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OBSTRUCTIVE SLEEP APNEA IS ASSOCIATED WITH ALTERED MIDBRAIN CHEMICAL CONCENTRATIONS

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Abstract—Obstructive sleep apnea (OSA) is accompanied by altered structure and function in cortical, limbic, brainstem, and cerebellar regions. The midbrain is relatively unexamined, but contains many integrative nuclei which mediate physiological functions that are disrupted in OSA. We therefore assessed the chemistry of the midbrain in OSA in this exploratory study. We used a recently developed accelerated 2D magnetic resonance spectroscopy (2D-MRS) technique, compressed sensing-based 4D echo-planar J-resolved spectroscopic imaging (4D-EP-JRESI), to measure metabolites in the midbrain of 14 OSA (mean age ± SD:54.6 ± 10.6 years; AHI:35.0 ± 19.4; SAO_2 min:83 ± 7%) and 26 healthy control (50.7 ± 8.5 years) subjects. High-resolution T1-weighted scans allowed voxel localization. MRS data were processed with custom MATLAB-based software, and metabolite ratios calculated with respect to the creatine peak using a prior knowledge fitting (ProFit) algorithm. The midbrain in OSA showed decreased *N*-acetylaspartate (NAA; OSA:1.24 ± 0.43, Control:1.47 ± 0.41; $p = 0.03$; independent samples *t*-test), a marker of neuronal viability. Increased levels in OSA over control subjects appeared in glutamate (Glu; OSA:1.23 ± 0.57, Control:0.98 ± 0.33; $p = 0.03$), ascorbate (Asc; OSA:0.56 ± 0.28, Control:0.42 ± 0.20; (50.7 ± 8.5 years; $p = 0.03$), and myo-inositol (mI; OSA:0.96 ± 0.48, Control:0.72 ± 0.35; $p = 0.03$). No differences between groups appeared in γ-aminobutyric acid (GABA) or taurine. The midbrain in OSA patients shows decreased NAA, indicating neuronal injury or dysfunction. Higher Glu levels may reflect excitotoxic processes and astrocyte activation, and higher mI is also consistent with glial activation. Higher Asc levels may result from oxidative stress induced by intermittent hypoxia in OSA. Additionally, Asc and Glu are involved with glutamatergic processes, which are likely upregulated in the midbrain nuclei of OSA patients. The altered metabolite levels help explain dysfunction and structural deficits in the midbrain of OSA patients. © **2017 Published by Elsevier Ltd on behalf of IBRO.**

Neurochemical abbreviations with conventional capitalization: Asc, ascorbate; Asp, aspartate; Ch, choline; GABA, gamma-aminobutyric acid; Gln, glutamine; Glu, glutamate; Gly, glycine; GPC, glycerophosphorylcholine; GSH, glutathione; mI, myo-inositol; NAA, *N*-acetylaspartate; NAAG, *N*-acetylaspartate glutamate; PCh, phosphocholine; PE, phosphoethanolamine; Scy, scyllo-inositol; Tau, taurine; Thr, threonine.

Key words: intermittent hypoxia, autonomic, sleep-disordered breathing, periaqueductal gray, respiration, magnetic resonance spectroscopy.

INTRODUCTION

The defining feature of obstructive sleep apnea (OSA) is the occurrence of repeated airway collapses leading to successive cessation and restoration of airflow during sleep, with resultant exposure of the brain to intermittent hypoxia, excessive CO_2 levels, and repeated, large swings in blood pressure. Such exposures lead to overall and regional sites of brain injury, which presumably are responsible for multiple deficits in cardiovascular, hormonal, cognitive, and memory functions in the syndrome (Berry et al., 2012; Hudgel et al., 2012; Franklin and Lindberg, 2015). The processes underlying failure of the upper airway musculature during sleep with continued diaphragmatic efforts remain unclear, but several of the necessary sensory and motor control elements required for eupneic breathing lie within the midbrain; sites within the midbrain show injury or impaired responses to challenges in OSA (Harper et al., 2003; Macey et al., 2003, 2006, 2008).

The damaged midbrain sites are unlikely to be solely responsible for the wide range of neural influences that

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Abbreviations: 4D-EP-JRESI, accelerated 4D echo-planar J-resolved spectroscopic imaging; AHI, apnea–hypopnea index; BOLD, blood oxygen level dependent; CPAP, continuous positive airflow pressure; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; MSNA, muscle sympathetic nerve activity; NUS, non-uniform undersampling; OSA, obstructive sleep apnea; TE, echo time; TR, repetition time; VOI, volume of interest.

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contribute to the physiological deficits emerging in OSA. The midbrain is the recipient of projections from more-rostral and more-caudal sites that are also damaged in the syndrome, and serve essential timing and modulating influences on those descending and ascending projections. Among the damaged rostral sites projecting to the midbrain, the insular and ventromedial frontal cortices (Macey et al., 2008; Yaouhi et al., 2009; Joo et al., 2013; Kumar et al., 2014; Tummala et al., 2016) exert influences on cardiovascular and breathing systems; the midbrain contains many nuclei with major cardiovascular and breathing modulation and timing roles. While the final common pathways for outflow to upper airway and diaphragmatic respiratory musculature and for sympathetic and parasympathetic outflow to the cardiovascular system lie within the medulla, many modulatory systems, including those of the midbrain, affect those outflows (Davis et al., 1996; Subramanian, 2013). Principal drives to both the diaphragm and the upper airway muscles are CO_2 and O_2, followed by transient changes in blood pressure (Trelease et al., 1985). Sites within the midbrain play significant roles to all three drives (Harper et al., 1998, 2000, 2005; Woo et al., 2005). People with a lack of CO_2 sensitivity, such as those with congenital central hypoventilation syndrome, show impaired functional MRI signals in portions of the midbrain to hypercarbia, hypoxia (Macey et al., 2005), and forced expiration (Macey et al., 2004, 2005). The midbrain periaqueductal gray (PAG), projects to medullary cardiovascular and respiratory areas, and includes lateral and dorsomedial subregions involved in respiratory musculature control (Faull et al., 2015). Neurons in the PAG are synchronized with breathing cycles during sleep (Ni et al., 1990), and the structure is involved with modulation of respiratory rhythm and activity (Dampney et al., 2013; Farmer et al., 2014; Subramanian and Holstege, 2014). In animal models, the midbrain red nuclei are involved in coordinating responses to hypoxia (Waites et al., 1996; Ackland et al., 1997), a significant issue in a condition associated with repeated, extreme hypoxia exposure (Berry et al., 2012), and is presumably mediated through projections to the cerebellum (Granziera et al., 2009); the cerebellum is also damaged in OSA (Macey et al., 2008), leaving potential for impaired coordination.

The evidence supporting a concern for midbrain structural and functional alterations in OSA is substantial, considering the injury sites and nature of challenges that elicited the functional outcomes. Functional MRI studies show increased activation of the ventral and dorsal midbrain during inspiratory loading exercises (Macey et al., 2006, 2003) and decreased activity during cold pressor and expiratory loading challenges in OSA (Harper et al., 2003; Macey et al., 2003). Elevated muscle sympathetic nerve activity (MSNA) correlates with altered Blood Oxygen-Level-Dependent (BOLD) signals in the midbrain in OSA, suggesting a midbrain role in eliciting the high sympathetic tone in the sleep disorder (Fatouleh et al., 2014; Lundblad et al., 2014). CBF is decreased in the right midbrain of OSA subjects (Yadav et al., 2013), which may reflect lower perfusion demand, perhaps from an altered functional state (for example, lower tonic activity), or impaired cerebral perfusion. Structural changes consistent with inflammatory or glial changes, namely diffusion decreases and volume increases, appear in the hypothalamus in OSA (Lundblad et al., 2014; Tummala et al., 2016), which projects heavily to the midbrain (Sakuma and Tada, 1984; Thompson and Swanson, 1998). The data suggest that the midbrain is compromised in OSA, potentially interfering with regulatory roles for cardiovascular and breathing control, as well as other physiological functions in the disorder. Although the MRI findings cannot further distinguish the nature of pathologies, we can gain understanding of what processes might lead to injury and dysfunction by examining neurochemical levels, such as markers of cellular integrity, neuronal concentration, oxidative stress, and neurotransmitter levels.

Magnetic resonance spectroscopy (MRS) allows examination of differences in OSA in relatively abundant metabolites. Previous OSA spectroscopy studies found differences in neurochemical levels, such as decreased *N*-acetylaspartate (NAA), a neuronal marker, and increased levels of the excitatory neurotransmitter glutamate, in limbic brain regions including the hippocampus, thalamus, and putamen (Sarma et al., 2014, 2016). These findings suggest neural injury and increased excitation. Using "2-dimensional" spectroscopy, we showed the insulae have altered neurotransmitter levels bilaterally, including low GABA and high glutamate in OSA (Macey et al., 2016). These large differences in neurochemical levels likely affect the function of the structure, presumably leading to a more excitatory state (lower inhibition from lower GABA, higher excitation from higher glutamate). The insulae, key autonomic regulatory regions, project to the hypothalamus and midbrain nuclei to help regulate sympathetic outflow (Otake et al., 1994; Kurth et al., 2010); altered midbrain neurochemical levels would compromise that sympathetic regulation, as well as other functional processes. The intermittent hypoxia of OSA leads to widespread excitotoxic processes, which should be accompanied by high levels of glutamate.

The objective was to assess multiple midbrain metabolites alterations in OSA subjects using 2D MRS spectroscopy, and to interpret how such changes might contribute to the structural and functional alterations, and to the symptoms of autonomic dysfunction. The evaluation required new procedures for evaluating neurotransmitter levels, with a short scan time for often-ill patients, while still collecting spectral characteristics of multiple neurotransmitters. Assessment of NAA, glutamate, GABA, and other neurochemicals related to oxidative stress can help understand dysfunction and possible neurodegenerative processes that may be present in OSA. Measurements of levels of neurotransmitters contributing to function in the midbrain (see Kazemi, 2006 for review) could help determine processes mediating the previously found structural injury, assisting in understanding of mechanisms of dysfunction in OSA.

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