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A resource of potential drug targets and strategic decision-making for obstructive sleep apnoea pharmacotherapy

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ABSTRACT

There is currently no pharmacotherapy for obstructive sleep apnoea (OSA) but there is no principled a priori reason why there should not be one. This review identifies a rational decision-making strategy with the necessary logical underpinnings that any reasonable approach would be expected to navigate to develop a viable pharmacotherapy for OSA. The process first involves phenotyping an individual to quantify and characterize the critical predisposing factor(s) to their OSA pathogenesis and identify, a priori, if the patient is likely to benefit from a pharmacotherapy that targets those factors. We then identify rational strategies to manipulate those critical predisposing factor(s), and the barriers that have to be overcome for success of any OSA pharmacotherapy. A new analysis then identifies candidate drug targets to manipulate the upper airway motor circuitry for OSA pharmacotherapy. The first conclusion is that there are two general pharmacological approaches for OSA treatment that are of the most potential benefit and are practically realistic, one being fairly intuitive but the second perhaps less so. The second conclusion is that after identifying the critical physiological obstacles to OSA pharmacotherapy, there are current therapeutic targets of high interest for future development. The final analysis provides a tabulated resource of 'druggable' targets that are relatively restricted to the circuitry controlling the upper airway musculature, with these candidate targets being of high priority for screening and further study. We also emphasize that a pharmacotherapy may not cure OSA *per se*, but may still be a useful adjunct to improve the effectiveness of, and adherence to, other treatment mainstays.

Key words: neurobiology, obstructive sleep apnoea, phenotype, sleep, translational medical research.

Abbreviations: 5-HT$_{2c}$, 5-hydroxytryptamine 2c; AHI, apnoea–hypopnoea index; CFTR/MRP, Cystic fibrosis transmembrane conductance regulator/Multidrug resistance-associated protein; CPAP, continuous positive airway pressure; FDA, Food and Drug Administration; GABA: γ-aminobutyric acid; GPCR, G-protein coupled receptor; HMN, hypoglossal motor nucleus; ISH, in situ hybridization; Kir2.4, inward-rectifying potassium 2.4; nCPAP, nasal CPAP; NMDA3B, N-methyl-D-aspartate receptor 3B; OSA, obstructive sleep apnoea; PaCO$_2$, partial pressure of carbon dioxide; PIP3, phosphatidylinositol 3,4,5 trisphosphate; PRE-HMN, premotor HMN; REM, rapid eye movement.

INTRODUCTION

Strategic decisions are long-term complex choices that affect the subsequent course of action for a large entity like a business or a field. Strategic decision-making in business can result in a corporation breaking new ground and becoming a market leader. Strategic decision-making in physiology and medicine can lead to breaking new ground for a medical problem in a distinct patient population, leading to new, or better, treatment options or outcomes.

Obstructive sleep apnoea (OSA) is a common and serious breathing problem with significant clinical, social and economic consequences.[1–3] There is currently no pharmacotherapy for OSA. However, there is no principled a priori reason why there should not be one—unless the biology simply cannot support it because of the complexity and individuality of OSA pathogenesis. The main thrust of this review is to identify a decision-making strategy with the necessary logical underpinnings that any reasonable approach would be expected to navigate in order to develop a viable pharmacotherapy for OSA—while also avoiding the identifiable and sometimes predictable pitfalls. We

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then identify rational strategies to manipulate those critical predisposing factor(s), and the typically overlooked barriers that have to be overcome for success of any OSA pharmacotherapy. This review also provides a new analysis that identifies candidate drug targets to manipulate the upper airway motor circuitry for OSA pharmacotherapy. This analysis will be a valuable resource for the field moving forward.

It is also necessary at the outset to state upfront that this review does *not* take the stance that potential pharmacotherapy for OSA is automatically an alternate for mainstays of treatment such as nasal continuous positive airway pressure (nCPAP). Although treatment of OSA with nCPAP is effective when the prescribed pressures are tolerated and present throughout the night, suboptimal adherence and effectiveness are common.[4,5] In this context, a pharmacotherapy for OSA may be viewed in the first instance as a useful adjunct to help improve upper airway stability and/or stabilize breathing. Such an intervention may, therefore, improve the effectiveness of, and adherence to, other treatment mainstays, for example, by reducing the absolute pressure required for effective nCPAP therapy or the amount of jaw repositioning for effective oral appliance therapy. We also recognize that other strategies to increase tongue muscle tone via non-pharmacological means, for example, surgically implanted upper airway stimulation devices,[6–8] may also prove effective for the treatment of OSA.

PHENOTYPING OSA PATIENTS IS CRITICAL TO TARGETED THERAPY

Regardless of OSA severity, all OSA patients present with a trio of characteristic features: (i) repeated episodes of upper airway obstruction that occur *only* during sleep, accompanied by (ii) repeated episodes of obstruction-related asphyxia, plus (iii) sleep disturbance. The simplicity and reproducibility of these *presenting* features in all patients, however, masks the complexity and individuality of the underlying physiology in each patient that *initiates* the repetitive cycles of OSA in the first place. The first key point to be made here is that varying combinations of predisposing factors contribute to the pathogenesis of OSA within a given individual: *that is, different individuals have OSA for different reasons and these reasons (traits) can now be identified and quantified.*[9–14]

The main predisposing factors to the manifestation of OSA include: (i) an anatomical predisposition to upper airway obstructions; (ii) diminished effectiveness of the upper airway neural compensatory mechanisms that are either already present to prevent an obstruction or recruited to reopen the airspace; (iii) enhanced loop gain that predisposes to respiratory control instability and sleep apnoea; (iv) arousals from sleep that also destabilize breathing and can precipitate further sleep apnoea after an initial event has occurred; (v) decreased functional residual capacity that occurs in sleep, and is exacerbated by obesity, that increases upper airway collapsibility; and/or (vi) rostral fluid shifts when adopting the supine position that may also increase upper airway collapsibility.[9–13,15]

The second key point related to these physiological factors that can initiate and sustain OSA is that any one (or combination) of them can be critical to pathogenesis within and between patients. More specifically, while the presence of one or more of these factors may be the tipping point for OSA pathogenesis in a particular patient, the combination of critical factors may be quite different for another. For example, Patients A and B may have similar OSA severities as judged by their respective apnoea–hypopnoea indices (AHIs). However, the key elements to OSA pathogenesis in Patient A may be an anatomical predisposition to upper airway obstructions plus diminished pharyngeal dilator muscle compensatory reflexes that are insufficient to reopen the airspace once closed. In contrast, the key predisposing factors causing OSA in Patient B may be enhanced loop gain and heightened arousal responses, both of which destabilize breathing and can precipitate recurrent apnoeas.[9–13] Treatment with nCPAP would be beneficial to both patients but if they are non-compliant, what is the alternative? The principle that arises from this discussion is that in failed or restricted nCPAP users, personalized therapy targeting the primary factors(s) contributing to OSA pathogenesis would benefit those individual patients. Importantly, however, the choice of personalized treatment other than nCPAP for Patient A would be different than for Patient B. Patient A would likely benefit the most from oral appliance therapy, whereas Patient B may be better served by a pharmacological intervention to lower loop gain and arousal threshold.[10,16,17] Also importantly, swapping therapy between patients would be particularly ineffective (e.g. Patient A instead receiving a pharmacological intervention to lower loop gain and arousal threshold and Patient B receiving oral appliance therapy), as the interventions would not target the primary causative factor in those individuals.

The outcome of this initial discussion is that *any* clinical trial of *any* potential therapeutic option in unselected patients with OSA (other than with nCPAP) will likely show some responders, partial responders and non-responders within the cohort. This mix of outcomes is entirely predictable because different individuals have OSA for different reasons. Even if the pharmacotherapy is 100% effective in modulating the mechanism it is designed for, the response will be successful only in a subset of patients because only in those will the targeted mechanism be relevant to their OSA. It is possible (even likely), therefore, that some potentially useful therapeutic strategies may have been prematurely dismissed because the intervention was tested in a group of unselected OSA patients, many of whom would not be expected to respond given the differing factors that are responsible for their particular OSA phenotype.

It is for these reasons that here we identify the first strategic requirement before moving forward to developing a potential pharmacotherapy for OSA: *First, phenotype an individual OSA patient to characterize and quantify their critical predisposing factors and identify, a priori, if the patient is likely to benefit from a pharmacotherapy that targets their major predisposing factor(s).* Once a patient has been phenotyped and the critical factors predisposing to their OSA have been identified, the second necessary key strategic decision is: *Identify current rational and viable pharmacologic targets(s) and the barriers to be overcome for their success.* Finally, we

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identify and discuss the third strategic decision: *Identify the next generation of targets and approaches that may be brought to bear on this problem.* The physiological bases for these three key strategic decisions of potential therapeutic value are discussed in the following sections.

PHENOTYPING PATIENTS WITH OSA AND POTENTIAL PHARMACOLOGICAL TARGETS ARISING

Here, we identify a procedure for measuring and modelling the phenotypic traits causing OSA. While this procedure is not the only way of quantifying the phenotypic traits, it is detailed here for several reasons. It will serve as a platform for defining each of the phenotypic traits. It will also demonstrate how the different phenotypic traits interact to produce OSA and thus will also serve as a review of the multifactorial nature of this disorder. Finally, it will also provide the framework for categorizing different potential pharmacological approaches to treating OSA. It is also noted that while the techniques presented here may currently be too costly for routine clinical sleep studies, less expensive methods for phenotyping are being developed and tested.[18–21] These less expensive phenotyping methods are based on the same principles presented here but differ in subtle ways, making the derived parameters accessible from the clinical polysomnogram.

The main aim of this section of the review is to emphasize that, in the opinion of these authors, there are two general pharmacological approaches to OSA treatment that are of the most potential benefit and practically realistic. The first approach is to improve the anatomy and/or pharyngeal dilator muscle activation. This approach is fairly intuitive but anatomical approaches using surgery and mechanical devices are not universally effective or tolerated, and the pharmacological agents and targets to achieve this goal have remained elusive (although there are rational and newly identified strategic directions that are identified in later sections). The second approach to the pharmacological treatment of OSA is perhaps less intuitive and appreciated, but may be more practically realistic in the short term given currently available pharmacological agents. This second approach is to reduce the ventilatory control sensitivity and/or raise the arousal threshold. It is the aim of this review to summarize the logical and physiological framework for these approaches in OSA patients.

The components of the phenotype model

The phenotypic traits associated with OSA (as described here) can be quantified as four 'ventilation levels'.[11] As outlined below and illustrated schematically in Figure 1, these four ventilation levels can be measured by adjusting nCPAP during sleep and then measuring the associated changes in ventilation. Following the description of each of the ventilation levels and how they relate to the phenotypic traits, a physiological model of breathing will be described. It is this model that will make clear what the potential therapeutic targets are for OSA.

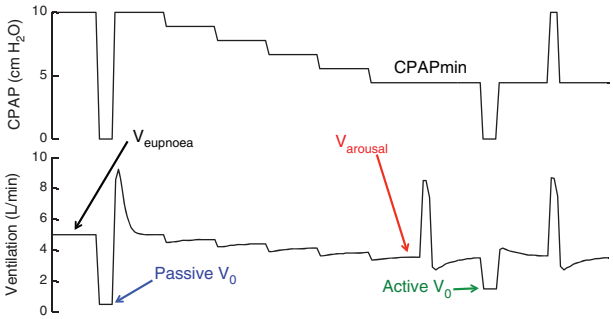

Figure 1 Schematic drawing of the continuous positive airway pressure (CPAP) manipulations used to measure the phenotypic traits. Active V_0, ventilation off CPAP (CPAP of 0 cm H_2O) when the upper airway muscles are active; CPAPmin, minimum tolerable CPAP, which is the minimum CPAP that an individual can tolerate before having respiratory effort-related arousals; Passive V_0, ventilation off CPAP (i.e. at a CPAP of 0 cm H_2O) when the upper airway muscles are passive; $V_{arousal}$, the ventilation at which arousal occurs or the 'minimum tolerable ventilation'; $V_{eupnoea}$, eupnoeic ventilation on optimum CPAP, which is a measure of the patient's ventilatory requirements under resting conditions when the airway is completely open.

$V_{eupnoea}$

The first ventilation level determined in the phenotyping procedure is the eupnoeic ventilation during sleep when the airway is open (abbreviated $V_{eupnoea}$). This value is the patient's ventilatory demand or ventilatory requirement during sleep, based on their metabolic rate and dead space ventilation. It is obtained from measuring the minute ventilation on optimum nCPAP. In the simulated data in Figure 1, the $V_{eupnoea}$ is 5 L/min. Note that, while the $V_{eupnoea}$ as well as the other phenotypic components presented in Figure 1 are presented as constant values, in reality these variables vary over the course of the night in an individual, for example, by $\pm$ 0.5 L/min.[11]

Passive V_0

Next, the nCPAP is abruptly dropped from an optimum level to 0 cm H_2O for five breaths. This intervention is repeated several times. The resulting ventilation at 0 cm H_2O is the ventilation that the patient can achieve through a passive airway, and it is a measure of their *passive airway collapsibility*. This designation is because the stiffness of the upper airway is affected by pharyngeal muscle activation, and nCPAP reduces this activation. Furthermore, the muscles remain relatively inactive, or 'passive', for the few breaths after nCPAP is abruptly dropped;[22] hence the term 'Passive V_0' defined as the ventilation achieved through this passive upper airway at 0 cm H_2O. In the accompanying example, the Passive V_0 is 0.5 L/min. This low level of Passive V_0 indicates a significantly collapsible airway.

$V_{arousal}$

After several Passive V_0 measurements are made, nCPAP is slowly reduced by 1 cm H_2O for every 1–2 min. During this slow nCPAP reduction, the airway becomes progressively narrower, leading to gradually

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reduced ventilation and increased $PaCO_2$ (partial pressure of carbon dioxide). The increased $PaCO_2$ and ventilatory drive eventually leads to an arousal from sleep. Provided that the ventilation was reduced slowly enough, the ventilation prior to arousal is a measure of how much ventilation can be decreased before the patient arouses from sleep, that is $V_{arousal}$ is the patient's 'minimum tolerable ventilation' during sleep. The $V_{arousal}$ is influenced by two factors; the *arousal threshold* and the *sensitivity of the ventilatory control system*. The lower the arousal threshold, or the more sensitive the ventilatory control system, the higher the $V_{arousal}$ will be, thus indicating that the patient can only tolerate a small reduction in ventilation before waking up. Therefore, $V_{arousal}$ is a composite measurement of both arousal threshold and ventilatory control sensitivity. In Figure 1, $V_{arousal}$ is 3.5 L/min, which is 1.5 L/min below $V_{eupnoea}$ and represents a relatively low $V_{arousal}$ compared with $V_{eupnoea}$.

Active V_0

The fourth phenotypic trait is the amount of ventilation the patient can achieve through an activated pharyngeal airway. This value is termed the 'Active V_0', or the ventilation achieved at 0 cm H_2O when the pharyngeal muscles are activated as much as possible without arousing from sleep. Active V_0 is measured as follows. After the measurement of $V_{arousal}$ (described above), the nCPAP level is maintained at the same low level (termed the 'CPAPmin') for 1–3 min to see if arousal occurs. If not, then the nCPAP is decreased to 0 cm H_2O for five breaths to measure how much ventilation can be achieved when ventilatory drive to the upper airway is maximal. The difference between Active V_0 and Passive V_0 is an indicator of the *ability of the patient's upper airway to compensate* (i.e. to reopen) by itself based on reflex compensatory mechanisms and/or pharmacologically driven increased pharyngeal muscle tone. The simulated patient depicted in Figure 1 has mild compensatory ability, as indicated by the Active V_0 being only 1 L/min above Passive V_0.

Manipulating the components of the phenotype model

Measuring the phenotypic traits is only the first step; the second step is to determine which of the traits should be treated. To this end, the four ventilation levels are plotted (Fig. 2), and this representation of the phenotype model illustrates how the phenotypic traits interact to produce OSA. Figure 2 can be 'read' as follows. Starting at the top, $V_{eupnoea}$ is the minute ventilation measured on optimum nCPAP. If nCPAPs were to be turned off abruptly (i.e. simulating sleep onset), then ventilation would decrease to Passive V_0. Breathing at Passive V_0 causes the $PaCO_2$ and ventilatory drive to increase. The increase in ventilatory drive stimulates the upper airway muscles and increases ventilation according to the reflex upper airway compensatory effectiveness of the particular patient. If the compensatory ventilation, or Active V_0, cannot be brought above the minimum tolerable ventilation (i.e. $V_{arousal}$), then the patient arouses from sleep,

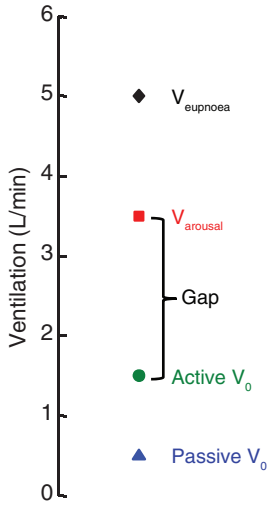

Figure 2 The phenotype model showing the relationship between the four ventilation variables and the 'Gap' that must be overcome to achieve stable breathing. Active V_0, ventilation off continuous positive airway pressure (CPAP) (CPAP of 0 cm H_2O) when the upper airway muscles are active; Passive V_0, ventilation off CPAP (i.e. at a CPAP of 0 cm H_2O) when the upper airway muscles are passive; $V_{arousal}$, the ventilation at which arousal occurs, or the 'minimum tolerable ventilation'; $V_{eupnoea}$, eupnoeic ventilation on optimum CPAP, which is a measure of the patient's ventilatory requirements under resting conditions when the airway is completely open.

hyperventilates temporarily until sleep resumes and the cycle repeats. This cyclical pattern of breathing defines their OSA. If, however, the Active V_0 *can* be brought above $V_{arousal}$, then stable breathing occurs, that is there is no OSA. Therefore, to eliminate OSA, the maximum achievable ventilation during sleep (Active V_0) of a patient must be brought above the minimum tolerable ventilation ($V_{arousal}$). The difference between $V_{arousal}$ and Active V_0 is the 'gap' that must be overcome to prevent OSA.

Having provided measured estimates of the phenotypic traits in an individual and placed them on the model, it is then possible to *simulate treatment* and determine which therapy would be the most appropriate based on these measures (Fig. 3). For example, previous studies have shown that acetazolamide reduces the sensitivity of the ventilatory control system, which would lower $V_{arousal}$, that is the patient can tolerate lower ventilation during sleep.[23] In addition, the hypnotic eszopiclone raises the arousal threshold,[24] which also has the effect of lowering $V_{arousal}$. These two treatments can be simulated by moving $V_{arousal}$ down by some amount based on the effect size of these treatments on $V_{arousal}$ (see the downward arrow in Fig. 3). Therefore, it is important to know the effect size of a particular treatment on a particular trait in order to predict responsiveness to that treatment within an individual; for example, 'Patient B' as introduced in the *Phenotyping OSA patients is critical to targeted therapy* Section.

To simulate an anatomical intervention, such as oral appliance therapy or a pharmacotherapy that stimulates the pharyngeal muscles, the Passive V_0 and Active

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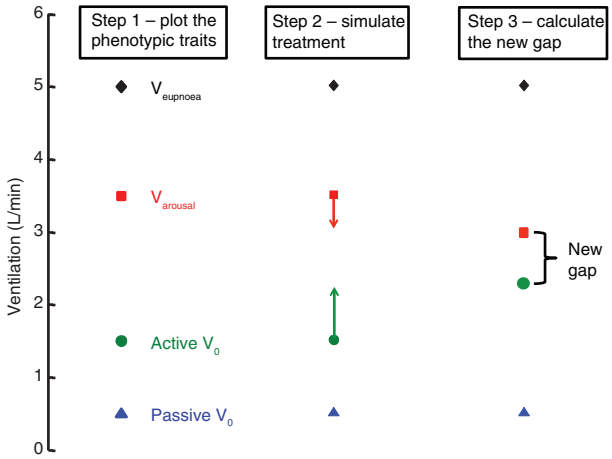

Figure 3 Predictions can be made about the response to a particular therapy by simulating treatment on the model diagram and then calculating the new gap between key parameter values. In this example, the new gap, which is the difference between the new $V_{arousal}$ and the new Active V_0, is +0.8 L/min (i.e. a slightly positive value). Therefore, the model predicts that the patient would likely fail this particular treatment. Active V_0, ventilation off continuous positive airway pressure (CPAP) (CPAP of 0 cm H_2O) when the upper airway muscles are active; Passive V_0, ventilation off CPAP (i.e. at a CPAP of 0 cm H_2O) when the upper airway muscles are passive; $V_{arousal}$, the ventilation at which arousal occurs, or the 'minimum tolerable ventilation'; $V_{eupnoea}$, eupnoeic ventilation on optimum CPAP, which is a measure of the patient's ventilatory requirements under resting conditions when the airway is completely open.

V_0 can be increased by some amount, again based on experimental data. The third step is to calculate the 'new gap', which is calculated as follows: New gap = Simulated $V_{arousal}$ – Simulated Active V_0. If the new gap is positive, then the patient is predicted to still have OSA on the treatment in question. Alternatively, if the new gap is negative, then it is predicted that OSA will be attenuated or resolved with the treatment. In theory, the more negative the new gap, the greater the likelihood of success with a particular treatment. The more positive the new gap, however, the less likelihood that the treatment will be clinically effective.

This modelling approach identifies the two general strategies that can be undertaken to treat OSA. The first strategy, which is the most obvious, involves treatments that increase the size of the pharynx using devices or surgeries, and/or drugs, to stimulate the pharyngeal muscles. The second strategy, which is less intuitive but potentially easier to target pharmacologically, involves reducing the ventilatory requirements of the patient. It is also worth mentioning that the second strategy would *only* be appropriate in patients who can (at least partially) open their airway during sleep via reflex compensatory mechanisms; otherwise, harmful levels of hypoventilation may occur. Nevertheless, it is a potentially viable pharmacotherapy for those patients that arouse prematurely in response to a ventilatory stimulus. Moreover, it might be useful as a combination therapy with the first strategy to achieve greater effects on OSA. For example, the use of an existing or newly discovered pharmacotherapy or mechanical device to open the upper airway may prove incompletely effective in some individuals. In such circumstances, the addition of a drug that suppresses arousals or permits lower ventilation during sleep might stabilize breathing and thus eliminate or substantially reduce OSA.

More research is needed to identify a pharmacotherapy or combination of pharmacotherapies that can manipulate the particular phenotypic traits identified as significant to the pathogenesis of OSA within an individual patient, and to test the predictive ability of modelling tools such as the one described above. In addition, the procedures for identifying the different OSA phenotypes will need to be made clinically feasible. To this end, research has begun on techniques for estimating surrogate measures of the traits from spontaneously breathing subjects during clinical sleep studies.[18–21] There may also be other ways to phenotype OSA using additional measurements. However, those approaches would most likely require measurements of 'ventilatory drive' using an oesophageal catheter or diaphragm electromyogram, both of which would be difficult to implement routinely in a clinical setting. Other potential ways to phenotype (non-invasively) are to try to fit the ventilation data to a physiological model[21] or perform extra CPAP manoeuvres to measure 'loop gain' or 'arousal threshold'.[11] It is important that these methodologies be developed in concert with the search for new rational pharmacological targets for OSA, to identify who may benefit the most from particular interventions. This combination of phenotyping and targeted therapy is the strategy of choice compared with performing trials that include all patients a subset of which, a priori, would not be expected to benefit because the therapeutic target is not relevant to their OSA phenotype.

STRATEGIC DIRECTIONS FROM THE PHENOTYPE MODEL AND IDENTIFYING KEY BARRIERS FOR OSA PHARMACOTHERAPY

With a view to targeting the critical predisposing factor(s) for an individual's OSA pathogenesis, agents that modify key elements of the 'phenotype model' are of the highest strategic interest. To this end, one of the two major strategic directions is to decrease $V_{arousal}$, that is the patient's minimum tolerable ventilation during sleep. Given that $V_{arousal}$ is a composite measure of ventilatory control sensitivity and arousal threshold, a decrease in the former and/or an increase in the latter would result in a beneficial decrease in $V_{arousal}$ in those individuals in whom this factor is significant to their OSA pathogenesis. Interventions targeting ventilatory control sensitivity and/or arousal threshold, for example, using acetazolamide and eszopiclone, respectively,[23–25] were introduced in the previous section and are the subject of recent reviews[26,27] and other studies (e.g. Refs 28–32). For this reason, this section will mainly focus on other developments that identify targets of high interest relevant to the second major strategic direction in OSA treatment by pharmacotherapy. This second strategic direction is to increase

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Active V_0 using agents that target an effector that increases pharyngeal muscle tone and/or reflex compensatory mechanisms. Here, the aim is to effectively increase the size and compensatory effectiveness of the pharyngeal airway in those individuals in whom this factor is critically significant to their OSA pathogenesis. Importantly, however, there are significant physiological obstacles that are typically overlooked but need to be explicitly identified and overcome for any pharmacological approach to OSA to be successful. If these impediments are not overcome, then the likelihood of a successful pharmacotherapy is low.

In a previous article in *Respirology* that focused on controversies and future directions in OSA pathogenesis and treatment, the critical barriers that have to be overcome for any success of a potential OSA pharmacotherapy were identified.[33] Here, we update and further discuss these barriers, and identify rational 'druggable' targets(s) that may be on the horizon to overcome them. This approach is necessary for this review because previous attempts at OSA pharmacotherapy have largely been ineffective (for reviews, see Refs 33–38) and in many ways this has been predictable based on the targets chosen, and because the trials were comprised of unphenotyped OSA patients, only a subset of which would be expected to benefit even if the pharmacological target was rational and effectively manipulated (discussed in *Manipulating the components of the phenotype model* Section). More recent clinical studies, however, using agents targeting the significant mechanisms of state-dependent pharyngeal muscle control identified from basic science experiments,[39–42] have proved beneficial when administered to phenotyped patients,[43,44] with other studies ongoing.[45–47]

The process of identifying an effective OSA pharmacotherapy involves satisfying four overarching requirements: (i) *effectively administering* a pharmacotherapy that (ii) *selectively targets* the critical mechanisms involved in an individual's OSA pathogenesis (iii) at the *required times* (i.e. throughout the sleep period) and (iv) with *minimal* side effects.[33] The latter point is significant because the major neurotransmitters involved in modulating pharyngeal muscle tone across sleep–wake states[40,48] are also involved in sleep–wake regulation and other components of cognition, mood, as well as brain and autonomic functions (e.g. Ref. 49), such that some off-target effects are likely.

Once potential therapeutic approaches and targets have been identified that can satisfy the above overarching requirements, there are an additional set of more specific obstacles that need to be overcome for an effective OSA therapy. These obstacles are identified as: (i) the *delivery problem*, defined as the agent effectively getting to the desired target site(s) to exert its beneficial effect; (ii) the *specificity problem*, defined as the agent exerting minimal effects at other sites to obscure, or oppose, the beneficial response; (iii) the *pharmacological problem*, identified as any efficacious response being obscured by unwanted off-target effects due, for example, to issues of dose and/or specificity for the intended versus unintended receptors/ion channels; (iv) the *neurobiology problem*, in this case identified as different responses to the pharmacotherapy occurring in non-rapid eye movement (non-REM) versus REM sleep because of the different brain chemistry comprising these states; and (v) the *variability problem*, which has to take into account that the physical sites of upper airway obstruction vary *within* and *between* patients both across non-REM and REM sleep, and in the supine and lateral body positions, such that the pharmacotherapy may be effective at some times and not others, even in the same patient.[50]

Based on the identification of the overarching requirements for an OSA pharmacotherapy, this review will conclude by discussing potential therapeutic targets of high interest in the circuitry controlling upper airway motor output for the purpose of modulating Active V_0.

MAPPING POTENTIAL DRUG TARGETS IN THE CIRCUITRY CONTROLLING UPPER AIRWAY MOTOR OUTPUT

It is not clear whether the difficulty in treating OSA pharmacologically reflects a deficiency in effective targets or a deficiency in target identification. The pharmacological tractability of a disorder depends on the differential expression of druggable targets in the underlying physiological systems that can be modulated for clinical benefit whilst limiting toxicity. There is now sufficient data to support genome-wide analyses of the differential expression of druggable targets in the circuitry controlling motor output to the upper airway musculature, and this review provides that analysis.

The approach used to identify the candidate drug targets

For the purposes of this review, we used the differential search function of the Allen Mouse Brain Atlas' database of ~25 000 in situ hybridization experiments to isolate genes that differentiate the hypoglossal motor nucleus (HMN) and the primary afferents of the HMN from the rest of the brain.[51,52] Separate differential searches were performed for the HMN and a grouping of HMN premotor structures (premotor HMN (PRE-HMN)). A listing of the primary hypoglossal afferents was taken from previous tracing studies.[53,54] These studies exploit the retrograde transport capabilities of viral tracers to produce an especially accurate map of the hypoglossal premotor circuitry. Those studies identify the primary sources of afferent inputs to the HMN as the central tegmental field, Kölliker-Fuse region, supra-trigeminal, inter-trigeminal and principal sensory trigeminal nuclei, nucleus subcoeruleus, parvicellular reticular formation, dorsal medullary reticular fields, Probst's region and the spinal trigeminal nucleus caudalis (see Appendix S1 (Supplementary Information) for a list of structural equivalents used in the Allen Mouse Brain Atlas).[52] We created a database of the genes identified as having at least a twofold greater expression in the HMN and/or PRE-HMN relative to the rest of the brain (Appendix S1 (Supplementary Information)). The mean fold-change was used in cases

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where multiple in situ hybridization experiments had been performed for a given gene.

A recent bioinformatics assessment of the 'druggability' of the human genome generated a database of all the protein targets for the drugs approved for clinical use by the U.S. Food and Drug Administration (FDA).[55] Their analysis confirmed a previously identified trend[56] that proteins targeted by approved drugs tend to cluster in four privileged families: G-protein coupled receptors (GPCRs), ligand-gated ion channels, nuclear receptors and kinases. Because, for the purposes of this review, we are interested in potential drug targets capable of modulating cellular activity to increase hypoglossal motor output, we classified the gene products in our database according to their status as modulators of neuronal activity and according to their inclusion in GPCR and ion channel protein families. These classifications were made by screening the differentially expressed mouse genes for those with human orthologues, followed by cross-referencing the resulting list with the Gene Ontology Consortium's database of Molecular Function and Biological Process annotations of the human genome[57,58] (AmiGO 2 version: 2.4.24). Final classifications were made using Boolean keyword/phrase searches of the gene ontology terms assigned to the genes in our database (the list of the inclusion and exclusion keywords/phrases used are available in Appendix S1 (Supplementary Information)). The database was screened for false-positive results by manually examining the gene ontology terms and in situ hybridization results for all differentially expressed genes classified as GPCRs, ion channels and/or modulators of neuronal activity. Finally, we cross-referenced our database with the database of protein targets and FDA-approved drugs[55] to match FDA-approved drugs to genes with enhanced expression in the HMN and/or PRE-HMN. The list differentially expressed HMN and PRE-HMN drug targets matched to FDA-approved drugs is available in Appendix S1 (Supplementary Information).

The identified candidate drug targets

We identified 1492 genes from the Allen Mouse Brain database of in situ hybridization experiments that showed enhanced expression at the HMN and/or PRE-HMN relative to the rest of the brain; gene expression in the HMN was enhanced as high as 33-fold. Of those 1492 genes, the expression of 1168 are specifically enhanced in the HMN, the expression of 88 are specifically enhanced in the PRE-HMN and 236 exhibit enhanced expression in both the HMN and PRE-HMN. A total of 99 genes were classified as being probable modulators of neuronal activity, of which 18 were classified as GPCRs and 37 were classified as ion channels (i.e. part of the privileged protein families for drug discovery). These 99 targets are listed in Table 1 and are mapped in Figure 4. Protein products of 26/99 genes are targets of 175 FDA-approved drugs. Of those drugs trialled for the treatment of OSA, a total of 9 act pharmacologically on 10 of the targets (a list of drugs trialled for the treatment of OSA[28,38,44–46] annotated with protein targets[55] is available in Appendix S1 (Supplementary Information)). Importantly, although the identified targets, or combinations thereof, may not ultimately prove effective for OSA pharmacotherapy, our analysis does reveal significant *unexplored* potential in terms of trialling approved drugs and developing new drugs for differentially expressed targets in the circuitry critical for OSA pathogenesis and that modulate Active V_0.

Figure 4 shows that the GPCR group of differentially expressed genes is associated with the greatest number of relatively specific approved drugs ('specific' in this case refers to drugs having four or less preferred protein targets). Differentially expressed ion channel genes are associated with a large number of approved drugs; however, many of those drugs are non-specific and exert their clinical effects by acting on large groups of similar ion channels (e.g. dalfampridine (4-aminopyridine)[47] acts on 40 voltage-gated potassium channel targets). Moreover, of the top 10 differentially expressed ion channel genes only 2 are associated with approved drugs (Table 1). Table 1 also includes the inward-rectifying potassium 2.4 (Kir2.4) channel (listed as potassium inwardly rectifying channel, J, 14 on line 20) that, in the brain, is expressed almost exclusively in the cranial motor pools that modulate pharyngeal muscle tone,[41,59] although there is also expression in the spinal cord and in other non-nervous tissue such as kidney and heart.

Overall, the ion channel class of targets could be a focus of drug discovery efforts for OSA pharmacotherapy. However, the lack of specific ion channel modulators can be taken as an indication of the difficulty in developing specific drugs within this protein target class. Nevertheless, the fact that Kir2.4 channels exhibit markedly lower barium sensitivity compared with other Kir channels[59] indicates that there is something structurally and/or biochemically different about Kir2.4. This difference may be tractable for medicinal chemists to develop small molecule inhibitors.

One desired outcome of an OSA pharmacotherapy would be to sustain pharyngeal muscle activity during sleep at normal waking levels. If this effect can be achieved, then the potential of therapeutic efficacy is realistic. Manipulation of certain potassium channels at the hypoglossal motor pool can activate the tongue musculature throughout sleep to waking levels.[40,42] Importantly, it has been recently shown that introducing a 'designer' receptor into the hypoglossal motor pool, and selectively modulating it with a 'designer' drug, led to significant and sustained increases in tongue muscle activity and increases in upper airway size in preclinical rodent models.[60,61] Moreover, the increases in tongue muscle activity during sleep persisted for 8–10 h, were of physiological pattern and magnitude and were specific and selective for the tongue with no effects on diaphragm or postural muscle activities or sleep–wake states.[60] These results support targeting a selective and restricted druggable target at the hypoglossal motor pool to activate tongue motor activity during sleep. Such candidate targets are present, and candidate drugs can also be explored and tested using the database of resources provided in this review (Table 1, Fig. 4 and Appendix S1 (Supplementary Information)).

Using this database of candidate drug targets

There are several factors that ought to be considered when using this database as a tool for the identification of potential drug targets for OSA. Here, we report

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Table 1 Differential expression of neuronal activity-related genes in the HMN and PRE-HMN regions

ID	Gene #	Gene name	Fold-change HMN	Fold-change PRE-HMN	FDA drug
GPCRs					
1	552	Arginine vasopressin receptor 1A	15.9	—	Y
2	257313	Urotensin II domain containing	12.6	—	—
3	1269	Cannabinoid receptor 2	6.8	—	—
4	7201	Thyrotropin releasing hormone receptor	6.3	—	Y
5	6869	Tachykinin receptor 1	5.1	—	Y
6	4160	Melanocortin 4 receptor	4.9	—	—
7	5726	Taste receptor, type 2, member 138	4.7	—	—
8	1395	Corticotropin releasing hormone receptor 2	4.2	—	—
9	8325	Frizzled homolog 8	4.2	—	—
10	10936	G protein-coupled receptor 75	4.0	2.0	—
11	148	Adrenergic receptor, alpha 1a	3.7	—	Y
12	1129	Cholinergic receptor, muscarinic 2, cardiac	3.6	2.4	Y
13	3350	5-Hydroxytryptamine (serotonin) receptor 1A	2.7	—	Y
14	3061	Hypocretin (orexin) receptor 1	—	2.5	Y
15	8811	Galanin receptor 2	2.3	—	—
16	10866	Neuropeptide FF receptor 2	2.1	—	—
17	5745	Parathyroid hormone 1 receptor	—	2.0	Y
18	3358	5-Hydroxytryptamine (serotonin) receptor 2C	—	2.0	Y
Ion channels					
19	116444	Glutamate receptor, ionotropic, NMDA3B	33.4	—	Y
20	3770	Potassium inwardly rectifying channel, J, 14	28.0	—	—
21	3773	Potassium inwardly rectifying channel, J, 16	16.1	—	—
22	51305	Potassium channel, subfamily K, member 9	12.7	—	Y
23	1184	Chloride channel 5	9.0	2.4	—
24	53405	Chloride intracellular channel 5	8.5	—	—
25	7417	Voltage-dependent anion channel 2	8.0	2.3	—
26	9127	Purinergic receptor P2X, ligand-gated ion channel, 6	6.9	2.2	—
27	59341	Transient receptor potential cation channel, V, 4	6.8	—	—
28	54499	Transmembrane and coiled-coil domains 1	6.5	—	—
29	93107	Potassium voltage-gated channel, G, 4	5.8	2.1	Y
30	2741	Glycine receptor, alpha 1 subunit	5.7	8.8	Y
31	84230	Leucine-rich repeat-containing 8 family, member C	5.2	—	—
32	610	Hyperpolarization-activated, cyclic nucleotide-gated K2	3.7	3.2	Y
33	196527	Anoctamin 6	3.7	—	—
34	8514	Potassium voltage-gated channel, shaker-related, β2	3.6	3.2	—
35	441509	Glycine receptor, alpha 4 subunit	3.5	5.6	—
36	309	Annexin A6	3.4	—	—
37	3748	Potassium voltage-gated channel, Shaw-related, 3	3.3	2.3	Y
38	9992	Potassium voltage-gated channel, Isk-related, gene 2	3.2	—	—
39	55129	Anoctamin 10	3.2	—	—
40	5026	Purinergic receptor P2X, ligand-gated ion channel, 5	3.2	2.5	—
41	56666	Pannexin 2	3.0	3.1	—
42	2697	Gap junction protein, alpha 1	2.8	—	—
43	59284	Calcium channel, voltage-dependent, gamma subunit 7	2.5	—	Y
44	6337	Sodium channel, non-voltage-gated 1 alpha	2.5	—	Y
45	348980	Hyperpolarization-activated, cyclic nucleotide-gated K1	2.4	—	Y
46	1185	Chloride channel 6	2.4	—	—
47	3738	Potassium voltage-gated channel, shaker-related, 3	—	2.3	Y
48	2705	Gap junction protein, beta 1	2.3	—	—
49	53822	FXYD domain-containing ion transport regulator 7	2.3	—	—
50	7419	Voltage-dependent anion channel 3	2.1	—	—
51	6323	Sodium channel, voltage-gated, type I, alpha	2.1	2.2	Y
52	3785	Potassium voltage-gated channel, subfamily Q, 2	2.1	—	Y
53	57113	Transient receptor potential cation channel, C, 7	2.0	—	—
54	6336	Sodium channel, voltage-gated, type X, alpha	2.0	—	Y
55	2743	Glycine receptor, beta subunit	—	2.0	—

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Table 1 Continued

ID	Gene #	Gene name	Fold-change HMN	Fold-change PRE-HMN	FDA drug
Other					
56	201780	Solute carrier family 10, 4	17.0	2.1	—
57	4129	Monoamine oxidase B	11.4	—	Y
58	706	Translocator protein	9.3	—	—
59	6522	Solute carrier family 4 (anion exchanger), member 2	6.9	2.8	—
60	83697	Solute carrier family 4, sodium bicarbonate cotransporter, 9	6.2	3.9	—
61	1244	ATP-binding cassette, sub-family C (CFTR/MRP), 2	6.1	—	—
62	284129	Solute carrier family 26, member 11	6.1	—	—
63	147798	Transmembrane channel-like gene family 4	5.8	—	—
64	60482	Solute carrier family 5 (choline transporter), 7	5.6	4.1	—
65	55089	Solute carrier family 38, member 4	4.7	—	—
66	9628	Regulator of G-protein signalling 6	4.5	—	—
67	10057	ATP-binding cassette, sub-family C (CFTR/MRP), 5	4.3	—	—
68	80243	PIP3-dependent Rac exchange factor 2	4.2	—	—
69	6569	Solute carrier family 34 (sodium phosphate), 1	4.2	2.7	—
70	7421	Vitamin D receptor	4.1	—	Y
71	9446	Glutathione S-transferase omega 1	4.0	—	—
72	84258	Synaptotagmin III	4.0	2.0	—
73	2745	Glutaredoxin	3.3	—	—
74	43	Acetylcholinesterase	3.2	2.3	Y
75	2946	Glutathione S-transferase, mu 7	3.1	—	—
76	2273	Four and a half LIM domains 1	3.0	—	—
77	11060	WW domain-containing E3 ubiquitin protein ligase 2	2.9	—	—
78	6569	Solute carrier family 6 (glycine transporter), 9	2.8	2.2	—
79	392862	Glutamate receptor, ionotropic, δ2-interacting protein 1	2.9	—	—
80	89869	Phospholipase C, zeta 1	2.9	—	—
81	140679	Solute carrier family 32, (GABA vesicular transporter) member 1	2.8	—	—
82	3799	Kinesin family member 5B	2.7	2.7	—
83	6860	Synaptotagmin IV	2.6	—	—
84	10518	Calcium and integrin-binding family member 2	2.6	2.0	
85	2539	Glucose-6-phosphate dehydrogenase X-linked	2.6	—	—
86	128414	Na^+/K^+ transporting ATPase-interacting 4	2.6	—	—
87	7915	Aldehyde dehydrogenase family 5, subfamily A1	2.5	—	Y
88	114789	Solute carrier family 25, 25	2.5	—	—
89	206358	Solute carrier family 36 (H^+/amino acid symporter), 1	2.5	—	—
90	1836	Solute carrier family 26 (sulphate transporter), 2	2.3	—	—
91	127833	Synaptotagmin II	2.3	2.2	—
92	57084	Solute carrier family 17, 6	2.3	2.9	—
93	285195	Solute carrier family 9 (sodium/hydrogen exchanger), 9	2.3	—	—
94	9725	Transmembrane protein 63a	—	2.3	—
95	9990	Solute carrier family 12, member 6	2.2	—	—
96	84679	Solute carrier family 9 (sodium/hydrogen exchanger), 7	2.2	—	—
97	81539	Solute carrier family 38, member 1	2.1	—	—
98	23315	Solute carrier family 9 (sodium/hydrogen exchanger), 8		2.1	—
99	54946	Solute carrier family 41, member 3	2.2	—	—

A list of genes having at least twofold greater expression in the HMN and/or PRE-HMN relative to the brain-at-large are classified as probable modulators of neuronal activity. Numbers under the column identification heading 'ID' refer to locations of the corresponding gene information in Figure 1. Gene identifiers under column heading 'Gene #' refer to the human orthologues of mouse genes. See Appendix S1 (Supplementary Information) for a listing of the target structures included in the PRE-HMN group as well as contrast structures used to compute expression fold-changes using the Allen Mouse Brain Atlas differential search function of RNA in situ hybridization experiments. Under the column heading 'FDA Drug', 'Y' denotes genes associated with at least one FDA-approved drug.

CFTR/MRP, Cystic fibrosis transmembrane conductance regulator/Multidrug resistance-associated protein; FDA, Food and Drug Administration; GABA: γ-aminobutyric acid; GPCR, G-protein coupled receptor; HMN, hypoglossal motor nucleus; NMDA3B, N-methyl-D-aspartate receptor 3B; PIP3, phosphatidylinositol 3,4,5 trisphosphate; PRE-HMN, premotor HMN; —, none.

differences in RNA expression determined by in situ hybridization experiments, which are not strictly indicative of differences in protein expression. Secondly, we did not screen the list of FDA-approved drugs associated with genes in our database according to the likelihood of their having clinically beneficial effects on

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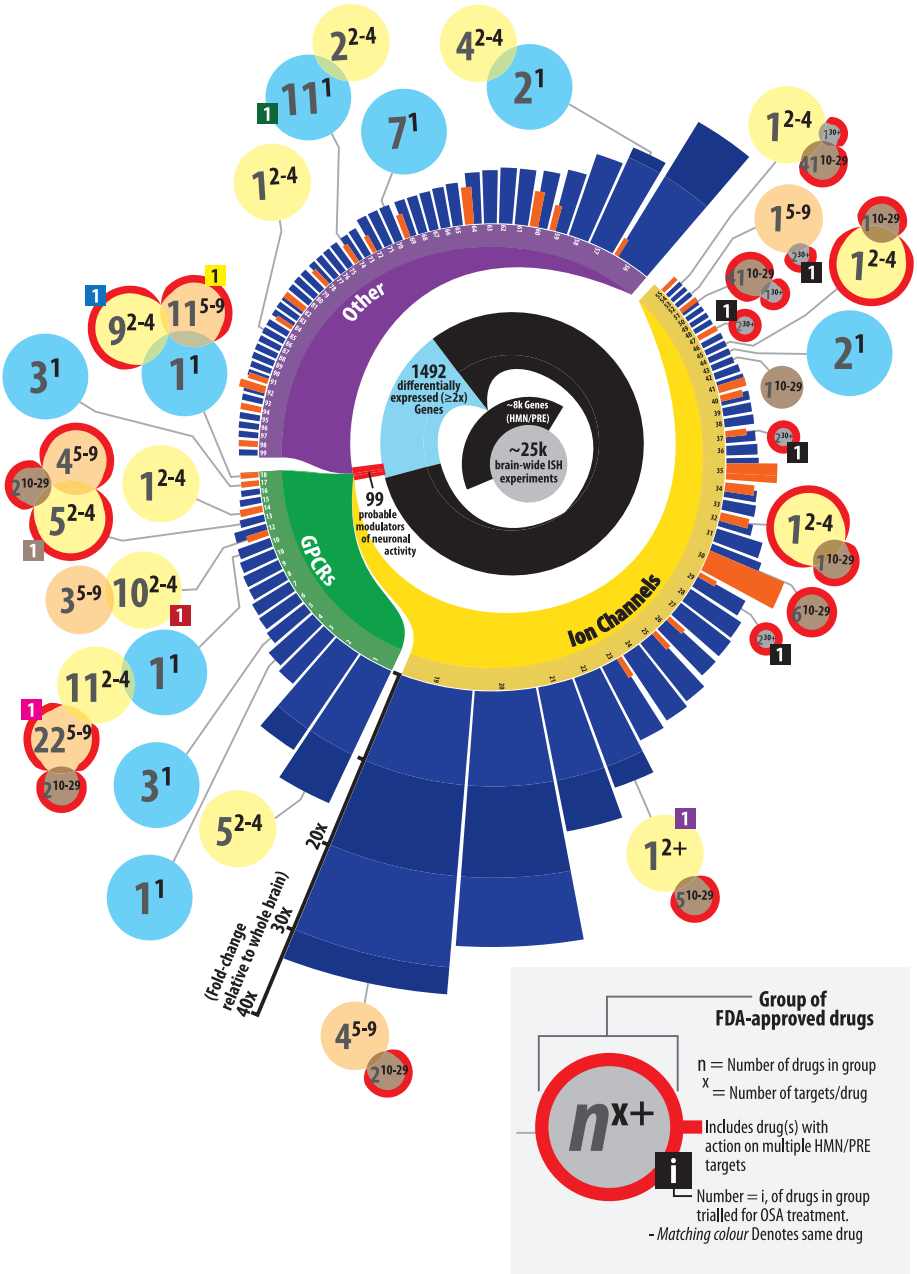

Figure 4 A map of potential drug targets differentially expressed in the circuitry controlling upper airway motor output. This figure shows a mapping of the data listed in Table 1. The numbers associated with individual bars in the radial bar chart refer to the position of the corresponding gene in Table 1. Genes associated with the FDA list of approved drugs are indicated by connections with bubble graphs. Each bubble represents a drug or group of drugs; bubble size is inversely related to drug specificity (i.e. the number of protein targets per drug). The notation in the bubbles is indicated on the figure. Fold-change in gene expression relative to the whole brain: ■, HMN; ■, group of HMN primary premotor structures (PRE-HMN). FDA, Food and Drug Administration; GPCR, G-protein coupled receptor; HMN, hypoglossal motor nucleus; ISH, in situ hybridization; PRE-HMN, premotor HMN.

hypoglossal motor output. For example, expression of the alpha 1a adrenergic receptor gene is enhanced at the HMN, and noradrenergic inputs to the HMN mediate an important component of the state-dependent modulation of tongue muscle activity.[39] We list a total of 36 FDA-approved drugs capable of targeting this receptor, 14 of which are antagonists whose direct effects on the HMN would be expected to negatively impact upper airway motor tone. Nevertheless, the number and selectivity of drugs associated with a given target is useful information, not least because it can be taken as an indicator of the relative druggability of that target. Moreover, initial studies have shown that desipramine has beneficial effects on genioglossus muscle tone, upper airway collapsibility and OSA severity.[43,44] Desipramine is a tricyclic antidepressant with strong noradrenergic and some anti-muscarinic effects. It was selected for those studies with the aim of preserving the excitatory noradrenergic tone to the pharyngeal motor pools that is normally withdrawn in sleep,[39,62] and to counter the strong muscarinic cholinergic receptor-mediated inhibition of the tongue musculature that occurs in REM sleep.[40]

This database is also an important resource for investigators working in preclinical animal models, because in the case of several targets preclinical studies will be required to evaluate their potential clinical utility. For instance, expression of the gene for 5-hydroxytryptamine 2c (5-HT_{2c}) receptors is enhanced in the PRE-HMN but not in the HMN (Table 1), yet we do not know what effect the selective agonism of these receptors would have on hypoglossal motor output, or

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if 5-HT_{2c} receptor-positive neurons innervate the HMN. Similarly, the effect of modulating the activity of urotensin II receptors on hypoglossal motor output has not been determined but would be worth investigating given that urotensin II receptor RNA shows a high degree of differential expression at the HMN (being 12.6-fold higher than for the rest of the brain), and that urotensin II receptor activation has potent excitatory effects on central cholinergic neurons.[63–65]

We recognize that, for the purposes of this review, our analysis focused specifically on identifying targets for the activation of the upper airway musculature. This focus on the circuitry critical for OSA pathogenesis and the modulation of Active V_0 is, however, only one of the several possible pharmacological strategies for the treatment of OSA. Nevertheless, consulting databases of this kind will be important regardless of the ultimate stratagem employed, because when screening candidate drugs for OSA pharmacotherapy it is prudent to consider the potential positive or negative effects of drug action on the targets present in the HMN and PRE-HMN.

FUTURE DIRECTIONS

This review identifies and builds on two major platforms that can be used to support and facilitate future research. The first platform supports the further development, refinement, automation and advanced validation of the phenotyping tools and algorithms used to identify the principal factors precipitating OSA in individual patients. As these approaches mature, the aim will be to develop practical and cost-effective phenotyping tools and devices that are amenable to broad and routine use by sleep researchers and clinicians.

The second major platform is the resource of candidate targets and drugs that was generated for this review (Table 1, Fig. 4 and Appendix S1 (Supplementary Information)). These resources are made freely available to explore, select and test such candidates in preclinical experimental models and early phase clinical studies. The resources include identifying differentially expressed genes at critical sites in the upper airway motor circuitry (HMN and PRE-HMN), as well as FDA-approved drugs associated with the differentially expressed genes that are classified as probable modulators of neuronal activity, and drugs that have undergone trials for the treatment of OSA and their protein targets. It is our hope that this resource will not only be widely accessed but also shared to spur new discovery science for OSA pathophysiology and possible pharmacotherapy. Moreover, the resource of candidate targets and drugs for the HMN and PRE-HMN may also be more broadly applicable to other disorders of upper airway motor function such as motor neuron diseases and dysphagia.

Overall, this review identifies a rational decision-making strategy with the necessary logical underpinnings that any reasonable approach would be expected to navigate to develop a viable pharmacotherapy for OSA. It can also be used to identify the critical barriers that have to be overcome for any success of a potential OSA pharmacotherapy, and to understand why previous attempts at OSA pharmacotherapy have largely been ineffective for predictable reasons. In the *Introduction* Section of this review, we stated: *There is currently no pharmacotherapy for OSA. However, there is no principled a priori reason why there shouldn't be one—unless the biology simply cannot support it because of the complexity and individuality of OSA pathogenesis.* The field is at the stage now—with the tools, barriers and candidate targets and drugs identified—to now design and perform experiments and clinical trials to critically test how amenable the upper airway motor circuitry and OSA may be to pharmacotherapy.

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Supplementary Information

Additional supplementary information can be accessed via the *html* version of this article at the publisher's website.

Appendix S1 This file contains the database of differentially expressed hypoglossal motor nucleus (HMN) and premotor HMN (PRE-HMN) genes, drugs and drug targets. The database is separated into four parts on separate spreadsheets: (i) differentially expressed HMN genes; (ii) differentially expressed PRE-HMN genes; (iii) Food and Drug Administration (FDA)-approved drugs associated with differentially expressed HMN and PRE-HMN genes classified as probable modulators of neuronal activity; and (iv) drugs that have undergone trials for the treatment of obstructive sleep apnoea and their protein targets. Gene expression data were obtained from the Allen Mouse Brain Atlas database of in situ hybridization experiments.[51,52] Parts (i) and (ii) include a listing of the target and contrast structures used for the differential searches. Biological process and molecular function gene annotations are from the Gene Ontology Consortium's AmiGO 2 database[57,58] (AmiGO 2 version: 2.4.24). Protein target annotations of FDA-approved drugs are from the Santos *et al.*'s database.[55]

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