds, and analyze the global
characteristics of human brain networks in each “Nose” and “Mouth” breathing condition, as well as in the “Nose>Mouth” and “Mouth>Nose” contrasts. The connectome maps are obtained at a FDR-corrected height threshold of $P < 0.05$.

**Results**

In the seed-to-voxel map, DMN hubs including the MPFC, PCC, and right and left LLP areas significantly and positively correlated during the resting-state “Nose” and “Mouth” breathing conditions (Fig. 1 and Fig. 2). The ratio and number of cluster voxels in the frontal medial cortex (FMC) during the “Nose” condition were 7% of the FMC and 68 voxels (Fig. 1a), while those during the “Mouth” condition were 37% of the FMC and 367 voxels (Fig. 1b). In the precuneus, the ratio and number of cluster voxels during “Nose” condition were 93% of the precuneus and 5211 voxels (Fig. 2a), while those during “Mouth” condition was 92% of the precuneus and 5147 voxels (Fig. 2b). Additionally, neither “Nose>Mouth” nor “Mouth>Nose” contrast made a statistical difference on the seed-to-voxel FC analysis.

Fig. 3 and Fig. 4 showed the connectome maps obtained from the ROI-to-ROI group analysis. There were more resting connections between all ROI seeds during the “Mouth” breathing condition compared to the “Nose” condition (Fig. 3). As shown in Fig. 4, there were 14 seeds and 14 connection pairs in “Mouth>Nose” contrast, but only 7 seeds and 4 connection pairs in the “Nose>Mouth” contrast. In particular, in the “Mouth>Nose” contrast, the right superior parietal lobule (SPL) seed was connected to 5 seeds: The posterior superior temporal gyrus (pSTG) of the language network, the
right planum temporale (PT), both right and left temporooccipital middle temporal gyrus (toMTG), and the left pSTG. Statistical results for the connection pairs, including $T$ and $P$ values, are summarized in Table 1.

**Discussion**

To demonstrate whether mouth breathing changes resting-state FC in the human brain, we examined FC networks in both “Mouth>Nose” and “Nose>Mouth” contrasts using seed-to-voxel and ROI-to-ROI analyses at 3T fMRI. To the best of our knowledge, no experimental study has been reported investigating FC differences between nasal and mouth breathing during the resting-state using fMRI. In the seed-to-voxel second-level results (Fig. 1 and Fig. 2), the PCC seed positively correlated with other DMN hubs not only in the ”Nose” but also the “Mouth” breathing condition, as previously shown in many seed-based correlation analysis studies (Fransson & Marrelec, 2008; Gusnard et al., 2001; Raichle et al., 2001).

The resting connection patterns based on connectome maps from “Nose” and “Mouth” breathing conditions were different, as shown in Fig. 3. In order to confirm the statistical difference between the two breathing conditions, we additionally conducted ROI-to-ROI analysis in both “Nose>Mouth” and “Mouth>Nose” contrasts. For ROI-to-ROI FC connectome analysis, in the “Mouth>Nose” contrast in the entire brain, the right SPL seed had the maximum number of connections (Table 1 and Fig. 4). In detail, the seed was linked to right PT, right and left toMTG, and left pSTG, but was mostly located in the temporal parietal cortex. This connecting network indicates that mouth breathing is deeply correlated with communication between the limbic system and the
posterior regions (temporal and parietal cortices), as shown in recent studies (Park & Kang, 2017; Zelano et al., 2016). Although previous studies have used task-based fMRI and intracranial EEG, which differ from the present experiment, the results proved that the limbic system, including e.g. the hippocampus, was associated with and/or influenced by a cognitive task, in a differential way between nasal and mouth breathing. Furthermore, nasal breathing is a critical source of the production of nitric oxide (NO) that is an essential vasodilator that regulate on vascular smooth muscles and then oxygen delivery, and increases the oxygen transport throughout the human body (Džoljić et al., 2015; Jon O. Lundberg et al., 2015). However, mouth breathers who have a limit to produce NO due to the pathway blockade have been shown to have a lower oxygen concentration in their blood than nasal breathers (J. O. Lundberg et al., 1996; Jon O. Lundberg et al., 2015).

In this study, mouth breathing during resting-state produced the more BOLD-based connection pairs between the limbic system (temporal cortex) and the posterior part (parietal cortex), but not the anterior part (frontal cortex), compared to conventional nasal breathing during resting-state. In the previous study, however, the oxygen load in the PFC was correlated with different patterns between nasal and mouth breathing (Sano et al., 2013). Disparity between these studies may come from the different signal examined in this study, namely the connection pattern between contrasts.

Although the present result provides a new finding about effects of mouth breathing on the brain, we should note that no physiological data, including real respiratory and cardiac signals, have been used as covariates for FC analysis in resting-state fMRI studies (Birn, 2012; Birn, Murphy, et al., 2008; Birn, Smith, et al., 2008; Khalili-Mahani et al., 2013). However, this type of noise would not have produced false positive results...
in the current experiment, since heart rate has only a local effects due to the beating of blood vessels (Chang et al., 2009; Dagli et al., 1999), and small fluctuations in end-tidal CO\textsubscript{2} during normal breathing at rest occurred at a frequency range of 0 to 0.05 Hz (Wise et al., 2004). Further studies should be undertaken using resting-state fMRI without controlled respiration cycles (closed eyes), to focus on voluntary self-control respiration through nose or mouth. In addition, breath-dependent brain activation should be investigated in further studies with multiple and appropriate task-rest sessions to examine how brain activation is affected by breathing type.

In conclusion, here we examined the resting-state FC during mouth versus nasal breathing, demonstrating that mouth breathing induced significantly increased FC in several ROIs in the entire brain compared to nasal breathing. When we investigated the effect of mouth breathing using the FC analysis, the node with more connections with ROIs in mouth breathing, and specifically during the resting-state with controlled respiration, was revealed to be the SPL, suggesting that habitual mouth breathing could affect the functional brain relationships of those regions. Therefore, our result suggests that the role played by acute mouth breathing in the resting brain is unexpected but crucial, since it is widely known that mouth breathing due to nasal obstruction has adverse health effects. As a consequence of long-term (habitual) mouth breathing, irreversible effects on brain function can induce cognitive problems. These considerations could underline the opportunity of undertaking further investigations of the principles underlying the observed resting-state difference of the two breathing manners for both research and clinical purposes.
Acknowledgements

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Conflicts of Interest

We declare that there is no conflict of interest regarding the publication of this paper.

Authors’ Contributions

Conceptualization, Author names [all authors]; Methodology, Author names [Kang CK, Park CA]; Investigation, Author names [Park CA]; Writing – Original Draft, Author names [Kang CK, Park CA]; Writing – Review & Editing, Author names [Kang CK, Park CA]; Funding Acquisition, Author names [Lee YB, Kang CK]; Resources, Author names [Lee YB, Kang CK]; Supervision, Author names [Kang CK]
References


Table 1. ROI-to-ROI connections of the entire brain using a second-level group analysis of all selected ROI seeds (one-sided positive and seed-level FDR-corrected threshold of \( P < 0.05 \), “Nose>Mouth” and “Mouth>Nose” contrasts).

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Pair connection</th>
<th>Statistics (T)</th>
<th>Uncorrected P</th>
<th>FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nose&gt;Mouth</td>
<td>PaCiG (R) – Visual Lateral(^\d)</td>
<td>5.99</td>
<td>0.0001</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>aPaHC (R) – Accumbens (L)</td>
<td>5.70</td>
<td>0.0001</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>Salience Anterior Insula(^\d) – OP (L)</td>
<td>5.46</td>
<td>0.0001</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td>Visual Lateral(^\d) – Fronto Parietal PPC(^\d)</td>
<td>4.72</td>
<td>0.0004</td>
<td>0.033</td>
</tr>
<tr>
<td>Mouth&gt;Nose</td>
<td>SPL (R) – Language pSTG(^\d)</td>
<td>6.42</td>
<td>0.0000</td>
<td>0.0047</td>
</tr>
<tr>
<td></td>
<td>SPL (R) – PT (R)</td>
<td>6.10</td>
<td>0.0001</td>
<td>0.0047</td>
</tr>
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<td>SPL (R) – toMTG (R)</td>
<td>5.55</td>
<td>0.0001</td>
<td>0.0066</td>
</tr>
<tr>
<td></td>
<td>SPL (R) – pSTG (L)</td>
<td>4.75</td>
<td>0.0004</td>
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<td>SPL (R) – toMTG (L)</td>
<td>4.19</td>
<td>0.0009</td>
<td>0.0305</td>
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<tr>
<td></td>
<td>Precuneus – aMTG (R)</td>
<td>5.82</td>
<td>0.0001</td>
<td>0.0136</td>
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<tr>
<td></td>
<td>Precuneus – Cerebellar Anterior(^\d)</td>
<td>4.54</td>
<td>0.0005</td>
<td>0.0440</td>
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<td>Dorsal Attention IPS(^\d) – toMTG (R)</td>
<td>5.80</td>
<td>0.0001</td>
<td>0.0141</td>
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<td>Dorsal Attention IPS(^\d) – toMTG (L)</td>
<td>5.30</td>
<td>0.0002</td>
<td>0.0143</td>
</tr>
<tr>
<td></td>
<td>Dorsal Attention IPS(^\d) – Language pSTG(^\d)</td>
<td>4.36</td>
<td>0.0007</td>
<td>0.0387</td>
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<tr>
<td></td>
<td>Default Mode PCC(^\d) – Cerebellar Anterior(^\d)</td>
<td>5.18</td>
<td>0.0002</td>
<td>0.0274</td>
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<td>Default Mode PCC(^\d) – aMTG (R)</td>
<td>4.85</td>
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<td></td>
<td>PostCG (R) – Cereb45 (R)</td>
<td>5.04</td>
<td>0.0003</td>
<td>0.0413</td>
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<tr>
<td></td>
<td>toMTG (R) – TOFusC (L)</td>
<td>4.24</td>
<td>0.0009</td>
<td>0.0463</td>
</tr>
</tbody>
</table>

Footnotes
Abbreviations: FDR, false discovery rate; ROI, region of interest; R and L, right and left; PaCiG, paracingulate gyrus; aPaHC, parahippocampal gyrus (anterior division); OP, occipital pole; PPC, posterior parietal cortex; SPL, superior parietal lobule; pSTG, superior temporal gyrus (posterior division); PT, planum temporale; toMTG, middle temporal gyrus (temporooroccipital part); aMTG, middle temporal gyrus (anterior division); IPS, intraparietal sulcus; PCC, posterior cingulate cortex, PostCG, postcentral gyrus; Cereb45, cerebellum 45; TOFusC, temporal occipital fusiform cortex; \(^\d\), network.
Fig. 1 Seed-to-voxel maps using the main effect of PCC in the DMN during “Nose” (a) and “Mouth” (b) breathing conditions. The maps are obtained at a height threshold FDR of $P < 0.05$ and cluster-sized extent threshold FDR of $P < 0.05$, with 1000 simulations for non-parametric statistics, in the axial view. The internal distance between adjacent slices is 3 mm. The color bar represents statistical $T$ values.

Abbreviations: A, P, R, and L, anterior, posterior, right and left; PCC, posterior cingulate cortex; DMN, default mode network; FDR, false discovery rate.
**Fig. 2** Seed-to-voxel maps using the main effect of PCC in the DMN during “Nose” (a) and “Mouth” (b) breathing conditions. The maps are obtained at height threshold FDR of $P < 0.05$ and cluster-sized extent threshold FDR of $P < 0.05$, with 1000 simulations for non-parametric statistics, in the coronal view. The internal distance between adjacent slices is 6 mm. The color bar represents statistical $T$ values.

Abbreviations: A, P, R, and L, anterior, posterior, right and left; PCC, posterior cingulate cortex; DMN, default mode network; FDR, false discovery rate.
**Fig. 3** ROI-to-ROI functional connectivity based three-dimensional rendering connectome maps during “Nose” (a) and “Mouth” (b) breathing conditions. The rings are obtained at a FDR-corrected height threshold of $P < 0.05$. The color bar represents statistical $T$ values.

Abbreviations: FDR, false discovery rate; ROI, region of interest.
Fig. 4 ROI-to-ROI three-dimensional rendering connectome maps in “Nose>Mouth” (a) and “Mouth>Nose” (b) contrasts. The rings are obtained at a FDR-corrected height threshold of $P < 0.05$. The color bar represents statistical $T$ values.

Abbreviations: FDR, false discovery rate; ROI, region of interest; R and L, right and left; PaCiG, paracingulate gyrus; aPaHC, parahippocampal gyrus (anterior division); OP, occipital pole; PPC, posterior parietal cortex; SPL, superior parietal lobule; pSTG, superior temporal gyrus (posterior division); PT, planum temporale; toMTG, middle temporal gyrus (temporoccipital part); aMTG, middle temporal gyrus (anterior division); IPS, intraparietal sulcus; PCC, posterior cingulate cortex, PostCG, postcentral gyrus; Cereb45, cerebellum 45; TOFusC, temporal occipital fusiform cortex; §, network.