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Reversal of functional changes in the brain associated with obstructive sleep apnoea following 6 months of CPAP

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ABSTRACT

Obstructive sleep apnoea (OSA) is associated with an increase in the number of bursts of muscle sympathetic nerve activity (MSNA), leading to neurogenic hypertension. Continuous positive airway pressure (CPAP) is the most effective and widely used treatment for preventing collapse of the upper airway in OSA. In addition to improving sleep, CPAP decreases daytime MSNA towards control levels. It remains unknown how this restoration of MSNA occurs, in particular whether CPAP treatment results in a simple readjustment in activity of those brain regions responsible for the initial increase in MSNA or whether other brain regions are recruited to over-ride aberrant brain activity. By recording MSNA concurrently with functional Magnetic Resonance Imaging (fMRI), we aimed to assess brain activity associated with each individual subject's patterns of MSNA prior to and following 6 months of CPAP treatment. Spontaneous fluctuations in MSNA were recorded via tungsten microelectrodes inserted into the common peroneal nerve in 13 newly diagnosed patients with OSA before and after 6 months of treatment with CPAP and in 15 healthy control subjects while lying in a 3 T MRI scanner. Blood Oxygen Level Dependent (BOLD) contrast gradient echo, echo-planar images were continuously collected in a 4 s ON, 4 s OFF (200 volumes) sampling protocol. MSNA was significantly elevated in newly diagnosed OSA patients compared to control subjects (55 ± 4 vs 26 ± 2 bursts/min). Fluctuations in BOLD signal intensity in multiple regions covaried with the intensity of the concurrently recorded bursts of MSNA. There was a significant fall in MSNA after 6 months of CPAP (39 ± 2 bursts/min). The reduction in resting MSNA was coupled with significant falls in signal intensity in precuneus bilaterally, the left and right insula, right medial prefrontal cortex, right anterior cingulate cortex, right parahippocampus and the left and right retrosplenial cortices. These data support our contention that functional changes in these suprabulbar sites are, via projections to the brainstem, *driving* the augmented sympathetic outflow to the muscle vascular bed in untreated OSA.

1. Introduction

Obstructive sleep apnoea (OSA) is characterized by repetitive complete or partial collapse of the upper airway and cessation of airflow during sleep. It is associated with significantly increased daytime muscle sympathetic nerve activity (MSNA; Carlson et al., 1993, 1996; Elam et al., 2002; Fatouleh and Macefield, 2014a; Hedner et al., 1988, 1995; Imadojemu et al., 2007; Narkiewitz et al., 1998, 1999; Narkiewicz and Somers, 2003; Somers et al., 1995), thought to result from the repetitive intermittent periods of hypoxemia during sleep and brain alterations that likely result (Lanfranchi and Somers, 2001; Nieto et al., 2000; Peppard et al., 2000). Despite the detrimental effects of increased MSNA on an individual's health, and the elevated risk of future adverse cardiovascular events, very little is known about the underlying mechanisms responsible for increased daytime MSNA in individuals with OSA.

We have recently used functional Magnetic Resonance Imaging (fMRI), coupled with concurrent recordings of MSNA, to show that the elevated MSNA in OSA subjects is associated with altered signal intensity in a number of brain regions (Fatouleh et al., 2014b; Lundblad et al., 2014). These include discrete sites within the prefrontal, cingulate and precuneus cortices, as well as the retrosplenial cortex, caudate nucleus, hippocampus and parahippocampal regions (Fatouleh et al., 2014b), as well as within the dorsolateral pons, rostral ventrolateral medulla, medullary raphe and midbrain (Lundblad et al., 2014). In most of these regions, signal intensity covaried with each burst of MSNA in

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OSA subjects but not in controls. Surprisingly, none of these areas of altered signal displayed altered grey matter volume, suggesting that the altered cortical activity associated with increased MSNA in OSA subjects does not result directly from gross structural changes as a consequence of repetitive hypoxic events during sleep.

It has been shown by a number of investigators that treatment of OSA with continuous positive airway pressure (CPAP) is associated with a decrease in daytime MSNA towards control levels, as long as patients are compliant with the requirement to use CPAP every night (Fatouleh et al., 2014a; Hedner et al., 1995; Imadojemu et al., 2007; Narkiewicz et al., 1999; Waradekar et al., 1996). Indeed, patients with OSA who did not receive CPAP and were followed up after 6 and 12 months showed no changes in baseline MSNA (Narkiewicz et al., 1999). It remains unknown how this reduction in MSNA occurs, in particular whether CPAP treatment results in a readjustment in activity of those brain regions responsible for the initial increase in MSNA, or whether other brain regions are recruited to over-ride the aberrant brain activity. Elucidating the underlying mechanisms is important as it may provide evidence that changes in brain activity in individuals with conditions such as OSA are reversible by effective treatment regimens. The aim of this investigation was to determine the effects of CPAP on resting MSNA and associated brain activity patterns in individuals with OSA. This was achieved by using concurrent recording of MSNA and fMRI to assess brain activity associated with each individual subject's sympathetic burst patterns, prior to and following 6 months of CPAP treatment. We hypothesized that activity in those brain regions associated with increased spontaneous MSNA, such as the prefrontal, cingulate, and parahippocampus, would return to control levels as the augmented MSNA seen in OSA returned to control levels following CPAP treatment.

2. Methods

2.1. Subjects

Data were obtained from 13 newly diagnosed patients with OSA (11 males, mean ± SEM age 53 ± 3, range 35–67 years) and 15 healthy controls (12 males, age 53 ± 3, 35–68 years). These patients were a subset of 17 reported previously (Fatouleh et al., 2014b; Lundblad, 2014). All patients had been evaluated at the sleep laboratory of the Prince of Wales hospital for one night, 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. Oxyhaemoglobin saturation was recorded overnight 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 study was analysed offline and apnoeas and hypopnoeas defined according to the international classification of sleep disorders, by using Alice (Philips Medical Systems, The Netherlands) and Somonologica (Medcare 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 apnoeic events. Subsequently, subjects were treated at home with a CPAP machine individually calibrated for their optimal pressure (Series 9, ResMed, Sydney, Australia). Compliance with prescribed CPAP therapy was based on an automated download of the CPAP machine at 1 and 6 months, analysed using ResScan software.

All control subjects undertook an overnight assessment using an in-home device that monitored nasal airflow and oxygen saturation (ApneaLink™; ResMed, Sydney, Australia). All procedures were approved by the 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.

2.2. MRI and MSNA acquisition

Subjects lay supine on an MRI bed with their knees supported on a foam cushion. An insulated tungsten microelectrode was inserted percutaneously into a muscle fascicle of the common peroneal nerve to record multiunit muscle sympathetic nerve activity (MSNA). A second uninsulated microelectrode inserted subdermally nearby (1–2 cm) acted as a reference electrode. Neural activity was amplified (gain 100, band pass 0.1–5.0 kHz) using an MR compatible stainless steel isolated headstage (NeuroAmp Ex. ADInstruments, Sydney, Australia) and further amplified and filtered (total gain 2×10^4, band pass 0.3–5.0 kHz). Data were recorded using a computer-based data acquisition and analysis system (PowerLab 16S; ADInstruments, Sydney, Australia).

In the laboratory ECG (0.3–1.0 kHz) was recorded with Ag–AgCl surface electrodes on the chest and sampled at 2 kHz. Continuous non-invasive blood pressure (BP) was recorded using radial arterial tonometry (Colin 7000 NIBP, Colin Corp., Aichi, Japan), sampled at 400 Hz, and respiration was monitored from 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 a fingerpad, and respiration was monitored via an MR-compatible piezoelectric transducer around the abdomen.

With each subject relaxed and enclosed in a 32-channel SENSE head coil, a continuous series of 200 gradient echo echo-planar images, sensitive to Blood Oxygen Level Dependent (BOLD) contrast were collected (46 axial slices, TR = 8 s, TE = 40 ms, flip angle 90°, raw voxel size = 1.5 mm^3) using a 3 Tesla MRI whole body scanner (Achieva, Philips Medical Systems). A 4 s-ON, 4 s-OFF protocol was used, with MSNA measured during the 4 s-OFF period, and a whole-brain, 46 slice axial volume were collected during the subsequent 4 s-ON period. A high-resolution 3D T1-weighted anatomical image set was also collected (turbo field echo; TE = 2.5 ms, TR = 5600 ms, flip angle = 8°, voxel size 0.8 mm^3).

2.3. MSNA processing

All MSNA signals were RMS-processed (root mean square, 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 per 100 heart beats). Analysis of variance, coupled with Tukey's multiple comparisons test, was used to assess statistical significance across each group. Significant differences between controls and OSA subjects prior to CPAP treatment were determined (two-tailed, two sample t-test, $p < 0.05$), and between OSA subjects prior to and following CPAP treatment (two-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 numbers of MSNA bursts for each 1 s epoch was determined.

2.4. MRI processing

Details on the analytical approach can be found in our earlier studies (James et al., 2013; Macefield and Henderson, 2010). Briefly, using

SPM8 (Friston et al., 1995), fMRI images were realigned, spatially normalized to the Montreal Neurological Institute (MNI) template, and intensity normalized to eliminate slow signal intensity drift. No subject displayed movement parameters greater than 1 mm in any direction and, as a result, all subjects were used in the final analysis. Scans were then smoothed by a 6 mm full-width half-maximum Gaussian filter. BOLD signal intensity changes were measured during the subsequent 4 s ON period to take into account the ~5 s neurovascular coupling delay and the ~1 s required for the conduction of the sympathetic bursts from the forebrain to the peripheral recording site (Fig. 1). Brain images were collected in a caudal to rostral sequence, extending from the medulla to the vertex of the cerebral cortex. This meant that the first set of scans in the 46-slice scanning sequence included the brainstem, while subsequent slices included progressively more rostral structures. Given that we had previously performed high-resolution imaging of the brainstem in OSA patients (Lundblad et al., 2014), in the current investigation we focused on brain regions rostral to the midbrain (seconds 2, 3 and 4). Based on the 4 s temporal delay noted above, in each individual subject the number of MSNA bursts during the corresponding 1 s epoch in each of these 1 s periods was determined and a 200 time point model derived for each individual subject for the 2nd, 3rd and 4th second time periods. That is, for each brain volume, if an MSNA burst occurred a "1" was entered into the model and if no MSNA burst occurred a "0" was entered. In this way a 200 volume model was created for the 2nd second of the 200, 4 s fMRI collection periods. The same procedure was then performed for the 3rd and 4th second periods so that, for each subject, three ×200 volume MSNA models were used to determine signal intensity changes during MSNA bursts in each corresponding caudal-rostral section of the brain. It is important to note that this was not a region-of-interest (ROI) study: any change in BOLD signal intensity occurring in the volume of brain captured in one of the 1-s periods simply "popped-out" because its signal was temporally coupled to the corresponding burst of MSNA.

Changes in BOLD signal intensity that matched each individual subject's MSNA burst model were then determined. For each subject, the 6 direction realignment parameters as well as cerebrospinal fluid signal intensity derived from a 3 mm sphere place in the lateral ventricle were included as nuisance variables. Second level, random effects analyses were performed to compare signal intensity changes during each MSNA burst in OSA subjects prior to and following 6 months of compliant CPAP treatment. We have previously shown that the anterior cingulate cortex, medial prefrontal cortex, precuneus, retrosplenial cortex and parahippocampus all displayed significantly elevate signal intensity in OSA subjects prior to CPAP treatment compared with controls (Fatouleh et al., 2014b). Given this we hypothesized that signal intensity in these brain regions would be reversed and return towards controls levels following 6 months of CPAP treatment. As a consequence we used small volume corrections ($p < 0.05$) to reduce the likelihood of Type 1 errors. In addition, we used a minimum cluster extent of 20 contiguous voxels to reduce Type 1 errors.

Since we were essentially correlating on-going fluctuations in signal intensity with spontaneous fluctuations in MSNA, and given that bursts of MSNA were significantly more frequent in OSA subjects prior to CPAP treatment than after treatment and in controls, it is possible that differences in contrast values between OSA before and after CPAP, and compared with controls, may have been partially due to differences in search models. To ensure that this was not the case, for each significant cluster we extracted the raw signal intensity changes and compared signal intensity during bursts of MSNA to signal intensity during periods where there were no bursts. We also extracted signal intensity changes from these clusters in control subjects to assess differences between all three groups. Significant differences in signal intensity between controls

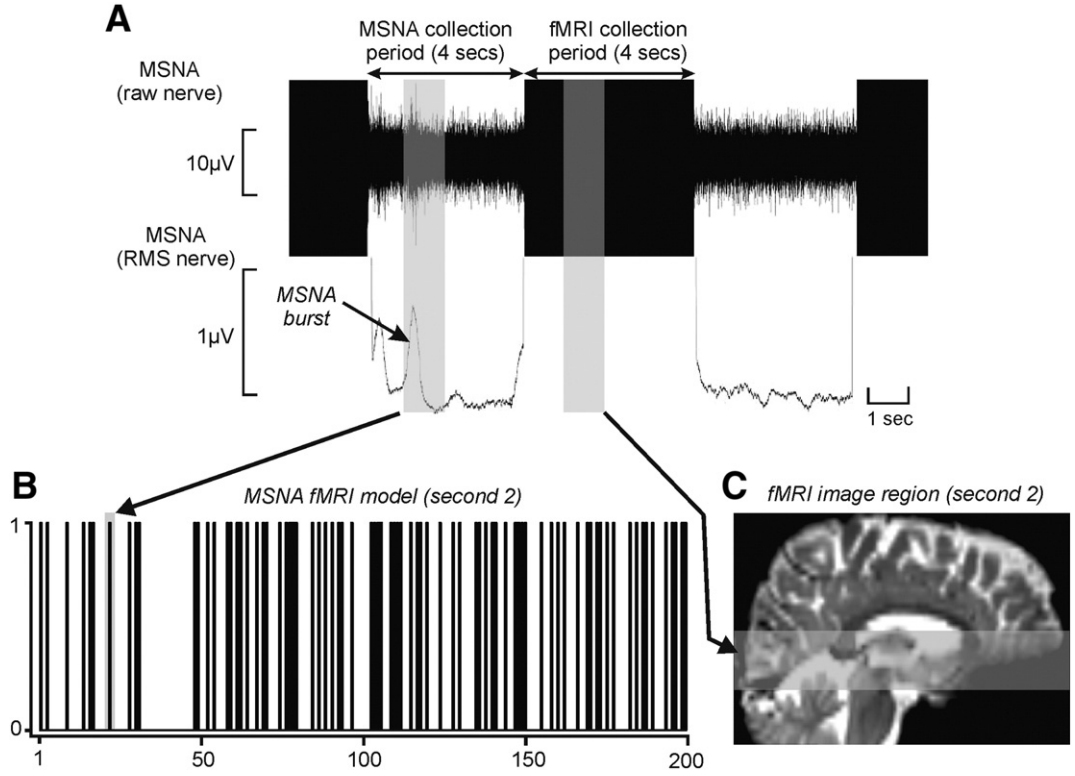

Fig. 1. Concurrent microneurography and functional magnetic resonance imaging (fMRI) procedure. (A) Upper trace shows a raw muscle sympathetic nerve activity (MSNA) recording in an individual subject. Note that during each 8 s period, the initial 4 s does not contain scanner artifact (MSNA recording period) whereas the subsequent 4 s does (fMRI collection period). The lower trace shows RMS (root mean square) nerve activity in which MSNA bursts are clearly visible. During an 8 s period, the corresponding MSNA and fMRI periods are indicated by the grey shading. (B) A plot of the 200 volume repeated box-car model indicating when MSNA bursts occurred during the 2nd second period for each of the two hundred fMRI Volumes. Each MSNA burst is represented by a single box-car. (C) A sagittal fMRI slice indicating the region during which the 2nd second collection period occurred.

and OSA subjects pre-CPAP treatment and between controls and OSA subjects post-CPAP were determined (two-tailed, two sample *t*-test, $p < 0.05$), and also between OSA subjects prior to and following CPAP treatment (two-sample, paired *t*-test, $p < 0.05$).

3. Results

Based on overnight polysomnography, all OSA subjects were diagnosed with mild to severe OSA. Based on apnoea–hypopnoea indices (AHI: mild = 5–15 events/h, moderate = 15–30, severe = >30), two subjects were diagnosed with mild, one with moderate and 10 with severe OSA (AHI range 7–62; AHI 38 ± 5). In OSA subjects, the minimum SaO_2 on the night of polysomnography was 83 ± 2% (range 67–93%), baseline SaO_2 during wakefulness was 95 ± 1% (range 91–99%), and the baseline Epworth Sleep Scale score was 9 ± 1 (range 3–19). We monitored compliance during the 6 months CPAP treatment period and found that OSA patients used CPAP for an average of 6.0 ± 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 months of compliant treatment (AHI range 1–21; AHI 4 ± 2). Furthermore, during the CPAP treatment period, there was no significant change in BMI in OSA subjects (BMI: pre-CPAP 31 ± 2, post-CPAP 33 ± 2; $p = 0.7$).

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, while the majority had an AHI of 1–3, three of the control subjects had an AHI of 8, 9 and 10. Nevertheless, these subjects were otherwise healthy and did not identify as snorers or report being tired during the day; one subject reported drinking on the night of the test, which may well have affected his sleep patterns. Given the absent clinical history of sleep disorders, it was not deemed necessary to undertake a full polysomnographic assessment in these individuals because they were symptom-free and normotensive. Although there was no significant difference in age between OSA and control subject groups (two sample *t*-test; $p > 0.05$), as expected there was a significant difference in body mass index (BMI: pre-CPAP 31 ± 2, controls 24 ± 1, $p = 0.0005$).

3.1. Physiology

Cardiovascular parameters are provided in Table 1. During the physiology recording session, compared to controls, OSA patients prior to CPAP treatment had significantly elevated systolic and diastolic pressures and MSNA burst frequency and burst incidence. In contrast, there was no significant difference in heart rate. Six months of compliant CPAP treatment resulted in a significant reduction in resting MSNA burst frequency and burst incidence in all 13 OSA subjects. Nevertheless, despite a significant reduction in MSNA, CPAP treatment did not have a significant effect on blood pressure or heart rate. Fig. 2 shows a recording of MSNA during fMRI in one subject with OSA prior to and following 6 months of CPAP; it can be seen that there are dramatically fewer bursts of MSNA after CPAP treatment.

Table 1
Cardiovascular parameters (mean ± SEM) of healthy control subjects, obstructive sleep apnoea (OSA) patients prior to (OSA-0M), and following 6 months (OSA-6M) of treatment with continuous positive airway pressure (CPAP) collected immediately prior to the MRI scanning session. Statistical comparisons (ANOVA) of cardiovascular parameters in OSA subjects compared with the control group ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$) and compared with the OSA-0M group ($^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$, $^{++++}p < 0.0001$).

	Controls ($n = 15$)	OSA-0M ($n = 13$)	OSA-6M ($n = 13$)
Systolic pressure (mm Hg)	118 ± 4	141 ± 5***	136 ± 5*
Diastolic pressure (mm Hg)	67 ± 3	82 ± 2**	76 ± 3
Heart rate (beats/min)	67 ± 4	72 ± 3	70 ± 2
Burst incidence (bursts/100 beats)	40 ± 2	80 ± 6****	56 ± 4* $^{++}$
Burst frequency (burst/min)	26 ± 2	55 ± 4****	39 ± 2** $^{+++}$

3.2. Changes in BOLD signal intensity

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 regions (Fig. 3, Table 2). Significantly reduced signal-intensity changes post-CPAP compared with pre-CPAP occurred in the region of the precuneus bilaterally, the left and right insula, right medial prefrontal cortex (mPFC), right anterior cingulate cortex (ACC), right parahippocampus and the left and right retrosplenial cortices.

Direct comparison of percentage changes in signal intensity during MSNA bursts, compared with periods of no bursts, showed that CPAP significantly reduced signal intensity within all of these brain regions (Fig. 4; mean ± SEM signal intensity pre-CPAP vs post-CPAP: *precuneus*: 0.22 ± 0.05 vs −0.06 ± 0.05, $p = 0.0001$; *left insula*: 0.18 ± 0.03 vs −0.09 ± 0.06, $p = 0.001$; *right insula*: 0.18 ± 0.04 vs −0.05 ± 0.03, $p = 0.0005$; *right mPFC*: 0.11 ± 0.05 vs −0.03 ± 0.06, $p = 0.02$; *right ACC*: 0.07 ± 0.04 vs −0.06 ± 0.05, $p = 0.01$; *right parahippocampus*: 0.08 ± 0.04 vs −0.10 ± 0.06, $p = 0.008$; *left retrosplenial cortex*: 0.18 ± 0.04 vs −0.04 ± 0.03, $p = 0.0006$; *right retrosplenial cortex*: 0.16 ± 0.03 vs −0.12 ± 0.05, $p = 0.0003$).

In all of these brain regions, with the exception of the left insula and right parahippocampus, MSNA-correlated signal intensity was significantly increased in OSA subjects pre-CPAP compared with controls (controls vs OSA pre-CPAP: *precuneus*: −0.04 ± 0.07 vs 0.22 ± 0.05, $p = 0.004$; *left insula*: 0.09 ± 0.08 vs 0.18 ± 0.03, $p = 0.19$; *right insula*: 0.01 ± 0.07 vs 0.18 ± 0.04, $p = 0.03$; *right mPFC*: −0.02 ± 0.05 vs 0.11 ± 0.05, $p = 0.04$; *right ACC:* −0.07 ± 0.06 vs 0.07 ± 0.04, $p = 0.03$; *right parahippocampus*: 0.01 ± 0.05 vs 0.08 ± 0.04, $p = 0.14$; *left retrosplenial cortex*: −0.10 ± 0.07 vs 0.18 ± 0.04, $p = 0.002$; *right retrosplenial cortex*: −0.09 ± 0.07 vs 0.16 ± 0.03, $p = 0.006$). Furthermore, within all of these brain regions signal intensity returned towards control levels post-CPAP, with signal intensity in the left insult being below control levels (controls vs OSA post-CPAP: *precuneus*: $p = 0.45$; *left insula*: $p = 0.04$; *right insula*: $p = 0.22$; *right mPFC*: $p = 0.38$; *right ACC*: $p = 0.44$; *right parahippocampus*: $p = 0.29$; *left retrosplenial cortex*: $p = 0.24$; *right retrosplenial cortex*: $p = 0.37$).

4. Discussion

We have shown that the functional changes in the brain in patients with obstructive sleep apnoea return to control levels after 6 months of compliant CPAP treatment. We had previously reported, in the same patients, that CPAP caused a significant fall in MSNA (Fatouleh et al., 2014a), as had been shown by others (Hedner et al., 1995; Narkiewicz et al., 1999; Waradekar et al., 1996). However, the reduction in MSNA was not associated with a significant fall in blood pressure, as had been shown previously by some investigators (Hedner et al., 1995; Narkiewicz et al., 1999) but not others (Becker et al., 2003). While MSNA fell significantly following 6 months of CPAP, it did not return completely to control levels. Importantly, the fall in resting MSNA following CPAP was associated with significant changes in BOLD signal intensity within a number of brain regions, including precuneus bilaterally, the left and right insula, right medial prefrontal cortex (mPFC), right anterior cingulate cortex (ACC), right parahippocampus and the left and right retrosplenial cortices.

We have previously used concurrent recording of MSNA and fMRI to determine cortical and subcortical sites underlying the increase in MSNA in OSA patients (Fatouleh et al., 2014b; Lundblad et al., 2014). The results from this study confirm these previous findings and show that the increase in MSNA in OSA before treatment is associated with

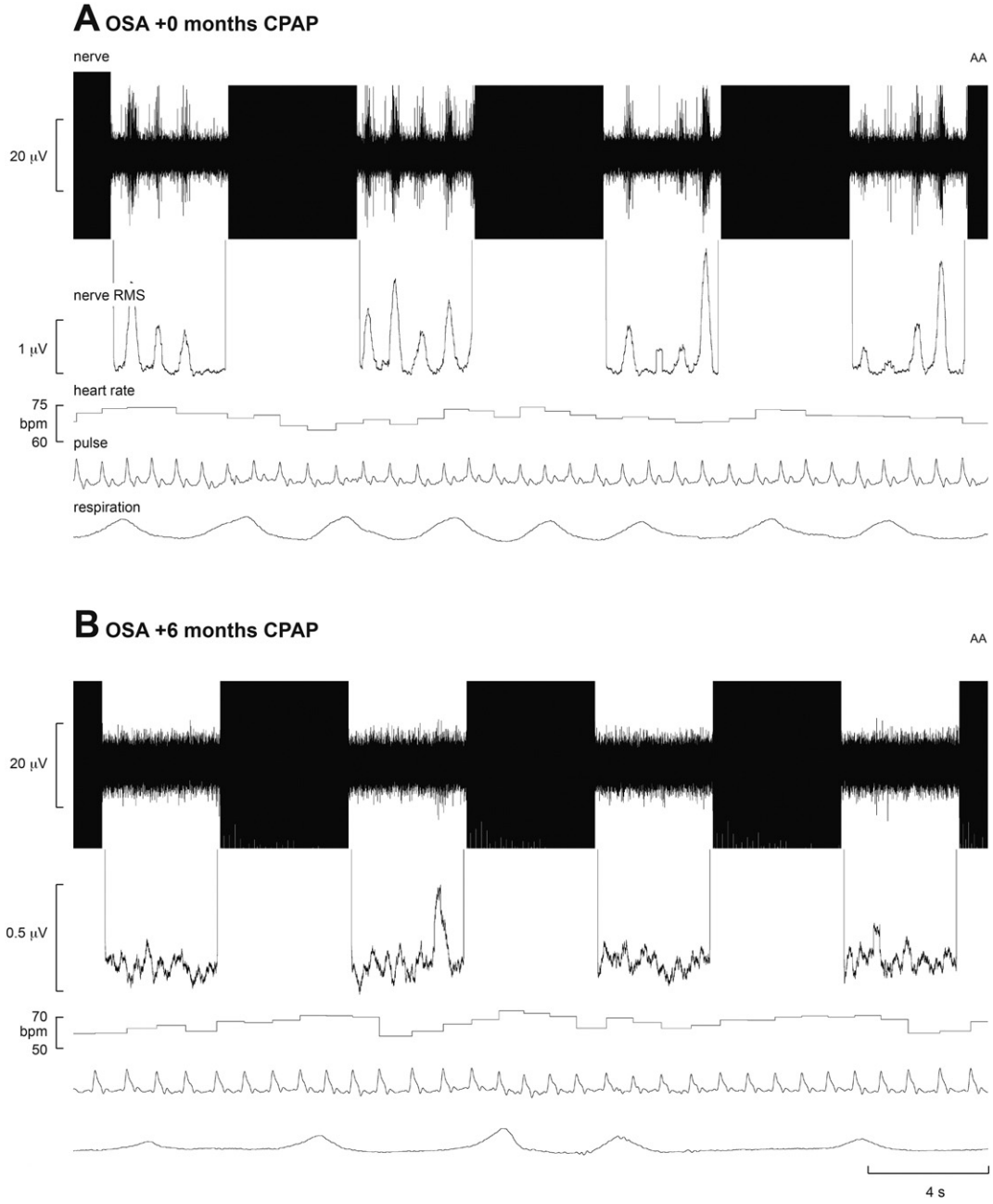

Fig. 2. Multiunit recording of muscle sympathetic nerve activity (MSNA) from a 50-year-old male patient with obstructive sleep apnoea (OSA) during scanning of the brain, obtained prior to (A) and following (B) 6 months of CPAP treatment. The mean-voltage neurogram is shown in the nerve RMS (root mean square) trace; this was used to quantify the number of sympathetic bursts. The black areas represent the scanning artifacts. MSNA bursts were measured during the OFF periods. Resting levels of MSNA were greatly reduced following CPAP.

increases in BOLD signal intensity within the precuneus, retrosplenial cortex, mPFC, ACC and parahippocampus. Importantly, these increases in signal intensity were reversed by 6 months of CPAP treatment. That these signal intensity changes following CPAP treatment essentially paralleled the changes in MSNA, despite the fact that blood pressure remained elevated, strongly suggests that the functional changes in the brain are responsible for the changes in muscle sympathetic outflow.

We found a reduction in signal intensity in mPFC after CPAP treatment of OSA to control levels. Previously, we showed an increase in signal intensity in mPFC which covaried with MSNA in OSA subjects (Fatouleh et al., 2014b). The mPFC is known to send projections to specific nuclei in the brainstem related to control of muscle sympathetic outflow, such as the nucleus tractus solitarius (NTS) and rostral ventrolateral medulla (RVLM) (Sica et al., 2000a; Weisz et al., 2001). Similarly, the precuneus is functionally coupled to the RVLM at rest in healthy controls, suggesting that activity within these cortical regions contributes to resting MSNA via the RVLM (James et al., 2013). In summary, the reduction in MSNA with CPAP is accompanied by reduced activity in mPFC and the precuneus.

In addition to the functional changes in mPFC after therapy, CPAP treatment displayed a reduction in MSNA-related signal intensity within the ACC and the retrosplenial cortex. The role of ACC in sympathetic control is of great interest (Beissner et al., 2013; Critchley et al., 2003; Kimmerly et al., 2013): pyramidal neurons within the ACC project directly and indirectly to subcortical regions associated with homeostasis and autonomic control, including the hypothalamus (Ongür et al., 1998). Electrical stimulation of this brain region in experimental

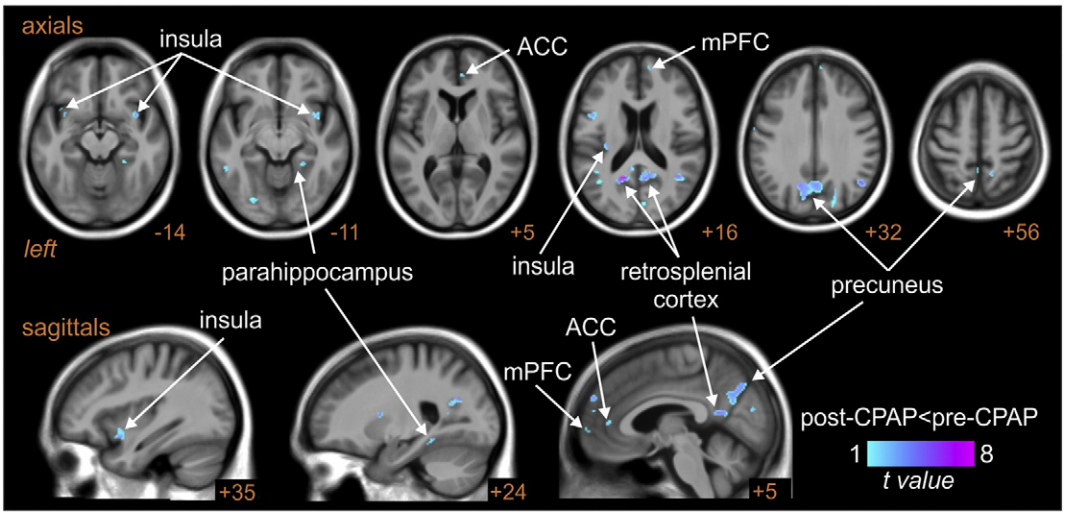

Fig. 3. Brain regions in which fluctuations in BOLD signal intensity coupled to fluctuations in muscle sympathetic nerve activity (MSNA) were significantly different in subjects with obstructive sleep apnoea (OSA) prior to and following 6 months of continuous positive airway pressure (CPAP) treatment. Cool color scale represents regions in which changes in signal intensity during each burst of MSNA were significantly reduced following CPAP treatment. Significant clusters are overlaid onto a mean T1-weighted anatomical template image. Slice locations in Montreal Neurological Institute space are indicated at the lower right of each image. ACC: anterior cingulate cortex, mPFC: medial prefrontal cortex.

animals evokes autonomic responses affecting heart rate and blood pressure (Ward, 1948) and ACC stimulation in humans causes prompt and usually complete arrest of respiration, bradycardia and a gradual increase in blood pressure (Pool and Ransohoff, 1949). Orthostatic stressors which unload baroreceptors and increase MSNA are also associated with increased ACC activity (Goswami et al., 2012; Macefield et al., 2006), and lesions encompassing the ACC are associated with disrupted sympathetic cardiovascular regulation and impaired generation of cardiovascular arousal during cognitive efforts (Critchley et al., 2003). Moreover, it has been shown that heart rate variability is associated with changes in activity within both the ACC and retrosplenial cortex (Critchley et al., 2003).

In an animal model of OSA, 30 days of chronic intermittent hypoxia evokes c-fos expression in the cingulate cortex (Sica et al., 2000b), and in OSA subjects manoeuvres that evoke significant increases in blood pressure and heart rate result in aberrant cardiovascular responses associated with altered activity changes in brain regions, including the ACC and retrosplenial cortex (Harper et al., 2003; Macey et al., 2013). This combined evidence of the key role for the ACC and retrosplenial cortex in autonomic circuitry raises the possibility that dysfunction in these brain regions contributes to the sympathoexcitation in OSA; the normalization of signal intensity within these brain regions following CPAP treatment further demonstrates their potential contribution to the marked sympathoexcitation seen in OSA.

Table 2
Location, *T*-score and cluster size for regions showing significant signal intensity differences that were coupled to spontaneous muscle sympathetic nerve activity in subjects with obstructive sleep apnoea prior to, compared with after 6 months of continuous positive airway pressure treatment. Cluster locations are given in Montreal Neurological Institute (MNI) space.

Brain regions	X	Y	Z	*T*-score	Cluster size
Insula	38	8	−16	4.04	116
Right	−38	11	−17	3.81	29
Left	−30	−24	16	4.49	70
Right anterior cingulate cortex	6	42	6	4.32	20
Right parahippocampus	24	−42	−11	3.64	26
Right medial prefrontal cortex	8	60	−2	3.78	22
	11	53	19	4.16	20
Retrosplenial cortex					
Right	9	−52	13	6.30	252
Left	−14	−55	16	6.62	348
Precuneus	2	−63	30	5.22	923

In contrast to the regional differences described above, although signal intensity decreased significantly following CPAP treatment in the left insula and right parahippocampus, it was not different to controls prior to treatment. Somewhat surprisingly, signal intensity declined after CPAP treatment in both these regions to control levels in the right parahippocampus, and even significantly more than controls in the left insula. The insular cortex has been considered to play an important role in autonomic control in humans (Oppenheimer et al., 1990) as well as in animals; indeed, we know that baroreceptors project to the insular cortex in the rat and monkey (Zhang and Oppenheimer, 1997, 2000a, b), and that electrical stimulation of the anterior insula can elicit changes in arterial pressure, heart rate and sympathetic activity (Butcher and Cechetto, 1995). The insular cortex has been implicated in cardiovascular regulation (Harper et al., 2003; Oppenheimer et al., 1996; Woo et al., 2005, 2007); cardiovascular challenges — such as the Valsalva manoeuvre, inspiratory-capacity apnoea, cold-pressor test, hand-grip exercise and loaded breathing — all evoke increases in signal intensity in the anterior insula (Harper et al. 2000, 2003; Henderson et al. 2002, 2004; Macey et al. 2002, 2003, 2012; Sander et al. 2010; Wong 2007a,b; Woo et al. 2007). However, the precise route by which the insula alters sympathetic outflow remains unknown. Although the insula does not project directly to RVLM (Saper, 1982), electrical stimulation of the insula excites some RVLM sympathoexcitatory neurons (Sun, 1992), so it must achieve this via a polysynaptic pathway.

The parahippocampus has been always associated with cognitive functions. However, electrical stimulation of this region in monkeys evokes marked decreases in arterial pressure and bradycardia (Wall and Davis, 1951). Additionally, animal experiments have documented the presence of sympathetic-related neurons in this structure (Westerhaus and Loewy, 2001), which suggest that this region forms part of the higher-brain central autonomic network. Indeed, a recent fMRI study showed altered signals in this brain region in OSA patients, in comparison to a healthy control group, during static handgrip exercise (Macey et al., 2013). Therefore, it is not unreasonable to posit that the pathophysiological changes seen in this brain region in OSA might also contribute to the elevated muscle sympathetic outflow. That BOLD signal intensity was back to control levels after CPAP treatment further supports the idea that this region is functionally related to the altered sympathetic outflow in OSA.

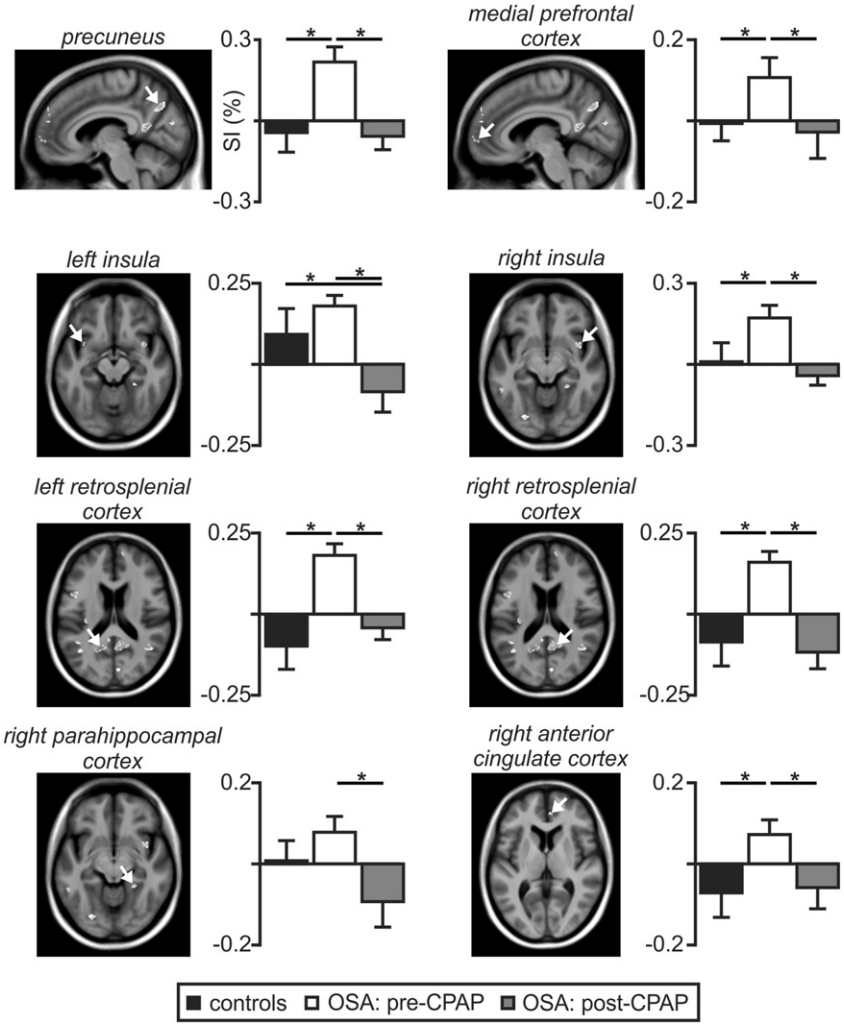

Fig. 4. Plots of percentage signal intensity (SI) changes in regions in which MSNA-locked changes in BOLD signal intensity were significantly different in subjects with obstructive sleep apnoea (OSA) prior to and following 6 months continuous positive airway pressure (CPAP) treatment. Graphs show mean (±SEM) SI changes during MSNA bursts compared with periods of no bursts in controls (black), OSA pre-CPAP (white) and OSA post-CPAP (grey). Note that CPAP treatment results in a significant reduction in signal intensity during each MSNA burst in all seven brain regions. Furthermore, these signal reductions return to control levels in all regions except for the left insula. * indicates $p < 0.05$.

5. Conclusions

Given that MSNA is temporally coupled to BOLD signal intensity in various discrete sites within the brain, that the activity within these regions increased in OSA with the increase in MSNA, and that activity in these areas *decreased* with the decrease in MSNA following CPAP treatment, our current observations lend further support to our conclusion that these suprabulbar sites are *driving* sympathetic outflow to the muscle vascular bed via the brainstem. The findings also reinforce the importance of CPAP in reducing the pathophysiology associated with untreated OSA.

Conflicts of interest

The authors report no competing interests.

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