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RODENT MODELS OF SLEEP APNEA

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Abstract

Rodent models of sleep apnea have long been used to provide novel insight into the generation and predisposition to apneas as well as to characterize the impact of sleep apnea on cardiovascular, metabolic, and psychological health in humans. Given the significant body of work utilizing rodent models in the field of sleep apnea, the aims of this review are three-fold: first, to review the use of rodents as natural models of sleep apnea; second, to provide an overview of the experimental interventions employed in rodents to simulate sleep apnea; third, to discuss the refinement of rodent models to further our understanding of breathing abnormalities that occur during sleep. Given mounting evidence that sleep apnea impairs cognitive function, reduces quality of life, and exacerbates the course of multiple chronic diseases, rodent models will remain a high priority as a tool to interrogate both the pathophysiology and sequelae of breathing related abnormalities during sleep and to improve approaches to diagnosis and therapy.

Keywords

Breathing; central sleep apnea; intermittent hypoxia; mice; obstructive sleep apnea; rats; upper airway

1. INTRODUCTION

Sleep apnea is a highly prevalent condition affecting 4% of men and 2% of women (Young et al., 1993) which, if left untreated, is associated with increased cerebrovascular, cardiovascular, and cancer morbidity and mortality (Marin et al., 2005; Nieto et al., 2012; Punjabi et al., 2009; Yaggi et al., 2005). Sleep apnea is defined by an increased apnea-hypopnea index of >5 with the associated symptom of daytime somnolence, and in the simplest terms can be classified as obstructive or central in nature. Obstructive sleep apnea is characterized by repetitive obstructions of the upper airway during sleep, despite increasing effort from respiratory pump muscles, which results in cessation or reduction in airflow. In central sleep apnea, there is cessation of effort from respiratory pump muscles

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that induces apnea in the absence of airway obstruction. Thus, sleep apnea is a multifaceted presentation of breathing abnormalities during sleep, which is common and, if present, leads to an increase in morbidity and mortality in patients.

Due to the confounding effects of obesity, cardiovascular disease and other co-morbid conditions on respiratory control and upper airway function during sleep in humans, animal models have long been used to investigate the physiological basis and the pathological implications of sleep apnea. Over the last twenty years, rodent models have provided novel insight into the generation and predisposition to apneas as well as the downstream effects of sleep apnea particularly on cardiovascular, metabolic, and psychological health (Campen et al., 2005; Fletcher et al., 1992; Li et al., 2005; Row et al., 2002). Rodent models have greatly enhanced our understanding of sleep apnea in humans with implications for who is at risk for developing sleep apnea and identifying mechanisms linking sleep apnea to adverse health outcomes.

Given the significant body of work utilizing rodent models in the field of sleep apnea, the aims of this review are three-fold: first, to review the use of rodents as natural models of sleep apnea; second, to provide an overview of the experimental interventions employed in rodents to simulate sleep apnea; third, to discuss the refinement of rodent models to further our understanding of breathing abnormalities that occur during sleep.

2. RODENTS AS NATURAL MODELS OF SLEEP APNEA

2.1 Classification of central apneas during sleep in rodents

Reports that adult rats exhibit periodic cessations of respiratory effort during the daytime date back as early as 1988 (Mendelson et al., 1988). Subsequently, apneas in rodents were reported to occur almost exclusively in absence of diaphragmatic electromyography (EMG) activity (Sato et al., 1990). Systematic characterization of apneas in rats was performed by Christon et al. (1996) as they observed apneas under normoxic conditions, as well as during hypoxia, hypercapnia, and hyperoxia. They assessed breathing patterns throughout the sleep-wake cycle by combining whole body plethysmography, to measure respiratory patterns, with electrical recordings of EEG and nuchal EMG signals, to characterize sleep. Rats breathe on average 4–5 times more rapidly than humans. Thus, the authors defined a central apnea as a cessation of respiratory effort for at least 2.5 seconds (corresponding proportionally to the accepted duration of 10 seconds used to define an apnea in humans (Berry et al., 2012)). Central apneas were subcategorized as (i) postsigh apneas if the preceding breath was at least 25% above the average amplitude of prior breaths or (ii) spontaneous apneas if there was no evidence of a preceding sigh. Under normoxic conditions, central apneas occurred at a higher rate during REM sleep (approximately 11 apneas/hour) compared to NREM sleep. Post-sigh apneas were four times more commonly noted than spontaneous apneas. Hyperoxic conditions increased the frequency of apneas whereas hypoxia and hypercapnia both decreased the rate of apneas. Thus, sleep in rats is associated with the generation of spontaneous central apneas and the apnea rate is responsive to alterations in inspired gas concentrations.

Similarly, the impact of inspired gases on central apnea rates during sleep was assessed in mice by Nakamura et al. (2003). Sleep was measured in adult 129/Sv mice via interpretation of EEG and nuchal EMG electrical signals and breathing was determined by plethysmography as well as by intercostal muscle EMG electrodes. A central apnea was defined as a cessation of plethysmographic signal for at least two respiratory cycles (chosen to mimic a pause of >10 seconds in humans). Again, central apneas were characterized as either spontaneous or post-sigh based on the same criteria used in the rat studies. The results indicate that 32 instances of apnea occurred on average during the 6 hour recording period under room air conditions. Interestingly, postsigh apneas only occurred during NREM sleep whereas spontaneous apneas (altogether much less common) occurred only during REM sleep. All apneas were associated with a disappearance of the intercostal EMG trace, consistent with the apneas being central, not obstructive events. To determine a rate of apneas, they calculated an apnea occurrence index, defined as the number of apneic episodes per hour of each sleep stage. Spontaneous apneas during REM sleep had a higher occurrence index (14.8) than postsigh apneas during NREM sleep (9.1). The investigators exposed the mice to hypoxic, hyperoxic, and hypercapnic environments and demonstrated that hypoxia induced a 50% increase in postsigh apneas, whereas hyperoxia and hypercapnia resulted in a 60–70% decrease in postsigh apneas compared to room air. Taken together with the characterization of apneas during sleep in rats, the number, type of apnea, and response to altered ambient environments may differ between the two species of rodents. However, both species clearly have evidence of central apneas during sleep and can serve as models for further investigation into the relationship between central sleep apnea and various disease states.

2.2 Central apnea rates in rat strains with abnormal blood pressure

Extending on initial studies characterizing central apneas in rats, the Carley and Radulovacki laboratory assessed apnea rates after manipulating blood pressure to examine the complex relationship between cardiovascular health and abnormalities in breathing during sleep. In both lean and obese Zucker rats, hypotension led to a reduction in spontaneous apneas (lean rats: approximately 6 apneas/hour at baseline to less than 1 apnea/hour after hypotension; obese rats: approximately 2 apneas/hour at baseline to less than 1 apnea/hour after hypotension) and post-sigh apnea rates (both lean and obese rats: approximately a 70% reduction in apneas after hypotension) during NREM sleep (Radulovacki et al., 1996). Additionally, they noted an increase in respiratory rate and minute ventilation in the setting of hypotension which suggested that the impact of hypotension on apnea severity may be a result of modulation of the underlying respiratory drive. Conversely, spontaneously hypertensive rats, a substrain of Wistar Kyoto rats, demonstrated an increase in spontaneous apneas during NREM sleep (from approximately 1 to 17 apneas/hour) as well as an increase during REM sleep (from approximately 4 to 45 apneas/hour) compared to controls (Carley et al., 2000). Interestingly, sustained administration of antihypertensive medication normalized the blood pressure of the hypertensive rats but did not lead to changes in apnea rates. The authors postulated that there is a genetic predeterminant for central sleep apnea in the spontaneously hypertensive rats which is independent of the effects of blood pressure. In summary, perturbations in blood pressure impact sleep apnea rates in rats, supporting the

concept that rodents serve as useful models to further explore the mechanistic basis for the development of central sleep apnea in cardiovascular disease.

2.3 Central apnea rates in different mouse strains

In an effort to examine genetic pre-determinants for sleep apnea, several studies have investigated central apnea rates and breathing variability between inbred mouse strains or between genetically modified mouse strains and their wildtypes. Han et al. (2002) measured periodic breathing in C57BL/6J and A/J mice during wakefulness after a brief hypoxic challenge to assess for strain differences in mice. Isocapnic hypoxia (3% CO₂–10% O₂) or hypocapnic hypoxia (8% O₂) was administered to A/J mice and B6 mice for 5 minutes followed by a rapid transition to either normoxia or hyperoxia (100% O₂) to induce breathing instability through rapid withdrawal of chemoreceptor input. Periodic breathing was assessed by plethysmography as cyclic fluctuations in tidal volume and respiratory frequency interrupted by periods of apnea (defined as a pause greater than twice the average breath duration). The B6 mice exhibited periodic breathing upon reoxygenation with either air or 100% O₂, whereas none of the A/J mice showed clear oscillations in breathing patterns. A/J mice were subsequently used in a model of cerebral infarction to test for induction of periodic breathing. Koo et al. (2010) induced a stroke by surgical occlusion of the middle cerebral artery in A/J mice and demonstrated that breathing patterns were much more variable in the mice with stroke vs. controls. Thus, although A/J mice have less variability in breathing than B6 mice, periodic breathing is inducible experimentally in the context of a clinical state that predisposes to central sleep apnea.

Subsequently, the hypothesis that different mouse strains experience varying breathing patterns during wakefulness was extended into sleep (Friedman et al., 2004). A/J mice were again compared to B6 mice and plethysmography was performed for an average of 5 hours, at least 72 hours after surgical placement of EEG and EMG electrodes to allow for simultaneous assessment of sleep and breathing. Consistent with the findings of differing breathing patterns during wakefulness (Han et al., 2002), frequency of breathing differed between A/J and B6 animals throughout all stages of sleep, with A/J mice breathing more slowly within each sleep state. Although apneas and variability of breathing were not reported in this analysis, the study does highlight fundamental strain differences in breathing that occur during wakefulness are also manifest during sleep.

2.4 Genetically modified mouse models and central apnea generation

Looking beyond strain differences, several investigators have explored the impact of specific candidate genes on control of breathing and apnea generation in mice. For example, the monoamine serotonin (5-HT) is known to play a role in the control of ventilation, with increased levels leading to greater apnea generation (Joseph et al., 2002). Thus, a monoamine oxidase A (MAO-A) knockout mouse was studied to assess the impact of increased levels of 5-HT on apnea generation (Real et al., 2007). Ventilation was measured by whole body plethysmography during daylight (sleeping) hours simultaneously with EEG and nuchal EMG signals to determine sleep. Using the same definitions of apneas as Nakamura (2003), Real et al. (2007) demonstrated significantly higher apnea rates (3-fold increase) during both NREM and REM sleep in MAO-A knockout mice compared to

wildtype mice. Furthermore, administration of the MAO-A inhibitor, clorgyline, to wildtype mice similarly increased apnea rates in NREM and REM sleep.

A second example of the use of knockout mice to evaluate sleep and breathing is seen in studies focusing on orexin (Nakamura et al., 2007). Although orexin knockouts are commonly thought of as models of human narcolepsy, axons of orexin-containing neurons project from the hypothalamus to brainstem regions implicated in the control of breathing (Young et al., 2005). Orexin-knockout mice exhibited more spontaneous, but not post-sigh, apneas compared to wildtype controls, suggesting that orexin may exert an inhibitory effect on the genesis of spontaneous central sleep apneas.

As a third example, breathing parameters were evaluated in newborn mice heterozygous for the transcription factor Phox2b (Durand et al., 2005). Central congenital hypoventilation syndrome (CCHS) is a rare autosomal dominant syndrome characterized by abnormal control of breathing during sleep and has been linked to mutations in Phox2b (Amiel et al., 2003). Phox2b-expressing neurons in the retrotrapezoid nucleus, for example, have been shown to be necessary for CO₂ chemosensitivity and adequate ventilation. Loss of these neurons leads in part to the development of CCHS (Goridis et al., 2010; Stornetta et al., 2006). To test the hypothesis that Phox2b knockout mice express abnormal control of breathing, newborn mice with heterozygous deletion of Phox2b were studied at age 5 days for short periods of time (15 min) by plethysmography (Durand et al., 2005). As anticipated, Phox2b knockout mice showed significantly longer apnea time during sleep than the wild-type controls without change in sleep-wake state durations. A final example, also of a developmental defect impacting breathing regularity, is the X-linked methyl-CpG binding protein 2 gene (Mecp2) knockout mouse that models the human neurodevelopmental disorder Rett Syndrome (Stettner et al., 2007). Similar to human patients who can experience breathing arrhythmias, the mice with targeted deletion of the Mecp2 gene exhibit dysregulated post-inspiratory activity that leads to a more than doubling of the unprovoked central apnea rate. Taking the four genetically modified models described above, as well as the studies demonstrating strain differences in breathing during sleep, mice represent an essential tool to study the impact of genomic background and specific candidate genes on altered respiratory control and generation of central sleep apnea.

2.5 Pharmacologic induction of central apnea

Rodents have been used to study the neurochemical pathways involved in apnea generation. For example, Carley and Radulovacki (1999a) investigated the impact of serotonergic pathways on central apnea generation in rats based on complementary work demonstrating systemic serotonin administration increases apnea expression (Carley and Radulovacki, 1999b) and blockade of the 5-HT₃ specific serotonin receptor reduces apnea expression in rats (Radulovacki et al., 1998). The investigators chose mirtazapine as a test agent since it is known to enhance serotonin at 5-HT₁ receptors in the brain while also acting as an antagonist at 5-HT₃ receptors. Mirtazapine significantly reduced apnea expression in rats by more than 50% in both NREM and REM sleep, while increasing minute ventilation throughout all sleep/wake stages. Support for a role of 5-HT₁ receptors in apnea genesis is also evident in mice, which exhibit a significant decrease in spontaneous apneas in the

presence of the receptor agonist 8-OH-DPAT (Stettner et al., 2008). As a final example, Fenik et al. (2001) studied the local and systemic effects of alterations in the serotonergic pathway in rats on hypoglossal nerve function, suggesting that serotonin pathways have potential to impact the development of both central and obstructive apneas. Taken together, these studies demonstrate a complex role for serotonin in apnea generation and provide model approaches for testing potential therapeutic agents in rodents.

2.6 Obese rodents and altered control of breathing

Given the recent obesity epidemic, a variety of obese rodent models have been studied to provide insight into abnormal control of breathing and potentially to model obstructive sleep apnea. For example, the Zucker rat and ob/ob mouse have been used to study the impact of obesity on pulmonary function and respiratory control. Both obese rodents exhibited depressed ventilatory responses to either hypoxic or hypercapnic stimuli compared to lean controls (Farkas and Schlenker, 1994; O'Donnell et al., 1999). Furthermore, under resting conditions, leptin-deficient ob/ob mice exhibited a higher PaCO₂ than lean mice, consistent with a phenotype of obesity hypoventilation. Three-day leptin replacement in the ob/ob mice stimulated ventilation across all sleep wake states; a response that was independent of any weight change. Neither study in obese mice or rats comments specifically on the presence or differential expression of apneas between the groups. However, the findings indicate that rodents provide an important tool for examining molecular pathways that contribute to ventilatory depression in obesity.

2.7 Obese rodents and obstructive sleep apnea

To date, the English bulldog (Hendricks et al., 1987), with its distinct craniofacial abnormalities, is the animal that most closely models human obstructive sleep apnea. Since obesity is a major risk factor for obstructive sleep apnea, the upper airway of obese mice has been characterized using imaging and physiologic approaches. Volumetric MRI assessment in the obese New Zealand mouse demonstrated a phenotype of increased visceral fat, neck fat, and increased volume of the tongue, soft palate, and lateral pharyngeal walls compared to lean mice (Brennick et al., 2009). Additionally, leptin-deficient ob/ob mice have defects in upper airway neuromechanical control which is reversed with leptin administration (Polotsky et al., 2012), which suggests that leptin protects against upper airway obstruction in obese mice, and that mice deficient in leptin may be vulnerable to the development of obstructive apnea. In a follow-up study, Hernandez et al. (2012) used plethysmography and polysomnography to obtain short-term characterization of sleep and breathing in the New Zealand obese mouse. With adaptation of an infant blood pressure cuff, respiratory movement was estimated and compared to signals of airflow to determine whether reduced upper airway flow occurred in the presence or absence of respiratory movement. They report evidence of a progressive decrease in inspiratory airflow despite increasing inspiratory effort, which is suggestive of inspiratory flow limitation. In contrast, obese rats did not exhibit any increase in genioglossus muscle activity, despite a narrower airway, compared to lean rats, and their upper airway remained stable even during REM sleep (Sood et al., 2007). Taken together, obese rodents have imaging suggestive of airway narrowing and can secrete adipokines that impact upper airway collapsibility, but it remains inconclusive whether obesity induces functional obstruction or airflow limitation in rodents.

2.8 Summary of natural models of sleep apnea

Taking the above studies together with many unmentioned in this review, it is clear that rodents have contributed significantly to our understanding of the genesis of sleep apnea and its complex relationship with co-morbid disease processes. Under natural conditions, both rats and mice exhibit central sleep apnea with differing apnea rates depending on sleep state, much like humans, although there is no evidence to date that central apneas in rodents lead to downstream tissue organ or pathology. The expression of apnea varies by strain, genetic manipulation, and pharmacologic intervention, leading to a greater understanding of respiratory control and highlighting potential therapeutic approaches to reduce central sleep apnea rates in the clinical setting. Obese mice exhibit an obesity hypoventilation phenotype, have impairments in upper airway structure and function, and potentially may simulate obstructive sleep apnea in humans.

3. EXPERIMENTALLY-INDUCED MODELS OF SLEEP APNEA

3.1 Intermittent hypoxia

In the natural rodent models of sleep apnea, central events have been consistently reported, but there is no compelling evidence as yet that rodents exhibit spontaneous obstructive apneas. Given the high prevalence of obstructive sleep apnea in humans and its associated adverse impact on cardiovascular, metabolic, psychological and other health outcomes, experimental models of obstructive sleep apnea have been developed in rodents. The classic model involves administration of intermittent hypoxia (IH) to simulate the repetitive brief periods of hypoxia and reoxygenation that characterize obstructive sleep apnea. Fletcher et al. (Fletcher et al., 1992) pioneered the use of IH in Wistar rats in 1992 in a study examining the impact of episodic hypoxia on systemic blood pressure. Based on observational studies in humans with obstructive sleep apnea, it was hypothesized that IH would lead to acute, as well as chronic, elevations in blood pressure. Rats were housed in hypoxic chambers that allowed for timed administration of nitrogen to reduce the ambient concentration of oxygen to 3–5% for approximately 3–6 seconds followed by a gradual return to a normal 21% oxygen concentration. The cycle was repeated twice per minute over 6–8 hours per day for 35 consecutive days. Rats treated with IH exhibited increased systemic blood pressure as well as evidence of left ventricular hypertrophy. These initial studies performed by Fletcher and colleagues demonstrate a shared phenotype of systemic hypertension in both human obstructive sleep apnea and rodent IH, thus providing early evidence that the rodent model serves as a useful tool to study the pathologic sequelae of obstructive sleep apnea.

Patients with obstructive sleep apnea commonly exhibit arousals that result from oxyhemoglobin desaturation and increasingly negative intrapleural pressure swings, which act to disrupt and fragment sleep architecture and contribute to daytime sleepiness (Gstaaut et al., 1966). Rodent studies have examined the impact of IH on sleep architecture to determine if sleep is similarly disrupted (Decker et al., 2003; Gozal et al., 2001; Veasey et al., 2004). For example, Polotsky et al. (2006) compared sleep architecture in mice exposed to five days of IH to a comparable rate of non-hypoxic sleep fragmentation using high-flow air blasts to produce an auditory/tactile stimulus. Analysis of individual events demonstrated that whenever sleep was present at the start of the hypoxic episode (44% to 64% of the time

from day 1–5, respectively), the nadir of a hypoxic event resulted in an arousal response effectively 100% of the time which closely simulates human obstructive sleep apnea. On the macro level, NREM sleep was reduced approximately 30% during the light phase (when IH was delivered) and was followed by a rebound of NREM sleep during the dark phase (when room air was delivered) throughout the first day of exposure. Over the course of the 5 day study, there was a normalization of total NREM sleep during both the light and dark phases, but individual hypoxic events continued to produce arousals from sleep. IH effectively eliminated REM sleep during the light phase exposure period (from approximately 6% at baseline to less than 1% of the light period), and rebounded significantly during the recovery dark period. Non-hypoxic sleep fragmentation resulted in similar disruptions in sleep architecture although the findings were in general less severe than for IH, particularly during REM sleep. Similarly, Gozal et al. (2001) exposed rats to intermittent hypoxia and studied the resultant impact on neurocognitive morbidity. They report a reduction in NREM and REM sleep during the first day of episodic hypoxia which normalizes by day three. The persistent reductions in REM sleep described by Polotsky et al. (2006) throughout the five days of analysis may be due to differences in the pattern and severity of intermittent hypoxic exposure.

In a separate study (Veasey et al., 2004), it was demonstrated that long-term exposure to IH (8 weeks) produced a 'sleepy' phenotype based on a novel development of a mouse 'sleep latency test.' Moreover, the phenotype persisted for two weeks after completion of the IH exposure and was associated with damage to selected wake-promoting regions of the brainstem and basal forebrain, consistent with reported brain structural defects in patients with obstructive sleep apnea (Macey et al., 2008; Morrell et al., 2003). Thus, closely mimicking human obstructive sleep apnea, the mouse model of IH results in an arousal with each hypoxic event, disrupts overall sleep architecture, suppresses cycling into REM sleep, and induces a 'sleepy' phenotype.

3.2 Caveats to intermittent hypoxia models in rodents

Since the initial studies on systemic blood pressure responses in rats, there have been numerous studies on the impact of IH on a wide range of pathological outcomes. However, in part due to the equipment and technology used to produce IH, there is significant variability in the protocols used to deliver IH. For example, one study investigating sex differences in blood pressure responses to IH utilized a protocol which exposed rats to 3 minutes of 10% oxygen alternating with 3 minutes of 21% oxygen for 8 hours a day over the course of 1 week (Hinojosa-Laborde and Mifflin, 2005). Another such protocol exposed rats to IH over the 12 hour light period cycling from 10 to 21% oxygen every 90 seconds during the light/sleeping period for seven days (Row et al., 2003) to investigate the impact of IH on spatial learning in rats. Other studies have utilized much more severe degrees of hypoxia, including Chen et al. (2005) who targeted a nadir concentration of 4–5% O₂ cycling every 60 seconds in a protocol that more closely resembled the original studies of the Fletcher laboratory (1992). Similarly in mouse studies, the IH protocols have varied in the degree of hypoxemia, rate of exposure, and duration of exposure. Using a comparable model of nadir hypoxia and rate of exposure, studies in mice have varied from as short as nine hours, studying acute effects on insulin resistance (Iiyori et al., 2007), to as long as twelve weeks,

studying the chronic effects of atherosclerosis (Savransky et al., 2007). Likely, the duration of IH exposure will differentially impact specific physiologic or pathologic processes.

The vast majority of rodent IH studies have attempted to replicate pathologic processes associated with clinical obstructive apnea as a first step in ascribing causality. However, recent cardiovascular studies of IH in mice indicate that particular exposure times may actually induce physiologic compensatory improvements in function rather than pathologic defects. Park and colleagues tested the hypothesis that chronic IH exacerbates myocardial ischemia-reperfusion injury by subjecting mice to a standard IH protocol over the course of 1, 2, or 4 weeks (Park and Suzuki, 2007). The duration of IH was shown to be important in that mice treated with 4 weeks of IH had reduced cardiac damage compared to mice treated for shorter durations. Similarly, a protective effect on left ventricular function has been described in a genetically modified murine model of heart failure when the animals were exposed to four weeks of IH (Naghshin et al., 2012). In summary, there has been heterogeneity in IH studies, with outcomes, in general, affected more by the duration of exposure rather than the hypoxic nadir or the rate of hypoxic cycling.

Whether the variability in IH protocols has enhanced or detracted from the rodent model of obstructive sleep apnea is debatable. Indeed, obstructive sleep apnea in humans is a heterogeneous disorder with variable phenotypic expression of the degree, frequency, and depth of hypoxia. Thus, by extrapolating the findings from rodent models of IH into human obstructive sleep apnea, a certain degree of variability is in fact representative of the disease process and has been arguably more informative in the long run than if laboratories had attempted to standardize and replicate IH protocols.

3.3 The addition of hypercapnia to the intermittent hypoxia model

A common criticism of the IH model is that it does not simulate the hypercarbia that occurs acutely in patients during obstructive apneic episodes. However, there is evidence that the acute increase in sympathetic nerve activity that occurs during obstructive apnea is significantly attenuated by removal of the hypoxic stimulus (breathing 100% O₂ immediately prior to apnea) with the hypercapnic stimulus remaining present (O'Donnell et al., 1996). These data supporting a dominant role for intermittent hypoxia over intermittent hypercapnia on sympathetic nerve activity are corroborated by an earlier study from the Fletcher laboratory showing similar increases in systemic blood pressure in rats exposed to IH under hypocapnic, eucapnic, and hypercapnic conditions (Fletcher et al., 1995). A more recent paper (Norton et al., 2011) suggests that after four weeks exposure to IH there is a differential response in nitric oxide-dependent vasodilation of pulmonary vascular smooth muscle based on the presence of eucapnia versus hypocapnia. Although these latter data indicate that background PaCO₂ may impact physiologic responses to IH, there is a need for further studies targeting the physiologic and pathologic responses to the more clinically relevant combination of simultaneous intermittent hypoxia and hypercapnia.

3.4 Airway obstruction in rodents

Another defining feature of obstructive sleep apnea that is not modeled by IH is the collapse of the upper airway and associated increased negative pleural pressure swings. The first

mechanically obstructed rodent model described in the literature was performed in 1991 and employed two years later to assess the impact of chronic upper airway obstruction on right and left ventricular hypertrophy in rats (Salejee et al., 1993; Tarasiuk et al., 1991). Upper airway obstruction was created by placing a plastic cannula around the trachea to create a tracheal band. The band was tightened until the intrathoracic pressure oscillations were twice that of control animals. Continuous tracheal banding led to higher PaCO₂ levels, increased intrathoracic pressure swings, and increased ventricular mass in rats compared to non-obstructed controls. Despite the conceptual attraction of tracheal banding to induce upper airway obstruction, the inability to specifically link the obstruction to periods of sleep and hypoxia detracts from its generalizability as a model of obstructive sleep apnea.

More recently, an alternative model for inducing upper airway collapse in anesthetized rats was developed to more closely simulate obstructive apneas in humans (Almendros et al., 2008). Using a customized mask attached to a dual lumen cannula, the investigators applied recurrent negative pressure swings to the upper airway while maintaining patency to the lower airway, inducing 15 second periods of obstruction at a rate of 60 events per hour for five hours. They demonstrated that recurrent collapse and reopening of the upper airway induced a local inflammatory response. A subsequent paper suggested that under acute conditions (one hour) airway obstruction differed from IH in cerebral oxygenation responses (Almendros et al., 2011), suggesting model selection may be important for studying specific pathologic outcomes. The same group has now gone on to develop a model to induce airway obstruction in mice using an electronically controlled airbag system located at the animal's snout (Carreras et al., 2011). However, the inability to apply these innovative methods in sleeping mice or rats for extended durations of weeks to months currently limits their applicability as models of obstructive sleep apnea.

3.5 Intermittent hypoxia specifically during sleep

A limitation of both the airway obstruction and IH models in rodents is that the intervention is not linked to sleep. In the IH model, the stimulus is delivered during the light or sleeping phase to maximize the likelihood the animal will be asleep. However, there will be significant intervals during the light period when animals will be exposed to IH while awake, and significant intervals during the dark period when animals will be asleep but not exposed to IH. To address limitations in the IH model, techniques have been developed using automated real-time sleep scoring and feedback to gas control systems to expose rats (Hamrahi et al., 2001) and mice (Tagaito et al., 2001) to hypoxia only during periods of sleep.

In mice, delivery of sleep-induced hypoxia (a term used by the authors to describe the model of intermittent hypoxia delivered specifically during sleep) was used to examine the genetic impact on 'event expression' by comparing two different inbred mouse strains with divergent chemoresponsiveness (Rubin et al., 2003). The A/J mouse strain possesses small carotid bodies and exhibits a depressed hypoxic ventilatory responsiveness relative to the DBA/2J mouse strain. During five consecutive days of sleep-induced hypoxia, the chemo-depressed A/J mice exhibited longer and more severe hypoxic events than DBA/2J mice. However, despite the more severe hypoxic insult, the A/J mice surprisingly had less sleep

disruption than the DBA/2J mice and consequently developed a higher rate of hypoxic events. Thus, genetic background can influence the degree and frequency of hypoxic events as well as impacting total sleep time, suggesting that when upper airway collapse occurs in a patient with obstructive sleep apnea the subsequent oxyhemoglobin desaturation and apnea index are, at least in part, genetically determined responses.

Although sleep-induced IH is likely a more clinically relevant model than the commonly used non-sleep dependent IH, it does have inherent limitations. Namely, the technical requirements for automated on-line detection software and feedback control limit the number of animals that can be exposed at one time. Moreover, the length of the exposure period is constrained by the ability to maintain viable polysomnographic signals. Thus, for studies that are focused on pathologic outcomes of obstructive sleep apnea, particularly over extended periods of time, the non-sleep dependent IH exposure still remains the most practical approach.

3.6 Summary of experimentally-induced models of sleep apnea

Rodent models of sleep apnea have greatly enhanced our understanding of control of breathing and the pathophysiologic consequences of IH. Intermittent hypoxia is now established as the dominant rodent model in the field, and refinements in the areas of sleep-induced IH, the addition of hypercapnic stimuli, and development of models of mechanical obstruction of the upper airway continue to provide important and novel findings moving forward.

4. CONCLUSIONS AND FUTURE DIRECTIONS

A body of work over the last two decades has utilized rodents as either natural models of sleep apnea or experimentally-induced models of sleep apnea. For the IH model, the short duration of repetitive hypoxic episodes (e.g. two minutes or less) administered for weeks to months separates the field from other models of hypoxic/ischemic preconditioning (e.g. less than 10 brief hypoxic episodes) and models of altitude 'live high/train low' performance enhancement (e.g. hypoxia lasting hours at a time), which are also commonly referred to as 'intermittent hypoxia.' It is not surprising given the physiologic benefits ascribed to hypoxic/ischemic preconditioning and 'live high/train low' regimens, that intermittent hypoxia modeling sleep apnea has induced a range of outcomes from adaptive to pathologic responses. The variability in outcomes is in part likely to be related to organ exposure and hypoxic vulnerability to different IH regimens (Reinke et al., 2011), as well as the effects of IH on body weight and respiratory muscle exercise. Although it is tempting to suggest that laboratories should adopt common IH protocols, it can be argued that the field as a whole has gained more from the diversity of protocols and approaches than it has lost from the difficulties inherent in comparing between studies using disparate protocols.

The study of natural models of sleep apnea in rodents remains focused on the genesis and modulation of central sleep apnea. The models have relevance across a range from the underlying molecular basis of respiratory control and apnea generation to the development of central apneas in pathologic states including heart disease and stroke. However for accurate classification of apneas and to maximize the translational applicability of the data it

is imperative that rodent studies of breathing stability and apnea generation are accompanied by simultaneous classification of sleep state using polysomnography.

Development of natural models of obstructive sleep apnea or inducible airway obstruction in non-anesthetized rodents would greatly move the field forward. Historically, apneas in rodents have been described as exclusively central events and the upper airway anatomy of mice and rats is more stable than the upper airway of humans. Although the recent work by Hernandez et al. (2012) suggests that obese mice can demonstrate flow limitation of the upper airway, it is still unclear whether clinically relevant obstructive apneas occur in sleeping rodents. A potential approach to addressing the question of whether or not rodents have obstructive events would be to utilize diaphragm and/or intercostal EMGs to measure respiratory effort simultaneously with airflow measurements. In future, detailed assessment of breathing during sleep via interpretation of airflow signals, diaphragm muscle activity, and whole body plethysmography will be needed to definitively uncover the presence of obstructive apneas in rodents.

If obese rodents do not exhibit obstructive apneas, other creative approaches may hold promise. Increasing the mechanical load of the upper airway (e.g. depositing substances such as silicon around the tissues of the upper airway) or decreasing the neuromuscular input to the upper airway (e.g. denervating specific upper airway dilator muscles) could potentially change flow limitation of the upper airway in obese mice to a state of upper airway collapse during sleep. The development of a naturally occurring rodent model of obstructive sleep apnea would provide a much needed platform for screening and testing of therapeutic drugs directed at the pathology of upper airway collapsibility. A second approach is to build on the nascent models of inducible airway obstruction recently described in rats and mice. However, unless these inducible airway obstruction models can be developed in non-anesthetized sleeping rodents and applied for prolonged periods of time their potential application may be limited.

Overall, great progress has occurred in the development, application, and translation of rodent models of sleep apnea. In the setting of high morbidity and mortality in patients with sleep apnea, rodents will continue to serve as invaluable tools to help uncover the pathophysiology and sequelae of this heterogeneous disorder and maintain a vital role in translating new discoveries into improved diagnosis and treatment of patients.

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HIGHLIGHTS

- Rodents are an established and evolving tool for modeling sleep apnea in humans
- We review the use of rodents as natural models of central sleep apnea
- We examine the simulation of obstructive sleep apnea in rodent models
- We explore possibilities for future advances in rodent models of sleep apnea