NeuroImage: Clinical 6 (2014) 275–283

Contents lists available at ScienceDirect

NeuroImage: Clinical

journal homepage: www.elsevier.com/locate/ynicl

Functional and structural changes in the brain associated with the increase in muscle sympathetic nerve activity in obstructive sleep apnoea

Rania H. Fatouleh[a], Elie Hammam[a], Linda C. Lundblad[a], Paul M. Macey[c], David K. McKenzie[b,d], Luke A. Henderson[e], Vaughan G. Macefield[a,b,*]

[a]University of Western Sydney, School of Medicine, Sydney, Australia
[b]Neuroscience Research Australia, Sydney, Australia
[c]UCLA School of Nursing and Brain Research Institute, Los Angeles, CA, USA
[d]Prince of Wales Hospital, Department of Respiratory Medicine, Sydney, Australia
[e]Discipline of Anatomy and Histology, University of Sydney, Sydney, Australia

ARTICLE INFO

Article history:
Received 18 June 2014
Received in revised form 13 August 2014
Accepted 22 August 2014
Available online 27 August 2014

Keywords:
fMRI
Microneurography
Muscle sympathetic nerve activity
Obstructive sleep apnoea

ABSTRACT

Muscle sympathetic nerve activity (MSNA) is greatly elevated in patients with obstructive sleep apnoea (OSA) during daytime wakefulness, leading to hypertension, but the underlying mechanisms are poorly understood. By recording MSNA concurrently with functional Magnetic Resonance Imaging (fMRI) of the brain we aimed to identify the central processes responsible for the sympathoexcitation. Spontaneous fluctuations in MSNA were recorded via tungsten microelectrodes inserted percutaneously into the common peroneal nerve in 17 OSA patients and 15 healthy controls 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. Fluctuations in BOLD signal intensity covaried with the intensity of the concurrently recorded bursts of MSNA. In both groups there was a positive correlation between MSNA and signal intensity in the left and right insulae, dorsolateral prefrontal cortex (dlPFC), dorsal precuneus, sensorimotor cortex and posterior temporal cortex, and the right mid-cingulate cortex and hypothalamus. In OSA the left and right dlPFC, medial PFC (mPFC), dorsal precuneus, anterior cingulate cortex, retrosplenial cortex and caudate nucleus showed augmented signal changes compared with controls, while the right hippocampus/parahippocampus signal intensity decreased in controls but did not change in the OSA subjects. In addition, there were significant increases in grey matter volume in the left mid-insula, the right insula, left and right primary motor cortices, left premotor cortex, left hippocampus and within the brainstem and cerebellum, and significant decreases in the mPFC, occipital lobe, right posterior cingulate cortex, left cerebellar cortex and the left and right amygdala in OSA, but there was no overlap between these structural changes and the functional changes in OSA. These data suggest that the elevated muscle vasoconstrictor drive in OSA may result from functional changes within these brain regions, which are known to be directly or indirectly involved in the modulation of sympathetic outflow via the brainstem. That there was no overlap in the structural and functional changes suggests that asphyxic damage due to repeated episodes of nocturnal obstructive apnoea is not the main cause of the sympathoexcitation.

1. Introduction

Obstructive sleep apnoea (OSA) is characterized by repetitive complete or partial cessation of airflow during sleep, owing to collapse of the upper airway. OSA is associated with significantly increased muscle sympathetic nerve activity (MSNA; Hedner et al., 1988, 1995; Carlson et al., 1993, 1996; Somers et al., 1995; Narkiewicz et al., 1998; Narkiewicz & Somers, 2003; Elam et al., 2002; Imadojemu et al., 2007; Fatouleh et al., 2014), which leads to neurogenic hypertension, and it is thought that the repetitive episodes of intermittent hypoxia during sleep are largely responsible for this sustained sympathoexcitation during daytime wakefulness (Lanfranchi & Somers, 2001; Nieto et al., 2000; Peppard et al., 2000). However, despite the substantial negative effects of increased MSNA on health, little is known about the underlying mechanisms responsible for the increased MSNA in OSA.

Over the past decade, a number of investigations in humans have begun to describe both anatomical and functional brain changes associated with OSA. These studies have found that OSA is associated with

* Corresponding author at: School of Medicine, University of Western Sydney, Locked Bag 1797, Penrith, NSW 2751, Australia.
E-mail address: v.macefield@uws.edu.au (V.G. Macefield).

http://dx.doi.org/10.1016/j.nicl.2014.08.021

significant functional and grey matter changes in a number of regions, including those that can modulate MSNA (Canessa et al., 2011; Harper et al., 2003, 2012; Macey et al., 2002, 2003; Morrell et al., 2010; Joo et al., 2010, 2013). Although these studies investigated neural substrates responsible for *evoked* changes in sympathetic drive, none have explored brain function in structures responsible for the *increased MSNA at rest* and the hypertension associated with OSA.

The aim of the current investigation was to identify brain sites potentially responsible for the increased MSNA associated with OSA. We used concurrent recordings of MSNA and functional Magnetic Resonance Imaging (fMRI) to assess brain activity associated with the pattern of an individual subject's MSNA, as described previously (James et al., 2013; Macefield and Henderson, 2010). Furthermore, we assessed regional grey matter changes using voxel-based morphometry (VBM) (Ashburner & Friston, 2000). We hypothesised that the increase in MSNA in OSA would be associated with altered function and anatomy in higher brain regions that modulate MSNA, including the cingulate cortex, prefrontal cortex, insula and hypothalamus.

2. Methods

2.1. Subjects

Seventeen subjects with obstructive sleep apnoea (15 males, mean ± SEM age 55 ± 3, range 35–69 years) and 15 healthy controls (12 males, age 53 ± 3, 35–68 years) were recruited. All OSA subjects were evaluated and diagnosed based on an overnight sleep study (polysomnography), which included both standard respiratory and SaO_2 measurements, as well as electroencephalographic (EEG), electrooculographic (EOG) and electromyographic (EMG) measurements, at the sleep laboratory of Prince of Wales Hospital. 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). An uninsulated microelectrode was inserted nearby subdermally (1–2 cm) 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 (Neuro Amp EX ADInstruments, 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, Australia).

In the laboratory continuous blood pressure (BP) was recorded using radial arterial tonometry (Colin 7000 NIBP; Colin Corp., Aichi, Japan), ECG (0.3–1.0 kHz) was recorded from the chest using Ag—AgCl surface electrodes, and respiration was monitored from a piezoelectric transducer around the abdomen (Pneumotrace, UFI). Spontaneous MSNA, heart rate and BP were recorded continuously for 10 min of undisturbed rest, of which the final 5 min was 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 fingerpad and respiration was monitored via the 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 the blood oxygen level dependent 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 was 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 and fMRI 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^{-1}) 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 (Prism 6.0, GraphPad Software, USA). All values are expressed as means and standard errors, and $p < 0.05$ was considered statistically significant.

During the fMRI scanning period, MSNA bursts were manually measured from RMS-processed nerve signal during the 4 s inter-scan OFF period. This period was divided into 4 × 1 s intervals and the number of MSNA burst for each 1 s epoch was determined.

Using SPM8 (Friston et al., 1995), fMRI images were realigned, spatially normalized to the Montreal Neurological Institute (MNI) template and intensity normalized to eliminate any slow drift in signal intensity. Scans were then smoothed by a 6 mm full-width at half-maximum (FWHM) Gaussian filter. Signal intensity changes were measured during the subsequent 4 s ON period corresponding to a measurement concurrent with the MSNA recording based on the ~5 s neurovascular coupling delay and the ~1 s required for conduction of the sympathetic bursts from the brain to the peripheral recording site. Brain images were collected in a caudal to rostral sequence, extending from the rostral midbrain to the vertex of the cerebral cortex. Given that we have previously performed brainstem-specific analyses, we focused this investigation on brain regions rostral to the midbrain (seconds 2, 3 and 4). Therefore, in each individual subject, the number of MSNA bursts during 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, a value of the total number of MSNA bursts that occurred during the 2nd second of the 4 second TR was entered into a search model. The same analysis was then performed for the 3rd and 4th second periods.

Signal intensity changes that matched each individual subject's MSNA burst model were identified. Second-level analyses were then performed to determine in which brain regions signal intensity increased or decreased during each MSNA burst in both control and OSA subjects (random effects, $p < 0.005$, uncorrected, minimum cluster size 20 voxels). In addition, regions in which changes in signal intensity were significantly different between control and OSA subjects were determined (random effects, $p < 0.005$, uncorrected, minimum cluster size 20 voxels).

Since we were essentially correlating on-going signal intensity with spontaneous bursts of MSNA, and that these bursts were significantly more frequent in OSA subjects than controls, it is possible that differences in contrast values between OSA and 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 when bursts were present to signal intensity during periods where there were no bursts. Significant differences in signal intensity between controls and OSA subjects were then determined ($p < 0.05$, two sample t-test).