Functional Neuroanatomy and Sleep-Disordered Breathing: Implications for Autonomic Regulation

RONALD M. HARPER,1,2* RAJESH KUMAR,1 PAUL M. MACEY,2,3 JENNIFER A. OGREN,3 AND HEIDI L. RICHARDSON1

1Department of Neurobiology, David Geffen School of Medicine at UCLA, University of California at Los Angeles, Los Angeles, California
2Brain Research Institute, University of California at Los Angeles, Los Angeles, California
3UCLA School of Nursing, University of California at Los Angeles, Los Angeles, California

ABSTRACT

A major concern with sleep-disordered breathing conditions, which include obstructive sleep apnea (OSA), central apnea, and congenital central hypoventilation syndrome (CCHS), is the high incidence of accompanying autonomic dysfunction and metabolic disorders. Patients with OSA show exaggerated sympathetic tone, leading to hypertension, cardiac arrhythmia, profuse sweating, impaired cerebral perfusion, and stroke. In addition, OSA appears in 86% of obese Type II diabetic patients, suggesting common deleterious processes. Autonomic deficiencies also appear in CCHS patients, who are often hypoglycemic. The impaired autonomic control may stem from injury to central sympathetic and parasympathetic regulatory areas resulting from apnea-related inflammation, hypoxia, or perfusion-related consequences in OSA, and genetic mutation repercussions in CCHS. Disturbed sleep organization from apnea arousals may also disrupt hormonal release. Brain areas affected in both OSA and CCHS include cortical and limbic regions that influence hypothalamic-regulated sympathetic control and hormone release, essential for glycemic regulation, as well as parasympathetic nuclei influencing the pancreas and other viscera, and raphé serotonergic sites, important for thermal and vascular regulation. Brain injury and altered functional responses appear in OSA and CCHS, assessed with magnetic resonance imaging techniques, in areas which show regional gray matter loss, alterations of free water within tissue, loss of axonal integrity, and disruption of functional responses to autonomic and ventilatory challenges. Evaluation of neural injury and distortion in functional signals to autonomic challenges in localized brain areas can provide insights into common pathological mechanisms for dysregulation of hormonal release and autonomic processes in sleep-disordered breathing and metabolic disorders. Anat Rec, 00:000–000, 2012. © 2012 Wiley Periodicals, Inc.

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The incidence of sleep-disordered breathing, including both obstructive sleep apnea (OSA) and central apnea, is remarkably high, with at least 5%, and perhaps as much as 10% of the United States adult population affected (Young et al., 2002, Hiestand et al., 2006, Lee et al., 2008). Obstructive sleep apnea, the repeated collapse of the upper airway with continued diaphragmatic efforts, leads to a rapid decline in tissue oxygenation for periods that can last for a minute or more, and is terminated by an arousal, restoration of upper airway muscle tone, and re-oxygenation. Apneic episodes, with loss of upper airway muscle tone, may appear initially in rapid eye movement (REM) sleep periods, affecting postural, and abdominal muscles (but not the entire diaphragm), and later extend to quiet sleep periods as well, greatly disrupting normal sleep patterning with repeated arousals. The condition is associated with very high levels of baseline sympathetic discharge, which likely leads to the high incidence of hypertension in OSA, with moderate sleep apnea resulting in a three-fold increased risk for hypertension and higher risk for arrhythmia, myocardial infarction, and stroke (Peppard et al., 2000, Shahar et al., 2001, Gami et al., 2004). In addition, each apneic period is accompanied by an even further increase in sympathetic discharge, and transient elevations in blood pressure appear with each breathing cessation, potentially also contributing to cardiovascular problems (Somers et al., 1995, Macefield et al., 1999). Central apnea, a failure of both diaphragmatic and upper airway musculature activity leading to cessation of airflow, can accompany OSA, and is commonly found in heart failure patients, either in isolation or with OSA (Bradley and Floras, 2003a,b, Redeker et al., 2010). Central apneic events are accompanied by rapid oxygen desaturation as well, but often, termination of the apnea is resolved with initiation of breathing rather than a major arousal.

An additional, but fortunately rare, sleep-disordered breathing condition is congenital central hypoventilation syndrome (CCHS), resulting from defects in the Paired-Like Homeobox 2B (PHOX2B) gene, which encodes a transcription factor that affects autonomic nervous system development. The syndrome is characterized by a loss of drive to breathe during sleep, requiring mechanical nocturnal and sometimes 24-hr ventilatory support. The condition is also accompanied by severe autonomic disruptions, with increased symptom severity related to extent of gene mutation (Matera et al., 2004, Berry-Kravis et al., 2006). Extensive brain injury appears in autonomic regulatory areas (Kumar et al., 2005, Kumar et al., 2006a, 2008b, 2010), but the processes contributing to injury are unclear, with initial damage possibly stemming from underdevelopment of neurons arising directly from PHOX2B mutations, and ongoing injury from failed perfusion resulting from aberrant autonomic function due to compromised development targeted by PHOX2B, and from hypoxic exposure, a near-unavoidable outcome from inadvertent loss or insufficient ventilatory support.

**DIABETES AND OSA**

Both Type II diabetes mellitus (T2DM) and OSA are significant health problems, with each disease leading to increased morbidity and mortality (Marshall et al., 2008, Young et al., 2008). A major concern is the high incidence of OSA in metabolic syndrome and T2DM (Coughlin et al., 2004, Aronsohn et al., 2010), with over 86% of obese T2DM patients showing an apnea-hypopnea index >5, and over 22% showing severe OSA (Foster et al., 2009). The relationship between OSA and altered glucose metabolism is apparently independent of obesity (Tasali et al., 2008). The pathological mechanisms underlying the comorbidity of the two conditions are unclear; a portion of the glycemic alterations may result from the disturbed hormonal release accompanying the sleep-disordered breathing condition, including thyrotropin-releasing hormone (TRH) and ghrelin and leptin regulation (Popovic and Duntas, 2005, Amin et al., 2011, Steiger et al., 2011), which have major effects on appetite and metabolism (Allison et al., 2005). In addition, the exaggerated sympathetic tone in OSA can directly affect glucagon release (Patel, 1984); several of these aspects have been reviewed elsewhere (Aurora and Punjabi, 2007). Pancreatic functions are influenced by the vagus as well, with insulin release modified by parasympathetic tone; substantial parasympathetic changes accompany obstructive events of OSA (Leung, 2009). Sudden unexplained death, although principally associated with Type I diabetes, is also a concern with adult onset glucose dysregulation (Curb et al., 1995).

Dynamic changes in autonomic influences are also impacted in OSA (Fig 1); sympathetically-mediated heart rate responses to transient blood pressure changes are late and blunted, and recovery patterns are attenuated (Harper et al., 2003); the late and dampened responses are of concern if sympathetically-mediated glucose recovery from transient challenges show analogous patterns, and timely delivery of brain cellular nourishment is compromised.

**NEURAL INJURY WITH SLEEP-DISORDERED BREATHING**

The significant hypoxic exposure during apneic events of OSA, combined with rapid oxygenation on ventilatory
restoration, and the extreme changes in the vasculature accompanying large swings in sympathetic discharge have the potential to induce ischemic- or reperfusion-related neural tissue injury. In addition to the hypoxia and perfusion concerns, inflammatory processes may also contribute to neural damage (Ohga et al., 2003). The long-term consequences of OSA, which include a range of cardiovascular issues, elevated levels of depression and anxiety (Berry et al., 1986, Bedard et al., 1991, Sforza et al., 2002), and significant memory deficits (Ferini-Strambi et al., 2003, Felver-Gant et al., 2007) suggest that central brain injury occurs in OSA, especially to autonomic, affective, and cognitive regulatory areas. Moreover, not all of the consequences accompanying OSA are resolved with intervention for the breathing disorder. Hypertension is reduced only by minor levels, for example, after treatment with continuous positive airway pressure, the “Gold Standard” intervention (Barbe et al., 2010), and certain memory deficits also remain (Naeglele et al., 1998, Ferini-Strambi et al., 2003, Saunamaki et al., 2010), indicating continued presence of injury.

The first demonstration that OSA is accompanied by significant brain injury emerged from studies examining regional gray matter loss across the entire brain using voxel-based morphometry (VBM) procedures. After separating gray from white matter and cerebrospinal fluid tissue types, we demonstrated gray matter volume losses appearing in classic autonomic regulatory areas in OSA subjects (Macey et al., 2002), as well as sites which serve a range of cognitive, verbal expression, and sensory and motor skills.

Of interest to autonomic regulation, injury appeared in many of the brain loci, which influence hypothalamic action, and thus sympathetic outflow and hormone release, and included regions within the cingulate cortex, amygdala, ventral medial prefrontal cortex, and hippocampus. Moreover, injury also appeared in the cerebellar deep nuclei, including the fastigial “autonomic” nuclei (Lutherer et al., 1983), and cerebellar cortex.

The VBM procedure, although offering an overall view of brain tissue loss and providing the first indications that OSA can induce significant brain tissue injury, suffers from significant resolution and other methodological issues (Macey and Harper, 2005). Regional tissue loss within brain structures can also be assessed by examining local atrophy with 3D surface morphometry techniques, which involve assessment of a reconstructed surface from successive slices through the structure, determining a central line from which measures to the surface can be taken, and evaluating local differences in such measures from the central line to the surface (Thompson et al., 2004). The procedure shows localized atrophy on the hippocampal surface in OSA (Fig. 2A), principally in CA1 areas. Later studies of OSA also used manual volumetric measurements from high-resolution structural MR images (Kumar et al., 2008a), and diffusion tensor imaging (DTI)-based procedures (Macey et al., 2008), which can evaluate injury in both gray and white matter sites.

Manual assessment of a principal output structure of the hippocampus, the mammillary bodies, shows a substantial, and heavily lateralized loss of volume in OSA (Kumar et al., 2008a), Fig. 2B,C; a scattergram of mammillary body volumes of individual control and OSA subjects is shown in Fig. 2E. The mammillary body volume loss appears greater in OSA patients with T2DM (Fig. 2C vs. D); however, this latter diabetes finding must be verified with a larger diabetic sample.
The brain injury in OSA subjects is reflected as gray matter volume loss, altered metabolites corresponding to functional impairment, increased free tissue water content, and altered diffusion characteristics, including fractional anisotropy (FA, an index of fiber integrity), in those affected sites (Kamba et al., 2001, Macey et al., 2002, Morrell et al., 2003, Kumar et al., 2006b, Macey et al., 2008). In addition, we found injury to hypothalamic-projecting fibers from the insular cortex and to the cerebellar climbing fibers, hippocampal projections to the mammillary bodies, and ponto-cerebellar projections. Other autonomic regulatory fiber bundles are also injured, including the cingulum bundle (major projections to the insula, amygdala and hypothalamus), and caudal raphé (major projections to the spinal cord, nucleus of the solitary tract and to the cerebellum). Significant brain injury also appears in cortical and suprapontine areas that influence hypothalamic regulation of autonomic action, as well as hormone release in both OSA and CCHS patients.

Medullary areas regulating autonomic outflow have been relatively unexplored until recently, since VBM procedures are inappropriate for the limited spatial dimensions and mixed fiber and neuronal tissue in these regions. Diffusion tensor imaging procedures, however, allow evaluation of both gray matter and axonal injury, and provide remarkable insights into structural changes accompanying sleep-disordered breathing conditions. DTI-based mean diffusivity measures have been used to assess tissue injury in both CCHS and OSA (Kumar et al., 2006a, 2012), and extensions of other DTI procedures can reveal specific white matter injury, distinguishing between myelin and axonal injury (Kumar et al., 2008b, 2010), outlining axonal paths with fiber tractography (Kumar et al., 2011), as well as showing overall fiber integrity with FA procedures (Macey et al., 2008).

Medullary injury in CCHS appears in both dorsal and ventrolateral medullary areas (Fig. 3C, D), the former a primary integration site for autonomic regulation (nucleus of the solitary tract), and the latter, part of the final output path for projection to the spinal intermediolateral sympathetic neurons. In addition, the locus coeruleus, the major source of adrenergic fibers is affected, as is the caudal midline raphé, source of serotonergic fibers; the ventrolateral medulla and raphé are also affected in OSA (Kumar et al., 2012). The injury appears as decreased mean diffusivity values (Kumar et al., 2012), decreased FA values (Macey et al., 2008), and regional tissue loss, measured by VBM (Macey et al., 2002). Some of the injury appears in limbic sites that exert a wide range of influences on both hypothalamic and cognitive functions; for example, the hippocampus showed major injury, as did key recipients of its projections, the mammillary bodies, and the fibers composing those projections (Macey et al., 2002, 2008, Kumar et al., 2008a). We showed that OSA results in significant tissue loss or injury to brain sympathetic and parasympathetic regulatory sites (Macey et al., 2002, 2008), including the ventromedial prefrontal cortex, cingulate cortex, and insular areas influencing the hypothalamus; axons between those areas and the hypothalamus are also affected (Wu et al., 2010). In a comparable fashion, cerebellar sites that limit or dampen the extremes of sympathetic elevation or depression (Lutherer et al., 1983), and dorsal and ventral medullary areas that regulate sympathetic and parasympathetic tone, as well as caudal raphé serotonergic neurons, which modify vascular dilation, temperature, and other functions indirectly modifying glycemic regulation are affected (Harper et al., 2000). Findings of neural injury in OSA are not confined to our group; several other laboratories describe structural damage in limbic structures, especially in the hippocampus and anterior cingulate cortex, frontal and parietal cortices, and in the cerebellum.
Metabolite changes have also been outlined, and some of these changes remain after treatment (O'Donoghue et al., 2012). Similarly, CCHS subjects show significant injury, indicated by T2-relaxometry procedures, which evaluate free water content within tissue; deviations from normal T2-relaxation values can show tissue damage (Kumar et al., 2005). Figure 3, Left, demonstrates some of the brain areas which show increased T2-relaxation values in CCHS, illustrating the affected regions in the hypothalamus (i), mid and posterior cingulate cortex (ii), subgenu and medial prefrontal cortex (iii), and cerebellar cortex (iv). Figure 3, Right, shows injury, based on axial diffusivity procedures with increased values, in A, cerebral peduncles and periaqueductual gray, B, midline raphe, and C, D, ventrolateral medulla.

AFFECTIVE DEFICITS

Most of the structural studies have principally focused on physiological consequences of autonomic regulation, such as baseline and dynamic alterations in blood pressure, heart rate, and sweating. However, OSA is accompanied by a very high incidence of depressive symptoms, with over half of subjects affected (Berry et al., 1986, Bedard et al., 1991, Sforza et al., 2002). Moreover, over a third of OSA patients show high anxiety symptoms (Sforza et al., 2002). The latter

Fig. 4. Left: Decreased FA values in 41 OSA subjects versus 69 controls superimposed on T1-weighted background; ACC = anterior cingulate, CC = corpus callosum, CB = cingulum bundle (Macey et al., 2008). The lowest panel represents outlines superimposed on a diffusion direction map. Right: The injury in the anterior cingulate is also reflected as gray matter volume loss (21 OSA vs. 21 Controls) based on VBM procedures (Macey et al., 2002).
relationship of sleep-disordered breathing with anxiety is of interest for CCHS investigators, since CCHS patients show a remarkable absence of concern for perceptions with life-threatening implications; they show no concern for the normal feelings of breathlessness encountered with high CO₂ or low O₂ levels, and engage in unreasonable risk behaviors, for example, underwater competitions with normal playmates for breathholding duration. The neural basis for depression and anxiety regulatory deficits have been described for non-OSA individuals, and point to specific brain sites of tissue injury. We examined OSA subjects with and without depressive symptoms (Fig. 5), and found enhanced brain injury in OSA subjects with increased depression symptoms in the insular, cingulate, and ventral frontal cortices, hippocampus, and amygdala, among other areas above that found in OSA-alone subjects (Cross et al., 2008). Similarly, OSA subjects with high anxiety levels showed similar exaggerated injury over subjects with OSA alone (Kumar et al., 2009).

CONSEQUENCES OF STRUCTURAL INJURY ON FUNCTIONAL SIGNAL RESPONSES

The structural injury found in both OSA and CCHS has consequences as to how the affected brain structures respond to autonomic or ventilatory challenges, and we used functional magnetic resonance imaging (fMRI) procedures to assess such responses in structurally-affected areas. Muted and time-altered functional responses are found in multiple structures, and especially in the insular and cerebellar cortices (Fig. 6). The time distortion found in both those areas are of importance for blood pressure regulation, since the right insular cortex principally mediates sympathetic influences, and the left, parasympathetic action (Oppenheimer et al., 1992, Macey et al., 2012), although both sides interact. The inability of the insular cortex to respond appropriately to a challenge, illustrated by the Valsalva maneuver in Fig. 6, would modify influences to the hypothalamus; if the neural response is reduced in time or magnitude, the outcome would likely alter activity of the hypothalamus. Similarly, the inability of the deep cerebellar nuclei to adequately respond and coordinate blood pressure would interfere with momentary control of the cardiovascular system. Altered timing of the central responses, found in the cerebellar and deep nuclei to autonomic challenges would especially affect blood pressure responses to challenges; late responses to modulate blood pressure to a postural change, for example, might lead to syncope.

T2DM AND OSA

The principal structure in the forebrain underlying TRH and other hormonal release and autonomic action is the hypothalamus, with anterior and posterior portions serving separate parasympathetic and sympathetic
action. Neurons in the paraventricular hypothalamic nucleus produce TRH, and catecholamines increase the set point for TRH inhibition by T3, promoting higher thyroid hormone levels and greater thermogenesis. The hypothalamic functional and anatomical organization has been well-described by others (Saper, 2002, Saper et al., 2005). Although the hypothalamus is an essential integrative site for hormone and catecholamine release, the key to that integration derives from cortical and subcortical regions exerting influences on its output. The insular cortex is one such structure, exerting major influences on the hypothalamus, and communicating with amygdala, cingulate, and medial prefrontal cortical areas, as well as medullary sites. The right insula is principally involved in sympathetic influences on the hypothalamus, and the left, parasympathetic action. The influences are substantial, and can be demonstrated by right-sided anterior insular excitation by electrical stimulation (Oppenheimer et al., 1992), and posterior insular stimulation eliciting arrhythmia (Oppenheimer et al., 1991). We mapped the lateralized insular gyral projections to the hypothalamus in humans to describe the anatomical basis for topographical lateralization of autonomic regulatory functions (Wu et al., 2010), and outlined the anterior-posterior right and left side insular distributions to the hypothalamus (Macey et al., 2012); those functional distributions, we found, were impaired in OSA (Wu et al., 2011).

A number of brain imaging studies have examined gray and white matter integrity and metabolic aspects of diabetic patients, and have documented presence of lacunar infarcts and cerebral atrophy. However, specific relationships between autonomic and glycemic-regulatory structures remain unclear, since many of the studies have combined Type I and II diabetic subjects, essential aspects of vascular characteristics, such as hypertension, have been uncontrolled, and especially, the presence of sleep-disordered breathing has not been partitioned. The particular involvement of autonomic regulatory structures that control sympathetic and parasympathetic action has not been evaluated, although cortical atrophy, and injury in the cerebellar cortex, hippocampus, and amygdala has been noted (Araki et al., 1994, den Heijer et al., 2003, Biessels et al., 2006, Hsu et al., 2012). A positive correlation between alterations of long-term glucose levels and overall extent of atrophy has been described (Araki et al., 1994, Biessels et al., 2006).

We found that injury, based on T2-relaxometry, in multiple structures in OSA subjects with T2DM was greatly enhanced over subjects with OSA alone. Those findings are of importance, because the increased injury in these areas that influence the hypothalamus may contribute to distorted hormone release or altered autonomic regulatory patterns that could influence the development of diabetes. The enhanced injury appears in the insular and cingulate cortices, as well as the putamen and claustrum, hippocampus, and mammillary bodies (Fig. 7). The insular and cingulate cortices play significant roles modulating hypothalamic output.

HYPOGLYCEMIA AND CCHS

Because CCHS patients show injury in a number of forebrain structures similar to OSA subjects, it would be expected that comparable problems in glycemic regulation would also appear. However, CCHS patients who do show regulatory problems (depending on severity of PHOX2B gene mutations), usually show hypoglycemia. The nature of brain injury in autonomic regulatory sites...
in CCHS differs somewhat from OSA; parasympathetic action is severely affected in CCHS, as found in pupillary adjustment deficits, absence of vagally mediated heart rate variation (Woo et al., 1992, Weese-Mayer et al., 2010), and frequent intestinal motility issues. Although sympathetic deficits are also prominent, injury to a large number of sites, including periaqueductal gray, raphé, medullary autonomic regulatory areas, as well as the cerebellum, have the potential to significantly affect parasympathetic influences to pancreatic sites.

**LATERALIZED INJURY IN OSA**

A remarkable aspect of both gray and white matter injury in OSA is lateralization of damage, with more right-sided insula cortex and cingulum bundle injury than left, greater volume loss in the left mammillary bodies, unilateral ventrolateral medullary damage, significantly asymmetrical cerebellar cortex injury, and unilateral diminished numbers of ponto-cerebellar fibers. The asymmetric nature of injury has several implications for hormonal and autonomic regulation. More right-sided injury appears in the insular cortex in OSA, which should affect, that is, preferentially disinhibit, sympathetic outflow more over parasympathetic action (Oppenheimer et al., 1992, Oppenheimer, 2006, Macey et al., 2012). The potential for asymmetric action, especially on the hypothalamus, enhances the possibility for influencing hormone and sympathetic levels contributing to diabetes, for example, TRH and glucagon levels, and may alter left versus right brain perfusion, potentially furthering neural injury. Exaggerated lateralized sympathetic outflow enhances risk for potentially fatal arrhythmia (Oppenheimer, 2006).
CONCLUSIONS

Sleep-disordered breathing is accompanied by significant injury to brain areas that are implicated for autonomic and hormonal regulation. Autonomic, and especially sympathetic dysfunctions, appear in OSA, and OSA is present in a very high proportion of T2DM subjects. We suggest that autonomic dysregulation in OSA develops from damage to central autonomic regulatory areas, and this dysregulation extends to release of hormones essential for glycemic regulation. Analogous injury in other autonomic regulatory areas in CCHS may lead to the frequent hypoglycemia in that syndrome. Both abnormal autonomic regulation in OSA and emergence of OSA in T2DM may stem from common injury to neural areas modulating hypothalamic functions. Sleep-disordered breathing, brain injury, and impaired glycemic regulation co-occur, and understanding the common mechanisms may lead to improved treatment of the overlapping conditions, as well as determination of approaches to prevent further brain injury.

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LITERATURE CITED


