Flinders UNIVERSITY
ADELAIDE · SOUTH AUSTRALIA

Personalized, physiology-based treatment for obstructive sleep apnea

By

Ludovico Messineo

Thesis
Submitted to Flinders University
for the degree of Doctor of Philosophy

Supervisor
Prof. Danny Eckert

Doctor of Philosophy

College of Medicine and Public Health
March 2022

CONTENTS

LIST OF FIGURES

LIST OF TABLES

PUBLICATIONS FROM THIS THESIS

I. **Messineo L**, Eckert DJ. Pathogenesis of Sleep Apnea. Published in: Obesity Hypoventilation Syndrome, 1st Edition, From Physiologic Principles to Clinical Practice. Elsevier, 2020, pp 55-56, ISBN 9780128152904.

II. **Messineo L**, Eckert DJ. Obstructive Sleep Apnea Phenotyping to Understand Pathophysiology and Improve Treatment and Outcomes. Editor(s): Sam M Janes, Encyclopedia of Respiratory Medicine (Second Edition), Academic Press, 2022, Pages 22-33, ISBN 9780081027240.

III. **Messineo L**, Eckert DJ, Lim R, Chiang A, Azarbarzin A, Carter SG, Carberry JC. Zolpidem increases sleep efficiency and the respiratory arousal threshold without changing sleep apnoea severity and pharyngeal muscle activity. J Physiol. 2020;598(20):4681-4692. doi: 10.1113/JP280173.

IV. Taranto-Montemurro L, **Messineo L**, Wellman A. Targeting endotypic traits with medications for the pharmacological treatment of obstructive sleep apnea. A review of the current literature. J Clin Med. 2019;8(11):1846. doi: 10.3390/jcm8111846.

V. **Messineo L**, Carter SG, Taranto-Montemurro L, Chiang A, Vakulin A, Adams, RJ, Carberry JC, Eckert DJ. The addition of zolpidem to combination therapy with atomoxetine-oxybutynin increases sleep efficiency and the respiratory arousal threshold in obstructive sleep apnea: A randomized trial. Respirology. 2021;26(9):878-886. doi: 10.1111/resp.14110. Epub 2021 Jun 23.
**This article was accompanied by an Editorial: Ip MSM, Chung KF. Pharmacotherapy for obstructive sleep apnoea: Is the magic bullet in sight? Respirology 2021;26(9):835-836.*

VI. **Messineo L**, Eckert DJ, Taranto-Montemurro L, Vena D, Azarbarzin A, Hess L, Calianese N, White DP, Wellman A, Gell L, Sands SA. Ventilatory drive withdrawal rather than reduced genioglossus compensation as a mechanism of obstructive sleep apnea in REM. Am J Respir Crit Care Med. 2022;205(2):219-232. doi: 10.1164/rccm.202101-0237OC.
**This article was accompanied by an Editorial: Jordan AS, Kairaitis K. Dreaming of New Obstructive Sleep Apnea Treatments. Am J Respir Crit Care Med. 2022;205:148-149.*

VII. **Messineo L**, Loffler K, Chiang A, Osman A, Taranto-Montemurro L, Eckert DJ. The combination of betahistine and oxybutynin increases respiratory control sensitivity (loop gain) in people with obstructive sleep apnea: a randomized, placebo-controlled trial. [submitted]. 2022.

PUBLISHED WORKS OUTSIDE OF THIS THESIS

I. Landry SA, Mann DL, Djumas L, **Messineo L**, Terrill PI, Thomson LDJ, Beatty CJ, Hamilton G, Mansfield D, Edwards BA, Joosten SA. Laboratory performance of CPAP circuit with entrained oxygen and novel snorkel device. Respirology. 2020;25(12):1309-1312. doi: 10.1111/resp.13922

II. **Messineo L**, Lonni S, Magri R, Pedroni L, Taranto-Montemurro L, Corda L, Tantucci C. Lung air trapping lowers respiratory arousal threshold and contributes to sleep apnea pathogenesis in COPD patients with overlap syndrome. Respir Physiol Neurobiol. 2020;271:103315. doi:10.1016/j.resp.2019.103315

III. **Messineo L**, Perger E, Corda L, Joosten SA, Fanfulla F, Pedroni L, Terrill PI, Lombardi C, Wellman A, Hamilton GS, Malhotra A, Vailati G, Parati G, Sands SA. Breath-holding as a novel approach to risk stratification in COVID-19. Crit Care. 2021;25(1):208. doi: 10.1186/s13054-021-03630-5.

IV. Taranto-Montemurro L, **Messineo L**, Azarbarzin A, Vena D, Hess LB, Calianese N, White DP, Wellman A, Sands SA. Effects of the combination of atomoxetine and oxybutynin on OSA endotypic traits. Chest. 2020;S0012-3692(20)30135-5. doi:10.1016/j.chest.2020.01.012

V. Lim R, **Messineo L**, Grunstein RR, Carberry JC, Eckert DJ. The noradrenergic agent reboxetine plus the antimuscarinic hyoscine butylbromide reduces sleep apnoea severity: a double-blind, placebo-controlled, randomised crossover trial. J Physiol 2021; 599: 4183-4195.

PUBLISHED ABSTRACTS FROM THIS THESIS

I. Lim R, **Messineo L**, Grunstein R, Carberry JC, Eckert DJ. Reboxetine and hyoscine butylbromide reduce obstructive sleep apnoea severity. Journal of sleep research, 2019.

II. **Messineo L**, Eckert DJ, Taranto-Montemurro L, Azarbarzin A, Calianese N, White DP, Wellman A, Sands SA. Ventilatory drive withdrawal rather than reduced genioglossus compensation as a mechanism of obstructive sleep apnea in REM. [accepted at ATS 2020] doi.org/10.1164/ajrccm-conference.2020.201.1_MeetingAbstracts.A6437.

III. **Messineo L,** Carter SG, Chiang A, Carberry J, Eckert DJ. Zolpidem Increases Sleep Efficiency and the Respiratory Arousal Threshold in Obstructive Sleep Apnea. [accepted at ATS 2020] doi.org/10.1164/ajrccm-conference.2020.201.1_MeetingAbstracts.A6453

SUMMARY

Obstructive sleep apnea (OSA) is a multi-factorial disorder with several altered traits that contribute its pathogenesis. These include increased pharyngeal collapsibility, reduced pharyngeal muscle responsiveness, increased loop gain, and reduced respiratory arousal threshold. A reduced arousal threshold (i.e., level of ventilatory drive immediately prior to a respiratory-event-related arousal) is common in OSA with >30% of patients estimated to wake up too easily to progressive pharyngeal narrowing. This promotes increased risk of apnea/hypopnea cycling. A recent clinical trial performed in OSA and healthy participants sleeping on continuous positive airway pressure (CPAP) demonstrated that zolpidem, a "z-drug" class hypnotic, increased the arousal threshold and improved pharyngeal muscle responsiveness. Hence, zolpidem may be an ideal agent to stabilize sleep and breathing for certain OSA patients.

Accordingly, I ran two double-blind, randomized, crossover trials with zolpidem during my candidature.

In the first detailed physiology study, I demonstrated that a standard dose (10mg) of zolpidem increased the arousal threshold and improved sleep efficiency in 19 OSA patients off-CPAP but did not change OSA severity or upper airway muscle responsiveness. Explanations include an insufficient increase of the arousal threshold to consistently stabilize breathing during sleep. Zolpidem did not lead to deterioration of next-day perceived sleepiness or objective alertness. Overall, these results demonstrate that zolpidem is safe in certain OSA patients and may be useful for those with reduced sleep efficiency, such as patients with comorbid insomnia.

In the second study, I investigated the effects of the addition of zolpidem 10mg in 12 OSA patients undergoing simultaneous acute administration of atomoxetine and oxybutynin (Ato-Oxy), two drugs recently shown to greatly reduce OSA severity. I demonstrated that, *vs.* Ato-Oxy alone, the triple combination increased the arousal threshold and sleep efficiency. The magnitude of the increase was similar to that obtained with zolpidem alone *vs.* placebo. However, this magnitude of the increase in arousal threshold was insufficient to systematically change OSA severity. Importantly, certain components of next-day alertness (assessed via a driving-simulation test) were impaired with the triple combination.

In the attempt to find new strategies to activate pharyngeal muscles during sleep, in my third study I tested the effects of betahistine, a histaminergic drug, in combination with oxybutynin on OSA severity, in a double-blind, placebo-controlled, crossover trial. Despite no overall effect on AHI, this drug combination increased loop gain. This finding provides new insight into the role of histaminergic processes on respiratory control and opens new potential lines of intervention for disorders characterized by reduced chemosensitivity.

Finally, I ran a detailed physiological study to assess common drive withdrawal to both pump and pharyngeal muscles or a preferential loss of upper airway function during REM sleep and the potential role of these mechanisms on REM OSA. Contrary to common knowledge, I demonstrated that REM-related vulnerability to pharyngeal obstruction importantly relies on withdrawal of common ventilatory drive rather than preferential loss of ventilation or genioglossus activity in REM *vs.* non-REM. This is clinically important, as REM-related loss of general respiratory drive may be a key target for OSA therapy.

DECLARATION

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text. I also declare that the intellectual content of this thesis is the product of my own work, except to the extent that assistance from others in the project`s design and conception or in style, presentation and linguistic expression is acknowledged.

_____

Ludovico Messineo

January 2022

ACKNOWLEDGEMENTS

All right, let's do this one last time. This is my third thesis in less than ten years. Someone might think it is getting a little repetitive, but I cannot say that I am not enjoying the process. After my MD and my specialisation, this is the degree I got done in the quickest time, yet not with the least struggle. Living far from home (especially this far!) does not help and keeping on with being a student at plus 30 years old is not easy as it might seem. Or maybe it is, and I am just the perfect prototype of the nostalgic Italian who misses his family and friends. I am not sure what the answer is, but one thing I do know. This entire experience was a hell of a ride. The whole package. With its downs and ups, all the people that I met, drank or danced with, all the places that I visited and the things that I learnt. So, I feel like I owe some thanking here and there.

I would obviously start with Professor Danny Eckert. We both know it was a tough run, so thank you for being always so supportive and encouraging and for opening the door of your office anytime I knocked. This entire time I enjoyed our science conversations and admired your professionalism and your capability to make things working. I came to Australia only because I respected your career successes and knowledge and was eager to learn from you, and, despite all the difficulties, I can say I am beyond happy of what we achieved and would do it again all the way from the beginning. Hope the research we made will make you look back with proudness and a smile. But most of all, I hope that the best science, with new exciting collaborations, is yet to come.

I am very appreciative to Richard Lim, Alan Chiang, Benjamin Tong, Atqiya Aishah and Amal Osman for their great support and for the late night laughs.

Thanks to all my Australian friends and all the people that found room into my memories: Yan, Renè, Sandra, Erica, Valentina, Tom K, Jenny, and the Adelaide company of the good fellas (obviously all Italians: Luca & Roberta, Simone Ferraro, Simone Gelsinari, Diego, I know you are Spanish but it does not matter, Francesca, Luigi and Edoardo). That means you also gained a forever-lasting spot in my heart.

Huge appreciation to Giulia, who walked me through the quarantine, took me company along some misery days, accepted to be my pet (and my patter) at times, assisted me during my tantrums and, most of all, gave me a lot, a lot of love. I am not sure I would not have stayed this long without you.

I am so lucky to have people supporting me all around the world, thus I cannot avoid to mention the invaluable advice that I received throughout this time from my mentors, who I also have the privilege to call friends, Luigi Taranto-Montemurro, Andrew Wellman and Scott Sands. Especially this one guy, Scott, whose brain is something really close to perfection, was the key to get this degree done (and not only this degree) in such a short time; thanks Scott, I really owe you. And obviously thanks to the best scooper I could ever ask for, Nicole. I do not think that I would have made it through this journey without you guys.

I clearly cannot avoid to mention the friends of the "Parco" clan, Pratta, Barabba, Piddu, and Enzo and Giancazzo (*ad honorem*). Parco means garden in Italian, but there is no way I can translate that word in English

with an equivalent meaning, as Parco is not just a word, but the thing that shaped us into the inseparable friends (at least figuratively) that we grew up into. I used to tell them they were my friends for life, now I tell them I hope our kids will share the same feelings that we did and we do every day (man, I have literally spoken with these hammerheads every single day ever since I made it to Australia), because our legacy is too bright not to keep shining on. A big shout out also to my little Kimo and the Glorious House Enzo.

Thanks to my ex colleagues Luciano Corda, Sara Lonni and "Maestro" Toninelli who assisted me with any kind of advice prior to my departure and during this whole time, every time I needed them, and to Claudio Tantucci, one of the best people I found in the medical field, who guided me through the Residency maze, made a better doctor and a better man out of me, and never failed to support me and show me the right path to follow.

Finally, a huge thanks to my family, the best possible family. My sisters Anna and Biancamaria were always there when I needed a word of comfort or wanted to spend some time chatting. My mom and dad came all the way from Italy twice, going through a twenty-four hours trip only to keep me company and support me during the time I needed them the most (my dad never travelled for more than 8 hours and that only time he spent 8 hours flying was to come visit me in Boston). I cannot even mention all the things they did for me and the ways they found to be close and caring even if distant, to make me feel their vicinity in every hard moment and their appreciation after every good result. You are really the best example of what every human should be and I hope to one day become at least the half of the incredible persons that you are. Thank you. I love you to the moon and back a million times.

CHAPTER ONE:
INTRODUCTION AND LITERATURE REVIEW

Declaration

I hereby certify that I contributed to the majority of the articles (>50%) that I used for writing this chapter, including conceptualisation, realisation and documentation, in accordance with the Research Publication, Authorship and Peer Review Policy.

The articles that I used are: "Pathogenesis of Sleep Apnea", published in "Obesity Hypoventilation Syndrome, 1st Edition, From Physiologic Principles to Clinical Practice", Elsevier, 2020, written with Danny J Eckert; "Obstructive Sleep Apnea Phenotyping to Understand Pathophysiology and Improve Treatment and Outcome", published in "Encyclopedia of Respiratory Medicine (Second Edition)", Academic Press, 2022, written with Danny J Eckert. I also used some passages that I personally wrote from the following article, which I have co-written with Luigi Taranto-Montemurro (who I equally share the contribution with), under the supervision of Andrew Wellman: "Targeting endotypic traits with medications for the pharmacological treatment of obstructive sleep apnea. A review of the current literature", published in "J Clin Med", 2019.

Each of the other authors provides permission for use of this work to be included in the thesis.

Sleep apnea: definition and consequences

Sleep apnea is characterized by repetitive interruptions of breathing during sleep. These interruptions result from a decline or absence of breathing effort, a partial or total obstruction of the upper airway in the presence of increased respiratory effort, or both. These interruptions are classified clinically as central (absence breathing effort), obstructive (upper airway collapse), or mixed apneas, during which elements of both central and obstructive apnea occur[3]. During an apnea, PCO_2 typically increases and PO_2 decreases. Apneas are frequently associated with cortical arousals[4] with consequent sleep fragmentation and daytime symptomatology such as sleepiness, fatigue, and lack of concentration[5-7]. Untreated sleep apnea increases the odds of car accidents[8], metabolic morbidity[9], cardiovascular morbidity[10], and mortality[1].

Treatment with continuous positive airway pressure (CPAP) normalizes breathing interruptions during sleep in most people with OSA[11]. However, only half of the patients prescribed CPAP are able to use it regularly, and the other half remains partially treated or without treatment[12,13]. As a result, many patients with sleep apnea remain exposed to serious sequelae. Given these shortcomings and the heterogeneity of sleep apnea pathogenesis, there has recently been a focus on personalized medicine for sleep apnea[14]. This approach hinges on targeting one or more of the altered pathogenic traits responsible for the occurrence of OSA in a particular patient[15-19]. Thus, understanding sleep apnea pathophysiology is essential for the development of more effective and precise therapy.

Sleep apnea: pathogenesis

At least four altered endotypic traits contribute to the pathogenesis of sleep apnea (Figure 1)[20-24]. Wellman and colleagues described an innovative technique to calculate these traits. This technique involves inducing a respiratory disturbance by an abrupt 3-minute CPAP drop from a therapeutic to a subtherapeutic level[21] or by a progressive decrease of CPAP to sub therapeutic levels (CPAP dial downs)[25]. All the traits can be plotted together as a function of the change in ventilation (i.e. the disturbance) and the subsequent variation of ventilatory drive (i.e. the response)[21]. Abnormalities in one or more of these traits may be inherited, acquired, or both[24].

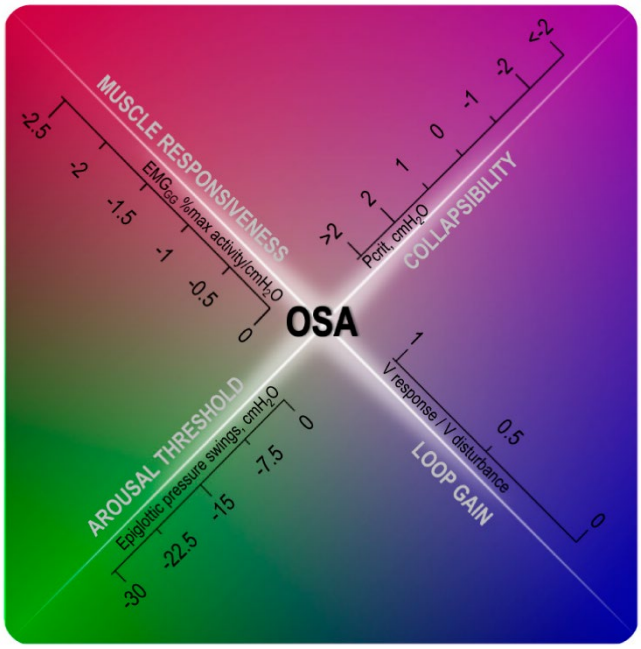

Figure 1. Sleep apnea endotypes. Diagram showing the interaction of four endotypic traits on obstructive sleep apnea (OSA) pathogenesis in the presence of mild-to-moderate collapsibility, indicated by a critical collapsing pressure (Pcrit) between −2 and 2 cmH_2O, other non-anatomical traits play a role in OSA pathophysiology. The inability to recruit the upper airway dilator muscle in response to negative pharyngeal pressure swings during an obstructive event (% activity/cmH_2O close to 0), a low arousal threshold (epiglottic pressure swings before the arousal above −15 cmH_2O) and a high loop gain (close to or above 1) will contribute to different degrees of OSA development. Note that the boundaries between the four traits are intentionally blurred to show that OSA presence and severity is often determined by the interaction of these pathogenic traits. EMG_{GG}: genioglossus electromyography, V response/V disturbance: ratio between the ventilatory response to a preceding ventilatory disturbance, dimensionless.

The extent of trait impairment varies widely between patients with sleep apnea[20]. For example, it is unlikely that OSA develops in the absence of any anatomical impairment of the upper airway. Yet, several non-anatomical physiological mechanisms contribute to the development of OSA or determine its severity. Similarly, many central sleep apnea (CSA) syndromes cannot emerge without a certain degree of impairment of the respiratory control system (Figure 2).

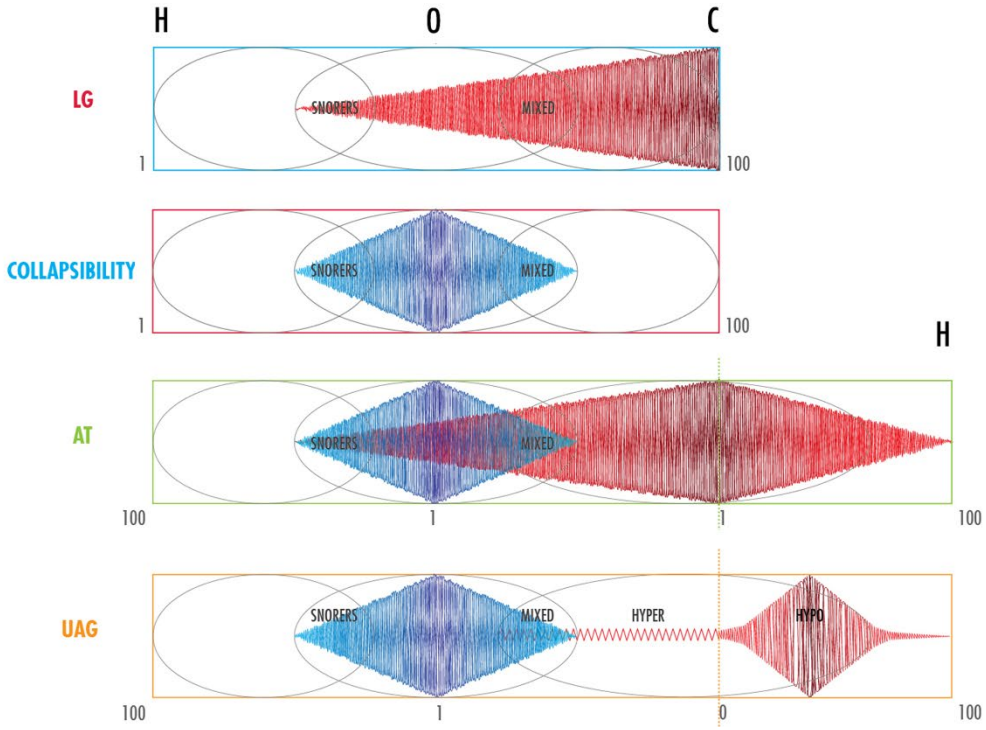

Figure 2. Endotypes interactions. The four key endotypic traits are illustrated in *amplification* boxes. 1 indicates no amplification and 100 is maximal amplification. On top of the boxes are three conditions: healthy without sleep-disordered breathing (H), obstructive sleep apnea (O), and central sleep apnea (C) with their respective area indicated by the adjacent ovals within each box. The closer to the corresponding letter the more severe the condition (or lack of disease for H). Overlapping pencil-like oscillatory lines symbolize the alteration of an overlaying trait from zero to maximal (full box amplitude). In the top box, the likelihood for loop gain (LG) to contribute to (hypocapnic) central sleep apnea (right circled box) increases with its amplification. If the anatomic trait (red pencil-like oscillations) overlaps, an OSA pattern would be more likely to emerge. The degree of anatomic alteration leads to variations in disease severity: 1) mild collapsibility and a mildly amplified loop gain would likely produce only snoring (overlapping part between the right circled box, corresponding to healthy without sleep-disordered breathing, and middle circled box, OSA), 2) an elevated collapsibility would produce OSA predominance with mild-to-moderate alteration of loop gain, 3) mild collapsibility, in the presence of higher loop gain magnitudes, would produce mixed predominant sleep apnea (overlapping part of OSA and CSA circled box). In the top middle box, the situation is reversed, with snoring more likely to emerge in the case of a mildly amplified loop gain (blue pencil-like oscillations) and a mild collapsibility, OSA in a setting of a vulnerable upper airway (moderate-to-severe collapsibility) plus a somewhat altered loop gain, mixed predominant sleep apnea in the case of progressively increasing loop gain values, and CSA predominant only with loop gain at its full magnification (in this latter case, OSA would be prevented only if there was excellent upper airway muscle compensation). In the low middle box, the alteration of a third trait, arousal threshold, is presented. A progressively reduced arousal threshold would increase the likelihood to develop both OSA and CSA. OSA would emerge in case of an enhanced collapsibility and/or a mild-to-moderate amplified loop gain. CSA would manifest with peak values of loop gain. Snoring would develop with mild impairment of anatomy and/or arousal threshold and/or mildly amplified loop gain. Notably, with the alteration of all the aforementioned factors, compensation of the fourth trait, muscle responsiveness, would protect the upper airway from full collapse. Mixed sleep apnea would develop when the predominant trait is loop gain, but an impairment of the pharyngeal anatomy still remains. Of note, CSA could manifest also in the absence of a reduced arousal threshold. In the bottom box, a decrease upper airway gain (UAG) of the muscles could contribute either to OSA or to hypercapnic CSA. For the onset of the former, an anatomy vulnerability is also mandatory, while, for the onset of the latter, a combination of a complete absence of muscle responsiveness and/or a decreased loop gain (zigzagged red pencil-like line) is necessary. With increasing loop gain and UAG values, hypocapnic (or non-hypercapnic) CSA develops. Note: for simplicity, several potential links between the traits and various manifestations of OSA have been omitted. Refer to the text for further details.

Increased upper airway collapsibility

Anatomical impairment of the upper airway is the most evident trait that contributes to the pathogenesis of sleep apnea. Indeed, narrowing or obstruction of the upper airway is observed in both obstructive and central

apnea[26]. The narrowing or obstruction occurs at one or more susceptible or *floppy* segments of the pharynx. Airway patency is determined by the transmural pressure, that is the difference between intraluminal and extraluminal tissue pressure[27]. Whenever the transmural pressure falls below zero, the upper airway narrows or occludes and ventilatory requirements are no longer met. During these periods of partial airway narrowing/collapse, "airflow-limitation" occurs whereby airflow generally plateaus despite increasing respiratory effort, similar to a Starling resistor model[28]. The elastic segment of the upper airway is necessitated by its roles in talking and swallowing but this property also renders it vulnerable to the undesired narrowing and collapse during sleep[29].

Airway crowding (i.e., the increased tissue volume within anatomical confines of the upper airway) is another important factor that increases the propensity for upper airway narrowing and collapse by reducing the cross sectional area and thus, increases the possibility for the transmural pressure to fall below zero[30]. The factors that can contribute to airway crowding include increased fat tissue deposition around the neck[31] and within the tongue[32] as well as altered pharyngeal or craniofacial structures[33], such as retrognathia[34] and variations in hyoid bone position[35].

Thus, for OSA to develop, the upper airway anatomy must be impaired to some degree[20]. One measure of airway collapsibility is the pharyngeal critical pressure (Pcrit) which is the airway pressure below which the pharynx closes. Pcrit is higher in patients with OSA than in individuals without OSA, in whom suction (a negative Pcrit) is required to close upper airway[36]. The extent of upper airway impairment varies among patients with OSA depending on the contribution of the non-anatomical traits. This explains why Pcrit in patients with OSA ranges from -5 cmH_2O to more than +5 cmH_2O[20,37]. A Pcrit of −5 cmH_2O usually corresponds to absence of OSA.

Although there are clear pathophysiological links between OSA and CSA[38], impaired upper airway anatomy is not the primary cause of CSA. The case of patients with heart failure is illustrative. Both CSA and OSA are common in patients with heart failure. Their severity correlates with the volume of fluid that redistribute rostrally from the legs during sleep[39]. To explain the dual effect of rostral fluid redistribution, it has been proposed that fluid that redistributes to the neck worsens OSA by increasing pharyngeal narrowing/collapsibility, and that fluid that redistributes to lungs worsens CSA by activating inhibitory breathing reflexes (i.e. Hering-Breuer reflex, stimulation of juxtacapillary fibers with cycles of hypo-hyperventilation)[40].

Altered control of breathing or *loop gain*

The term loop gain was adopted from engineering systems to describe the negative feedback circuit for the control of ventilation. In this context, the components of loop gain encompass pulmonary, circulatory, and central responses to precisely regulate blood gas tension levels within narrow limits[41]. Within this feedback

loop, any change in blood gas tension (increase or decrease) results in a series of responses that eventually lead to a corresponding change in ventilation.

The magnitude of the ventilatory response to hypercapnia and hypoxemia is an expression of chemosensitivity (controller gain). The resultant changes in blood gas tensions from the sudden change in ventilation depend on the properties of the lungs (plant gain) and circulation (mixing gain). This information is then fed back to the controller to complete the loop. The delay from the time it takes for a change in breathing to complete these steps and reach the chemoreceptors and mount a response (circulation delay) is also a crucial component of unstable breathing as outlined below.

Although many nonchemical-behavioral influences on breathing are lost during sleep[42], recent data indicate that estimates of loop gain during wakefulness closely track loop gain during sleep[43]. In a dynamic system, an unstable chemical control of breathing, when (dynamic) loop gain is elevated[44], results in an exaggerated breathing response to a given continuous breathing disturbance such as apneas, so that the response to disturbance ratio is ≥1. This large response creates a new disturbance and propagates instability at a rate determined by the plant (i.e. lungs) and with an amplitude determined by the controller (i.e. chemoreceptors) such that loop gain = plant gain × controller gain[45]. In contrast, a stable chemical control of breathing, when loop gain is low, responds less vigorously to a given breathing disturbance and dampens any breathing disturbance with progressively smaller breathing responses (response to disturbance ratio <1)[46]. However, an abnormally blunted response to changes in blood gases, when loop gain is very low, can contribute to sleep hypoventilation syndromes.

Elevated loop gain is the key determinant of non-hypercapnic CSA. Similar to the key role that upper airway anatomical impairment plays in the genesis of OSA, there would be no CSA without high loop gain (i.e., unstable respiratory control). Indeed, increasingly high values of loop gain likely predict CSA development. High loop gain can result from altered controller gain, plant gain, or both. These factors coincide to increase ventilatory output and decrease PCO_2 below the apnea threshold[47]. Since CO_2 is the main driver of ventilation during sleep, ventilation decreases as CO_2 decreases and ceases when CO_2 falls below a critical level: the apnea threshold[47]. In patients with congestive heart failure, the gap between the sleep CO_2 set point and the CO_2 apnea threshold is very narrow[48], and very small respiratory disturbances (e.g. a minor ventilatory response that reduces CO_2) can reduce the CO_2 below the apnea threshold and cause central apnea.

Plant gain

Plant gain (plant gain= ΔPCO_2 / Δventilation) represents the effectiveness of the lungs to alter blood gases. Factors that increase (dynamic) plant gain, such as reduced lung volume, promote less effective elimination of CO_2 from the lungs and thus increases of CO_2 tension in the pulmonary capillaries and result in a higher loop gain[49]. Factors such as alveolar and systemic PCO_2 may also influence plant gain to meet the ventilatory demand according to the characteristics of the metabolic hyperbola[24]. However, they are unlikely to contribute

to the pathogenesis of sleep apnea in the absence of alterations in blood gas diffusion rate or abnormalities in circulation as in heart failure[50].

For a given duration of ventilatory disturbance (i.e. cycle frequency), large swings in CO_2 (i.e. increased plant gain) may result in an amplified ventilatory response (i.e. bigger than the disturbance that caused the variation of PCO_2 with an increased CO_2 excretion through the lungs)[51]. However, different durations of disturbances have a major impact on the swings in gas tension, the longer the disturbance, the larger the swing. Plant gain, through its determinants such as lung volume, mediates the rate of the respiratory response. Factors such as a small lung volume can lead to poor efficiency of the lungs to process CO_2. This increases likelihood to produce blood gas tension imbalance and ventilatory instability as a small change in breathing can cause a comparatively large change in CO_2[52]. Accordingly, increased plant gain can be an important promoter of CSA[46].

Circulatory delay

There is a physiologic delay for gas swings to reach peripheral and central chemoreceptors (circulation time). Circulation time increases when cardiac output decreases[53,54]. Circulation time influences how long each CSA cycle lasts[50]. Indeed, with increased circulatory time, a breathing disturbance is sensed later by the chemoreceptors. This leads to a delayed response to the initial breathing disturbance which perpetuates breathing instability during sleep[45]. In fact, if considering the disturbance and the response as two sinusoids, the *ideal* time that the response needs to be efficiently contrasting to the disturbance is when it is delayed minimally so that it completely or partially offset the disturbance[45].

Controller gain

Chemosensitivity comes from both peripheral[55,56] and central chemoreceptors[57] and is the most powerful determinant of periodic breathing[58]. It converts the sensed variation of gas tension into a corresponding change in ventilation (controller gain = Δventilation / ΔPCO_2)[21]. Intuitively, with increasing magnitude of a ventilatory response, an individual will have greater fluctuations in CO_2 levels and increased probability of crossing the CO_2 apnea threshold and the occurrence of CSA. Chemoreflex control can be inherently elevated[59] or acquired as in heart failure[58,60,61] or during ascent to high altitude[62].

Transmissibility

Transmissibility is a function of the biological non-chemical noise, such as ataxic opioid-induced ventilatory fluctuations[63] or ventilatory fluctuations in rapid eye movement (REM) sleep[64]. It has been demonstrated to amplify loop gain (Transmissibility = 1 / |1 – loop gain|) and expose patients with heart failure to periodic breathing during wakefulness or REM sleep[45].

OSA and loop gain

At least one third of patients with OSA have elevated loop gain[20]. Elevated loop gain promotes OSA periodicity by several mechanisms[20,21,65,66]. First, the frequent oscillations in drive to the respiratory pump muscles lead to breathing instability with intermittent periods of reduced *mechanical drive* (see also next paragraph) to both

the pharyngeal dilator and pump muscles[67]. Second, the increased ventilatory instability after arousals from sleep can propagate apnea-reventilation cycling[68]. Third, the large negative inspiratory pressure swings in response to small increases in CO_2 are able to suck the pharyngeal airway when the pharyngeal dilator response is inefficient and the pharyngeal compliance is high. Consequently, as effort increases, flow does not plateau but decreases, a behavior known as *negative effort dependence*[67,69].

Decreased upper airway muscle function

Upper airway dilator muscles, such as genioglossus, and respiratory pump muscles, such as the diaphragm, receive neural drive from pattern generator neurons within the brainstem. This drive is then relayed to activate respiratory muscles (i.e. *mechanical output*) via the spinal cord. Sensory feedback from respiratory muscle afferents is relayed back to pattern generator neurons via nuclei of the tractus solitarius. As the upper airway lacks a rigid bony support, impairment of the dilator muscles of the upper airway is a key mechanism in the pathogenesis of OSA. In contract, impaired respiratory pump muscle function can be key contributor to hypercapnic CSA pathogenesis in certain disease states. Indeed, a hypercapnic CSA breathing pattern can emerge with disease that affects one or more of the components of this process as well as the neuromuscular junction. Examples include: congenital central hypoventilation syndrome, amyotrophic lateral sclerosis, myasthenia gravis, and myopathies/chest wall syndromes[70].

Although the structural pathways than underpin breathing tend to be intact in patients with OSA, there can be a mismatch between drive to the respiratory pump muscles and the upper airway dilators that results in *decreased dilator muscle responsiveness*. In other cases, drive to the upper airway does not translate into an increase in airflow resulting in a *reduced mechanical efficiency* of the upper airway dilators. The term *muscle effectiveness* can combine elements of either muscle responsiveness, mechanical efficiency, or both.

At sleep onset and during the lighter stages of sleep, the activity of the upper airway dilator muscles decreases and can lead to OSA in the presence of an anatomically vulnerable airway or a predisposition to unstable control of breathing[71,72]. On the other hand, respiratory drive increases during the deeper stages of sleep[73]. Although this is usually protective from OSA, it is not always so. One explanation is that some patients with OSA are unable to sense the narrowing of a collapsible pharyngeal airway[74,75]. This can lead to inadequate recruitment of dilator muscles (i.e., poor muscle responsiveness) to cause further narrowing of the airway. This mechanism is observed in at least one third of patients with OSA[20].

Enhanced muscle responsiveness during sleep can protect an individual with an anatomically vulnerable upper airway from OSA during non-REM sleep[76]. The same individual, however, may have OSA during REM sleep when muscle activity decreases dramatically[77,78].

Individuals with poor muscle responsiveness also have impaired muscle effectiveness because they do not have enough neural drive to adequately activate the pharyngeal dilators. Other patients with OSA have

substantial increases in pharyngeal muscle activity for a given level of increased respiratory drive[20,79]. However, this does not translate into increased airflow (i.e., poor muscle effectiveness). The reasons for this are not well understood but may include impaired airway mechanics and poorly coordinated drive across dilator muscles[80-83].

Altered arousal threshold

The respiratory arousal threshold is the level of respiratory drive or *effort* required to awaken an individual in response to airway narrowing from sleep[84]. Arousals were thought to be life-preserving events in OSA[85]. This was the prevailing understanding until Younes and colleagues demonstrated that after an apnea or a hypopnea airflow can be restored before a cortical arousal occurs or without the occurrence of arousal[86,87]. Specifically, Younes delivered ventilatory disturbances to provoke upper airway collapse and subsequent reopening in patients with OSA and investigated the impact of the arousal on the subsequent airflow response[87]. He showed that 1) the temporal relation between arousal and airway reopening is inconsistent, 2) the arousal is not required for an adequate flow response, 3) the effect of the arousal on the upper airway is not manifest until there is a clear high-frequency change in electroencephalogram, and 4) the occurrence of cortical arousal at airway opening is associated with a greater flow overshoot and a greater subsequent undershoot. The reason that many respiratory events are associated with arousal in adults is that the same stimuli that recruits pharyngeal dilators also triggers arousal and often at similar thresholds[41]. Given the important role that ventilatory drive plays in mediating arousals, the arousal threshold can interact with other traits such as loop gain and upper airway responsiveness.

Rather than being protective, a low threshold for arousal can promote both OSA and CSA in several ways. First, if the arousal happens before the respiratory drive reaches the threshold for the upper airway muscle to open the airway, the individual will wake up prematurely without the possibility of effective compensation to the ventilatory disturbance and restoration of adequate airflow[41]. Thus, frequent arousals to minor airway narrowing can increase the rate of respiratory event (i.e., insufficient time to stabilize the ventilatory pattern). Second, arousals can elicit ventilatory instability from the overshoot in ventilation following the arousal and propagate breathing oscillations from the ensuing changes in ventilatory pattern and gas tensions. Specifically, transient wakefulness-to-sleep changes in PCO_2 create intermittent periods of relative hypercapnia and subsequent hyperventilation, potentially driving PCO_2 levels down below the apnea threshold[46,68,88]. Third, sleep fragmentation prevents deeper sleep stages that are generally protective against unstable breathing[89,90].

Traditionally, it was thought that patients with OSA have an increased threshold for respiratory arousal[91]. On average, many patients with OSA require larger amounts of respiratory drive to arouse from sleep than healthy controls[20]. This is likely to be partly due to sleep fragmentation and sleep deprivation. However, it is now clear that at a low respiratory arousal threshold plays a role in the pathogenesis in at least one third of patients with OSA.[20] Other evidence to support this concept stems from findings that 1) drugs that increases arousal threshold can reduce OSA severity[92,93], and that 2) short respiratory events, that might be due to a low arousal

threshold, predicted mortality in the Sleep Heart Health Study cohort[94]. In patients with CSA and heart failure, arousal is more likely to occur at peak hyperpnea[95] than at event termination[96], therefore leading to more ventilatory instability.

Although increasing the respiratory arousal threshold may decrease OSA severity in certain patients[15,20,92,93], a low arousal threshold is potentially the last line of defense against severe life-threatening events in patients with poor muscle compensation[11,83,97,98]. A patient that cannot properly sense the upper airway narrowing because of an arousal threshold that is too high would stay asleep independent of the extent of oxygen desaturation and its potential dangerous sequelae.

Arousal intensity (i.e. its duration or wave frequency of cortical activation) can contribute to the pathogenesis of OSA because it is a determinant of ventilatory overshoot[87]. Higher levels of arousal intensity are associated with larger respiratory responses and increased probability of subsequent respiratory instability that predisposes to OSA[99] or CSA[100].

Different ways of phenotyping

Clinical OSA phenotypes have been recently defined to help categorize patients according to a single or a combination of distinct disease features[101-105]. The goal of this approach is to use these categories to derive clinically meaningful attributes such as symptoms, responses to therapy, health outcomes and quality of life[104]. These categories are derived using unsupervised (i.e. new phenotypes generated based on associations between features not evident in highly multidimensional data) or supervised (i.e. the aforementioned generated phenotypes, or common "a priori" disease characteristics are which are studied to assess the validity of specific hypotheses) statistical approaches[104]. Clinical phenotyping approaches may begin by differentiating patient subgroups according to presence or absence of key symptoms and/or other health related consequences and linking to a specific clinically available definition of disease severity. The overarching objectives of these approaches are to facilitate precision medicine via improved physiological understanding of disease mechanisms, earlier diagnosis and risk stratification using personalized treatment[14,106,107]. The aims of personalized treatment are to tailor efficacious therapy or therapies to the individual to achieve high rates of acceptance, that are minimally invasive, and cost effective. The challenge with clinical phenotyping is that subgrouping tends to be quite broad and several clinical features may overlap between categories. This limits the ability to deliver precision-based, individualized solutions.

Symptom based

One of the most common symptoms in OSA is excessive daytime sleepiness (EDS)[108]. Thus, clustering people with this complaint in an OSA-with-EDS phenotype may help to expedite diagnosis and treatment. However, EDS is not invariably present[105], poorly correlates to OSA severity using traditional metrics such as the AHI[109], and is vulnerable to reporting bias and other confounding factors such as age, gender, comorbidities, psychological factors, and fatigue[110]. Despite these limitations, EDS in OSA is associated with higher risk of death[111], cardiovascular morbidity[112,113], development of systemic hypertension[114-116], insulin resistance[117], and metabolic syndrome[118] compared with non-sleepy patients. CPAP can reduce EDS[119] and may reduce blood pressure[120] and glucose dysregulation[121] in sleepy but potentially not in non-sleepy patients[120,122,123].

This target group has all of the aforementioned properties of a clinically valuable phenotype: easy recognition (i.e., invasive tests are not necessary to identify EDS), reversible target (i.e., EDS can be reduced with therapy), within-category homogeneous behavior (i.e., OSA people with EDS are different to than those without EDS), and potentially responds consistently to treatment with a decrease in associated risk factors. Other possible therapeutic strategies include: 1) decrease in BMI, since EDS directly relates to weight change[124], and 2) behavioral/pharmaceutical approaches[15].

Ethnicity

Ethnicity influences OSA prevalence. Although eastern Asian individuals appear more anatomically predisposed to OSA due to craniofacial bony restrictions[33,125], the within-category homogeneity requirement is not met. Indeed, there is no clear relationship linking eastern Asian ethnicity with higher cardiovascular

morbidity and mortality than Caucasians[126,127]. However, positional OSA appears more common in people of Asian ethnicity[128] and thus, may be a target for therapy in this patient-population.

Emerging research highlights the high prevalence of OSA in African-Americans[129,130], particularly in specific age groups and after adjusting for covariates[131,132]. Blacks are more prone to EDS[133] and are more likely to manifest AHI-dependent low-grade systemic inflammation[134]. Furthermore, they carry a disproportional burden of cardiovascular disease with marked discrepancies in hypertension[135], sudden cardiac death rate and significantly lower life expectancy[136,137].

Accordingly, all of the conditions to generate a clinical phenotype are met. However, the most challenging component may be access to diagnosis and treatment. Indeed, African-Americans have reduced access to health care[138] and are less likely to have a sleep study after a recommendation to do so[139]. Thus, these individuals remain exposed to the various consequences of untreated OSA[140,141]. However, when treatment and diagnosis barriers are overcome, CPAP acceptance rates are comparable to Caucasians[142].

Age

Younger versus older people can be considered as two distinct phenotypes[143]. OSA in older people potentially has different pathogenic causes such as increased upper airway collapsibility in those over >60 years[143]. Increasing age with OSA may also increase the risk for incident hypertension[144], atrial fibrillation[145] and cognitive decline[146], possibly through altered regional cerebral blood flow[147]. Conversely, OSA in younger people with OSA (<65 years) is associated with higher cancer mortality[148]. Moreover, middle-aged people (~40 years) are more likely to experience daytime sleepiness[149] (i.e. EDS phenotype).

Sex

OSA is more in men[150] and associated with higher mortality[151] for disparate reasons[152-154]. However, post-menopausal women have equivalent OSA risk to men[150] and increased cardiovascular risk[155]. Hence, hormone replacement therapy may theoretically be possible/beneficial[156]. However, this remains speculative[157]. Male sex is also associated with reduced occurrence of EDS in an age-dependent fashion[149].

Sleep stage

REM-isolated OSA is quite common[158]. It may be more prevalent in women[150], is associated with poor objective sleep quality indexes[159], higher risk of hypertension[160], and is characterized by specific pathophysiologic features (i.e., low loop gain and muscle responsiveness)[161,162]. Although this homogeneous behavior seems to support REM-related OSA into a distinct phenotype, whether to treat REM OSA is debated[160]. This may be particularly due to lack of studies with full night CPAP usage and insufficient responses to alternative therapies[163]. On the contrary, non-REM-isolated OSA could be a separate phenotype[164] and may respond more favorably to tailored alternative treatments.

To inform the development of new targeted therapies, including potential sleep stage dependent interventions, increased knowledge of the mechanisms that contribute to sleep stage specific OSA is required. For example, REM-related OSA is largely believed to be driven by a generalized REM-dependent muscle hypo/atonia, which is thought to also reduce pharyngeal muscle function[77,165]. However, a REM-related decline of

pharyngeal muscle responsiveness *per se* in humans has not been quantified. There is evidence to suggest that the diminished dilator muscle activity during REM may be also explained by withdrawal of common drive (to both the diaphragm and the pharyngeal dilator muscles)[166-168]. Nevertheless, most REM-related mechanistic research has focused on hypoglossal nuclei to investigate the origin of upper airway muscle hypotonia during REM sleep[169]. Indeed, although not thoroughly studied in people with OSA, the available evidence suggests that drive to the respiratory pump muscles such as the diaphragm seems relatively spared during REM sleep[170-172]. Thus, this requires further investigation.

Sleep apnea: treatment

The recent identification of multiple endotypes (pathogenic mechanisms) underlying this disorder has oriented pharmacological research towards tailored therapies targeting specific pathophysiological traits that contribute differently to cause OSA in each patient. For example, recently introduced drugs for weight loss that modify upper airway anatomy may play an important role in the management of OSA in the near future[173]. Promising results have also been obtained with drugs that increase upper airway muscle activity during sleep and reduce loop gain[174]. The lack of a medication that can effectively increase the arousal threshold makes this strategy less encouraging, although recent studies have shown that the use of certain sedatives do not worsen OSA severity and could actually improve patients' sleep quality[175]. Furthermore, the use of combined therapies that target more than one trait simultaneously offers considerable promise for certain patient subgroups[15,18].

Drugs targeting upper airway anatomy

CPAP mainly targets increased pharyngeal collapsibility, splinting the airway open to resolve OSA in the majority of cases. As CPAP works downstream from the different OSA endotype mechanisms, if tolerated, CPAP decreases OSA severity regardless of the underlying endotype profile for a given individual. However, CPAP is burdened by a number of side effects (e.g. facial lesions, gastric tension, secondary insomnia, etc.) that negatively affects its usage and uptake[12,13]. Other non-CPAP therapies have variable efficacy, including upper airway surgery, mandibular advancement devices and other medications. Drugs with clinical potential to improve upper airway collapsibility and thus, reduce OSA severity are disparate and range from nasal decongestants[176], to medications for weight-loss[173,177] or those that reduce upper-airway edema[178].

Drugs targeting loop gain

The main medical therapies that target ventilatory instability include: 1) carbonic anhydrase inhibitors such as acetazolamide, which has been shown to decrease resting PCO_2 via generating a transient metabolic acidosis and relative hyperventilation. The lower PCO_2 reduces PCO_2 variations for a given change in ventilation[179]. 2) Oxygen treatment converts the sensed variation of gas tension into a smaller change in ventilatory drive[180].

Drugs that target pharyngeal muscle responsiveness

Since the activity of upper airway dilator muscles is regulated by specific neurotransmitters whose concentration varies between wake and sleep states, it should be theoretically possible to manipulate the airway muscle tone by identifying the monoamines responsible for the muscle activation and administering them to OSA patients during sleep to prevent upper airway muscle relaxation. In the last decade, many advances in basic science have helped to refine the choice of receptor targets to stimulate the upper airway muscles[181-191]. Attempts to translate these findings into OSA patients are ongoing[192].

Noradrenergic and antimuscarinic drugs

Nevertheless, the most promising results to date have been obtained with noradrenergic drugs in combination with antimuscarinics. Noradrenergic agents such as desipramine can increase genioglossus muscle activity[193] and reduce upper airway collapsibility during sleep in humans[194]. However, when taken alone, noradrenergic

drugs such as norepinephrine reuptake inhibitors only mildly reduce OSA severity, and only in selected patients. The tricyclic antidepressants protriptyline[195,196] and desipramine[194], as well as the selective norepinephrine reuptake inhibitor atomoxetine[197], have all been tested in patients with OSA, with modest success in reducing the severity of the disorder. According to Richard Horner's group in Toronto, noradrenergic withdrawal is not the only mechanism involved in sleep-related loss of genioglossus activity. For example, noradrenergic stimulation with phenylephrine at the hypoglossal motor nucleus (HMN) failed to reverse REM sleep-related tongue muscle atonia[198]. This finding suggests that an additional inhibitory mechanism may be involved. This inhibitory mechanism was identified in a follow up study to be predominantly muscarinic. Grace et al. delivered the muscarinic receptor antagonist scopolamine into the HMN in rats and demonstrated that there is progressive muscarinic inhibition of drive to the HMN from wakefulness to non-REM and REM sleep. Muscarinic receptor antagonism had a particularly strong restorative effect on genioglossus activity in REM sleep. These findings have been recently applied to humans in a preliminary proof-of concept study. In 20 OSA patients, the combination of atomoxetine and the antimuscarinic oxybutynin was administered before bedtime for one night and compared to placebo[174]. Atomoxetine-plus-oxybutynin increased by ~3-fold the genioglossus muscle responsiveness to negative esophageal pressure swings and lowered the AHI by 63%. The combination reduced the AHI in both REM and non-REM sleep with an accompanying improvement in oxygen saturation parameters. A similar combination consisting of the adrenergic agent reboxetine and the antimuscarinic drug hyoscine butylbromide was tested by Lim and coworkers on 12 healthy subjects in a double-blind, placebo-controlled, randomized, cross-over fashion[199]. The combination increased the activity of the tensor palatini muscle, a representative tonic upper airway dilator muscle, and reduced pharyngeal resistance during sleep. However, it did not increase the phasic activity of the genioglossus muscle. Nevertheless, when these drugs were tested in 12 OSA participants, the AHI decreased by approximately 35% from placebo, with a concurrent increase in nadir oxygen saturation[200].

Other drug targets with potential to activate pharyngeal dilator muscles

The hypoglossal motor pool expresses multiple other receptors which, potentially, could be targeted with drugs to activate pharyngeal dilator muscles and, consequently, reduce OSA severity[192]. Another novel emerging approach is Designer Receptors Exclusively Activated by Designer Drugs (DREADS) which have the potential to specifically target key upper airway motor neurons[190,192]. Another potential mechanistic target that has not yet been carefully investigated in humans is histaminergic stimulation of the brainstem. Animal studies have shown that histamine is a key neurotransmitter at the hypoglossal motor nuclei level[201-203] and that histamine administration increases genioglossus muscle activity[204,205]. Thus, human studies to investigate the role of histaminergic mechanisms and their effects on OSA severity are warranted.

Drugs that target the arousal threshold

Sedative use in OSA has historically been discouraged because of concerns that delayed arousal would produce further blood gas derangement. Furthermore, there has been concern that many of these drugs have myorelaxant properties that could worsen obstruction. However, no studies show worsening of OSA severity

with sedatives as measured via the AHI and some show improvement, even in individuals with moderate OSA[175,206,207]. Moreover, recent studies indicate that certain sedatives might be beneficial for the recruitment of upper airway dilator muscles during sleep[208-210]. Therefore, the belief that all sedatives are contraindicated in all patients with OSA is not supported by the literature[175] However, it is still not clear if the changes in respiratory arousal threshold can modify the severity of sleep-disordered breathing. While most data collected to date are neutral, only a few OSA trials have measured the arousal threshold on and off hypnotics, and more data need to be collected on selected patients with low arousal threshold endotype. Moreover, the drugs tested to date were unable to increase the arousal threshold by more than 20-30% (see below for a detailed description). Ideally, a drug that mimics the effects of slow wave sleep (a state in which the arousal threshold is elevated) would be ideal for reducing OSA severity, given that this is a relatively protected stage of sleep in regards to OSA[90,211-213].

The drugs with hypnotic properties that have been studied for OSA can be grouped in two main classes, as follows.

Benzodiazepines

Several benzodiazepines have been tested in OSA patients. Cirignotta et al compared the acute effect of flurazepam 30 mg *vs.* placebo in 2 different crossover, placebo-controlled trials[214,215] (total of 24 patients). In one trial flurazepam significantly worsened the oxygen saturation nadir compared to placebo. In another study, the same investigators tested the effect of brotizolam 0.25 mg *vs.* placebo[215] and showed no effect on OSA severity. Barry et al[216] found that triazolam 0.25 mg significantly increased the arousal threshold by ~20% in 12 patients with severe OSA during a randomized placebo-controlled trial. The AHI in this group remained unchanged, but the obstructive events were longer and led to lower oxygen saturation. Nitrazepam was tested by Hoijer et al on 11 patients with mild-to-moderate OSA in the doses of 5 and 10 mg. These investigators compared the results to placebo in a 1-night crossover study[207]. There was no significant change in AHI or oxygen saturation indices, although there was substantial individual variability in the response: 3 patients had a worsening of OSA whereas 6 seemed to improve at the higher dose. Endotypic traits were not measured so it is uncertain if the improvement was in patients with a low arousal threshold.. Temazepam was tested in 3 studies. The first one from Camacho et al[217] included mild OSA patients ≥60 years of age with insomnia. This was a placebo-controlled randomized trial with 2 parallel groups and a total of 15 patients. The RDI on temazepam at the dose of 15 or 30 mg was not significantly different from placebo after 8 weeks of treatment. The second study by Wang et al[206] investigated the effect of temazepam 10 mg compared to placebo in a single-night crossover trial including 20 patients, mostly with mild-to-moderate OSA. Once again, the AHI did not change systematically on drug but there was high inter-individual variability in the response. The authors attempted to characterize patients by their baseline chemosensitivity but could not find any relationship between the change in AHI and the baseline response to CO_2. Arousal threshold was not determined in this study. Lastly, Carberry et al tested the effect of Temazepam in a 4-arm, randomized, placebo-controlled crossover study that assessed several endotypic variables: upper airway collapsibility, arousal threshold and genioglossus muscle responsiveness. The three hypnotics tested were Zolpidem, Zopiclone and Temazepam. Twenty-one individuals with and without OSA attended an overnight study on placebo and on the drugs.

Temazepam did not significantly vary either the arousal threshold or the collapsibility. However, in contrast to conventional wisdom, Temazepam did not adversely affect the genioglossus muscle responsiveness to negative epiglottic pressure swings[218].

Z-drugs

George et al tested the effect of zolpidem 10 mg for a single night in a group of 42 patients with an AHI between 10 and 40 events/h in a randomized crossover trial[219]. Compared to placebo, these investigators found no difference in AHI or oxygen saturation. Zolpidem 10 mg was again recently tested by Carberry et al[220] in an open label pilot study including 12 OSA patients administered the drug for a single night after a diagnostic polysomnography. There was no systematic effect on oxygen saturation or AHI, but sleep efficiency significantly improved from 77 to 84% on the drug night. In another physiology trial including 21 individuals with and without OSA, the same group recently showed that 10 mg of zolpidem increased the arousal threshold by 25% and, unexpectedly, also the genioglossus response to pharyngeal negative pressure as compared to placebo. This finding suggests that this medication is at least not harmful for OSA patients[218]. Nonetheless, it is important to note that a previous trial by Cirignotta et al[214] that tested the effects of zolpidem at the higher dose of 20 mg in a crossover trial of 1 night in 12 patients showed a lowered mean O_2 saturation from 91.7 to 88.6%, and a worsened nadir SaO_2 from 85.2 to 76.8%.

Among other Z-drugs, eszopiclone and zopiclone were studied in OSA patients to measure their effect on the arousal threshold. Rosenberg et al[221], in a double blinded crossover trial of 2-days treatment, showed that, compared to placebo, eszopiclone did not change the AHI in 21 patients, whereas it improved sleep efficiency and reduced spontaneous arousals. Eckert et al[92], in a 1-night crossover trial including 17 OSA patients, showed that eszopiclone increased the arousal threshold by ~30%. Participants with a low arousal threshold at baseline (8/17) had a 43% reduction in AHI (~25% for the group overall). Carter et al[97] showed that zopiclone 7.5 mg administered for 1 night (n=12) increased the arousal threshold by 20% but did not significantly change the AHI compared to placebo. A subsequent parallel-arm trial from the same group, testing zopiclone (n=14) *vs.* placebo (n=16), also showed significant changes in AHI between the two groups after 30 days of treatment[222]. In the aforementioned four-arm trial by Carberry and coworkers[218] zopiclone 7.5 mg significantly increased arousal threshold (but not AHI) *vs.* placebo in a group of 21 healthy individuals and OSA patients.

Interestingly, a recent double-blind, placebo-controlled, randomized, cross-over trial from the same group showed that doubling the dose of zopiclone (15 mg) for one night did not have any effect on the arousal threshold (or the AHI), suggesting it is not a dose-dependent effect. This dosage of zopiclone also did not affect next-day sleepiness or alertness measures[223].

Summary of study aims and hypotheses of this thesis

This thesis aims to investigate novel, physiology-based ways to inform the development of new treatments for OSA.

The aim of **experiment #1** is to evaluate the effect of zolpidem on OSA severity, sleep efficiency, physiological traits such as the respiratory arousal threshold and the upper airway muscle responsiveness, and next-day alertness and sleepiness in a population of OSA participants.
Based on the prior recent findings, I hypothesize that zolpidem will increase the respiratory arousal threshold, sleep efficiency, and genioglossus muscle responsiveness to reduce OSA severity in appropriately selected participants (i.e., those with a low arousal threshold endotype).

The aim of **experiment #2** is to assess the effect of zolpidem on sleep efficiency, OSA severity, the respiratory arousal threshold and next-day alertness and sleepiness in a population of OSA participants undergoing acute treatment with atomoxetine and oxybutynin.
I hypothesize that the addition of zolpidem to combination therapy with atomoxetine and oxybutynin will increase the respiratory arousal threshold and sleep efficiency which may yield further reductions in OSA severity in appropriately selected participants without impairing next-day alertness or sleepiness.

The aim of **experiment #3** is to investigate the effect of betahistine, a histaminergic agent, and oxybutynin on OSA severity and endotypes.
I hypothesize that histamine agonism with betahistine when combined with oxybutynin will reduce OSA severity via an increase in pharyngeal muscle responsiveness during sleep while preserving overall sleep quality.

The aim of **experiment #4** is to evaluate the physiological mechanisms that contribute to REM-dependent upper airway collapse/OSA.
I hypothesize that REM OSA relies principally on common withdrawal of drive to both pump and dilator muscles rather preferential inhibition of upper airway motor activity.

CHAPTER TWO:
ZOLPIDEM INCREASES SLEEP EFFICIENCY AND THE RESPIRATORY AROUSAL THRESHOLD WITHOUT CHANGING SLEEP APNOEA SEVERITY AND PHARYNGEAL MUSCLE ACTIVITY

Abstract

Rationale

A recent physiology study performed using continuous positive airway pressure (CPAP) manipulations indicated that the hypnotic zolpidem increases the arousal threshold and genioglossus responsiveness in people with and without obstructive sleep apnoea (OSA). Thus, zolpidem may stabilise breathing and reduce OSA severity without CPAP. Accordingly, we sought to determine the effects of zolpidem on OSA severity, upper airway physiology and next-day sleepiness and alertness.

Methods

Nineteen people with OSA with low-to-moderate arousal threshold received 10mg zolpidem or placebo according to a double-blind, randomised, cross-over design. Participants completed two-overnight in-laboratory polysomnographies (1-week washout), with an epiglottic catheter, intramuscular genioglossus electromyography, nasal mask and pneumotachograph to measure OSA severity, arousal threshold and upper airway muscle responsiveness. Next-morning sleepiness and alertness were also assessed.

Results

Zolpidem did not change the apnoea-hypopnoea index versus placebo (40.6±12.3 *vs.* 40.3±16.4 events/h mean±SD, p=0.938) or nadir oxyhemoglobin saturation (79.6±6.6 *vs.* 79.7±7.4%, p=0.932), but was well tolerated. Zolpidem increased sleep efficiency by 9±14% (83±11 *vs.* 73±17%, p=0.010). Arousal threshold increased by 15±5% with zolpidem throughout all sleep stages (p=0.010), whereas genioglossus muscle responsiveness did not change. Next-morning sleepiness and alertness were not different between nights.

Conclusions

In summary, a single night of 10mg zolpidem is well-tolerated and does not cause next-day impairment in alertness or sleepiness, or overnight hypoxemia in OSA. However, despite increases in arousal threshold without any change in pharyngeal muscle responsiveness, zolpidem does not alter OSA severity. It does, however, increase sleep efficiency by ~10%, which may be beneficial in people with OSA and insomnia.

Introduction

Obstructive sleep apnoea (OSA) is a heterogeneous disorder. There are at least four different traits that contribute to OSA pathogenesis. These include increased pharyngeal collapsibility, unstable respiratory control (high loop gain), reduced upper airway muscle responsiveness during sleep and a low respiratory arousal threshold[20]. Approximately 30% of people with OSA have a low respiratory arousal threshold[20]. This can contribute to OSA pathogenesis via: 1) frequent cortical arousals and sleep fragmentation to mild airway narrowing that prevents accumulation of chemical stimuli required to activate the upper airway dilators[224], 2) ventilatory instability[225], and 3) prevention of deeper, more stable sleep[87,226]. Thus, increasing the arousal threshold with hypnotics may stabilise breathing and reduce OSA severity in selected patients. However, to date, studies that have tested hypnotics in OSA have yielded variable findings, with either reduction in the apnoea/hyopnoea index (AHI) [92,93] or no systematic change in OSA severity[97,220,222,227,228]. These differences may be explained, at least in part, by 1) differences in the ability to shift the arousal threshold, 2) the effects on pharyngeal muscle activity between agents and 3) variability in underlying pathophysiology between study participants[174].

Zolpidem is an imidazopyridine "z-drug" that specifically binds to the non-benzodiazepinic alpha-1 subunit of the GABA-A receptor[229]. It is widely prescribed to decrease sleep onset latency and acutely manage insomnia[230]. "Z-drugs", similar to other hypnotics, induce sleep[231] and can increase the respiratory arousal threshold[92,97,218]. Zolpidem was recently found to not only increase the threshold for arousal to airway closure or narrowing during transient continuous positive airway pressure (CPAP) reductions, but to also increase upper airway muscle responsiveness three-fold in people with and without OSA[218]. This indicates that zolpidem improves two key contributors to OSA (arousal threshold and muscle responsiveness), and may therefore, have superior therapeutic potential to reduce OSA severity compared to other common hypnotics. Our recent open-label pilot study in 12 unselected OSA patients indicated that zolpidem was safe and well-tolerated[220].

Accordingly, the aim of this randomized, double-blind, placebo-controlled, crossover trial was to investigate the effects of zolpidem on OSA severity, the respiratory arousal threshold, genioglossus muscle responsiveness, objective sleep quality and next-day perceived sleepiness, and objective alertness in patients with low-to-moderate respiratory arousal thresholds, without major overnight hypoxia during naturally occurring sleep (without CPAP).

Methods

Ethical approval

This study conformed to the standards set by the latest revision of the Declaration of Helsinki, was approved by the South Eastern Sydney Local Health District Human Ethics Committee, and prospectively registered on the Australian New Zealand Clinical Trials Registry (ACTRN12618001287224). All patients provided written informed consent upon enrolment.

Participants

Twenty otherwise healthy people with OSA (AHI >10 events/h) aged 18 to 64 years were recruited. Inclusion criteria included a low-to-moderate (>-25cmH_2O) respiratory arousal threshold estimated according to diagnostic polysomnographic parameters[232] and a nadir overnight arterial blood oxygen saturation >70%. Exclusion criteria were pregnancy, hypersensitivity to lignocaine/phenylephrine/zolpidem and medications which may impact breathing control or upper airway muscle function.

Protocol

Participants completed two overnight sleep studies approximately one week apart. Participants received either a placebo pill or a standard dose of zolpidem (10 mg) orally, immediately prior to lights-out, in a double-blind, randomized cross-over design (Figure 3). Time of lights out was based on the patients' usual bedtime and kept constant between the two study nights. Participants were instructed to sleep on their back and given an 8-hour sleep opportunity.

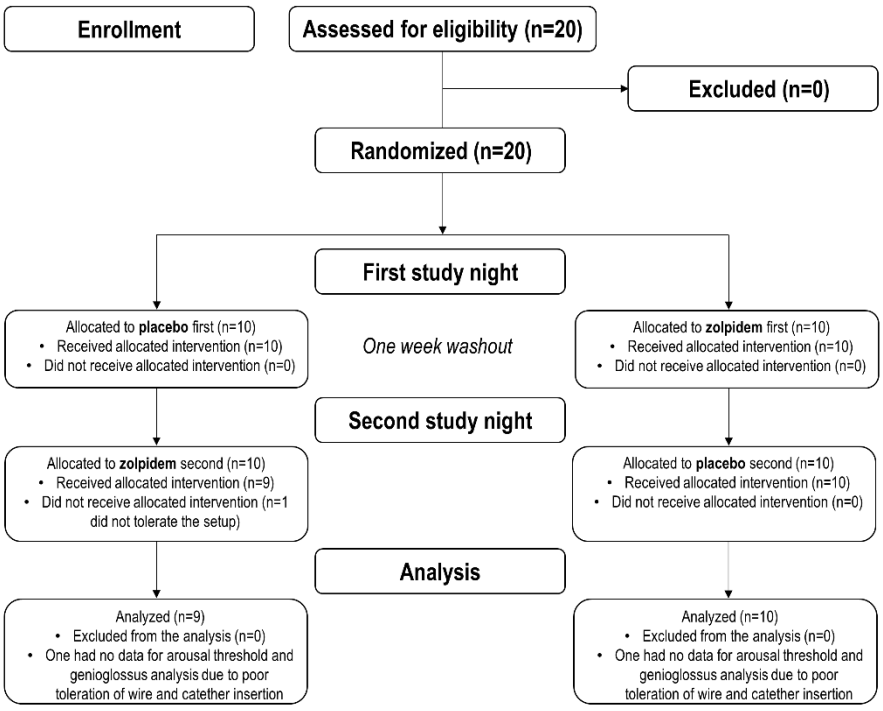

Figure 3. CONSORT diagram. This indicates recruitment, randomization, and analysis procedures for the trial.

Prior to sleep, on either one of the study nights, baseline loop gain via breath-holding technique[43] and the upper airway collapsibility index via negative pressure pulse administration[233] were quantified.

Participants completed the Insomnia Severity Index (ISI) and Epworth Sleepiness Scale (ESS) questionnaires on their first night ~30 min after arrival.

On the following morning of each visit, participants completed the Karolinska Sleepiness Scale (KSS) questionnaire and a 30-min driving simulation task (AusEd)[228] ~ 30 min after wake. Participants were asked which night they thought they were given the study drug on the morning of the final visit after all testing was complete. Systemic blood pressure was measured at bedtime and each morning after waking.

Equipment and key measurements

In addition to standard polysomnographic setup[234], participants were fitted with a nasal mask attached to a pneumotachometer (Hans-Rudolph, Kansas City, MO, USA). Mask pressure (Validyne, Northridge, CA, USA) and end-tidal partial pressure for carbon dioxide (PCO_2) (Vacumetrics Inc., Ventura, CA, USA) were measured via sensors attached to ports in the nasal mask. Epiglottic pressure was measured with a pressure-tipped catheter (Millar, TX, USA) inserted into a decongested and anaesthetised nostril. This was advanced ~1.5–2 cm caudally below the base of the tongue.

Bipolar electromyography (EMG) was used to determine genioglossus muscle activity. Two stainless-steel Teflon-coated intramuscular fine-wire electrodes (no. 791500; A-M Systems Inc., Sequim, WA, USA) were inserted sublingually (~3-4cm either side of the frenulum) using a 25-gauge needle, following surface anaesthesia (1% lignocaine), as described previously[174]. EMG data were acquired at a sample rate of 2,000Hz and filtered at 20 to 500Hz.

Data analysis

All analyses were conducted blinded to the study intervention. Respiratory events and arousals were scored using standard American Academy of Sleep Medicine 2012 guidelines[235] by an experienced sleep technician.

Raw data were generated at Neuroscience Research Australia. The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to restrictions, e.g. they contain information that could compromise the privacy of research participants.

The respiratory arousal threshold was calculated as the average nadir epiglottic pressure immediately prior to arousal during each obstructive event (apnoeas and hypopnoeas) using custom designed semi-automated software[236]. Breath-by-breath activation of genioglossus was assessed during obstructive events. Genioglossus EMG was rectified, moving-time averaged (100ms) and expressed as percentage of maximum activity obtained during a swallow or tongue protrusion conducted prior to sleep and the study intervention. Genioglossus muscle responsiveness was quantified as the slope of a linear regression of peak (highest value during

inspiration) and tonic (nadir during expiration) genioglossus EMG against nadir epiglottic pressure (%/cmH_2O)[227]. Breaths which included arousal or other artefact were excluded from arousal threshold and muscle analyses.

Arousal intensity was calculated on both nights using a validated algorithm[237] from the available EEG leads. The hypoxic burden was defined as the total area under the respiratory event-related desaturation curve divided by the sleep duration expressed as (%min)/h[238].

Statistical analysis

A power calculation was performed prior to the study with AHI as the primary outcome. We conservatively estimated that 18 participants would be required to detect a >7 events/h difference between placebo and zolpidem (ΔSD= 10 events/h)[174] to achieve >80% power (two-tailed paired Student's t-test, alpha=0.05). Thus, we allowed for recruitment of up to 24 patients to account for a potential dropout rate of 25% for these detailed physiological studies.

Data are expressed as mean±SD or median [interquartile range]. Statistical significance was inferred if $p<0.05$. Data were analyzed using paired Student's *t*-test or Wilcoxon signed-rank test according to normal or non-normal data distribution for respiratory, sleep, sleepiness and alertness parameters. Mixed model analysis, with sleep stage and treatment condition as fixed effects, and the patient as a random effect, was performed to test the effect of zolpidem on the arousal threshold and genioglossus muscle responsiveness across the sleep stages. Where significant main effects were detected, post-hoc analyses were performed using Bonferroni adjustment for multiple comparisons. Statistical analyses were performed using Graph Pad Prism 6.0 (Graph Pad Software, La Jolla, CA) and SPSS 23.00 (IBM, Armonk, NY).

Results

Nineteen of the twenty participants recruited for the study completed both nights (Figure 3). Anthropometric and baseline data are summarised in Table 1. One participant withdrew from the study due to discomfort with the polysomnography equipment on his first night (placebo).

Characteristic	
Age, years	47 ±12
Female gender, n (%)	2 (11)
Neck circumference, cm	39.8 ± 3.8
Waist circumference, cm	101.5 ± 11.3
Body mass index, kg/m^2	29.4 ± 4.5
Apnoea-hypopnoea index, events/h	27.2 ± 16.1
Loop gain (from breath-holds)*	0.53 ± 0.15
Upper airway collapsibility index#, %	42.2 ± 28.4
Epworth Sleepiness Scale	7 ± 4
Insomnia Severity Index	5 ± 4

Table 1. Participants baseline characteristics. Six participants had an Insomnia Severity Index consistent with insomnia (i.e. total score ≥8). Data are mean ± standard deviation unless indicated otherwise. Apnoea-hypopnoea index data are derived from the baseline diagnostic polysomnography used for study inclusion prior to enrollment. N=19 except: *indicates data available for 17 patients and # indicates data available for 18 patients (due to insufficient time).

Effects of zolpidem on physiological parameters

Respiratory arousal threshold

Overall, zolpidem increased the arousal threshold (Δmean = −2.5 ± 0.9 cmH_2O) and there was a sleep stage effect (Figure 4). A progressive increase in arousal threshold with deeper sleep (N1 *vs.* N3, Δmean = −4.5 ± 1.5 cmH_2O, p=0.024) and a decrease in REM (REM *vs.* N2, Δmean = 4.9 ± 1.2 cmH_2O, p=0.001; REM *vs.* N3, Δmean = 7.3 ± 1.6 cmH_2O, p <0.001) occurred with zolpidem. There was no interaction effect (i.e. the increase in arousal threshold due to zolpidem was not specifically related to sleep stage changes; p=0.573; Figure 4).

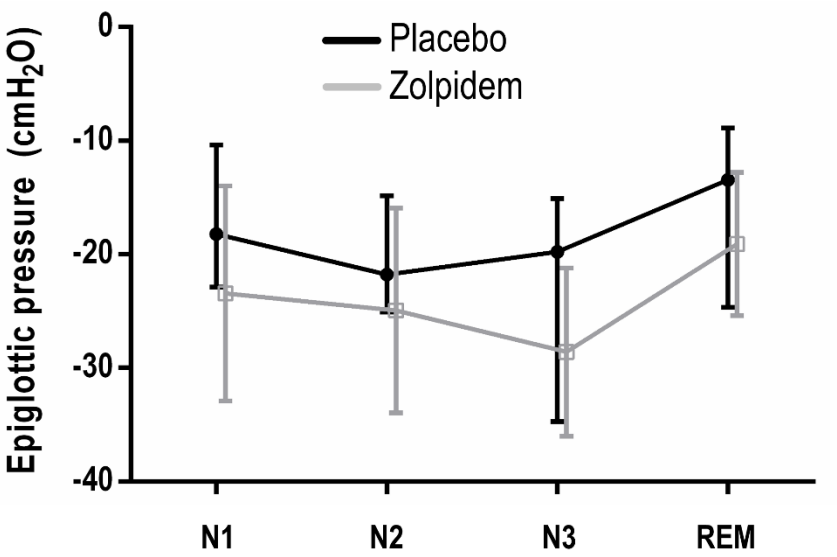

Figure 4. Arousal threshold changes between placebo and zolpidem across sleep stages. More negative values correspond to a higher arousal threshold. A mixed model analysis showed that zolpidem increased the arousal threshold by a mean value of 2.5 ± 0.9 cmH_2O (p=0.010) across all sleep stages. Arousal threshold was lowest in REM and highest in N3 (see text for further details). Arousal threshold data were available in 17 patients. Dots indicate median values and lines indicate 25th (low) and 75th (top) percentiles. REM, rapid eye movement; NON-REM, non-rapid eye movement.

Genioglossus activity

Muscle responsiveness was not different with zolpidem, or across sleep stages, with no interaction effect (Figure 5). Maximal maneuver values were not different between zolpidem and placebo nights (325.1 ± 65.6 *vs.* 330.5 ± 50.0 μV; p=0.936). Similarly, zolpidem had no effect on either peak (p=0.155) or tonic activity (p=0.216) when muscle responsiveness was expressed in absolute values (i.e. $\mu V/cmH_2O$).

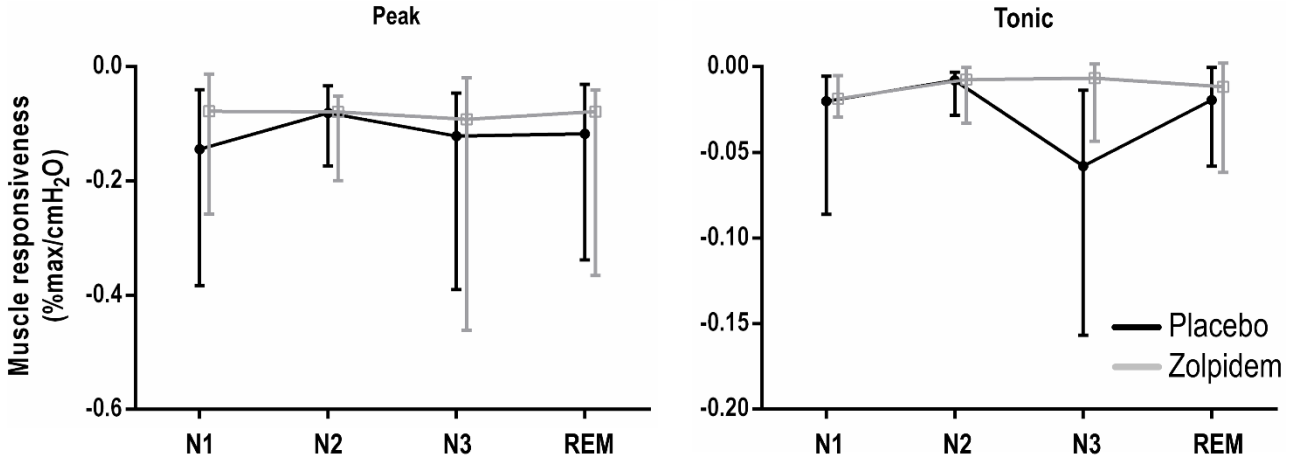

Figure 5. Muscle responsiveness changes between placebo and zolpidem across the sleep stages. Left and right panels represent peak and tonic activity, respectively, as a percentage of maximum genioglossus activity (e.g. tongue protrusion). More negative values correspond to a higher muscle responsiveness. A mixed model analysis revealed that there was no change in muscle responsiveness due to zolpidem (p=0.422, p=0.095 for peak and tonic, respectively), sleep stage (p=0.246, p=0.078 for peak and tonic, respectively) or interaction effect (p=0.515, p=0.253 for peak and tonic, respectively). Data expressed as absolute values (uV/cmH2O) led to similar results (see text for details). Data were available from 17 patients. Dots indicate median values and lines indicate 25th (low) and 75th (top) percentiles. REM, rapid eye movement; NON-REM, non-rapid eye movement.

The number of breaths used for arousal threshold and muscle responsiveness analyses is shown in Table 2.

Analysis	Placebo	Zolpidem	*p* value
Arousal threshold			
N1, n	16 ± 4	18 ± 5	0.738
NON-REM (N2+N3), n	21 ± 5	22 ± 4	0.782
REM, n	5 ± 1	6 ± 2	0.495
Muscle responsiveness			
N1, n	72 ± 20	77 ± 22	0.870
NON-REM (N2+N3), n	162 ± 49	144 ± 33	0.665
REM, n	57 ± 18	88 ± 40	0.428

Table 2. Average number of breaths used for the arousal threshold and muscle responsiveness analyses. Data are mean ± standard deviation and were compared with paired Student's t-test. N=17 participants. NON-REM, non-rapid eye movements.

Effects of zolpidem on OSA severity and sleep parameters

Zolpidem did not reduce OSA severity. Total AHI, AHI during non-REM and REM sleep were unaltered on the drug night compared to placebo (Figure 6).

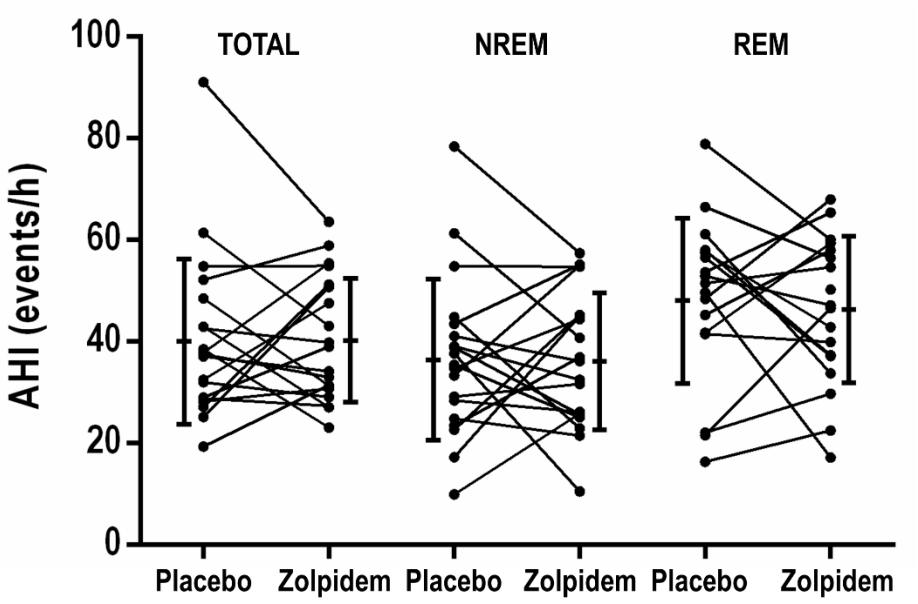

Figure 6. Effect of zolpidem on OSA severity. Total, non-REM (non-rapid eye movement) and rapid eye movement (REM) apnea-hypopnea index (AHI) between the two conditions. Zolpidem had no effect on obstructive sleep apnea severity p=0.938, p=0.933 and p=0.524 respectively (paired Student's t-tests). Bars represent mean ± standard deviation.

Supine AHI was also unchanged (Table 3).

Characteristic	Placebo	Zolpidem	*p* value
Supine AHI, events/h	37.2 [18.0]	33.8 [25.6]	0.609
Obstructive apnea index, events/h	5.9 [12.1]	6.4 [16.4]	0.980
Mixed apnea index, events/h	0 [0.2]	0 [0.4]	0.203
Central apnea index, events/h	0 [0.6]	0 [1.1]	0.550
Percent supine, % sleep time	100.0 [9.9]	98.5 [19.7]	0.625
Sleep onset latency, min	6.0 [12.2]	6.5 [11.2]	0.832
N1, % sleep time	30.7 ± 15.5	24.4 ± 11.1	**0.043**
N2, % sleep time	38.3 ± 10.9	39.7 ± 6.4	0.581
N3, % sleep time	11.4 [19.5]	19.3 [11.9]	**0.019**
REM sleep, % sleep time	16.1 ± 9.0	17.0 ± 9.6	0.789
Arousal index, events/h	45.5 ± 17.9	41.4 ± 14.6	0.147
NON-REM arousal index, events/h	39.8 ± 15.4	35.3 ± 14.0	0.106
REM arousal index, events/h	32.7 ± 14.2	34.1 ± 16.1	0.762
Respiratory event duration, sec	24.6 ± 3.7	25.7 ± 5.2	0.254
Mean SpO_2 during sleep, %	93.0 [2.7]	92.8 [3.3]	0.113
SpO_2 nadir during sleep, %	79.6 ± 7.4	79.7 ± 6.6	0.932
Arousal intensity, 1-10 scale	3.79 [0.19]	3.69 [0.15]	0.234
Hypoxic burden, (%min)/h*	69.6 [64.2]	67.2 [39.0]	0.865
Systolic blood pressure, mmHg*	132 ± 13.2	130 ± 17	0.534
Diastolic blood pressure, mmHg*	90 ± 14.2	90 ± 16.0	0.865

Table 3. Polysomnography parameters. Data are mean ± standard deviation or median [interquartile range] where appropriate and were compared with paired Student's t-test or Wilcoxon signed rank test accordingly. NON-REM, non-rapid eye movements; AHI, apnea hypopnea index; SpO2, oxyhemoglobin saturation. N=19 except: * indicates data available for 18 participants (hypoxic burden: due to absence of data sampled at the desired frequency in one participant's night; blood pressure: the procedure was not in the protocol yet). Paired data for N3 and REM sleep were available for 18 and 17 participants, respectively (three patients did not have any N3 (N=1) and REM (2) sleep).

Five participants had OSA amelioration on the drug night, with an AHI drop of >15 events/h during NON-REM sleep, while five participants experienced a >15 event/h increase in AHI. Within the subgroups of "responders" and "non-responders", participants did not present any shared characteristics (i.e. low/high basal loop gain, baseline arousal threshold or baseline pharyngeal collapsibility, comparable increase/decrease of arousal threshold or genioglossus responsiveness).

Zolpidem increased sleep efficiency by 9.0±13.8% and total sleep time by 1.1±1.2 h versus placebo (Figure 7). Additionally, wake after sleep onset was reduced on the drug by −31.7±52.3 min (Figure 7). Improvements in sleep architecture were driven by a decrease in N1 (Δmean = −6.2 ± 2.9 min) and an increase in N3 on the drug (Δmean = 3.6 ± 8.7 min, Table 3).

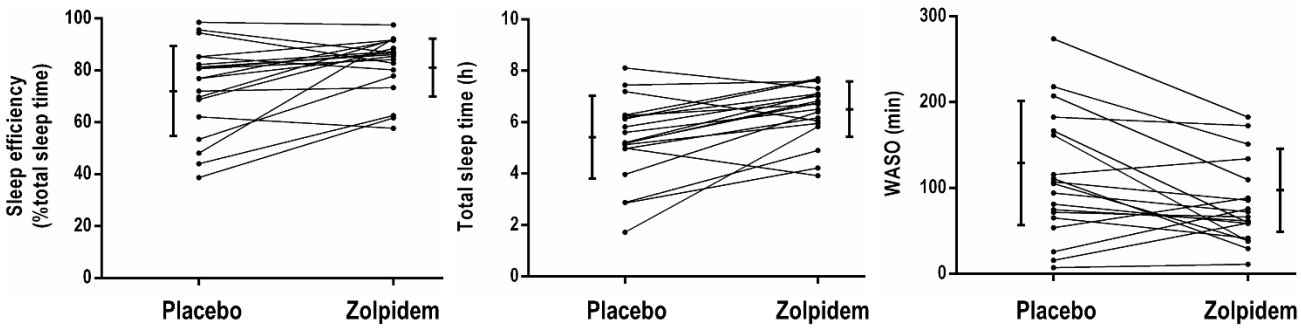

Figure 7. Effect of zolpidem on sleep efficiency. Compared to placebo, zolpidem improved sleep efficiency (left panel, p=0.010) and total sleep time (middle panel, p=0.001) and reduced WASO (right panel, p=0.017) (paired Student's t-test). * highlight statistical significance p<0.05. Bars represent mean ± standard deviation. WASO, wake after sleep onset.

There was no effect of zolpidem on nadir oxyhemoglobin saturation and mean oxygen saturation during sleep (Table 3). The hypoxic burden was also unchanged on the drug compared to the placebo (Table 3).

Effect of zolpidem on next-day perceived sleepiness, alertness and safety

Next day sleepiness and alertness were not different following zolpidem compared to placebo as shown by the Karolinska sleepiness scale and driving simulator performance indicators (Table 4).

Characteristic	Placebo	Zolpidem	*p* value
Karolinska Sleepiness Scale (1-9 scale)	5.2 ± 1.7	4.7 ± 2.0	0.325
AusEd driving simulator*			
Deviation from median of lane, cm	47.6 [27.4]	50.6 [31.3]	0.252
Deviation from 60–80 km/h, km/h	1.4 [2.5]	1.9 [2.0]	0.542
Braking reaction time, s	0.94 [0.21]	1.00 [0.40]	0.594
Crashes, n	0 [1]	0 [1]	0.266

Table 4. Effects of zolpidem on next day perceived sleepiness, objective alertness and safety. Data are mean ± standard deviation or median [interquartile range] where appropriate and were compared with paired Student's t-test or Wilcoxon signed rank test accordingly. N= 19 except: *indicated data available for 16 participants (three participants could not undertake the test due to vision impairment (N=2) and insufficient time (1)).

Only 6 participants (32%) correctly determined the drug night when surveyed on the morning of the final visit at the end of the study. Of these 6 individuals, 5 reported worse subjective sleep quality (next-morning complaints were present in 2 cases with drowsiness and sore throat), whereas 1 reported improvement in next-day alertness. No additional complaints were reported on the drug night. During the overnight study, one participant experienced a few minutes of apparent dream enactment, whereas another had enuresis. Both occurred on the zolpidem night. After these events, which were associated with mild confusion, these participants resumed sleep within five minutes and did not have any other episodes during the night, nor any next day complaints/awareness.

Discussion

The main findings of this study are that a standard 10mg dose of zolpidem does not alter OSA severity, increases the respiratory arousal threshold and improves sleep efficiency in predominantly severe OSA patients with a low to-moderate-arousal threshold and mild overnight hypoxia at baseline. Zolpidem also improves sleep architecture with a shift away from light N1 to deeper N3 sleep. Contrary to a recent physiology study with transient CPAP reductions, zolpidem does not increase pharyngeal muscle responsiveness during naturally occurring respiratory events. From a safety perspective, zolpidem was generally well tolerated in this patient group and did not result in increases in perceived next day sleepiness or alertness.

Effects of zolpidem on physiological parameters

An ~15% increase in the arousal threshold with zolpidem is quite modest compared with previous studies of "z-drugs"[92,97,218] or other hypnotic agents[216,227] that show increases of ~30%.

Arousal threshold changes with sleep stage, with highest values during N3 (i.e. the negative swings in epiglottic pressure are larger) and lowest during REM[239]. Although we confirmed that arousal threshold was lower in REM *vs.* N2/N3 and in N1 *vs.* N3, the sleep stage differences did not impact the effect of zolpidem on the arousal threshold which was consistent across sleep stages. This property may be advantageous in the context of combination therapy with other agents that may reduce the arousal threshold or disrupt sleep architecture[199].

In contrast to recent findings with CPAP dial-downs[218], zolpidem did not improve genioglossus muscle responsiveness during naturally occurring respiratory events. Rather, the current findings are consistent with previous zopiclone investigations which had no effect on genioglossus muscle activity[97]. Activation of $GABA_A$ receptors enhance Cl^--mediated inhibition at the hypoglossal motor nucleus and would not be expected to lead to excitatory motor activity[240]. However, a positive effect of zolpidem, when administered systemically, on genioglossus activity has been demonstrated in rats[208], via an increased arousal threshold that facilitated greater CO_2-mediated respiratory stimulation during sleep.

Thus, the apparent disparity in genioglossus muscle responsiveness in the current study versus the recent physiology study may relate to differences in participant characteristics (both OSA and non-OSA in the previous study *vs.* only OSA in the current study) and important methodological differences. For example, CPAP inhibits genioglossus activity[241]. Genioglossus responsiveness was measured from a low but consistent level with stable blood gases just prior to the transient CPAP reductions in the prior study[218]. At a CPAP level sufficient to abolish airflow limitation, genioglossus behaves similarly in OSA and controls[242]. However, CPAP dial-downs may reveal abnormal upper airway muscle responses in OSA patients[243]. Conversely, spontaneous respiratory events were examined in the current study, with variable pre-event blood gas levels. This may lead to different upper airway responses. Nonetheless, the current study reflects "real world" circumstances and there was no evidence for a beneficial effect on muscle responsiveness or OSA severity under these conditions.

Effect of zolpidem on OSA severity and sleep parameters

The lack of a sytematic effect of zolpidem on AHI is consistent with our pilot study findings [220] and earlier preliminary reports[214,219]. It is also consistent with recent acute single dose, 1 month of nightly use and acute high dose findings with zopiclone[97,222,223]. The lack of effect on OSA severity is in line with the modest increase in arousal threshold and absence of a benifical effect on genioglossus muscle responsiveness in the current study. While there was some interindividual variability changes in OSA severity with zolpidem, these were not explained by differences in key OSA phenotypic traits. However, caution is warrented given the relatively small sample size for these mechanistic comparisons.

Nonetheless, based on the current study findings and other recent evidence on the role of common hypnotics on sleep and breathing in OSA[218,223,244], it would appear that the extent to which most available hypnotic agents increase the arousal threshold is insufficient to stabilise breathing in the majority of patients. Whether this means that the arousal component contributes less to OSA pathophysiology than previously thought remains uncertain and likely depends on the combination of other influential pathophysiological factors (e.g. pharyngeal muscle characteristics and anatomy) which vary between individuals. It is possible that a higher dose of zolpidem may yield greater increases in the arousal threshold and therefore, therapeutic benefit in appropriately selected patients. However, as the unexpected findings from a recent randomised trial with zopiclone indicate, higher doses do not necessarily yield greater increases in arousal threshold or changes in OSA severity as anticipated[223].

Consistent with our pilot study[220], zolpidem increased sleep efficiency by approximately 10% to within a normal range (~80% of total time in bed). This was driven by increased total sleep time, reduced WASO and accompained by improved sleep architeture. Comparable improvements in sleep efficiency are seen with Cognitive-Behavioral Treatment (CBT), the first line treatment for insomnia (~9-17% increase, depending on the method of administration and selected population[245,246]). Zolpidem also increases sleep efficiency in patients with insomnia[247]. Insomnia symptoms and OSA often coexist[248]. Insomnia symptoms also tend to worsen CPAP usage and quality of life in OSA patients[248], regardless of resolution of OSA with CPAP treatment[249]. CBT, the recommended therapeutic intervention for chronic insomnia, not only improves insomnia symptoms but also ameliorates CPAP adherence in people with comorbid OSA and insomnia[250]. Thus, given the subtantial improvements in sleep efficiency and architeture with zolpidem (without AHI or next day funtion changes) in people with OSA in the current study, it would be of interest to investigate if zolpidem yields similar benefits to CBT in relation to CPAP usage in people with comorbid OSA and insomnia. If equally beneficial, this approach would have the advantage of being more time and cost effective and more readily accessible than traditional CBT approaches. Indeed, acute use of the "z-drug" eszopiclone showed potential to improve CPAP tolerance in people with OSA[251]. However, zolpidem only addresses one symptomatic component of insomnia whereas CBT provides a more comprehensive approach that incoprorates sleep education and sleep hygiene components to target the underlying causes. Furthermore, CBT in people with insomnia and OSA has recently been shown to not only improve insomnia but also modestly reduce OSA severity[252] and thus may be the preferred therapy.

Effects of zolpidem on next-day sleepiness, alertness and safety

While there were no favourable effects of a standard dose of zolpidem on AHI in this selected group, the safety profile in terms of perceived sleepiness and driving simulator performance was similar between conditions. This challenges the contraindications for zolpidem use in OSA based on potential harmful effects on the upper airway muscles and respiratory drive. Indeed, the latest revision of Food and Drug Administration guidelines recommends that zolpidem be prescribed cautiously to sleep apnoea patients[253] due to the potential negative effects on respiratory drive, arousal index and oxyhemoglobin saturation[254]. The Therapeutic Good Administration in Australia classifies sleep apnoea as a contraindication for zolpidem usage[255]. These recommendations are based largely on case reports[256], early works on unselected patients[257] and animal studies[258] with concern that these potential negative effects would translate clinically into worse subjective sleep quality and daytime symptoms, with car accidents as a potential life-threatening consequence[8,259]. However, zolpidem has less next-morning residual effect when compared to benzodiazepines[231]. Furthermore, as assessed in other recent randomised trials, zopiclone, given acutely at standard and high doses[97,228] or over 1 month at a standard dose[222], does not increase next day sleepiness or impair alertness in people with OSA.

Other studies raised concern that z-drugs may decrease blood oxygen concentration through a decrease in respiratory drive[97] or via lenghtening in respiratory event duration[18]. Indeed, high doses of zolpidem can worsen oxygen saturation in unselected patients with OSA[214] or in those with heart failure[260]. Conversly, overnight oxygenation was not different in the current study between conditions and there was no alteration of systemic blood pressure on drug. Thus, while there is currently no evidence to prescribe zolpidem to reduce OSA severity, the majority of evidence indicates that zolpidem is not deleterious, at least in people with low-to-moderate arousal thresholds who do not have major overnight hypoxemia at baseline. Nonetheless, it is important to note that these acute physiolgy studies were conducted over a single night in a relaitvely small number of selected people with OSA. Thus, the long term effects of zolpidem in the broader OSA population, including the eldery, more obese and patients with comobidities, remain unknown and require further investigation. However, treatment efficacy, including increases in sleep efficiency, remain stable for at least 8 months of nightly use[261] without the need for dose escalation[262] in people with primary insomnia.

Methodological considerations

We studied 19 patients and performed detailed measurements of sleep and upper airway physiology after a single, standard dose of zolpidem. Thus, while these findings provide novel insight and represent the largest randomised trial on the effects of zolpidem in people with OSA conducted to date, long term trials with an increased number of participants, including those with insomnia and OSA, are needed to further assess the safety profile and potential efficacy of chronic use of zolpidem in people with OSA.

Conclusions

A standard single dose of zolpidem substantially improves sleep efficiency, total sleep time and promotes deeper sleep and modestly increases the respiratory arousal threshold with no major deleterious effects on respiratory event duration, overnight hypoxemia, pharyngeal muscle activity, or next day sleepiness or alertness in people with predominately severe OSA and low-to-moderate arousal thresholds. Zolpidem does not however, reduce the frequency of respiratory events. Given these properties, further work to investigate potential beneficial effects of short-term zolpidem in people with comorbid OSA and insomnia, including to facilitate CPAP tolerance and adherence, are warranted. Additionally, increasing arousal threshold with zolpidem might be of benefit in the context of new combination therapy approaches to treat OSA, some of which have unwanted wake promoting properties[263].

CHAPTER THREE:
THE ADDITION OF ZOLPIDEM TO COMBINATION THERAPY WITH ATOMOXETINE-OXYBUTYNIN INCREASES SLEEP EFFICIENCY AND THE RESPIRATORY AROUSAL THRESHOLD IN OBSTRUCTIVE SLEEP APNEA: A RANDOMIZED TRIAL

Abstract

Rationale

Atomoxetine combined with oxybutynin (Ato-Oxy) has recently been shown to reduce obstructive sleep apnea (OSA) severity by >60%. This offers promise for development of pharmacotherapy for OSA. However, Ato-Oxy also slightly reduced the respiratory arousal threshold which may decrease sleep quality/efficiency. To determine whether the addition of zolpidem with Ato-Oxy combination therapy increases sleep efficiency and the arousal threshold. We also aimed to investigate the effects of the addition of zolpidem to Ato-Oxy on OSA severity and other polysomnography parameters, next-day sleepiness and alertness.

Methods

Twelve people with OSA received 10mg zolpidem plus Ato-Oxy (80mg and 5mg respectively) on one occasion and Ato-Oxy plus placebo on another just prior to overnight in-laboratory polysomnography according to a double-blind, randomized, cross-over design (1-week washout). Participants were fitted with an epiglottic catheter, a nasal mask and pneumotachograph to quantify arousal threshold and airflow. Next-day sleepiness and alertness were assessed via the Karolinska Sleepiness Scale and a driving simulation task.

Results

The addition of zolpidem increased sleep efficiency by 9±13% (80.9±16.9 *vs.* 88.2±8.2 %, p=0.037) and the respiratory arousal threshold by 17±18% (−26.6±14.5 *vs.* −33.8±20.3 cmH2O, p=0.004) versus Ato-Oxy+placebo. Zolpidem did not systematically change OSA severity. Combination therapy was well-tolerated, and zolpidem did not worsen next-day sleepiness. However, median steering deviation during the driving simulator task increased following the zolpidem combination.

Conclusions

Zolpidem improves sleep efficiency via an increase in the respiratory arousal threshold to counteract potential wake-promoting properties of atomoxetine in OSA. These changes occur without altering the rate of respiratory events or overnight hypoxemia. However, while the addition of zolpidem does not increase next-day perceived sleepiness, caution is warranted given the potential impact on next-morning objective alertness.

Introduction

Obstructive sleep apnea (OSA) is a common disorder with multi-factorial pathogenesis. Key traits include a collapsible pharyngeal airway, elevated loop gain (unstable respiratory control), poor upper-airway dilator muscle responsiveness and a low respiratory arousal threshold (waking up too easily to minor airway narrowing)[20]. Identification of the impaired trait or traits has the potential to inform customized treatment to increase efficacy and acceptance of existing non-continuous positive airway pressure (CPAP) therapies and emerging pharmacotherapy[263]. This is an important objective as CPAP, although efficacious regardless of underlying pathogenesis, is often poorly tolerated[12]. The combination of atomoxetine, a noradrenergic drug, and the antimuscarinic oxybutynin (Ato-Oxy) has recently been shown to reduce OSA severity by 63% in unselected patients[174]. Improvements were largely driven by an increase in upper airway dilator muscle responsiveness[264]. Despite no change in total sleep time and sleep architecture compared to placebo, in accordance with the pharmacodynamics of atomoxetine, a mild wake promoting agent, Ato-Oxy also counterproductively reduced the respiratory arousal threshold during sleep by ~9%[264].

Zolpidem, a commonly prescribed non-benzodiazepine hypnotic, increases the respiratory arousal threshold by ~15-25% in people with OSA[218,265]. The magnitude of this increase is comparable to other "z-drug" hypnotics[92,97]. In addition, unlike other z-drugs, zolpidem also increases sleep efficiency by ~10% in people with OSA without impairing next-day sleepiness or alertness[265] and, in one study, also improved genioglossus muscle responsiveness during sleep[218]. Given these properties, the addition of zolpidem to Ato-Oxy could alleviate counterproductive reductions in arousal threshold with the potential to increase therapeutic efficacy versus Ato-Oxy alone.

Accordingly, we conducted a double-blind, randomized, crossover trial to investigate the additional effects of zolpidem to Ato-Oxy combination therapy on sleep efficiency (primary outcome) in unselected people with OSA. Secondary aims were to investigate the effects of the addition of zolpidem to Ato-Oxy on the respiratory arousal threshold, OSA severity (i.e. apnea-hypopnea index [AHI] and nadir oxygen saturation), other polysomnographic parameters (i.e. arousal index), next-day perceived sleepiness, and objective alertness .

Methods

Participants

Thirteen people with a previous diagnosis of OSA (AHI >10 events/hr) aged 18 to 75 years were recruited via social media advertisement, placement of flyers on local clinics and institutional notice boards and review of our volunteer registry. Participants on CPAP (N=6) were asked to suspend treatment during the trial and for one week prior to the first study visit. General exclusion criteria were: any acute disease and hypersensitivity to the drugs (further exclusion criteria details provided in the online supplement). The study was approved by the Southern Adelaide Clinical Research Ethics Committee, and prospectively registered (ACTRN12619001427167). All participants provided written informed consent prior to enrolment. Participants were studied at Adelaide Institute for Sleep Health, Flinders University.

Protocol

Participants were asked to come to the laboratory twice, one week apart, to undertake two overnight sleep studies. Prior to lights-out (based on the participant's usual bed time and kept constant between the nights), they received Ato-Oxy (80-5 mg) plus placebo on one occasion and the combination of Ato-Oxy and zolpidem 10mg (order randomized) separated by one week (Figure 8). Participants and investigators were blinded to the study interventions. Participants were also instructed to sleep on their back for as much as possible and were given an 8-hour sleep opportunity during each study visit.

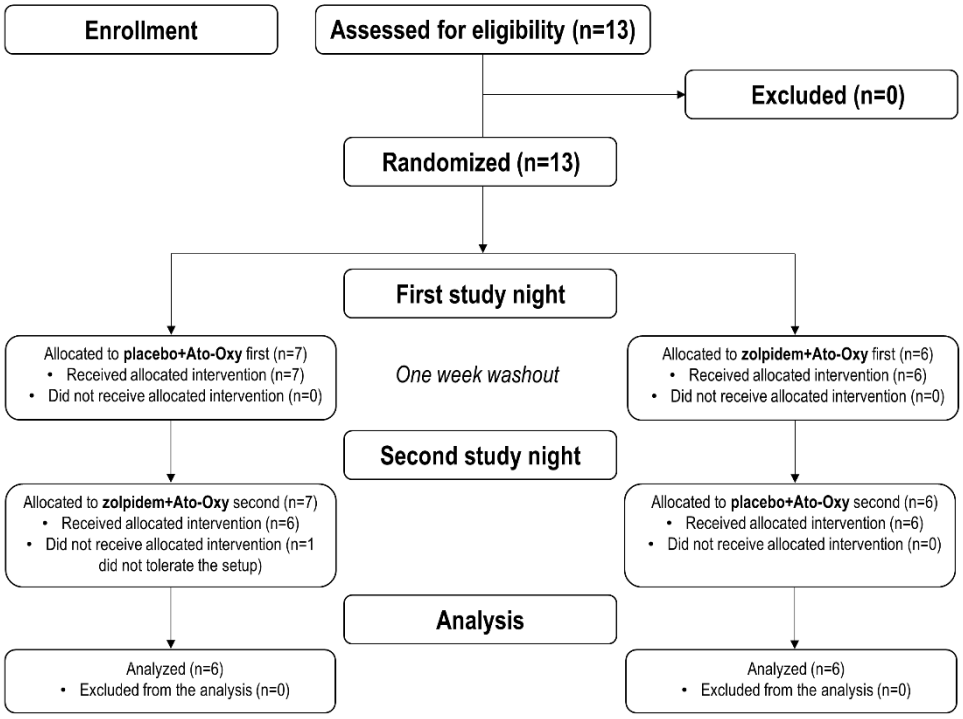

Figure 8. CONSORT diagram. This indicates recruitment, randomization, and analysis procedures for the trial.

On the first night, ~30 mins after arrival, participants completed the Insomnia Severity Index (ISI) and the Epworth Sleepiness Scale (ESS) questionnaires. Systemic blood pressure was also recorded. The Karolinska Sleepiness Scale (KSS) and blood pressure were also recorded in the morning approximately 30 mins after

wake time. This was followed by a 30-min driving simulation task using the AusEd driving simulator[266]. Potential side effects (e.g. sleepiness, dysuria etc.) were also investigated in the morning after each visit. After the final visit, participants were asked which night they thought they were given the hypnotic and whether they felt that their sleep improved.

Equipment

Participants were equipped with standard polysomnographic (PSG) equipment[234], along with a sealed nasal mask attached to a pneumotachometer (Hans-Rudolph, Kansas City, MO, USA), and pressure transducer (Validyne, Northridge, CA, USA). A pressure-tipped catheter (Millar, TX, USA) was inserted into a decongested and anaesthetised nostril and advanced ~1.5–2cm caudally below the base of the tongue to allow for direct quantification of the respiratory arousal threshold (nadir epiglottic pressure just prior to arousal)[265].

Data analysis

All analyses were conducted blinded to the study interventions. Respiratory events and arousals were scored by experienced sleep technicians, using standard American Academy of Sleep Medicine 2020 criteria[267].

OSA endotypes (i.e. loop gain, Vpassive, Vactive, Vcompensation and ventilatory response to arousal) were estimated using previously validated algorithms[2,268]. Briefly, Loop gain was calculated from polysomnographic flow signals as the response to a one cycle/min disturbance. Vpassive was defined as the ventilation during sleep at eupneic ventilatory drive when the pharyngeal muscles are relatively passive. Vactive was defined as the level of ventilation at maximum drive (i.e., arousal threshold). Vcompensation was taken as the difference between ventilation at maximal Vdrive and Vpassive.

Statistical analysis

An *a priori* power calculation was performed with sleep efficiency as the primary outcome. We estimated that 12 participants would be required to detect a >6% change in sleep efficiency between Ato-Oxy with versus without zolpidem ($\Delta SD = 6.5$)[265] with >80% power. Data were expressed as mean±SD or median [interquartile range] for non-normality distributed data. Statistical significance was inferred if $p<0.05$. In accordance with our statistical analysis plan, data were analyzed using two-tailed paired Student's *t*-tests or a Wilcoxon signed-rank test as appropriate. Chi-squared analysis was used to compare the percent of people who crashed during the driving simulation task between conditions. Analyses were performed using Graph Pad Prism 6.0 (Graph Pad Software, La Jolla, CA) and SPSS 23.00 (IBM, Armonk, NY).

Additional information are provided in the Thesis Supplement.

Results

Twelve of the thirteen participants recruited successfully completed both overnight studies as shown in Figure 8. One individual withdrew from the protocol after the first overnight (placebo) due to discomfort related to the study procedures. Baseline participant characteristics are shown in Table 5. Patients were recruited from November 2019 to June 2020.

Characteristic	
Age, years	55 ± 14
Female sex, n (%)	2 (17)
Neck circumference, cm	37 ± 6
Waist circumference, cm	98 ± 17
Body mass index, kg/m2	27 ± 5
Insomnia Severity Index	9 ± 4
Epworth Sleepiness Scale	9 ± 5

Table 5. Baseline characteristics of the study participants. Data are mean ± standard deviation.

Effect of combination therapy on arousal threshold, sleep efficiency and architecture

The addition of zolpidem to Ato-Oxy increased sleep efficiency by 9±13% (p=0.037; Figure 9) and total sleep time by roughly 1 hour (Table 6) compared to Ato-Oxy alone. Additionally, wake after sleep onset trended to be reduced on the zolpidem night (Table 6). There was no difference in either sleep architecture or arousal index or intensity between visits (Table 6).

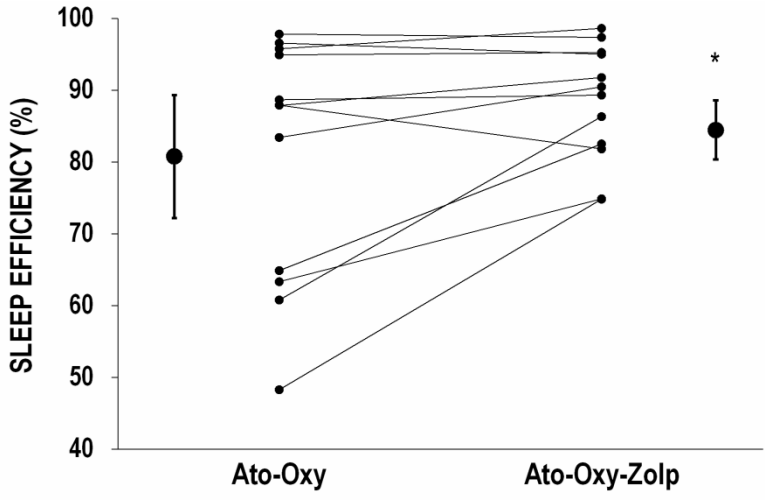

Figure 9. Additional effect of zolpidem on sleep efficiency. Zolpidem increased sleep efficiency compared to Ato-Oxy alone (p=0.037). Note: the greatest improvements in sleep efficiency occurred in those with the lowest sleep efficiency values on Ato-Oxy alone (~10-30% absolute improvements). Bars represent mean ± standard deviation. * indicates a significant difference between conditions (80.9±16.9 vs. 88.2±8.2 %, *p*=0.037).

Characteristic	Ato-Oxy	Ato-Oxy-Zolp	p value
Total sleep time, h	6.0 ± 1.3	6.9 ± 0.5	0.023
Percent supine, % sleep time	100 [28.2]	87.7 [43.1]	0.383
Obstructive apnea index, events/h	2 [25]	10 [26]	0.206
Mixed apnea index, events/h	0 [0]	0 [0]	>0.999
Central apnea index, events/h	0.0 [0.2]	0.0 [0.2]	>0.999
O2 desaturation index, events/h	34 [31]	37 [44]	0.791
Sleep onset latency, min	15 ± 19	8 ± 8	0.149
Wake after sleep onset, min	72 ± 61	48 ± 37	0.052
N1, % total sleep time	33.1 ± 17.9	28.3 ± 13.1	0.187
N2, % total sleep time	48.5 ± 16.8	50.0 ± 14.3	0.720
N3, % total sleep time	8.4 [13.2]	7.4 [11.8]	0.850
REM sleep, % total sleep time	2.6 [10.3]	8.2 [17.1]	0.322
Arousal index, events/h	33 ± 21	37 ± 16	0.461
Respiratory event duration, s	20 ± 4	20 ± 5	0.368
Nadir O2 saturation, %	82.0 ± 7.6	82.8 ± 6.5	0.509
Arousal intensity, 1-10 scale	4 [2.0]	3.5 [1.7]	0.125
Hypoxic burden, (%min)/h	48.4 [101.5]	61.8 [163.1]	0.077
Systolic blood pressure, mmHg	133 ± 22	140 ± 16	0.159
Diastolic blood pressure, mmHg	84 ± 11	87 ± 9	0.144
Heart rate during sleep, beats/min	71 ± 17	76 ± 14	0.256

Table 6. Polysomnography parameters. Data are mean ± standard deviation or median [interquartile range] where appropriate and were compared with a two-tailed paired Student's t-test or a Wilcoxon signed rank test accordingly. Note: rapid-eye movement (REM) sleep was absent in 7 overnight studies (2 participants did not have any REM sleep on either visit and 3 other participants did not have REM sleep on one of the study nights). Blood pressure measurements refer to next-day values.

The non-REM respiratory arousal threshold increased by 17±18% on the zolpidem night % as a proportion of the Ato-Oxy condition on the zolpidem night (Figure 10). Zolpidem also increased the arousal threshold during N1 sleep (−22.3±11.1 vs. −30.7±15.7cmH_2O, p=0.006).

Effect of combination therapy on OSA severity and endotypes

Participants slept predominantly supine on both nights (Table 6). Zolpidem did not alter OSA severity as shown by no systematic changes in AHI between nights (P=0.4; Figure 11). Event duration and markers of oxygenation such as oxygen desaturation index, nadir oxygen saturation during sleep, and hypoxic burden were also not different between nights (Table 6). 42% of the participants had AHI values of ~15 events/h sleep or less on combination therapy (Figure 11). OSA endotypes estimated from the pneumotachograph-derived flow signal did not vary between nights (Table 7).

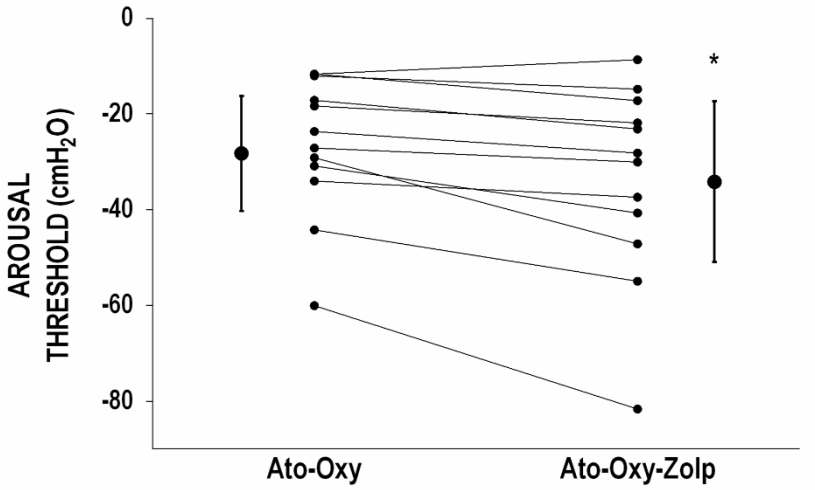

Figure 10. Additional effect of zolpidem on the respiratory arousal threshold. Zolpidem systematically increased the respiratory arousal threshold compared to Ato-Oxy alone (p=0.004). Notably, on the zolpidem night, the arousal threshold increased in 11 of the 12 subjects (92%). Bars represent mean ± standard deviation. * indicates a significant difference between conditions (−26.6±14.5 vs. −33.8±20.3 cmH_2O, *p*=0.004).

Characteristic	Ato-Oxy	Ato-Oxy-Zolp	p value
Loop gain	0.54 [0.11]	0.49 [0.16]	0.233
Vpassive (%Veupnea)	92.4 [16.7]	89.8 [36.1]	0.092
VActive (%Veupnea)	106.5 [40.1]	105.6 [41.1]	0.204
VCompensation (%Veupnea)	11.2 [20.3]	10.5 [10.0]	0.850
Ventilatory response to arousal (L/min)	14.4 [16.9]	19.6 [21.8]	0.266

Table 7. Polysomnography-estimated OSA endotypes. Data are median [interquartile range] and were compared with a Wilcoxon signed rank test. Vpassive represents passive upper airway collapsibility, namely when the upper airway muscles are not activated (ventilation at eupneic ventilatory drive). Vactive indicates active upper airway collapsibility, when the pharyngeal muscles are activated (immediately prior to arousal). Vcompensation is the difference between Vpassive and Vactive and provides an estimate the pharyngeal muscle response to improve airflow during a respiratory event. These three values are expressed as a % of ventilation during stable, unobstructed breathing during sleep (Veupnea).

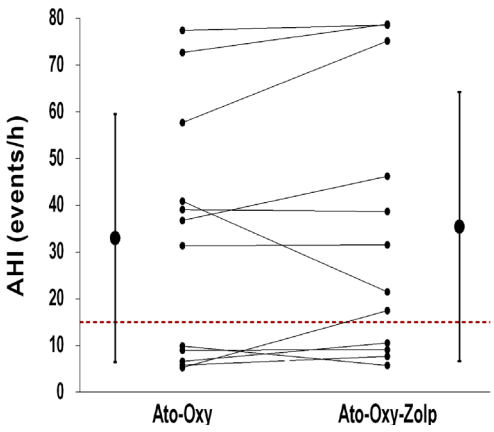

Figure 11. Additional effect of zolpidem on OSA severity. There was no difference in the apnea-hypopnea index (AHI) between the study nights. However, half of the participants were below the threshold of mild-moderate OSA severity (red dotted line) on combination therapy. Bars represent mean ± standard deviation.

Effects of zolpidem on next-day perceived sleepiness, objective alertness and safety

Next-day sleepiness as measured by KSS was not different between the nights (Table 8).

Characteristic	Ato-Oxy	Ato-Oxy-Zolp	p value
Karolinska Sleepiness Scale (1-9 scale)	5 ± 2	5 ± 2	0.693
AusEd driving simulator*			
Deviation from median of lane, cm	36.4 [29.0]	72.7 [71.7]	0.003
Deviation from 60–80 km/h, km/h	1.1 [1.6]	2.2 [3.7]	0.233
Braking reaction time, s	1.0 [0.3]	1.3 [0.8]	0.052
Crashes, n	1 [3]	1 [8]	0.242

Table 8. Next-day perceived sleepiness, objective alertness, and safety. Data are mean ± standard deviation or median [interquartile range]. Conditions were compared with a two-tailed paired Student's t-test or a Wilcoxon signed rank test as appropriate.

Although overall performance in two of the four key measurements during the driving simulation task was comparable between conditions (Table 8), steering deviation from median lane position was significantly higher (p=0.052, Figure 12) and breaking time tended to be delayed following the addition of zolpidem (Table 8). Importantly, however, there was no difference in car crashes between the two groups, also when testing whether the addition of zolpidem increased the number of individuals who experienced at least one car crash.

Combination therapy was generally well-tolerated with no major adverse events.

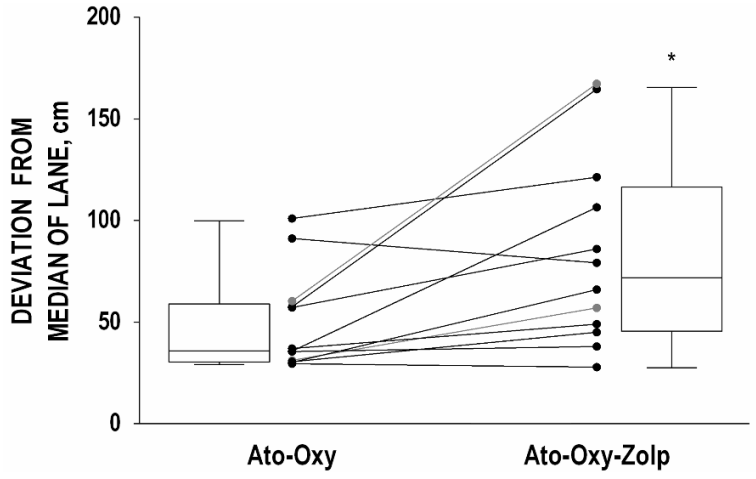

Figure 12. Deviation from median of lane prior to and after zolpidem administration. Black circles and lines indicate participants who were older than 50 years, grey indicate those who were younger than 50 years. Note that one of the largest increases in median steering deviation with the addition of zolpidem occurred in one of the youngest participants (64% increase) in a participant aged 35 years. Overall, there was no relationship between change in steering deviation between conditions and age (linear regression: r=0.201, p=0.501). The age range of the participants was 25-75 years. Boxes represent median [IQR]. *Indicates a significant difference between conditions.

Additional information are provided in the Thesis Supplement.

Discussion

The main finding of this study is that the addition of 10mg of zolpidem to the combination of the noradrenergic agent atomoxetine plus the antimuscarinic oxybutynin improves sleep efficiency by ~9% and the respiratory arousal threshold by ~17%. Thus, zolpidem may be beneficial in OSA patients in reverting the wake promoting properties of Ato-Oxy to potentially improve its tolerability in those with poor sleep quality. Approximately half of the participants with OSA had <15 events/h on both combination therapy study arms. However, the addition of zolpidem did not increase therapeutic efficacy. While combination therapy was well-tolerated and the addition of zolpidem did not change next-day perceived sleepiness, markers of objective alertness and driving simulator performance were worse with zolpidem. These findings are important for ongoing targeted pharmacotherapy development for OSA.

Effect of combination therapy on arousal threshold, sleep efficiency and architecture

The ~17% increase in arousal threshold with the addition of zolpidem in the current study is consistent with our previous single-night zolpidem studies in which zolpidem increased the arousal threshold by ~15-25%[218,265]. The increase in arousal threshold with zolpidem was highly consistent, with 11 of 12 participants (92%) manifesting an increase. In contrast to previous studies, the participants of this research were not selected to have a low-to-moderate arousal threshold. This indicates that increases in arousal threshold and improvements in sleep quality with zolpidem occur in people with OSA regardless of baseline arousal threshold category.

The ~9% increase in sleep efficiency is comparable with recent studies where zolpidem was administered alone in people with OSA[244,265]. This was coupled with an ~1h increase in total sleep time during the 8h sleep opportunity in the current study. Individual increases in sleep efficiency with zolpidem (up to ~30%) were most pronounced in the 30% of participants who had low sleep efficiency (<70%) on Ato-Oxy, whereas those who already had high sleep efficiency had intuitively very little change. This effect was independent of the baseline levels of self-perceived sleep quality assessed by the Insomnia Severity Index. Thus, the addition of zolpidem may be a therapeutic option for those who have poor sleep quality with Ato-Oxy to treat OSA. Improvements in sleep efficiency with zolpidem may also promote favorable cardiovascular effects[269]. However, larger, longer-term studies are required and consideration of potentially deleterious effects on next-day alertness with zolpidem as outlined below are required before this can be recommended clinically.

Effect of combination therapy on OSA severity and endotypes

Despite substantial increases in sleep duration and sleep efficiency, zolpidem did not yield additional beneficial effects on OSA severity compared with Ato-Oxy alone. This is consistent with previous acute zolpidem studies in people with OSA in which there were no systematic changes in AHI[214,219,244,265]. Absence of a reduction in OSA severity with zolpidem is also consistent with recent studies with another z-drug, zopiclone, on the AHI, either after acute (with standard or high doses) or prolonged (1-month) nightly administration[97,222,270]. One possible explanation for a lack of an additional benefit is that OSA was already effectively "treated" (AHI below 15 events/h) in almost half (42%) of the study participants in the Ato-Oxy alone night (i.e. floor effect).

However, as highlighted in Figure 11, there was no consistent change in AHI in the remaining participants, all of whom had AHI values >15 events/h on the Ato-Oxy alone night. Alternatively, it is possible that the magnitude of the increase in arousal threshold was not large enough to produce major stabilization of breathing as measured by the AHI. Although eszopiclone had a larger increase (~30%) in arousal threshold with a concurrent decrease in OSA severity[92], most hypnotics with similar (~30%) increases in arousal threshold[97,218,222,227,270] did not systematically alter the AHI. Thus, either the arousal threshold may be a relatively less important contributor to OSA pathogenesis versus other traits and/or the magnitude of increase in arousal threshold with existing hypnotics is insufficient to yield therapeutic benefit as measured via the AHI. A third possibility is that zolpidem worsens other OSA endotypes (e.g. muscle responsiveness, loop gain or airway collapsibility), such that any therapeutic improvement in AHI driven by the arousal threshold was counterbalanced. However, this is not supported by recent physiology studies[218,265] and the current data that show either no change or improvement in other OSA endotypes with zolpidem. At an individual level, 4 participants had a >5 event/h increase while one had an ~20 event/h decrease in AHI during the zolpidem night. Consistent with OSA phenotyping concepts, the 4 participants who had an increase in AHI had the highest arousal threshold values on the Ato-Oxy alone night (all < $-30cmH_20$), whereas the participant who improved with zolpidem had the lowest arousal threshold ($-11.7cmH_2O$).

Effects of zolpidem on next-day perceived sleepiness, objective alertness and safety

Previous studies have highlighted potential deleterious effects of zolpidem on oxygen saturation during sleep at higher doses[214] or in patients with heart failure[260]. In contrast, similar to a recent report [265], zolpidem was well-tolerated in the current study and the majority of the participants had self-reported improvements in sleep quality. Indeed, consistent with findings with zopiclone at standard[97] and high doses[270], zolpidem also did not have any deleterious effects on polysomnographic parameters including respiratory event duration or overnight hypoxemia.

Daytime sleepiness and impaired alertness in people with OSA increases the risk of traffic accidents in the absence of an efficacious treatment[8]. Thus, anything that may further worsen daytime sleepiness or alertness in people with OSA is typically contraindicated. Accordingly, the main concern of zolpidem administration in OSA is its potential "hangover" effects[271,272]. However, consistent with previous findings on zolpidem[265] and zopiclone alone[97,270], zolpidem in combination with Ato-Oxy does not have any effect on subjective sleepiness. However, unlike an earlier report in people without OSA[259] and other recent studies with zolpidem and zopiclone in people with OSA[265,270], objective next-day alertness as measured via driving simulation task showed worse steering deviation after the zolpidem night. Indeed, the magnitude of the increase in steering deviation after zolpidem was comparable to the effect of moderate alcohol intake in healthy people and those with untreated OSA[266,273]. Although braking reaction time also tended to be slower following zolpidem addition, the number of crash events on the driving simulator task did not change significantly. Conversely, while the majority of driving simulator parameters did not change in a recent high dose zopiclone study in people with OSA versus placebo, there was a tendency for increased crash risk[270]. The reasons for the apparent conflicting findings are unclear. Potential explanations include a zolpidem dose-dependent effect, with

possible alterations of daytime alertness in some participants and not others[274]. In people over 50 years, reduced metabolism and sleepiness side effects with zolpidem may be more pronounced. However, while most participants were over 50 years old (10/12), there was no relationship between change in median steering deviation and age in current study (see online supplement). The addition of an objective sleepiness test (e.g. MSLT) could have provided additional insight. However, this was beyond the scope of the current investigation. While caution is warranted, the potential for an adverse interaction effect between zolpidem and Ato-Oxy would appear unlikely[275].

Methodological considerations

This study was designed to investigate the effects of the addition of zolpidem to Ato-Oxy combination therapy in participants with OSA. The rigorous clinical trial design allowed us to definitively address the key study questions. However, as the study enrolment criteria required a prior diagnosis of OSA rather than a formal diagnostic study using the same equipment in conjunction with the current study, we are unable to make valid comparisons between the two study arms and baseline polysomnography parameters (see online supplement for further detail). In addition, while an *a priori* sample size calculation was performed and allowed us to detect key differences in our primary outcomes, the relatively small number of participants (that included only 2 female volunteers) and the short-term, single-night design warrant future investigation in a larger, longer-term trial to further evaluate the safety and efficacy of the triple combination therapy during chronic administration in men and women with OSA.

Additional information are provided in the Thesis Supplement.

Conclusions

Compared to the combination of atomoxetine and oxybutynin alone, the addition of zolpidem improves sleep efficiency and increases the respiratory arousal threshold in people with OSA. These effects may be beneficial to counteract the excitatory properties of atomoxetine in patients who experience reduced sleep efficiency with atomoxetine and oxybutynin. However, while the addition of zolpidem was well-tolerated and perceived sleepiness and other polysomnographic parameters were unchanged, next-day driving simulator performance was impaired with zolpidem. Thus, further studies in larger populations including more women are required to determine the wider generalizability, long-term safety and efficacy profile of this triple combination before considering prescribing zolpidem in combination with Ato-Oxy clinically, especially in high-risk populations (i.e. commercial drivers and those who are already sleepy).

CHAPTER FOUR:
THE COMBINATION OF BETAHISTINE AND OXYBUTYNIN INCREASES RESPIRATORY CONTROL SENSITIVITY (LOOP GAIN) IN PEOPLE WITH OBSTRUCTIVE SLEEP APNEA: A RANDOMIZED, PLACEBO-CONTROLLED TRIAL

Abstract

Rationale

There are widespread histaminergic projections throughout the brain, including hypoglossal nuclei, that control pharyngeal muscle tone and respiratory control centers. However, the effects of histaminergic agents on upper airway stability and respiratory control in people with obstructive sleep apnea (OSA) have been minimally investigated. Antimuscarinics increase pharyngeal tone during REM sleep in rats and when combined with noradrenergic agents have recently been shown to reduce OSA severity. Here we aim to test the effects of betahistine, an H3-autoreceptor antagonist, in combination with the antimuscarinic oxybutynin (Beta-Oxy), on OSA severity, OSA endotypes, polysomnography parameters and next-day sleepiness and alertness.

Methods

Thirteen otherwise healthy OSA people received either Beta-Oxy (96-5 mg) or placebo according to a randomized, crossover, double-blind design, prior to an overnight, in-laboratory, clinical PSG. Participants completed the Karolinska Sleep Scale and Leeds Sleep Evaluation Questionnaire and performed a driving simulation task (AusEd) to record next-day sleepiness and alertness, respectively. OSA endotypes were estimated through validated algorithms using the PSG records.

Results

Compared to placebo, Beta-Oxy increased respiratory control sensitivity (loop gain) (0.52[0.24] *vs*. 0.60[0.34], median[IQR], P=0.021) without systematically changing OSA severity (34.4±17.2 *vs*. 40.3±27.3 events/h, mean±SD, P=0.124), sleep efficiency, arousal index or markers of hypoxemia. Beta-Oxy was well tolerated and did not worsen next-day sleepiness or alertness.

Conclusions

Rather than stabilize breathing during sleep, Beta-Oxy increases respiratory control sensitivity, which is likely to be deleterious for most people with OSA. However, in certain sleep disordered breathing conditions characterized by blunted respiratory control (e.g., obesity hypoventilation syndrome) interventions to increase loop gain may be beneficial.

Introduction

Obstructive sleep apnea (OSA) is a common breathing disorder characterized by repetitive narrowing or occlusion of the upper airway during sleep[276]. Impairment in the anatomical components of the upper airway (e.g. a narrow/collapsible pharynx) and other physiological traits/endotypes, such as respiratory control stability/loop gain, the respiratory arousal threshold, and upper airway muscle function, play a major role in mediating the propensity for OSA[277]. However, current treatments, such as continuous positive airway pressure (CPAP), mandibular advancement devices and surgery, primarily target the anatomical endotype, with variable efficacy, compliance and patient outcomes[278].

Recent research has shown that pharmacotherapy that targets one or more of the non-anatomical OSA endotypes can reduce OSA severity and thus may have a potential future role in personalized treatment for OSA[174,263,278-280]. Key background findings stemmed from animal studies that demonstrated several neuro transmitters play a major role in upper airway stabilization during sleep[198,281]. Hypoglossal nuclei express abundant concentrations of H1-receptors in rats[201] and guinea pigs[202,203]. Histamine administration at the hypoglossal motor nucleus significantly increased tonic (i.e. expiratory) activity of the largest upper airway dilator muscle, genioglossus, in both non-REM and REM sleep via activating H1-receptors in rats[204,205] and cats [282]. However, histamine neurons become largely silent at sleep onset during natural sleep[283,284]. In humans, knowledge on the role of histaminergic stimulation on upper airway stability is limited. Desipramine, which reduces upper airway collapsibility in healthy controls and people with OSA, has a wide, non-specific spectrum of target activity, including antagonism of histaminergic receptors[193,194]. Pitolisant, an H3-autoreceptor inverse agonist with wake promoting properties, increases daytime alertness in people with OSA[285,286]. However, the effects of histaminergic mechanisms on upper airway stability and respiratory control in people with OSA has not been investigated.

Betahistine, a drug commonly used in clinical practice for Ménière syndrome, is a mild H1-agonist and a potent H3-autoreceptor antagonist/inverse agonist[287]. H3-autoreceptor blockage increases brain levels of histamine[288]. Increased histaminergic tone facilitates G-protein-coupled inward rectifier potassium channels blocking[289], a path involved in enhanced genioglossus muscle responsiveness in animals[187]. Additionally, H3 antagonism can potentiate the activity of other neurotransmitters in the central nervous system, including norepinephrine[290], highly expressed at the hypoglossal motor nuclei, and acetetylcholine[291].

Recent studies indicate that noradrenergic agents, which like histamine agonists also have wake promoting properties, when combined with an antimuscarinic can reduce OSA severity[174,280,292]. Animal data suggest that antimuscarinics directly increase pharyngeal muscle tone during REM sleep[293]. In addition, antimuscarinics also have mild sleep promotion effects[174,280,292,294]. This property may be beneficial to counteract, at least in part, the wake promoting properties of other agents when used in combination therapy for OSA.

In this randomized, double-blinded, placebo controlled, crossover study we aimed to test the effects of betahistine combined with the antimuscarinic oxybutynin (Beta-Oxy) on OSA severity (primary outcome).

Secondary outcomes were to investigate the effects of the combination on OSA endotypes, other standard polysomnography parameters and next-day sleepiness and alertness.

Methods

Participants

Thirteen people with a diagnosis of OSA within the past year (apnea/hypopnea index [AHI] >15 events/hr) aged 18 to 75 years were recruited. Participants on CPAP therapy were asked to suspend treatment during the trial and for one week prior to the first study visit. Exclusion criteria included any acute or chronic condition other than controlled hypertension and hypercholesterolemia, hypersensitivity to the study drugs, class 3 obesity, any medication known to influence breathing, sleep/arousal, muscle physiology, or to interact with mono amino oxidases, current treatment with tricyclic antidepressants.

The study was approved by the Southern Adelaide Clinical Research Ethics Committee (248.20), and prospectively registered (ACTRN12621000158864). All participants provided written informed consent prior to enrolment. Participants were studied at Adelaide Institute for Sleep Health, Flinders University.

Protocol

Participants were asked to come to the sleep laboratory twice, one week apart, to undertake two overnight sleep studies. Prior to lights-out (based on the participant's usual bedtime and kept constant between study nights), participants received 96mg betahistine plus 5mg oxybutynin or placebo according to a double-blind, randomized, crossover design (Figure 13).

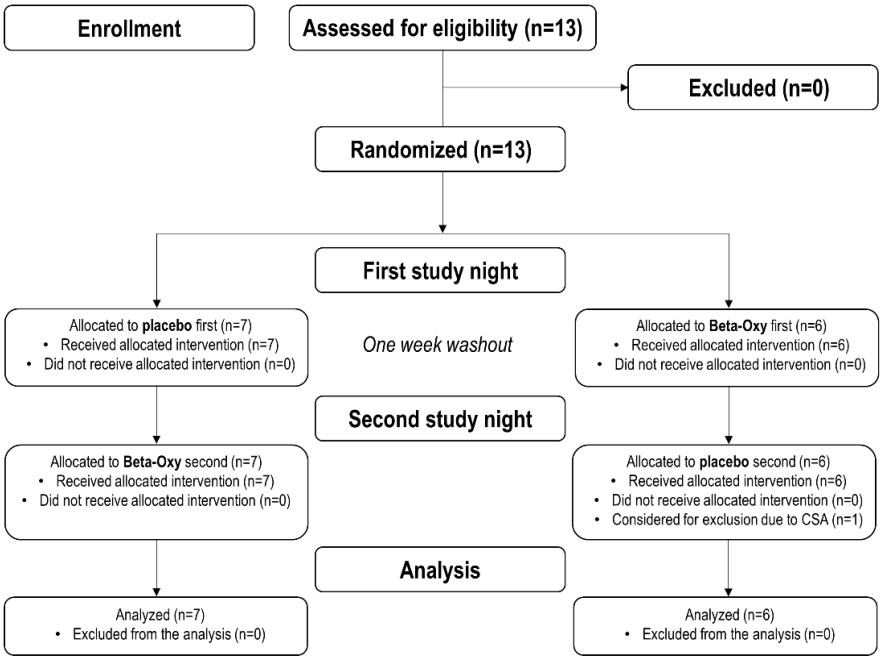

Figure 13. CONSORT diagram. Diagram shows recruitment, randomization, and analysis procedures for the trial.

The study pharmacist, separate to the study site, provided the randomization code and maintained allocation concealment throughout the study. Prior to sleep, participants were instructed to sleep on their back for as much as possible and were given a standardized 8-hour sleep opportunity during each study visit.

Participants slept with standard clinical PSG equipment including a nasal cannula attached to a pressure transducer to estimate airflow[234].

On the first night, ~30 mins after arrival, participants completed the Insomnia Severity Index and the Epworth Sleepiness Scale questionnaires. Systemic blood pressure and Karolinska Sleepiness Scale (KSS) were recorded in both treatment arms, ~30 min before bed and ~30 min after wake time. In addition, participants completed the AusEd driving simulator for alertness[266] and the Leeds Sleep Evaluation Questionnaire (LSEQ) on the next morning following each study night. Potential side effects (e.g. dry mouth, dysuria etc.) were also investigated in the morning after each visit.

Data analysis

All analyses were conducted blinded to the study interventions. Respiratory events and arousals were scored using standard American Academy of Sleep Medicine 2020 criteria[267]. OSA endotypes (i.e. loop gain, arousal threshold, upper airway collapsibility ($V_{passive}$), markers of pharyngeal muscle compensation (V_{active} and $V_{compensation}$) and the ventilatory response to arousal) were estimated from the PSG-derived flow signal using previously validated algorithms[2,268,295]. In brief, loop gain was calculated as the response to disturbance of different frequencies: one cycle/minute (i.e. loop $gain_1$) and the frequency that would lead to periodic breathing onset (i.e. loop gain at natural frequency [loop $gain_n$]). Arousal threshold was calculated as the average estimated ventilation during sleep prior to arousals (i.e. maximum ventilatory drive). $V_{passive}$ was defined as the ventilation during sleep at eupneic ventilatory drive when the pharyngeal muscles are relatively passive. V_{active} was defined as the level of ventilation at maximum drive (i.e. arousal threshold). $V_{compensation}$ was taken as the difference between ventilation at maximal drive and as an estimate of pharyngeal muscle compensation. All traits, expect for loop gain, which is dimensionless, were expressed as percent of the estimated eupneic ventilation (V_{eupnea}).

Statistical analysis

An a priori power calculation indicated that 12 participants were required to detect a minimally important change in AHI of 10 events/hour (SD=10) with >80% power at an alpha level = 0.05 (two-tailed paired t-test), including a 20% drop-out rate.

Continuous data were expressed as mean±SD, or median [interquartile range] for non-normaly distributed data. Statistical significance was inferred if $p<0.05$. According to our statistical analysis plan, data were analyzed using two-tailed paired Student's *t*-tests or a Wilcoxon signed-rank test as appropriate. A mixed model analysis was also carried out (random effect: participants; fixed effects: treatment and percent supine sleep) to explore potential effects of sleep positions on the AHI (effect size [confidence interval]). Exploratory linear regression assessed the association between baseline loop gain and change in AHI between the nights. Analyses were performed using Graph Pad Prism 6.0 (Graph Pad Software, La Jolla, CA) and SPSS 23.00 (IBM, Armonk, NY).

Results

Thirteen participants were recruited to allow for a potential drop out to reach our recruitment target of n=12. However, all 13 participants successfully completed both nights and were included in the analyses (Figure 13). Baseline participant characteristics are shown in Table 9. Participants were recruited from October 2020 to May 2021.

Characteristic	
Age, years	61±6
Sex, M:F	6:7
Neck circumference, cm	39.1±3.9
Waist circumference, cm	106.9±17.3
Body mass index, kg/m^2	31.1±5.2
Mallampati index	3±1
Insomnia severity index	11±5
Epworth sleepiness scale	7±4

Table 9. Baseline characteristics. Data are mean ± SD

Effect of Beta-Oxy on OSA severity

Beta-Oxy did not systematically alter OSA severity versus placebo (Figure 15), including when separated according to sleep stage (Table 10).

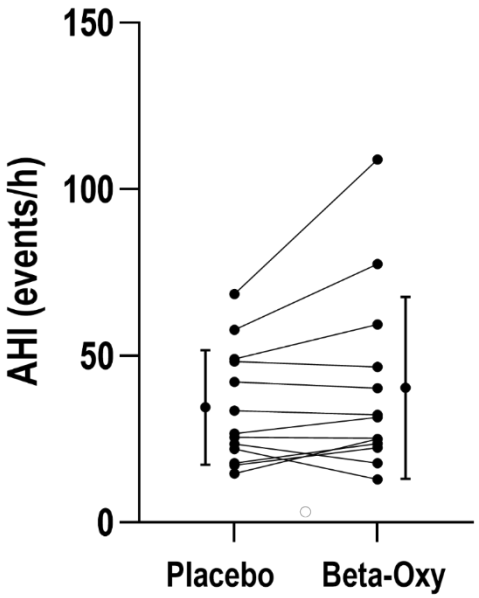

Figure 14. Effect of Beta-Oxy on OSA severity. There was no systematic difference in apnea-hypopnea index (AHI) between study nights (34.4±17.2 *vs*. 40.3±27.3 events/h sleep, P=0.124). Bars represent mean ± standard deviation.

Characteristic	Placebo	Beta-Oxy	p value
NON-REM AHI, events/h	41.7±20.0	46.6±24.7	0.096
REM AHI, events/h	32.0±20.3	38.4±29.9	0.268
Total sleep time, h	7.1±0.9	7.0±0.8	0.641
Sleep efficiency	84.4±8.7	83.4±9.0	0.608
Sleep onset latency, min	6.5 [6.0]	10.0 [7.7]	0.719
Wake after sleep onset, min	59.5 [36.2]	60.5 [41.2]	0.893
Percent supine, % total sleep time	49.5±38.6	52.4±31.6	0.707
Obstructive apnea index, events/h	1.8 [3.6]	0.9 [2.5]	0.825
Mixed apnea index, events/h	0 [0]	0 [0.0]	0.500
Central apnea index, events/h	0 [0]	0 [0.0]	0.500
Hypopnea index, events/h	24.7 [23.0]	24.7 [22.4]	0.366
N1, % total sleep time	9.4 [18.9]	15.5 [17.7]	**0.049**
N2, % total sleep time	42.6±8.6	41.1±8.2	0.537
N3, % total sleep time	22.1±9.5	22.1±10.7	0.988
REM sleep, % total sleep time	21.3±5.6	19.3±7.2	0.252
Arousal index, events/h	23.3 [9.6]	23.5 [9.9]	0.735
Respiratory event duration, s	24.4±6.7	23.6±4.9	0.515
O_2 desaturation index, events/h	18.7±14.0	27.6±26.0	0.093
Mean O_2 saturation during sleep, %	94.0±1.0	93.9±1.7	0.721
Nadir O_2 saturation, %	87.0 [7.0]	83 [8.0]	0.064
Sleep time spent <90%SpO_2, %total	0.9 [1.9]	1.0 [1.5]	0.464
Systolic blood pressure, mmHg	133.9±15.2	133.2±12.9	0.807
Diastolic blood pressure, mmHg	88.8±8.7	87.7±8.3	0.988
Heart rate during sleep, beats/min	71.5±12.1	70.1±10.8	0.704

Table 10. Polysomnography parameters. Data are mean ± standard deviation or median [interquartile range] where appropriate and were compared with a two-tailed paired Student's t-test or a Wilcoxon signed rank test accordingly. Blood pressure measurements refer to next-day values. REM, rapid eye movements; NON-REM, non REM

The supine AHI also did not change between study nights (51.0±22.8 on placebo versus 51.0±31.1 events/h on Beta-Oxy, P=0.37). When adjusting the AHI for sleeping body position (Table 10) and missing values in the supine position (in N=2 nights there was no recorded supine sleep data), there was no mixed model effect of the combined drugs on OSA severity (+6.33 [–0.93, 13.60] events/h, mean [CI], P=0.08). Overnight desaturation profiles were also not significantly different between the two treatment arms (Table 10).

Effect of Beta-Oxy on OSA endotypes

Beta-Oxy significantly increased loop $gain_1$ compared to placebo (Table 11). Loop $gain_n$ was also greater on Beta-Oxy versus placebo in all but one of the study participants (Figure 15). The other OSA endotypes did not change between nights (Table 11).

Characteristic	Placebo	Beta-Oxy	p value
Loop gain$_1$ (at 1 cycle/min frequency)	0.52 [0.24]	0.60 [0.34]	**0.021**
Arousal threshold (%V_{eupnea})	116.4 [18.2]	120.8 [11.4]	0.057
$V_{passive}$ (%V_{eupnea})	93.9 [2.7]	93.6 [5.9]	0.216
V_{Active} (%V_{eupnea})	102.2 [10.8]	105.3 [13.2]	0.414
$V_{Compensation}$ (%V_{eupnea})	8.0 [9.6]	11.5 [12.2]	0.216
Ventilatory response to arousal (%V_{eupnea})	36.4±23.7	36.0±13.1	0.938

Table 11. Polysomnography-estimated OSA endotypes. Data are mean ± standard deviation or median [interquartile range] where appropriate and were compared with a two-tailed paired Student's t-test or a Wilcoxon signed rank test accordingly. $V_{passive}$ represents passive upper airway collapsibility, namely when the upper airway muscles are not activated (ventilation at eupneic ventilatory drive). V_{active} indicates active upper airway collapsibility, when the pharyngeal muscles are activated (immediately prior to arousal). $V_{compensation}$ is the difference between $V_{passive}$ and V_{active} and provides an estimate the pharyngeal muscle responsiveness. Except for loop gain (dimensionless), values are expressed as a % of ventilation during stable, unobstructed breathing during sleep (V_{eupnea}).

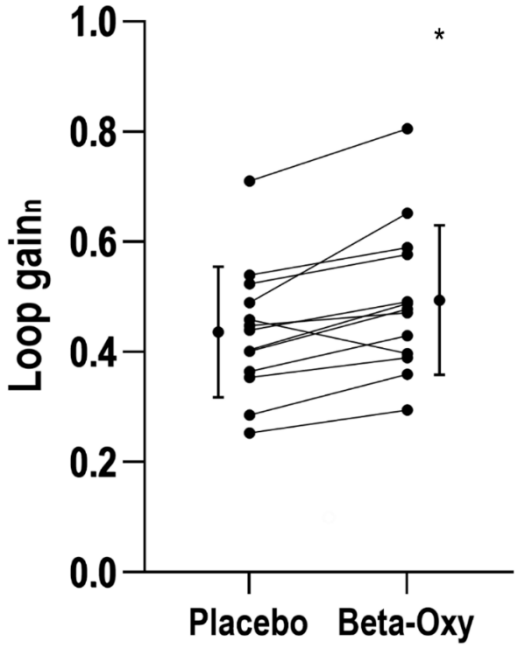

Figure 15. Effect of Beta-Oxy on loop gain. Beta-oxy significantly increased loop gain at the natural frequency of resonance (loop gain$_n$) by a physiologically meaningful margin (>10%; Δchange=0.06±0.05 [mean±SD], P=0.001, asterisk).

Notably, the change in AHI between the nights was directly associated with loop gain on the placebo night such that OSA severity increased in those with higher loop gain values (Figure 16).

Effect of Beta-Oxy on OSA endotypes

Sleep architecture was slightly worse on Beta-Oxy, with a modest increase in N1 sleep versus placebo (Table 13). No other significant differences were detected between nights for overall sleep efficiency, total sleep time, wake after sleep onset or arousal index (Table 12).

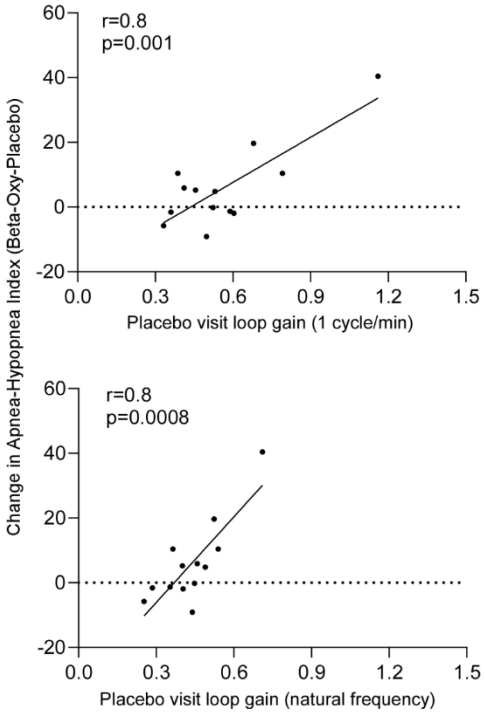

Figure 16. Relationship between change in apnea-hypopnea index (AHI) and loop gain (placebo visit). Change in AHI and loop gain$_1$ (upper panel) and loop gain$_n$ (lower panel) is illustrated. Individual participants are indicated by dots and solid line indicates the calculated relationship from linear regression

Characteristic	Placebo	Beta-Oxy	p value
Karolinska sleepiness scale	6±1	5±2	0.180
Leeds Sleep Evaluation Questionnaire			
Getting to sleep	5.0±0.7	5.0±1.18	0.984
Quality of sleep	4.5±1.8	4.5±1.4	0.889
Awake following sleep	4.3±1.3	5.2±1.2	0.079
Behavior following sleep	4.2±1.2	4.4±1.1	0.647
AusEd driving simulator			
Deviation from median of lane, cm	39.5±27.6	38.7±19.9	0.932
Deviation from 60–80 km/h, km/h	0.5±2.0	0.7±1.6	0.952
Breaking time, s	1.0±0.3	1.1±0.3	0.503
Crashes, n	0±1.0	0±0.5	0.937

Table 12. Next-day perceived sleepiness and objective alertness. Data are mean ± standard deviation or median [interquartile range]. Conditions were compared with a two-tailed paired Student's t-test or a Wilcoxon signed rank test as appropriate.

Three participants reported feeling rested/wide awake after Beta-Oxy single-night treatment versus one after placebo (P=0.194). Similarly, only one participant reported feeling very tired after Beta-Oxy versus three participants after the placebo night (P=0.139). Four participants on Beta-Oxy versus six on placebo felt that the corresponding laboratory night was worse/much worse than sleeping at home (P=0.619). Perceived sleep latency was comparable between placebo and Beta-Oxy (29±18 min *v*. 44±60 respectively, P=0.397).

Discussion

This is the first proof-of-concept, mechanistic study to investigate the effects of a histaminergic agent, together with an antimuscarinic, on OSA severity in humans. Beta-Oxy led to a physiologically important increase in loop gain, without changing the AHI, other sleep parameters or next-day sleepiness/alertness. These findings provide novel physiological insight and, if confirmed in larger follow-up studies, may have implications for certain respiratory diseases where blunted respiratory control is a feature.

Novel physiological insights

The response to a respiratory disturbance during sleep (reduced ventilation) consists of an accumulation of ventilatory drive that generally matches increased hypoxic—and hypercapnic—demand to generate a subsequent ventilatory compensation response[296]. The main effectors of this process, a constituent component of loop gain[43], are the chemoreceptors, that project to the nucleus tractus solitarii in the brainstem and are influenced via a wide supply of neurotransmitters, including histamine[297]. In rats, histaminergic modulation through H1-receptor stimulation[297] or H3-receptor blockade in the brain[298], augments chemoreflex control. A similar effect was observed in goats[299] and cats[300]. However, its human translatability was only putative. Thus, the findings of this study that show that loop gain is almost invariably increased with Beta-Oxy in people with OSA, indicate that this potent chemosensitivity excitatory modulation is also present in humans during sleep.

Conversely, it is not clear as to why Beta-Oxy did not have an effect on estimated pharyngeal muscle compensation despite the strong neurobiological rational behind our study hypothesis. One explanation could be that the dose of betahistine was not high enough to produce a detectable effect on this endotype. 96 mg was selected in this study as it is twice as high as a typical dose administered clinically, although up to 200 mg was well tolerated in other studies with no significant complications[301]. A second explanation is that betahistine *did lead* to an increase in genioglossus muscle activity (not directly measured in this study) but its potential beneficial effect on OSA was offset by the more pronounced increase in loop gain and thus was not detectable via our indirect measurement technique. Indeed, $V_{compensation}$ is the most challenging PSG-estimated endotype to accurately quantify[2]. Accordingly, it will be important in future studies to directly measure pharyngeal muscle activity, including different doses, to investigate the effects of betahistine definitively

Clinical and physiological implications

A drug that increases loop gain without altering pharyngeal pathophysiology could have disparate effects in clinical practice. The prokinetic domperidone has been shown to increase chemosensitivity and loop gain in animal models[302,303] and early reports in healthy humans[304]. However, this effect is presumably only mediated at the peripheral chemoreceptors as domperidone poorly penetrates the blood brain barrier[305]. Betahistine could exert its effects on either central or peripheral chemoreceptors, and this may unveil therapeutic implications for conditions in which central chemosensitivity is impaired or depressed, such as obesity hypoventilation syndrome[306], congenital central hypoventilation[307] and opioid-induced respiratory depression[308-311]. Thus, the use of betahistine in these conditions is worthy of further investigation in light of the current novel findings on respiratory control.

Conversely, although there was no overall increase in AHI in the current study, any agent that increases loop gain in people with OSA where blunted respiratory control is not a feature, especially in the more than one third of patients who already have high loop gain[277], is likely to be deleterious. Our finding that Beta-Oxy was associated with increased OSA severity in participants with high loop gain on placebo and vice versa provides initial support for this concept. Thus, the use of betahistine in most people with OSA should be cautioned until further endotype specific studies are performed to separate out the characteristics of those who may experience beneficial versus deleterious effects.

Despite well-known histamine-related arousal facilitation[312], Beta-Oxy did not worsen sleep efficiency or increase the arousal index or the arousal threshold. However, there was a small increase in lighter N1 stage sleep. While these findings may provide support that betahistine (widely used worldwide at any time of the day) is unlikely to disrupt sleep, it may also be that potential sleep disruption effects were alleviated by oxybutynin which can serve as a mild sleep promotion aid[174,280,292,294,313]. This will require further investigation with betahistine studied in isolation rather than in combination with oxybutynin

Methodological considerations

This study has several limitations. 1) As highlighted, betahistine and oxybutynin were not tested separately, and we cannot, therefore, confidently discriminate the effects of the single drugs. However, when studied in a single drug trial, oxybutynin alone did not increase loop gain[174]. Thus, the effect on loop gain observed in this study is likely to be solely attributable to the increase in histaminergic tone. 2) Due to the clinical setting of the PSG, we did not record end-tidal CO_2 and the different components of loop gain (e.g. plant gain, controller gain) were not calculated. Yet, based on the neurobiological signaling associated to H3-receptor blockade from animal data, betahistine effects on loop gain are likely to be predominantly influenced by increased chemosensitivity (controller gain). 3) We also did not assess whether the histaminergic mediated changes took place in the central, peripheral chemoreceptors or both. 4) All endotypes were collected using PSG-derived signals that provide estimates of gold-standard measurements. As highlighted, the current findings provide initial physiological insight to guide more detailed physiological investigations in future research.

Conclusions

The combination of betahistine, a histaminergic drug, and the anticholinergic oxybutynin increases loop gain in people with OSA, without major accompanying systematic effects on OSA severity, sleep architecture or next-day alertness and sleepiness.

CHAPTER FIVE:
VENTILATORY DRIVE WITHDRAWAL RATHER THAN REDUCED GENIOGLOSSUS COMPENSATION AS A MECHANISM OF OBSTRUCTIVE SLEEP APNEA IN REM

Abstract

Rationale

Rapid eye movement sleep (REM) is associated with reduced airflow and greater obstructive sleep apnea (OSA) severity versus non-REM (non-REM) for reasons not fully elucidated. Here we use direct physiological measurements to determine whether the pharyngeal compromise in REM OSA is most consistent with 1) *withdrawal* of neural ventilatory drive or 2) drive-independent deficits in pharyngeal pathophysiology (i.e. increased *collapsibility*, decreased muscle *responsiveness*).

Methods

63 OSA participants completed sleep studies with gold-standard measurements of ventilatory "drive" (calibrated intra-esophageal diaphragm EMG), ventilation (oronasal "ventilation"), and genioglossus EMG (EMGgg). Drive *withdrawal* was assessed by examining these measurements at nadir drive (1st decile drive within stage). Pharyngeal physiology was assessed by examining *collapsibility* (lowered ventilation at eupneic drive) and *responsiveness* (ventilation-drive slope). Mixed model analysis compared REM *vs*. non-REM; sensitivity analysis examined phasic REM.

Results

REM (≥10 min) was obtained in 25 patients. Compared with non-REM, drive in REM dipped to markedly-lower nadir values (1st decile: −21.8[−31.2,−12.4]%$_{eupnea}$; P<0.0001, estimate[95%CI]), with an accompanying reduction in ventilation (−25.8[−31.8,−19.8]%$_{eupnea}$; P<0.0001). However, there was no effect of REM on collapsibility (ventilation at eupneic drive), baseline EMGgg activity, or *responsiveness*. REM was associated with increased OSA severity (+10.1[1.8,19.8] events/hr), but not after adjusting for nadir drive (+4.3[−4.2,14.6]). Drive withdrawal was exacerbated in phasic REM.

Conclusions

In OSA patients, the pharyngeal compromise characteristic of REM is explained by a ventilatory drive withdrawal rather than a preferential decrement in muscle activity or responsiveness. Preventing drive withdrawal may be the leading target for REM OSA.

Introduction

Obstructive sleep apnea (OSA) is a highly prevalent disorder with major consequences for neurocognitive and cardiovascular health[8,238,314,315] and is most severe in rapid-eye-movement sleep (REM)[316]. In particular, REM sleep is accompanied by more frequent and longer obstructive events, that yield more profound hypoxemia than during non-REM (non-REM). Exaggerated OSA severity in REM is the consequence of a REM-related reduction in pharyngeal dilator muscle activity[161,281,317,318], particularly during phasic eye movements[161,319]. However, the pathophysiological mechanism underlying the REM-related loss of pharyngeal muscle activity (hypotonia)—and accompanying increase in OSA severity—remains unknown. Specifically, it is unclear whether the dominant mechanism of pharyngeal compromise involves 1) *withdrawal* of neural ventilatory drive, providing common inhibition of both pump and pharyngeal muscles, or 2) preferential loss of neural drive to pharyngeal dilator muscles, manifest as reduced *collapsibility* (per decreased baseline activity) and dilator muscle *responsiveness.* Understanding the primary mechanisms responsible is needed for focused investigation into promising avenues for therapeutic interventions.

In REM, the decrement in pharyngeal dilator muscle activity occurs in conjunction with a generalized REM-related hypotonia[320] characterized by inhibition of postural[321] and accessory inspiratory muscle activity[170,171]. Available evidence indicates a reduction in genioglossus *responsiveness* to neuromechanical stimuli, as seen in animals (in response to CO_2[172]) and in humans (in response to pharyngeal negative pressure pulses[77,165] and increasing negative epiglottic pressure swings following CPAP manipulation[317]); however, a REM-related decline in pharyngeal responsiveness to ventilatory drive stimuli during spontaneously breathing human patients has not been demonstrated. Alternatively, a major transient *withdrawal* of common ventilatory drive (quantified by diaphragm EMG, referred to as "drive" herein) is established in REM[166-168], particularly during phasic REM (active eye movements)[322], and may explain diminished dilator muscle activity and pharyngeal compromise in REM. At present, however, the prevailing view is that REM produces a disproportionate loss of pharyngeal dilator muscle activity compared with drive—which is thought to be spared from generalized REM inhibition[170-172]—leading investigators to focus on discovery of the mechanisms of hypotonia originating at the hypoglossal motor neuron pool[169]. However, this view has not been directly tested in humans.

We contend that the pharyngeal neuromechanical compromise observed in REM (loss of ventilation—referred to as "flow" herein—and genioglossus activity) in patients with OSA[317-319], could be predominantly accounted for by the common withdrawal of drive (Figure 17) rather than drive-independent deficits in pharyngeal pathophysiology. Accordingly, we studied patients with OSA during natural sleep using direct physiological measurements to assess whether reduced flow and genioglossus muscle activity (EMGgg) in REM *vs.* non-REM is accounted for by accompanying effects of REM on drive. Primary analysis compared pathophysiological variables in REM *vs.* non-REM; additional analysis examined phasic REM specifically. Further analysis examined whether drive withdrawal explains the exacerbation of OSA in REM *vs.* non-REM.

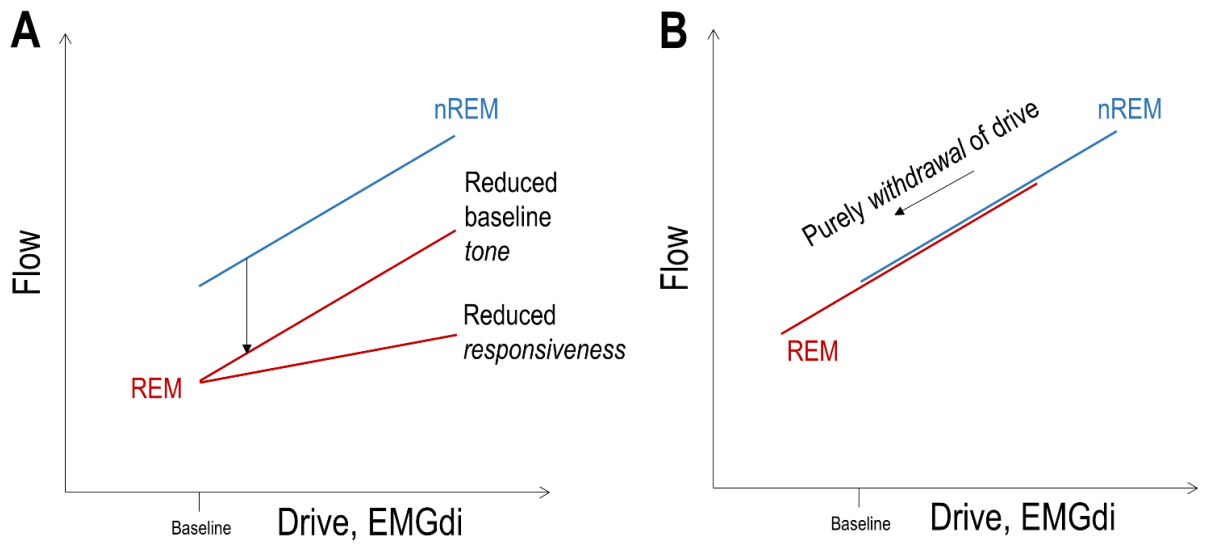

Figure 17. Initial hypothesis. Conceptual diagram illustrating the candidate mechanisms of REM-related pharyngeal compromise, manifest as reduced ventilation through a flow-limited pharyngeal airway *vs*. non-REM (non-REM). A) The prevailing view is that there is a preferential loss of output to the pharyngeal dilator muscles, which would manifest as increased pharyngeal collapsibility in REM (lower ventilation at eupneic drive, i.e. diaphragm activity). Such an effect would be seen as a downward parallel shift in the ventilation-drive relationship characterizing sleep apnea pathophysiology (and a downward shift in the genioglossus EMG-drive relationship). Further, a reduced responsiveness to rising ventilatory drive stimuli is presumed in REM, which would manifest as a reduced ventilation-drive slope. B) We contend that that pharyngeal compromise is explained by a common reduction in drive (neural output to both the diaphragm and pharyngeal dilators), which would be seen as a reduction in the position on the same ventilation-drive relationship.

Methods

Participants

Sixty-three patients with suspected or diagnosed OSA were recruited for the parent study designed to investigate OSA pathophysiology[2]. Exclusion criteria were: use of respiratory stimulants or depressants, heart failure, lung disease or other major organ system disease, central sleep apnea, and pregnancy. Participants provided written informed consent and approval was granted by the Partners' Institutional Review Board. For the current investigation, additional inclusion criterion was a minimum apnea-hypopnea index (AHI) of 5 events/hr in either non-REM or REM, plus successful collection of spontaneous breathing data for at least 10 min in both states. Overall, N=25 satisfied these additional criteria and provided data for analysis.

Protocol

Patients were studied during overnight polysomnography in-laboratory with additional physiological measurements (see below). During the parent study, CPAP was applied during a portion of the night; here only data *off CPAP* were analyzed. The technique used to quantify pharyngeal pathophysiology was based on breath-by-breath measurement of ventilation (tidal volume × respiratory rate, "flow"), and ventilatory drive measured with gold-standard intraesophageal diaphragm EMG (EMGdi, "drive") as previously described[2]. We also measured genioglossus EMG (EMGgg) with sublingual insertion of two fine-wire electrodes[265]. Patients were asked to sleep supine for as long as they could (*n.b*. sensitivity analysis adjusting for position had no impact on results).

Equipment

In addition to routine polysomnography setup[234], ventilatory flow was assessed via a pneumotachograph (Hans Rudolf, Shawnee KS, USA; Validyne Engineering, Northbridge CA, USA) attached to a sealed oronasal mask (AirFit small, Resmed Inc., San Diego, CA, USA). To assess ventilatory drive, we measured intraesophageal diaphragm EMG (Servo-i ventilator; Maquet Getinge Group) via a catheter (2.7 mm of diameter) inserted through a lidocaine-anesthetized nostril such that the center of its electrode array (nine circumferential electrodes, 16 mm apart) lay at the level of the crural diaphragm. Bipolar electromyography of the genioglossus was recorded after the oral insertion of two stainless-steel Teflon-coated intramuscular electrodes (no. 791500; A-M Systems Inc., Sequim, WA, USA) using a 25-gauge needle, following surface anaesthesia (1% lidocaine)[265].

Data analysis

Sleep, arousals, and respiratory events were scored according to American Academy of Sleep Medicine criteria[235].

Pharyngeal Collapsibility, Responsiveness and Drive Withdrawal

In both REM and non-REM sleep, pharyngeal pathophysiology was was assessed by first plotting ventilation against ventilatory drive using an established method[2]: All available spontaneous breath-by-breath data for a given state (arousals excluded) were divided into 10 bins (per drive deciles) that provided a 10-point plot of

the *ventilation-drive* relationship[2,323]. From this relationship, *collapsibility* was quantified as the ventilation at eupneic drive levels ("$V_{passive}$"; lower values indicate greater collapsibility given flow-limited conditions), and upper airway muscle *responsiveness* was quantified as the slope of the ventilation-drive relationship (greater slope indicates greater functional responsiveness[25]). Eupneic ventilation for all analyses of REM was based on data from non-REM (below) to allow comparisons at a constant drive.

To characterize *baseline* EMGgg activity (EMGgg at eupneic drive) and EMGgg *responsiveness* to increasing drive[324], the above analysis was repeated to plot a EMGgg-drive relationship (responsiveness was based on peak EMGgg). Conceptually, a lower baseline pharyngeal muscle activity in REM might be responsible for increased collapsibility in the background of flow limitation[317,325]. Likewise, lowered EMGgg responsiveness might be responsible for reduced functional responsiveness (ventilation-drive slope). Additional analysis examined EMGgg tonic levels (i.e. lowest values of EMGgg, near end-expiration).

Drive *withdrawal* was quantified using the 1st decile value of drive for all breaths in a given state (referred to as "nadir drive"). The consequences of drive withdrawal were assessed by examining the accompanying 1st decile values of ventilation and EMGgg.

All ventilation and drive data from REM and non-REM were expressed as a percentage of eupneic ventilation during non-REM to facilitate interpretation. Eupneic ventilation was taken as the mean non-REM ventilation in L/min, including events and arousals)[295]. Genioglossus EMG data were expressed as a percentage of peak EMGgg calculated at eupneic drive during non-REM.

Primary analysis compared REM against non-REM data; additional analysis examined phasic REM *vs.* non-REM.

Additional analysis of OSA pathophysiology

Arousal threshold was calculated as the median value of drive preceding arousals from sleep[84]. We also assessed loop gain (drive response to spontaneous changes in flow)[268] and reported values for a one-cycle/minute disturbance (LG_1) and at the natural cycling frequency (LG_n). Values were not separated into phasic and tonic REM.

Signal processing and calibration

To calculate drive, raw diaphragm EMG (EMGdi) was root-mean-squared and smoothed to provide an integrated signal for analysis. For calibration, EMGdi swings were normalized using the median wake value of *flow:EMGdi* to provide a drive signal in L/min, such that (on average) drive = flow during wakefulness. Genioglossus EMG (EMGgg) was rectified, smoothed (time constant = 100 ms[227]) and expressed as percent of peak genioglossus activity at eupneic ventilatory drive in non-REM. Periods with pervasive, non-physiological signal artifact (noise/interference, partial/complete signal dropout) were manually rejected prior to analysis.

Phasic and Tonic REM classification

Primary analysis compared data in REM against non-REM. Additional analyses examined phasic REM *vs.* non-REM. For phasic REM analysis (based on continuous-time signals no shorter than 3 s, below), breaths entirely within each substate were included for analysis. In brief, periods of manually-scored REM sleep were subdivided into phasic and tonic REM based on the presence or absence of conjugate eye movements respectively using an automated approach (visually confirmed). Left and right electrooculogram signals were bandpass filtered (0.25-to-7 Hz). In 6-s sliding windows (0.25-s steps), we assessed signal magnitude (rectified, median, sum of left and right) and left-right correlation. A single signal describing the amplitude of the conjugate movements was then generated (total power gated by correlation <-0.5). Subsequently, automated thresholding identified candidate phasic REM periods. Subsequently, arousals were excluded, and periods of 3-s or less between scored phasic REM periods were merged. Tonic REM was then scored as the absence of phasic REM or arousals (minimum duration 3 s). Continuous tonic and phasic REM signals were then used to determine whether individual breaths belonged to phasic REM, tonic REM, or otherwise.

Statistical analysis

Primary statistical analysis used mixed model analyses of ventilation and drive (10 decile levels of ventilation and drive per state per patient) to examine the following: Drive withdrawal was assessed by examining the effect of REM on drive at the first decile. Analysis was then repeated for accompanying effects on ventilation and EMGgg. A potential deleterious effect of REM on collapsibility per se was assessed by examining whether REM lowers ventilation at eupneic drive. A potential deleterious effect of REM on responsiveness was assessed by examining whether REM reduces the slope of the ventilation-drive relationship (REM×drive interaction). Compared with non-REM, a significantly greater withdrawal of drive (at $P<0.05$) with an accompanying significant reduction in ventilation (at $P<0.05$), without a significant accompanying increase in collapsibility or a decrease in functional responsiveness was taken as evidence of a primary drive-dependent mechanism of pharyngeal compromise in REM. EMGgg provided secondary data to explain the primary functional (ventilation-based) findings.

The primary mixed model analysis approach used binned decile data rather than employing individual breath-level data to minimize the impact of measurement noise (23) and ensure each patient contributes similarly to the model estimates. The mixed model approach was chosen over a single point-estimate method (below) to take advantage of the multiple representative data points making up the 10-point ventilation-drive relationship, and ultimately provide results that were robust to measurement noise. This approach also provided a necessary means to account for the curvilinear ventilation-drive relationship in comparing responsiveness between REM and non-REM, achieved by adjusting for a drive^2 term and comparing slopes at eupneic ventilatory drive. Alternatively, single point-estimates of collapsibility (Vpassive = ventilation interpolated at drive = 100%) and responsiveness (2-point slope between drive = 100% and arousal threshold) [2] were also calculated to facilitate presentation of individual data points (Results).

Further analysis examined whether REM remains associated with increased OSA severity (apnea hypopnea index, AHI) after adjusting for the lower nadir ventilatory drive, and/or the reduced arousal threshold in REM.

Statistical analyses were performed using Matlab (Mathworks, Natick, MA, USA).

Additional information are provided in the Thesis Supplement.

Results

Baseline characteristics for the 25 patients studied are detailed in Table 13. Compared with non-REM, REM was associated with an increase in OSA severity (higher AHI) accompanied by greater obstructive apnea frequency, longer respiratory events, reduced arousal frequency, and a marked increase in OSA-related hypoxemia (hypoxic burden).

Characteristic	**Value (N=25)**		
Age, years	53 ± 13		
Sex, M:F	16:9		
Black:White:Asian:Hispanic	7:15:2:1		
Neck circumference, cm	40.6 ± 4.1		
Body mass index, kg/m2	29.9 ± 5.2		
Characteristics by state	**All Sleep**	**non-REM**	**REM**
Apnea hypopnea index, events/h	22.7 ± 22.4	21.2 ± 24.3	31.4 ± 20.5*
Obstructive apnea index	10.7 ± 15.9	9.0 ± 15.0	15.2 ± 22.3*
Central apnea index	0.1 ± 0.4	0.1 ± 0.3	0.0 ± 0.3
Mixed apnea index	0.1 ± 0.5	0.1 ± 0.4	0.1 ± 0.3
Obstructive hypopnea index	9.4 ± 8.8	9.2 ± 10.0	7.7 ± 9.7
Central hypopnea index	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Hypoxic burden, %.min/hr	27.6 ± 29.7	18.8 ± 21.6	87.5 ± 118.5*
Arousal index	40.0 ± 20.6	41.8 ± 22.9	29.7 ± 16.3*
Event duration (mean), s	25.1 ± 6.4	23.9 ± 6.4	31.1 ± 10.6*
Sleep duration, min	220 ± 79	189 ± 76	32 ± 20
Supine, %sleep	89 ± 22	90 ± 22	89 ± 25

Table 13. Polysomnography parameters. Data are represented as mean ± SD. REM, rapid-eye movement; NON-REM, non-REM. Compared to non-REM, REM had significantly higher apnea-hypopnea index and apnea index, but lower arousal index ($P<0.05$, t-test). All apnea-hypopnea index, arousal index, and hypoxic burden values were square-root transformed for normality and have been back-transformed for presentation (SD is based on mean of back-transformed upper and lower levels). Five patients had a non-REM AHI<5. Sleep duration describes the time off CPAP available for analysis. Hypoxic burden describes the event-related area under oxygen saturation curve per hr^{1}

Example physiological signals during REM and non-REM (Figure 18) illustrate an overall reduction of drive (reaches visibly lower nadir values, i.e. *withdrawal*) in REM versus non-REM; dips in drive were accompanied by reduced ventilation and EMGgg.

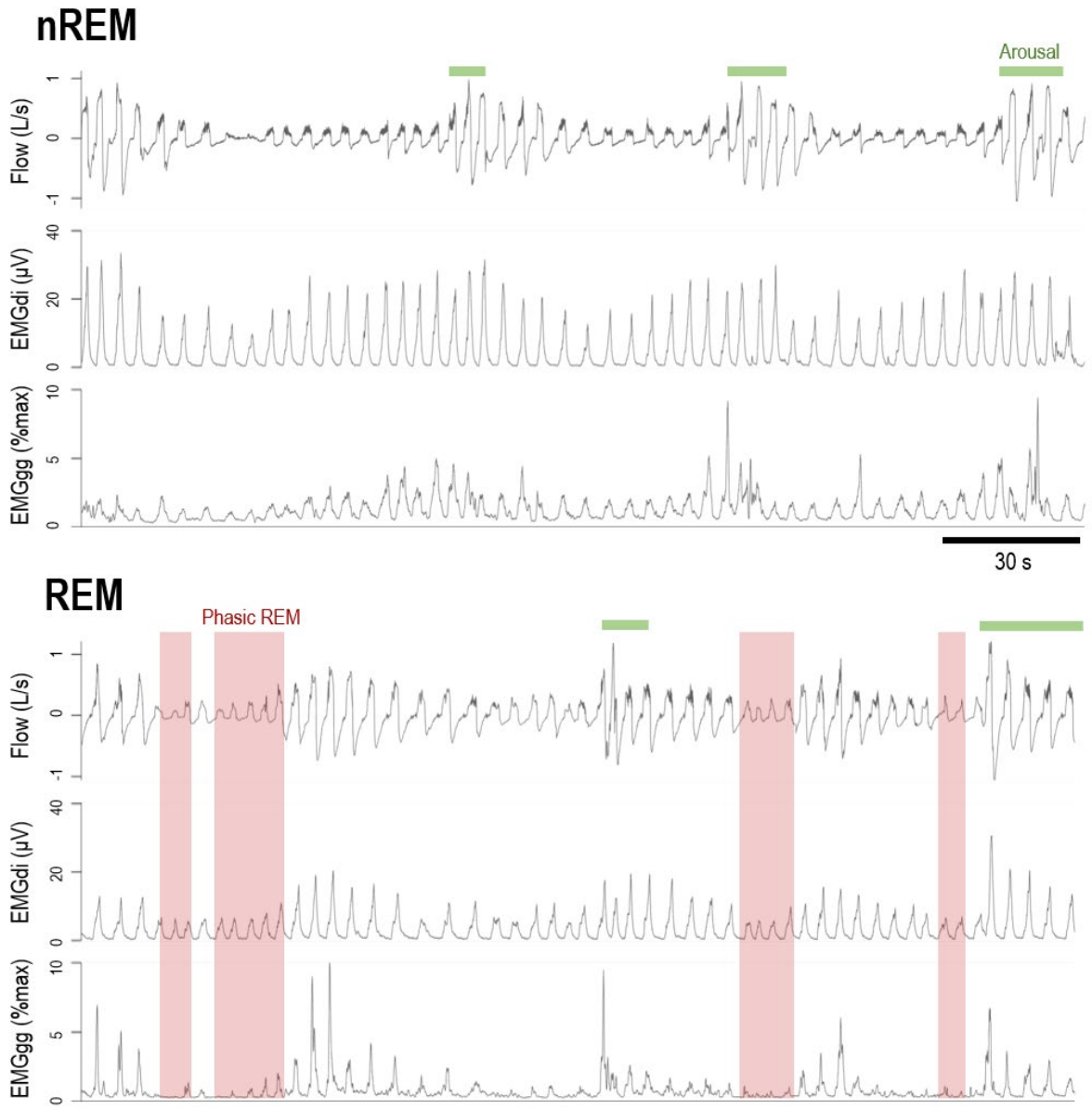

Figure 18. Example traces. This example illustrates the effect of REM on flow (ventilatory flow waveform shown), drive (diaphragm EMG moving time average, EMGdi, shown) and genioglossus activity (EMGgg). Compared to non-REM, drive is visibly reduced, and is transiently withdrawn to substantially lower values in REM; drive withdrawal occurs contemporaneously with lowered EMGgg and flow. Note drive withdrawal is particularly evident during phasic REM periods (shaded). The example window of non-REM shown is ~1 hr following the example window of REM.

Ventilatory drive withdrawal

Group data plots of the ventilation-drive relationships (Figure 19) show that these relationships clearly overlap for REM and non-REM (Figure 19A), i.e. ventilation does not appear reduced at any given level of drive that would support an increased collapsibility or reduced responsiveness (quantified below). Instead, during REM, drive appears reduced (i.e. *withdrawal*); i.e. during REM ventilatory drive fluctuations spanned a lower range of drive (along a similar ventilation-drive relationship). The EMGgg-drive relationships during sleep (Figure 19B) also visibly overlap with no overt indication of a reduction in baseline EMGgg or a reduced responsiveness in REM.

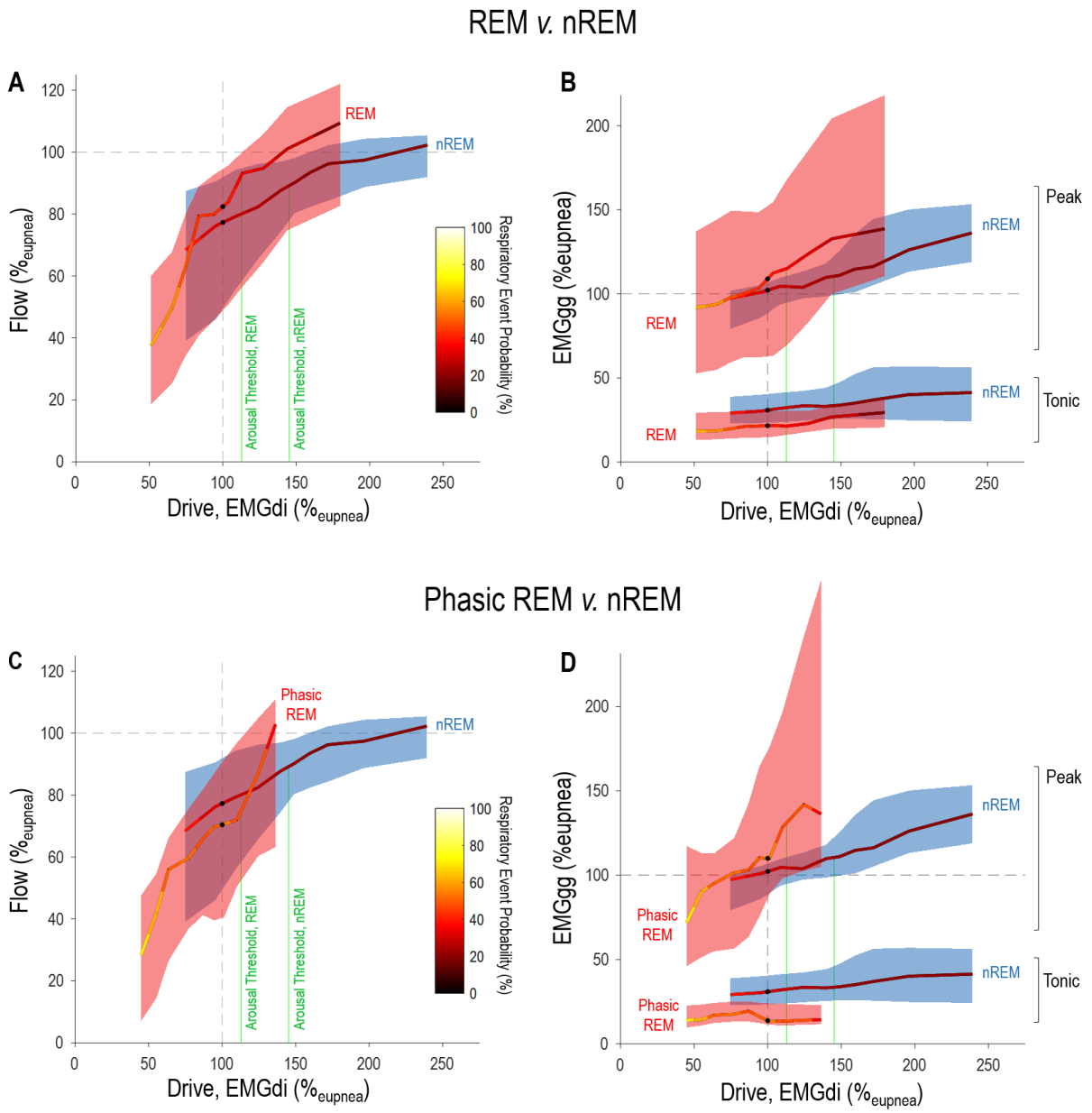

Figure 19. REM *vs.* non-REM Endograms. This figure shows breath-by-breath values of flow (i.e. ventilation, *left*) and genioglossus activity (EMGgg, right) at different (decile) levels of drive (per diaphragm EMG). Solid lines show the group median values for each level of ventilatory drive (10 values per line); color varies to illustrate increased likelihood of a respiratory event at lower ventilatory drive (see color bar). Shading denotes interquartile range in REM (red) and non-REM (blue), shown to illustrate heterogeneity). Black solid dots illustrate differences measured at eupneic drive (i.e. "$V_{passive}$" in the left panels [higher values reflect reduced collapsibility]). Panels A-B show data for REM, Panels C-D show REM data when analysis is isolated to Phasic REM; non-REM data are shown for comparison. Note that the flow-drive profiles overlap for REM and non-REM; there is no evidence of a reduction in flow for any given level of drive in REM *vs.* non-REM (curve is not shifted downwards). Likewise, peak EMGgg is not shifted downwards in REM. Tonic EMGgg, however is reduced in REM *vs.* non-REM. Note there is also no reduction in the flow-drive slope (or peak EMGgg-drive slope) to support a reduced *responsiveness* in REM. Alternatively, note the clear reduction in drive in REM *vs.* non-REM (*withdrawal*), particularly in phasic REM (median nadir drive [decile 1] is 51% in REM *vs.* 75% in non-REM). Note also the lower arousal threshold in REM (vertical green lines). REM, rapid eye movement sleep; non-REM, non-REM sleep. Flow and drive data are expressed as a percentage of eupneic ventilation during non-REM (original units: L/min); EMGgg data (peak and tonic) are expressed as a percentage of peak EMGgg values at eupneic drive during non-REM.

Formal analysis demonstrated that drive in REM dipped to markedly-lower values (1st decile "nadir drive": −21.8 [−31.2, −12.4] $\%_{eupnea,}$ change from non-REM, estimate [95%CI]; Table 14). Ventilation measured at the nadir drive was also markedly reduced (−25.8 [−31.8, −19.8] $\%_{eupnea}$, Table 14).

Variable	Drive Level	non-REM (reference)	Effect of REM	Effect of Phasic REM	Effect of Tonic REM
Drive (%eupnea)	Nadir (1st decile)	79.1 [64.3, 93.8]	−21.8 [−31.2, −12.4]* P<0.00001	−24.4 [−34.5, −14.4] P<0.00001	−15.5 [−25.0, −6.0] P=0.001
	Median	144.3 [131.0, 157.6]	−45.0 [−50.1, −40.0]* P<0.00001	−60.8 [−66.3, −55.3] P<0.00001	−37.7 [−42.8, −32.5] P<0.00001
	Maximal (10th decile)	232.3 [218.1, 246.5]	−68.3 [−77.9, −58.7]* P<0.00001	−97.2 [−107.3, −87.0] P<0.00001	−59.8 [−69.5, −50.1] P<0.00001
Flow (%eupnea)	Nadir (1st decile)	64.5 [53.6, 75.4]	−25.8 [−31.8, −19.8]* P<0.00001	−32.2 [−38.7, −25.7] P<0.00001	−18.4 [−24.4, −12.4] P<0.00001
	Median	79.2 [68.9, 89.4]	−10.4 [−13.6, −7.2]* P<0.00001	−22.0 [−25.5, −18.4] P<0.00001	−3.1 [−6.3, 0.1] P=0.058
	Maximal (10th decile)	93.8 [83.0, 104.7]	+5.0 [−1.0, 11.0]* P=0.1	−11.7 [−18.2, −5.2] P=0.0002	+12.1 [6.1, 18.1] P=0.00008
GG_{peak} (%eupnea)	Nadir (1st decile)	92.8 [64.3, 93.8]	−1.8 [−16.3, 12.7] P=0.8	−19.9 [−37.4, −2.4] P=0.026	+17.8 [1.0, 34.5] P=0.037
	Median	115.2 [94.4, 136.0]	+13.5 [5.6, 21.3]§ P=0.0009	+8.8 [−0.9, 18.4] P=0.074	+23.1 [13.9, 32.2] P<0.00001
	Maximal (10th decile)	137.6 [115.1, 160.0]	+28.7 [14.2, 43.2]§ P=0.0001	+37.5 [20.0, 55.0] P=0.00003	+28.4 [11.7, 45.1] P=0.0009
GG_{tonic} (%eupnea)	Nadir (1st decile)	32.3 [23.9, 40.7]	−8.4 [−11.4, −5.3]* P<0.00001	−12.7 [−16.4, −9.1] P<0.00001	−6.5 [−9.4, −3.6] P=0.00001
	Median	37.3 [29.1, 45.5]	−10.1 [−11.7, −8.4]* P<0.00001	−16.5 [−18.5, −14.5] P<0.00001	−8.6 [−10.2, −7.0] P<0.00001
	Maximal (10th decile)	42.3 [33.9, 50.7]	−11.7 [−14.8, −8.7]* P<0.00001	−20.3 [−23.9, −16.7] P<0.00001	−10.7 [−13.6, −7.8] P<0.00001

Table 14. Decile analysis. Table describes model coefficients (estimate [95%CI]) for mixed model analysis of the effect of REM on decile-specific levels of drive, flow, genioglossus activity. Red illustrates significant reductions with REM *vs*. non-REM; Green illustrates significant increases in REM *vs*. non-REM. For each variable, a single model described the effects of REM across multiple drive levels (rather than use of multiple separate models for different decile levels); equations followed the structure e.g.: Drive ~ Decile + $Decile^2$ + REM + REM×Decile + Subject, where Decile values are given by 1-10 (1=1st decile, 10=10th decile). The effect of REM was estimated from each model at different estimated drive levels (decile = 1, 5.5, and 10 representing nadir, median and maximal drive levels respectively). Subject is modelled as a random effect. Data were binned rather than employing individual breath-level data to minimize the impact of measurement noise[2] and ensure each patient contributes similarly to the model estimates. Analysis of REM was repeated using REM data isolated to Phasic and Tonic periods only. For each model, each subject provided 20 rows of data containing values of drive (10 rows in non-REM and 10 in REM; data were binned into deciles based on levels of drive, bins were represented by their median values). Drive denotes calibrated diaphragm EMG (to units of L/min) expressed as a percentage of non-REM eupneic ventilation. Flow denotes ventilation (tidal volume × respiratory rate, expressed as a percentage of non-REM eupneic ventilation). The $Decile^2$ term was included to account for the observation that the effect of REM on drive (and other parameters) is curvilinear (i.e. note the impact of REM on drive was greater at maximal drive than at nadir drive). Note that median ventilation during qualifying breaths (sleep without arousals) is generally below eupneic values while median drive is generally above eupneic levels, as expected in the presence of pharyngeal obstruction. Focused sensitivity analysis was performed for REM *vs.* non-REM: *Findings were similar using ventilatory effort in place of ventilatory drive in a separate analysis (estimated inspiratory muscle pressure *Pmus* from esophageal pressure in N=21 patients); §Findings were non-significant using ventilatory effort in place of drive.

Individual data are shown in Figure 20.

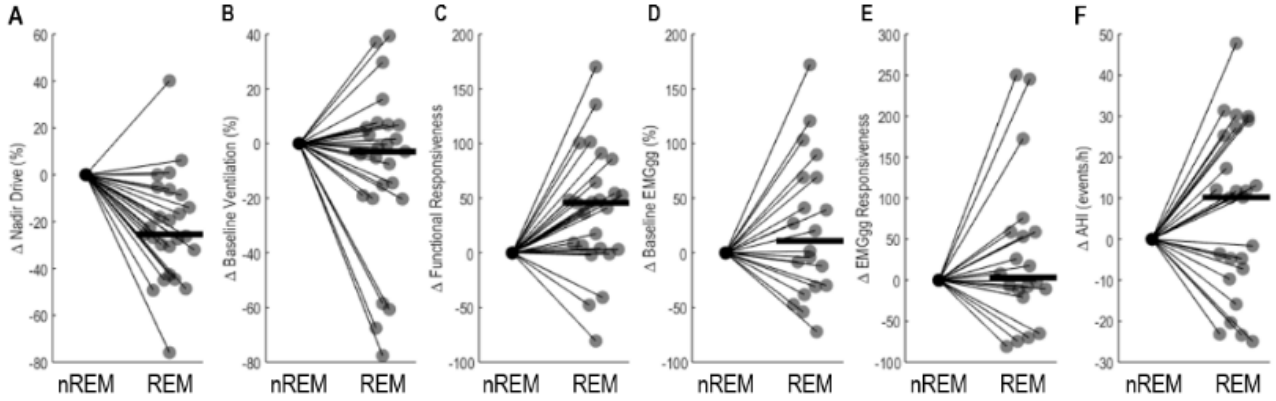

Figure 20. Individual data. Effect of REM v. non-REM: Individual differences. (A) REM is associated with a lower nadir drive (1st decile value of diaphragm EMG, $\%_{eupnea}$), i.e. drive *withdrawal*. However, when measured at a common eupneic drive, there was no evidence of a preferential effect of REM on pharyngeal mechanics (ventilation was not reduced, B) or baseline genioglossus activity (EMGgg in C; results of peak EMGgg shown). Baseline ventilation at eupneic drive is referred to as "Vpassive"; lower values of ventilation indicate greater collapsibility. Moreover, there was no evidence of a reduction in responsiveness to drive stimuli in REM, whether measured as the ventilation-drive slope (functional responsiveness in D) or the EMGgg-drive slope (responsiveness in E). (F) Apnea-hypopnea index was increased in REM. Of the candidate mechanisms of increased AHI in REM (panels A-E), only a deficit in nadir drive (A) was observed. Data are presented as absolute changes from non-REM. Bars with asterisks indicate significant difference (P<0.05). Responsiveness units describe the change in flow or EMGgg ($\%_{eupnea}$) accompanying a change in drive by $+100\%_{eupnea}$.

Pharyngeal pathophysiology

When drive was not considered, REM was associated with a reduction in ventilation v. non-REM (−10.4 [−14.6, −6.2] $\%_{eupnea}$, change from non-REM; Table 15, Model A).

Characteristic	Intercept	Drive	REM	REM×Drive	Interpretation
A. Effect of REM on flow *Flow ~ REM + Subject*	79.2 [68.8, 89.5]		**−10.4** **[−14.6,−6.2]** **P<0.00001**		REM lowers flow*
B. Effect of drive on flow *Flow ~ Drive + Drive² + Subject*	69.0 [59.2, 78.9]	**+38.8** **[34.7,43.0]** **P<0.00001**			Higher drive increases flow*
C. Effect of REM on baseline flow *Flow ~ Drive + Drive² + REM + Subject*	68.2 [58.15, 78.321	+39.4 [35.0, 43.8] P<0.00001	**−1.4** **[−2.2,4.9]** **P=0.44**		REM does not lower flow independent of drive
D. Effect of REM on responsiveness *Flow ~ Drive + Drive² + REM + REM×Drive + Subject*	71.0 [60.6, 81.3]	+29.0 [23.3, 34.6] P<0.00001	−2.7 [−6.4,1.0] P=0.16	**+18.1** **[11.7, 24.5]** **P<0.00001**	REM does not reduce the slope (effect of drive on flow)*

Table 15. Influence of REM on flow. This table describes model coefficients (estimate±95%CI) for serial modelling analysis. Each subject provided 20 rows of data containing values of flow and drive (10 rows in non-REM and 10 in REM; data were binned into deciles based on levels of drive, bins were represented by medians within each decile). Flow denotes ventilation (tidal volume × respiratory rate, expressed as a percentage of non-REM eupneic ventilation). Drive denotes calibrated diaphragm EMG (expressed as a percentage of non-REM eupneic ventilation). Drive data were centered at 100% such that model coefficients (intercept and slope [drive coefficient] for models B-D) reflect the local values at eupneic drive. Subject is included as a random effect in each model (akin to repeated measures analysis). Bold denotes the coefficient that is the focus of each model analysis (described in interpretation). *Findings are similar using ventilatory effort in place of ventilatory drive in separate analysis (estimated inspiratory muscle pressure *Pmus* from esophageal pressure). Analysis (models B to D) adjusted for Drive² because the flow-drive slope falls with increasing drive (i.e. avoiding potential bias towards observing higher slopes at lower drive in REM): coefficients for models B-D were −11.7 [−13.9,−9.4], −11.7 [−14.0,−9.5], and −8.4 [−10.9,−5.9] respectively.

Yet, when adjusting for drive (note the clear ventilation-drive relationship in Table 15, Model B), there was no longer evidence of increased collapsibility in REM: Specifically, at eupneic drive, ventilation ($V_{passive}$: +2.4 [−0.8, 5.5] $\%_{eupnea}$, Table 15, Model C) was not lower in REM. In support of this observation, we also observed no loss of baseline genioglossus activity (EMGgg peak +26.9 [18.4, 35.4] $\%_{eupnea}$, Table 16) in REM. Individual data are shown in Figure 20B-C. Moreover, we observed no REM-related loss of *responsiveness* based on the slope of the ventilation-drive relationship (+22.7 [17.3, 28.1] $\%_{ventilation/drive,}$ Table 15, Figure 19A), or the slope of the EMGgg-drive relaionship (+48.1 [33.6, 62.6] $\%_{eupnea}$, Table 16, Figure 19B). Individual data are shown in Figure 20D-E

Characteristic	Intercept	Drive	REM	REM×Drive	Interpretation
A. Effect of REM on flow $GG_{peak} \sim REM + Subject$	115.2 [94.3, 136.1]		**+13.5** **[4.7,22.3]** **P=0.003**		REM does not lower GG_{peak} *
B. Effect of drive on flow $GG_{peak} \sim Drive + Drive^2 + Subject$	119.4 [100.6, 138.2]	**+39.5** **[28.4, 50.6]** **P<0.00001**			Higher drive increases GG_{peak} *
C. Effect of REM on baseline flow $GG_{peak} \sim Drive + Drive^2 + REM + Subject$	105.3 [86.6, 124.0]	+49.3 [38.1, 60.5] P<0.00001	**+25.5** **[16.7,34.2]** **P<0.00001**		REM does not lower GG_{peak}* independent of drive
D. Effect of REM on responsivenes $GG_{peak} \sim Drive + Drive^2 + REM + REM \times Drive + Subject$	111.8 [93.0, 130.5]	+22.3 [8.5, 36.1] P=0.002	+14.0 [4.9,23.1] P=0.003	**+46.4** **[31.4, 61.4]** **P<0.00001**	REM does not reduce the slope* (effect of drive on GG_{peak})

Table 16. Influence of REM on GGpeak. GGpeak denotes peak genioglossus activity presented as a percentage of GGpeak at eupneic drive during non-REM. Table describes model coefficients (estimate±95%CI) for serial modelling analysis. Each subject provided 20 rows of data containing values of GGp and drive (10 rows in non-REM and 10 in REM; data were binned into deciles based on levels of drive, bins were represented by medians within each decile). See footnote for Table S1 for additional details. Bold denotes the coefficient that is the focus of each model analysis (described in interpretation). Analysis (models B to D) adjusted for $Drive^2$ because the EMGgg-drive slope falls with increasing drive (i.e. avoiding potential bias towards observing higher slopes at lower drive in REM): coefficients for models B-D were −16.9 [−23.4, −10.4], −18.2 [−24.5, −11.9], and −9.8 [−16.4, −3.3] respectively. *Indicates findings are similar using ventilatory effort in place of ventilatory drive in separate analysis (estimated inspiratory muscle pressure *Pmus* from esophageal pressure).

Lower nadir drive as a mechanism of OSA exacerbation in REM

OSA severity was exacerbated in REM vs. non-REM (+10.1 [1.8, 19.8] events/hr). Notably, this difference was markedly attenuated (and no longer significant) after adjusting for nadir ventilatory drive (+4.3 [−4.2, 14.6] events/hr, Table 17). Likewise, when drive was not considered, REM was associated with increased event likelihood during sleep (odds ratio=2.9 [2.4, 3.6]), see color bars in Figure 19; adjusting for drive greatly attenuated this association (odds ratio=1.7).

Influence of body position on REM pathophysiology

Sensitivity analysis demonstrated no effect of body position on the REM-vs.-non-REM difference in ventilation measured at eupneic drive or in *responsiveness*.

Characteristic	Intercept	Drive	REM	Interpretation
A. Effect of REM on AHI *AHI ~ REM + Subject*	21.2 [13.8, 30.2]		**+10.1** **[1.8, 19.8]** **P=0.016**	REM increases AHI
B. Effect of Nadir Drive on AHI *AHI ~ Drive + Subject*	22.6 [15.6, 31.0]	**−19.7** **[−22.6, −9.4]** **P=0.003**		Lower nadir drive increases AHI
C. Effect of REM on AHI adjusting for Nadir Drive *AHI ~ Drive + REM + Subject*	21.2 [14.0, 29.9]	−16.4 [−21.2, −0.7] P=0.043	**+4.3** **[−4.2, 14.6]** **P=0.3**	REM does not increase AHI independently of drive

Table 17. Influence of REM on OSA severity. AHI denotes apnea hypopnea index. AHI data were square-root transformed for normality prior to analysis; results shown are back-transformed for presentation. Table describes model coefficients (estimate±95%CI) for serial modelling analysis. Each subject provided 2 rows of data containing values of AHI and drive (1 rows in non-REM and 1 in REM). Bold denotes the coefficient that is the focus of each model analysis (described in interpretation).

Phasic REM

In phasic REM (Figure 19C-D), drive *withdrawal* was particularly evident (−24.4 [−34.5,−14.4] $\%_{eupnea}$, Table 15) with accompanying dips in ventilation (−32.2 [−38.7,−25.7] $\%_{eupnea}$), peak EMGgg (−19.9 [−37.4,−2.4] $\%_{baseline}$) and tonic EMGgg (−12.7 [−16.4,−9.1] $\%_{baseline}$, Table 14). Notably, the ventilation-drive relationship clearly overlapped with non-REM; that is, there was no loss of ventilation at eupneic drive (i.e. unchanged *collapsibility*), and no loss of peak EMGgg that might contribute to greater collapsibility.

Tonic genioglossus activity during REM

One observation that might support an increased collapsibility in REM *vs.* non-REM was a clear reduction in tonic EMGgg activity at eupneic drive (−7.9 [−9.4, −6.5] $\%_{eupnea}$, Table 18B). However, this deficit was not accompanied by evidence of accompanying pharyngeal compromise (ventilation, Figure 19A).

Characteristic	Intercept	Drive	REM	REM×Drive	Interpretation
A. Effect of REM on flow GG_{tonic} *~ REM + Subject*	37.3 [29.1, 45.5]		**−10.1** **[−11.8, −8.3]** **P<0.00001**		REM lowers GG_{tonic}*
B. Effect of drive on flow GG_{tonic} *~ Drive + Drive² + Subject*	30.6 [22.6, 38.7]	**+8.4** **[6.0, 10.9]** **P<0.00001**			Higher drive increases GG_{tonic}*
C. Effect of REM on baseline flow GG_{tonic} *~ Drive + Drive² + REM + Subject*	35.1 [22.6, 38.7]	+5.3 [2.9, 7.6] P=0.00001	**−8.1** **[−9.9, −6.3]** **P=0.00001**		REM lowers GG_{tonic} independent of drive*
D. Effect of REM on responsivenes GG_{tonic} *~ Drive + Drive² + REM + REM×Drive + Subject*	35.0 [26.9, 43.1]	+5.7 [2.7, 8.7] P=0.0002	−7.9 [−9.9, −5.9] P=0.00001	**−0.8** **[−4.1, 2.4]** **P=0.6**	REM does not reduce the slope (effect of drive on GG_{tonic})*

Table 18. Influence of REM on GG_{tonic}. GG_{tonic} denotes tonic genioglossus activity presented as a percentage of GG_{peak} at eupneic drive during non-REM. Table describes model coefficients (estimate±95%CI) for serial modelling analysis. Each subject provided 20 rows of data containing values of GG_{tonic} and drive (10 rows in non-REM and 10 in REM; data were binned into deciles based on levels of drive, bins were represented by medians within each decile). See footnote for Table S1 for additional details. Bold denotes the coefficient that is the focus of each model analysis (described in interpretation). Analysis (models B to D) adjusted for $Drive^2$ because we considered the possibility that EMGgg-drive slopes could fall with increasing drive (i.e. avoiding any potential bias towards observing higher slopes at lower drive in REM): coefficients for models B-D were not significant and given by −0.7 [−2.1, 0.8], −0.2 [−1.5, 1.1], and −0.4 [−1.8, 1.0] respectively. *Indicates findings are similar using ventilatory effort as per figure above.

Arousal threshold and ventilatory control

REM was associated with a (deleterious) reduction in the arousal threshold (drive prior to arousal, 114.1 [48.7] *vs.* 145.2 [49.5], P=0.024) and an (advantageous) reduction of loop gain *vs.* non-REM (LG_1: 0.51 [0.27] *vs.* 0.64 [0.17], P=0.021; LG_n: 0.38 [0.14] *vs*. 0.46 [0.14], P=0.010). Lower arousal threshold in REM was also considered as a potential additional determinant of the elevated AHI in REM. Additional inclusion of arousal threshold (to Table 17 model) further attenuated the effect of REM on AHI (from +4.3 [−4.2, 14.6] events/hr to −2.1 [−15.4, 12.4] events/hr). Note that *lower* arousal threshold was independently associated with *higher* AHI in this analysis. Lower loop gain was not considered as a potential additional determinant of the elevated AHI in REM, because low loop gain is considered an advantageous trait with respect to AHI. Inclusion of loop gain had no impact on the effect of REM on AHI.

Additional information are provided in the Thesis Supplement.

Discussion

Using direct physiological measurement, the current study demonstrates that the primary pathophysiological mechanism underlying pharyngeal compromise and exacerbated OSA in REM *vs.* non-REM lies with the common *withdrawal* of neural ventilatory "drive" (to pump and upper airway muscles) rather than preferential deficits in pharyngeal muscle control. Specifically, when measured at a common eupneic drive, there was no observed loss of ventilation ("flow") in REM *vs.* non-REM (i.e. constant collapsibility) and no loss of peak genioglossus activity. Likewise, rising drive during REM was accompanied by increasing flow and genioglossus activity just as seen during non-REM, indicating no deficit in spontaneous responsiveness to rising ventilatory drive stimuli. Instead, REM leads to a shift to a lower position along a shared flow-drive profile capturing pharyngeal pathophysiology as seen in non-REM. Drive withdrawal was particularly evident during the phasic eye movements that characterize REM. Finally, we show that the greater ventilatory drive withdrawal in REM provides an explanation for exacerbated OSA severity (per AHI) in this state (with lowered arousal threshold providing an additional contribution). Understanding the neurobiological factors underlying the REM-related loss of common drive to the genioglossus and diaphragm—rather than mechanisms of direct inhibition at the hypoglossal-motor-pool—can now be prioritized for investigation as the leading avenue for obviating OSA exacerbation in REM.

Novel physiologic insights

Baseline activity/collapsibility

It is well established that REM sleep is characterized by a reduction in genioglossus activity[161,166,172,317-319,322], particularly during phasic REM[161,166,319,322], plus downstream adverse effects on flow[89,317], OSA severity[316,326], and hypoxemia[316]. A preferential deficit in pharyngeal control is supported by data in rats illustrating a REM-related reduction in genioglossus activity despite preserved diaphragm EMG[172]. However, human data demonstrating REM-related pharyngeal compromise have not shown preferential pharyngeal deficits *independent of ventilatory drive*. Specifically, in humans with and without OSA, genioglossus activity was found to be approximately halved in REM *vs.* non-REM despite minor reductions in tidal volume[319], although findings were in the context of CPAP administration which disproportionately suppresses genioglossus activity versus ventilatory drive[211]. Findings that pharyngeal collapsibility is greater in REM *vs.* non-REM, accompanied by lower genioglossus activity[317], were also observed alongside smaller epiglottic pressure swings (3 *vs.* 5-8 cmH_2O) suggesting reduced ventilatory drive. During spontaneous obstructive events OSA, Jordan et al identified lower ventilation and tonic genioglossus activity in REM[89], again with reduced epiglottic pressure swings. Finally, Joosten *et al* recently showed that patients with REM OSA exhibit an increased collapsibility (per Vpassive) in REM *vs.* non-REM[327], observations that could again be explained by lower drive.

In the current study, we sought to determine whether there is a REM-related compromise in pharyngeal function independent of drive. Our study demonstrated no REM-related deficits in baseline pharyngeal mechanical function, i.e. collapsibility, when assessed at baseline eupneic drive. Notably, the significantly

lower flow identified in REM (−10 $\%_{eupnea}$) was no longer observed after adjusting for drive (−1 $\%_{eupnea}$). We did, however, observe an independent reduction in tonic EMGgg (evident at any given level of drive, Figure 18B and Table 18–row 3), yet this reduction was not accompanied by an observed functional deficit (i.e. lower flow), possibly because of a counterbalancing rise in peak EMGgg (Figure 18B and Table 18–row 3). Thus, taken together, the available evidence demonstrates that global output of the pharyngeal dilator muscles is not preferentially inhibited in REM *independent of drive* in patients with OSA. Hence, the prevailing view that there is a special REM-related loss of dilator muscle activity (e.g. via muscarinic inhibition of the hypoglossal motor pool) does not appear to apply to spontaneously breathing human patients with OSA.

Responsiveness

Our laboratory and others have commonly invoked a reduced pharyngeal dilator muscle *responsiveness* to neuromechanical stimuli as the explanation for REM-related mechanical deficits[165,172,317]. Indeed, we often describe that patients with greater dilator muscle responsiveness in non-REM are at risk of sleep apnea exacerbation in REM[326,328,329] because muscle *responsiveness* in REM is reduced. However, our study shows that flow--drive and genioglossus- drive profiles are fully preserved in REM *vs.* non-REM, demonstrating no preferential (drive-independent) loss of dilator muscle activity or pharyngeal compromise. Thus, any loss of responsiveness to local mechanical (i.e. negative pressure) stimuli observed experimentally[165,172,317] is not typically translated into a failure to raise EMGgg and flow whenever drive rises spontaneously within REM.

Withdrawal

Data from the current study imply that the primary mechanism of REM-related mechanical deficit and genioglossus activity in REM is a transient withdrawal of drive, observed particularly during phasic REM For the first time to our knowledge, in patients with OSA, drive was found to dips to lower nadir levels in REM (1st decile=51$\%_{eupnea}$ in REM and 45$\%_{eupnea}$ in phasic REM, *vs.* 75$\%_{eupnea}$ in non-REM), consistent with prior reports in non-OSA subjects[166,322,330]. Accompanying transient drive withdrawal in phasic REM, we observed severe pharyngeal compromise (flow=28$\%_{eupnea}$) alongside lowered peak genioglossus activity. Taken together with the absence of preferential effects on collapsibility (baseline EMGgg) and dilator muscle responsiveness, these data support our central contention that pharyngeal compromise in REM is best explained by a withdrawal of common drive.

Finally, withdrawal of ventilatory drive was shown to provide an explanation for exacerbated OSA severity (increased AHI) in REM *vs.* non-REM. OSA severity was 50% greater in REM *vs.* non-REM (+10 events/hr), but this difference was no longer significant (>50% attenuation) after adjusting for nadir ventilatory drive (1st decile value). Thus, greater ventilatory drive withdrawal appears to mediate the exacerbated OSA in REM *vs.* non-REM.

Physiological implications

The current study provides insight into previously perplexing observations: First, a drive withdrawal mechanism of OSA exacerbation in REM may appear reminiscent of central apneas and hypopneas in patients with central sleep apnea, which is characterized by falling drive as a primary pathophysiological mechanism

without a meaningful contribution of pharyngeal obstruction. The current work provides new evidence that lowered drive is a major determinant of OSA, where a loss of drive stimuli to the hypoglossal motor pool promotes hypotonicity (EMG) and lost functional output (flow) of the pharyngeal dilator muscles. The apparent paradox—that REM is accompanied by more severe prolonged obstruction (Figure 16) but simultaneously characterized by lower drive and central hypoventilation—can be reconciled by recognizing that lowered common drive to hypoglossal nerves promotes more severe pharyngeal obstruction. Second, REM is also associated with a lower arousal threshold[84,265], which we consider to be an important additional mechanism of increased AHI in this state; yet the lower arousal threshold (ventilatory drive at arousal) may appear incongruent with a substantial lengthening of events and a nearly 5-fold increase in the severity of hypoxemia. However, in REM *vs.* non-REM, drive not only falls to lower levels to promote lower ventilation (i.e. lower starting point), but ventilatory drive stimuli rise more slowly due to the lower chemosensitivity (lowered loop gain) in this state. Thus, a low arousal threshold (per ventilatory drive) can co-exist with longer and more severe events in the presence of lower background ventilatory drive levels.

Clinical implications

REM OSA is notoriously difficult to treat and contributes disproportionately to OSA-related hypoxemia (Table 14) and potentially the adverse health outcomes of OSA[8,238]. While CPAP is efficacious, therapeutic pressure requirements are higher in REM, and incomplete adherence later in the night commonly leaves REM periods undertreated. New therapeutic options for patients are needed, but development is hampered by limited knowledge on the neurobiological and pathophysiological pathways of disease. Our data implicating a loss of central drive as the upstream mechanism of exacerbated OSA in REM, rather than preferential genioglossus inhibition, suggests that current pharmacological efforts targeting a reversal of the REM-related inhibition of the hypoglossal motor pool may have limited yield. On the basis that drive inputs to the dilator muscle centers are intact during REM, these inputs could potentially be leveraged in novel therapeutic strategies to raise drive or augment/sustain its impact on genioglossus activation. Our study also provides treating physicians with a primary mechanism for the more severe and prolonged obstruction and attendant hypoxemia observed ubiquitously in patients during REM; knowledge that lowered common respiratory drive is a key factor may provide further impetus to minimize medications (e.g. opioids) and lifestyle factors (e.g. alcohol) that could cause respiratory depression in those with evidence of REM OSA.

Methodological considerations

The current study has several limitations: 1) REM-related changes to pharyngeal pathophysiology were variable between individuals (Figure 19), with four individuals exhibiting a physiologically-meaningful increase in collapsibility (despite no group effects). Specific patterns of ventilatory drive fluctuations during REM are also heterogenous; patterns in one patient may not be representative of others Further analysis of subgroups would be possible with a larger sample but would likely require less-invasive measurements. 2) We measured genioglossus activity as a representative upper airway dilator muscle; it is possible that other pharyngeal muscles (e.g. tensor palatini) might exhibit a drive-independent loss of muscle activity with REM; however, since there was no drive-independent reduction in flow, it is not likely that unmeasured sources of

pharyngeal stiffness could have provided an important additional source of pharyngeal compromise[317]. 3) Relationships between EMGgg were assessed as a function of drive but equally could be presented as a function of pressure inputs. Since the goal was to explain the REM-related loss of flow, and esophageal pressures are confounded by obstruction-related loss of tidal volume, diaphragm EMG measurement of drive was considered the state-of-the-art for the current investigation. Alternative analysis using esophageal pressure signals did not meaningfully change the findings. 4) We consider changes in EMGgg and ventilation occur in *response* to increases in drive based on established physiological principles, yet the source of ventilatory drive fluctuations may be more complex in REM *vs.* non-REM In REM, drive fluctuations likely originate from intermittent central inhibition as well as chemical drive responses to prior hyperventilation. We emphasize that 1) chemoreflexes are only modestly dampened in REM *vs.* non-REM (~25% reduction in loop gain, per results here and prior experimental data[331]), and 2) the surges in drive observed in tonic REM generally appear as "crescendo" chemoreflex-like responses to prior hypoventilation (Figure 17, see also Figure 1 in ref [89]). Regardless of the origin—and whether one should interpret the genioglossus output as strictly a *response* per se—we show that rising drive is accompanied by an elevation in dilator muscle activity during REM that is equal to (and sometimes greater than) that seen during non-REM.

Additional information are provided in the Thesis Supplement.

Conclusions

REM-related pharyngeal neuromechanical compromise appears to originate from a REM-related withdrawal of ventilatory drive rather than preferential drive-independent loss of pharyngeal muscle activity or responsiveness. Withdrawal of ventilatory drive, as well as a reduction in the ventilatory drive threshold for arousal, provide explanations for the REM-related exacerbation of OSA. We now consider drive withdrawal as a primary avenue for novel strategies to ameliorate OSA during REM and its sequelae.

SUMMARY AND INTERPRETATION

Targeting specific altered pathophysiological traits during non-REM and REM sleep offers a promising alternative approach to advance knowledge on OSA pathophysiology to inform the development of new treatments and to ultimately overcome some of the shortcomings of existing OSA therapies. However, it remains challenging to identify a drug/device intervention capable of consistently resolving OSA given the multifactorial underlying pathophysiology. The discovery that atomoxetine and oxybutynin, a combination of noradrenergic and antimuscarinic properties, offers promise that targeting specific key pathophysiological traits in OSA is possible and may be efficacious for a substantial proportion of patients. However, many questions remain. For example, Ato-Oxy acts primarily via increasing pharyngeal muscle function in non-REM, likely through noradrenergic stimuli to the hypoglossal motor pool, and REM, possibly via inhibition of muscarinic outputs at the pre-motor hypoglossal area. Although the neurobiological path of upper airway muscle recruitment during Ato-Oxy treatment is still unclear, it is evident that Ato-Oxy decreases the respiratory arousal threshold, another key trait responsible for OSA genesis, thus potentially promoting residual breathing instability and consequent possible apnea cycling and sleep fragmentation.

This thesis describes a way to pharmacologically increase the respiratory arousal threshold, with or without the simultaneous administration of Ato-Oxy (**chapters two** and **three**), in an attempt to provide further protection from OSA. The studies I conducted with the selected agent, zolpidem, aimed to determine its effect on a number of important respiratory and sleep parameters (in both REM and non-REM) that are altered in OSA patients, such as the number of respiratory events and the sleep efficiency as well as the underlying mechanisms/ impact on key OSA endotypes.

Specifically, **Chapter two** investigates the effects of zolpidem 10 mg *vs.* placebo on OSA severity (per AHI) according to a randomized, double-blind, crossover protocol performed in 19 OSA selected to have a low-to-moderate arousal threshold. Zolpidem, that was previously shown to improve both the respiratory arousal threshold and the upper airway muscle responsiveness, increases the respiratory arousal threshold by ~15% throughout all the sleep stages (including REM), and improves sleep efficiency and sleep architecture in OSA patients. However, contrary to earlier physiology studies conducted during transient CPAP reductions, it has no effects either on pharyngeal muscle responsiveness or on OSA severity during naturally occurring sleep. Explanations include an insufficient increase of the arousal threshold to determine consistent ventilatory stability. However, the lack of effect on the respiratory event rate, apnea length, oxygen desaturation, next day perceived sleepiness and objective alertness, along with the absence of side effects, suggests that zolpidem may be safe in OSA patients with these phenotypic characteristics. Future studies will also need to investigate the physiological and potential therapeutic effects of zolpidem and potentially other hypnotics (including over longer duration) in the clinically relevant group of people who have both OSA and insomnia. This work will be important, as the Food and Drug Administration currently recommends caution with zolpidem prescription and Therapeutic Good Administration guidelines, discourages its use in OSA patients.

Chapter three takes the next, logical step and tests the additional effect of zolpidem 10 mg plus Ato-Oxy *vs.* Ato-Oxy alone on sleep efficiency according to a randomized, double-blind, crossover protocol run in 12 unselected OSA participants. The addition of zolpidem to Ato-Oxy increases the respiratory arousal threshold by ~15% and sleep efficiency by ~10%, without deteriorating overnight hypoxia or apnea duration. Again, the addition of zolpidem does not lead to amelioration of OSA severity. This finding sheds further light on the fact that the respiratory arousal threshold might be a less important contributor than other physiological traits to OSA pathogenesis. The triple combination does not worsen next-day subjective alertness. However, it reduces the performance of certain key parameters on a driving simulation task (two of the four key measured parameters, steering deviation from median lane position and breaking time, were worse with the addition of zolpidem *vs.* Ato-Oxy alone). Therefore, while zolpidem may help to counteract the negative effects of Ato-Oxy on sleep quality and likely improve the longer-term adherence to the noradrenergic antimuscarinic combination therapy, zolpidem-dependent deleterious effects on the objective next-day alertness needs to be carefully considered before this combination approach can be considered clinically. Future investigations to enhance the sleep promotion effects and minimize potential next day alertness decrements remain a research priority.

Chapter four uncovers the unknown effect of histaminergic central stimulation on OSA severity. This study is of particular importance as previous research demonstrated 1) abundant concentrations of histaminergic receptors at the hypoglossal nucleus in the brainstem, and 2) improvements of OSA symptoms after administration of histaminergic drugs. With a double-blind, crossover, placebo-controlled trial design, the combination of betahistine, a histaminergic drug with inverse agonism on the auto-receptors H3, plus oxybutynin, given to counteract the waking action of histamine due to its hypnotic properties, does not reduce OSA severity. Unexpectedly, however, it does have an action on a key OSA endotype via an increase in loop gain/respiratory control instability. The effect of Beta-Oxy on loop gain has several implications. For example, caution is warranted regarding the use of such agents in people with OSA, particularly those with high loop gain, as it may worsen disease severity. Conversely, diseases characterized by a depression of respiratory drive (e.g., obesity hypoventilation syndrome) may benefit from a targeted increase in loop gain. It is not clear, however, why Beta-Oxy does not have an effect on pharyngeal compensation. This requires further investigation in appropriately designed mechanistic studies.

Chapter five addresses a question of major question for respiratory and sleep physicians: why is OSA highly prevalent in REM sleep? OSA is notoriously most prevalent during REM, where it is often accompanied by profound hypoxemia. Many patients—particularly women—often exhibit OSA in REM even when breathing is stable during the rest of the night. Despite the well-known problem, very little is known about OSA pathophysiology in REM beyond established observations of REM-related genioglossus hypotonia. In a detailed physiology study in instrumented human patients, I show that REM OSA relies on a withdrawal of common neural ventilatory "drive" to both pump (per diaphragm EMG) and upper airway muscles (per genioglossus EMG), and that, at a common eupneic iso-drive level, ventilation and genioglossus muscle activity are relatively preserved in REM. Indeed, falling drive levels may largely explain the loss of ventilation

in REM, and exacerbation of OSA severity. The implications of this discovery are broad: a loss of central drive as the upstream mechanism implies that loss of output to the hypoglossal motor pool is dependent on upstream inputs from ventilatory drive centers in the medulla, rather than special local REM-related inhibition. There are also major implications for drug therapies targeting OSA, that are currently based on knowledge from animal studies that suggest a specific REM-related inhibition of hypoglossal motor output. REM-related common drive preservation, rather than a reversal of the REM-related inhibition of the hypoglossal motor pool, can now be viewed as the primary avenue for OSA therapy in this sleep stage. Further mechanistic work, including with targeted agents to increase drive, will be important in expanding knowledge of this concept and its potential therapeutic implications.

Overall, the intent of this thesis was to explore new strategies to target physiologic traits in OSA. Although zolpidem does not have a therapeutically beneficial effect in OSA in terms of reducing the AHI, its arousal threshold-promoting property is confirmed, together with its safety for administration in certain OSA patients.. Similarly, betahistine may be of use in certain sleep disordered breathing patients with decreased chemosenstivity due to its effect on loop gain, while deleterious to others. In REM, preservation of ventilatory drive (thus near to arousal threshold levels) may be an important therapeutic target to treat REM OSA. Studies with interventions that alter common drive are needed to validate this physiologic finding and better understand the therapeutic potential.

BIBLIOGRAPHY

1. Azarbarzin A, Sands SA, Stone KL, et al. The hypoxic burden of sleep apnoea predicts cardiovascular disease-related mortality: the Osteoporotic Fractures in Men Study and the Sleep Heart Health Study. Eur Heart J 2018;14:1149-47.
2. Sands SA, Edwards BA, Terrill PI, et al. Phenotyping Pharyngeal Pathophysiology using Polysomnography in Patients with Obstructive Sleep Apnea. Am J Respir Crit Care Med 2018;197:1187-97.
3. Ito E, Inoue Y. [The International Classification of Sleep Disorders, third edition. American Academy of Sleep Medicine. Includes bibliographies and index]. Nihon Rinsho 2015;73:916-23.
4. Rees K, Spence DP, Earis JE, Calverley PM. Arousal responses from apneic events during non-rapid-eye-movement sleep. Am J Respir Crit Care Med 1995;152:1016-21.
5. Punjabi NM, O'Hearn D J, Neubauer DN, et al. Modeling hypersomnolence in sleep-disordered breathing. A novel approach using survival analysis. Am J Respir Crit Care Med 1999;159:1703-9.
6. Kim HC, Young T, Matthews CG, Weber SM, Woodward AR, Palta M. Sleep-disordered breathing and neuropsychological deficits. A population-based study. Am J Respir Crit Care Med 1997;156:1813-9.
7. Young T, Blustein J, Finn L, Palta M. Sleep-disordered breathing and motor vehicle accidents in a population-based sample of employed adults. Sleep 1997;20:608-13.
8. Teran-Santos J, Jimenez-Gomez A, Cordero-Guevara J. The association between sleep apnea and the risk of traffic accidents. Cooperative Group Burgos-Santander. N Engl J Med 1999;340:847-51.
9. Punjabi NM, Shahar E, Redline S, et al. Sleep-disordered breathing, glucose intolerance, and insulin resistance: the Sleep Heart Health Study. Am J Epidemiol 2004;160:521-30.
10. Peppard PE, Young T, Palta M, Skatrud J. Prospective study of the association between sleep-disordered breathing and hypertension. N Engl J Med 2000;342:1378-84.
11. Sullivan CE, Issa FG, Berthon-Jones M, Eves L. Reversal of obstructive sleep apnoea by continuous positive airway pressure applied through the nares. Lancet 1981;1:862-5.
12. Weaver TE, Grunstein RR. Adherence to continuous positive airway pressure therapy: the challenge to effective treatment. Proc Am Thorac Soc 2008;5:173-8.
13. Wolkove N, Baltzan M, Kamel H, Dabrusin R, Palayew M. Long-term compliance with continuous positive airway pressure in patients with obstructive sleep apnea. Can Respir J 2008;15:365-9.
14. Carberry JC, Amatoury J, Eckert DJ. Personalized Management Approach for OSA. Chest 2018;153:744-55.
15. Messineo L, Magri R, Corda L, Pini L, Taranto-Montemurro L, Tantucci C. Phenotyping-based treatment improves obstructive sleep apnea symptoms and severity: a pilot study. Sleep Breath 2017;21:861-8.
16. Landry SA, Joosten SA, Sands SA, et al. Response to a combination of oxygen and a hypnotic as treatment for obstructive sleep apnoea is predicted by a patient's therapeutic CPAP requirement. Respirology 2017;22:1219-24.
17. Sands SA, Edwards BA, Terrill PI, et al. Identifying obstructive sleep apnoea patients responsive to supplemental oxygen therapy. Eur Respir J 2018;52.
18. Edwards BA, Sands SA, Owens RL, et al. The Combination of Supplemental Oxygen and a Hypnotic Markedly Improves Obstructive Sleep Apnea in Patients with a Mild to Moderate Upper Airway Collapsibility. Sleep 2016;39:1973-83.
19. White DP. Pharmacologic Approaches to the Treatment of Obstructive Sleep Apnea. Sleep Med Clin 2016;11:203-12.
20. Eckert DJ, White DP, Jordan AS, Malhotra A, Wellman A. Defining phenotypic causes of obstructive sleep apnea. Identification of novel therapeutic targets. Am J Respir Crit Care Med 2013;188:996-1004.
21. Wellman A, Eckert DJ, Jordan AS, et al. A method for measuring and modeling the physiological traits causing obstructive sleep apnea. J Appl Physiol (1985) 2011;110:1627-37.
22. White DP, Younes MK. Obstructive sleep apnea. Compr Physiol 2012;2:2541-94.
23. White DP. The pathogenesis of obstructive sleep apnea: advances in the past 100 years. Am J Respir Cell Mol Biol 2006;34:1-6.
24. White DP. Pathogenesis of obstructive and central sleep apnea. Am J Respir Crit Care Med 2005;172:1363-70.
25. Wellman A, Edwards BA, Sands SA, et al. A simplified method for determining phenotypic traits in patients with obstructive sleep apnea. J Appl Physiol (1985) 2013;114:911-22.

26. Badr MS, Toiber F, Skatrud JB, Dempsey J. Pharyngeal narrowing/occlusion during central sleep apnea. J Appl Physiol (1985) 1995;78:1806-15.
27. Gold AR, Schwartz AR. The pharyngeal critical pressure. The whys and hows of using nasal continuous positive airway pressure diagnostically. Chest 1996;110:1077-88.
28. Schwartz AR, Smith PL. CrossTalk proposal: the human upper airway does behave like a Starling resistor during sleep. J Physiol 2013;591:2229-32.
29. Strohl KP, Butler JP, Malhotra A. Mechanical properties of the upper airway. Compr Physiol 2012;2:1853-72.
30. Neelapu BC, Kharbanda OP, Sardana HK, et al. Craniofacial and upper airway morphology in adult obstructive sleep apnea patients: A systematic review and meta-analysis of cephalometric studies. Sleep Med Rev 2017;31:79-90.
31. Li Y, Lin N, Ye J, Chang Q, Han D, Sperry A. Upper airway fat tissue distribution in subjects with obstructive sleep apnea and its effect on retropalatal mechanical loads. Respir Care 2012;57:1098-105.
32. Kim AM, Keenan BT, Jackson N, et al. Tongue fat and its relationship to obstructive sleep apnea. Sleep 2014;37:1639-48.
33. Schorr F, Kayamori F, Hirata RP, et al. Different Craniofacial Characteristics Predict Upper Airway Collapsibility in Japanese-Brazilian and White Men. Chest 2016;149:737-46.
34. Imes NK, Orr WC, Smith RO, Rogers RM. Retrognathia and sleep apnea. A life-threatening condition masquerading as narcolepsy. JAMA 1977;237:1596-7.
35. Genta PR, Schorr F, Eckert DJ, et al. Upper Airway Collapsibility is Associated with Obesity and Hyoid Position. Sleep 2014;37:1673-8.
36. Gleadhill IC, Schwartz AR, Schubert N, Wise RA, Permutt S, Smith PL. Upper airway collapsibility in snorers and in patients with obstructive hypopnea and apnea. Am Rev Respir Dis 1991;143:1300-3.
37. Kirkness JP, Schwartz AR, Schneider H, et al. Contribution of male sex, age, and obesity to mechanical instability of the upper airway during sleep. J Appl Physiol 2008;104:1618-24.
38. Eckert DJ, Malhotra A, Jordan AS. Mechanisms of apnea. Prog Cardiovasc Dis 2009;51:313-23.
39. Yumino D, Redolfi S, Ruttanaumpawan P, et al. Nocturnal rostral fluid shift: a unifying concept for the pathogenesis of obstructive and central sleep apnea in men with heart failure. Circulation 2010;121:1598-605.
40. Arzt M, Eckert DJ. Is fluid overload a target to treat sleep disordered breathing in patients with end-stage renal disease, and what are the underlying mechanisms? Eur Respir J 2017;49.
41. Younes M, Ostrowski M, Atkar R, Laprairie J, Siemens A, Hanly P. Mechanisms of breathing instability in patients with obstructive sleep apnea. J Appl Physiol (1985) 2007;103:1929-41.
42. Skatrud JB, Berssenbrugge AD. Effect of sleep state and chemical stimuli on breathing. Prog Clin Biol Res 1983;136:87-95.
43. Messineo L, Taranto-Montemurro L, Azarbarzin A, et al. Breath-holding as a means to estimate the loop gain contribution to obstructive sleep apnoea. J Physiol 2018;596:4043-56.
44. Hudgel DW, Gordon EA, Thanakitcharu S, Bruce EN. Instability of ventilatory control in patients with obstructive sleep apnea. Am J Respir Crit Care Med 1998;158:1142-9.
45. Sands SA, Mebrate Y, Edwards BA, et al. Resonance as the Mechanism of Daytime Periodic Breathing in Patients with Heart Failure. Am J Respir Crit Care Med 2017;195:237-46.
46. Khoo MC, Kronauer RE, Strohl KP, Slutsky AS. Factors inducing periodic breathing in humans: a general model. J Appl Physiol Respir Environ Exerc Physiol 1982;53:644-59.
47. Dempsey JA. Crossing the apnoeic threshold: causes and consequences. Exp Physiol 2005;90:13-24.
48. Xie A, Skatrud JB, Puleo DS, Rahko PS, Dempsey JA. Apnea-hypopnea threshold for CO2 in patients with congestive heart failure. Am J Respir Crit Care Med 2002;165:1245-50.
49. Sands SA, Edwards BA, Kee K, et al. Loop gain as a means to predict a positive airway pressure suppression of Cheyne-Stokes respiration in patients with heart failure. Am J Respir Crit Care Med 2011;184:1067-75.
50. Sands SA, Owens RL. Congestive Heart Failure and Central Sleep Apnea. Sleep Med Clin 2016;11:127-42.
51. Younes M, Ostrowski M, Thompson W, Leslie C, Shewchuk W. Chemical control stability in patients with obstructive sleep apnea. Am J Respir Crit Care Med 2001;163:1181-90.
52. Cherniack NS, Longobardo GS. Mathematical models of periodic breathing and their usefulness in understanding cardiovascular and respiratory disorders. Exp Physiol 2006;91:295-305.
53. Javaheri S. A mechanism of central sleep apnea in patients with heart failure. N Engl J Med 1999;341:949-54.

54. Wilcox I, Grunstein RR, Collins FL, Berthon-Jones M, Kelly DT, Sullivan CE. The role of central chemosensitivity in central apnea of heart failure. Sleep 1993;16:S37-8.
55. White DP, Douglas NJ, Pickett CK, Weil JV, Zwillich CW. Hypoxic ventilatory response during sleep in normal premenopausal women. Am Rev Respir Dis 1982;126:530-3.
56. Douglas NJ, White DP, Weil JV, et al. Hypoxic ventilatory response decreases during sleep in normal men. Am Rev Respir Dis 1982;125:286-9.
57. Douglas NJ, White DP, Weil JV, Pickett CK, Zwillich CW. Hypercapnic ventilatory response in sleeping adults. Am Rev Respir Dis 1982;126:758-62.
58. Solin P, Roebuck T, Johns DP, Walters EH, Naughton MT. Peripheral and central ventilatory responses in central sleep apnea with and without congestive heart failure. Am J Respir Crit Care Med 2000;162:2194-200.
59. Xie A, Rutherford R, Rankin F, Wong B, Bradley TD. Hypocapnia and increased ventilatory responsiveness in patients with idiopathic central sleep apnea. Am J Respir Crit Care Med 1995;152:1950-5.
60. Francis DP, Willson K, Davies LC, Coats AJ, Piepoli M. Quantitative general theory for periodic breathing in chronic heart failure and its clinical implications. Circulation 2000;102:2214-21.
61. Solin P, Bergin P, Richardson M, Kaye DM, Walters EH, Naughton MT. Influence of pulmonary capillary wedge pressure on central apnea in heart failure. Circulation 1999;99:1574-9.
62. White DP, Gleeson K, Pickett CK, Rannels AM, Cymerman A, Weil JV. Altitude acclimatization: influence on periodic breathing and chemoresponsiveness during sleep. J Appl Physiol (1985) 1987;63:401-12.
63. Farney RJ, Walker JM, Cloward TV, Rhondeau S. Sleep-disordered breathing associated with long-term opioid therapy. Chest 2003;123:632-9.
64. Rostig S, Kantelhardt JW, Penzel T, et al. Nonrandom variability of respiration during sleep in healthy humans. Sleep 2005;28:411-7.
65. Deacon NL, Sands SA, McEvoy DR, Catcheside PG. Daytime loop gain is elevated in obstructive sleep apnea but not reduced by CPAP treatment. J Appl Physiol (1985) 2018.
66. Salloum A, Rowley JA, Mateika JH, Chowdhuri S, Omran Q, Badr MS. Increased propensity for central apnea in patients with obstructive sleep apnea: effect of nasal continuous positive airway pressure. Am J Respir Crit Care Med 2010;181:189-93.
67. Eckert DJ, Wellman A. Physiological phenotypes. In: Barbé F, Pépin JL, eds. Sleep Apnoea: European Respiratory Society Monograph. Plymouth, UK: ERS educational publications; 2015:9-23.
68. Edwards BA, Connolly JG, Campana LM, et al. Acetazolamide attenuates the ventilatory response to arousal in patients with obstructive sleep apnea. Sleep 2013;36:281-5.
69. Genta PR, Owens RL, Edwards BA, et al. Influence of pharyngeal muscle activity on inspiratory negative effort dependence in the human upper airway. Respir Physiol Neurobiol 2014;201:55-9.
70. Malhotra A, Berry RB, White DP. Central sleep apnea. In: Carney PR, Berry RB, Geyer JD, eds. Clinical sleep disorders. Philadelphia, PA: Lippincott Williams and Wilkins; 2004:331-46.
71. Nicholas CL, Jordan AS, Heckel L, et al. Discharge patterns of human tensor palatini motor units during sleep onset. Sleep 2012;35:699-707.
72. Wilkinson V, Malhotra A, Nicholas CL, et al. Discharge patterns of human genioglossus motor units during sleep onset. Sleep 2008;31:525-33.
73. Basner RC, Ringler J, Schwartzstein RM, Weinberger SE, Weiss JW. Phasic electromyographic activity of the genioglossus increases in normals during slow-wave sleep. Respir Physiol 1991;83:189-200.
74. Tun Y, Hida W, Okabe S, et al. Inspiratory effort sensation to added resistive loading in patients with obstructive sleep apnea. Chest 2000;118:1332-8.
75. Kimoff RJ, Sforza E, Champagne V, Ofiara L, Gendron D. Upper airway sensation in snoring and obstructive sleep apnea. Am J Respir Crit Care Med 2001;164:250-5.
76. Sands SA, Eckert DJ, Jordan AS, et al. Enhanced Upper-Airway Muscle Responsiveness Is a Distinct Feature of Overweight/Obese Individuals without Sleep Apnea. Am J Respir Crit Care Med 2014;190:930-7.
77. Shea SA, Edwards JK, White DP. Effect of wake-sleep transitions and rapid eye movement sleep on pharyngeal muscle response to negative pressure in humans. J Physiol 1999;520 Pt 3:897-908.
78. Horner RL, Innes JA, Morrell MJ, Shea SA, Guz A. The effect of sleep on reflex genioglossus muscle activation by stimuli of negative airway pressure in humans. J Physiol 1994;476:141-51.
79. Dotan Y, Pillar G, Schwartz AR, Oliven A. Asynchrony of lingual muscle recruitment during sleep in obstructive sleep apnea. J Appl Physiol (1985) 2015;118:1516-24.
80. Owens RL, Edwards BA, Sands SA, et al. The classical Starling resistor model often does not predict inspiratory airflow patterns in the human upper airway. J Appl Physiol (1985) 2014;116:1105-12.

81. Wellman A, Genta PR, Owens RL, et al. Test of the Starling resistor model in the human upper airway during sleep. J Appl Physiol (1985) 2014;117:1478-85.
82. Owens RL, Edwards BA, Sands SA, et al. Upper airway collapsibility and patterns of flow limitation at constant end-expiratory lung volume. J Appl Physiol (1985) 2012;113:691-9.
83. Bilston LE, Gandevia SC. Biomechanical properties of the human upper airway and their effect on its behavior during breathing and in obstructive sleep apnea. J Appl Physiol (1985) 2014;116:314-24.
84. Gleeson K, Zwillich CW, White DP. The influence of increasing ventilatory effort on arousal from sleep. Am Rev Respir Dis 1990;142:295-300.
85. Phillipson EA, Sullivan CE. Arousal: the forgotten response to respiratory stimuli. Am Rev Respir Dis 1978;118:807-9.
86. Eckert DJ, Gandevia SC. The human upper airway: more than a floppy tube. J Appl Physiol 2014;116:288-90.
87. Younes M. Role of arousals in the pathogenesis of obstructive sleep apnea. Am J Respir Crit Care Med 2004;169:623-33.
88. Longobardo GS, Gothe B, Goldman MD, Cherniack NS. Sleep apnea considered as a control system instability. Respir Physiol 1982;50:311-33.
89. Jordan AS, White DP, Lo YL, et al. Airway dilator muscle activity and lung volume during stable breathing in obstructive sleep apnea. Sleep 2009;32:361-8.
90. Ratnavadivel R, Stadler D, Windler S, et al. Upper airway function and arousability to ventilatory challenge in slow wave versus stage 2 sleep in obstructive sleep apnoea. Thorax 2010;65:107-12.
91. Guilleminault C, Rosekind M. The arousal threshold: sleep deprivation, sleep fragmentation, and obstructive sleep apnea syndrome. Bull Eur Physiopathol Respir 1981;17:341-9.
92. Eckert DJ, Owens RL, Kehlmann GB, et al. Eszopiclone increases the respiratory arousal threshold and lowers the apnoea/hypopnoea index in obstructive sleep apnoea patients with a low arousal threshold. Clin Sci (Lond) 2011;120:505-14.
93. Smales ET, Edwards BA, Deyoung PN, et al. Trazodone Effects on Obstructive Sleep Apnea and Non-REM Arousal Threshold. Ann Am Thorac Soc 2015;12:758-64.
94. Butler MP, Emch JT, Rueschman M, et al. Apnea-Hypopnea Event Duration Predicts Mortality in Men and Women in the Sleep Heart Health Study. Am J Respir Crit Care Med 2018.
95. Simms T, Brijbassi M, Montemurro LT, Bradley TD. Differential timing of arousals in obstructive and central sleep apnea in patients with heart failure. J Clin Sleep Med 2013;9:773-9.
96. Ruttanaumpawan P, Logan AG, Floras JS, Bradley TD, Investigators C. Effect of continuous positive airway pressure on sleep structure in heart failure patients with central sleep apnea. Sleep 2009;32:91-8.
97. Carter SG, Berger MS, Carberry JC, et al. Zopiclone Increases the Arousal Threshold without Impairing Genioglossus Activity in Obstructive Sleep Apnea. Sleep 2016;39:757-66.
98. Bosi M, De Vito A, Kotecha B, et al. Phenotyping the pathophysiology of obstructive sleep apnea using polygraphy/polysomnography: a review of the literature. Sleep Breath 2018;22:579-92.
99. Amatoury J, Azarbarzin A, Younes M, Jordan AS, Wellman A, Eckert DJ. Arousal Intensity is a Distinct Pathophysiological Trait in Obstructive Sleep Apnea. Sleep 2016;39:2091-100.
100. Xie A, Wong B, Phillipson EA, Slutsky AS, Bradley TD. Interaction of hyperventilation and arousal in the pathogenesis of idiopathic central sleep apnea. Am J Respir Crit Care Med 1994;150:489-95.
101. Zinchuk AV, Jeon S, Koo BB, et al. Polysomnographic phenotypes and their cardiovascular implications in obstructive sleep apnoea. Thorax 2018;73:472-80.
102. Bailly S, Destors M, Grillet Y, et al. Obstructive Sleep Apnea: A Cluster Analysis at Time of Diagnosis. PLoS One 2016;11:e0157318.
103. Lacedonia D, Carpagnano GE, Sabato R, et al. Characterization of obstructive sleep apnea-hypopnea syndrome (OSA) population by means of cluster analysis. J Sleep Res 2016;25:724-30.
104. Zinchuk AV, Gentry MJ, Concato J, Yaggi HK. Phenotypes in obstructive sleep apnea: A definition, examples and evolution of approaches. Sleep Med Rev 2017;35:113-23.
105. Ye L, Pien GW, Ratcliffe SJ, et al. The different clinical faces of obstructive sleep apnoea: a cluster analysis. Eur Respir J 2014;44:1600-7.
106. Sutherland K, Kairaitis K, Yee BJ, Cistulli PA. From CPAP to tailored therapy for obstructive sleep Apnoea. Multidiscip Respir Med 2018;13:44.
107. Zinchuk A, Edwards BA, Jeon S, et al. Prevalence, Associated Clinical Features, and Impact on Continuous Positive Airway Pressure Use of a Low Respiratory Arousal Threshold Among Male United States Veterans With Obstructive Sleep Apnea. J Clin Sleep Med 2018;14:809-17.

108. Bjorvatn B, Lehmann S, Gulati S, Aurlien H, Pallesen S, Saxvig IW. Prevalence of excessive sleepiness is higher whereas insomnia is lower with greater severity of obstructive sleep apnea. Sleep Breath 2015;19:1387-93.
109. Ishman SL, Cavey RM, Mettel TL, Gourin CG. Depression, sleepiness, and disease severity in patients with obstructive sleep apnea. Laryngoscope 2010;120:2331-5.
110. Kendzerska TB, Smith PM, Brignardello-Petersen R, Leung RS, Tomlinson GA. Evaluation of the measurement properties of the Epworth sleepiness scale: a systematic review. Sleep Med Rev 2014;18:321-31.
111. Gooneratne NS, Richards KC, Joffe M, et al. Sleep disordered breathing with excessive daytime sleepiness is a risk factor for mortality in older adults. Sleep 2011;34:435-42.
112. Kendzerska T, Gershon AS, Hawker G, Leung RS, Tomlinson G. Obstructive sleep apnea and risk of cardiovascular events and all-cause mortality: a decade-long historical cohort study. PLoS Med 2014;11:e1001599.
113. Mazzotti DR, Keenan BT, Lim DC, Gottlieb DJ, Kim J, Pack AI. Symptom Subtypes of Obstructive Sleep Apnea Predict Incidence of Cardiovascular Outcomes. Am J Respir Crit Care Med 2019.
114. Wang Q, Zhang C, Jia P, et al. The association between the phenotype of excessive daytime sleepiness and blood pressure in patients with obstructive sleep apnea-hypopnea syndrome. Int J Med Sci 2014;11:713-20.
115. Ren R, Li Y, Zhang J, et al. Obstructive Sleep Apnea With Objective Daytime Sleepiness Is Associated With Hypertension. Hypertension 2016;68:1264-70.
116. Kapur VK, Resnick HE, Gottlieb DJ, Sleep Heart Health Study G. Sleep disordered breathing and hypertension: does self-reported sleepiness modify the association? Sleep 2008;31:1127-32.
117. Barcelo A, Barbe F, de la Pena M, et al. Insulin resistance and daytime sleepiness in patients with sleep apnoea. Thorax 2008;63:946-50.
118. Fu Y, Xu H, Xia Y, et al. Excessive daytime sleepiness and metabolic syndrome in men with obstructive sleep apnea: a large cross-sectional study. Oncotarget 2017;8:79693-702.
119. Zhao YY, Wang R, Gleason KJ, et al. Effect of Continuous Positive Airway Pressure Treatment on Health-Related Quality of Life and Sleepiness in High Cardiovascular Risk Individuals With Sleep Apnea: Best Apnea Interventions for Research (BestAIR) Trial. Sleep 2017;40.
120. Barbe F, Duran-Cantolla J, Sanchez-de-la-Torre M, et al. Effect of continuous positive airway pressure on the incidence of hypertension and cardiovascular events in nonsleepy patients with obstructive sleep apnea: a randomized controlled trial. JAMA 2012;307:2161-8.
121. Iftikhar IH, Hoyos CM, Phillips CL, Magalang UJ. Meta-analyses of the Association of Sleep Apnea with Insulin Resistance, and the Effects of CPAP on HOMA-IR, Adiponectin, and Visceral Adipose Fat. J Clin Sleep Med 2015;11:475-85.
122. McEvoy RD, Antic NA, Heeley E, et al. CPAP for Prevention of Cardiovascular Events in Obstructive Sleep Apnea. N Engl J Med 2016;375:919-31.
123. Robinson GV, Smith DM, Langford BA, Davies RJ, Stradling JR. Continuous positive airway pressure does not reduce blood pressure in nonsleepy hypertensive OSA patients. Eur Respir J 2006;27:1229-35.
124. Ng WL, Orellana L, Shaw JE, Wong E, Peeters A. The relationship between weight change and daytime sleepiness: the Sleep Heart Health Study. Sleep Med 2017;36:109-18.
125. Lam B, Ip MS, Tench E, Ryan CF. Craniofacial profile in Asian and white subjects with obstructive sleep apnoea. Thorax 2005;60:504-10.
126. Jose PO, Frank AT, Kapphahn KI, et al. Cardiovascular disease mortality in Asian Americans. J Am Coll Cardiol 2014;64:2486-94.
127. Ueshima H, Sekikawa A, Miura K, et al. Cardiovascular disease and risk factors in Asia: a selected review. Circulation 2008;118:2702-9.
128. Mo JH, Lee CH, Rhee CS, Yoon IY, Kim JW. Positional dependency in Asian patients with obstructive sleep apnea and its implication for hypertension. Arch Otolaryngol Head Neck Surg 2011;137:786-90.
129. Olafiranye O, Akinboboye O, Mitchell JE, Ogedegbe G, Jean-Louis G. Obstructive sleep apnea and cardiovascular disease in blacks: a call to action from the Association of Black Cardiologists. Am Heart J 2013;165:468-76.
130. Dudley KA, Patel SR. Disparities and genetic risk factors in obstructive sleep apnea. Sleep Med 2016;18:96-102.
131. Redline S, Tishler PV, Hans MG, Tosteson TD, Strohl KP, Spry K. Racial differences in sleep-disordered breathing in African-Americans and Caucasians. Am J Respir Crit Care Med 1997;155:186-92.

132. Ancoli-Israel S, Klauber MR, Stepnowsky C, Estline E, Chinn A, Fell R. Sleep-disordered breathing in African-American elderly. Am J Respir Crit Care Med 1995;152:1946-9.
133. O'Connor GT, Lind BK, Lee ET, et al. Variation in symptoms of sleep-disordered breathing with race and ethnicity: the Sleep Heart Health Study. Sleep 2003;26:74-9.
134. Geovanini GR, Wang R, Weng J, et al. Association between Obstructive Sleep Apnea and Cardiovascular Risk Factors: Variation by Age, Sex, and Race. The Multi-Ethnic Study of Atherosclerosis. Ann Am Thorac Soc 2018;15:970-7.
135. Redmond N, Baer HJ, Hicks LS. Health behaviors and racial disparity in blood pressure control in the national health and nutrition examination survey. Hypertension 2011;57:383-9.
136. Roger VL, Go AS, Lloyd-Jones DM, et al. Executive summary: heart disease and stroke statistics--2012 update: a report from the American Heart Association. Circulation 2012;125:188-97.
137. Mitchell JE, Hellkamp AS, Mark DB, et al. Outcome in African Americans and other minorities in the Sudden Cardiac Death in Heart Failure Trial (SCD-HeFT). Am Heart J 2008;155:501-6.
138. Mensah GA, Mokdad AH, Ford ES, Greenlund KJ, Croft JB. State of disparities in cardiovascular health in the United States. Circulation 2005;111:1233-41.
139. Jean-Louis G, von Gizycki H, Zizi F, Dharawat A, Lazar JM, Brown CD. Evaluation of sleep apnea in a sample of black patients. J Clin Sleep Med 2008;4:421-5.
140. Chen X, Wang R, Zee P, et al. Racial/Ethnic Differences in Sleep Disturbances: The Multi-Ethnic Study of Atherosclerosis (MESA). Sleep 2015;38:877-88.
141. Johnson DA, Thomas SJ, Abdalla M, et al. Association Between Sleep Apnea and Blood Pressure Control Among Blacks: Jackson Heart Sleep Study. Circulation 2018.
142. Scharf SM, Seiden L, DeMore J, Carter-Pokras O. Racial differences in clinical presentation of patients with sleep-disordered breathing. Sleep Breath 2004;8:173-83.
143. Edwards BA, Wellman A, Sands SA, et al. Obstructive sleep apnea in older adults is a distinctly different physiological phenotype. Sleep 2014;37:1227-36.
144. Guillot M, Sforza E, Achour-Crawford E, et al. Association between severe obstructive sleep apnea and incident arterial hypertension in the older people population. Sleep Med 2013;14:838-42.
145. Lin GM, Colangelo LA, Lloyd-Jones DM, et al. Association of Sleep Apnea and Snoring With Incident Atrial Fibrillation in the Multi-Ethnic Study of Atherosclerosis. Am J Epidemiol 2015;182:49-57.
146. Gosselin N, Baril AA, Osorio RS, Kaminska M, Carrier J. Obstructive Sleep Apnea and the Risk of Cognitive Decline in Older Adults. Am J Respir Crit Care Med 2019;199:142-8.
147. Baril AA, Gagnon K, Arbour C, et al. Regional Cerebral Blood Flow during Wakeful Rest in Older Subjects with Mild to Severe Obstructive Sleep Apnea. Sleep 2015;38:1439-49.
148. Martinez-Garcia MA, Campos-Rodriguez F, Duran-Cantolla J, et al. Obstructive sleep apnea is associated with cancer mortality in younger patients. Sleep Med 2014;15:742-8.
149. Morrell MJ, Finn L, McMillan A, Peppard PE. The impact of ageing and sex on the association between sleepiness and sleep disordered breathing. Eur Respir J 2012;40:386-93.
150. Lin CM, Davidson TM, Ancoli-Israel S. Gender differences in obstructive sleep apnea and treatment implications. Sleep Med Rev 2008;12:481-96.
151. Barford A, Dorling D, Davey Smith G, Shaw M. Life expectancy: women now on top everywhere. BMJ 2006;332:808.
152. Malhotra A, Huang Y, Fogel R, et al. Aging influences on pharyngeal anatomy and physiology: the predisposition to pharyngeal collapse. Am J Med 2006;119:72 e9-14.
153. Mohsenin V. Gender differences in the expression of sleep-disordered breathing : role of upper airway dimensions. Chest 2001;120:1442-7.
154. Jordan AS, Wellman A, Edwards JK, et al. Respiratory control stability and upper airway collapsibility in men and women with obstructive sleep apnea. J Appl Physiol (1985) 2005;99:2020-7.
155. Ossewaarde ME, Bots ML, Verbeek AL, et al. Age at menopause, cause-specific mortality and total life expectancy. Epidemiology 2005;16:556-62.
156. Bixler EO, Vgontzas AN, Lin HM, et al. Prevalence of sleep-disordered breathing in women: effects of gender. Am J Respir Crit Care Med 2001;163:608-13.
157. Manber R, Kuo TF, Cataldo N, Colrain IM. The effects of hormone replacement therapy on sleep-disordered breathing in postmenopausal women: a pilot study. Sleep 2003;26:163-8.
158. Conwell W, Patel B, Doeing D, et al. Prevalence, clinical features, and CPAP adherence in REM-related sleep-disordered breathing: a cross-sectional analysis of a large clinical population. Sleep Breath 2012;16:519-26.

159. Khan A, Harrison SL, Kezirian EJ, et al. Obstructive sleep apnea during rapid eye movement sleep, daytime sleepiness, and quality of life in older men in Osteoporotic Fractures in Men (MrOS) Sleep Study. J Clin Sleep Med 2013;9:191-8.
160. Mokhlesi B. REM-related obstructive sleep apnea: to treat or not to treat? J Clin Sleep Med 2012;8:249-50.
161. McSharry DG, Saboisky JP, Deyoung P, et al. Physiological mechanisms of upper airway hypotonia during REM sleep. Sleep 2014;37:561-9.
162. Landry SA, Andara C, Terrill PI, et al. Ventilatory control sensitivity in patients with obstructive sleep apnea is sleep stage dependent. Sleep 2018;41.
163. Sutherland K, Takaya H, Qian J, Petocz P, Ng AT, Cistulli PA. Oral Appliance Treatment Response and Polysomnographic Phenotypes of Obstructive Sleep Apnea. J Clin Sleep Med 2015;11:861-8.
164. Yon MI, Kokturk O. Is NREM-predominant obstructive sleep apnea syndrome a different clinical entity? Turk J Med Sci 2018;48:967-72.
165. Eckert DJ, McEvoy RD, George KE, Thomson KJ, Catcheside PG. Genioglossus reflex inhibition to upper-airway negative-pressure stimuli during wakefulness and sleep in healthy males. J Physiol 2007;581:1193-205.
166. White JE, Drinnan MJ, Smithson AJ, Griffiths CJ, Gibson GJ. Respiratory muscle activity during rapid eye movement (REM) sleep in patients with chronic obstructive pulmonary disease. Thorax 1995;50:376-82.
167. de Melo CM, Taranto-Montemurro L, Butler JP, et al. Stable Breathing in Patients With Obstructive Sleep Apnea Is Associated With Increased Effort but Not Lowered Metabolic Rate. Sleep 2017;40.
168. White DP. Occlusion pressure and ventilation during sleep in normal humans. J Appl Physiol (1985) 1986;61:1279-87.
169. Horner RL, Hughes SW, Malhotra A. State-dependent and reflex drives to the upper airway: basic physiology with clinical implications. J Appl Physiol (1985) 2014;116:325-36.
170. Phillipson EA, Bowes G. Control of Breathing During Sleep. In: Terjung R, ed. Comprehensive Physiology2011:649-89.
171. Tusiewicz K, Moldofsky H, Bryan AC, Bryan MH. Mechanics of the rib cage and diaphragm during sleep. Journal of applied physiology: respiratory, environmental and exercise physiology 1977;43:600-2.
172. Horner RL, Liu X, Gill H, Nolan P, Liu H, Sood S. Effects of sleep-wake state on the genioglossus vs.diaphragm muscle response to CO(2) in rats. J Appl Physiol (1985) 2002;92:878-87.
173. Winslow DH, Bowden CH, DiDonato KP, McCullough PA. A randomized, double-blind, placebo-controlled study of an oral, extended-release formulation of phentermine/topiramate for the treatment of obstructive sleep apnea in obese adults. Sleep 2012;35:1529-39.
174. Taranto-Montemurro L, Messineo L, Sands SA, et al. The Combination of Atomoxetine and Oxybutynin Greatly Reduces Obstructive Sleep Apnea Severity. A Randomized, Placebo-controlled, Double-Blind Crossover Trial. Am J Respir Crit Care Med 2019;199:1267-76.
175. Carter SG, Eckert DJ. Effects of hypnotics on obstructive sleep apnea endotypes and severity: Novel insights into pathophysiology and treatment. Sleep Med Rev 2021;58:101492.
176. Koutsourelakis I, Minaritzoglou A, Zakynthinos G, Vagiakis E, Zakynthinos S. The effect of nasal tramazoline with dexamethasone in obstructive sleep apnoea patients. Eur Respir J 2013;42:1055-63.
177. Blackman A, Foster GD, Zammit G, et al. Effect of liraglutide 3.0 mg in individuals with obesity and moderate or severe obstructive sleep apnea: the SCALE Sleep Apnea randomized clinical trial. Int J Obes (Lond) 2016;40:1310-9.
178. Fiori CZ, Martinez D, Montanari CC, et al. Diuretic or sodium-restricted diet for obstructive sleep apnea-a randomized trial. Sleep 2018;41.
179. Edwards BA, Sands SA, Eckert DJ, et al. Acetazolamide improves loop gain but not the other physiological traits causing obstructive sleep apnoea. The Journal of physiology 2012;590:1199-211.
180. Wellman A, Malhotra A, Jordan AS, Stevenson KE, Gautam S, White DP. Effect of oxygen in obstructive sleep apnea: role of loop gain. Respir Physiol Neurobiol 2008;162:144-51.
181. Mendelson WB, Maczaj M, Holt J. Buspirone administration to sleep apnea patients. J Clin Psychopharmacol 1991;11:71-2.
182. Marshall NS, Yee BJ, Desai AV, et al. Two randomized placebo-controlled trials to evaluate the efficacy and tolerability of mirtazapine for the treatment of obstructive sleep apnea. Sleep 2008;31:824-31.
183. Berry RB, Yamaura EM, Gill K, Reist C. Acute effects of paroxetine on genioglossus activity in obstructive sleep apnea. Sleep 1999;22:1087-92.
184. Hanzel DA, Proia NG, Hudgel DW. Response of obstructive sleep apnea to fluoxetine and protriptyline. Chest 1991;100:416-21.

185. Schmidt HS. L-tryptophan in the treatment of impaired respiration in sleep. Bulletin europeen de physiopathologie respiratoire 1983;19:625-9.
186. Suratt PM, Wilhoit SC, Brown ED, Findley LJ. Effect of doxapram on obstructive sleep apnea. Bull Eur Physiopathol Respir 1986;22:127-31.
187. Taranto-Montemurro L, Sands SA, Azarbarzin A, et al. Effect of 4-Aminopyridine on Genioglossus Muscle Activity during Sleep in Healthy Adults. Ann Am Thorac Soc 2017;14:1177-83.
188. Prasad B, Radulovacki MG, Carley DW. Proof of concept trial of dronabinol in obstructive sleep apnea. Front Psychiatry 2013;4:1.
189. Zevin S, Swed E, Cahan C. Clinical effects of locally delivered nicotine in obstructive sleep apnea syndrome. Am J Ther 2003;10:170-5.
190. Fleury Curado T, Pho H, Freire C, et al. Designer Receptors Exclusively Activated by Designer Drugs Approach to Treatment of Sleep-disordered Breathing. Am J Respir Crit Care Med 2021;203:102-10.
191. Horton GA, Fraigne JJ, Torontali ZA, et al. Activation of the Hypoglossal to Tongue Musculature Motor Pathway by Remote Control. Sci Rep 2017;7:45860.
192. Horner RL, Grace KP, Wellman A. A resource of potential drug targets and strategic decision-making for obstructive sleep apnoea pharmacotherapy. Respirology 2017;22:861-73.
193. Taranto-Montemurro L, Edwards BA, Sands SA, et al. Desipramine Increases Genioglossus Activity and Reduces Upper Airway Collapsibility during Non-REM Sleep in Healthy Subjects. Am J Respir Crit Care Med 2016;194:878-85.
194. Taranto-Montemurro L, Sands SA, Edwards BA, et al. Desipramine improves upper airway collapsibility and reduces OSA severity in patients with minimal muscle compensation. Eur Respir J 2016;48:1340-50.
195. Brownell LG, West P, Sweatman P, Acres JC, Kryger MH. Protriptyline in obstructive sleep apnea: a double-blind trial. N Engl J Med 1982;307:1037-42.
196. Smith PL, Haponik EF, Allen RP, Bleecker ER. The effects of protriptyline in sleep-disordered breathing. The American review of respiratory disease 1983;127:8-13.
197. Bart Sangal R, Sangal JM, Thorp K. Atomoxetine improves sleepiness and global severity of illness but not the respiratory disturbance index in mild to moderate obstructive sleep apnea with sleepiness. Sleep Med 2008;9:506-10.
198. Chan E, Steenland HW, Liu H, Horner RL. Endogenous excitatory drive modulating respiratory muscle activity across sleep-wake states. American journal of respiratory and critical care medicine 2006;174:1264-73.
199. Lim R, Carberry JC, Wellman A, Grunstein R, Eckert DJ. Reboxetine and hyoscine butylbromide improve upper airway function during nonrapid eye movement and suppress rapid eye movement sleep in healthy individuals. Sleep 2019;42.
200. Lim R, Messineo L, Grunstein R, Carberry J, Eckert D. Reboxetine and hyoscine butylbromide reduce obstructive sleep apnoea severity. Presented in the form of Abstract at ASA 2019, Sydney 2019.
201. Kivipelto L, Majane EA, Yang HY, Panula P. Immunohistochemical distribution and partial characterization of FLFQPQRFamidelike peptides in the central nervous system of rats. J Comp Neurol 1989;286:269-87.
202. Traiffort E, Leurs R, Arrang JM, et al. Guinea pig histamine H1 receptor. I. Gene cloning, characterization, and tissue expression revealed by in situ hybridization. J Neurochem 1994;62:507-18.
203. Bouthenet ML, Ruat M, Sales N, Garbarg M, Schwartz JC. A detailed mapping of histamine H1-receptors in guinea-pig central nervous system established by autoradiography with [125I]iodobolpyramine. Neuroscience 1988;26:553-600.
204. Bastedo T, Chan E, Park E, Liu H, Horner RL. Modulation of genioglossus muscle activity across sleep-wake states by histamine at the hypoglossal motor pool. Sleep 2009;32:1313-24.
205. Liu ZL, Wu X, Luo YJ, et al. Signaling mechanism underlying the histamine-modulated action of hypoglossal motoneurons. J Neurochem 2016;137:277-86.
206. Wang D, Marshall NS, Duffin J, et al. Phenotyping interindividual variability in obstructive sleep apnoea response to temazepam using ventilatory chemoreflexes during wakefulness. J Sleep Res 2011;20:526-32.
207. Hoijer U, Hedner J, Ejnell H, Grunstein R, Odelberg E, Elam M. Nitrazepam in patients with sleep apnoea: a double-blind placebo-controlled study. Eur Respir J 1994;7:2011-5.
208. Park E, Younes M, Liu H, Liu X, Horner RL. Systemic vs. central administration of common hypnotics reveals opposing effects on genioglossus muscle activity in rats. Sleep 2008;31:355-65.

209. Younes M, Park E, Horner RL. Pentobarbital sedation increases genioglossus respiratory activity in sleeping rats. Sleep 2007;30:478-88.
210. Eikermann M, Eckert DJ, Chamberlin NL, et al. Effects of pentobarbital on upper airway patency during sleep. Eur Respir J 2010;36:569-76.
211. Taranto-Montemurro L, Sands SA, Grace KP, et al. Neural memory of the genioglossus muscle during sleep is stage-dependent in healthy subjects and obstructive sleep apnoea patients. J Physiol 2018;596:5163-73.
212. Ratnavadivel R, Chau N, Stadler D, Yeo A, McEvoy RD, Catcheside PG. Marked reduction in obstructive sleep apnea severity in slow wave sleep. J Clin Sleep Med 2009;5:519-24.
213. Taranto-Montemurro L, Sands SA, Edwards BA, et al. Effects of tiagabine on slow wave sleep and arousal threshold in patients with obstructive sleep apnea. Sleep 2017;40.
214. Cirignotta F, Mondini S, Zucconi M, Gerardi R, Farolfi A, Lugaresi E. Zolpidem-polysomnographic study of the effect of a new hypnotic drug in sleep apnea syndrome. Pharmacol Biochem Behav 1988;29:807-9.
215. Cirignotta F, Mondini S, Gerardi R, Zucconi M. Effect of brotizolam on sleep-disordered breathing in heavy snorers with obstructive apnea. Current therapeutic research 1992;51:360-6.
216. Berry RB, Kouchi K, Bower J, Prosise G, Light RW. Triazolam in patients with obstructive sleep apnea. Am J Respir Crit Care Med 1995;151:450-4.
217. Camacho ME, Morin CM. The effect of temazepam on respiration in elderly insomniacs with mild sleep apnea. Sleep 1995;18:644-5.
218. Carberry JC, Fisher LP, Grunstein RR, et al. Role of common hypnotics on the phenotypic causes of obstructive sleep apnoea: paradoxical effects of zolpidem. Eur Respir J 2017;50.
219. George CF, Feldman N, Inhaber N, et al. A safety trial of sodium oxybate in patients with obstructive sleep apnea: Acute effects on sleep-disordered breathing. Sleep Med 2010;11:38-42.
220. Carberry JC, Grunstein RR, Eckert DJ. The effects of zolpidem in obstructive sleep apnea - An open-label pilot study. J Sleep Res 2019:e12853.
221. Rosenberg R, Roach JM, Scharf M, Amato DA. A pilot study evaluating acute use of eszopiclone in patients with mild to moderate obstructive sleep apnea syndrome. Sleep Med 2007;8:464-70.
222. Carter SG, Carberry JC, Cho G, et al. Effect of 1 month of zopiclone on obstructive sleep apnoea severity and symptoms: a randomised controlled trial. Eur Respir J 2018;52.
223. Carter SG, Carberry JC, Grunstein RR, Eckert DJ. Randomized Trial on the Effects of High-Dose Zopiclone on OSA Severity, Upper Airway Physiology, and Alertness. Chest 2020.
224. Basner RC, Onal E, Carley DW, Stepanski EJ, Lopata M. Effect of induced transient arousal on obstructive apnea duration. J Appl Physiol (1985) 1995;78:1469-76.
225. Younes M. Role of respiratory control mechanisms in the pathogenesis of obstructive sleep disorders. J Appl Physiol (1985) 2008;105:1389-405.
226. Saboisky J, Eckert D, Malhotra A. Stable breathing through deeper sleeping. Thorax 2010;65:95-6.
227. Eckert DJ, Malhotra A, Wellman A, White DP. Trazodone increases the respiratory arousal threshold in patients with obstructive sleep apnea and a low arousal threshold. Sleep 2014;37:811-9.
228. Carter SG, Carberry JC, Grunstein RR, Eckert DJ. Randomized trial on the effects of high-dose zopiclone on OSA severity, upper airway physiology and alertness. Chest 2020.
229. Pritchett DB, Seeburg PH. Gamma-aminobutyric acidA receptor alpha 5-subunit creates novel type II benzodiazepine receptor pharmacology. J Neurochem 1990;54:1802-4.
230. Dang A, Garg A, Rataboli PV. Role of zolpidem in the management of insomnia. CNS Neurosci Ther 2011;17:387-97.
231. Uchimura N, Nakajima T, Hayash K, et al. Effect of zolpidem on sleep architecture and its next-morning residual effect in insomniac patients: a randomized crossover comparative study with brotizolam. Prog Neuropsychopharmacol Biol Psychiatry 2006;30:22-9.
232. Edwards BA, Eckert DJ, McSharry DG, et al. Clinical predictors of the respiratory arousal threshold in patients with obstructive sleep apnea. Am J Respir Crit Care Med 2014;190:1293-300.
233. Osman AM, Carberry JC, Burke PGR, Toson B, Grunstein RR, Eckert DJ. Upper airway collapsibility measured using a simple wakefulness test closely relates to the pharyngeal critical closing pressure during sleep in obstructive sleep apnea. Sleep 2019;42.
234. Sleep-related breathing disorders in adults: recommendations for syndrome definition and measurement techniques in clinical research. The Report of an American Academy of Sleep Medicine Task Force. Sleep 1999;22:667-89.

235. Berry RB, Budhiraja R, Gottlieb DJ, et al. Rules for scoring respiratory events in sleep: update of the 2007 AASM Manual for the Scoring of Sleep and Associated Events. Deliberations of the Sleep Apnea Definitions Task Force of the American Academy of Sleep Medicine. J Clin Sleep Med 2012;8:597-619.
236. Nguyen CD, Amatoury J, Carberry JC, Eckert DJ. An automated and reliable method for breath detection during variable mask pressures in awake and sleeping humans. PLoS One 2017;12:e0179030.
237. Azarbarzin A, Ostrowski M, Hanly P, Younes M. Relationship between arousal intensity and heart rate response to arousal. Sleep 2014;37:645-53.
238. Azarbarzin A, Sands SA, Stone KL, et al. The hypoxic burden of sleep apnoea predicts cardiovascular disease-related mortality: the Osteoporotic Fractures in Men Study and the Sleep Heart Health Study. Eur Heart J 2019;40:1149-57.
239. Berry RB, Gleeson K. Respiratory arousal from sleep: mechanisms and significance. Sleep 1997;20:654-75.
240. Morrison JL, Sood S, Liu H, Park E, Nolan P, Horner RL. GABAA receptor antagonism at the hypoglossal motor nucleus increases genioglossus muscle activity in NREM but not REM sleep. J Physiol 2003;548:569-83.
241. Fogel RB, Malhotra A, Pillar G, et al. Genioglossal activation in patients with obstructive sleep apnea versus control subjects. Mechanisms of muscle control. Am J Respir Crit Care Med 2001;164:2025-30.
242. Jordan AS, Wellman A, Heinzer RC, et al. Mechanisms used to restore ventilation after partial upper airway collapse during sleep in humans. Thorax 2007;62:861-7.
243. McGinley BM, Schwartz AR, Schneider H, Kirkness JP, Smith PL, Patil SP. Upper airway neuromuscular compensation during sleep is defective in obstructive sleep apnea. J Appl Physiol (1985) 2008;105:197-205.
244. Carberry JC, Grunstein RR, Eckert DJ. The effects of zolpidem in obstructive sleep apnea - An open-label pilot study. J Sleep Res 2019;28:e12853.
245. Geiger-Brown JM, Rogers VE, Liu W, Ludeman EM, Downton KD, Diaz-Abad M. Cognitive behavioral therapy in persons with comorbid insomnia: A meta-analysis. Sleep Med Rev 2015;23:54-67.
246. Morin CM, Vallieres A, Guay B, et al. Cognitive behavioral therapy, singly and combined with medication, for persistent insomnia: a randomized controlled trial. JAMA 2009;301:2005-15.
247. Roth T, Roehrs T, Vogel G. Zolpidem in the treatment of transient insomnia: a double-blind, randomized comparison with placebo. Sleep 1995;18:246-51.
248. Bjornsdottir E, Janson C, Gislason T, et al. Insomnia in untreated sleep apnea patients compared to controls. J Sleep Res 2012;21:131-8.
249. Bjornsdottir E, Janson C, Sigurdsson JF, et al. Symptoms of insomnia among patients with obstructive sleep apnea before and after two years of positive airway pressure treatment. Sleep 2013;36:1901-9.
250. Sweetman A, Lack L, Catcheside PG, et al. Cognitive and behavioral therapy for insomnia increases the use of continuous positive airway pressure therapy in obstructive sleep apnea participants with comorbid insomnia: a randomized clinical trial. Sleep 2019;42.
251. Lettieri CJ, Shah AA, Holley AB, Kelly WF, Chang AS, Roop SA. Effects of a short course of eszopiclone on continuous positive airway pressure adherence: a randomized trial. Ann Intern Med 2009;151:696-702.
252. Sweetman A, Lack L, McEvoy RD, et al. Cognitive behavioural therapy for insomnia reduces sleep apnoea severity: a randomised controlled trial. ERJ Open Res 2020;6.
253. AMBIEN ® (zolpidem tartrate)[package insert]. U.S. Food and Drug Administration website. https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/019908s046lbl.pdf. Revised August 2019. Accessed October 11, 2019.
254. Burdon JG, Pain MC, Rubinfeld AR, Nana A. Chronic lung diseases and the perception of breathlessness: a clinical perspective. Eur Respir J 1994;7:1342-9.
255. Stilnox (zolpidem tartrate)[package insert]. Australian Therapeutic Goods Administration website. https://www.ebs.tga.gov.au/ebs/picmi/picmirepository.nsf/pdf?OpenAgent&id=CP-2010-PI-05721-3&d=201910111016933. Revised May 2018. Accessed October 11, 2019.
.
256. Mendelson WB, Garnett D, Gillin JC. Flurazepam-induced sleep apnea syndrome in a patient with insomnia and mild sleep-related respiratory changes. J Nerv Ment Dis 1981;169:261-4.
257. Dolly FR, Block AJ. Effect of flurazepam on sleep-disordered breathing and nocturnal oxygen desaturation in asymptomatic subjects. Am J Med 1982;73:239-43.
258. Bonora M, St John WM, Bledsoe TA. Differential elevation by protriptyline and depression by diazepam of upper airway respiratory motor activity. Am Rev Respir Dis 1985;131:41-5.

259. Leufkens TR, Lund JS, Vermeeren A. Highway driving performance and cognitive functioning the morning after bedtime and middle-of-the-night use of gaboxadol, zopiclone and zolpidem. J Sleep Res 2009;18:387-96.
260. Gatti RC, Burke PR, Otuyama LJ, Almeida DR, Tufik S, Poyares D. Effects of Zolpidem CR on Sleep and Nocturnal Ventilation in Patients with Heart Failure. Sleep 2016;39:1501-5.
261. Randall S, Roehrs TA, Roth T. Efficacy of eight months of nightly zolpidem: a prospective placebo-controlled study. Sleep 2012;35:1551-7.
262. Roehrs TA, Randall S, Harris E, Maan R, Roth T. Twelve months of nightly zolpidem does not lead to dose escalation: a prospective placebo-controlled study. Sleep 2011;34:207-12.
263. Taranto-Montemurro L, Messineo L, Wellman A. Targeting Endotypic Traits with Medications for the Pharmacological Treatment of Obstructive Sleep Apnea. A Review of the Current Literature. J Clin Med 2019;8.
264. Taranto-Montemurro L, Messineo L, Azarbarzin A, et al. Effects of the Combination of Atomoxetine and Oxybutynin on Obstructive Sleep Apnea Endotypic Traits. Chest 2020.
265. Messineo L, Eckert DJ, Lim R, et al. Zolpidem increases sleep efficiency and the respiratory arousal threshold without changing sleep apnoea severity and pharyngeal muscle activity. J Physiol 2020.
266. Vakulin A, Baulk SD, Catcheside PG, et al. Effects of alcohol and sleep restriction on simulated driving performance in untreated patients with obstructive sleep apnea. Ann Intern Med 2009;151:447-55.
267. Berry RB, Brooks R, Gamaldo C, et al. AASM Scoring Manual Updates for 2017 (Version 2.4). J Clin Sleep Med 2017;13:665-6.
268. Terrill PI, Edwards BA, Nemati S, et al. Quantifying the ventilatory control contribution to sleep apnoea using polysomnography. Eur Respir J 2015;45:408-18.
269. Huang Y, Mai W, Cai X, et al. The effect of zolpidem on sleep quality, stress status, and nondipping hypertension. Sleep Med 2012;13:263-8.
270. Carter SG, Carberry JC, Grunstein RR, Eckert DJ. Randomized trial on the effects of high-dose zopiclone on OSA severity, upper airway physiology and alertness. Chest 2020;Accepted 22 Feb.
271. Danjou P, Paty I, Fruncillo R, et al. A comparison of the residual effects of zaleplon and zolpidem following administration 5 to 2 h before awakening. Br J Clin Pharmacol 1999;48:367-74.
272. Drover DR. Comparative pharmacokinetics and pharmacodynamics of short-acting hypnosedatives: zaleplon, zolpidem and zopiclone. Clin Pharmacokinet 2004;43:227-38.
273. Roehrs T, Beare D, Zorick F, Roth T. Sleepiness and ethanol effects on simulated driving. Alcohol Clin Exp Res 1994;18:154-8.
274. Verster JC, Volkerts ER, Schreuder AH, et al. Residual effects of middle-of-the-night administration of zaleplon and zolpidem on driving ability, memory functions, and psychomotor performance. J Clin Psychopharmacol 2002;22:576-83.
275. von Moltke LL, Weemhoff JL, Perloff MD, et al. Effect of zolpidem on human cytochrome P450 activity, and on transport mediated by P-glycoprotein. Biopharm Drug Dispos 2002;23:361-7.
276. Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S. The occurrence of sleep-disordered breathing among middle-aged adults. N Engl J Med 1993;328:1230-5.
277. Eckert DJ, White DP, Jordan AS, Malhotra A, Wellman A. Defining phenotypic causes of obstructive sleep apnea. Identification of novel therapeutic targets. Am J Respir Crit Care Med 2013;188:996-1004.
278. Pepin JL, Eastwood P, Eckert DJ. Novel avenues to approach non-CPAP therapy and implement comprehensive OSA care. Eur Respir J 2021.
279. Messineo L, Carter SG, Taranto-Montemurro L, et al. Addition of zolpidem to combination therapy with atomoxetine-oxybutynin increases sleep efficiency and the respiratory arousal threshold in obstructive sleep apnoea: A randomized trial. Respirology 2021;26:878-86.
280. Lim R, Messineo L, Grunstein RR, Carberry JC, Eckert DJ. The noradrenergic agent reboxetine plus the antimuscarinic hyoscine butylbromide reduces sleep apnoea severity: a double-blind, placebo-controlled, randomised crossover trial. J Physiol 2021;599:4183-95.
281. Grace KP, Hughes SW, Horner RL. Identification of the mechanism mediating genioglossus muscle suppression in REM sleep. Am J Respir Crit Care Med 2013;187:311-9.
282. Neuzeret PC, Sakai K, Gormand F, et al. Application of histamine or serotonin to the hypoglossal nucleus increases genioglossus muscle activity across the wake-sleep cycle. J Sleep Res 2009;18:113-21.
283. Vanni-Mercier G, Gigout S, Debilly G, Lin JS. Waking selective neurons in the posterior hypothalamus and their response to histamine H3-receptor ligands: an electrophysiological study in freely moving cats. Behav Brain Res 2003;144:227-41.
284. Haas HL, Sergeeva OA, Selbach O. Histamine in the nervous system. Physiol Rev 2008;88:1183-241.

285. Dauvilliers Y, Verbraecken J, Partinen M, et al. Pitolisant for Daytime Sleepiness in Patients with Obstructive Sleep Apnea Who Refuse Continuous Positive Airway Pressure Treatment. A Randomized Trial. Am J Respir Crit Care Med 2020;201:1135-45.
286. Pepin JL, Georgiev O, Tiholov R, et al. Pitolisant for Residual Excessive Daytime Sleepiness in OSA Patients Adhering to CPAP: A Randomized Trial. Chest 2021;159:1598-609.
287. Lacour M, Sterkers O. Histamine and betahistine in the treatment of vertigo: elucidation of mechanisms of action. CNS Drugs 2001;15:853-70.
288. Arrang JM, Garbarg M, Quach TT, Dam Trung Tuong M, Yeramian E, Schwartz JC. Actions of betahistine at histamine receptors in the brain. Eur J Pharmacol 1985;111:73-84.
289. Sahlholm K, Nilsson J, Marcellino D, Fuxe K, Arhem P. The human histamine H3 receptor couples to GIRK channels in Xenopus oocytes. Eur J Pharmacol 2007;567:206-10.
290. Schlicker E, Fink K, Hinterthaner M, Gothert M. Inhibition of noradrenaline release in the rat brain cortex via presynaptic H3 receptors. Naunyn Schmiedebergs Arch Pharmacol 1989;340:633-8.
291. Clapham J, Kilpatrick GJ. Histamine H3 receptors modulate the release of [3H]-acetylcholine from slices of rat entorhinal cortex: evidence for the possible existence of H3 receptor subtypes. Br J Pharmacol 1992;107:919-23.
292. Perger E, Taranto Montemurro L, Rosa D, et al. Reboxetine Plus Oxybutynin for OSA Treatment: A 1-Week, Randomized, Placebo-Controlled, Double-Blind Crossover Trial. Chest 2021.
293. Grace KP, Hughes SW, Horner RL. Identification of the Mechanism Mediating Genioglossus Muscle Suppression in REM Sleep. Am J Respir Crit Care Med 2013;187:311-9.
294. Lim R, Carberry JC, Wellman A, Grunstein R, Eckert DJ. Reboxetine and hyoscine butylbromide improve upper airway function during nonrapid eye movement and suppress rapid eye movement sleep in healthy individuals. Sleep 2019;42:pii: zsy261.
295. Sands SA, Terrill PI, Edwards BA, et al. Quantifying the Arousal Threshold Using Polysomnography in Obstructive Sleep Apnea. Sleep 2018;41.
296. Feldman JL, Mitchell GS, Nattie EE. Breathing: rhythmicity, plasticity, chemosensitivity. Annu Rev Neurosci 2003;26:239-66.
297. Dutschmann M, Bischoff AM, Busselberg D, Richter DW. Histaminergic modulation of the intact respiratory network of adult mice. Pflugers Arch 2003;445:570-6.
298. Lazarov N, Rozloznik M, Reindl S, Rey-Ares V, Dutschmann M, Gratzl M. Expression of histamine receptors and effect of histamine in the rat carotid body chemoafferent pathway. Eur J Neurosci 2006;24:3431-44.
299. Haxhiu MA, Cherniack NS, Altose MD, Kelsen SG. Effect of histamine on respiratory chemosensitivity in conscious goats. Respiration 1983;44:411-8.
300. Del Rio R, Moya EA, Koenig CS, Fujiwara K, Alcayaga J, Iturriaga R. Modulatory effects of histamine on cat carotid body chemoreception. Respir Physiol Neurobiol 2008;164:401-10.
301. Moorthy G, Sallee F, Gabbita P, Zemlan F, Sallans L, Desai PB. Safety, tolerability and pharmacokinetics of 2-pyridylacetic acid, a major metabolite of betahistine, in a phase 1 dose escalation study in subjects with ADHD. Biopharm Drug Dispos 2015;36:429-39.
302. Nemati S, Edwards BA, Sands SA, et al. Model-based characterization of ventilatory stability using spontaneous breathing. J Appl Physiol (1985) 2011;111:55-67.
303. Edwards BA, Sands SA, Skuza EM, et al. Increased peripheral chemosensitivity via dopaminergic manipulation promotes respiratory instability in lambs. Respir Physiol Neurobiol 2008;164:419-28.
304. Delpierre S, Fornaris M, Guillot C, Grimaud C. Increased ventilatory chemosensitivity induced by domperidone, a dopamine antagonist, in healthy humans. Bull Eur Physiopathol Respir 1987;23:31-5.
305. Schinkel AH, Wagenaar E, Mol CA, van Deemter L. P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. J Clin Invest 1996;97:2517-24.
306. Sampson MG, Grassino K. Neuromechanical properties in obese patients during carbon dioxide rebreathing. Am J Med 1983;75:81-90.
307. Carroll MS, Patwari PP, Kenny AS, Brogadir CD, Stewart TM, Weese-Mayer DE. Residual chemosensitivity to ventilatory challenges in genotyped congenital central hypoventilation syndrome. J Appl Physiol (1985) 2014;116:439-50.
308. Weil JV, McCullough RE, Kline JS, Sodal IE. Diminished ventilatory response to hypoxia and hypercapnia after morphine in normal man. N Engl J Med 1975;292:1103-6.
309. Robinson RW, Zwillich CW, Bixler EO, Cadieux RJ, Kales A, White DP. Effects of oral narcotics on sleep-disordered breathing in healthy adults. Chest 1987;91:197-203.

310. Altree TJ, Chung F, Chan MTV, Eckert DJ. Vulnerability to Postoperative Complications in Obstructive Sleep Apnea: Importance of Phenotypes. Anesth Analg 2021;132:1328-37.
311. Martins RT, Carberry JC, Wang D, Rowsell L, Grunstein RR, Eckert DJ. Morphine alters respiratory control but not other key obstructive sleep apnoea phenotypes: a randomised trial. Eur Respir J 2020;55.
312. Lacour M. Betahistine treatment in managing vertigo and improving vestibular compensation: clarification. J Vestib Res 2013;23:139-51.
313. Taranto-Montemurro L, Messineo L, Azarbarzin A, et al. Effects of the Combination of Atomoxetine and Oxybutynin on OSA Endotypic Traits. Chest 2020;157:1626-36.
314. Schafer H, Koehler U, Ploch T, Peter JH. Sleep-related myocardial ischemia and sleep structure in patients with obstructive sleep apnea and coronary heart disease. Chest 1997;111:387-93.
315. Dyken ME, Im KB. Obstructive sleep apnea and stroke. Chest 2009;136:1668-77.
316. Findley LJ, Wilhoit SC, Suratt PM. Apnea duration and hypoxemia during REM sleep in patients with obstructive sleep apnea. Chest 1985;87:432-6.
317. Carberry JC, Jordan AS, White DP, Wellman A, Eckert DJ. Upper Airway Collapsibility (Pcrit) and Pharyngeal Dilator Muscle Activity are Sleep Stage Dependent. Sleep 2016;39:511-21.
318. Okabe S, Hida W, Kikuchi Y, Taguchi O, Takishima T, Shirato K. Upper airway muscle activity during REM and non-REM sleep of patients with obstructive apnea. Chest 1994;106:767-73.
319. Eckert DJ, Malhotra A, Lo YL, White DP, Jordan AS. The influence of obstructive sleep apnea and gender on genioglossus activity during rapid eye movement sleep. Chest 2009;135:957-64.
320. Arrigoni E, Chen MC, Fuller PM. The anatomical, cellular and synaptic basis of motor atonia during rapid eye movement sleep. J Physiol 2016;594:5391-414.
321. Brooks PL, Peever JH. Identification of the transmitter and receptor mechanisms responsible for REM sleep paralysis. J Neurosci 2012;32:9785-95.
322. Wiegand L, Zwillich CW, Wiegand D, White DP. Changes in upper airway muscle activation and ventilation during phasic REM sleep in normal men. J Appl Physiol 1991;71:488-97.
323. Wellman A, Eckert DJ, Jordan AS, et al. A method for measuring and modeling the physiological traits causing obstructive sleep apnea. J Appl Physiol 2011;110:1627-37.
324. Loewen AH, Ostrowski M, Laprairie J, Maturino F, Hanly PJ, Younes M. Response of genioglossus muscle to increasing chemical drive in sleeping obstructive apnea patients. Sleep 2011;34:1061-73.
325. Eastwood PR, Szollosi I, Platt PR, Hillman DR. Collapsibility of the upper airway during anesthesia with isoflurane. Anesthesiology 2002;97:786-93.
326. Won CH, Reid M, Sofer T, et al. Sex Differences in Obstructive Sleep Apnea Phenotypes, the Multi-Ethnic Study of Atherosclerosis. Sleep 2019.
327. Joosten SA, Landry SA, Wong AM, et al. Assessing the Physiologic Endotypes Responsible for REM and NREM Based OSA. Chest 2020.
328. Sands SA, Eckert DJ, Jordan AS, et al. Enhanced Upper-airway Muscle Responsiveness is a Distinct Feature of Overweight/Obese Individuals Without Sleep Apnea. Am J Respir Crit Care Med 2014.
329. Edwards BA, Eckert DJ, Jordan AS. Obstructive sleep apnoea pathogenesis from mild to severe: Is it all the same? Respirology 2017;22:33-42.
330. Millman RP, Knight H, Kline LR, Shore ET, Chung DC, Pack AI. Changes in compartmental ventilation in association with eye movements during REM sleep. J Appl Physiol (1985) 1988;65:1196-202.
331. Messineo L, Taranto-Montemurro L, Azarbarzin A, et al. Loop gain in REM versus non-REM sleep using CPAP manipulation: A pilot study. Respirology 2019;24:805-8.