Effects of mandibular advancement on brain activation during inspiratory loading in healthy subjects: a functional magnetic resonance imaging study

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REFLEX VENTILATORY COMPENSATION is essential for regulation of the upper airway to counteract the negative intraluminal pressure exerted during the inspiratory phase of respiration (9). In the normal upper airway, recovery from obstruction occurs promptly through the marked activation of airway-dilating muscles (26). In contrast, neuromuscular failure and/or anatomical complications of the upper airway sometimes result in recurrent nocturnal obstruction of the airway (28), i.e., obstructive sleep apnea (OSA). Continuous positive airway pressure (CPAP) has been regarded to be the gold standard in the treatment of OSA (35). It is noteworthy that many of these areas overlapped regions of previously demonstrated gray matter loss (36). Moreover, it has been reported that patients with OSA show aberrant cortical activity in association with abnormal cardiovascular responses to cold pressor effects (22) and inspiratory loading (37). Although the cause-and-effect relationship between anatomical and functional deficiencies has not been determined, the examination of neural sites that characterize OSA may reveal the neurogenic nature and/or consequences of the disorder (19). It should be noted that the pathological background of OSA is not straightforward, rather there is the variability in signs and symptoms and effectiveness of treatment alternatives (e.g., CPAP, OA), according to factors that included the severity of the disease, obesity, and gender (1, 17, 18, 23, 40, 42). Thus, for better understanding of the possible interaction between neural deficits and responses to the OA for a wide spectrum of OSA, it would be necessary to collect the baseline knowledge regarding mandibular advancement in healthy subjects. Furthermore, it seems interesting to investigate whether the change in neural activities caused by respiratory loading would occur in association with mandibular advancement.

We hypothesized that the brain activation caused by respiratory stress, such as by resistive inspiratory loading, would be alleviated by advancement of the mandible in association with an improvement in the subjective rating of the level of discomfort. Therefore, the objective of this study was to use the blood...
oxygenation level-dependent (BOLD) fMRI to investigate the localization of normal brain activation associated with changes in the anteroposterior mandibular position in normal subjects to determine whether and how mandibular advancement affected the central nervous system. Resistive inspiratory loading was experimentally applied to simulate the clinical aspects of upper airway obstruction (5, 11, 20, 27, 35).

MATERIALS AND METHODS

Subjects

Twelve male volunteers participated in the study. The mean age was 28.3 (range: 25–36) yr, and the mean body mass index was 21.6 (range: 18.2–26.3) kg/m². All of the subjects were healthy and were not receiving medications at the time of the study, and none had a history of neurological or psychological illness. All of the subjects were strongly right-handed (45). The study conformed to the Declaration of Helsinki and was approved by the institutional ethical committee. Written informed consent was obtained from each subject before commencement of the study.

After informed consent was obtained from the subjects, their ability to anteriorly move the mandible was determined by using the George gauge (Great Lakes Orthodontics, Tonawanda, NY; Fig. 1A). A bite registration was then made for each subject with silicone putty (EXAFINE, GC, Tokyo, Japan) in two positions with minimal vertical jaw opening (Fig. 1B): one in the rest position [i.e., no mandibular protrusion (MAX0)] and the other at 67% (MAX67) of the maximum protrusive position (MAX100). The custom-made silicone bite registration for each subject was used in both the behavioral and fMRI studies.

Behavioral Study

All of the 12 subjects participated in the study. Each subject was asked to lie comfortably in the supine position on a dental chair with the silicone bite in place and to breathe via a face mask (no. 6850, Vital Sign, Totowa, NJ) through two one-way non-rebreather respiratory valves (no. 1644/no. 1655, Hudson RCI, Temecula, CA) to regulate the direction of the inspired and expired gases. The inspiratory limb of the non-rebreather valve was connected to a silicone tube (4 mm in internal diameter). The dead space of the system was estimated to be ~0.9 liter, and resistance to airflow was 10 cmH₂O/s at flow between 5 and 30 l/min, a condition that was considered to exert “low loading” (LL) on the upper airway. In contrast, a plastic tube (2.5 mm in diameter) was also fitted to the end of the silicone tube of the inspiratory limb. This condition was considered to exert “high loading” (HL) on the upper airway. A constant resistance of ~30 cmH₂O/s at flow between 5 and 30 l/min was obtained in the HL condition. During spontaneous breathing, each subject randomly underwent four different (i.e., LL/MAX0, LL/MAX67, HL/MAX0, and HL/MAX67) 40-s conditions, four times each.
Breathlessness was evaluated by the subject every 40 s with the use of a visual analog scale (VAS), which was formed from a standard 10-cm VAS with anchors at “0” and “100” (41, 49). The subject was asked to grade on an arbitrary “point” scale, with a minimum of 0 and a maximum of 100. After completion, average scores for the four conditions were calculated from the 16 ratings for each subject. After VAS scores of LL/MAX67, HL/MAX0, and HL/MAX67 were standardized to that of LL/MAX0, changes in the VAS score were compared among the four conditions. Friedman’s χ² r-test and the Student-Newman-Keuls test were used to establish statistical significance. Statistical significance was set at P < 0.01. All procedures were performed with commercially available statistical software (Excel 2003, Microsoft, Redmond, WA).

fMRI Study Design

First, a pilot study (experiment 1) was undertaken in 12 subjects. The task used a block design (Fig. 1C). Each task block consisted of eight periods of scan time (3.1 s) and eight periods of no-scan time (1.9 s). Therefore, the repetition time (TR) totaled 5.0 s, whereas the effective TR was 3.1 s. Immediately after each task block, an auditory instruction to change the mandibular position was presented to the subject through a loudspeaker. The verbal instructions were either “back” or “forward,” which indicated either MAX0 or MAX67, respectively. Each subject changed the mandibular position within the last no-scan time (1.9 s) to eliminate motion artifacts and maintained the mandible at rest until the next instruction (40 s later). An fMRI session began with three dummy scans to stabilize the effect of T1 time (15 s), and this was followed by the LL/MAX0 block. Therefore, LL/MAX0 was regarded as the baseline condition; it was repeated 16 times in every second block. The second and fourth blocks were HL/MAX67 and HL/MAX0, respectively. Thereafter, HL/MAX0 and HL/MAX67 tasks were randomly repeated eight times in every second block. The duration of each fMRI session was 21 min 35 s, including dummy scans.

Brain Scanning and Analysis

Several measures were taken to minimize motion artifacts; we used a vacuum pillow, placed form pads on either side of the subject’s head, and secured the forehead of the subject to the head/neck coil using a strap. Moreover, each subject was exposed to the same experimental protocol in the behavioral study, which had been undertaken before the fMRI study, and was trained to perform the maneuver while concentrating on keeping his head stationary. Thirty transaxial slices with a gradient-echo echo-planar T2* sequence (echo time = 60 ms, flip angle = 90°, matrix = 64 × 64 mm, field of view = 256 × 256 mm, voxel size = 3 × 3 × 5 mm) covering the entire brain were acquired at an effective TR of 3.1 s using a 1.5-T apparatus (Magnetom Vision, Siemens, Erlangen, Germany). Therefore, eight scans were obtained during each block. The initial three scans in each session were dummy scans to equilibrate the state of magnetization and were excluded from the analysis. All preprocessing procedures and data analyses were performed by using Statistical Parametric Mapping 99 (SPM99) software (Wellcome Department of Cognitive Neurology, London, UK) implemented in MATLAB (Mathworks, Natick, MA) and customized software. The effect of head motion across the scans was corrected by realigning all of the scans to the first one, using a least sum squares method with three-dimensional sinc interpolation (14). If head motion exceeded 1.0 mm in any of three dimensions, the data were excluded. The data were then spatially normalized to a Talairach space (51) and classified into the gray

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Table 1. Raw VAS scores in the four conditions

<table>
<thead>
<tr>
<th>Conditions</th>
<th>LL/MAX0</th>
<th>LL/MAX67</th>
<th>HL/MAX0</th>
<th>HL/MAX67</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAS score</td>
<td>25.8 (16.9–36.7)</td>
<td>19.3 (14.6–36.7)</td>
<td>46.0 (23.1–56.2)</td>
<td>30.3 (18.5–42.8)</td>
</tr>
</tbody>
</table>

Both the 25th and 75th percentiles (%) are shown in parentheses. VAS, visual analog scale; LL, low-inspiratory loading; HL, high-inspiratory loading; MAX0, resting mandibular position (no mandibular protrusion); MAX67, 67% of maximum mandibular protrusion.

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Fig. 2. Changes in the visual analog scale score in the 4 conditions. Solid circles depict individual values. Values in the LL/MAX67, HL/MAX0, and HL/MAX67 conditions were standardized to those at LL/MAX0. NS, not significant. **P < 0.01.
matter, white matter, and cerebrospinal fluid. Only the gray matter images were used for the subsequent preprocessing and analysis. Next, each subject’s functional data were smoothed with a Gaussian kernel in a spatial domain (10-mm full-width at half-maximum) to improve the signal-to-noise ratio. Moreover, data were detrended by using customized software (38) to account for global signal changes.

Statistical tests were performed for the group and for each individual to determine whether regional BOLD signal changes were significantly related to the task. Low-frequency confounding effects were removed by using an appropriate high-pass filter (99 s). A model was hypothesized in which the hemodynamic response of the activation fields to the MAX0 and MAX67 blocks in experiment 1 and the LL/MAX0, HL/MAX0, and HL/MAX67 blocks in experiment 2 was assumed to be a convolution of the hemodynamic response function that SPM99 provides and a box-car function that equals 1 during the respective block and is otherwise 0. Multiple regression analyses were performed on each voxel on an inrasubjective basis to detect regions in which changes in the magnetic resonance signal were correlated to the convolution of the hemodynamic response function and box-car function to obtain the partial correlation coefficients of each voxel during each MAX0 and MAX67 block in experiment 1 and each LL/MAX0, HL/MAX0, and HL/MAX67 block in experiment 2. Subtraction images were obtained from the contrast of MAX0-MAX67 in experiment 1. Regions of task-related differences in fMRI signal intensity were represented by maps of the T-statistics generated by SPM99 ($P < 0.05$, corrected for multiple comparisons). In experiment 2, subtraction images were obtained from the contrasts of HL/MAX0-LL/MAX0, HL/MAX0-HL/MAX67, and HL/MAX67-HL/MAX0. Intersubject maps were then constructed by performing a one-sample $t$-test ($P < 0.05$, corrected for multiple comparison) on the partial correlation coefficients of each voxel of each image to identify the voxels that had significantly large partial correlation coefficients.

A volume of interest analysis was performed on the adjusted images. The average intensity of the voxels for two conditions (HL/MAX0 and HL/MAX67) within each subject’s volume of interest was extracted, resulting in time trends of the signal within the activated region of intersubject maps.

RESULTS
Subjective Evaluation of Breathlessness Using the VAS Score

Table 1 and Fig. 2 show the changes in the VAS score obtained from the 12 subjects under four conditions. With regard to a change in the VAS score in association with the change in inspiratory loading in a given mandibular position, the VAS score significantly increased with HL (median ratio: 1.45) compared with that with LL when the mandible was at MAX0. Similarly, the VAS score significantly increased with HL (median ratio: 1.14) compared with that with LL (median ratio: 0.96) in MAX67. Thus the VAS score increased in association with an increase in the inspiratory loading, regardless of the mandibular position. With regard to the change in the VAS score in association with a change in the mandibular position during the given inspiratory loading, there were no significant differences in the VAS score between MAX0 and MAX67 during LL. On the other hand, the VAS score significantly decreased when the mandible was advanced from MAX0 to MAX67 during HL.

Effects of Changes in Mandibular Position on Cortical Activation

For this and the following fMRI studies, data from all of the subjects were included, as the head motion of the subjects did not exceed 1.0 mm in any of the three dimensions. No significant ($P < 0.05$, corrected for multiple comparison) activation was observed in the contrast MAX0-MAX67 and MAX67-MAX0.

Changes in Cortical Activation in Association With a Change in Inspiratory Loading

Areas that showed significant signal increases in response to inspiratory loading without a change in mandibular position are indicated in Fig. 3. Significant activations were found in the right cingulate gyrus, left middle frontal gyrus, cerebellum, bilateral basal ganglia, parahippocampal gyr, inferior frontal gyr, insula, and inferior parietal lobule.

Changes in Cortical Activation in Association With a Change in the Mandibular Position During HL

The results of the relative BOLD contrasts of HL/MAX0-HL/MAX67 are shown in Fig. 4. This reveals that there were significant activations in several cortical regions, including the left cingulate gyrus and middle frontal gyr in both hemispheres. Time trends of percent BOLD signal changes in these cortical regions are shown in Fig. 5. The stereotaxic coordinates of the activation maxima within these regions, their level of significance, and the size of activated foci are shown in Table 2. On the other hand, no other significant activation was observed in the contrast of HL/MAX67-HL/MAX0.

DISCUSSION

The present results showed that inspiratory loading was perceived as breathing discomfort under both the anterior and posterior positions of the mandible. Moreover, mandibular advancement did not significantly affect breathlessness in LL. In contrast, mandibular advancement did significantly amelio-
rate the subjective perception of breathlessness in HL. Furthermore, several cortical regions were activated in association with posterior positioning of the mandible during inspiratory loading. Thus these activations were diminished by mandibular advancement. Regions that showed enhanced BOLD signal changes followed by deactivation through mandibular advancement during resistive inspiratory loading coincided with previously recognized sites that control the activities of upper airway muscles, the initiation of inspiratory effort, and cardiovascular function. To the best of our knowledge, this is the first study to visualize the effect of mandibular advancement during respiratory loading. This study shed some light on the neural underpinnings or neural consequences of treatment using mandibular advancement.

**Methodological Considerations**

The behavioral study demonstrated that breathlessness was tempered in association with mandibular advancement upon inhalation during HL. MAX67 was selected as a protrusive position of the mandible for placement of the OA in the treatment of OSA, as MAX67 is the most popular anterior position of the mandible to start an OA (33). Morphologically, MAX67 significantly enlarges the velopharyngeal dimension compared with MAX0 in both subjects with OSA and healthy controls (25, 53). Functionally, mandibular advancement from MAX0 to MAX67 has been shown to be associated with a significant reduction in nasal resistance (44). In addition, MAX67 has been reported to be associated with fewer side effects, such as discomfort or strain to the temporomandibular joint and jaw muscle, compared with MAX100 on a 2- to 4-yr basis (7, 50, 56). On the other hand, three conditions (i.e., LL/MAX0, HL/MAX0, and HL/MAX67) were included in the fMRI study based on several findings in the behavioral study. First, there were no significant differences in the VAS score between MAX0 and MAX67 during LL. This was interpreted to mean that the subject felt no marked difficulty in breathing during LL. Second, an attempt was made to simulate the collapsible upper airway by applying HL to evaluate the functional changes in the brain, since there was a significant decrease in the VAS score when the mandible was advanced during HL.

Motion artifacts are a critical issue in the fMRI environment. To minimize motion artifacts, several measures were taken. The success of these procedures was made evident by the
finding that at no time did any subject’s head move >1.0 mm in any dimension. In addition, motion correction was performed on the image sets of all of the subjects. All of the above measures contributed to minimize motion-related brain activation during scanning. Moreover, each subject was instructed to change his mandibular position as quickly as possible within the no-scan time and to secure his teeth in the bite registration once the mandibular position was changed to either MAX0 or MAX67. This instruction was effective for avoiding activation of the primary motor cortex, which is associated with mandibular movement.

Although we did not record physiological data during inspiratory loading, subjects were likely to be hypercapnic during inspiratory loading, which produced large global signal changes. Since fMRI detects regional changes in the BOLD signal corresponding to neuronal responses, these regional signal changes often occur in the presence of global signal changes that are of no interest in this study. These global effects need to be removed or partitioned in subsequent analyses. Therefore, the data were analyzed by using a voxel-level linear model of the global signal (LMGS) (38), which removes both low- and high-frequency components, along with both large-amplitude global effects induced by hypercapnia and small-amplitude effects induced by phenomena such as scanner drift. LMGS was a tremendously useful technique in our study for correcting unstable time trends of the global BOLD signal and for determining true cortical activations.

Activation in Cortical and Subcortical Regions Associated With Inspiratory Loading

The structures in basal ganglia that are involved in somatomotor control (2) showed a significant change in the fMRI signal. It has been shown that respiratory loading is associated with the activation of basal ganglia (5, 8, 11, 20, 27, 31, 35, 47). Limbic areas, including the parahippocampal gyrus, insula, and cingulate gyrus, participate in emotive and visceral sensorimotor functions. Indeed, activation of these structures in response to respiratory challenges has been previously reported (5, 8, 11, 31, 35, 47). The prefrontal cortex, including the middle and inferior frontal gyri, was also activated during respiratory challenge, as in previous studies (5, 11, 31, 46). The cerebellum, which receives vagal inputs (24), plays an essential role in coordinating sensory aspects of loads with respiratory motor output. It has been reported that respiratory loading tasks are associated with activation of the cerebellum (11, 20, 27, 35, 46, 47), as in our study. Parietal lobules are somatosensory association areas and are particularly concerned with vigilance. It is reasonable that these regions are activated, because respiratory loading (27, 31) induced activation of the inferior parietal lobule.

Deactivation in Cortical and Subcortical Regions Associated With Mandibular Advancement

Limbic area. The cingulate cortex forms the circuit of Papez and is involved in functions of the gut and emotion. This structure is also related to the control of certain autonomic

Table 2. Group results of neural activation in the contrast of HL/MAX0-HL/MAX67

<table>
<thead>
<tr>
<th>Anatomic Location</th>
<th>BA</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Z Score</th>
<th>Cluster Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal lobe - Middle frontal gyrus</td>
<td>6</td>
<td>-32</td>
<td>2</td>
<td>44</td>
<td>5.02</td>
<td>477</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>-24</td>
<td>-6</td>
<td>44</td>
<td>4.13</td>
<td></td>
</tr>
<tr>
<td>Limbic lobe - Cingulate gyrus</td>
<td>24</td>
<td>-12</td>
<td>-16</td>
<td>46</td>
<td>4.64</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>-14</td>
<td>-12</td>
<td>38</td>
<td>3.88</td>
<td></td>
</tr>
<tr>
<td>Frontal lobe - Middle frontal gyrus</td>
<td>8</td>
<td>28</td>
<td>14</td>
<td>36</td>
<td>4.80</td>
<td>490</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>44</td>
<td>12</td>
<td>42</td>
<td>4.33</td>
<td></td>
</tr>
</tbody>
</table>

Locations, Brodmann’s areas (BAs), three-dimensional coordinates, Z scores, and cluster sizes of peak activations in anatomic regions activated in the contrast of HL/MAX0-HL/MAX6 (P < 0.05 corrected for multiple comparison) are summarized. The coordinates x, y, and z are expressed in millimeters, corresponding to the atlas of Talairach and Tournoux (51). The cluster sizes are expressed in mm3. Regions with statistically significant differences (Z score > 3.88) are shown.
functions, including respiration, blood pressure, and salivary secretion. With regard to respiration, it has been shown that some cingulate neurons discharge in pace with respiration (15). Moreover, cingulate areas are activated by dyspnea (47), breathlessness (5), and emotion related to a need for air (11). Indeed, it has been demonstrated that the cingulate cortex has a complex and indirect relationship to central networks that control respiration (8, 15). Therefore, we should expect that deactivation of the cingulate cortex might be observed in association with a relief from breathing difficulty by mandibular advancement. The cingulate cortex is also linked to central cardiovascular control mechanisms (15) and is activated by blood pressure challenges (21). Furthermore, it has been reported that there is a loss of gray matter in the cingulate cortex in subjects with OSA (36), who exhibit an increased risk of cardiovascular disorders and stroke. In addition, it was shown that the cingulate cortex in subjects with OSA was less active than that in healthy subjects (35). Several studies have reported a reduction in nocturnal cardiac ischemic episodes and an improvement in daytime blood pressure levels and left ventricular function with long-term nasal CPAP (nCPAP) treatment (30, 54). Furthermore, it has been shown that the treatment of OSA with an OA results in a significant reduction in hypertension (18), which is similar to that reported with nCPAP. Therefore, the deactivation of the cingulate cortex observed in this study may be related to changes in autonomic activity in association with mandibular advancement.

Prefrontal cortex. It is known that the frontal cortex adjacent to the primary motor cortex is related to motor control. Recent studies have provided evidence that this area is also involved in voluntary inspiratory effort (10, 11, 13). The premotor area indirectly innervates respiratory muscles and, therefore, does not contribute to respiratory drive on a breath-by-breath basis. Conversely, it is activated when subjects perform self-paced inspiratory or expiratory maneuvers (34). Moreover, the prefrontal cortex forms the so-called frontal-subcortical neuronal circuit with both the putamen and cingulate cortex, which mediate motor activity and behavior (52). It is notable that the prefrontal cortex displays reduced activity across all sleep stages and appears to be functionally disconnected during sleep from such a neuronal circuit, since this area is commonly regarded as the “hardest working” region of the brain during wakefulness, necessitating some time for recovery during sleep, with “recalibration” of the circuits that requires a lack of input interference from other brain regions (6). On the other hand, most other structures of the brain are active at some point during sleep. The central nervous system of subjects with OSA may be vulnerable to chemical and structural cellular injury due to not only sleep disruption and blood-gas abnormalities, but also restless overuse. Furthermore, neuroimaging studies in subjects with injured prefrontal cortex have revealed an impairment of motor execution (4, 39). A loss of gray matter (36) and less activity compared with healthy subjects (35) have been reported in the prefrontal cortex in subjects with OSA, suggesting some degree of impairment of psychophysiological function, such as amnesia, attention deficits and learning disabilities, daytime cognitive and behavioral deficits, etc. (3, 6, 32).

Clinical Implications

Treatment of OSA by nCPAP has been shown to significantly improve daytime vigilance and reduce cognitive dysfunction (12, 29). However, until now, the assessment of the efficacy of mandibular advancement by an OA for the treatment of OSA has depended solely on changes in clinical symptoms (17, 23, 40, 42). Moreover, the amount of mandibular advancement by an OA has been determined in the light of the best balance between clinical indications and contraindications (42, 48). Only one study has previously revealed that mandibular advancement with an OA significantly reduces clinical symptoms and improves cognitive function (43). Based on the findings in the present study, it may be possible to specifically determine the most efficient mandibular position by referring to the decrease in brain activation triggered by respiratory loading. Furthermore, it may be possible to visualize and quantitatively compare both the favorable and unfavorable effects of different amounts of mandibular advancement. Such prognostic quantification of treatment is important, as the conventional cognitive test battery is not sensitive enough to identify improvements in cognitive function (32). The establishment of neuroimaging as a useful tool may allow for tailored treatment, depending on the phenotypic variety of a respiratory disorder.

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