The multi-level impact of chronic intermittent hypoxia on central auditory processing

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\textbf{A R T I C L E   I N F O}

Keywords:
Functional magnetic resonance imaging
Auditory system
Chronic intermittent hypoxia
Sleep apnea
Rat

\textbf{A B S T R A C T}

During hypoxia, the tissues do not obtain adequate oxygen. Chronic hypoxia can lead to many health problems. A relatively common cause of chronic hypoxia is sleep apnea. Sleep apnea is a sleep breathing disorder that affects 3–7% of the population. During sleep, the patient’s breathing starts and stops. This can lead to hypertension, attention deficits, and hearing disorders. In this study, we apply an established chronic intermittent hypoxemia (CIH) model of sleep apnea to study its impact on auditory processing. Adult rats were reared for seven days during sleeping hours in a gas chamber with oxygen level cycled between 10% and 21% (normal atmosphere) every 90 s. During awake hours, the subjects were housed in standard conditions with normal atmosphere. CIH treatment significantly reduces arterial oxygen partial pressure and oxygen saturation during sleeping hours (relative to controls). After treatment, subjects underwent functional magnetic resonance imaging (fMRI) with broadband sound stimulation. Responses are observed in major auditory centers in all subjects, including the auditory cortex (AC) and auditory midbrain. fMRI signals from the AC are significantly increased after CIH by 0.13% in the contralateral hemisphere and 0.10% in the ipsilateral hemisphere. In contrast, signals from the neighboring inferior colliculus of the midbrain are significantly reduced by 0.39%. Signals from the neighboring inferior colliculus of the midbrain are relatively unaffected. Chronic hypoxia affects multiple levels of the auditory system and these changes are likely related to hearing disorders associated with sleep apnea.

\textbf{Introduction}

Oxygen is an essential component of life and the brain requires an adequate oxygen supply to function properly. Hypoxia occurs when the oxygen supply to tissues is insufficient and it may lead to brain damage and central nervous system disorders. Chronic hypoxia can occur in a number of health conditions and occupations. These include sleep breathing disorders and working or living at high altitude. Obstructive sleep apnea (OSA) is a relatively common sleeping breathing disorder that affects 3–7% of the population with higher incidence rates in certain subgroups (Punjabi, 2008). Subjects with OSA often have neurocognitive impairments such as difficulties with reasoning, attention, vigilance, learning, and memory (Lal et al., 2012). Chronic mountain sickness (CMS) is a clinical syndrome that affects people who spend long times, either for work or residence, at high altitudes (> 2500 m) (Leon-Velarde et al., 2005). CMS potentially affects many people as more than 140 million work/reside at high altitudes worldwide (Penaloza and Arias-Stella, 2007).

Despite the significant impact of hypoxia on cognitive function, to date, relatively few studies have investigated the impact of hypoxia on central auditory processing, which is critical for effective hearing. Subjects with chronic hypoxia or hypoxemia (low blood oxygen level) conditions have shown reduced auditory performance (Chen, 2002; Sheu et al., 2012), which indicates conditions such as central auditory processing disorder (CAPD), tinnitus, and hearing loss. OSA can
elevate the minimum detectible sound intensity (Casale et al., 2012), ie. hearing threshold. However, the threshold increase is small and it is important to understand how chronic hypoxia affects central processing. OSA also lowers pitch pattern sequence scores, a measure of central auditory function, even after adjusting for age, gender, obesity, and other variables (Hwang et al., 2011). OSA subjects perform worse in the dichotic digit test, which is an auditory processing assessment (Ziliotto et al., 2006). Furthermore, OSA leads to abnormal auditory evoked potentials, which indicate dysfunction in the central auditory system (Nean et al., 1996; Rumbaech et al., 1991; Vaclulin et al., 2012; Walzlen et al., 1989). CMS is also often associated with tinnitus, a brain-related hearing disorder where subjects hear phantom sounds (Leon-Velarde et al., 2005).

At present, studies have linked chronic hypoxia conditions with hearing disorders. However, relatively little is understood about the central auditory mechanisms affected by hypoxia. Functional magnetic resonance imaging (fMRI) is well suited to advancing this important research area. fMRI is noninvasive and offers whole brain field of view with relatively high spatial and temporal resolution. fMRI is typically performed with the intrinsic blood oxygenation-level dependent (BOLD) contrast (Ogawa et al., 1990). The BOLD contrast is a MRI measure of the hemodynamic response that follows neuronal activity. fMRI has been applied to study OSA (Archbold et al., 2009; Henderson et al., 2003; Kheirandish-Gozal et al., 2014; Li et al., 2015, 2016; Macey et al., 2006; Park et al., 2016; Zimmerman and Aloia, 2006) and people living/working at high altitude (X.A. Yan et al., 2011; X.D. Yan et al., 2011b).

fMRI has also been applied to study central auditory processing in humans (Maeder et al., 2001; Patterson et al., 2002) and animals (Bach et al., 2013; Baumann et al., 2011; Brown et al., 2013). The central auditory system consists of multiple centers, including the cochlear nucleus, superior olivary complex, lateral lemniscus, and inferior colliculus of the brainstem (Musiek and Baran, 2007). Higher up, the medial geniculate body of the thalamus and the auditory cortex are also important auditory centers. Our group has pioneered novel fMRI techniques for investigating the rat central auditory system (Cheung et al., 2012a, 2012b; Gao et al., 2015a, 2014, 2015b; Lau et al., 2015a, 2015b; Zhang et al., 2013). The rat auditory system is similar to that in humans (Malmierca, 2003). However, subcortical centers such as those in the brainstem and thalamus, are larger in rats relative to the brain size and are more superficially located. This facilitates fMRI studies.

In this study, we examine a chronic intermittent hypoxemia model of OSA (Xie et al., 2010; Xu et al., 2015) with BOLD fMRI and broadband sound stimulation. In the remainder of this paper, BOLD fMRI will be referred to as fMRI. Note that chronic continuous hypoxia, the situation at high altitudes, will not be directly examined. This study will enhance our understanding of central auditory processing after chronic hypoxia and facilitate future auditory studies of hypoxia conditions.

**Methods**

**Animal subjects**

All aspects of this study were approved by the animal ethics committees of the City University of Hong Kong, the Chinese University of Hong Kong, and the University of Hong Kong. Our group has extensive experience conducting investigations with rodent subjects, including investigations of chronic intermittent hypoxia (CIH) (Xie et al., 2010; Xu et al., 2015) and the central auditory system using functional magnetic resonance imaging (fMRI) (Cheung et al., 2012a, 2012b; Gao et al., 2015a, 2014, 2015b; Lau et al., 2015a, 2015b; Zhang et al., 2013). Sixty days old (P60) male Sprague-Dawley rats (N=26) were employed in this study. Subjects first underwent seven days of CIH treatment (CIH subjects) or sham treatment (controls). During this time, subjects were weighed daily and their behavior and food intake were qualitatively monitored. On the 7th day, arterial partial pressure of oxygen (PO$_2$) and oxygen saturation (SO$_2$) were measured from five subjects in each group. On the 8th day, fMRI was performed with sound stimulation on eight subjects per group. Separate subjects were employed for oxygen measurements and fMRI as the oxygen measurement procedures were invasive. Subjects were sacrificed after oxygen measurements or fMRI. Fig. 1 illustrates the experimental timeline for each subject.

**Chronic intermittent hypoxemia (CIH) treatment**

The chronic intermittent hypoxemia procedures in this study were adapted from our recent obstructive sleep apnea (OSA) studies (Xie et al., 2010; Xu et al., 2015). Subjects were placed in custom gas chambers (46×20×22 cm$^3$) at P60 from 9 am to 5 pm for seven days. The content of the air in the chamber was controlled by an oxygen profiler (Oxy cycles model A48XOV; Re ming Bioinstruments). For CIH subjects, the oxygen content inside the chamber was cycled between 10% and 21% with 90 s period. For controls, the oxygen content was kept at 21%. After the 8 h of treatment, both groups were returned to standard cages with normal room air. PO$_2$/SO$_2$ measurements and fMRI took place after the 7 day treatment period. See Fig. 1 for a description of the treatment timeline. In addition to regulating oxygen, the profiler also controlled nitrogen content in the chamber and humidity was maintained at 40–50%. The temperature inside the chamber was maintained between 22–24 °C and the CO$_2$ content was monitored. Subjects received ad libitum access to food and water and were housed in a 12 h light/dark cycle. The above CIH model mimicked the repeated episodes of airway obstruction in OSA without the complication or interference by sleep deprivation and fragmentation. The protocol was based on well reported rodent models of sleep apnea, which aimed to reproduce the overall cumulative hourly oxygen desaturation patterns routinely observed in moderately severe OSA patients (Gozal et al., 2001; Xu et al., 2004).

**Arterial blood oxygen**

Arterial PO$_2$ and SO$_2$ were measured on the 7th CIH treatment day with the subjects in the gas chamber. This measurement must be done in the low oxygen environment as PO$_2$ and SO$_2$ rapidly recover after the subject is returned to normal atmosphere. The subject was anesthetized using chloral hydrate and fixed on an operation plate. After the abdominal aorta was uncovered by surgery, the subject together with the operation plate, was taken back into the chamber for at least...
Fig. 2. (A) Body weight, (B) arterial PO2, and (C) arterial SO2 were measured from five CIH and five control subjects during the course of treatment (mean ± standard error). Body weight measurements were normalized to the measurement on day 0. Body weight differed significantly between the groups (p < 0.05) starting from day 2. PO2 and SO2 were significantly different when measured on day 7.

Fig. 3. (A) Block design acoustic stimulation paradigm used to perform fMRI. Twenty second periods with sound stimulation were interleaved between 40 s periods without stimulation. During a 20 s period, the sound stimulus was amplitude modulated at 10 Hz with duty cycle of 50% and 100% modulation depth. (B) Acoustic power spectrum of the sound stimulus. The 40 kHz low-passed noise had total sound pressure level (SPL) of 89 dB (each pulse). The spectrum was recorded at the ear end of the sound tube using a 50 kHz microphone (M50, Earthworks) and a 192 kHz recorder (FR-2, Fostex).

10 mins of treatment (either CIH or sham). To collect blood samples, we designed two dodge gates on the two opposite sides of a gas chamber for access. The abdominal aorta was penetrated under visual control and the blood was collected immediately using a 1 mL syringe which had previously been treated with 0.05 mL of heparin sodium solution. The blood sample was injected into three EG7+ cartridges and the oxygenation parameters were measured by an i-STAT handheld solution. The blood sample was placed in the prone position on a body holder with a head restraint and a tooth bar to restrict motion. Warm water was circulated through the holder and rectal temperature was monitored with a thermometer (SA Instruments). The water temperature was adjusted to maintain 37 °C throughout the imaging session. Heart rate and oxygen saturation were also monitored with a pulse oximeter (SA Instruments) attached to a hind paw. A 170 cm long custom sound tube (Zhang et al., 2013) with a narrow end (8 mm inner diameter) was next inserted into the left ear canal of the subject. The length of the tube was sufficient to place the speaker outside of the high magnetic field environment surrounding the MRI scanner. Cotton wool and vaseline were used to occlude the right ear canal to reduce the impact of scanner sounds. Lastly, the MRI surface coil was placed over the head above the midbrain. The subject and all supporting hardware were placed inside the bore of the scanner.

fMRI – acoustic stimulation

Acoustic stimulation was produced by a close-field magnetic speaker (MF1, Tucker-Davis Technologies) driven by an amplifier (SA1, Tucker-Davis Technologies). The speaker was placed at the far end of the tube outside of the scanner’s 5 G line. Subjects were stimulated with broadband noise pulses (40 kHz low-passed) amplitude modulated at 10 Hz with 50% duty cycle (i.e. pulse duration=50 ms) and 100% modulation depth. The total sound pressure level (SPL) of each pulse was 89 dB (see Fig. 3). The SPL and spectral properties were measured and calibrated at the tip of the tube entering the ear canal by an omnidirectional condenser microphone with uniform sensitivity up to 50 kHz (M50, Earthworks) and a 192 kHz recorder (FR-2, Fostex). The stimulus was presented in a standard block design paradigm consisting of an initial 40 s sound OFF following by four blocks of 20 s sound ON and 40 s sound OFF. This 280 s paradigm was temporally synchronized with image acquisition during each fMRI scan.

fMRI – image acquisition

Imaging was performed with a 7 T MRI scanner (PharmaScan70/16, Bruker Biospin GmbH) using a transmit-only birdcage coil in combination with an actively decoupled receive-only surface coil. Before the fMRI scans, scout images were acquired along the axial, coronal and sagittal views to determine the position and orientation of the brain relative to the scanner. Six 1.2 mm thick imaging slices with 0.2 mm interslice gap were oriented along the coronal view of the brain according to the rat brain atlas (Paxinos and Watson, 2005). The 2nd slice was centered on the inferior colliculus at bregma –8.5 mm. Anatomical images were then acquired on the above scan geometry using the following 2D Rapid Acquisition with Refocused Echoes (RARE) sequence: repetition time (TR)=4200 ms, echo time (TE)=36 ms, field of view (FOV)=32.0×32.0 mm2, data matrix=256×256 voxels, and RARE factor=8. fMRI images were acquired on the same geometry with the following Gradient-Echo Echo Planar Imaging (GE-EPI) sequence: single shot, TR=1000 ms, TE=20 ms, FOV=32.0×32.0 mm2, data matrix=64×64 voxels, flip angle=56°, repetitions=280. The fMRI scan duration was 280 s, matching the stimulation paradigm. Ten fMRI scans were performed per subject with one minute rest between scans.
fMRI – image processing

The EPI images acquired from all scans of a subject were rigid-body realigned to the mean image of the first scan using SPM8 (Wellcome Trust Center). The anatomical image was normalized to a template image acquired from a control subject using affine transformation with Gaussian smoothing (FWHM=0.5 mm) and non-brain structures masked out. The EPI images were then co-registered to the anatomical image. fMRI activation (t-value) maps were computed from the EPI images using the general linear model implemented in custom Matlab (The Mathworks) scripts and SPM8. An image voxel passed the activation threshold if \( p < 0.001 \) (equivalent to \( t > 3.1 \)). fMRI signals were determined for each voxel by computing the percent change in MRI signal going from sound OFF to sound ON periods.

Functionally-defined region of interest (ROI) analysis was performed to quantitatively analyze auditory brain centers affected by CIH. ROIs were defined for centers activated during fMRI. ROIs were defined as p < 0.05.

Data analysis

Body weight, PO\(_2\), and SO\(_2\) measurements were compared between CIH and control subjects using the standard two-tailed t-test. fMRI signals from the ROI of an auditory center were also compared between subject groups using the t-test. Statistically significant differences were defined as \( p < 0.05 \).

Results

Chronic intermittent hypoxemia (CIH) treated subjects have significantly reduced body weight relative to sham treated controls after 2 days of treatment (Fig. 2A). From the 3rd day onwards, the weight of CIH subjects stabilized while controls continued to grow. By the 7th day, CIH subjects are only 92.2 ± 2.7% (mean ± standard error) of their weight at day 0 before the start of treatment. In comparison, control subjects on day 7 are 108.0 ± 5.6% of their day 0 weight. Similarly, arterial oxygen partial pressure (PO\(_2\)) is significantly reduced by CIH treatment during the low oxygen period (9 am to 5 pm). In CIH subjects, PO\(_2\) on day 7 is 66.7 ± 5.2 mmHg (Fig. 2B). In controls, PO\(_2\) is significantly higher at 131.0 ± 9.8 mmHg. Arterial oxygen saturation (SO\(_2\)) is also significantly reduced by CIH treatment during the low oxygen period. In CIH subjects, SO\(_2\) on day 7 is 76.5 ± 9.9% (Fig. 2C). In controls, PO\(_2\) is significantly higher at 97.2 ± 1.2%. These measurements confirm that the CIH treatment induces hypoxemia and the subjects’ health is affected.

Broadband sound stimulation with pulsed 40 kHz low-passed noise (see Fig. 3) leads to fMRI responses across the central auditory system. Responses are observed in the lateral lemniscus (LL) and inferior colliculus (IC) of the midbrain contralateral to the stimulated ear (see Fig. 4). Higher up the auditory pathway, responses are observed in the auditory cortex (AC) in both brain hemispheres. Comparing with the rat brain atlas (Paxinos and Watson, 2005), the primary auditory cortex responds extensively in both hemispheres. The secondary auditory cortex, dorsal and ventral areas, also responds in the contralateral hemisphere. The strongest responses in the brain are observed in the LL and IC. These results are in good agreement with earlier fMRI studies of the rat auditory system (Cheung et al., 2012a; Zhang et al., 2013). Regions of interest (ROIs) were defined for the contralateral LL and IC, and both hemispheres of the AC, due to their robust responses in fMRI.

In both control and CIH treated subjects, the highest fMRI t-values are observed in voxels spanning the LL and IC while lower t-values are observed in voxels spanning the AC (see Fig. 5). Within the LL and IC, the highest t-value voxels are primarily in the dorsal nucleus and the central nucleus, respectively. CIH treatment reduces fMRI responses in the LL and IC of the midbrain but significantly increases responses in both AC hemispheres, relative to controls. When comparing fMRI responses voxel-by-voxel by taking the difference between t-value maps from CIH and control subjects, we see that responses from across the LL are reduced by CIH. Conversely, responses from across the AC are increased. In the IC, responses are increased in the dorsal portion and decreased elsewhere. Chronic hypoxia has differing effects on fMRI responses in the auditory cortex and midbrain.

![Fig. 4. fMRI activation (t-value) map obtained by averaging fMRI images from all subjects (controls and CIH treated) and thresholding at \( p < 0.001 \) as described in the methods section. The map is overlaid on an anatomical image of the brain acquired from a control subject. The positions of the image slices are indicated on a 3D brain rendering generated from an anatomical image using Amira (FEI). The positions are also indicated by bregma coordinates as in the rat brain atlas (Paxinos and Watson, 2005). The lateral lemniscus (LL) and inferior colliculus (IC) of the midbrain contralateral to the stimulated ear along with the ipsilateral (iAC) and contralateral (cAC) hemispheres of the auditory cortex are activated. The voxels with highest t-values are observed in the LL and IC, in accordance with earlier fMRI studies of the rat auditory system (Cheung et al., 2012a; Zhang et al., 2013). Functionally-defined regions of interest (ROIs) were defined on the LL (blue), IC (red), and both AC hemispheres (ipsilateral: green, contralateral: aqua) and indicated using different color scales.](Image 118x106 to 478x299)
The ROIs defined about the contralateral LL and IC, and both AC hemispheres, in Fig. 4 are applied to the fMRI data from CIH and control subjects. fMRI signals from the ROIs (see Fig. 6) show that CIH treatment significantly decreases signals in the LL of the midbrain relative to controls from 1.58 ± 0.15% to 1.19 ± 0.15% (p < 0.05). In contrast, in the larger midbrain center the IC, CIH has little effect on signals (1.66 ± 0.26% to 1.65 ± 0.23%). In both hemispheres of the AC, CIH significantly increases fMRI signals from 0.05 ± 0.02% to 0.18 ± 0.04% (contralateral, p < 0.05) and 0.02 ± 0.01% to 0.13 ± 0.02% (ipsilateral, p < 0.01). The impact of chronic hypoxia differs at different levels of the ascending auditory pathway from the auditory midbrain to the cortex.

**Discussion**

In this study, functional magnetic resonance imaging (fMRI) with broadband sound stimulation was performed on a rat chronic intermittent hypoxemia (CIH) model of obstructive sleep apnea (OSA). CIH treatment reduces body weight (relative to sham treated controls). CIH also reduces arterial oxygen partial pressure and oxygen saturation during sleeping hours. fMRI responses are observed in major auditory centers in all subjects, including the lateral lemniscus (LL) and inferior colliculus (IC) of the midbrain and the auditory cortex (AC). The midbrain responses are primarily in the hemisphere contralateral to the stimulated ear while the cortical responses are bilateral. fMRI signals from the AC are increased after CIH in both brain hemispheres.

In contrast, signals from the lateral lemniscus are reduced in the contralateral hemisphere. Chronic hypoxia affects multiple levels of the central auditory system and these changes are likely related to hearing disorders associated with sleep apnea.

**Auditory evoked potentials in OSA**

The fMRI signal increase in the auditory cortex following CIH is likely related to the increased P2 amplitude seen in auditory evoked potential (AEP) studies of OSA subjects (Vakulin et al., 2012). AEP signals primarily originate in the cortex and increased P2 suggests an abnormal stimulus classification response following chronic hypoxia. P2 is also positively correlated with the fMRI signal (Mayhew et al., 2010). Therefore, increased P2 amplitude is related to increased fMRI signal. Future fMRI studies can examine changes in central auditory processing in human OSA and high altitude subjects.

**Cerebral circulation in chronic hypoxia**

This fMRI signal reduction observed in the LL of CIH subjects is likely related to changes in cerebral circulation during chronic hypoxia (LaManna et al., 2004; Xu and Lamanna, 2006). After a few days of hypoxia, hemoglobin concentration and packed red blood cell volume increase. This increases the oxygen carrying capacity of the blood. After weeks of hypoxia, brain capillary density increases and intercapillary distance decreases (Boero et al., 1999). In this study, fMRI was
Performed shortly after subjects returned to a normal oxygen environment. Therefore, the brains of CHI subjects may be in a hyperoxia condition at the time of imaging. Hyperoxia has been shown to slightly reduce fMRI signals relative to normoxia (Sicard and Duong, 2005). Note that in the AC, the signal increase related to changes in P2 covers the decrease related to changes in circulation.

fMRI of chronic hypoxia conditions

Obstructive sleep apnea impairs cerebrovascular reactivity (CVR), which is the capability of blood vessels to meet the metabolic demands of the brain and to maintain sufficient perfusion during O2 and CO2 fluctuations (Prilipko et al., 2014). fMRI revealed that CVR changes in OSA, when compared with healthy controls, was not homogeneous. Their decreases in CVR were observed in left lentiform nucleus, left pulvinar and parahippocampal gyrus, left postcentral gyrus, bilateral superior frontal gyri, and left medial frontal gyrus (Prilipko et al., 2014). In children, significant reductions in CVR in OSA were observed in gray matter regions (Buterbaugh et al., 2015). OSA is also associated with cognitive impairments in working memory (Archbold et al., 2009). fMRI during working memory tasks showed that activation patterns in the right parietal lobe were positively correlated with OSA severity. In contrast, activations in the cerebellar vermis were negatively correlated. fMRI of an inspiratory loading task performed by OSA subjects revealed signal changes in brain areas mediating sensory and autonomic processes and motor timing (Macey et al., 2006). These areas overlap with regions of gray matter loss. fMRI of OSA subjects performing the Valsalva maneuver (attempting to exhale with the airway closed) observed signal changes in a range of brain areas mediating the Valsalva maneuver (Henderson et al., 2003). This is likely linked to dysfunction of neural sites that integrate afferent airway signals with autonomic and somatic outflow.

Continuous positive airway pressure treatment

Continuous positive airway pressure (CPAP) is one of the most effective methods for treating OSA. CPAP applies air pressure continuously to the airway during sleep to keep it open, significantly reducing hypoxia. The treatment of OSA with CPAP has been studied with AEPs and fMRI. OSA subjects were treated for two nights with CPAP and the CVR and working memory functions were measured. AEPs recorded from the sleep of OSA subjects treated with CPAP showed that treatment returned P3 latency to near normal but N2 latency remained abnormal. Another study treating for 6 weeks also observed P3 latency, along with N2-P3 amplitude and N1-P2 amplitude returning towards that of controls (Rumbach et al., 1991). A more recent study evaluated subjects after at least 2 months of treatment and observed P3 latency return to near normal in those under 45 years of age (Inoue et al., 2001). Another study conducted around the same time observed small changes in P3 latency (not statistically significant) towards normal after 4 weeks and 1 year of treatment (Neau et al., 1996). A recent study that carefully controlled for CPAP compliance also observed a return of P3 latency.
towards normal, but the latency remained significantly different from that of controls (Vakulin et al., 2012). P2 amplitude was largely unaffected by CPAP treatment.

OSA subjects were treated for two months with CPAP before undergoing fMRI with breath-hold stimulation (Prilipko et al., 2014) or a visuospatial task (Prilipko et al., 2012). Cerebrovascular reactivity, the BOLD signal during breath-hold stimulation, increased in the thalamus after CPAP and decreased in the medial frontal regions after sub-therapeutic CPAP. Further, the duration of nocturnal hypoxemia was negatively correlated with cerebrovascular intensity. For the visuospatial task, CPAP increased signals in task positive regions for all task levels and decreased signals in the anterior midline and medial temporal regions of the default mode network for the 3-back level. This was associated with significant improvements in manual response accuracy during the task. Sub-therapeutic CPAP led to less task positive activation and less default mode deactivation in temporal regions. A study treating for 12 months recorded muscle sympathetic nerve activity and fMRI signals without active stimulation at six and 12 months (Henderson et al., 2016). At both time points, signals were increased by CPAP in the caudal medullary raphe, left and right rostral medulla in the region of the rostral ventrolateral medulla, left and right dorsolateral pons in the region of the parabrachial nucleus, and ventral midbrain.

Technical considerations

Chronic hypoxia conditions such as OSA can increase blood pressure (BP) (Dopp et al., 2007), which in turn can affect fMRI signals (Wang et al., 2006). For chronic intermittent hypoxia models like the one in this study, BP does not change significantly until after 5 weeks of CIH treatment (Fletcher et al., 1992; Yin et al., 2012). CIH treatment lasted for 1 week in this study. Therefore, BP changes are not likely to be a significant factor in this study.

In this study, fMRI is performed in a normal atmosphere after CIH treatment. Therefore, the impact on fMRI signals is expected to be different from that due to acute or transient hypoxia (Duong, 2007; Sicard and Duong, 2005).

No qualitative differences are observed in subject behavior or food intake during the CIH treatment. The reduction in body weight in CIH subjects (see Fig. 2) is not contributed solely by less food intake, but reduced intake may play a role.

No medial geniculate body (MGB) responses are observed in the present study (see Figs. 4 and 5). Several of our recent rat studies has observed MGB responses (Cheung et al., 2012a; Lau et al., 2015b). These studies employed lower frequency acoustic stimulation (below approximately 30 kHz). The present study employs relatively high frequency stimulation, up to 40 kHz. The MGB (of mice) has been observed to primarily contain neurons with best frequency below approximately 25 kHz (Hackett et al., 2011). Therefore, we expect the earlier studies to yield greater MGB responses.

Conclusion

Functional magnetic resonance imaging (fMRI) with monaural sound stimulation was employed to study the impact of chronic intermittent hypoxia (CIH) on auditory processing. Using a rodent model of CIH, significantly increased fMRI signals were observed in both auditory cortex hemispheres. In contrast, reduced signals were observed in the contralateral lateral lemniscus and signals from the neighboring inferior colliculi were relatively unaffected. Chronic hypoxia affects multiple levels of the auditory system and these changes are likely related to hearing disorders associated with sleep apnea.

Acknowledgements

This research was supported by start-up funding from the City University of Hong Kong. The authors would also like to acknowledge Simon Chan of the School of Biomedical Sciences, The University of Hong Kong, for providing technical support to this project.

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