Brain stem activity changes associated with restored sympathetic drive following CPAP treatment in OSA subjects: a longitudinal investigation

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Submitted 29 January 2015; accepted in final form 11 May 2015

Lundblad LC, Fatouleh RH, McKenzie DK, Macefield VG, Henderson LA. Brain stem activity changes associated with restored sympathetic drive following CPAP treatment in OSA subjects: a longitudinal investigation. J Neurophysiol 114: 893–901, 2015. First published May 20, 2015; doi:10.1152/jn.00092.2015.—Obstructive sleep apnea (OSA) is associated with significantly increased muscle sympathetic nerve activity (MSNA), leading to hypertension and increased cardiovascular morbidity. Although little is known about the mechanisms responsible for the sympathoexcitation, we have recently shown that the elevated MSNA in OSA is associated with altered neural processing in various brain stem sites, including the dorsolateral pons, rostral ventrolateral medulla, medullary raphe, and midbrain. Given the risk associated with elevated MSNA, we aimed to determine if treatment of OSA with continuous positive airway pressure (CPAP) would reduce the elevated MSNA and reverse the brain stem functional changes associated with the elevated MSNA. We performed concurrent recordings of MSNA and blood oxygen level-dependent (BOLD) signal intensity of the brain stem, using high-resolution functional magnetic resonance imaging, in 15 controls and 13 subjects with OSA, before and after 6 mo CPAP treatment. As expected, 6 mo of CPAP treatment significantly reduced MSNA in subjects with OSA, from 54 ± 4 to 23 ± 3 bursts/min and from 77 ± 7 to 36 ± 3 bursts/100 heart beats. Importantly, we found that MSNA-coupled changes in BOLD signal intensity within the dorsolateral pons, medullary raphe, and rostral ventrolateral medulla returned to control levels. That is, CPAP treatment completely reversed brain stem functional changes associated with elevated MSNA in untreated OSA subjects. These data highlight the effectiveness of CPAP treatment in reducing one of the most significant health issues associated with OSA, that is, elevated MSNA and its associated elevated morbidity.

Muscle sympathetic nerve activity; rostral ventrolateral medulla; medullary raphe; continuous positive airway pressure; obstructive sleep apnea

Obstructive sleep apnea (OSA) is associated with significantly increased muscle sympathetic nerve activity (MSNA) (Carlson et al. 1996; Elam et al. 2002; Fatouleh et al. 2014a; Hedner et al. 1995, 1988; Imadojemu et al. 2007; Narkiewicz et al. 1998; Somers et al. 1995), leading to hypertension and increased cardiovascular morbidity (Nieto et al. 2000; Peppard et al. 2000). Although little is known about the underlying mechanisms responsible for this increased MSNA, using concurrent recordings of MSNA and functional magnetic resonance imaging (fMRI), we have recently shown that MSNA-related functional changes occur in cortical and brain stem regions in individuals with OSA (Fatouleh et al. 2014b; Lundblad et al. 2014). Within the brain stem we found that activity within the medullary raphe, rostral ventrolateral medulla (RVLM), and dorsolateral pons was significantly reduced in OSA subjects compared with controls (Lundblad et al. 2014). Furthermore, these functional changes were associated with significantly increased gray matter volume, which was correlated to an individual OSA subject’s level of MSNA. These results provide evidence that elevated MSNA in OSA subjects is associated with changes in brain stem function and anatomy and raises the question as to whether these changes can be reversed by treatments that reduce MSNA.

Continuous positive airway pressure (CPAP) is one treatment option that is effective at reducing the elevated MSNA associated with OSA (Fatouleh et al. 2014a). If a CPAP-driven reduction in MSNA is associated with a restoration of normal brain stem function, it would provide further support for the idea that elevated MSNA in individuals with untreated OSA results from altered brain stem function. The aim of this investigation was to determine whether functional changes in brain stem activity are associated with a reduction in resting MSNA during 6 mo of CPAP treatment. We used concurrent recording of MSNA and fMRI to assess regional brain stem activity associated with individual subject’s patterns of MSNA. We hypothesized that CPAP treatment would result in a restoration in brain stem function in regions of the medullary raphe, rostral ventrolateral medulla, and dorsolateral pons associated with the decrease in MSNA, providing further support to the idea that the elevated MSNA in OSA is related to disturbed brain stem function.

METHODS

Subjects. Thirteen subjects with OSA (10 males, age 54 ± 3 yr, mean ± SE, range 35–67 yr) and 15 healthy control subjects (12 males, age 50 ± 3 yr, 35–68 yr) were recruited for the study. As described previously (Fatouleh et al. 2014b), all OSA subjects were evaluated at the sleep laboratory of Prince of Wales hospital for one night. Patients were monitored continuously for 8 h using 12-channel polysomnography: EEG, ECG, and submental EMG recordings were obtained with surface electrodes; nasal and oral airflow were monitored by thermistor and chest and abdominal movements by respiratory inductive plethysmography. Oxyhemoglobin saturation was recorded all night by finger pulse oximetry, a microphone was placed on the lower neck to record snoring, and a camera sensitive to ultraviolet light recorded the patient movements during sleep. The overnight polysomnography study was analyzed offline, and apneas and hypopneas were defined according to the international classification of sleep disturbances.
disorders by using Alice (Philips Medical Systems, Eindhoven, The Netherlands) and Somonologica (Medicare Flaga, Reykjavik, Iceland) software. Therapeutic CPAP was determined during a full night with respiratory monitoring. The treating specialist determined the CPAP pressure that resulted in the cessation of the apneic events. Subsequently, subjects were treated at home with a CPAP machine individually calibrated for their optimal pressure (Series 9; ResMed, Sydney, Australia) and a mask that they felt comfortable with. Compliance with prescribed CPAP therapy was based on an automated download of the CPAP machine at 6 mo, analyzed using ResScan software. All control subjects undertook an overnight assessment using an in-home device that monitored nasal airflow and oxygen saturation (ApneaLink; ResMed). All procedures were approved by appropriate Human Research Ethics Committees of the University of Western Sydney and the University of New South Wales. Written consent was obtained from all subjects in accordance with the Declaration of Helsinki.

**MRI and MSNA acquisition.** Control subjects completed one MRI session, whereas all OSA subjects completed two MRI scanning sessions. One session was conducted immediately preceding continuous positive airway pressure (CPAP) treatment, and a second session was conducted after 6 mo of CPAP treatment. For each MRI session, subjects lay supine on an MRI bed with their knees supported on a foam cushion, and an insulated tungsten microelectrode was inserted into a muscle fascicle of the common peroneal nerve for recording MSNA. The common peroneal nerve was located at the fibular head by electrical stimulation through a surface probe (3–10 mA, 0.2 ms, 1 Hz; Stimulus Isolator, ADInstruments, Bella Vista, Australia). An insulated tungsten microelectrode (FHIC, Bowdoin, Maine) was inserted percutaneously into the nerve and manually guided into a muscle fascicle of the nerve while delivering weak electrical stimuli to evoke muscle twitches (0.01–1 mA, 0.2 ms, 1 Hz). A nearby subdermal microelectrode, with 1 mm of insulation removed, served as the reference electrode, and a surface Ag-AgCl electrode on the leg served as the ground electrode. Once a muscle fascicle had been entered, neural activity was amplified (gain 103, bandpass 0.3–5.0 kHz) using a low-noise, electrically isolated headstage (NeuroAmplifierEX; ADInstruments). The innervation territory of the muscle fascicle was identified by tapping over the muscle belly or relevant tendon, and the position of the microelectrode tip was manually adjusted until spontaneous bursts of MSNA were identified. Neural activity was acquired, RMS (root mean square)-processed (moving average, 200 ms), and analyzed on a computer-based data acquisition and analysis system (LabChart 7, PowerLab 16S; ADInstruments). A high-pass digital filter at 300 Hz was applied to the recorded signal to remove artifacts picked up by the cable from the MR-compatible stainless steel isolated headstage to the amplifier.

In the laboratory, ECG (0.3–1.0 kHz) was recorded with AG-AgCl surface electrodes, continuous noninvasive blood pressure (BP) using radial arterial tonometry (Colin 7000 NIBP; Colin, Aichi, Japan), and respiration with a piezoelectric transducer around the abdomen (Pneumotrace; UFI). Spontaneous MSNA, heart rate, respiration, and BP were recorded continuously for 10 min of undisturbed rest, of which the final 5 min were used for analysis. Following this period, the ECG electrodes were removed, the BP recording stopped, and the subject wheeled to the scanner with the microelectrode in situ. During scanning, heart rate was monitored via an MR-compatible piezoelectric pulse transducer on the finger pad, and respiration was monitored via the MR-compatible piezoelectric transducer around the abdomen.

Once a stable MSNA recording was achieved, a continuous series of 200 gradient echo echo-planar images, sensitive to blood oxygen level-dependent (BOLD) contrast and encompassing the entire brain stem, was collected (46 axial slices, repetition time (TR) = 8 s, echo time (TE) = 40 ms, flip angle = 90°, raw voxel size = 1.5 mm3) using a 3-Tesla MRI scanner (Achieva; Philips; 32-channel SENSE head coil). All 46 axial slices were collected during the first 4 s of the 8-s TR. A high-resolution three-dimensional T1-weighted anatomical image set was also collected (turbo field echo; TE = 2.5 ms, TR = 5,600 ms, flip angle = 8°, voxel size = 0.8 mm3).

**MSNA processing.** All MSNA signals were RMS-processed (moving average, time constant 200 ms). MSNA during the pre-MRI recording period was quantified according to standard time-domain analysis of the RMS-processed signal as burst frequency (bursts/min) and burst incidence (bursts/100 heart beats). Analysis of variance, coupled with Tukey’s multiple comparisons test, was used to assess statistical significance across each group. Significant differences were determined between controls and OSA subjects before CPAP treatment (2-tailed, 2-sample t-test, P < 0.05) and between OSA subjects before and after CPAP treatment (2-tailed, paired t-test, P < 0.05). During the fMRI scanning period, MSNA bursts were manually measured from the RMS-processed version of the filtered nerve signal during the 4-s inter-scan OFF period. This period was divided into 4 × 1-s intervals, and the total number of MSNA bursts for each 1-s epoch was determined.

**fMRI processing.** With the use of SPM8 software (Friston et al. 1995), the functional image sets for each individual subject were realigned and coregistered to their T1-weighted image set, and global signal intensity drifts were removed using a linear detrending method. Manual correction of the images was performed to create an accurate match between the functional and anatomical image sets. With the use of the SUIT toolbox (Diedrichsen 2006), the brain stem and cerebellum were isolated and the images spatially normalized into Montreal Neurological Institute space using a spatially unbiased atlas template of the cerebellum and brain stem. Note that we did not spatially smooth the images to maintain fine spatial detail.

For each of the 200 fMRI image volumes, the 4 s during which brain stem BOLD signals were recorded were related to the MSNA burst in the preceding 4-s period. This is possible because J) neurovascular coupling delays mean that changes in BOLD signal intensity lag the actual neuronal events in the brain by ~5 s (Logothetis et al. 2001), and 2) slow conduction along unmyelinated peripheral axons means it takes ~1 s for an individual sympathetic burst to travel from the brain to the peripheral recording site (Fagius and Wallin 1980); by taking these two factors into account, one can see that 3) an increase in BOLD signal intensity should appear ~4 s following an increase in neuronal activity within the brain. Moreover, since the scanning sequence was conducted in a caudal-to-rostral direction, extending from the upper cervical spinal cord to the thalamus, we could target specific regions in the brain stem on the basis of the temporal relationship between scanning slice and scanned structure (Fig. 1).

Therefore, in each subject, the brain stem was divided into 4 separate sections from caudal to rostral corresponding to the first, second, third, and fourth 1-s epochs, and a brain mask was created for each of the 4 × 1-s epochs. The fourth 1-s epoch was removed because it encompassed the region rostral to the brain stem. The MSNA recording was also then divided into corresponding 1-s epochs. For each 1-s epoch, if a MSNA burst occurred, a "1" was entered into an fMRI search model, and if no burst occurred, a "0" was entered. This was repeated for the entire 200-volume scanning period, resulting in a 200-volume fMRI search model for each of the remaining 3 × 1-s epochs in each individual subject. An example of this model creation is shown in Fig. 1.

A general linear model approach was used to determine changes in BOLD signal intensity that matched each individual subject’s MSNA burst pattern for each of the 3 × 1-s epochs. The hemodynamic delay function and the associated microtime resolution were removed, since we had already accounted for the hemodynamic delay in our methodological setup. Furthermore, in each individual subject’s analysis, the six directional movement parameters derived from the realignment step were added as nuisance variables. To eliminate the effects of heart rate, given the pulsatile nature of cerebrospinal fluid, we included signal changes derived from a 2-mm sphere placed in the center of the fourth ventricle in each individual subject as a nuisance variable. Following this, second-level random-effects analyses were performed...
to compare signal intensity changes during each MSNA burst in OSA subjects before and after 6 mo of CPAP treatment (random effects, displayed using a threshold of \( P < 0.005 \), uncorrected, minimum cluster size 3 voxels). During this second-level analysis, the resulting statistical maps were masked with the brain stem region corresponding to the first, second, or third 1-s epochs. Because we hypothesized that CPAP treatment would result in signal change restoration in regions of the medullary raphe, rostral ventrolateral medulla, and dorsolateral pons, we then employed small volume correction for multiple comparisons (\( P < 0.05 \)).

Since we were essentially correlating ongoing fluctuations in signal intensity with spontaneous fluctuations in MSNA, and given that bursts of MSNA were significantly more frequent in OSA subjects before CPAP treatment than after treatment and in controls, it is possible that differences in brain stem activation patterns may have been partially due to differences in the number of MSNA bursts and the resulting MSNA fMRI model. That is, differences in the number of ON and OFF periods could potentially influence the overall significance of the final contrast maps, which in turn may have influenced the second-level analyses. To ensure that this was not the case, for each significant cluster we extracted the raw signal intensity changes in OSA subjects before and after CPAP treatment, as well as in control subjects, and compared signal intensity during bursts of MSNA to signal intensity during periods where there were no bursts. Significant differences in signal intensity between controls and OSA subjects pre-CPAP treatment and between controls and OSA subjects post-CPAP were determined (2-tailed, 2-sample \( t \)-test, \( P < 0.05 \)), and also between OSA subjects before and after CPAP treatment (2-sample, paired \( t \)-test, \( P < 0.05 \)). Finally, for each significantly different cluster, linear relationships between percent change in fMRI signal intensity pre-CPAP compared with post-CPAP and the associated percent change in MSNA total bursts during the fMRI session were determined (\( P < 0.05 \)).

**T1 image processing.** The T1-weighted image from each subject was segmented and spatially normalized with a dedicated symmetrical brain stem template. In brief, the image was cropped and the brain stem masked before normalization such that no supratentorial gray matter can bias the results obtained using the SUIT toolbox. The subsequent normalization and reslicing process produces brain stem “maps” of gray matter probabilities, spatially normalized into the brain stem template space and modulated by the volume changes due to the normalization. Finally, the images were resliced into the new atlas space and smoothed (3-mm full-width at half-maximum). Significant differences in gray matter between OSA subjects before and after 6 mo of CPAP treatment were determined using a voxel-by-voxel analysis (paired \( t \)-tests; \( P < 0.05 \), false discovery rate corrected, minimum cluster size 3 voxels). Significant volume differences were then overlaid onto a T1-weighted template for visualization. To explore the direction and overall gray matter volume differences, individual gray matter volumes (probability volume) were extracted from clusters of difference from the OSA subjects before and after CPAP treatment, as well as from the control subjects, and the means compared between groups (\( P < 0.05 \), paired \( t \)-test).

**RESULTS**

**OSA subject characteristics.** Based on overnight polysomnography, OSA patients were diagnosed on the basis of their apnea-hypopnea index (AHI) values (apnea-hypopnea events...
per hour) as mild, moderate, or severe (mild: AHI 5–15; moderate: AHI 15–30; severe: AHI >30). Of the 13 OSA subjects, 1 subject had mild OSA, 1 subject had moderate OSA, and 11 subjects had severe OSA (AHI 41 ± 4; range 7–6). The minimum SaO2 during sleep was 82 ± 3% (range 67–93%); the baseline SaO2 during wakefulness was 95 ± 1% (range 91–99%), and the Baseline Epworth Sleep Scale score was 8 ± 1 (range 3–14). We monitored compliance during the 6-mo CPAP treatment period and found that OSA patients used CPAP for an average of 5.0 ± 0.4 h/night, as reported by the ResScan software. Also, as reported by the software, there was a significant reduction in AHI after 6 mo of compliant treatment (AHI 3 ± 2; range 1–21).

The mean AHI for the control subjects following an in-home overnight assessment of sleep patterns was 3 ± 1. Overnight monitoring of sleep was made at variable times after the scanning had been conducted and revealed that whereas the majority had an AHI of 1–3, two of the control subjects had an AHI of 8 and 10. We did not exclude these subjects because they were asymptomatic normotensive and did not report being tired during the day or snoring during sleep; we did not consider it necessary to undertake a full polysomnographic assessment in these two subjects. Although there was no significant difference in age between OSA and control subject groups (2-sample t-test; P > 0.05), those with OSA had a significantly higher body mass index (BMI: pre-CPAP 30 ± 2, controls 25 ± 1; P = 0.007). There was no significant difference in BMI between MRI sessions pre-CPAP and post-CPAP in the OSA subjects (pre-CPAP: 30 ± 2; post-CPAP: 31 ± 2; P = 0.6).

Physiology. During the laboratory recording session, compared with controls, OSA subjects had significantly elevated systolic (143 ± 5 vs. 121 ± 4 mmHg; P = 0.007) and diastolic (84 ± 2 vs. 68 ± 4 mmHg; P = 0.02) pressures before CPAP. Furthermore, MSNA burst frequency (54 ± 4 vs. 23 ± 3 bursts/min; P < 0.0001) and burst incidence (77 ± 7 vs. 36 ± 3 bursts/100 heart beats; P < 0.0001) were both significantly elevated before CPAP. Although there was no significant difference in heart rate before and after CPAP (72 ± 3 vs. 70 ± 2 beats/min; P = 0.55), the respiratory period was significantly longer (i.e., respiratory rate was slower) after CPAP (3.5 ± 0.4 vs. 4.3 ± 0.2 s; P = 0.038). Six months of CPAP treatment resulted in a significant reduction in resting MSNA, when measured as both burst frequency (36 ± 2 bursts/min; P < 0.0001) and burst incidence (54 ± 4 bursts/100 heart beats; P = 0.003) in all 13 OSA subjects. Despite this impressive fall in MSNA (a decrease of 31 bursts/min and 41 bursts/100 heart beats), CPAP treatment did not have a significant effect on systolic blood pressure (143 ± 5 vs. 131 ± 6 mmHg, P = 0.2) or heart rate (74 ± 4 vs. 69 ± 2 beats/min; P = 0.11) but did cause a significant fall in diastolic pressure (84 ± 2 vs. 73 ± 3 mmHg; P = 0.02). Although levels of resting MSNA cannot be predicted from baseline arterial pressure in healthy normotensive subjects below 40 yr of age, there is a linear relationship between MSNA and mean arterial pressure in subjects above age 40 (Narkiewicz et al. 2005). Nevertheless, despite the majority of our OSA subjects being over 40 yr of age, there were no significant correlations between MSNA, expressed as either burst frequency or burst incidence, and systolic or diastolic pressure, either before or after CPAP.
and in the controls. With the exception of two subjects, MSNA declined toward control levels in OSA subjects after 6 mo of CPAP treatment.

**fMRI signal intensity changes.** Voxel-by-voxel comparison of MSNA-related BOLD signal intensity changes pre- and post-CPAP treatment revealed that the reduction in resting MSNA was coupled with significant changes in signal intensity in a number of brain stem regions (Fig. 3, Table 1). Significantly increased signal intensity changes post-CPAP compared with pre-CPAP occurred in the region of the caudal medullary raphe, left RVLM, and left and right dorsolateral pons and in the ventral midbrain. Significantly decreased signal intensity changes occurred in the region of the right nucleus tractus solitarius (NTS). Direct comparison of percent changes in signal intensity during MSNA bursts, compared with periods of no bursts, showed that CPAP significantly increased signal intensity changes within the medullary raphe, RVLM, dorsolateral (dl) pons, and ventral midbrain (signal intensity pre-CPAP vs. post-CPAP: raphe: \(-0.04 \pm 0.06\) vs. \(0.39 \pm 0.14, P = 0.009\); left RVLM: \(-0.22 \pm 0.13\) vs. \(0.13 \pm 0.15, P = 0.02\); left dl pons: \(-0.10 \pm 0.08\) vs. \(0.50 \pm 0.20, P = 0.008\); right dl pons: \(0.04 \pm 0.10\) vs. \(0.37 \pm 0.12, P = 0.03\); ventral midbrain: \(-0.13 \pm 0.12\) vs. \(0.52 \pm 0.28, P = 0.02\)) and decreased signal intensity in the NTS (right NTS: \(0.15 \pm 0.10\) vs. \(-0.24 \pm 0.15, P = 0.009\)) (Fig. 4). Additionally, the percent increases in signal intensity within the medullary raphe and left RVLM during CPAP treatment were significantly correlated to the percent decrease in MSNA bursts during the fMRI scanning session (raphe: \(r = 0.80, P = 0.001\); left RVLM: \(r = 0.56, P = 0.04\); left dl pons: \(r = 0.12, P = 0.68\); right dl pons: \(r = 0.20, P = 0.51\); ventral midbrain: \(r = 0.02, P = 0.95\); right NTS: \(r = 0.28, P = 0.36\)).

Within the medullary raphe, left RVLM, and left dorsolateral pons, signal intensity was significantly decreased in OSA subjects pre-CPAP compared with controls (controls vs. OSA pre-CPAP: raphe: \(0.17 \pm 0.09\) vs. \(-0.04 \pm 0.06, P = 0.04\); left RVLM: \(0.26 \pm 0.14\) vs. \(-0.22 \pm 0.13, P = 0.01\); left dl pons: \(0.19 \pm 0.07\) vs. \(-0.10 \pm 0.08, P = 0.007\)). No significant difference between controls and OSA pre-CPAP occurred in the NTS, right dorsolateral pons, or ventral midbrain (controls vs. OSA pre-CPAP: NTS: \(0.23 \pm 0.09\) vs. \(0.15 \pm 0.10, P = 0.32\); right dl pons: \(0.19 \pm 0.05\) vs. \(0.04 \pm 0.10, P = 0.11\); ventral midbrain: \(0.12 \pm 0.12\) vs. \(-0.13 \pm 0.12, P = 0.08\)). Furthermore, within all of these brain stem regions, apart from the NTS, signal intensity returned to control levels post-CPAP (controls vs. OSA post-CPAP: NTS: \(P = 0.01\); raphe: \(P = 0.11\); left RVLM: \(P = 0.27\); right dl pons: \(P = 0.09\); left dl pons: \(P = 0.09\); ventral midbrain: \(P = 0.11\)).

**Functional overlap.** To determine if there was a restoration of brain stem regions in those brain stem regions that we have previously shown to be significantly reduced in OSA subjects pre-CPAP treatment compared with controls, we overlapped two statistical maps: controls > OSA pre-CPAP and OSA post-CPAP > OSA pre-CPAP. This resulted in three brain stem regions: the medullary raphe, left RVLM, and left dorsolateral pons (Fig. 5). Extraction of signal intensity changes during MSNA bursts compared with periods of no bursts confirmed that signal intensity returned to controls levels following CPAP treatment (signal intensity: controls, OSA pre-CPAP, OSA post-CPAP: raphe: \(0.16 \pm 0.08, -0.11 \pm 0.06, 0.27 \pm 0.15\); left RVLM: \(0.24 \pm 0.10, -0.13 \pm 0.11, 0.13 \pm 0.09\); left dl pons: \(0.18 \pm 0.08, -0.12 \pm 0.08, 0.48 \pm 0.19\)). For each of these three regions, signal intensity was significantly reduced in OSA subjects pre-CPAP compared with controls (raphe: \(P = 0.008\); left RVLM: \(P = 0.01\); left dl pons: \(P = 0.007\)), increased significantly in OSA post-CPAP compared with pre-CPAP (raphe: \(P = 0.04\); left RVLM: \(P = 0.02\); left dl pons: \(P = 0.006\)) to levels that were not signifi-

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**Table 1. Location, t score, and cluster size for regions showing significant differences in signal intensity changes coupled to spontaneous MSNA and in gray matter volume in OSA subjects before and after 6 mo of CPAP treatment**

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>MNI Coordinate</th>
<th>Cluster Size</th>
<th>t Score</th>
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<tbody>
<tr>
<td><strong>MSNA-coupled fMRI signal intensity differences</strong></td>
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<tr>
<td><strong>OSA post-CPAP &gt; pre-CPAP</strong></td>
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<td></td>
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<tr>
<td>Medullary raphe</td>
<td>-2 -38 -58</td>
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<td>Left RVLM</td>
<td>-6 -40 -46</td>
<td>5</td>
<td>3.85</td>
</tr>
<tr>
<td>Right dorsolateral pons</td>
<td>-6 -32 -28</td>
<td>3</td>
<td>3.10</td>
</tr>
<tr>
<td>Left dorsolateral pons</td>
<td>-4 -36 -28</td>
<td>7</td>
<td>2.97</td>
</tr>
<tr>
<td>Ventral midbrain</td>
<td>2 -20 -12</td>
<td>13</td>
<td>4.34</td>
</tr>
<tr>
<td><strong>OSA post-CPAP &gt; pre-CPAP</strong></td>
<td></td>
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<tr>
<td>Right NTS</td>
<td>-4 -46 -58</td>
<td>3</td>
<td>3.32</td>
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<tr>
<td><strong>Gray matter volume</strong></td>
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<tr>
<td><strong>OSA post-CPAP &gt; pre-CPAP</strong></td>
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<tr>
<td>Medullary raphe</td>
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<td>12 -39 -38</td>
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<tr>
<td>Left dorsolateral pons</td>
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<td>7.29</td>
</tr>
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</table>

Data are cluster locations, cluster sizes, and t scores for regions showing significant differences in signal intensity changes coupled to spontaneous muscle sympathetic nerve activity (MSNA) and in gray matter volume in obstructive sleep apnea (OSA) subjects before and after 6 mo of continuous positive airway pressure (CPAP) treatment. Cluster locations are given in Montreal Neurological Institute (MNI) space. fMRI, functional magnetic resonance imaging; RVLM, rostral ventrolateral medulla; NTS, nucleus tractus solitarius.

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Fig. 3. Brain stem regions in which fMRI signal intensity was significantly different in subjects with OSA before compared with after 6 mo of CPAP treatment. Hot and cool color scales indicate regions in which signal intensity changes were significantly increased or reduced after CPAP treatment, respectively. Significant clusters are overlaid onto a T1-weighted anatomical template image. Slice locations in Montreal Neurological Institute space are indicated at the top right of each image. Note that CPAP treatment resulted in signal intensity increases in regions of the medullary raphe, rostral ventrolateral medulla (RVLM), dorsolateral pons (dl pons), and ventral midbrain. Signal decreased occurred in the dorsal medulla in the region of the nucleus tractus solitarius (NTS).
Gray matter volume changes. Comparison of gray matter volumes in OSA subjects revealed that CPAP treatment resulted in a significant reduction in gray matter volume in the medullary raphe extending into the RVLM and in the right and left dorsolateral pons (probability volume: pre-CPAP vs. post-CPAP: raphe: 0.30 to 0.18, P < 0.01; right dl pons: 0.24 to 0.13, P = 0.00006; left dl pons: 0.20 to 0.10, P = 0.00009) (Fig. 6, Table 1). Furthermore, within all of these brain stem regions, gray matter was significantly different between controls and OSA pre-CPAP treatment (controls vs. OSA pre-CPAP: raphe: 0.25 ± 0.02 vs. 0.30 ± 0.02, P = 0.04; right dl pons: 0.19 ± 0.02 vs. 0.24 ± 0.02, P = 0.04; left dl pons: 0.16 ± 0.02 vs. 0.20 ± 0.01, P = 0.047) and significantly reduced in OSA subjects post-CPAP compared with controls (raphe: P = 0.01; right dl pons: P = 0.01; left dl pons: P = 0.003).

DISCUSSION

Given that resting MSNA is driven primarily from brain stem structures, it is not surprising that altered brain stem function is associated with elevated MSNA in individuals with OSA. In a previous investigation we found that OSA is associated with altered MSNA-related activity changes in brain stem regions: the medullary raphe, RVLM, and dorsolateral pons (Lundblad et al. 2014). We extend these earlier findings by showing that altered brain stem function in OSA subjects can be reversed by 6 mo of CPAP treatment, which is also associated with a significant reduction in OSA subjects post-CPAP compared with controls (raphe: P = 0.01; right dl pons: P = 0.01; left dl pons: P = 0.003).

We found that burst frequency fell by 31 bursts/min compared with only 10 bursts/min in a previous investigation by Narkiewitz et al. (1999). We suspect we achieved a more impressive fall in MSNA because our subjects were more compliant with their use of CPAP, using them on average for 5 h per night, as documented from the software within the

Fig. 4. Plots of percent signal intensity (SI) changes in regions in which SI changes correlated to MSNA were significantly different in subjects with OSA before compared with after 6 mo of CPAP treatment. Inset graphs show mean (±SE) SI changes during MSNA bursts compared with periods of no bursts in controls (black), OSA pre-CPAP (white), and OSA post-CPAP (gray). Note that CPAP treatment resulted in a significant reduction in SI during each MSNA burst in all 6 brain stem regions. Furthermore, these signal reductions returned to control levels in all regions except for the NTS. *P < 0.05.

Fig. 5. Brain stem regions in which SI changes correlated to MSNA were significantly reduced in OSA subjects before CPAP treatment compared with controls, which then increased significantly to control levels after CPAP treatment. Significant clusters are overlaid onto a T1-weighted anatomical template image. Slice locations in Montreal Neurological Institute space are indicated at the top right of each image. Inset graphs show mean (±SE) SI changes during MSNA bursts compared with periods of no bursts in controls (black), OSA pre-CPAP (white), and OSA post-CPAP (gray). * P < 0.05.
machines. Moreover, great care was taken to ensure that all patients had individually fitted masks that they felt comfortable with and were treated with pressures that were individually titrated. Compliance in the Narkiewicz et al. (1999) study was self-reported; although the authors state that patients reported >75% compliance, they do not know the number of sleeping hours each patient received CPAP therapy.

The MSNA changes associated with CPAP treatment were associated with functional and anatomical restoration in the medullary raphe encompassing the nucleus raphe obscures and pallidus. In anesthetized animals, direct chemical stimulation of this raphe region evokes significant changes in arterial pressure and sympathetic nerve activity (Coleman and Dampney 1995; Henderson et al. 1998), which is likely mediated by direct projections to the RVLM (Ellenberger and Feldman 1994; Zagon 1995) or the sympathetic preganglionic neurons in the intermediolateral cell column (Aicher et al. 1994; Allen and Cechetto 1994; Bacon et al. 1990). Although caudal medullary raphe activity does not appear to regulate arterial pressure at rest, our data strongly suggests that it regulates resting MSNA under pathological conditions, since signal intensity within this region is elevated at rest in controls and returns to an elevated level following CPAP treatment in OSA subjects. Furthermore, the activity restoration within this region during CPAP treatment was significantly correlated to the decrease in MSNA, strengthening our argument for a critical role of the raphe in OSA-related MSNA changes. Our data suggest that in individuals with untreated OSA, reduced medullary raphe activity results in elevated MSNA.

A similar pattern of signal intensity and anatomical changes also occurs in the region of the left and right dorsolateral pons. That is, signal intensity is reduced and gray matter elevated in untreated OSA, returning to control levels following CPAP treatment. Similar to the medullary raphe, although chemical stimulation of the same region can evoke increases or decreases in arterial pressure and sympathetic nerve activity (Dampney 1994; Hade et al. 1988; Miyawaki et al. 1991), inactivation of the dorsolateral pons does not alter either resting arterial pressure or heart rate (Shafer et al. 2011). These dorsolateral pons neurons do not appear to project directly to spinal preganglionic sympathetic neurons (Barman et al. 1999), although they do project directly to the RVLM (Dampney 1994). It appears that both the dorsolateral pons and medullary raphe contain neurons that can modulate sympathetic activity and whose activity corresponds to a 10-Hz resting sympathetic activity discharge (Barman et al. 1995). Altered activity within these two brain stem regions would almost certainly affect resting sympathetic outflow and potentially underlie the increases in MSNA that occur in OSA.

The region of the RVLM also displayed a return to control levels following 6 mo CPAP treatment, and this return was significantly correlated to the reduction in resting MSNA. In addition to its role in baroreflex function, the RVLM is the major output nucleus from which almost all brain regions, including the cerebral cortex, control arterial pressure (Dampney et al. 2002; Gabbott et al. 2005). We had previously shown that BOLD signal intensity within the human homolog of the RVLM covaries with MSNA in healthy subjects (Macefield and Henderson 2010) as well as in patients with OSA (Lundblad et al. 2014). Curiously, however, whereas we had predicted that BOLD signal intensity would be higher in OSA, which would fit with the elevated MSNA seen in OSA, it was actually lower than in controls. Nevertheless, as we had discussed previously (Lundblad et al. 2014), given that BOLD signal intensity is considered to reflect synaptic activity (Logothetis et al. 2001), our counterintuitive observation of a decrease in signal intensity within RVLM in patients with OSA may indicate that active inhibition of RVLM is lower in OSA. If this is the case, then the increase in MSNA-coupled signal intensity within RVLM following successful treatment with CPAP must reflect an increase in active inhibition of the RVLM, bringing its total activity, and hence MSNA, down toward control levels. Furthermore, since the medullary raphe and dorsolateral pons project directly to the RVLM, it could be the case that RVLM functional restoration results from restoration of afferent inputs from these brain stem regions.

Somewhat surprising were the signal changes within the region of the NTS, another brain stem region known to influence RVLM activity through direct excitatory projections. Unlike the RVLM, dorsolateral pons, or medullary raphe, signal intensity within the region of the NTS was not altered in individuals with OSA before CPAP treatment, but was significantly reduced by CPAP treatment to levels well below both control and pre-CPAP levels. Although this study provides the first evidence of restored brain stem function following CPAP, a number of studies have reported brain stem functional changes in the chronic intermittent hypoxia (CIH) model of OSA. More specifically, the elevated sympathetic activity and
increased arterial pressure in CIH (Bao et al. 1997; Fletcher et al. 1992) are associated with neural activation in the NTS and RVLM (Greenberg et al. 1999; Knight et al. 2011).

Our data cannot determine the underlying cellular changes that result in gray matter volume restorations and, indeed, decreases relative to controls in the medullary raphe, RVLM, and dorsolateral pons in OSA subjects following CPAP treatment. Since it has been observed that increased use can lead to an increase in grey matter volume within a relevant cortical region (Amunts et al. 1997; Maguire et al. 2000; Sluming et al. 2002), it is possible that the elevated MSNA in OSA subjects before CPAP treatment reflects a similar process. Although we cannot determine the mechanisms responsible for the increase and subsequent decrease in gray matter volume that occurs in OSA subjects, the finding that the medullary raphe, RVLM, and dorsolateral pons display both functional and anatomical changes that subsequently reverse following CPAP treatment, along with MSNA, strongly suggests that these changes are coupled.

Limitations. There are some limitations to the present investigation. We did not follow control subjects over a 6-mo period to examine reproducibility, and we did not include OSA subjects that did not undergo CPAP treatment; however, others have shown no MSNA change in OSA patients who did not receive CPAP when followed up over a year (Narkiewicz et al. 1999). Although the effects of such limitations cannot be examined, given the strong relationship in OSA subjects between the reduction in MSNA and functional and anatomical changes, we are confident that our results are not unduly influenced by these issues. Additionally, although we did not measure $S_{PO_2}$ or end-tidal $CO_2$ in the OSA subjects before and after CPAP, we know clinically that they are neither hypercapnic nor hypoxic in the awake state. Accordingly, we are confident that levels of carbon dioxide or oxygen in the blood did not unduly influence our results. Finally, although diastolic pressure fell significantly, systolic pressure did not. The elevated systolic pressure may reflect long-term vascular remodeling, since aortic stiffness increases (Cortuk et al. 2014) and arteriolar endothelium-dependent vasodilatation is reduced in OSA (Kato et al. 2000); both of these mechanisms may contribute to the persistent increase in systolic pressure despite a marked fall in neurally mediated vasoconstriction.

Conclusions. Our results show that 6 mo of CPAP treatment significantly reduces the elevated MSNA and the associated brain stem functional and anatomical changes associated with OSA. These data show that the underlying mechanisms responsible for elevated MSNA in OSA are indeed reversible and highlight the effectiveness of CPAP treatment in reducing one of the most significant health issues associated with OSA.

ACKNOWLEDGMENTS

We are grateful for the assistance provided by Elie Hammam in these experiments and to ResMed (Australia) for donating the CPAP machines for this study. All MRI scanning was conducted at Neuroscience Research Australia.

GRANTS

This work was supported by National Health and Medical Research Council of Australia Grant 1007557.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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